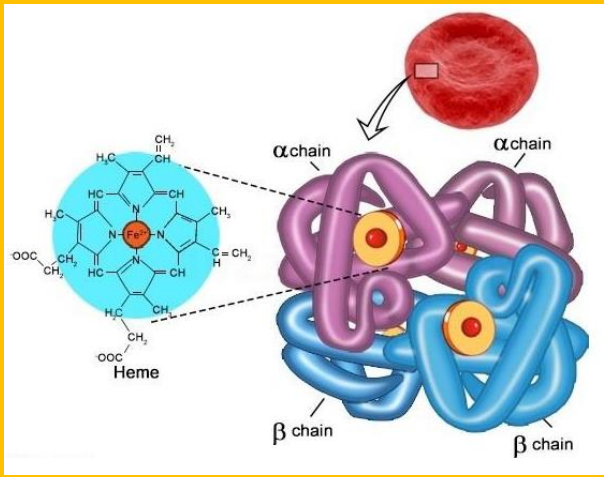
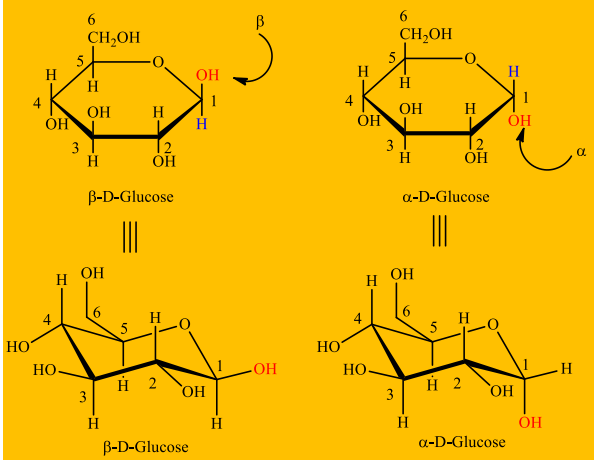
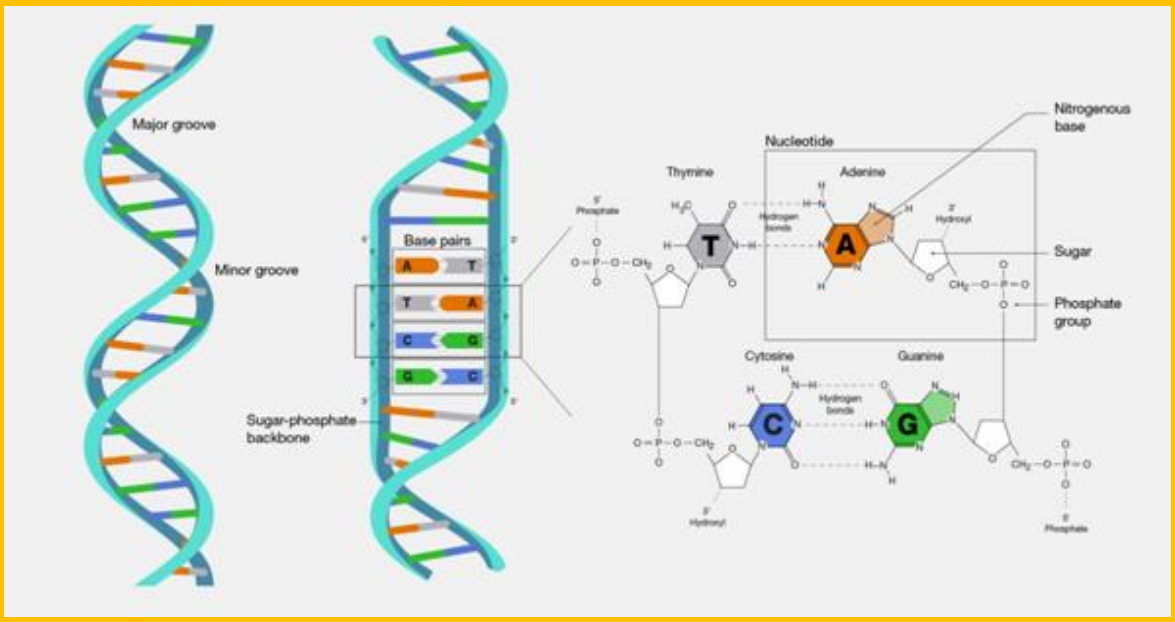
MSCCH-603

M.Sc. III Semester

BIO-INORGANIC, BIO-ORGANIC AND BIO-PHYSICAL CHEMISTRY







SCHOOL OF SCIENCES

DEPARTMENT OF CHEMISTRY

UTTARAKHAND OPEN UNIVERSITY

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BLOCK I: BIOINORGANIC CHEMISTRY

UNIT 1: METAL STORAGE AND TRANSPORT

CONTENTS:

1.1 INTRODUCTION

Living organisms store and transport transition metals both to provide appropriate concentrations of them for use in metalloproteins or cofactors and to protect them selves against the toxic effects of metal excesses; metalloproteins and metal cofactors are found in plants, animals, and microorganisms. The normal concentration range for each metal in biological systems is narrow, with both deficiencies and excesses causing pathological changes. The storage of transition metals and the creation of transporter molecules are not carried out by all types of cells in multicellular animals made up of a range of specialized cell types, but by particular cells that specialize in these functions. Metals are constantly ionic, although their oxidation states can change based on biological demands.

In order of decreasing abundance in living organisms, the transition metals iron, zinc, copper, molybdenum, cobalt, chromium, vanadium, and nickel are important for biological storage and transport. Despite the fact that zinc is not exactly a transition metal, it has many bioinorganic characteristics with them and is treated as such in this chapter. Iron storage and transportation are better understood than any other metal in the group. The goal of biochemistry is to use chemical principles to explain biological structure and function. Biomolecules are carbon-based compounds with various functional groups, therefore the chemistry of living organisms is centered on carbon. Carbon makes up more than half of a cell's dry weight. Hydrogen, oxygen, nitrogen, phosphorus, and sulfur are the other five most prevalent elements. Although organic chemistry is more commonly linked with molecular biology, inorganic elements and metal ions are equally crucial in biological processes. The importance of platinum in anticancer medicines, metals as natural components of proteins, metalloproteins, and mettaloenzyme in life processes are all significant.

In biology, alkali and alkaline earth ions, particularly Na^+, K^+, and Ca^{2+}, have a role in triggering cellular responses. Two of their critical activities are the fast influx of sodium ions across the cell membrane to fire neurons and the control of intracellular activity by calcium-binding proteins like calmodulin. Other activities that are critical for the organism's survival include bone calcification, blood coagulation, acid-base equilibrium, fluid balance, and osmatic control. Calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur are the seven major elements that make up roughly 70% of the inorganic substance in the human body. Iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium,

and chromium are all important trace elements. Some other trace elements like, nickel, vanadium and barium may be possibly essential in biological systems.

1.2 OBJECTIVE

The objective of this unit, you will be able to-

(i) What is the hemoglobin, its function and its structure

(ii) What is the myoglobin, its function and its structure

(iii) You will be able to function of ferrin and transferritin

(iv) After reading this unit you will be able to about photosynthesis process

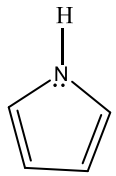
(v) And end of this unit will be able to what is nitrogen fixation

1.3 ELEMENTS IN LIVING SYSTEMS

The living matter is made up of six elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur (which make up roughly 90% of the dry weight of the human body). Other physiologically significant elements are Ca, K, Na, Cl, Mg, Cu, Co, I, Zn, F, Mo, and Se. Their body composition varies greatly, for example, Ca makes up about 2% of body weight whereas Co makes up only 0.00004 percent.

1.4 PORPHYRIN RING

A porphyrin is a large ring molecule made up of four pyrroles (smaller rings made up of four carbons and one nitrogen which is a heterocyclic compound (Fig.1.1). These pyrrole molecules are linked by a chain of single and double bonds, forming a huge ring.



Pyrrole ring

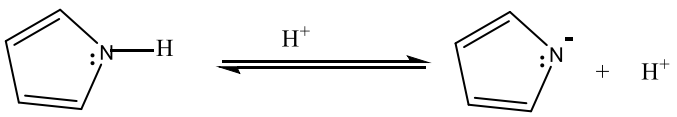
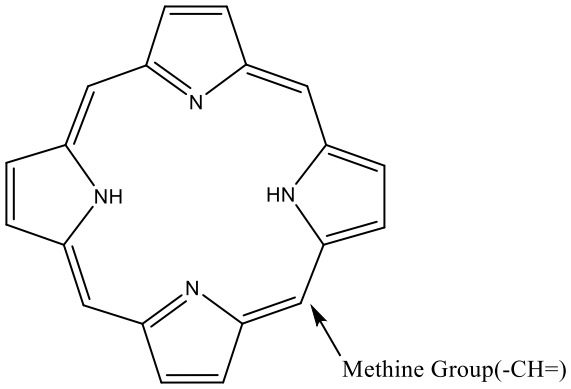


Fig. 1.1 Pyrrole ring

A tetrapyrrole is the technical term for four pyrroles linked together. The ring is flat in space and has a reasonably even distribution of electrons around its diameter. As a result, a porphyrin is classified as aromatics (Fig.1.2). A porphyrin molecule is highly stable in this shape. Porphin is the model of a universal porphyrin.



structure of porphyrin ring

Fig 1.2 Structure of porphyrin ring

The porphyrin pathway is found all across the biological world, acting as an assembly line for nature's most prevalent colors in both plants and animals. Porphyrin molecules are ring-shaped molecules that bind a variety of metal ions, each of which has a particular biological function. Chlorophylls bind magnesium, which is essential for photosynthesis. Heme binds iron to coordinate molecular oxygen and carbon dioxide transport, maintains the electron transport chains required for cellular respiration, and aids numerous enzymes in catalysis. Porphyrins bind nickel to produce coenzyme F430, which plays a crucial role in methane metabolism in bacteria (Fig.1.3).

Vitamin B_{12} is produced when cobalt binds to a porphyrin derivative; a deficiency in the vitamin can cause pernicious anemia and impair brain and nervous system function. These porphyrin-derived pigments can be referred to as the "colors of life" since they are required to maintain essential functions in virtually all organisms. Protoporphyrin, commonly known as heme, is an iron-containing molecule that plays a vital role in many biological processes binding of moleculer oxygen to its iron ion. Hememolecules' capacity to function depends on the ability of certain proteins, known as hemeproteins, to attach to them. The remarkable heme-iron coupling with gas molecules or particular amino acids is responsible for heme's natural action.

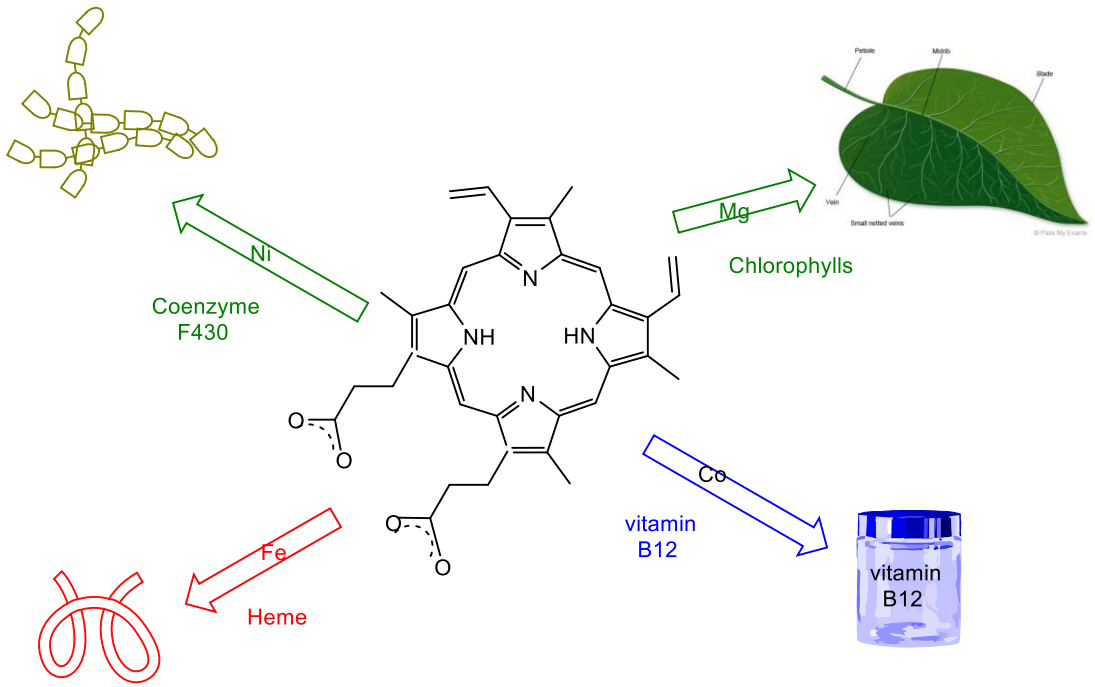


Fig 1.3 Combination of porphyrin ring with different type metal ions.

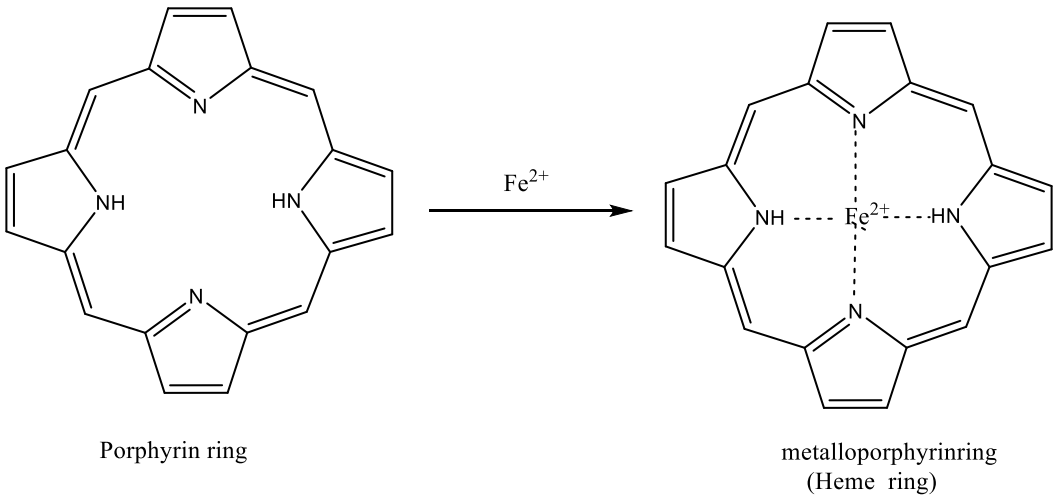


Fig 1.4 Formation of heme group

Other metal-containing protoporphyrins are not as common as the iron-containing protoporphyrin. Although there are many distinct types of hemeproteins (Fig.1.4) with various functions, such as hemoglobins, myoglobins, cytochromes, and peroxidases.

Hemeproteins are involved in all major electron transfer (ET) chain processes in nature, the most notable of which are our mitochondria's aerobic respiration system and chloroplasts' photosynthetic system. The redox of the Fe ion within the heme (Fe^{2+} , Fe^{3+}) shuttles electrons in ET processes, and its redox potential is strongly reliant on the heme-binding site within the protein.

key point

- *Porphyrin is a macrocyclic compound. Structurally, porphyrin ring consists of four pyrrole ring.*
- *Pyrrole ring is five membered heretocyclic compound consisting one nitrogen atom and four carbon atom.*
- *In porphyrin ring four pyrrole ring connected with each other by methine (-CH=) group.*
- *Porphyrin ring act as both diacidic as well as dibasic in nature.*
- *Porphyrin ring contain eleven (11) conjugative double bond.*
- *With a total of 26 pi-electrons, of which 18 pi-electron form a planar, continuous cycle, the porphyrin ring structure is often described as aromatic.*
- *When porphyrin ring combine with any metal (Fe, Co, Mg,) is known as metalloporphyrin.*
- *When Fe bind with porphyrin then it is kwon as heme, which is compound in red blood cells, participate in the oxygen storage and transportation.*
- *The electronic transition in porphyrin ring is $\pi - \pi *$.*
- *The complexes which containing porphyrin ring colour appears due to $\pi - \pi *$ electronic transition.*

1.5 METALLOPORPHYRIN

The metalloporphyrins include two important categories: the chlorophyll molecule and the molecules carrying the heme group. The ability of chlorophyll to absorb light is related to the conjugated polyene structure of the porphyrin ring. Magnesium ions that are coordinated to

the nitrogen atoms of the four pyrrole rings have at least two functions. They provide the necessary structural rigidity and they increase the rate of conversion of the singlet-excited state resulting from photon absorption into the triplet state that enables the transfer of the excitation energy into the redox chain. The two main functions of heme iron-containing proteins are the transport of oxygen and the mediation of electron transfer reactions. The heme group is in all cases associated with a protein molecule, as in hemoglobin, myoglobin, cytochromes, and enzymes such as catalase and peroxidase.

1.5.1 Some point noted about metalloporphyrin are:

i. Metalloprotein are cyclic structure or closed ring structure which is a micro cyclic compound.

ii. Metal ion that their size match with the cavity, fit inside the cavity.

iii. Matelloporphyrin ring the number of conjugated double bond is 11.

iv. Mattelloporphyrin ring is planar, rigid and conjugative hence it is aromatic compound because is obey huckel rule.

1.6 ROLE OF IRON IN LIVING SYSTEM

Iron is crucial for every kind of living organism, including plants, bacteria, animals and humans, to transport oxygen (through the haemoglobin in animals and humans) and to produce energy (through electron transfer in the mitochondrial respiratory chain). In living system iron is found in following types of compounds.

(a) Compound containing one or more heme group e.g. haemeglobin, myoglobin, cytochromes, cytochrome P450.

(b) Iron sulpher protein e.g. rubredoxin, ferridoxin, nitrogenase.

(c) Di-iron oxo bridged compound e.g. haemerythrene, ribonucleotide etc.

1.7 METAL STORAGE TRASNPORT AND BIOMINIRALISATION FERRITIN, TRANSFERRIN AND SIDERROPHORES

1.7.1 Introduction

In living organisms, particular organic surfaces of proteins and/or lipids are responsible for the synthesis of minerals with certain shapes and compositions. Biomineralization is a natural process that occurs in many places, including the ocean (Ca is involved in shell formation). The development of the ferritin core is another example. Ferritin is the primary non-haem Fe storage protein in mammals. Remember that the majority of Fe is found in haemoglobin and myoglobin. When ferritin is completely loaded, it comprises roughly 20% Fe by mass. No additional iron can enter the cell after the ferritin is saturated. Techniques exist in organisms to not only store and accumulate metal ions, but also to utilise them later. To transport iron, various complexion agents are utilised within the organisms.The iron binding protein transferrins transports iron through the bloodstream of higher animals. Transferrins transfer iron from the site of production of other iron-containing compounds, such as haemoglobin and chromosomes, to the porphyrin ring, where it is inserted by enzymes. Iron storage is important because it is required and utilised in significant amounts by cells, and inorganic iron is stored in intestine mucousal cells linked to the intracellular protein ferritin.

1.7.2 Storage of iron-ferritin

(i) Role of ferritin

The French scientist Laufberger discovered ferritin in 1937 when he extracted a novel protein from horse spleen that contained up to 23% iron by dry weight. Iron is stored mostly in ferritin, an iron storage protein. For the production of haemoglobin, myoglobin, and cytochrome, ferritin release tron is required. This is important because unbound free iron is extremely toxic and causes cellular damage by promoting the production of free radicals.In mammalian tissues such as the liver, spleen, and bone marrow, ferritin is a significant iron storage.

(ii) Structure of Ferritin

Inside and outside of cells, ferritin is an iron-binding protein. Apoferritin, as shown in the picture (Fig. 1.5), creates a roughly spherical container within which ferric iron is kept as the ferrihydrite mineral ferric iron (Apoferritin refers to the iron-free form of the protein; the iron-containing form is termed holoferritin or simply ferritin).

There are 24 subunits in the apoferritin shell. The two sorts of subunits are H and L. The ratio of these subunits varies a lot depending on the tissue type, and it can alter a lot under inflammatory and infectious situations. H-subunit-rich tissue ferritins (found primarily in the heart and kidney) to L-subunit-rich tissue ferritins (found predominantly in liver and spleen). Each apoprotein molecule has a diameter of around 450,000 d. The L monomer is made up of 174 amino acids and has a molecular weight of 174. The H monomer has a molecular mass of 21,000 d and is made up of 182 amino acids.

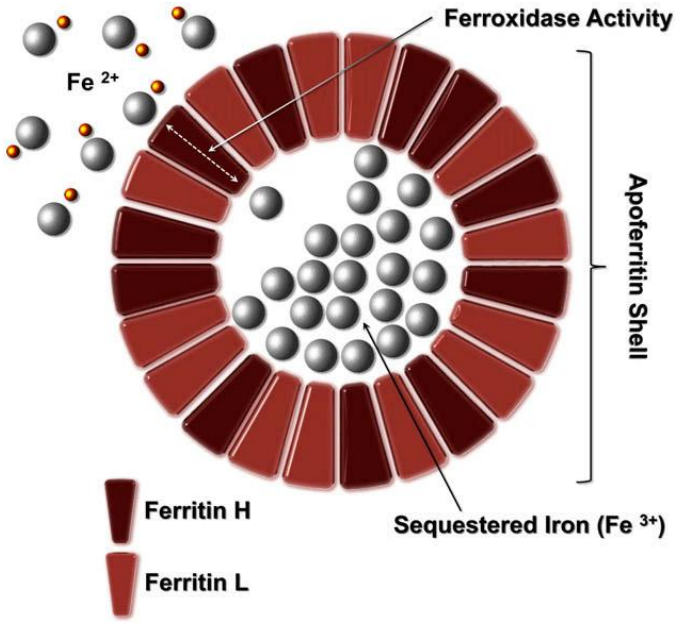


Fig 1.5 Structure of ferritin

1.7.3 Transport of iron-transferrins

Several structurally important and related Fe-protrins, all of which are glycoproteins, make up transferrins. Their molecular weights (molar masses) are approximately 80 k Da. New haemoglobin is produced in the bone marrow, while old red cells are destroyed in the spleen and liver. Transferrins [as a class of protein, serum protein, lactoferrin (milk), and ovatransferrin (egg)] are iron binding proteins that transport iron in higher animals via the bloodstream to the site of synthesis of other iron-containing compounds such as haemoglobin, cytochrome, and others, where it is inserted into the porphyrin ring via enzyme.

Key point

- *Transferrin binds Iron is in +3 oxidation stste.*
- *Fe^{2+} is oxidation to Fe^{+3} by ceruloplasmin (a blue copper protein).*
- *Transferrin binds Fe^{3+} (from stomach) and enters into blood and transport Fe^{3+} to bone marrows. In bone marrow, Fe^{3+} is reduced to Fe^{2+} and is delivered to ferritin. Before and after binding in transferrin, Fe in +2 oxidation state when binds to transferin, Fe in +3 oxidation state.*
- *Transferrin delivers ions when it is reduced to Fe^{+2} in bone marrow, Fe^{+2} is further oxidation by ceruloplasmin (ferroxidease) to Fe^{+3} and pickup by ferritin (ferritin find in bonemarrow, spleen and livers.*
- *Ferritin contain two compound,*

  I. Apoferritin- 24 protein chain each with 175 amino acid.

  II. Iron core- it contain 4500 Fe^{+3} ions.
- *Ferritin release iron for the synthesis of hemoglobin, myoglobin and cytochromes.*
- *Transferrin is the best scavengers of iron in the animals.*

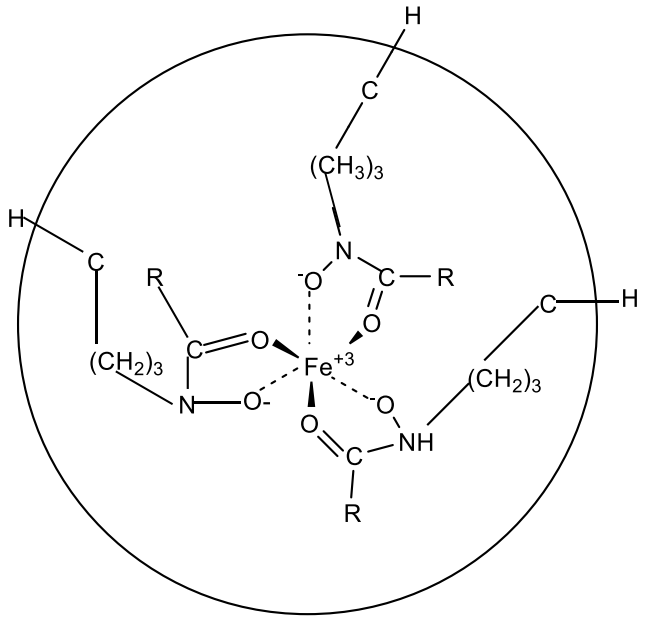
1.7.4 Siderophores

Despite the fact that iron in its ferric form is poorly soluble, it is necessary for microbial growth. As a result, most bacteria produce and release siderophores, which function as high-affinity chelators that enable iron into their cells. The production of siderophores is one of the most common strategies used by bacteria, fungi, and plants (from the Greek: "iron carriers"). Siderophores (400–2000 Da) are natural iron chelators with a low molecular weight. They are produced in the presence of an iron shortage to scavenge iron and form soluble iron(III) complexes. They are involved in pathogenic bacteria taking iron from host proteins because they allow organisms to access insoluble iron forms. Siderophores (400–2000 Da) are natural iron chelators with a low molecular weight. They are produced in the presence of an iron shortage to scavenge iron and form soluble iron(III) complexes. They are involved in

pathogenic bacteria taking iron from host proteins because they allow organisms to access insoluble iron forms. Iron is an essential component for almost all microorganisms.

In mammals, the serum proteins ferritin and transferrin bind and transport iron. Because microorganisms are unable to bisynthesize high-molecular-weight complex proteins, they gather iron from their surroundings via siderophores, solubilize and transport iron (III) by forming extremely stable octahedral complexes with Fe(III), and finally transport iron across the cell membrane.

The following considerations should be taken into consideration:



A ferrichrome complex showing the iron binding with three hydroxamate moieties. the structure of the cyclic peptide is shown by a circle ignoring peptide group for clarity.

Fig. 1.6 Structure of siderophores

- The siderophore is a chelating ligand that provides Fe(III) with a set of oxygen donors, and the complexes are considered to have six coordinates (III).
- Based on their chemical structure and how they chelate iron, siderophores are divided into various groups. A desferrichrome (Fig 1.6), for example, is a cyclic hexapeptide with three consecutive amino acid residues carrying side chains that terminate in hydroxamine moieties.

1.8 INTRODUCTION: IRON - CONTAINING PROTEINS WITH PORPHYRIN LIGAND SYSTEMS

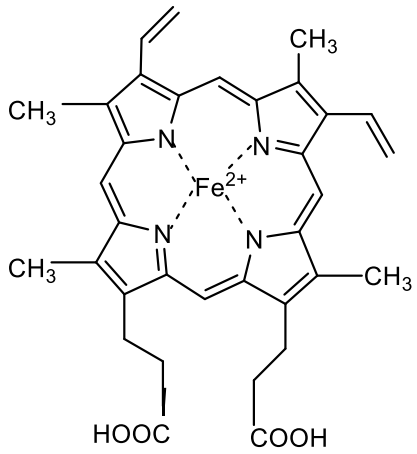
Iron is the most abundant transition metal found in biological systems with a percentage by weight in the human body - for instance, 5.0×10^{-3} %. Therefore, it is not surprising that iron - containing proteins and enzymes are found in huge numbers and varieties in all biological species. First, one might classify iron - containing species in two categories: those containing a porphyrin ligand system an iron containing heme moiety and those not containing porphyrin ligands non heme iron containing proteins.

1.9 HEAMOGLOBIN

Haemoglobin (molecular wieght 645000) can be consider an oxygen carrier in animals. In most of the animals, it is the pigment to provide colour of blood. The colour of blood is red if haemoglobin is present with oxygen. In the absence of oxygen in haemoglobin the colour of the blood is blue. It is due to the transfer of electron in between the π and π^* orbital of the ring and iron atom (Fig. 1.7). In human body near about 4 g iron is present. Out of thin approximately 0.8 g is used to produce red colour in RBC by haemoglobin. Remaining iron is store in the form of ferretin. Hemoglobin provides an excellence example of the Quaternary structure of a protein (association of two or more peptide chains in the complete protein). Hemoglobin is a collection of four heme units each bound to each of the four protein chains. Thus, there are four sub-units in hemoglobin two each of two slightly different peptide chains. These are held together by electrostatic and van der Waals force as well as hydrogen bonding. In each subunit of hemoglobin the heme prosthetic group is held in possition by the imidazole of histidine in the globin protein chain just as in myoglobin through a coordination histidine nitrogen atom.

1.9.1 Properties hemoglobin

- There are 5 billion red cells/Ml, 280 million Hb molecules/red cell, and 4 heme groups Hb molecule.
- Similar to Mb, each heme is tightly bound to a protein (globin) through about 80 hydrophobic interactions and a single coordinate bond between imidazole of the proximal histidine and Fe(II).
- Molecular weight 64,500, tetramer.
- Human Hb has four chains, $\alpha 2\ \beta 2$, two identical chains labeled α, with 141 amino acids, and two identical β-chains, with 146 amino acids.



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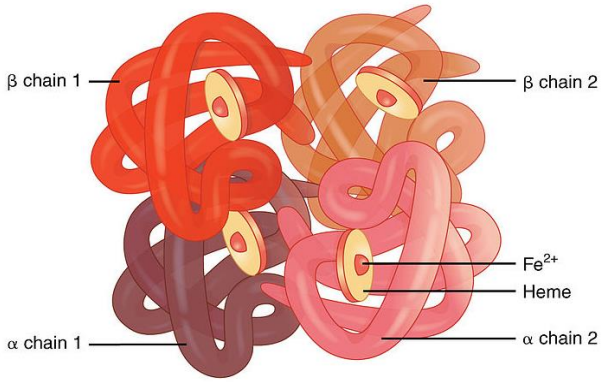

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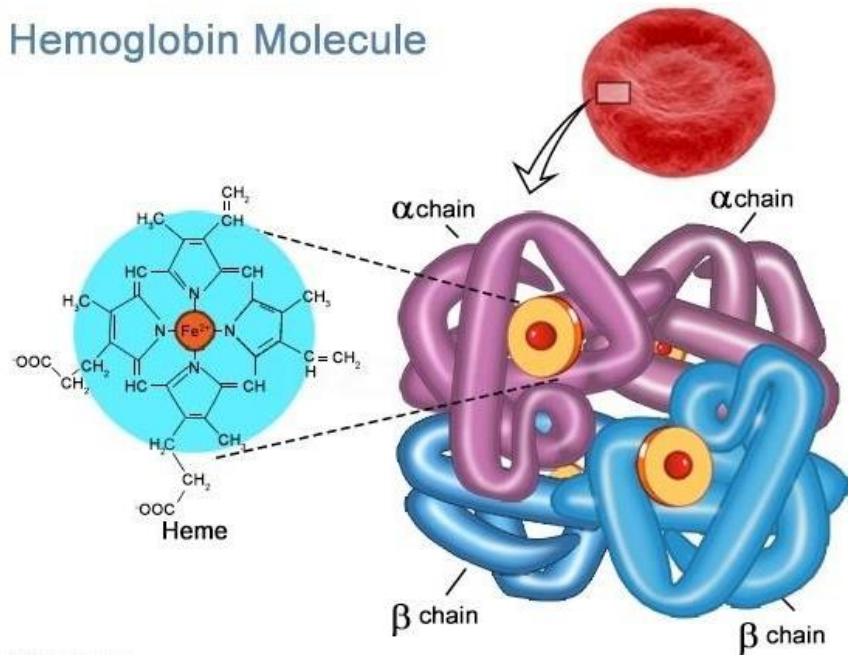


Fig. 1.7 structure of hemoglobin

1.9.2 Biological roles of the hemoglobin

(i) Hemoglobin binds O_2 to its heme iron at the lungs and delivers O_2 to myoglobin, which stores the O_2 until it is required for metabolic oxidation.

(ii) A second task of Hb is to bring the CO_2 by-product of oxidation back to the lungs to get rid of it:

$$R{-}NH_2 + CO_2 \longrightarrow R{-}NH{-}COO^- + H^+$$

CO_2, also buffering, as a result of the reversible formation of HCO3.

$$CO_2 + H_2O \longrightarrow HCO^-_3 + H^+$$

The function of hemoglobin is to take up oxygen in the lungs, and transport it to the various tissues in the body (Fig. 1.8).

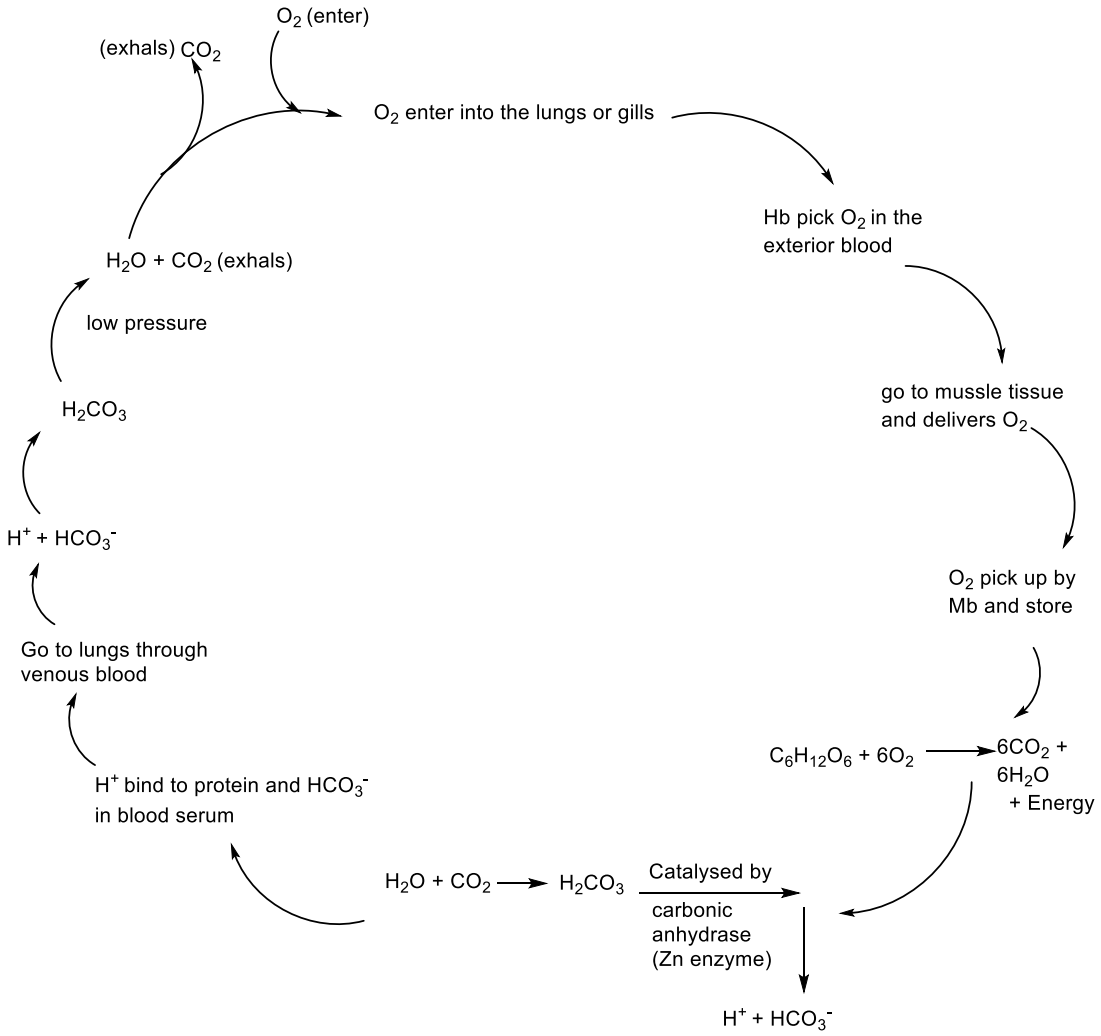


Fig 1.8 Mechanism of transportation of oxygen through hemoglobin

Myoglobin, present in muscle tissues, takes up the oxygen from the hemoglobin and stores it until it is needed for the production of adenosine triphosphate (ATP) in the mitochondria. One oxygen molecule binds to the iron atom of the heme moiety at the sixth position which is vacant in the deoxygenated form of these proteins.

1.9.3 Function of Hemoglobin in the Human Body

Following types function of the hemoglobin in human body are:

1. Hemoglobin is an oxygen carrier.

2. Hemoglobin is a carbon dioxide carrier.

3. Hemoglobin gives the red color to blood.

4. Hemoglobin maintains the shape of the red blood cells.

5. Hemoglobin acts as a buffer.

6. Hemoglobin interacts with other ligands.

7. Hemoglobin degradation accumulates physiologically active catabolites.

1.9.4 Structure of the hemoglobin

Hemoglobin is a conjugated protein. Its molecular weight is 64500. it is chromo protein. In this globin and heme two substances are present. Heme is metalloporphyrin and globin protein.

Heme + globin

Molecular formula of hemoglobin $C_{34}H_{32}N_4O_4Fe$. In the structure of hemoglobin two carcoxylic and two ethylenic grioup present. All the four pyroole ring in heme are conneced to ecah other by methine an (-CH=) group. All the four pyrrole ring has one methyl group each and iron present in hemeglobin to the centre of the heme.

Hemoglobin comprises four subunits, each having one polypeptide chain and one heme group (Fig 1.9). All hemoglobins carry the same prosthetic heme group iron protoporphyrin IX associated with a polypeptide chain of 141 (alpha) and 146 (beta) amino acid residues. the ferrous ion of the heme is linked to the N of a histidine.

The porphyrin ring is wedged into its pocket by a phenylalanine of its polypeptide chain. The polypeptide chains of adult hemoglobin themselves are of two kinds, known as alpha and beta chains, similar in length but differing in amino acid sequence. The alpha chain of all human hemoglobins, embryonic and adult, is the same. A expanded view of the Heme group reveals that it consists of an atom of ferrous iron (Fe^{2+}) and a surrounding porphyrin ring (four nitrogen-containing pyrrole molecules). The iron atom can reversibly bind with one

molecule of oxygen (O_2). Hemoglobin (Hb) transports oxygen, in blood, taking dioxygen from air in the lungs and delivering it to Mb in tissues. Hemoglobin is a multisubunit protein, with two *a* and two *p* polypeptide chains.Life of the hemoglobin molecule is approximate 16 week.

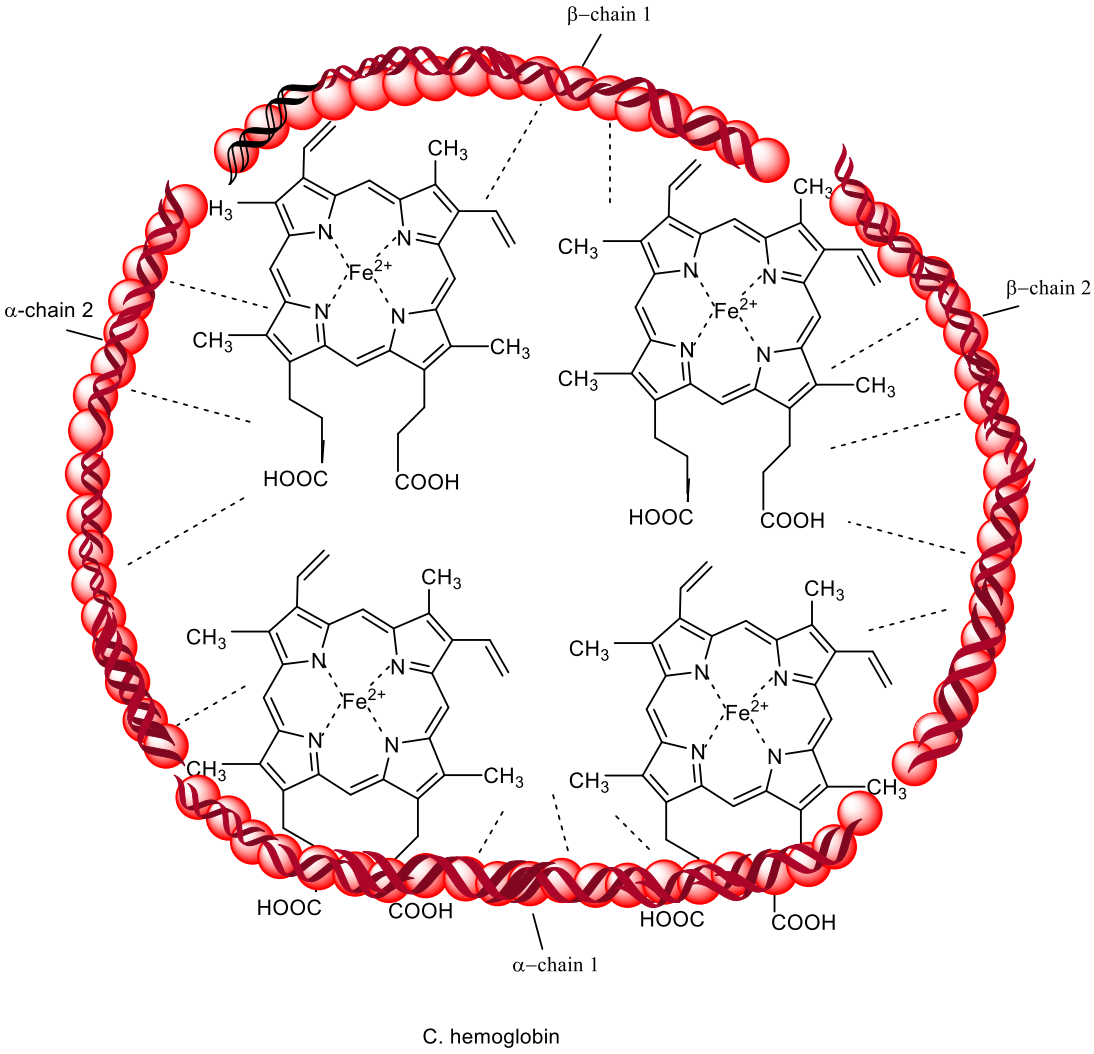


Fig 1.9 Structure of hemoglobin

The main role of the protein chain around the hemoglobin molecule and it provide the hydrophobic environment around of the Fe^{2+} ions and prevent it contact with water and its oxidation.Hemoglobin is present in human body in the two form:

Deoxyhemoglobin form of hemoglobin without oxygen, the predominant protein in red blood cells. Hemoglobin forms an unstable, reversible bond with oxygen. In its oxygen-loaded form it is oxyhemoglobin and is bright red. In the oxygen-unloaded form it is called deoxyhemoglobin and is purple-blue. In each chain there is an iron protoporphyrin IX

group held by a proximal histidine imidazole residue, as in Mb. In deoxy Hb, the iron lies 36 pm to 40 pm (Fig 1.10) out of the porphyrin ring plane but moves within 12 pm of the plane upon binding of dioxygen.

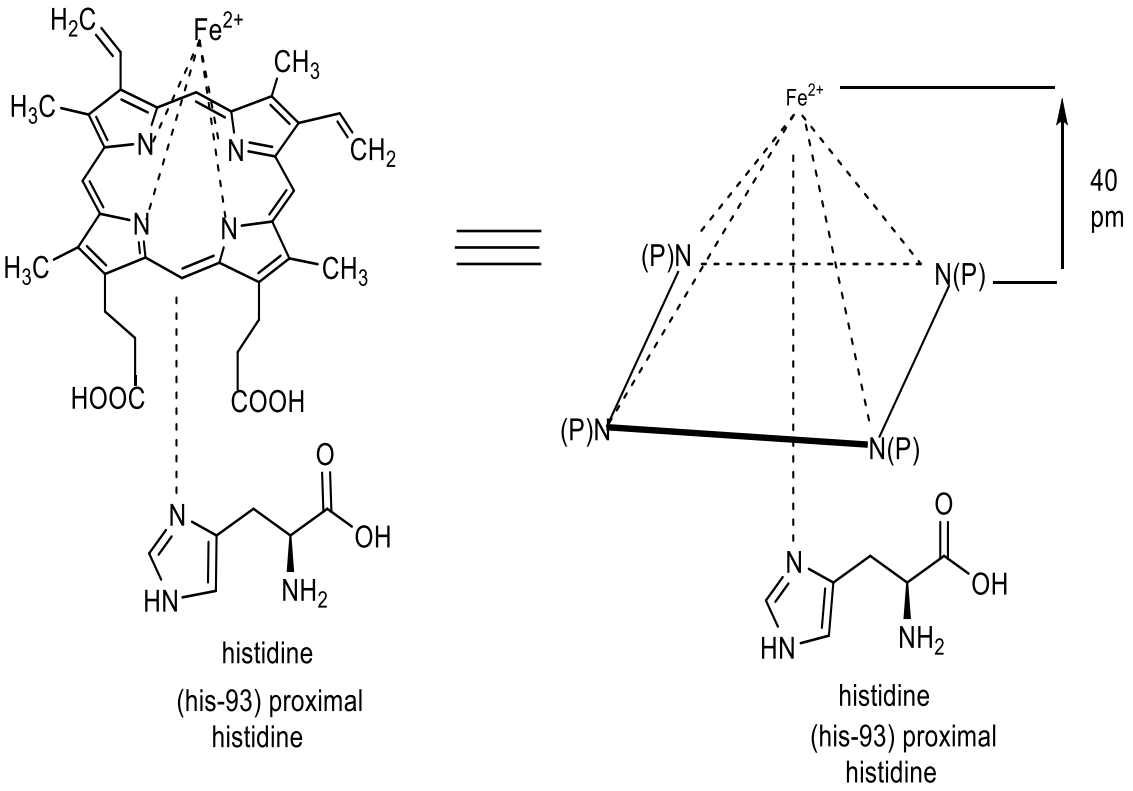


Fig. 1.10 Dome structure or square pyramidal

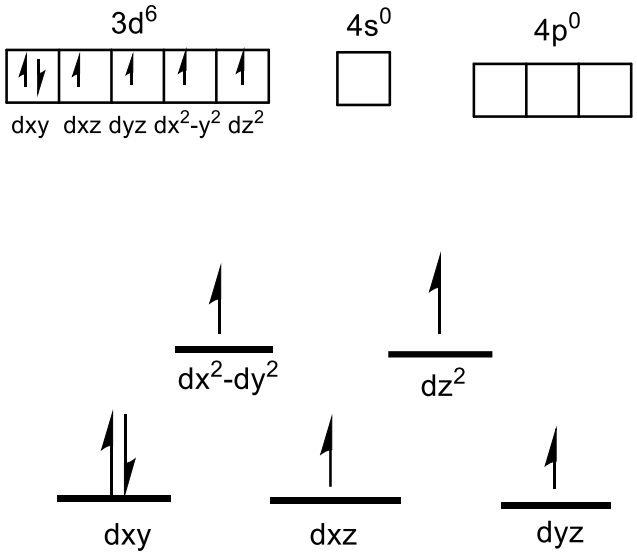
In deoxyhemoglobin (deox-Hb) the iron is coordinated to only five ligand since oxygen is not present. Thus in deoxy Hb and deoxy Mb has a pentacordinated iron (II) centre in the high spin state [iron (II) in d^6], with high spin (the square pyramidal arrangementof heme in myoglobin and hemoglobin can be considered an octahedron without the sex liigand) and therefore, the iron atom will not fit into the hole of the porphyrin ring (Fig. 1.11).

Electronic configuration of iron in free state

$Fe_{26} = 1s^2, 2s^2 2p^6, 3s^2 p^6 3d^6, 4s^2 4p^0$

Electronic configuration of iron in heme molecules

$Fe^{2+}_{26} = 1s^2, 2s^2 2p^6, 3s^2 p^6 3d^6, 4s^0 4p^0$



high spin complexes s= 2

2s+1 = 2 X 2 +1= 5

spin multiplicity(2s+1) = 5

Fig 1.11 high spin model of hemoglobin

In the case of deoxy Hb, the iron atom contain four unpaired electrons (scheme) which are repulsed by the non-bonded electron of the nitrogen atom (nitrogen atom of pyrrole ring), hence the iron atom in deoxy Hb molecules is 40 pm above the four pyrrole ring (porphyrin ring). Deoxy hemoglobin are the paramagnetic in nature due to the presence of four unpaired electron and their magnetic moment is 4.9 which measures by $\mu=\sqrt{n(n+2)}$ where n = Number of unpaired electrons

$$\mu=\sqrt{4(4+2)},$$

$$\mu=\sqrt{4(6)} = 4.9$$

Oxyhemoglobin the molecule of oxyhemoglobin, like that of carbonmonoxyhemoglobin, is found to have zero magnetic moment and to contain no unpaired electrons. Each iron atom is accordingly attached to the four porphyrin nitrogen atoms, the globin molecule, and the oxygen molecule by covalent bonds. In oxy Hb no unpaired present in the iron atom due to presence of strong field ligand (O_2), hence iron atom co-planner with the all four pyrrole ring (Fig 1.12)

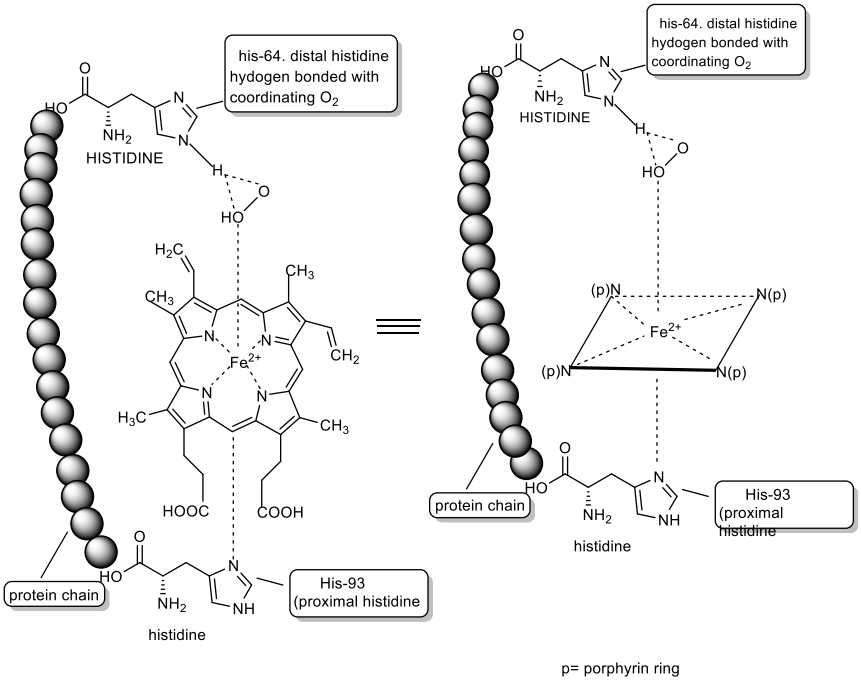


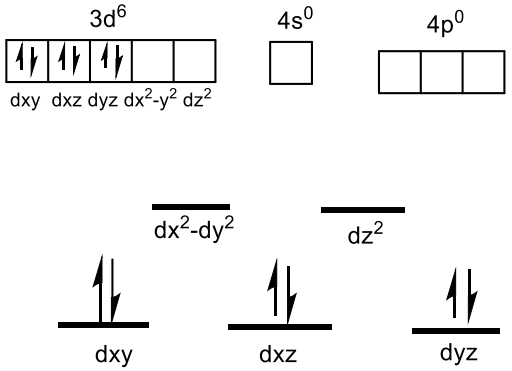
Fig. 1.12 Structure of the Oxyhemoglobin

Electronic configuration of iron in free state

$Fe_{26} = 1s^2, 2s^2 2p^6, 3s^2 p^6 3d^6, 4s^2 4p^0$

Electronic configuration of iron in heme molecules

$Fe^{2+}{}_{26} = 1s^2, 2s^2 2p^6, 3s^{23} p^6 3d^6, 4s^0 4p^0$



low spin complexes s= 0

2s+1 = 2 X 0+1= 1

spin multiplicity(2s+1) = 1

Fig 1.13. Low spin model of hemoglobin

In both Hb and Mb, there is a histidine situated in the Oxygen,-binding pocket on the side away (distal side) from the coordinated imidazole base. X-ray and, for oxy myoglobin, neutron diffraction studies indicate the formation of a hydrogen bond between the coordinated dioxygen molecule and the N-H proton of the distal histidine residue. This Fe-O-O-H-N unit is nicely accommodated, owing to the angular bend at the coordinated dioxygen atom. In carbon-monoxide adducts of Mb and Hb, however, the X-ray data reveal a distorted structure in which the Fe-CO angle is either bent or, more likely, tilted off the proximal histidine N-Fe bond axis to avoid steric clash with the distal histidine. Thus nature has cleverly tailored the iron- porphyrin centers in Hb and Mb to bind O_2, rather than its toxic surrogate CO, which often binds more tightly to metal complexes than does dioxygen.

Irreversible oxidation is another potentially catastrophic defect in the binding of dioxygen by heme. When free heme in aqueous solution is exposed to dioxygen, it forms a -oxo dimer known as hematin very instantly. The reactions are as follows, with the heme group 'PFe (II)'. The binding of the dioxygen molecule, as in haemoglobin, is the first step:

$$PFe^{II} + O_2 \rightleftharpoons PFe^{II}O_2 \leftrightarrow PFe^{III}O_2^- \qquad (1)$$

The bound dioxygen now coordinate with a second heme, forming a μ-peroxo complex:

$$PFe^{II}O_2 + Fe^{II} \rightarrow PFe^{III}–O–O–Fe^{III}P \qquad (2)$$

Cleavage of the peroxo complex results in two molecules of a ferryl complex with the iron in +4 formal oxidation state:

$$PFe^{III}–O–O–Fe^{III}P \rightarrow 2PFe^{III}–O. \leftrightarrow PFe^{IV}=O \qquad (3)$$

Finally, ferryl complex attract another heme to form hematin:

$$PFe^{IV}=O + PFe^{II} \rightarrow PFe^{III}–O–Fe^{III}P \qquad (4)$$

Obviously, living systems must find a means to stop processes (1) to (4) otherwise, instead of shutting electrons in the cytochromes or transporting dioxygen molecules in oxyhemoglobin and storing them in oxymyoglobin, all the heme should be precipitated as hematin.

1.10 SELF ASSISMENT QUESTIONS (SAQS)

Question 1.

A. **multiple choice**

1. The molecular weight of hemoglobin

A. 44,450, B. 54,450,

C. 64,500, D. 74,450

2.The porphyrins are cyclic compounds formed through methylene bridges by the linkages of pyrrole rings number.

A. 4 B. 3

C. 2 D. 1

3. Two alpha-chains of globin have identical amino acid composition of

A. 111 B. 121

C. 131 D. 141

4. Hemoglobin takes up the number of molecules of oxygen

A. 1 B. 2

C. 4 D. 6

5. Carboxyhemoglobin is formed by

A. CO B. CO_2

B. H_2CO_3 C. HCN.

6. In the porphyrin ring four pyrrole ring connected with each other via

A. Methane B. Methyl

C. Methine D. Methylene

7. In oxy-hemoglobin, the iron centre is best described by which of the following

A. high-spin Fe(III) B. high-spin Fe(II)

C. low-spin Fe(III) D. low-spin Fe(II)

B. Fill in the bling

1. The absence of oxygen molecule hemoglobin is known as…….

2. The α-chains of hemoglobin contains……. and β-chain contains…… amino acids.

3. Iron in the……state is bound to the…… atom of the pyrrole rings.

4. Iron is also internally linked to the nitrogen of the…….ring of …….of the polypeptide chains.

5. The main role of the protein chain around the hemoglobin molecule and it provide the ……around of the Fe^{2+} ions and prevent it contact with water and its …….

C. True and false:

1. Transferrin binds Iron is in +3 oxidation stste. True/False

2. Low spin form of hemoglobin is diamagnetic nature True/False

3. Life of the hemoglobin molecule is approximate 16 week True/False

4. Iron atom combine with the porphyrin ring it is called heme protein True/False

D. Match the following

i.	Hemoglobin	a.	Iron storage
ii.	Ferritin	b.	O_2 transport and iron
iii.	DeoxyHb	c.	Diacidic and dibasic
iv.	Porphyrin ring	d.	square pyramidal

1.11 MYOGLOBIN (MB)

Myoglobin (symbol Mb or MB) is an iron- and oxygen-binding protein found in the skeletal muscle tissue of vertebrates in general and in almost all mammals. myoglobin is a pigment as hemoglobin. It is constituted by heme and globin. It is alpha halical neuclear protein with 153 amino acids. The molecular weight of myoglobin is 17000. Myoglobin store oxygen in the muscles tissue and release when oxygen is required. Myoglobin is the monomer of the hemoglobin and contain Fe^{2+} ion in active site. Compared to hemoglobin, myoglobin has a higher affinity for oxygen and does not have cooperative-binding with oxygen like hemoglobin does. But at the core, it is an oxygen-binding protein in red blood cells. In humans, myoglobin is only found in the bloodstream after muscle injury. Iron within the heme group must be in the Fe^{+2} state to bind oxygen. If iron is oxidized to the Fe^{+3} state, met myoglobin is formed. The total amount of myoglobin in an animal depends on

body weight, degree of muscle development, and the myoglobin concentration in muscle, which varies between muscle types (red muscle is rich in myoglobin and white muscle is myoglobin poor).

1.11.1 Structure of the myoglobin

Myoglobin (Mb) is the oxygen binding protein found principally in muscle tissues of vertebrates. It consists of a single polypeptide chain of 153 amino acids called globin, made up of seven a-helical and six non helical segments (Fig 1.14). Attached to the chain by coordination to the imidazole ring of a histidine residue is the dioxygen-binding prosthetic group, iron(II) protoporphyrin IX. The structure of sperm-whale myoglobin has been determined in all three (deoxy, oxy, and met) forms.

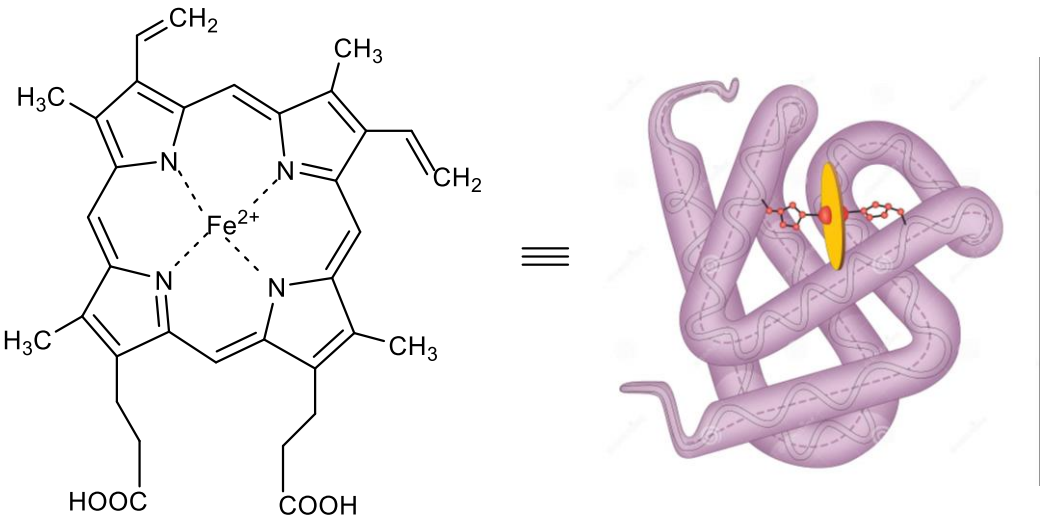


Fig.1.14 Structure of myoglobin

Deoxy Mb has a pentacoordinate iron(I1) center in which the metal atom lies 92 pm out of the plane of the four pyrrole-ring donor nitrogen atoms(sheme). It is displaced toward the bonded imidazole group, which is called the proximal side of the heme. Upon binding of dioxygen, the iron atom moves toward the FeN, planes. In oxy Mb, the dioxygen molecule is bonded end-on to iron, forming a bent structure with a Fe-0-0 bond angle of 115.

1.11.2 The dioxygen-binding reaction

Hb and Mb, probably the most thoroughly investigated metalloproteins, have been the subjects of innumerable spectroscopic, thermodynamic, and kinetic measurements. Their optical spectra are dominated by intense porphyrin ring π to π^* transitions in the 400-600 nm

range, known as the Soret, *a,* and bands that are sensitive to the state of oxygenation. From resonance Raman spectroscopic studies of the coordinated dioxygen molecule and its '80-substituted analogs, an O-O stretching band at - 1105 cm^{-1} has been identified. This value is characteristic of coordinated superoxide (O^{2-}) ion suggesting that MbO, and HbO, adducts might best be assigned as iron(II1) complexes of this ligand. Magnetic coupling between these ions leads to a diamagnetic, ***S*** = *0,* ground state. Thus coordination of dioxygen to deoxy Hb or Mb is accompanied by electron transfer to form a superoxide ion, which in turn is stabilized by hydrogen bonding to the distal imidazole proton, as indicated above.

1.12 COOPERATIVE EFFECT

The phenomena where the binding of one O_2 molecules to a sub unit encourage the binding of O_2 molecules to another sub unit is called cooperative effect.The cooperative effect describes the ability of the four identical haemoglobin sub-units to change their conformation. The cause of this change is the acceptance or release of an O_2 molecule by one of the sub-units, which increases the ability of the other haemoglobin domains to accept or release oxygen. Great importance to the physiological functions of Hb and Mb is the beautifully sophisticated bioinorganic system devised by nature in which dioxygen binds to Hb in the lungs and is transferred to Mb in tissues or is transferred to fetal Hb in the uterus of pregnant mammals. At the heart of this system is the motion of iron toward the plane of the porphyrin ring upon conversion of deoxy to oxy Hb, which serves as a trigger for cooperative binding of dioxygen by the multisubunit hemoglobin protein. The protein is presumed to have two different quaternary structures designated R, for relaxed, and T, for tense. The former has a high affinity for O_2,, similar to that of isolated subunits, whereas the latter, tense state has a diminished O_2, affinity. These two conformational states are in equilibrium with one another. In the T state, prevalent when all four sub units are a ligated, inter sub unit interactions are believed to constrain the proximal histidine to resist movement into the porphyrin-ring plane and diminish the O_2, binding constant.

1.13 BOHR'S EFFFECT

The Bohr effect is a phenomenon first described in 1904 by the Danish physiologist Christian Bohr. The Bohr effect generally describes the effect of pH on the blood-O_2 -binding affinity. The affinity of hemoglobin (Hb) towards the O_2 is pH dependent. The affinity of Hb towards

decreases with decreases in pH. Affinity of myoglobin (Mb) towards O_2 is pH independent. The Oxygen (O_2) competitively and reversibly binds to hemoglobin, with certain changes within the environment altering the affinity in which this relationship occurs (Fig. 1.15).

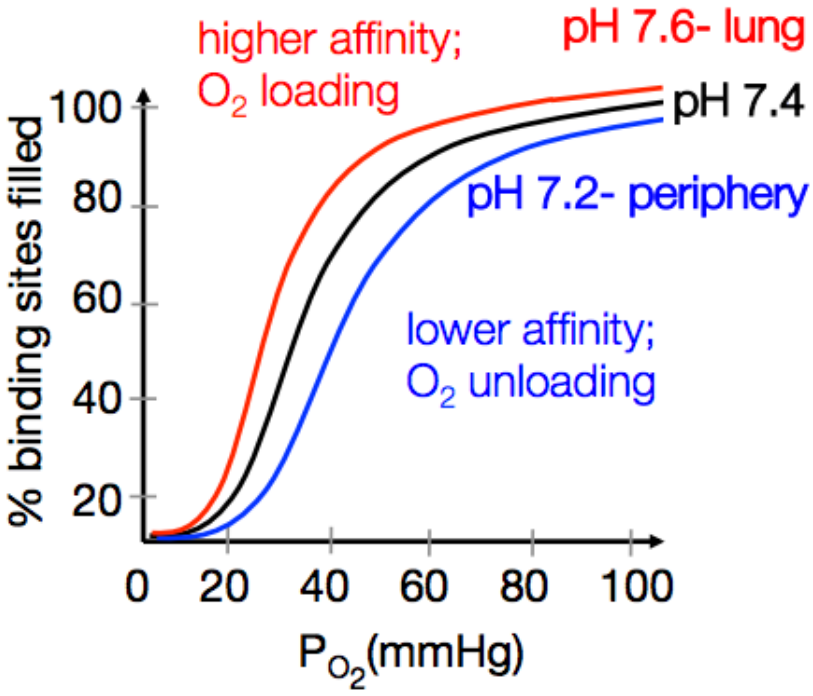


Fig.1.15 Partial pressure of the O_2 (PO_2 in mm Hg)

The sigmoidal shape of the oxygen dissociation curve illustrates hemoglobin's propensity for positive cooperativity, as hemoglobin undergoes conformational changes to increase its affinity for oxygen as molecules progressively bind to each of its four available binding sites. The Bohr effect describes hemoglobin's lower affinity for oxygen secondary to increases in the partial pressure of carbon dioxide and/or decreased blood pH. This lower affinity, in turn, enhances the unloading of oxygen into tissues to meet the oxygen demand of the tissue.

1.14 HEME MODELS

One has studied that hem group I hemoglobin and myoglobin has the striking ability to bind O_2 molecule and its subsequent release without the iron atom becoming permanently oxidized to the Fe(III) state. The following points are of significance:

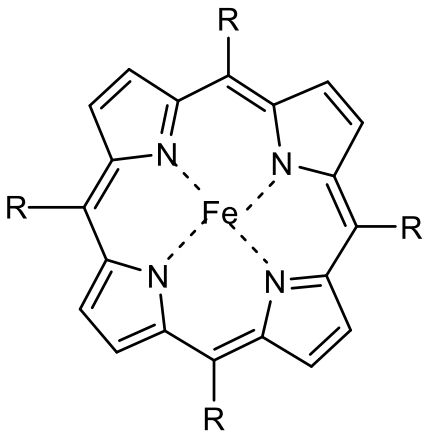
- Before O_2 binding, the Fe(II) centre is though to be located significantly out of plane of the porphyrin unit called doming effect.

This results in a constraint beetween the globin portion of the protein and iron atom. The movement of the iron atom of heme group on oxygen binding moves towards the plane of the heme which acts as a trigger and sets in extensive structure changes in other subunits in hemogobin.

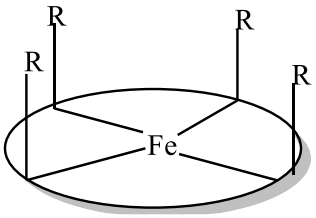
- In both hemoglobin and myoglobin the protein bulk folds around the heme units(s). this characteristic folding/protein structure around the heme unit leads to steric hindrance which checks any hematin formation by prohibiting approach of two iron porphyrin moieties.

In the synthetic models, bulk (like protein structure) has been added to sample iron porphyrin systems to block approach to the reactive iron centers. The following points may be noted:

- Imidazole and its derivatives play a role of good mimics for the protein histidine residue in the biological system



may be written as



- In the picket fence porphyrins bulky hydrophobic substituent are attached to the porphyrin core to give an upright fence around the Fe binding site. In the presence of 1-methylimidazole which serves as a base in the axial position on the other side of the ring from the pickets. The Fe(II) complex of this model porphyrin ring system binds O_2 is bound in the cavity created by picket fence

Substituent while the unhindered axial site can be occupied by a suitable nitrogen base e.g., imidazole (Fig 1.16)

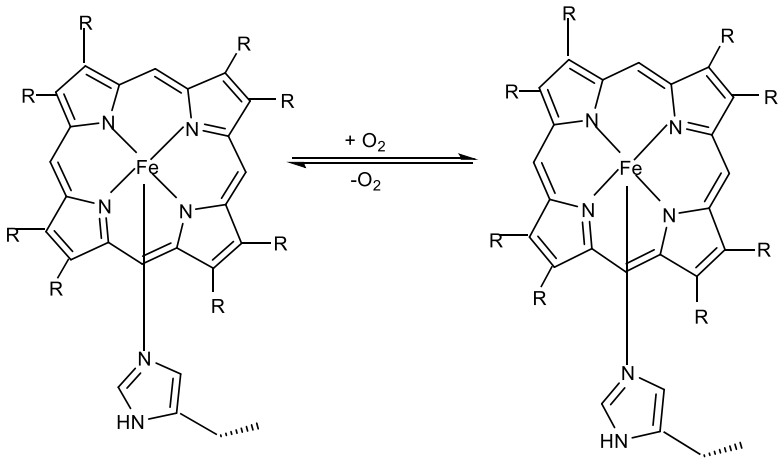


Fig.1.16 Synthetic models- picket fence models

1.15 PHOTOSYNTHESIS

1.15.1 Introduction

Plants produce their own food through a process called photosynthesis. Light energy is converted into chemical energy by them. CO_2 from the atmosphere and water are integrated into organic compounds with the help of this chemical energy. Instead of photosynthesis, it's sometimes termed CO_2 assimilation. Photosynthesis is a crucial process not just in terms of quality but also in terms of quantity. Photosynthesis transforms between 200 and 500 billion tonnes of carbon every year. As a result, photosynthesis is a quantitatively important activity as well. At the expense of solar energy, the light reaction of photosynthesis produces energy-rich NADPH and ATP. These products are utilized in the carbon-assimilation process, which reduces CO2 to produce carbohydrates and occurs in light or darkness. Green plants, algae, and photosynthetic bacteria use photosynthesis to capture solar energy and use it to fuel the synthesis of carbohydrates from carbon dioxide and water Fig.1.17.

$$6CO_2 + 6H_2O \xrightarrow[\text{CHLOROPHYLL}]{\text{SUNLIGHT}} C_6H_{12}O_6 + 6O_2$$

Fig.1.17 Synthesis of carbohydrates from carbon dioxide and water

1.15.2 Phase of photosynthesis

There are two phases to the photosynthesis reaction:

(1) The light process, which produces NADPH and ATP using light energy.

(2) A carbon-assimilation or carbon-fixation process in which NADPH and ATP are used to synthesise carbohydrate from CO_2 and H_2O.These reactions are frequently referred to as "dark reactions" (Fig.1.18)

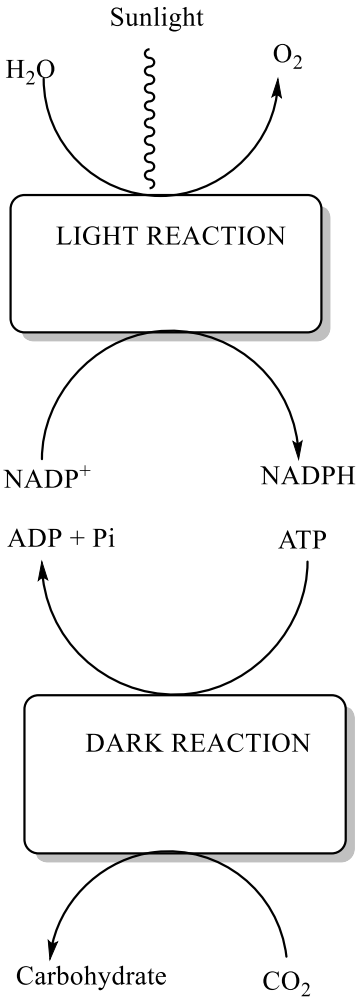


Fig.1.18 Dark reactions

1.15.3 Role of photosystem I and Photosystem II

Photosystem I (PSI) and photosystem II (PSII) are the two types of photosystems used by green plants and algae (PSII). PSI's reaction centre chlorophyll has an absorption maximum of 700 nm and is therefore known as P700 (P for pigment), while PSII's reaction centre chlorophyll has an absorption maximum of 680 nm and is thus known as P680. Other electron carriers, particularly the cytochrome bf complex, connect the two photosystems (Fig 1.19). The different components from the so-called Z scheme (Fig.1.20) when organised

according to their redox potential because the overall form of the redox diagram provides a look of z. Plastoquinone, plastocyanin, ferredoxin, and other highly mobile electron carriers are used in these events.

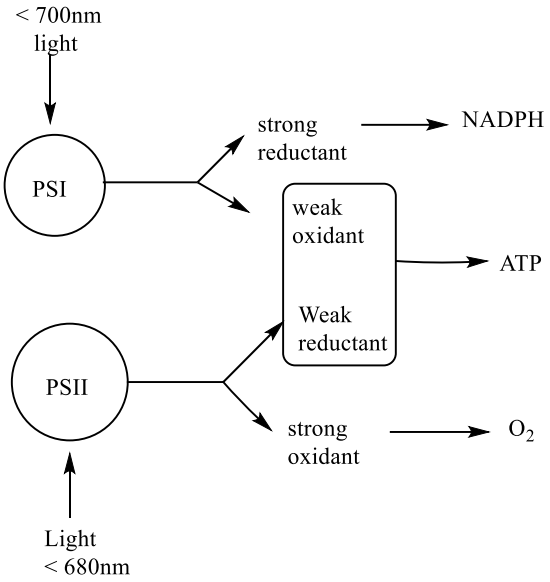


Fig 1.19 Interaction of PSI and PSII during photosynthesis.

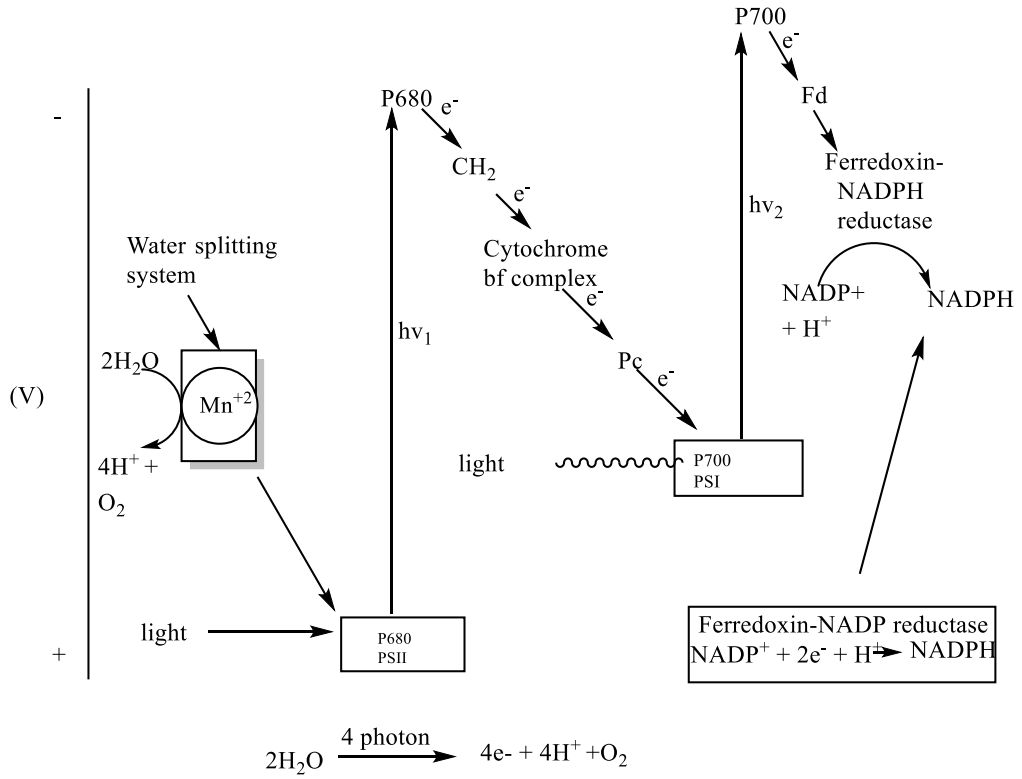


Fig 1.20 The Z scheme of photosynthesis.

1.15.4 Mechanism of light dependent reduction

In photosynthesis, electron transport pathways from H_2O to $NADP^+$ are defined. The absorption of light by PSII and PSII allows for this endergonic process. When a PSII chlorophyll in a PSII reaction centre is in its ground, unexcited state, it has no inclination to give up an electron. When a photon's energy reaches it through the antenna chlorophyll, it is excited, and it has a high inclination to pass on its excited electron. In reality, it is a reducing agent. The high-energy electron is transferred to plastoquinone (Q), a mobile quinone in the thylakoid membrane with a structure similar to ubiquinone in the mitochondrial electron transport chain. P680 is the $P680^+$ cation as a result of this. Plastoquinone is converted to plastoquinol(QH_2) by absorbing two electrons and two H^+ ions. It's worth noting the following:

i. The light-driven splitting of H_2O is catalysed by a Mn-containing protein complex, resulting in the production of O_2 (Fig. 1.21). This strong reductant transfers its electron to $NADP^+$ to create NADPH.

ii.The four electron abstracted from water do not pass directly to P680+, which can accept only one electron at a time. Instead, a remarkable molecular device, the water splitting complex,passes four electron one at a time to P680+.

iii.Reduced plastoquinone formed by PSII now passess electron into the cytochrome bf complex. Pc is a copper containing protein that accept electron by the copper cycling between Cu^{2+} and Cu^+ state.

A Mn-containing protein complex catalyses the light-driven splitting of H_2O, resulting in the production of O_2 (fig.1.21).This strong reductant donates an electron to $NADP^+$, resulting in NADPH.

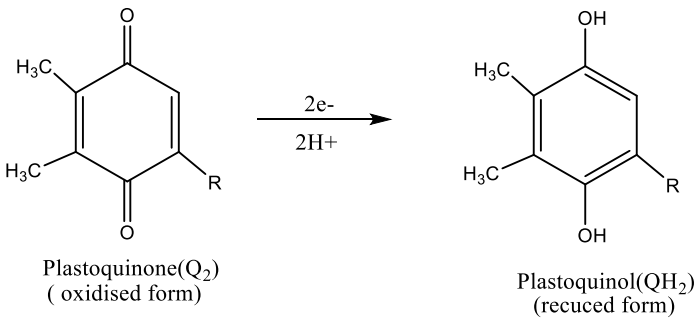


Fig. 1.21

In PSI, an electron was excited out of $P700^+$ and transferred to ferredoxin, resulting in the reduction of $NADP^+$. $P700^+$, on the other hand, has lost an electron and is now $P700^+$, an oxidising agent. It takes one electron from reduced plastocyanin (Pc) and returns to the unexcited state. Remember how light excited one electron out of P680, the PSII reaction centre pigments; this left P680+, which needed its electron restored so it could transition to the ground state, ready for another photon to start a new round of reaction. Water is the source of the electron (Fig.1.20).

1.15.5 Generation of ATP via cyclic electron flow

When ferredoxin has reduced virtually all of the $NADP^+$, it gives an electron to the cytochrome bf complex (Fig.1.22). The resultant proton gradient created by the H^+ pump, cytochrome bf complex, subsequently drives ATP production. ATP is generated during photophosporylation without the formation of NADPH, and no O_2 is produced since PSII is not engaged.

In summary, when electron transport through PSI AND PSII operates in a noncyclic mode, the products are NADPH and ATP. The sole result of cyclic electron transport, on the other hand, is ATP.

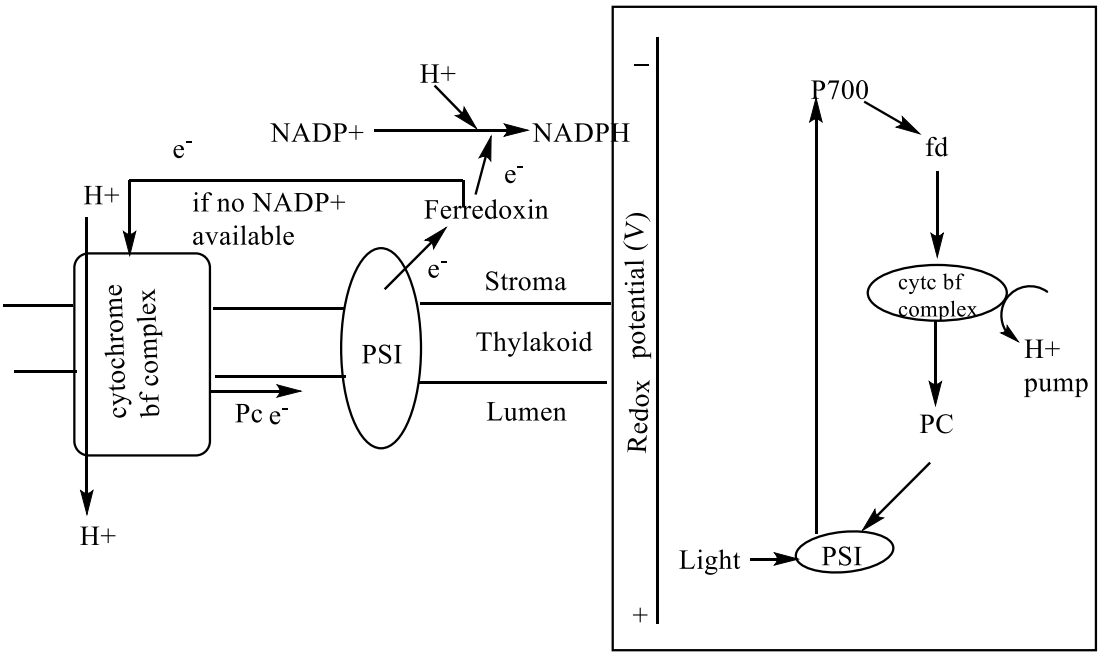


Fig 1.22. Cyclic electron flow

1.15.6 The Dark reaction of photosynthesis, the calvin cycle

The dark reaction, also known as the carbon-fixation process, converts CO_2 into carbohydrate using the ATP and NADPH generated by photosynthesis' light reaction. Sucrose and starch are the end products. The metabolite 3-phophogycerate is converted to glucose by a sequence of processes that are similar to glucogenesis in the liver, except that NADPH is employed as the reductant instead of NADH (Fig 1.23)

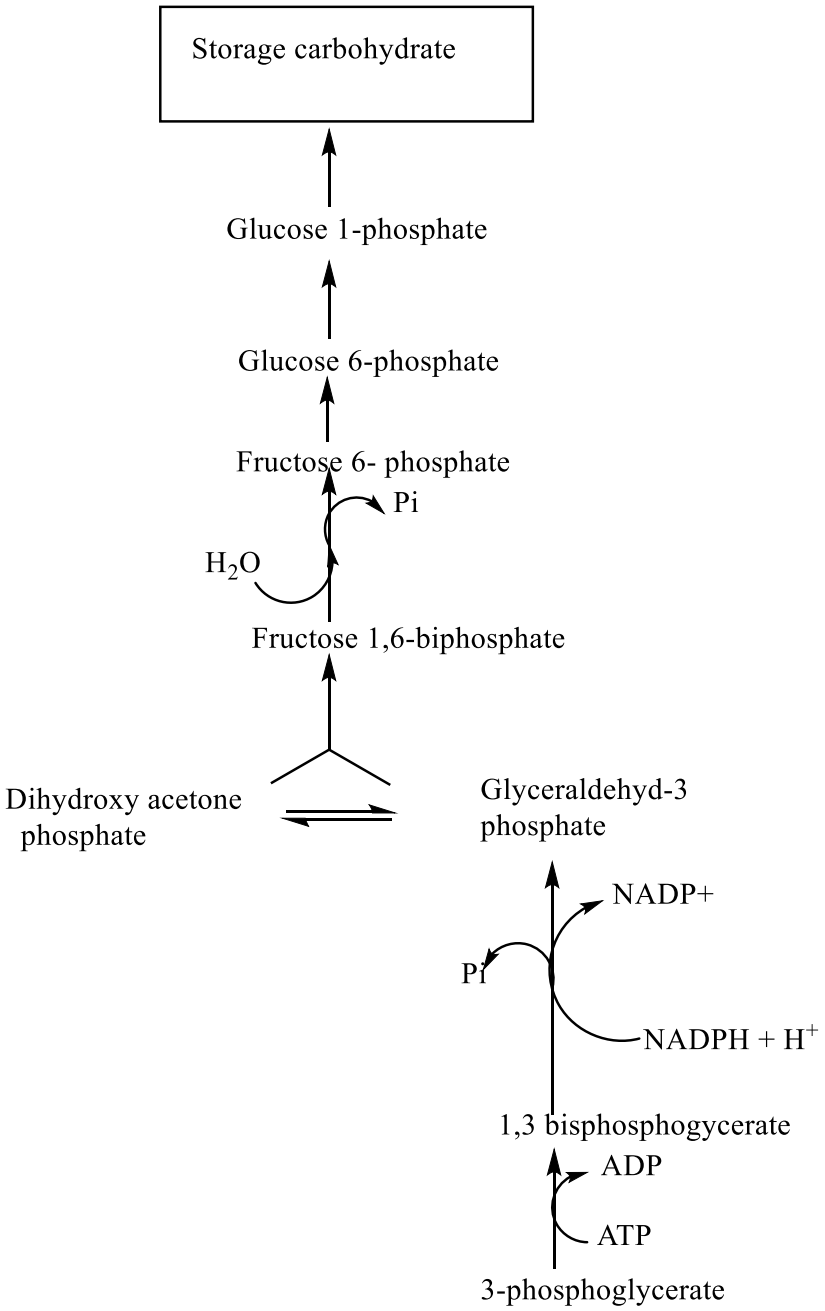


Fig. 1.23 Pathways for carbohydrate synthesis during photosynthesis

The main carbon fixation step is catalysed by the enzyme ribulose bidphosphate carboxylase, which is the most abundant single protein on the planet. It uses CO_2 to create 3-phosphoglycerate. The calvin cycle (dark phase of photosynthesis) begins with the interaction of CO_2 and ribulose 1,5-bisphosphate, which produces two molecules of 3-phosphpglycerate. The conversion of 3-phosphoglycerate to fructose and glucose 6-phosphate involves comparable enzymes to those involved in gluconeogenesis, with the exception that glyceraldehyde 3- phosphate degydrygenase in chloroplasts is selective for NADPH rather than NADH. Several complicated reactions are used to regenerate ribulose 1, 5-bisphosphate from fructose 6-phosphate, glyceraldehyde 3-phosphate and dihydroxoacetone phosphate. Several stages in ribulose 1, 5-bisphosphate regeneration are similar to those in the pentose phosphate pathway (Fig. 1.24).

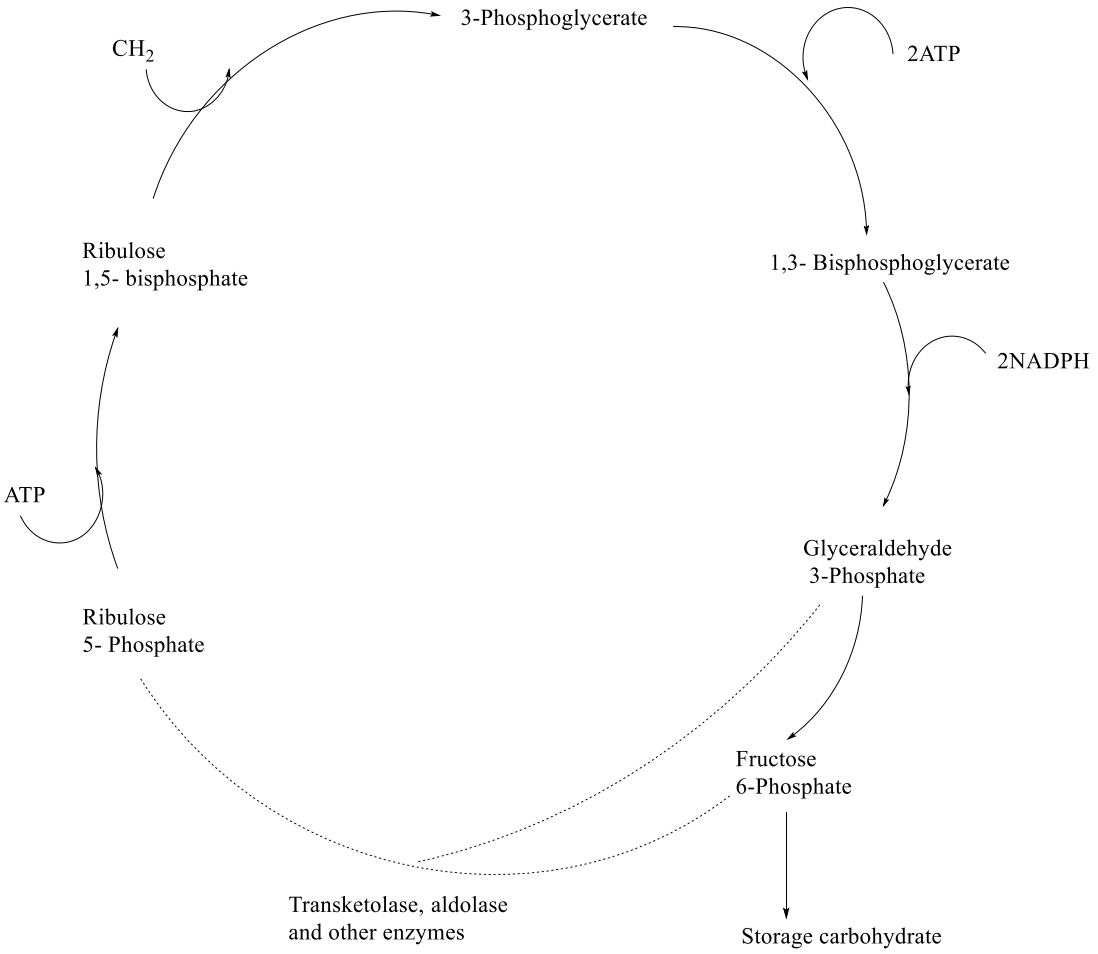


Fig. 1.24 The Calvin Cycle

For every CO_2 transformed into hexose, three ATP and two NADPH are used. Photosystem I absorbs four photons, while photosystem II absorbs another four, resulting in two NADPH and a proton gradient strong enough to cause the production of three ATP. The primary carbohydrate reserves of plants are starch in the chloroplast and sucrose in the cytosol.

Rubisco adds CO_2 to ribose 1, 5 bisphosphate under typical atmospheric conditions. It can then add O_2 if the CO_2 content is low. Phosphoglycolate and 3-phosphoglycerate are produced as a result of this process. Although the phosphoglycerate can be recovered and utilised in biosynthetic reactions, this method produces CO_2 and $NH4^+$ (wastage of metabolic energy). As a result of the net outcome of th this processis to consume O_2 and release CO_2 , it is called **photorespiration.** Solar energy is captured using bipyridine Ru(II) complexes, which act as a photochemical device and resemble green leaves. The following considerations should be taken into consideration:

• Green plants employ chlorophyll as a photosensitizer, and the photosynthetic protein complexes in green leaves capture light energy.

• As a result of the photoinduced electron, H_2O molecules split into H_2 and O_2, and CO_2 is converted to sugar.

• Synthetic bipyridine Ru(II) complexes act as photosensitizers for amines such as dimethyl aniline, which lose an electron that is captured by an Ir(III)-complexed ion connected to two bipyridine Ru(II) complexes through two pyridine rings.

• As a consequence, Ir (III) absorbs two electrons from two dimethyl aniline molecules, and Ir(III) is reduced to Ir(I).

• To recycle the electron-harvesting process, the Ir(I) state lowers CO_2 to formate and then oxidises back to Ir(III).

1.16 NITROGEN FIXATION

1.16.1 Introduction

Elemental nitrogen is highly irresponsible to ordinary chemical reaction. Nitrogen fixation is the process by which atmospheric nitrogen is converted by either a natural or an industrial means to a form of nitrogen such as ammonia. In nature, most nitrogen is harvested from the atmosphere by the microorganism to form ammonia nitrites, and nitrates that can be used by

plant. The biosynthesis of all nitrogen- containing organic compound, such as amino acid and nucleic acid required fixed inorganic nitrogen compound, so nitrogen fixation. is essential to life. Being a part of nitrogen cycle, it is essential for agriculture and the manufacture of fertilizer. It is also useful for the manufacture of all nitrogen chemical compounds, which includes some explosives, pharmaceuticals, and dyes

Biological nitrogen fixation was discovered by Jean-Baptiste Boussingault in 1938.

1.16.2 Type of nitrogen fixation

There are two type of nitrogen fixation:

I. Physical nitrogen fixation

II. Biological nitrogen fixation

1.16.2.1 Physical nitrogen fixation

The physical nitrogen fixation can be take place by two method:

1.Natural nitrogen fixation: N_2 and O_2 of the air react to form nitric oxide under the influence of lightening and thunder. The nitric oxide then again oxidized with oxygen to form nitrogen peroxide.

The reaction are as follows:

$$N_2 + O_2 \quad \text{lightening} \rightarrow \quad \text{Thunder } 2NO$$

$$2NO + O_2 \longrightarrow 2NO_2$$

When rain falls, NO_2 combines with rain water to form nitrous acid and nitric acid.The acids fall on the soil along with rain water react with the alkaline radical of the soil to form water soluble nitrates and nitrite.

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3;\ HNO_3 + \text{Ca or K}$$

$$\text{Salts} \longrightarrow \text{Ca or K nitrates}$$

The nitrates are soluble in water and are directly absorbed by the roots of the plants.

2- Industrial Nitrogen Fixation - In the industrial scale Ammonia is produced by direct combination of nitrogen with hydrogen at high temperature and pressure. Further it is converted into various kinds of fertilizer, such as urea etc.

1.16.2.2 Biological nitrogen fixation

The conversion of atmospheric nitrogen into the nitrogenous compound with the help of living organism is called as biological nitrogen fixation. The process is generally carried out by two main type of microorganism: those which live in close symbiotic association with other plants and those which are free living or non-symbiotic.

A- Free living nitrogen fixing bacteria:

- Azotobacter, Beijerinckia and clostridium are saprophytic bacteria that perform nitrogen fixation.
- These bacteria add approx 10-25 kg, of nitrogen/ ha/ annum.
- Many free living blue green algae also perform nitrogen fixation.
- They add 20-30 kg, of nitrogen/ ha/ annum.

B- Symbiotic Nitrogen Fixating bacteria:

- Several species of rhizobiom live in the soil but unable to fix nitogen by themselves.
- They undergo nitrogen fixation by only as symbionts in the association of roots of legumes.

1.16.2.3 Basic requirement of nitrogen fixation:

Basic requirement of nitrogen fixation are-

Nitrogenase enzyme complex, protective mecahnism against oxygen- leghaemoglobin, feerrodoxin, hydrogen releasing sysytem, constant supply of ATP, coenzyme and cofactor like CoA, inorganic phosphate and Mg^{+2}, Cobalt and molybdenum.

1.16.3 General Stuctural feature

- The nitrogenase complex, a highly conserved protein complex, is responsible for biological nitrogen fixation. Molybdnum nitrogenase, Vanadium nitrogenase, and iron-only nitrogenase are the three form of nitrogenase found in diverse nitrogen fixing bacteria. Molybdnum nitrogenase has been explored and characterised in more depth. All nitrogenase are made up of two protein: Fe protein dinitrogenase reductase and Mo-Fe protein dinitrogenase reductase. During catalysis, electron move from a pair of ATP

molecules within component II to the Fe-S cluster, where N_2 is reduced to NH_3 (Fig. 1.25).

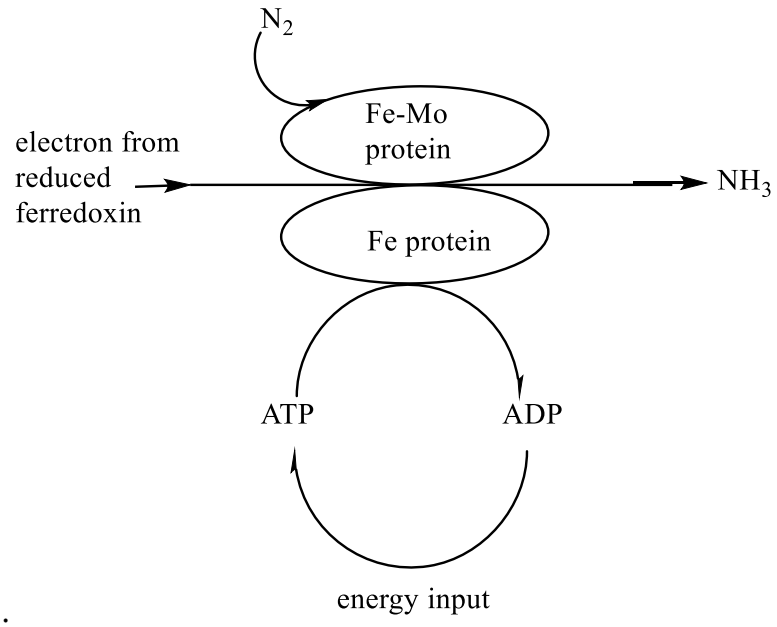


Fig. 1.25 Nitrogenase Complex

- The smaller protein has a molecular weight of 60000 and is often termed as iron protein. It contains a Fe_4S_4 cluster and called reductase.
- The other protein has a molecular weight of 240,000 and is called the Mo-Fe protein. This is an α_2-β_2 tetramer and contain two molybdenum atom, about 30 iron atom and around 30 inorganic/ labile sulphur.
- The iron sulphur cluster seems to act as redox centers. A soluble protein free cofactor containing molybdenum and iron has been isolated.
- Neither of the protein is separatly active, but on mixing them the activity is restored.

1.16.4 Specific structural feature

- The Fe- protein contains two identical subunit and a single Fe_4S_4 center is present in the protein which is bound between the subunit by forming Fe-S bonds to two cystein residues in each subunit. Furthermore, the single Fe_4S_4 centre is located at one end of the molecule and this is the only poin of any significant contact between the two subunit. The hydrolysis of ATP id carried out by these iron sulphur clusters, which operate as redox center (Fig. 1.26)

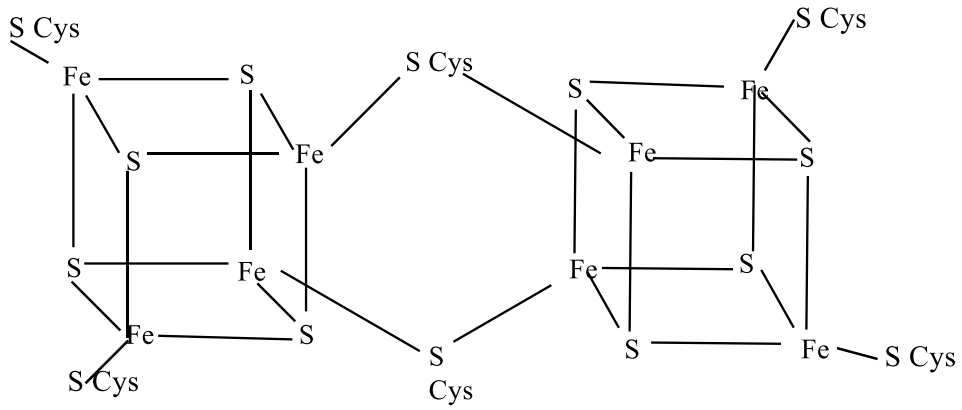


Fig.1.26 P-cluster

- The Fe-Mo protein is thaught to be immediatly responsible for substrate reduction.it is here in the FeMo protein where the actual conversion of N_2 to NH_3 occurs at the active site of this larger protein.in fact there are two type of centerwhich are present in the nitrogenous FeMo protein and are designated P cluster and Fe-Mo center.
- FeMo protein has four P cluster that are similar to Fe_4S_4 but not ferredoxin cluster. The P cluster has a double cubane structure. With all cystein ligands paired on one of the two non bridged iron atom in one bound Fe_4S_4 cluster. Its not uncommon for a Fe_4S_4 to have location of iron. The two Fe_4S_4 cluster are connected by two cystein ligand and two set of fe atom in a face sharing arrangment. A disulphide unit connects the two clusters. During nitrogenase cycle, this arrangment could be potentially redox active.
- The Fe-Mo is a novel iron- molybdenum cofactor. Ths cofactor is extremly insoluble and extremly air sensitive substance which contain 2Mo; 6-8 Fe; and < 6S atoms
- FeMo is thought as the site of substrate binding and for the actual conversion of N_2 to NH_3 .
- X- ray crystallography was recently used to derive the structure of Fe-Mo of molybdenum iron protein of nitrogenase, and the cluster core if composition Fe_3MoS_8 is represented by two cuboidal fragments. One of these components comprise five iron atoms, whereas the other has three iron atom plus a molybdenum atom. Two S-2 ions and an unknown ligand connect the two parts of the cluster.the fact that Mo is octahedrally coordinated whereas the iron atom at the interface has led to the hypothesis that dinitrgen is bound and activated for reduction at the clusters center by two or more iron atoms, implying that dinitrogen is not directly coordinated to molybdenum (Fig. 1.27).

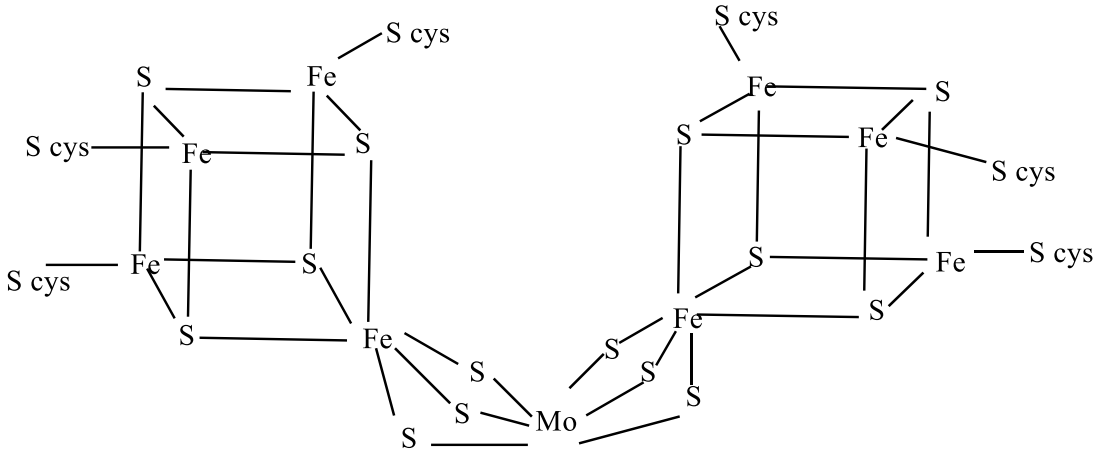


Fig. 1.27 Fe-Mo Protein

1.16.5 Mode of action of nitrogenase

The reduction of nitrogen to ammonia is an exergonic reaction.

$$N_2 + 3H_2 \longrightarrow 2NH_3$$

The bond of N_2 is very stable, so it can be understood that nitrogen fixation require a high activation energy due to presence of triple bond. So, atmospheric nitrogen is almost chemically inert under normal conditions. Biological nitrogen fixation however occurs at biological temperature and at 0.8 atm of nitrogen. The high activation barrier is overcome via several pathways and in part by binding and hydrolysis of ATP and represents the overall process

$$N_2 + 10H+ + 8e- + 16ATPN \rightarrow 2NH_4^+ + 16ADP + 16Pi + H_2$$

The biological nitrogen fixation is carried out with the help of enzyme nitrogenase compl and it requires six electrons to fix one molecule of N_2. The extra two electrons are needed to reduce $2H^+$ to H_2.

$$N_2 + 6e^- + 6H^+ \rightarrow 2NH_3$$

$$N_2 + 8e^- + 8H^+ \rightarrow 2NH_3 + H_2$$

A highly reduced version of Fe-Mo protein is responsible for nitrogen fixation, which necessitates eight electrons: six for N_2 reduction and two electrons are needed to produce one molecule of H_2. As a result, this protein in engeged in dinitrogen binding and reduction and numerous additional iron-sulphur clusters in the protein are implicated in electron transport.

In comparison to other molybdenum-containing enzymes, the molybednum site in FeMo is certainly unique.

Mechanism: Ferredoxin and Fe- protein transport electrons from the oxidation of pyruvate to Fe-Mo protein. (Fig. 1.28) show a schematic diagram of the nitrogenase complex, which is made up of Fe-Protein and Fe-Mo protein.

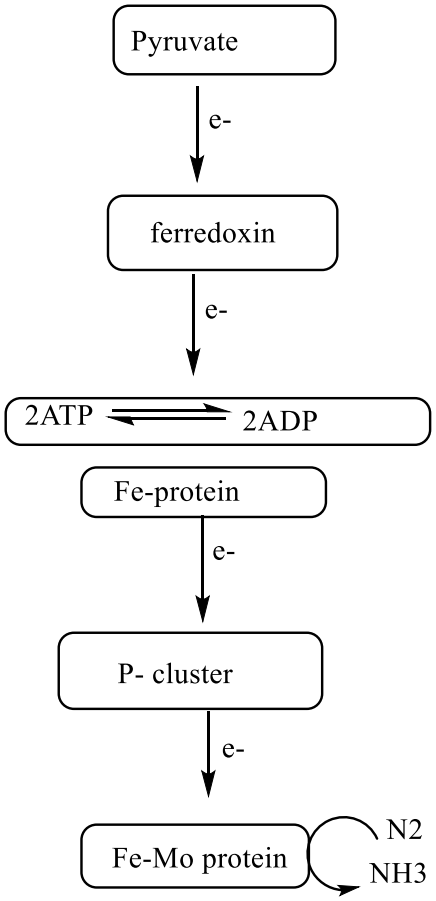


Fig.1.28 Nitrogen fixation by nitrogenase

During the conversion of N_2 to NH_3, the iron protein and nitrogenase component undergo cyclic association and dissociation. Fe-Mo protein is the name given to the nitrogenase component. Although neither of the teo protein can fix nitrogenon their own,they can be separated and jpined to do so. As a result, a reduced Fe-Protein bind to the Fe-Mo protein and transfer a singel electron. After which the oxidised Fe-protein dissociate from the Fe-Mo protein in a repating cycle. Two ATP molecules must be hydrolysed for each cycle. The job of ATP is to provide chemical energy and ATP hydrolysis cause conformational changes in the protein, which help to reduce the activation energy of the nitrogen fixation process. The Fe reduction proteins potential is shifted from 300-420 mV whwn two ATP molecule attach

to it. As a result, the reducing power of Fe-protein increases, making electron transfer to Fe-Mo protein easier.

It is the iron- molybdenum protein which is thought to be the actual site of N_2 coordination, since its mutants which have a defective Fe-Mo cluster co-factor can reduce acetylene alright but N_2 only poorly.

1.17 SUMMARY

The summary of the present chapter is:

- A Porphyrin is a large ring molecule made up of four pyrroles (smaller rings made up of four carbons and one nitrogen, which is a heterocyclic compound). These pyrrole molecules are linked by a chain of single and double bonds, forming a huge ring.
- In the absence of oxygen in haemoglobin the colour of the blood is blue and the colour of the blood is blue due to the transfer of electrons between the π and π^* orbital of ring and iron atom. A tetrapyrrole is the technical term for four pyroles linked together. It is flat in space and has a reasonably even distribution of electrons around its diameter.
- Haemoglobin (molecular wieght 645000) can be consider an oxygen carrier in animals. In most of the animals, it is the pigment to provide colour of blood.
- Myoglobin is an iron- and oxygen-binding protein found in the skeletal muscle tissue of vertebrates in general and in almost all mammals. myoglobin is a pigment as hemoglobin. It is constituted by heme and globin. It is alpha halical neuclear protein with 153 amino acids. The molecular weight of myoglobin is 17000.
- Plants produce their own food through a process called photosynthesis. Light energy is converted into chemical energy by them. CO_2 from the atmosphere and water are integrated into organic compounds with the help of this chemical energy.
- Instead of photosynthesis, it's sometimes termed CO_2 assimilation.Elemental nitrogen is highly un responsible to ordinary chemical reaction.
- Nitrogen fixation is the process by which atmospheric nitrogen is converted by either a natural or an industrial means to a form of nitrogen such as ammonia. In nature, most nitrogen is harvested from the atmosphere by the microorganism to form ammonia nitrites, and nitrates that can be used by plant.

1.18 SAQs TYPES QUESTIONS

A. Multiple choice question

1. Myoglobin contains the number of porphyrine ring

A. 1 B. 2

B. 3 D. 4.

2. Myoglobin binding of O_2 depends on ……

A. Hemoglobin B. O_2 concentration and affinity of myoglobin for O_2

C. Ka D. Kd

3. Myoglobin is a

A. Nitrogen fixation enzyme B. catalyst for epoxidation reaction

B. Component in photosynthetic system D. Di-oxygen binding metalloprotein

4. The active site of enzyme nitrogenase contains

A. Mo B. Mn

C. Fe D. Cu

5. In photosynthesis, the predominant metal present in the reaction centre of photosystem II is

A. Zn B. Mn

C. Cu D. Fe

6. The metals involved in nitrogenase are

A. Mo and K B. Mo and Fe

C.Fe and Mg C. Fe and K

7. The reduction of nitrogen to ammonia, carried out by the enzyme nitrogenase needs

A. 2 electrons B. 4 electrons

C. 6 electrons D. 8 electrons

8. The functions of myoglobin is

A. Storage of CO_2 B. storage of O_2

C. Storage of NO C. Storage of CO

9. Nitrogen is converted into NH_3 by enzyme

A. Uriage B. Invertage

C. Nimenase C. Nitrogense

10. Which element is present in major amount in human body

A. Zinc B. Copper

C. Iron D. Titanium

B. Match the following

A. Nitrogenase	i. Magnese
B. P.S II	ii. Iron
C. Myoglobin	iii. Molybdenium
D. Bohr effect	iv. Christian Bohr

C. Fill in the Blanks:

1. Myoglobins are the……pigments occurring in the……cells of vertebrates and invertebrates.

ii. The α-chains of hemoglobin A contains……..and β-chain contains…….amino acids.

iii. The porphyrins are…… compounds with……structure.

iv. Iron in the……state is bound to the…… atom of the pyrrole rings.

v. Iron is also internally linked to the nitrogen of the…….ring of …….of the polypeptide chains.

Ans. Imidazole; histidine

D. True/False

a. Chlorophyll is magnesium-containing porphyrin and the photosynthetic pigment of plants.

True/False

b. Chlorophyll and heme of hemoglobin are synthesized in living cells by different pathways.

True/False

c. The affinity of hemoglobin (Hb) towards the O_2 is pH dependent. True/False

d. Myoglobin is the monomer of the hemoglobin and contain Fe^{2+} ion in active site.

True/False

Answers Key

Question 1:

A. 1 a 2. b 3.d 4. c 5. c

B. 1. DeoxyHb 2. 141, 146 3. Fe^{2+}, nitrogen 4. cyclic, tetra pyrrole 5. hydrophobic environment, oxidation

C. 1. True 2. true 3. true 4. true

D. i-b ii-a iii-d iv-c

Question 2 :

A. 1 A 2 B 3 D 4 A 5 B 6 B 7 D 8 B 9 C 10 C

B. A iii B i C ii D iv

C. i Respiratory, Muscle ii 141, 146 iii Cyclic, tetra pyrrole iv Ferrous, nitrogen

v Imidazole, histidine

D. a True b False c True d True

1.19 GLOSSARY

ET= Electron transfer

Hb = Hemoglobin

ATP = Adenosin triphosphate

Mb = Myoglobin

DeoxyHb = Deoxyhemoglobin

Oxy-Hb = Oxy-hemoglobin

His = Histidine

PsI = Photosystem I

PsII = Photosystem

NADPH = Nicotinamide adenine dinucleotide phosphate

CoA = CoenzymeA

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1.22 TERMINAL QUESTION

Q.1 What the function of the ferritin and transferritine?

Q.2 Explain structure of the porphyrin ring with the help of the diagram.

Q.3 Give an account for the structure and its biological functions of myoglobin.

Q.4 How will you compares the myoglobin and aemoglobin.

Q.5 How metal ion complexes are helpful in biological system. Write down with special reference to the Iron atom.

Q.6 What is the nitrogen fixation.Discuss the mechanism of Nitragenase.

Q.7 What is the metalloporphyrins ? Write down the structure of metalloporphyrins with the example.

Q.8 What is the haemoglobin. Explain the structure of haemoglobin.

Q.9 Give the account for the biological function of haemoglobin in living.

Q.10 What is the cooperative effect. How it is helpful for the binding of oxygen in hemoglobin.

Q.11 What is the Bohr effect. How it is varies with pH.

UNIT 2: METALLOENZYMES

CONTENTS:

2.1 INTRODUCTION

In unit first we have discussed about the functions and role of hemoglobin and myglobin in human and other living organism and also discuss about other oxygen transfer and oxygen storage agency's. In this unit we also covered nitrogen fixation and enzymes involving in nitrogen fixation and what is the process of photosynthesis in the plant and type of the photosynthesis. In unit second we want to discuss the metalloenzymes and functions of metalloenzymes. Metal ions (metal cofactors) that are directly bound to the protein or to enzyme-binding nonprotein components are found in metalloenzymes (prosthetic groups). Metalloenzymes make up around a third of all enzymes discovered so far. Other metalloproteins, in addition to enzymes, are involved in non-enzyme electron transfer reactions (cytochromes), and can serve as storage (for example, ferritin for iron) or transport proteins (e.g., transferrin for iron). Metal storage is reversible in the latter groups of proteins, and the metal is only a transient component. In a larger sense, ribozymes, i.e. RNA molecules with enzyme function, may contain structurally and/or functionally significant metal ions (usually divalent metal ions like Mg^{2+}) and are thus referred to as metalloenzymes.

Natural metalloenzymes are well-known proteins that include one or more transition metal ions such as Fe, Cu, Zn, Ni, and Co. These metalloenzymes are capable of catalyzing a wide range of biosynthesis and metabolic events. These metal ions serve mostly as Lewis acids or redox-active sites. Furthermore, several enzymes have been used to manufacture important chemical molecules in both laboratory and industrial-scale operations. In one prominent case, nitrile hydratase, which possesses a Co(III) ion in the reactive site, has been utilized to produce acrylamide in commercial quantities. Metal complexes comprising valuable metals such as Ru, Rh, or Pd, on the other hand, have been used as catalysts in the creation of a wide range of chemicals and drug precursors. Several research groups have looked into altering such metal complexes to improve not only their individual catalytic reactivities, but also their stereo- and regio- selectivities, as well as their substrate specificity.

2.2 OBJECTIVE

In this unit you will be able to learn the-

(I) What is the function of zinc enzymes like, carboxypeptidase and carbonic anhydrase and how it is impoartant for all biological process of living object.

(II) Role of Iron enzymes in the life and main functions of catalase, peroxidase and cytochrome P-450 in biochemical reactions.

(III) Role of copper enzymes - superoxide dismutase, Molybdenum oxatransferase enzymes-xanthine oxidase in may oxidation-reduction.

(IV) Functions and catalatic activity of Coenzyme vitamin B_{12}in the biochemistry.

2.3 CARBOXYPEPTIDASE A (ZINC ENZYME)

Carboxypeptidase A is a zinc enzyme with a tetrahedral shape and a sp^3 hybridization. Its active site contains Zn^{2+}. The C-terminal peptide link in proteins and peptides is hydrolyzed by carboxypeptidase A, releasing the C-terminal amino acid. Regarding carboxypeptidase A, it's worth noting the following:

- In the active site of carboxypeptidase A, Zn^{2+} is present.
- Function catalyses the hydrolysis of the C-terminal end of peptide linkages or proteins.
- Tetrahedral structure with sp^3 hybridisation.
- The enzyme carboxypeptidase A, which is secreted by the pancreas and used to speed up the hydrolysis reaction, has a molecular mass of 34,800.
- This enzyme is made up of a single 307-amino-acid chain that folds into a compact, globular form with helices and pleated sheet regions.

2.3.1 Structure of carboxypeptidase A

Carboxypeptidase A (CPA) contains a zinc (Zn^{2+}) metal center in a tetrahedral geometry with amino acid residues in close proximity around zinc to facilitate catalysis and binding. Out of the 307 amino acids bonded in a peptide chain, the following amino acid residues are important for catalysis and binding; Glu-270, Arg-71, Arg-127, Asn-144, Arg-145, and Tyr-248. Figure 2.1 and 2.2 illustrates the tetrahedral zinc complex active site with the important amino acid residues that surround the complex.

The zinc metal is a strong electrophilic Lewis acid catalyst which stabilizes a coordinated water molecule as well as stabilizes the negative intermediates that occur throughout the hydrolytic reaction. Stabilization of both the coordinated water molecule and negative

intermediates are assisted by polar residues in the active site which are in close proximity to facilitate hydrogen bonding.

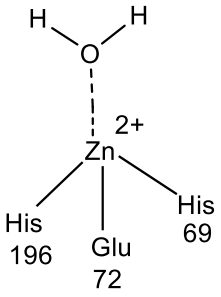


Fig. 2.1 structure of the carboxypeptidase A

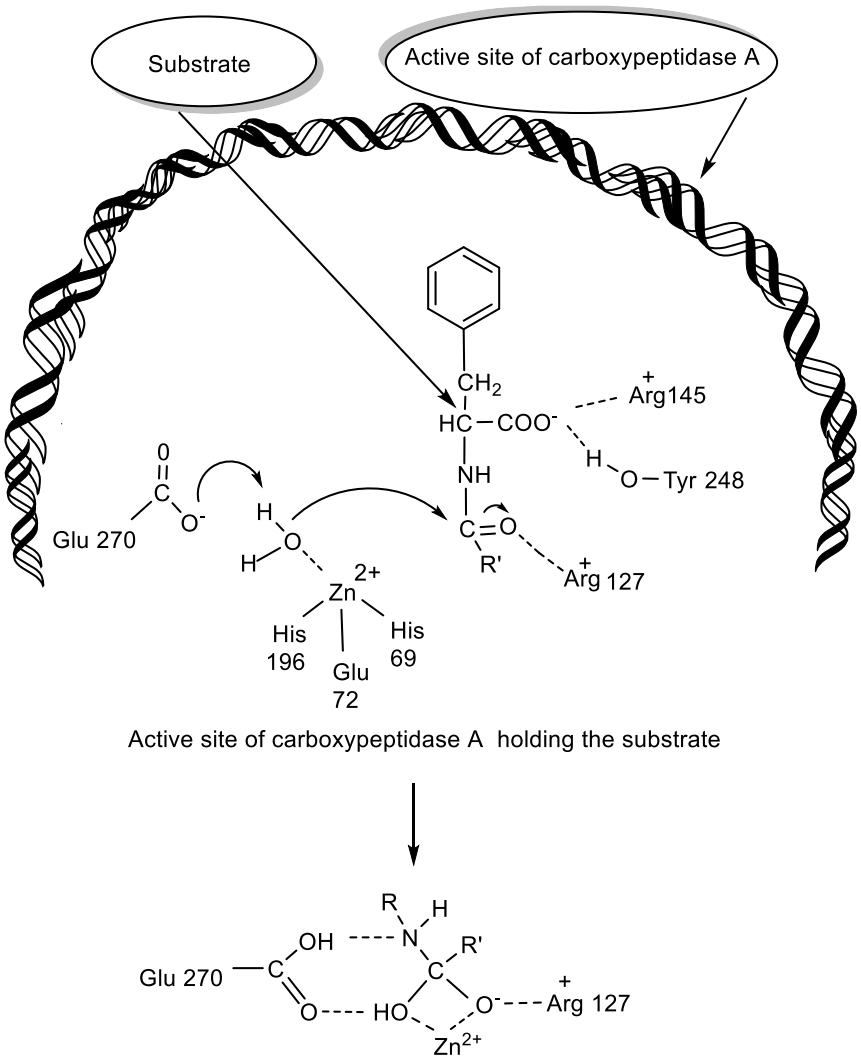


Fig. 2.2 mechanism of carboxipeptidase A with substrate

The following point may be noted :

- A Zn^{2+}ion is present at the active site of carboxypeptidase A and is held in place by a complex formed with Glu 72, His 196, and His 69, as well as water molecules.
- The reaction that occurs when an enzyme catalyses the breakdown of a protein's C-terminal peptide bond to liberate C-terminal amino acid is known as hydrolytic cleavage.

When Zn^{2+} binds to water, it becomes more acidic, making the nucleophile more like the OH ion. The negative charge that arises in the transition state is stabilised by Zn^{2+}, and the negative charge is also stabilised by Arg 127. Glu 270 is a general-purpose base catalyst. The substrate is held securely in place in the active site by Arg 145 and Tyr 248.

2.3.2 Mechanism of Carboxypeptidase A

Hydrolysis of the C-terminal peptide bond in peptides and protein and release the C-terminal amino acid (Fig. 2.3)

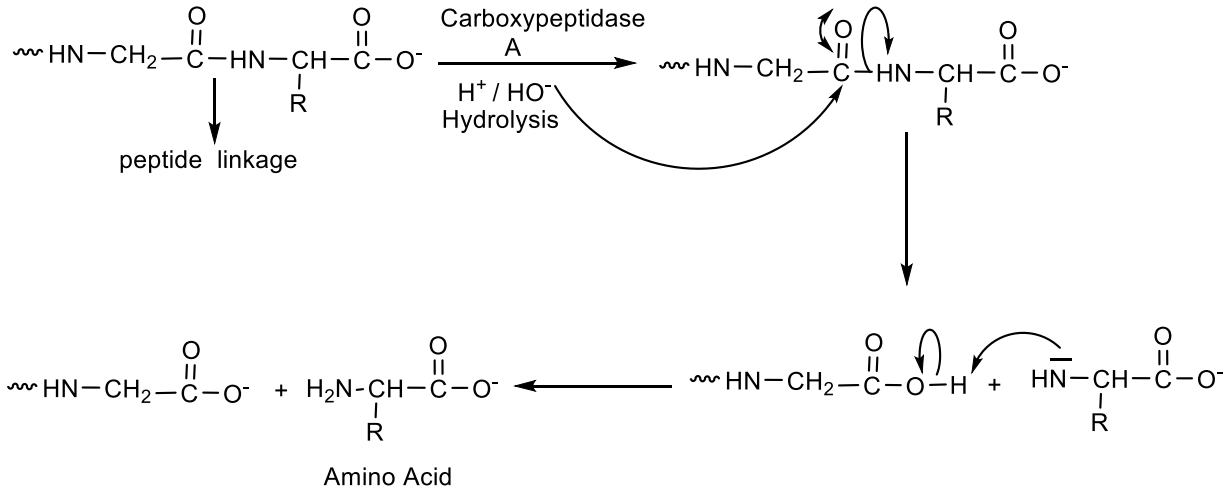


Fig 2.3. Hydrolysis of C- terminal end of amino acid.

2.4 CARBONIC ANHYDRASE

Carbonic anhydrase is an enzyme that catalyses the conversion of carbon dioxide (CO_2) to carbonic acid. It is present in red blood cells, stomach mucosa, pancreatic cells, and renal tubules (H_2CO_3). Carbonic anhydrase affects CO_2 transport in the blood and so plays a significant role in respiration. The uncatalyzed equilibrium takes a long time to reach.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

The forward (hydration) reaction happens in erythrocytes (red blood cells) during CO_2 uptake by the blood in tissue, whereas the backward (dehydration) reaction occurs when CO_2 is released in the lungs. The carbonic anhydrase enzyme multiplies the rate of this equilibrium by a million.

This enzyme has a molar mass of around 30,000 and a single protein unit of 260 amino acids. The active site contains a Zn^{2+} ion that is tetrahedrally coupled to three histidine imidazole nitrogen atoms (His-96 and His-119), as well as water molecules or hydroxide ions (Fig. 2.4). It comprises additional amino acids that work via hydrogen bonding, proton transfer and other mechanisms.

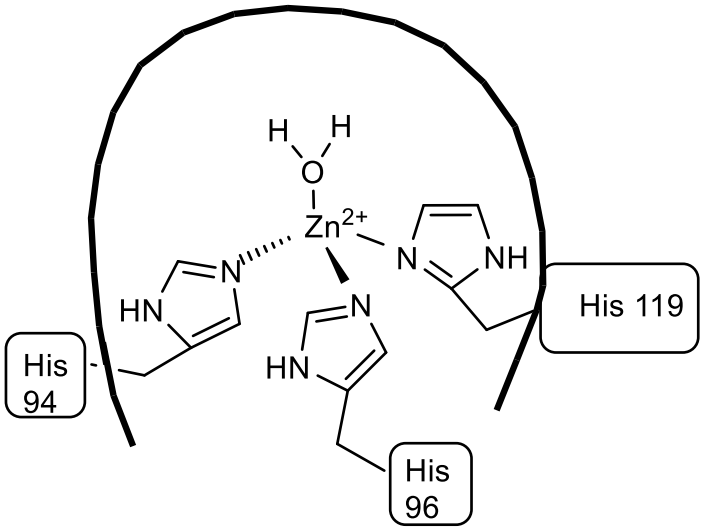


Fig. 2.4 Structure of carbonic anhydraase

As the pH rises, the forward and reverse reaction rates in the CO_2 hydration equilibrium increase. In carbonic anhydrase, the Zn^{2+} ion is more acidic than in carboxy peptidase. The inclusion of a neutral or liss basic histidine residue rather than a glutamase residue contributes to the acidity of the Zn^{2+} ion. The three histidine residues are also puleed back, causing the Zn^{2+} ion to become more electronegative and acidic as it approaches the fourth position. The nucleophilic OH^- then attacks the carbon atom of CO_2 trapped in the hydrophobic pocket near the Zn^{2+} ion, forming a temporary five coordinate Zn^{2+} ion in which a carbonato oxygen from HCO^-_3 coordinates to the Zn^{2+} ion. Following rearrangement, the HCO_3^- ligand is replaced by an H_2O ligand that is coupled to the Zn^{2+} ion, resulting in the regeneration of Zn-OH^-, which then attacks another CO_2 to complete the catakytic cycle.

2.4.1 Mechanism of action of carbonic anhydrase

The following point may be noted:

- The accepted explanation is based on the fact that the kinetics of the carbonic anhyydrase reaction are affected by pH (faster at high pH and when influenced by a group with an apparent pKa value of around 7.0).
- When compared to the value of 14.0 for free water, the pKa value for zinc coordinated water is around 7.0, which is quite low.

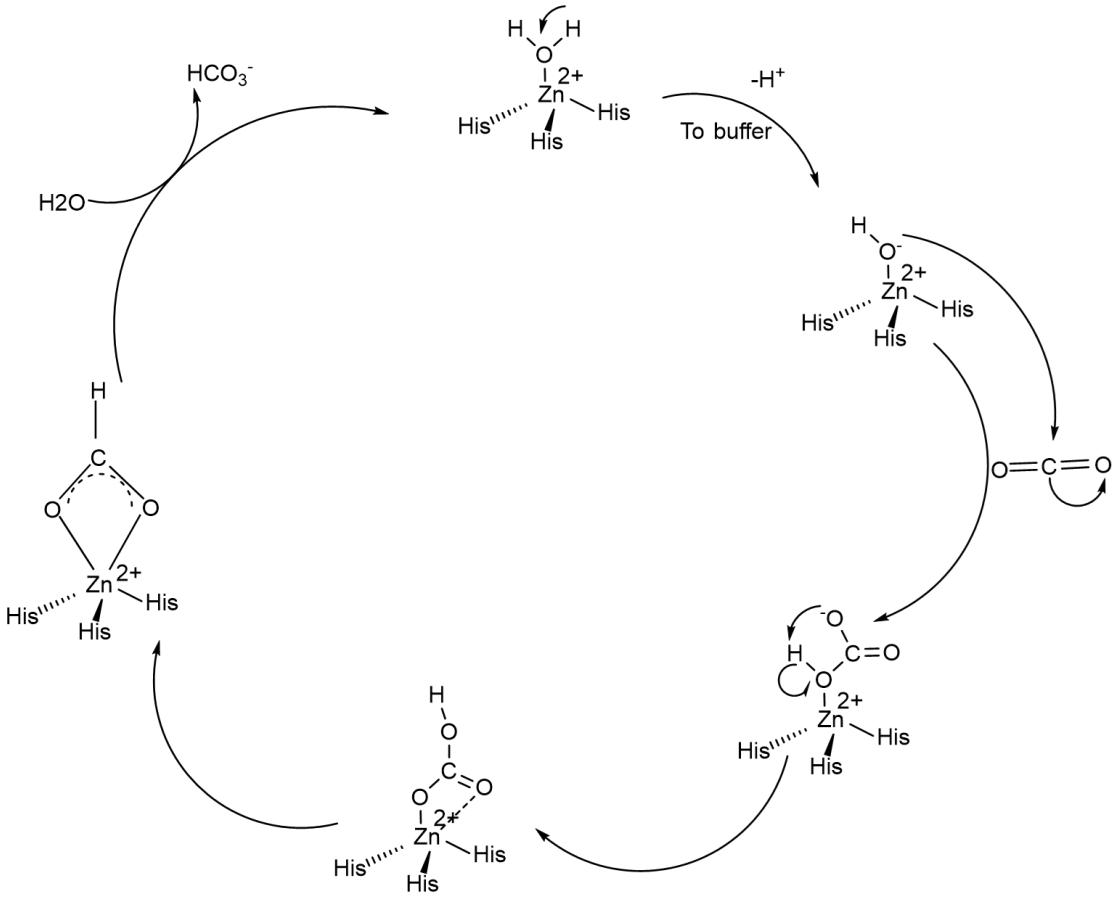


Fig. 2.5 Cyclic mechanism of carbonic anhydrase

- Because water attached to the zinc ion is swiftly transformed into hydroxide ion, the mechanism (Zn-hydroxide mechanism) begins with the creation of zinc bound hydroxide ion (Fig. 2.5). The nucleophilic hydroxide ion is positioned perfectly to attack carbon dioxide's carbon atom.

- The zinc ion also aids in the orientation of almost CO_2 to the active site (CO_2 is noncovalently bound), resulting in a high concentration of OH^-. As a result, the involvement of Zn^{2+} in carbonic anhydrase is crucial in bringing the substrates close together, which improves their reaction orientation.
- CO_2 attaches to metal-hydroxide once it forms, and the nucleophilic attack of ^-OH produces metal bound bicarbonate, which is then displaced by water.

2.5 CATALASES AND PEROXIDASES

Peroxide is a heme protein that catalyses the oxidation of a number of substrates by hydrogen peroxide, including ascorbate, ferrocynide, and cytochrome c. Almost every living entity contains the catalase enzyme. The disproportionation of hydrogen peroxide and organic peroxides was catalysed by catalases. They also catalyse the hydrogenperoxide oxidation of substrates. Cataleses have the largest turnover of any enzyme, with one catalyse molecule converting millions of molecules of H_2O_2 to water and oxygen every second.

The structure and reaction processes of peroxide and catalyses are similar. Both have a Fe(III) heme active site with a high spin, and the imidazole nitrogen of the residue occupies the fifth coordination site. In the resting enzyme, a water ligaand occupies the sixth coordination site.

The reaction is believed to occur in two steps:

$$[Fe^{III}—P]^+ + H_2O_2 \rightarrow [O = Fe^{IV}—P^+]^+$$

$$[O = Fe^{IV}—P^+]^+ + H_2O_2 \rightarrow [Fe^{III}—P]^+ + O_2$$

Where $Fe^{III}—P$ represents the enzyme's heme group and $O=Fe^{IV}—P^+$ represents the mesomeric form of $O=Fe^{V}—P$, indicating that Fe(III) is not entirely oxidised to Fe(V), but it does receive one electron from the porphyrin ring. It indicates that Fe(III) is oxidised to Fe(IV), and the porphyrin ring (P) is oxidised to porphyrin by one electron, and the porphyrin ring with one unpaired electron becomes a radical cation.

Tyr-357 (tyrosinate at position 357) in the fifth (axial) position can increase the reactivity of the iron core, assisting in the oxidation of Fe(III) to Fe(IV).Human catalase works best at a pH of around 7. Several metabolic activities produce hydrogen peroxide, which is a toxic byproduct. It must be transformed into H_2O and O_2 to avoid damage to cells and tissue. The π

to π* transition in the porphyrin ring gives oxidised and reduced versions of catalase their colour.

2.5.1 Define the biological role and the main properties of catalases

The following points may be noted :

- Catalases are found in, Erythrocyte, All plants, most aerobic bacteria.
- Catalases have the short C-conformation, in which both an axial position on iron and the porphyrin periphery are exposed.
- Catalases catalyze the decomposition of H_2O_2 into H_2O and O_2.
- Molecular weight 240,000.
- Catalases are made up of four identical subunits, each with one heme group at the active site and high-spin Fe^{3+}–porphyrin prosthetic groups.
- The enzyme's resting state is Fe^{3+}.
- Catalases and peroxidases are closely related enzymes.
- In the presence of cyanogen bromide (BrCN), absorption variations between native and modified catalase were barely evident, implying that there is no considerable change in the tyrosine environment.

2.5.2 Defifine the main biological role of peroxidases

The following points may be noted:

- Peroxidases are enzymes catalyzing the oxidation of a variety of organic and inorganic compounds by H_2O_2, also acting as dehydrogenases:
- Peroxidases and catalases are related enzymes; both are capable of promoting the oxidation of H_2O_2. The mechanism of this oxidation involves a similar enzymatic intermediate.
- Examples of peroxidases:

  (i) Chloroperoxidase: halogenates organic substrates.

  (ii) Lactoperoxidase: antibacterial, oxidizes NCS

  (iii) Cytochrome P-450: hydroxylates organic substrates.

(iv) Cytochrome c peroxidase: reduces H_2O_2.

- All the peroxidases purifified from plants contain the heme group (Fe^{3+}–protoporphyrin IX).
- Horseradish roots and the sap of fifig trees are the richest source of plant peroxidases. Cytochrome c peroxidase from baker's yeast also contains Fe^{3+}–protoporphyrin IX.
- Many of these enzymes are mixtures of isozymes of differing physical but similar catalytic properties.
- Most peroxidases are glycoproteins.

2.5.3 Mechanism and structural features

The following points may be noted:

- Beef liver catalase and horseradish b peroxidase have had their structures determined. The active sites of both enzymes have a high spin iron group.
- Catalase's peroxidase activity is generally preferred in most in vivo conditions. Catalase can be found in the blood, bone marrow, mucous membranes, kidneys, and liver, among other places. It is involved in the oxidation of H_2O produced by oxidases. As a result, enzymes that create H_2O_2 are grouped together with enzymes that degrade it.
- The catalase axial ligands are phenolate (tyrosine's deprotonated phenolic oxygen atom) and most likely a water molecule. The water molecule coupled to heme is assumed to be stored in the cavity of the enzyme's active site at the sixth position. On the active side, the phenolate moiety from a tyrosyl residue on the protein is attached to the heme and kept far away from the cavity (which holds water at the sixth position on the heme). During the catalytic activity of the enzyme, the water in the cavity at the active site is replaced by H_2O_2.
- In horse radish peroxidase the axial ligand is an imidazole from a histidyl residue on the protein.

Moreover, near thed active site of both enzyme are histidine and asparagine or arginine side chains which are suitably oriented to make part in the catalytic cleavage of O-O by the enzyme when H_2O_2 replaces water.

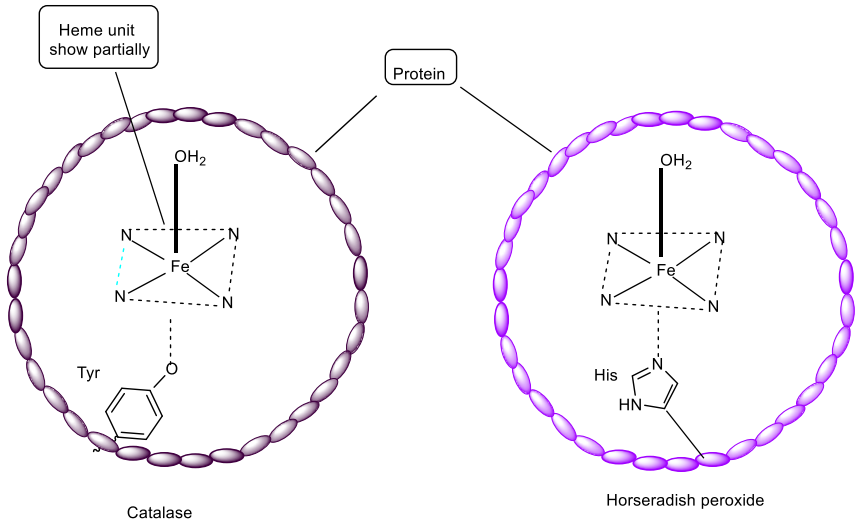
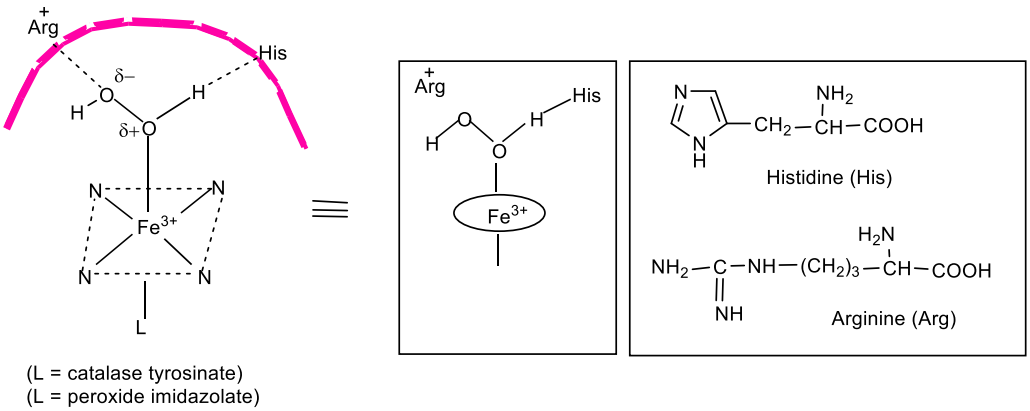


Fig. 2.6 Structure of catalase



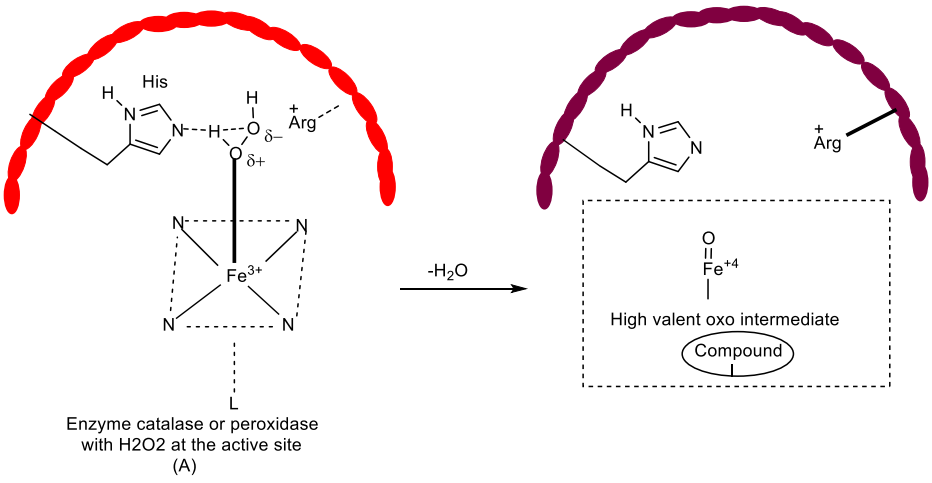


Fig. 2.7 Enzyme catalase action

Hydrogen peroxide binds to the ferric center and a subsequent heterolysis of the O-O bond takes place. This requires a prior positive and negative charge separation in the trasition state. The amini acid side chains (histidine and asparagine or arginine) near the active site perfoem this role. The basic imidazole group of histidine helps in the proton transfer from the oxygen atom of H_2O_2 attached with the iron to the departing oxygen atom and the arginine residue helps to stabilize the developing negative charge on the departing of oxygen atom. This results in O-O bond cleavage and the formation of the common high valent oxo intermediate known as compound 1. Compound 1 has tha ability to oxidize other soecies by two electrons. [Recall that in biological systems, like in chemical systems, oxidation (loss of electron) is always accompanied by reduction of an electron acceptor](Fig. 2.7).

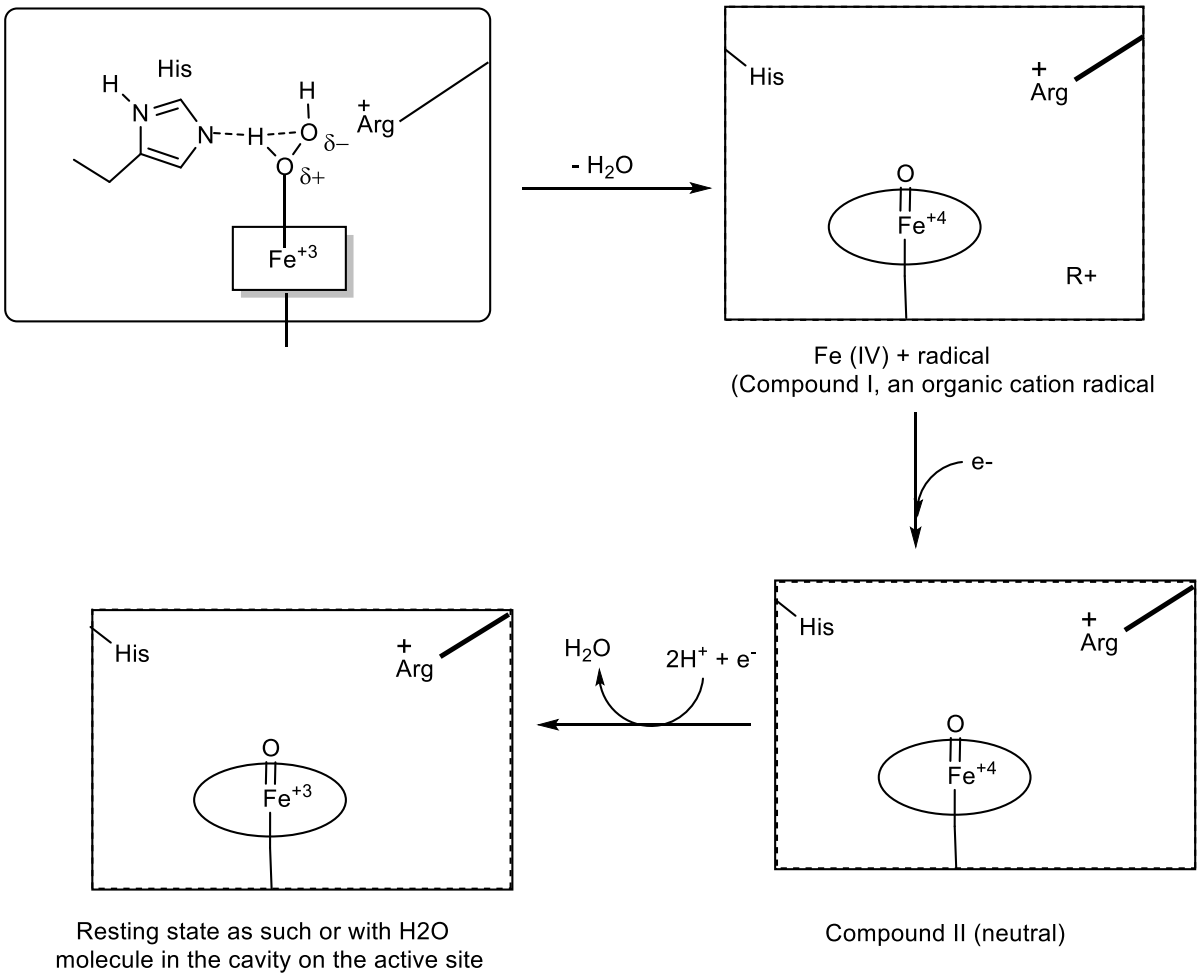


Fig. 2.8

The highly oxidising intermediate, formerly known as "compound I," is an organic cation radical that represents Fe(IV). In HRP, the radical is placed on the porphyrin ring, but in

bcytochrome c peroxidase, it is positioned on tryptophan-191, a peptide residue that is almost loated.Fe-O bonding can take many forms, ranging from Fe(IV)=O (also known as' ferryl') to Fe(IV)-O----H, in which the O atom is either protonated or attached to a donor group via a hydrogen bond. Two one-electron transfems are used to reduce compound (I) to the resting Fe(III) state, which can be accomplished with organic substrates or cytochrome c.In a variety of biological activities that involve oxygen, the Fe(IV) intermediates catalyse the elimination of harmful H_2O_2 [H_2O_2 (aq) + 2e-+ $2H^+$ (aq) $\rightarrow 2H_2O(l)$] (Fig.2.8)

- Using $H_2{}^{18}O_2$, it was discovered that the dioxygen generated in the catalase reaction is obtained from hydrogen peroxide. As a result, hydrogen peroxide disproportionation could take place in two stages. The reaction's substrate, H_2O_2, is reduced to water in the first step, and the resultant compound first represents the enzyme's oxidation (Fig.2.9).

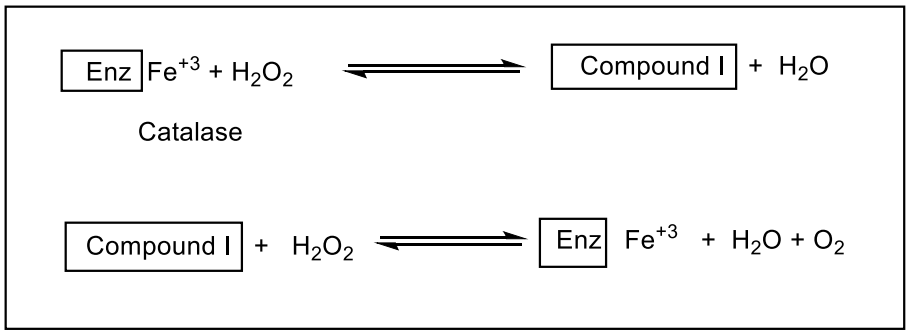


Fig. 2.9

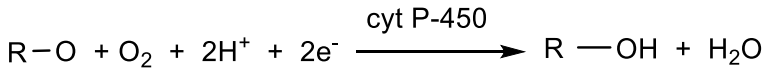
- The oxidation of H_2O_2 by compound I (the oxidation state enzyme) results in the formation of dioxygen and water. Fig. Formate, nitrate, and ethanol, as well as hydrogen peroxide, can all be oxidised by compound I. As a result, the iron centre in compound I is highly oxidised, and the job of the tyrossinate axial ligand phenolate O^-) in catalase is to stabilise such a centre. Similar stabilisation may be given by the histidyl imidazole ligand via deprotonation in the case of peroxidase.

2.6 CYTOCHROME P-450

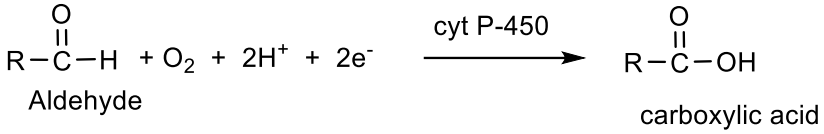
Cytochrome P-450 is a family of cytochromes that can be found in plants, animals, and microorganisms. It's called a pigment because its CO compounds absorb light at 450 nm. This is owing to the π-π^* (blue to red) transition, and this bond is known as the SORET bond. Cytochrome P-450 aids in O_2 cleavage and acts as a monooxygenase, facilitating the insertion of oxygen atoms into the substrate. Oxygenases are enzymes that add oxygen to a food

source. A monooxygenase introduces one oxygen atom into the substrate, while a dioxygenase inserts two oxygen atoms into the substrate. The C-H bond is transformed to C-OH groups in the most significant compounds. Here are a few examples:

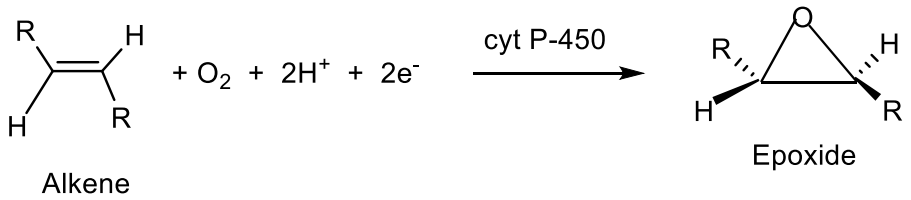
- Conversion of an hydrocrbon RH to ROH.



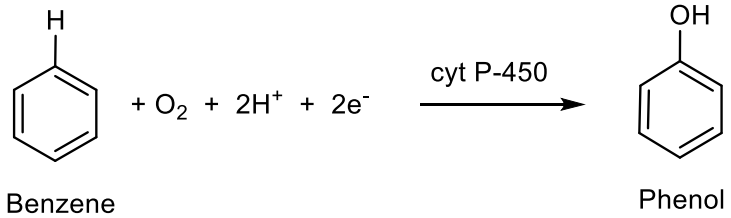
- Conversion of an aldehydeto the carboxylic acid.



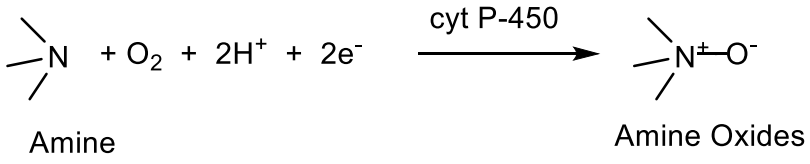
- Conversion of alkene into epoxide.



- Conversion of Benzene into Phenol



- Oxidation of amine into amine oxides.



- Oxidation of sulphide into sulphoxides.

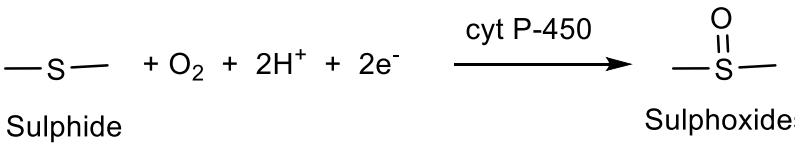


Fig.2.10 Different type catalytic reactions catalysed by cytochrome P-450

In the kidney, cytochrome P-450 enzymes convert insoluble hydrocarbons into water-soluble R-OH molecules, which are then excreted in the urine.An organic substrate receives one oxygen atom, which is then reduced to H_2O.The active site of cytochrome P-450 is heme, which is comparable to haemoglobin and myoglobin except for the following differences:

(i) Fe is present in Fe(III) state and it is low spin octahedral.

(ii) Sixth coordination site is occupied by H_2O.

(iii) One S-atom of cysteine is coordinated to Fe(III) instead of hisdine in the Proximal position.

The cytochrome P-450 enzyme have low spin octahedral Fe(III) active site.

Cytochrome P-450 belongs to a large group of heme-containing monooxygenases that oxygenate a wide range of substrates. Such enzymes catalyse the dioxygen oxidation of organic substrates and play a vital role in biosynthesis, metsbolism, and, most importantly, the deetoxification of hazardous chemicals. Water insoluble aliphatic or aromatic hydrocarbons, for example, are transformed into water soluble alcohols and expelled through the urine.

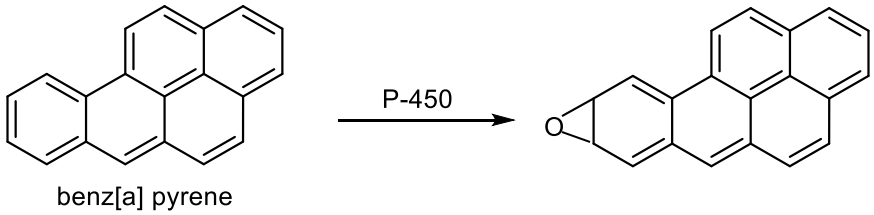


Fig. 2.11 Epoxidation of benz[a] pyrene

More arenes (including benzene) are epoxidized in humans, and this process is catalysed by a family of enzymes in the liver (cytochrome P-450). One of the compounds under investigation is benz[a] pyrene, a result of the reaction of a variety of organic materials, including tobacco, to produce an epoxide with carcinogenic characteristics.

2.6.1 Structure of cytochrome P-450

Cytochrome P-450 is a physiologically active protein that participates in a variety of oxidation processes. Cytochrome P-450 (heme B type) is a protein with the prosthetic group Fe(III) protoporphyrin IX.Cytochrome P-450, like myoglobin, has an oxygen-binding heme

unit, but instead of the axial histidine seen in myoglobin, a cystein thiolate residue is present. A single polypeptide (-halical) chain exists in cytochrome P-450.

A heme group b molecule is sandwiched between two helices of two axial ligands, one of which is a cystein ligand from a protein and the other of which is a water molecule. The CYPs are hemoproteins with a single haem prosthetic group in the active site and 400-500 amino acid residues. Low spin (LS), in which the five 3d electrons are maximally paired, and high spin (HS), in which the five 3d electrons are maximally unpaired, are the two spin states of iron in the ferric form (Fe^{3+}).According to spectral, NMR, and crystallographic evidence, a water molecule creates a sixth axial ligand of the Fe^{3+} in the substrate-free form, maintaining the LS state of the ion. When substrates bind to the enzyme, the iron-water molecule is displaced, changing the Fe^{3+} coordination state from six to five, where the Fe^{3+} travels out of the plane of the haem ring.

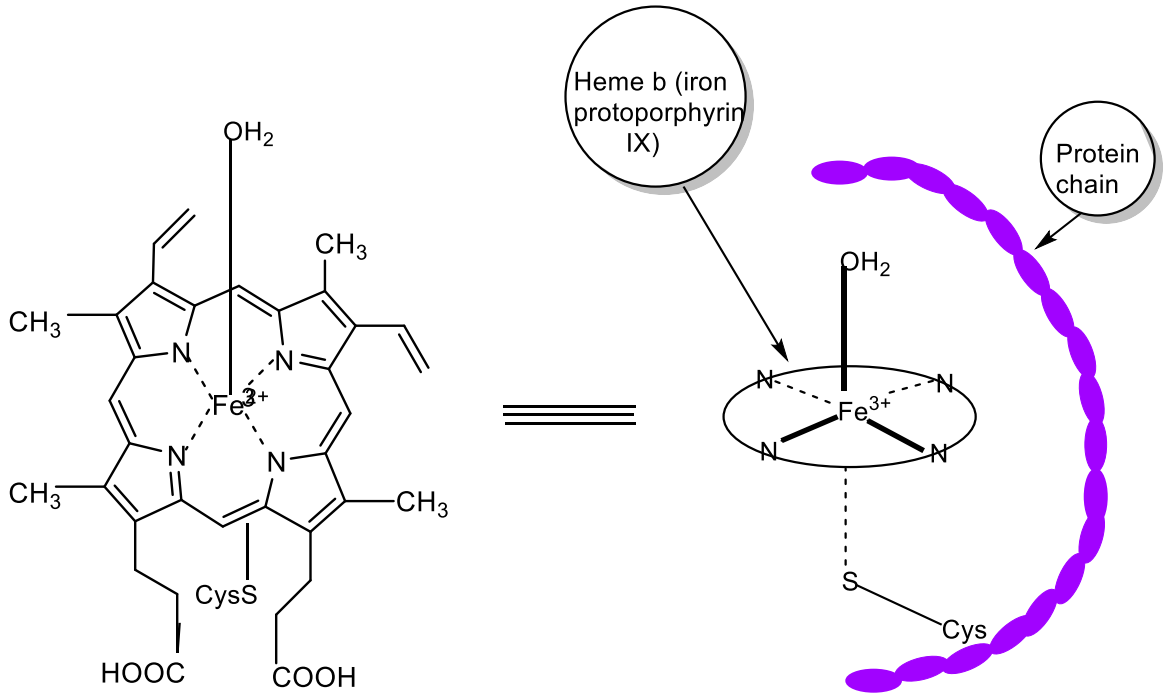


Fig.2.12 Structure of cytochrome P-450

Most of the information on P-450 is based on studies undertaken on an enzyme P-450 obtained from the bacterium Pseudomonas putida. This source (organism) makes use of camphor as its only source of carbon, the stage being. The oxygenation at the C5 position.

2.6.2 The mechanism of oxidation of a substrate with cytochrome P-450

The molar mass of the cytochrome P-450 enzyme is around 50,000. Figure 1 depicts the catalytic cycle for the action of the cytochrome P-450 enzyme.

The organic substrate enters a hydrophobic pocket of the protein at the Fe(III) center, expelling water molecules from the iron axial coordination site to form Fe(III) complexes, which are then reduced by another enzyme system to form high-spin Fe(II) complexes in the second step.

In step third, a dioxygen molecule forms a bond with a Fe(II) center, similar to haemoglobin and myoglobin, and one electron is transferred from Fe(II) to dioxygen, resulting in the formation of a Fe(III)-superoxo complex.In step four, another electron is added to form the Fe(III)-peroxo complex.In step five, the protonation of the Fe(III)-peroxo complex results in the removal of one oxide ion as water, resulting in an oxyferryl complex.

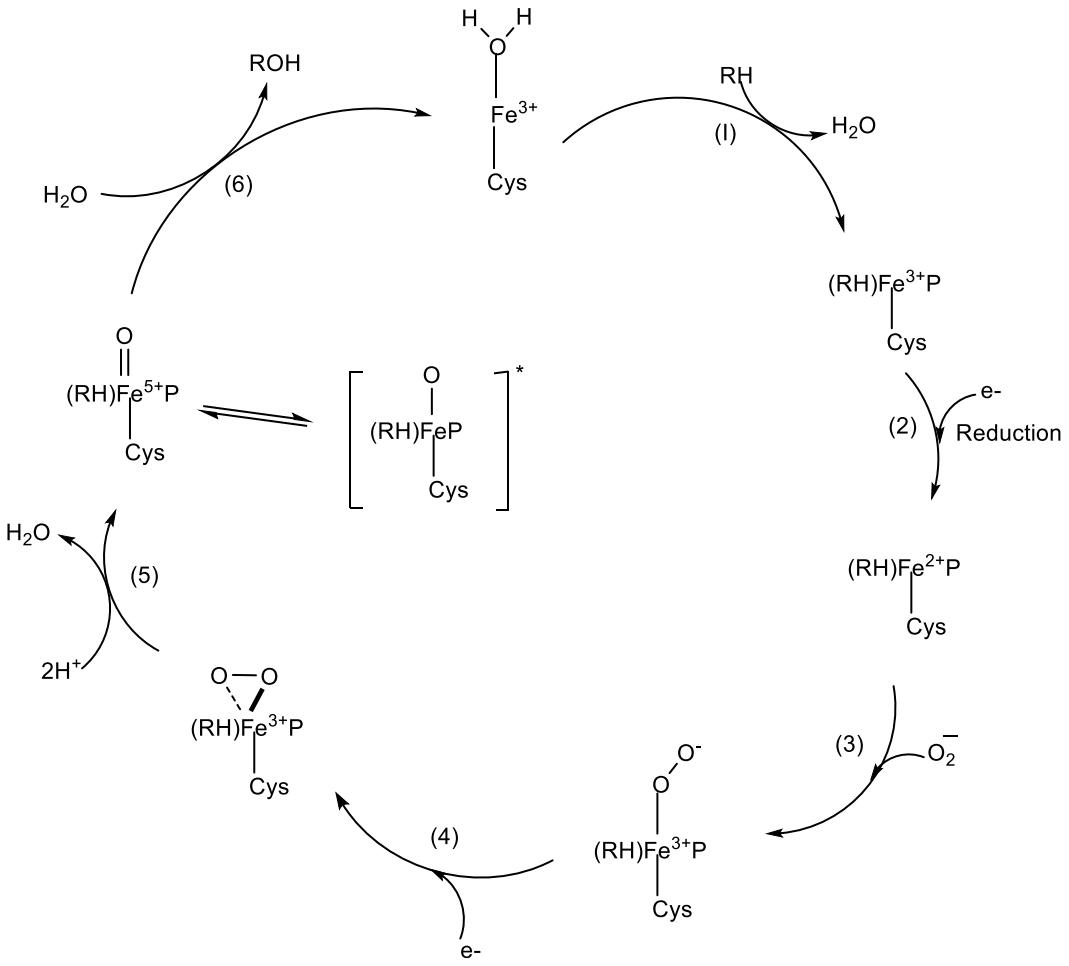


Fig. 2.13 Cyclic mechanism of cytochrome P-450

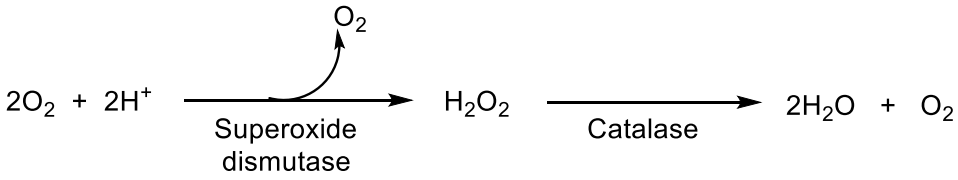
One electron has oxidised from the-HOMO of the porphyrin ring, leaving it as a radical cation. Fe(V) = O, or oxygen that has been double linked to Fe(IV).Step six involves oxidising the organic substrate (R-H) to R-OH while simultaneously binding an H2O ligand to the active site of the metalloenzyme, which now has a low spin Fe(III) center.

2.7 COPPER ENZYME

In yeast, copper is essential for iron intake, energy synthesis, and the protection against oxidative stress. The bulk of phenotypes found during copper deprivation are explained by three key copper enzymes in yeast: a multicopper oxidase, a copper heme oxidase, and a superoxide dismutase.

2.7.1 Superoxide dismutase (SOD) a copper enzyme

Superoxide dismutase is a catalytic enzyme that catalyses the elimination of the harmful superoxide anion, O_2^-, which is produced as a byproduct of oxidative metabolism. Superoxide is converted to molecular oxygen and hydrogen peroxide by this enzyme. The subsequent work of enzymes like catalase removes hydrogen peroxide, which is a potentially hazardous chemical. As a result of their collaboration, SOD and catalase help to safeguard organisms that use dioxygen from potentially hazardous byproducts of O_2 metabolism.



- Superoxide dismutase (SOD) is present in all aerotolerant organisms for the purpose of minimizing the concentration of superoxide, O2, and thus providing protection against oxygen toxicity.
- The SOD like, Ni SOD, either Fe or Mn SOD, which seem to be the same protein, and Cu/Zn SOD.
- Fe or Mn SOD is found in the prokaryotes or the mitochondria of eukaryotic cells, while Cu/Zn SOD is found in the cytoplasm of eukaryotic cells.

There are three type of superoxide dismutase :

(1) Copper- Zinc superoxide dismutase, CuZnSOD

(2) Manganese superoxide dismutase, MnSOD

(3) Iron superoxide dismutase, FeSOD

2.7.2 Structure of Cu-Zn superoxide dismutase

CuSOD (bovine superoxide dismutase) is present in the mitochondria of eukaryotic cells, while the other two are found in bacteria (prokaryotes). Cu^{2+} and Zn^{2+} ions are coupled to the imidazole of a histidine residue, according to a CuZnSOD crystal structure determination (fig. 2). The Cu^{2+} ion is a deformed square pyrimidal site bound to four imidazole histidine nitrogen atoms and a water molecule, whereas the Zn^{2+} ion is tetrahedrally coordinated to three imidazole histidine nitrogen atoms and oxygen from the asparatate residue.

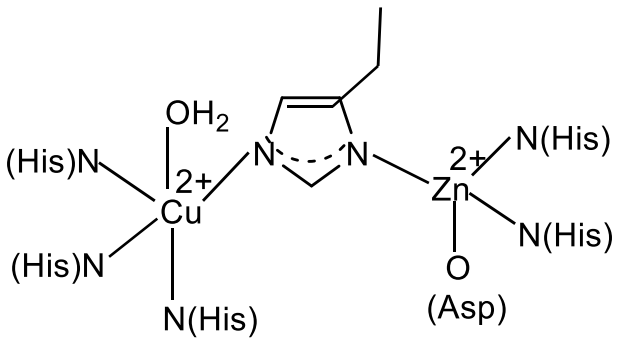


Fig.2.14 structure of Cu-Zn superoxide dismutase

The molar mass of copper-zinc dismutase is around 16,000. The Cu^{2+} ion has been demonstrated to be the functional one, whereas the Zn^{2+} ion serves as a structural stabiliser by holding the bridging imidazole histidine residue in place.

Cu^{2+} is a more important ion that cannot be substituted by another metal while maintaining activity. The Zn^{2+} ion, on the other hand, can be replaced with other divalent metals such as Co or Cd while maintaining the majority of the activity.

2.7.3 Mechanism Cu-Zn superoxide dismutase

The following point may be noted:

- When Cu(II) and Zn(II) are removed from an enzyme, its activity is diminished. Only the addition of Cu(II) reactivates the enzyme. As a result, the role of Zn(II) is only relevant from a structural standpoint. Furthermore, Zn(II) occupies all four coordination sites in

Cu-ZnSOD. The displacement of His 46 by superoxide is thought to be the mechanism by which it binds to Cu(II).

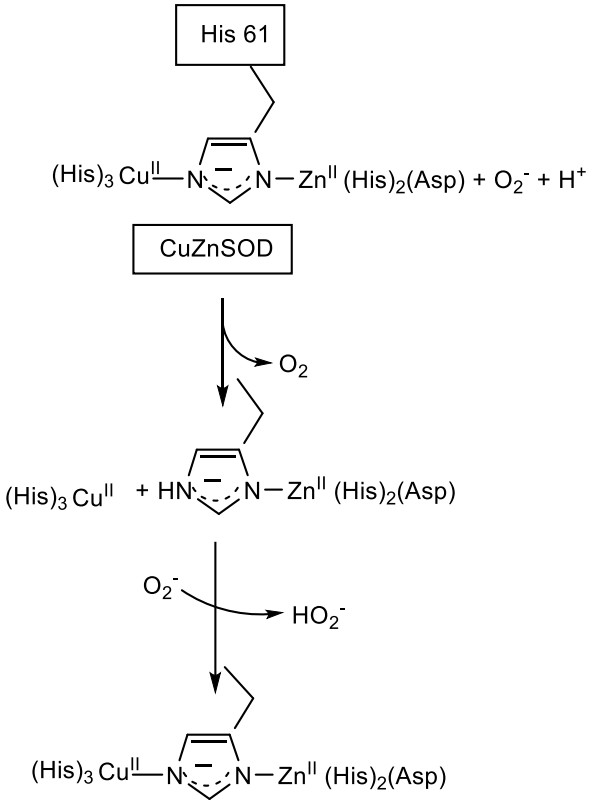


Fig.2.15 Mechanism Cu-Zn superoxide dismutase

- It has been proposed that the imidazole bridge breaks and reforms during each catalytic cycle of the reaction.
- The pKa of the other nitrogen atom is lowered when a histidine ring nitrogen atom is coordinated to Zn(II). As a result, it prefers to attach a proton rather than Cu (I).
- When a proton is transferred to a peroxide ion, which is then transformed to H_2O_2, the bridge is reformed. This idea also creates a location at the Cu(I) centre for substrate (O_2^-) binding, eliminating the necessity for a potentially high-energy five-coordinate transition state.
- The reaction is therodynamiccally favourable. The redox potential for the O_2/O_2^- coupleis -0.33V and for the O_2^-/H_2O_2 couple +0.89V. Any metal atom capable of one-electron redox chemistry between M^{n2+} and $M^{(n+1)+}$ states that has a potential between the limits $-0.3 \leq E^o \leq +0.9$ will be thermodynamically feasible to act as a superoxide dismutase. In

keeping with this, other single atom SOD's e.g. both Fe and Mn SOD also exist in nature.

2.8 MOLYBDENUMOXOTRANSFERASES-XANTHINE OXIDASE

It has been proposed that the imidazole bridge breaks and reforms during each catalytic cycle of the reaction.

The pKa of the other nitrogen atom is lowered when a histidine ring nitrogen atom is coordinated to Zn(II). As a result, it prefers to attach a proton rather than Cu (I).

When a proton is transferred to a peroxide ion, which is then transformed to H_2O_2, the bridge is reformed. This idea also creates a location at the Cu(I) centre for substrate (O_2^-) binding, eliminating the necessity for a potentially high-energy five-coordinate transition state.

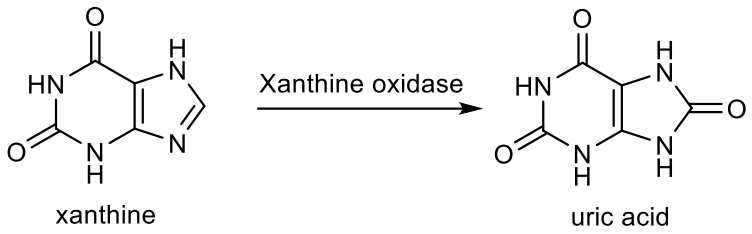


Fig.2.16 Oxidation of xanthine to uric acid

Gout is caused by an excess of uric acid in the body, which can be treated with xanthine oxidase inhibitors. The Mo(VI) site in xanthine oxidase performs the two-electron oxidation of xanthine to uric acid and then self-reduces to Mo. (IV). The Mo(VI) site is regenerated by transferring electrons to the Fe_2S_2 and FAD sites one at a time, making Mo(VI) ready for the oxidation of the next equivelnt of xanthine. The electron flow can be depicted as follows:

Xanthine → Mo(VI) → 2Fe_2S_2 → FAD → O_2

Fe_2S_2 sites in xanthine oxidase play the same electron-transfer role as the Fe2S2 ferroxine play in photosynthesis.

2.8.1 Structural features and mechanism

Molybdenum is the only second-row transition metal that has biological use.

It has a unique value and experiences two electron transfer reactions, mostly between the Mo(VI) and Mo(IV) oxidation states, as previously stated. It can also transfer an oxo atom to a substrate, as seen in fig.2.17

$$Mo^{VI}(O_2) + S \rightarrow S{=}O + Mo^{IV}O$$

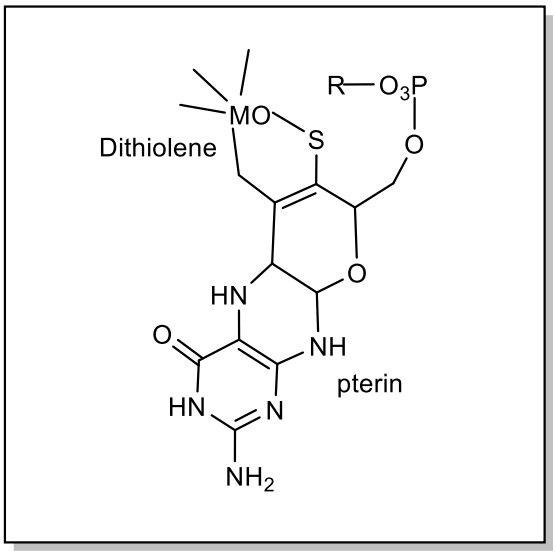


Fig.2.17 Structure of xanthine oxidase

The metal is usually coordinated by a ligand called molybdopterin in all Mo enzymes (fig. 2.18). A pair of S atoms from a ditholene group that is covalently bonded to a pterin serve as metal donors. A nucleoside base R, such as guanosine 5'-diphosphate, is linked to the phosphate group. The pterin group could have a function in assisting redox reactions by acting as an electron channel.

The mechanism of the reaction may be studied considering the following,

- One terminal molybdenum oxo (Mo=O) group, two thiolate-type sulphur ligands (pterin dithiolene side chain), and one terminal sulphido group (Mo=S) group characterise the oxidised form of the enzyme.
- The terminally attached sulphido group serves as a base to remove hydrogen from the reactant xanthine, while an oxygen atom is transferred from the Mo(VI)=O unit to xanthine.
- The Mo enzyme transfers an O atom directly to the substrate (xanthine). The O atom transported to the substrate is thus not via the Mo-promoted assault of solvent water or hydroxide on the substrate.
- The oxo group from the water molecule is repaired at the Mo centre via linked deprotonation and electron processes.

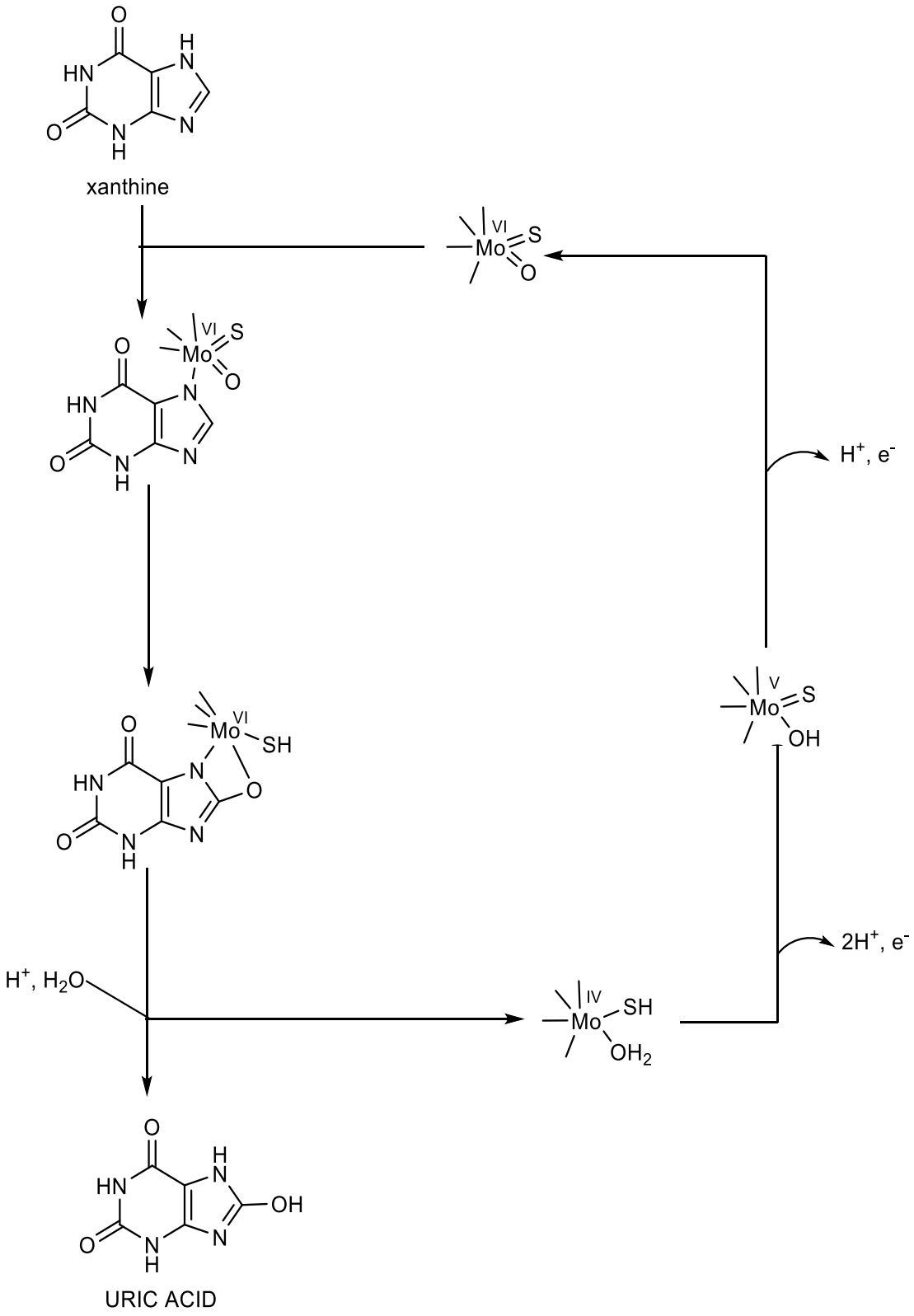


Fig.2.18 Mechanism of xanthine oxidase

- The Mo enzyme transfers an O atom directly to the substrate (xanthine). The O atom transported to the substrate is thus not via the Mo-promoted assault of solvent water or hydroxide on the substrate.
- The oxo group from the water molecule is repaired at the Mo centre via linked deprotonation and electron processes.
- Significantly, this oxygenation reaction varies from that of other Fe and Cu enzymes in that the oxo group transported by Mo enzymes is generated from water rather than molecular oxygen.

The Mo(VI) site [Mo cofactor (Molybdepterin)] in xanthine oxidase catalyses the two-electron oxidation of xanthine to uric acid, which is then reduced to Mo. (IV). Loss of electrons (one at a time) to the Fe_2S_2 and FAD sites regenerates the Mo(VI) species. Although the Fe_2S_2 sites of the enzyme are not directly involved in substrate reaction, they are critical to the enzyme's overall function. The Fe_2S_2 core of the enzyme has a role similar to Fe_2S_2 ferrodoxine's simple electron-transfer job in photosynthesis.

2.9 VITAMIN B_{12}

The only organometallic compounds found in nature are vitamin B_{12} and coenzyme B_{12}. Vitamin B_{12} was originally extracted from liver extracts, and it was discovered that human permicious anaemia is caused by a vitamin B_{12} or B_{12} coenzyme shortage. The Co(III) ion is linked to four N-atoms of a corrin ring in vitamin B_{12}. The corrin ring is a modified porphyrin ring with one fewer =CH- bridge connecting the two pyrrole rings than the porphyrin ring. As a result, the corrin ring is less symmetric and saturated than the porphyrin ring. An imidazole nitrogen and a cyanide ion occupy the fifth and sixth positions, respectively.

The cyanide ion, on the other hand, is absent in vivo, and the sixth place is occupied by a loosely attached water molecule.Cobalt can be reduced by one electron to give vitamin B_{12} [Co (II) complex] or by two electrons to give vitamin B_{12} [Co (I) complex] after incorporation of Co (III) into the corrin ring, altering the reduction potential of cobalt. Reduced ferredoxin can carry out these reductions in vivo. Because it is very nucleophilic, it is easily alkylated.

Some reaction of vitamin B12 are given below (Fig.2.19)

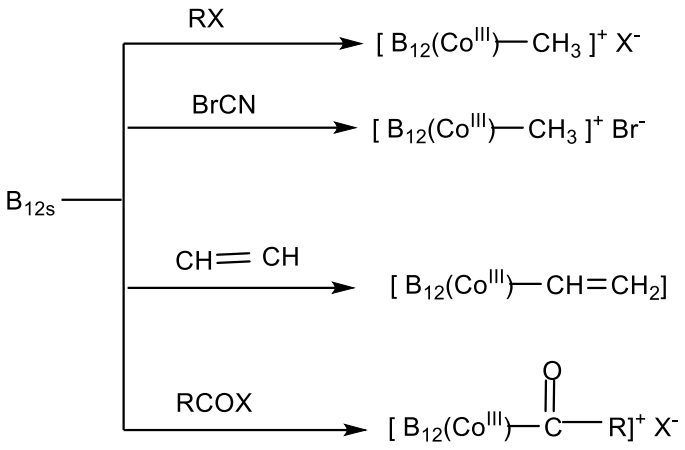


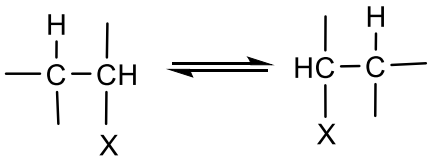
Fig.2.19 Catalytic reaction of vitamin B_{12}

The R sight is unoccupied in vitamin B_{12}, and the 5-coordinate cobalt (I) atom is extremely reactive. Cobalt is present in a +3 oxidation state in vitamin B_{12} and its various derivatives. Because the spin octahedral field of Co (III) in these compounds is low ($d_6 \rightarrow t_2^6 e_g^0$), they are diamagnetic and EPR inactive.

The red and brown colours of vitamin B_{12} and vitamin B_{12} are caused byπ-π* transitions in corrin rings. Due to the presence of an unpaired electron in the dz^2 orbital, the latter is EPR active. Vitamin B_{12r} has a low Co(II) spin.Co(I) in vitamin B_{12} is also EPR active due to the presence of two unpaired electronns, and its blue-green colour is due toπ-π* transitions.

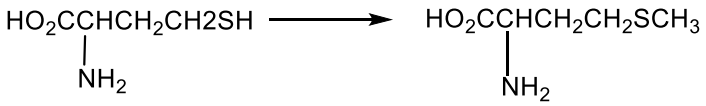
Unfortunately, certain bacteria may methylate not only sulphur in organic molecules, but also heavy metals including Hg, Sn, Pd, Pt, and Pd in aqueous solution, resulting in very hazardous species like Hg (CH_3) and Pd $(CH_3)_4$.

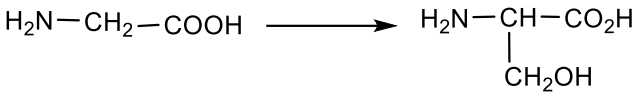
Adenosyl and cobalt form a direct cobalt-carbon bond when adenosine triphosphate (ATP) reacts with vitamin B_{12s}. B_{12} coenzyme is the chemical that results. It was the first time an organometallic compound was found in live organisms. Co(III) is a coordinated carbon atom of an adenosyl ligand that replaces the CN^- ligand in coenzyme B_{12}. This coenzyme catalyses 1,2 general type rearrangements.



Vitamin B_{12} with CN^- removed is called cobalamin, thereforee, vitamin B_{12} is called cyanocobalamin.

Coenzyme B_{12} takes a methyl or hydroxy methyl group (bound to Co) that can be transferred to a substrate to add a carbon. As ribonucleic acid (RNA) is converted to deoxyribonucleic acid, coenzyme B_{12} converts the -CH(OH) group to the -CH_2 group (DNA).





The breaking of the Co-C bond is the precise mechanism of these reactions. It's worth noting that given the right conditions, cobalt porphyrin analogues of vitamin B_{12} can be converted to the Co(I) state. As a result of the porphyrin ligand's failure to stabilise the Co(I), the corrin ring has been chosen in place of the porphyrin ring in the evolution of B_{12} cobalt complexes.

2.9.1 Structure and characteristic features of vitamin B_{12}

The following points may be noted:

- It is the only naturally occurring organometallic complex (a species with a direct metal to carbon connection) in biology, and it is the only vitamin that contains a metal ion.
- The molecule is an octahedral cobalt (III) complex with corrin, a 15-membered 4-nitrogen ring ligand.
- All of the side chains attached to corrin are acetamide and propionamide groups, with one of these being an isopropanol phosphate residue attached to a ribose. Finally, the molecule is terminated by a 5,6-dimethyl-benzimidazole, which coordinates.
- The majority of B_{12} coenzyme reactions are catalysed or initiated by homolytic cleavage of the CO-C bond, which is catalysed or launched by homolytic cleavage of the CO-C bond.

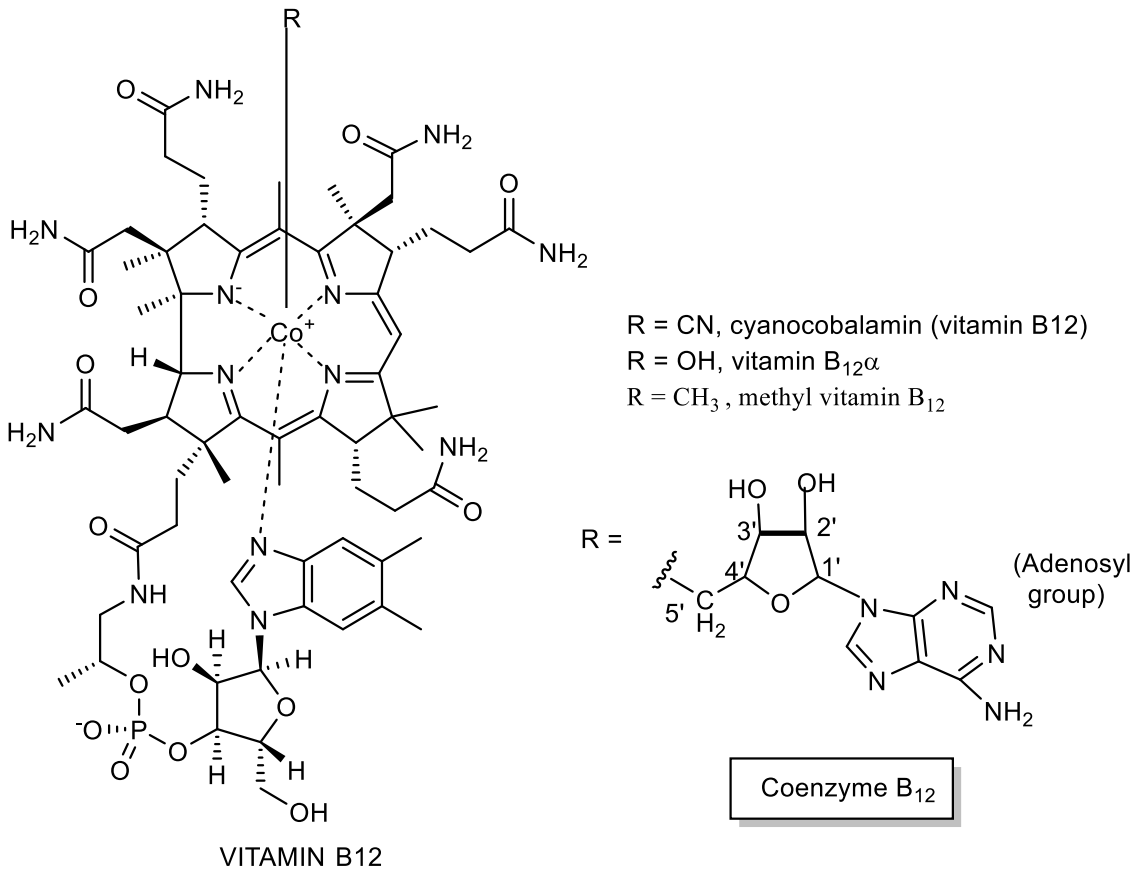


Fig. 2.20 Structure of vitamin B_{12}

2.9.2 Application of vitamin B_{12}

Vitamin B_{12} is a water-soluble vitamin found in meat, fish, and dairy products. It can also be manufactured in a laboratory and is frequently combined with other B_{12} vitamins.Many components of the body, including the brain, nerves, and blood cells, require vitamin B12 for proper function and development. The active form of vitamin B12 is methylcobalamin. The most common type used in supplements is cyanocobalamin, which must be converted by the body into the active form.

Vitamin B_{12} is widely used to treat vitamin B_{12} deficiency, cyanide poisoning, and excessive blood homocysteine levels. It's also used to treat canker sores, cataracts, Alzheimer's disease, osteoporosis, weariness, and a variety of other ailments, although most of these claims lack scientific backing. The following are some processes performed by enzymes that require coenzyme B_{12}:

A secondary methyl group is interted into a main chain e.g., shown in (Fig.2.21) or an amino

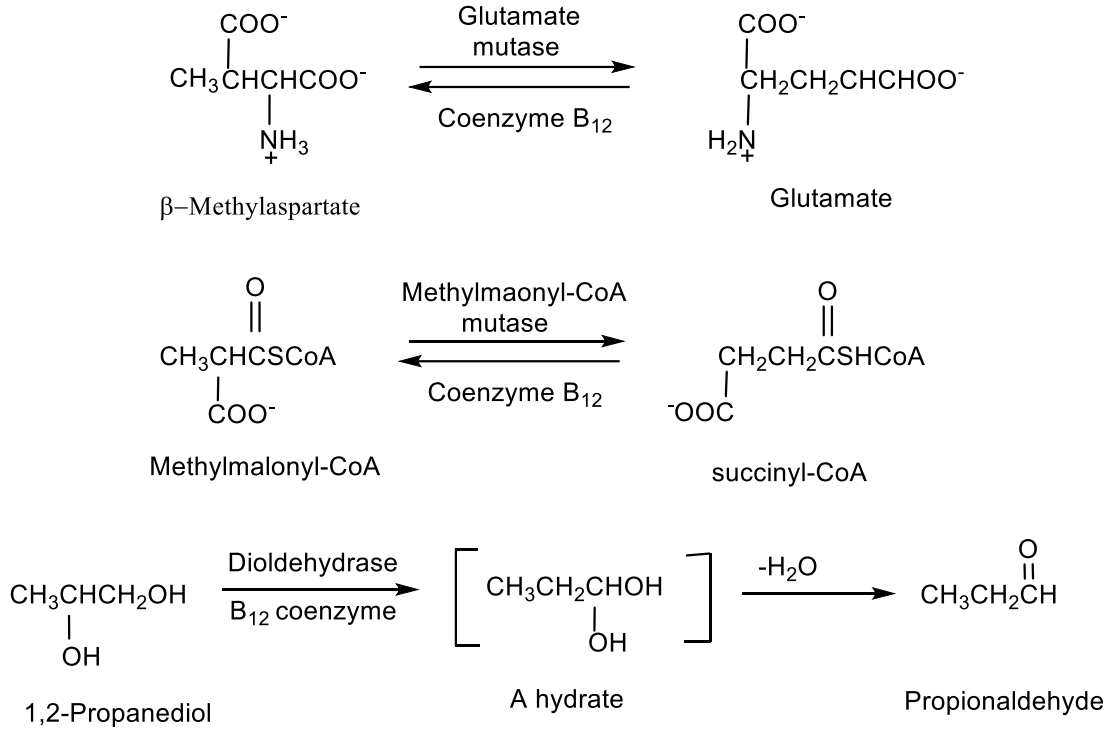


Fig. 2.21

Group can isomerize from a primary to a secondary carbon. Dioldehydrase B_{12} coenzyme produces propiopaldehyde from 1, 2-propanediol. The initial product of vicinal 1, 2-intercharge is a hydrate which loses water to give the product.

2.9.3 Vitamin B12 Deficiency

With age, it can become harder to absorb this vitamin. It can also happen if you have had weight loss surgery or another operation that removed part of your stomach, or if you drink heavily.You may also be more likely to develop vitamin B12 deficiency if you have:

- Atrophic gastritis, in which your stomach lining has thinned
- Pernicious anemia, which makes it hard for your body to absorb vitamin B12
- Conditions that affect your small intestine, such as Crohn's disease, celiac disease, bacterial growth, or a parasite
- Alcohol misuse or heavy drinking can make it harder for your body to absorb nutrients or prevent you from eating enough calories. One sign that you lack enough B12 may be glossitis, or a swollen, inflamed tongue.

- Immune system disorders, such as Graves' disease or lupus
- Been taking certain medications that interfere with the absorption of B12. This includes some heartburn medicines including proton pump inhibitors (PPIs) suchas esomeprazole (Nexium),
  lansoprazole (Prevacid), omeprazole (PrilosecOTC), pantoprazole (Protonix), and rabeprazole (Aciphex), H2 Blockers such as cimetidine (Tagamet) and famotidine (Pepcid AC); and certain diabetes medicines such as metformin (Glucophage).

2.9.4 Food Sources of Vitamin B_{12}

You can get vitamin B_{12} in animal foods, which have it naturally, or from items that have been fortified with it.Animal sources include dairy products, eggs, fish, meat, and poultry. If you're looking for a food fortified with B_{12}, check the product's Nutrition Facts label.

2.10 SUMMARY

The different essential aspects of enzymes are covered in the above unit. The following is a summary of the unit:

- Carboxypeptidase A is a zinc enzyme with a tetrahedral shape and a sp3 hybridization. Carbonic anhydrase is an enzyme that catalyses the conversion of carbon dioxide (CO_2) to carbonic acid.
- Cytochrome P-450 aids in O_2 cleavage and acts as a monooxygenase, facilitating the insertion of oxygen atoms into the substrate.
- In yeast, copper is essential for iron intake, energy synthesis, and protection against oxidative stress. The bulk of the phenotypes found during copper deprivation are explained by three key copper enzymes in yeast.
- Superoxide dismutase is a catalytic enzyme that catalyses the elimination of the superoxide anion, O_2, which is produced as a byproduct of oxidative metabolism. SOD and catalase help to safeguard organisms that use dioxygen from potentially hazardous byproducts of O_2 metabolism.
- Xanthine oxidase convert xanthinin into uric acid. Gout is caused by an excess of uric acid in the body, which can be treated with xanthine oxidase inhibitors.

- The only organometallic compounds found in nature are vitamin B_{12} and coenzyme B_{12}. Vitamin B_{12} was originally extracted from liver extracts, and it was discovered that human permicious anaemia is caused by a lack of B_{12} or B_{12} Coenzyme shortage. The Co(III) ion is linked to four N-atoms of a corrin ring - a modified porphyrin ring with one fewer =CH- bridge connecting the two pyrrole rings than the porphryin ring. Because it is nucleophilic, it is easily alkylated and can be reduced in vitro using reduced ferredoxin.

2.11 SAQs TYPE QUESTION

A. Multiple Choice Question

1. Which of the following mettaloenzyme converts oxygen to water

   a. Hemoglobin b. Catalase

   c. Cytochrome c-oxidase d. haloperoxidase

2. Zn in carbonic anhydrase is coordinated by three histidine and one water molecule. The reaction of CO_2 with the enzyme is an examples of,

   a. Nucleophilc addition b. Electrophilic addition

   c. Electron transfer d. Electrophilc substitution

3. Enzyme Carbonic anhydrase metal is present :

   a. Fe b. Zn

   c. Cu d. Co

4. The ligand present in Vitamin B_{12} is :

   a. Porphyrin ring b. Corrin

   c. Crown ether d. Phthalocyanin

5. Carboxypeptase contains :

   a. Mg(II) and Hydrolysis CO_2 b. Zn (II) and hydrolysis peptide bond

   c. Mg(II) hydrolysis peptide bond d. Zn(II) and Hydrolysis CO_2

6. The metal ion present in the active site of carboxypepdase enzyme is :

a. Iron b. Copper

c. Molybdenium d. Zinc

7. A well known naturally occuring organometallic compound is :

a. Hemoglobin b. Cytochrome P-450

c. Vitamin B_{12} d. Superoxide dismutase

8. Xanthine oxidase convert xanthinin into :

a. Citric acid b. Uric acid

b. Acetic acid d. Cinnamic acid

9. Normal blood pH is

a. 7.3 b. 7.2

c. 7.4d. 8.4

10. Loss of electrons can be termed as _____

a. Metabolism b. Anabolism

c. Oxidation d. Reduction

11. Which of the antineoplastic agent is metabolized by xanthine oxidase?

a. 6-mercaptopurine b. Vincristine

c. Chlorambucil d. 6-Thioguanine

12. Deffiency of Vitamine B12 is,

a. Asthma b. Noval covide-19

c. Penecius anemia None of these

13. Excess of uric acid

a. Gout formation b. Noval covide-19

c. Penecius anemia a. Asthma

14. Which of the following statements is not true about cytochrome P450 enzymes?

a. They ontain heme and Mg^{2+} b They called monooxygenases.

c.There are over 30 different Cyt P-450 d. They contain Fe^{3+} ion in active site

15. Which of the following groups is least susceptible to cytochrome P450 enzymes?

a. Terminal methyl groups b. Allylic carbons

c. Benzylic carbon atoms d. Quaternary carbon atoms

16. The enzyme carbonic anhydrase is of which type ?

a. Reversible b. Lyases

c. Unidirectional d. Isomerase

17. Carbonic anhydrase enzyme present in,

a. WBC b. RBC

c. Blood plasma d. Platelets

18. In production of which carbonic acid, Aspergillus niger is useful ?

a. Citric acid b. Acetic acid

c. Plamitic acid d. Butyric acid

B. Fill in the bling

(i) Cytochrome P-450 aids in O_2 cleavage and acts as a……. , facilitating the insertion of oxygen atoms into the substrate.

(ii) Vitamin B_{12} with CN^- removed is called…… , thereforee, vitamin B_{12} is Called cyanocobalamin.

(iii) Superoxide dismutase is a catalytic enzyme that catalyses the elimination of the harmful superoxide anion, O_2^-, which is produced as a byproduct of……

(iv) Cataleses have the largest…… of any enzyme, with one catalyse molecule converting millions of molecules of…… to water and oxygen every second.

(v) Carbonic anhydrase affects CO_2 transport in the blood and so plays a significant role in ……..

C. True/False

(i) The red and brown colours of vitamin B_{12} and vitamin B_{12} are caused by π-π^* transitions in corrin rings. True/False

(ii) Cytochrome P-450 called a pigment-450 because its CO compounds absorb light at 450 nm True/False

(iii) Carboxypeptidase A (CPA) contains a zinc (Zn^{2+}) metal center in a octahedral geometry with amino acid residues in close proximity around zinc to facilitate catalysis and binding. True/False

(iv) The disproportionation of hydrogen peroxide and organic peroxides was catalysed by catalases. True/False

(v) Coenzyme B_{12} takes a methyl or hydroxy methyl group (bound to Co) that can be transferred to a substrate to add a carbon. True/False

(vi) Carbonic anhydrase is an enzyme that catalyses the conversion of carban monoxide (CO) to uric acid. True/False

D. Match the following

i. Xanthine oxidase	a. Monoxygenase
ii.Carboxypepdase	b. Uric acid
iii.Cytochrome P-450	c. 1-2 methyl shift
iv. Vitamin B12	d. Peptide bond hydrolysis
v. Catalase	e. Disproportionation

Answer Key

A. 1 c 2 b 3 b 4 b 5 b 6 d 7 c 8 b 9 c 10 c 11 a 12 c 13 a 14 a 15 d 16a 17 b 18 a

B. i Monooxygenase, ii Cobalamin, iii oxidative metabolism, iv Turnover, H_2O_2,

v. respiration.

C. i True ii True iii False iv True v True vi False

D. i b, ii d, iii a, iv c, v e,

2.12 GLOSSARY

Glu = *Glutamic acid*

FAD = *F*lavin Adenine Dinucleotide

SOD = superoxide dismutase

CYP = Cytochrome

HS = High spin

LP = Low spin

Arg = Argene

CPA = Carboxypeptidase A

His = histidine

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2.15 TERMINAL QUESTIONS

Q1. What is the active metal in carborxypepdase A. What is the coordination number and how is it satisfied?

Q2. Write a reaction that is catalyzed by vitamin B12 and propose a mechanism for such reaction. What are the advantages of using vitamin B12?

Q3. Peroxidase, catalase and cytochrome-P450 all produce Fe(IV)=O as an important intermediate during the reaction with the substrates. This being the case, why are they so different in their biological activities?

Q4. Discuss about the diffence and similarities between cytochrome P-450 and other electron or oxygen-transfer functions of other heme proteins.

Q5. Explain that cytochrome P-450 is a monooxygenase. Discuss about the special features of cytochrome P-450 and its mechanism.

Q6. Explain with example the importance of metalloenzymes.

Q7. What are xanthine oxidase ? Discuss their role in formation of uric acid.

Q8. What is the Superoxide dismutase ? Describe the role of superoxide dismutase in oxidative metabilism.

Q9. What is Carboxypeptidase A ? Discuss about the structure and mechanism of action of carboxypeptidase A.

Q 10. What is carbonic anhydrase ? How it is helpful for the conversion of CO_2 into carbonic acid.

Q11. Discuss the structural features and mechanism of peroxidase and catalase.

Q12. Carboxypeptidase A with a bound peptide chain is a good example of a supramolecule explain.

UNIT 3: METAL-NUCLEIC ACID INTERACTIONS

CONTENTS:

3.1 INTRODUCTION

In Unit III we learn about DNA, RNA and role of metal ion in our living organism. In this we can understand use of metal ions in medical field. The toxicrole of metal ion in our environment. Metal ions are essential in many biological processes as catalyse

(enzyme).Components of our bodies Consider the elements that make up an average healthy person's constitution (weighing 70 kg). Oxygen accounts for more than half of an average man's overall weight. Table 1 lists the number of different elements in human bodies.

Table 1: Average concentration of various elements present in our body Elements Amount in our body

Element	**Amount in our body**
Oxygen	45.5 kg
Carbon	12.6kg
Hydrogen	7.0 kg
Nitrogen	2.10 kg
Phosphorous	700 g
Calcium	1050 g
Potassium	140 g
Sodium	105 g
Magnesium	35 g
Iron	4.2 g
Zinc	2.3 g

The rest of the metals, particularly Cu –0.11 g and Mn – 0.02 g, have a content of less than one gramme. Metals make up only 2% of the human body, but they play a critical role in human life. They bind to the substrate and orient it in relation to the active site's functional group, resulting in the creation of a redox reaction site. Enzymes contain some metal ions. Some are structural components, such as calcium in bones and teeth, while others are involved in transport, such as Fe^{2+} in haemoglobin and myoglobin, and still others are involved in control systems, such as $Na^{+}$and K^{+} in nerve transmission.

3.2 OBJECTIVE

After studying this Unit, you shall be able to know:

- What is the Constitutional structure of DNA molecule? Which type of bases and sugar are present in DNA and RNA biomolecule?
- Type interaction modes of binding and cleavageof DNA.
- We know about role of Metal ionin living organism and their ill effects.
- The metal complex used for diagnosis and chemotherapy.

3.3 DNA (DEOXYRIBOSE NUCLEIC ACID)

Deoxyribonucleic acid (DNA) is a very extensive macromolecule that holds genetic instructions for all known living organisms' development and function. It contains information in the form of a unique genetic code that can be passed through generations. Each DNA molecule is tightly twisted and bundled into chromosomes, which are thread-like structures that wrap around certain protein complexes (Fig. 3.1). Chromosomes are placed within the membrane-bound nucleus of eukaryotes (cells with a nucleus), while they are housed within the cellular cytoplasm of prokaryotes (cells without a nucleus). DNA is a polymeric biomolecule made up of nucleotides, which are linear chains of monomeric units. The nucleotide is made up of three parts: a phosphate, a pentose and a nitrogenous base. In DNA, the sugar is always 2'-deoxyribose.

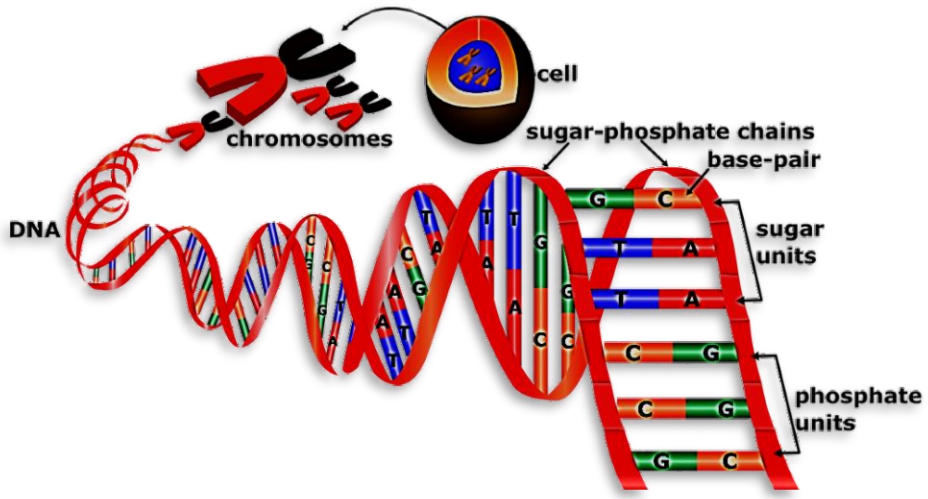
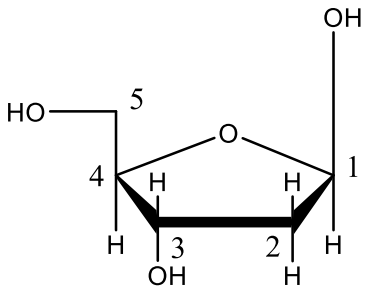


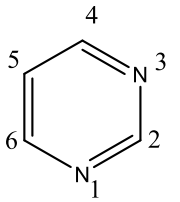
Fig. 3.1 Scheme show cell, chromosome, gene and DNA



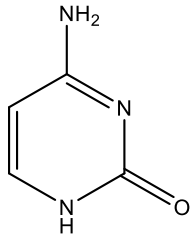
(2*R*,4*S*,5*R*)-5-(hydroxymethyl)tetrahydrofuran-2,4-diol

2' deoxyribose sugar

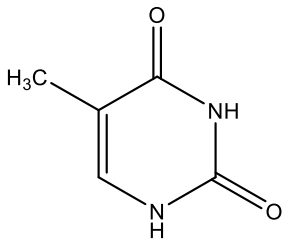
There are two types of nucleotide bases: pyrimidine and purine. Purines have a five-membered imidazole ring fused to a six-membered ring, while pyrimidines have a six-membered heterocyclic conjugated ring. Cytosine (C) and thymine (T) are two pyrimidine nucleotides found in DNA. N^1 links the pyridine bases to pentose.



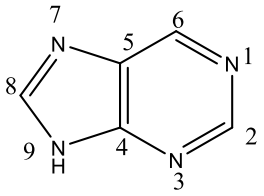
Pyrimidine ring



Cytosine

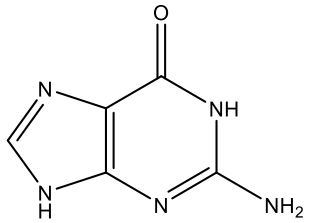


Thymine

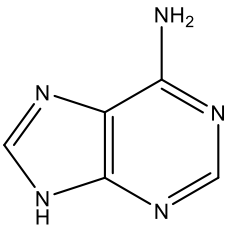


Purine ring

Adenine (A) and guanine (G) are the purine bases found in DNA (G).



Guanine



Adenine

The purine bases are attached to pentose sugar by N^9.

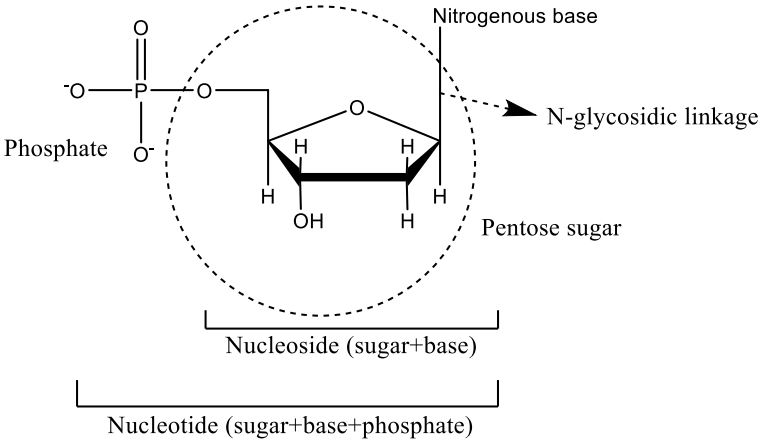


Fig. 3.2 Structure of nucleotide

3.3.1 Primary Structure of DNA

The fundamental structure of DNA is a single polynucleotide chain. The phosphate group on one nucleotide's 5' carbon is joined to the hydroxyl (OH) group on another nucleotide's 3' carbon to form a phosphodiester linkage, which results in a polynucleotide chain. The phosphodiester linkage creates the sugar phosphate backbone of the polynucleotide chain. At one end of the chain, a free 5' phosphate group exists, whereas at the other, a free 3' OH group exists.

The fundamental structure of DNA is:

Nucleic acids do not contain equal quantities of each nucleotide, as demonstrated by Erwin Chargaff and other biochemists in the 1940s. Chargaff extracted DNA from a variety of species and hydrolyzed it into individual nucleotides. After that, paper chromatography was used to separate the nucleotides. Chargaff claimed that for any given species, based on his experiments:

- A=T and G≡C
- Sum of purine bases (A+G) = Sum of pyrimidine bases (T+C)
- Ratio of A to T and G to C were close to one *i.e.* A/T=G/C ≈1

These facts arecalled**Chargaff's rule.**

3.3.2 Secondary structure of DNA

In 1947, William Astbury used the X-ray diffraction technique to study DNA fibres and discovered a 0.34 nm repeating unit within DNA. Rosalind Franklin's work on purified DNA samples (between 1950 and 1953) supported Astbury's findings and showed that DNA has a

helical structure. Using a combination of Franklin's data and Chargaff's guidelines. James Watson and Francis Crick proposed the double helical model of DNA in 1953.The following are the primary characteristics of DNA's double helix:

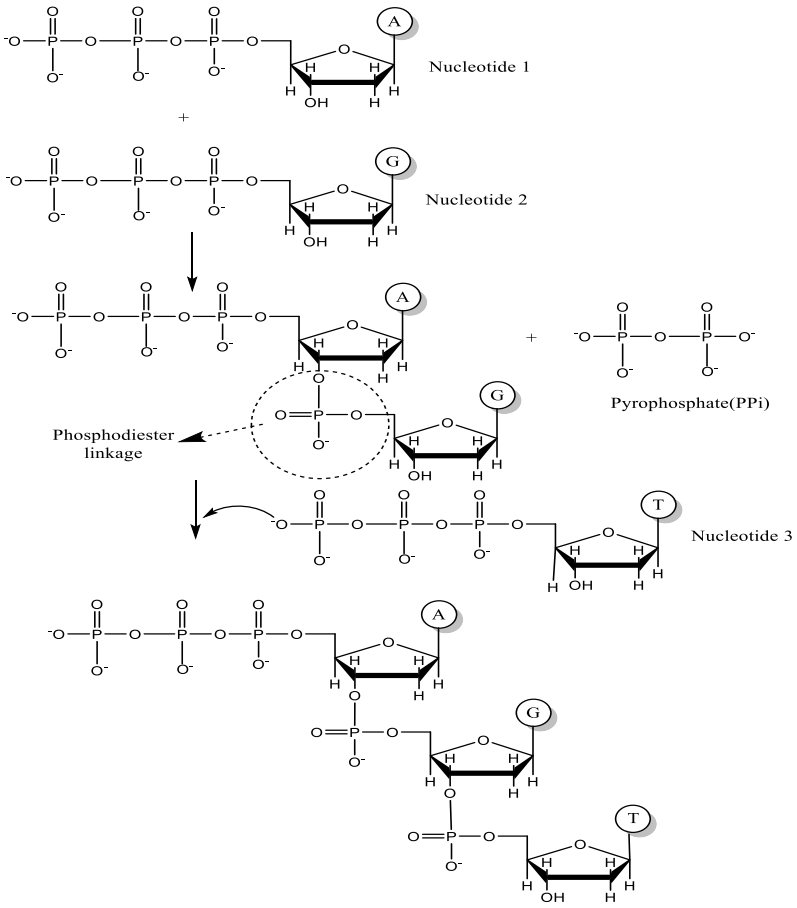


Fig. 3.3 Synthesis of polynucleotide chain of DNA

i) The DNA molecule is made up of two polynucleotide chains that coil around a central axis to form a right-handed double helix with a diameter of 20Å.

ii) The two chains run opposite direction, that is one chain run in 5' to 3' direction, while another run in 3' to 5' direction.

iii) The two anti-parallel polynucleotide chains are complimentary to each other in base sequence, that is Guanine (G) in one strand cab base pair with cytosine (C) in other strand, and similarly, Adenine (A) and Thymine are base4 paired. This is called complementary base pairing.

iv) Hydrogen bonds between the nitrogenous bases of opposing strands and base stacking interactions hold the two polynucleotide chains together.

v) The bases are flat structures which are stacked on one other, inside then helix (3.4 Å apart). They are lying perpendicular to the helix axis.

vi) Each base pair is rotated 36^o relative to next base pair around the helix axis.

vii) The hydrophilic sugar-phosphate backbone of the helix is on the outside and the base pairs lying on the inside of helix.

viii) The base pairing of two stands creates alternating major and minor groove on the surface of double helix. The major groove is wide, whereas the minor groove is narrow and deep.

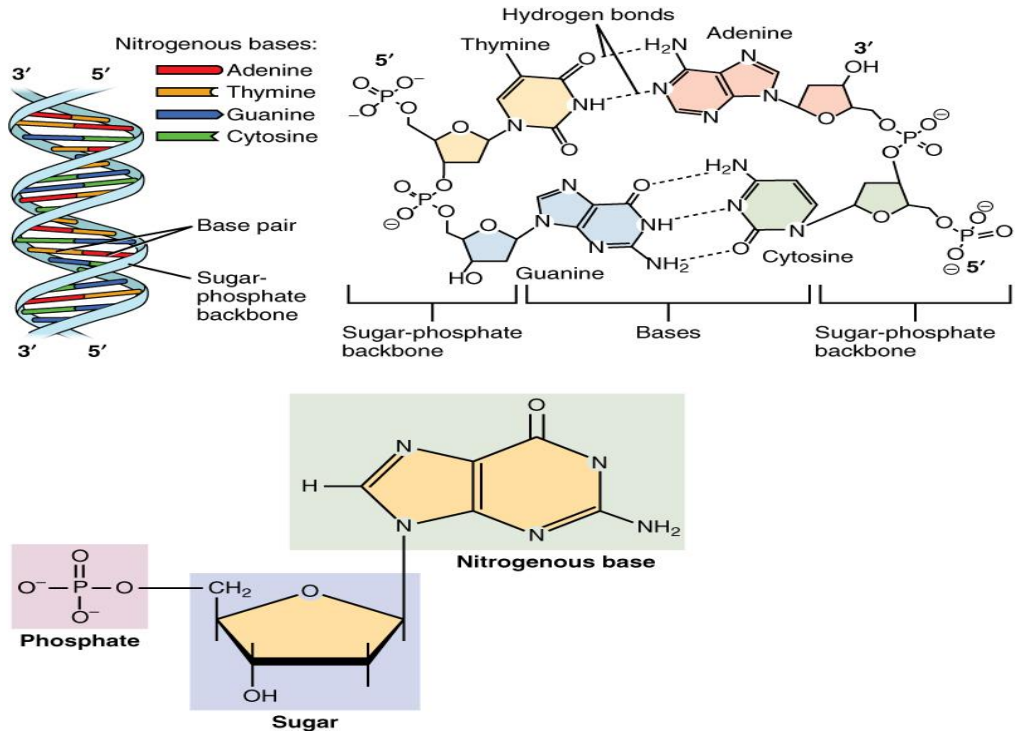


Fig. 3.4 The Wotson-Crick Model of B-DN

3.3.3 DNA Polymerization

The most crucial biological activity in all living species is DNA replication, which involves copying a double-stranded DNA molecule into two identical duplicates.DNA replication is the most important biological process that occurs in all living organisms by which a double stranded DNA molecule is copied to produce two identical replicas. DNA Polymerase is the enzyme responsible for DNA replication (Figure). This enzyme catalyses the synthesis of polynucleotide chains by adding nucleotides produced from deoxynucleoside triphosphates one after the other. It normally acts in pairs to split a single DNA molecule into two identical DNA strands. A DNA polymerase requires the following components for replication:

- ATP, GTP, TTP, and CTP are the triphosphate versions of four nucleotides.
- Single-stranded DNA is used as a template.
- A primer, an existing strand of nucleic acids with a free 3' end a primer.
- An existing strand of nucleic acids with a free 3' end

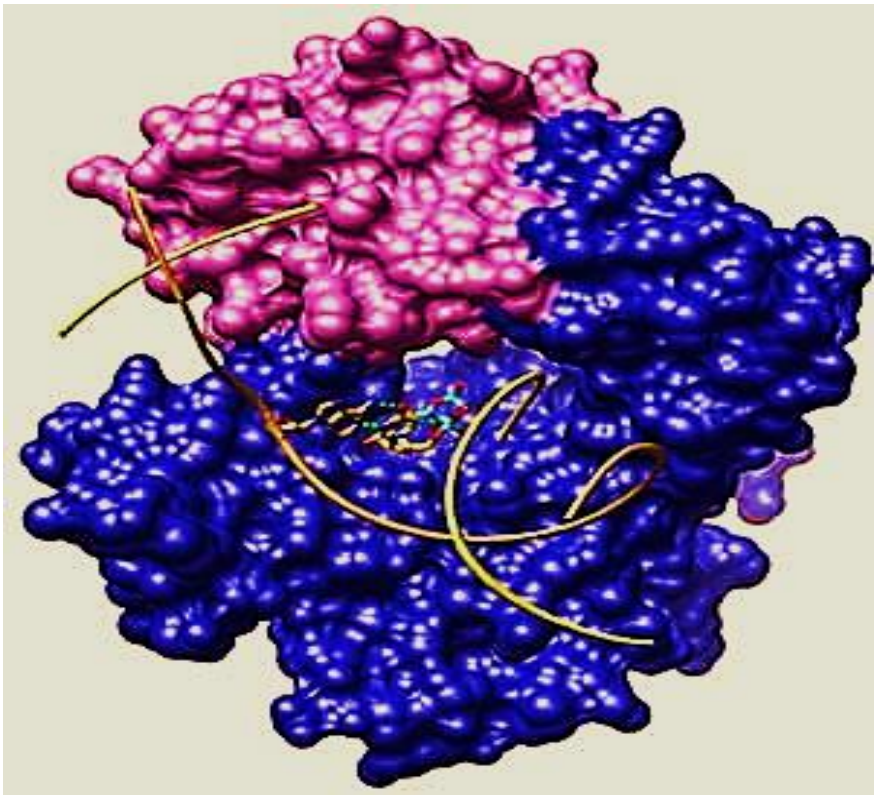


Fig 3.5 Human DNA polymerase (Pol), a key enzyme in the base excision repair (BER) process, is depicted on the surface.

DNA is usually composed of two polynucleotide chains coiled around each other in the form of doubler helix. DNA polymerization is the synthesis of polynucleotide chain by addition of successive nucleotides. The enzyme that catalyzes the synthesis of polynucleotide chain of DNA are known as DNA polymerases. DNA polymerases require three components for DNA synthesis-template, primer and four nucleotides (dATP, dGDP, dTTP, and dCTP). The template is a single stranded (ss) DNA that will direct addition complementary to 3' end template. The primer provides a free 3' OH group that extended by DNA polymerase by addition of nucleotides. A nucleotide is made up of three components- a nitrogenous base, a pentose sugar and three phosphate groups.The DNA polymerase

catalyzes the synthesis of DNA by addition of nucleotides to the 3ˈOHgroup of primer. The newly synthesized DNA have base complementary to the template DNA.

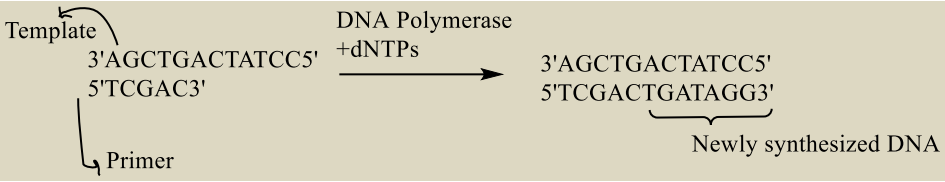


Fig. 3.6 DNA Polymerization by DNA polymerase

During DNA polymerization, the primer's 3' OH group assaults the incoming nucleotide's -P. When the incoming nucleotide is joined to the primer's 3' -OH group, the pyrophosphate is released. The hydrolysis of pyrophosphate into two inorganic phosphates by an enzyme known as pyrophosphatase provides the driving power for this process.

3.3.4 Model of DNA Polymerisation

After Watson and Crick's discovery of the double helix model of DNA, three models for DNA replication were presented. **(a) Conservative Model**

(a) **Conservative Model:**

The parental molecule guides the production of one daughter molecule with both parental DNA strands and another daughter molecule with DNA strands from all freshly synthesised material in this scenario.

(b) **Semiconservative Model:**

In this model, the parental molecule's two DNA strands split, with each strand serving as a template for the creation of a new DNA strand. The consequence of one round of replication is two DNA double helices, each with one parental and one new strand.

(c) **Dispersive Model:**

The parental double helix molecule is first broken down into double-stranded DNA segments, which are then randomly recombined in thismodel.

3.4.5 Catalytic mechanism of DNA polymerase

X-Ray the structure of various DNA polymerases suggest that they all have a similar DNA polymerization catalytic mechanism. Two metal ions, generally Mg^{2+}, are retained in position and orientation at the active site of a DNA polymerase by interactions with two conserved

aspartate residues. The (P) group of this dNTP interacts with the 3' OH group of primary, whereas the other metal ion (B) interacts with all three phosphate groups of this dNTP. The metal ion (A) activates the primer's 3' OH group, allowing it to attack the incoming dNTP's -(P) group nucleophilically. The metal ion (B) shields the negative charge that accumulates on the Penta co-ordinate transition state, causing the pyrophosphate to be released.

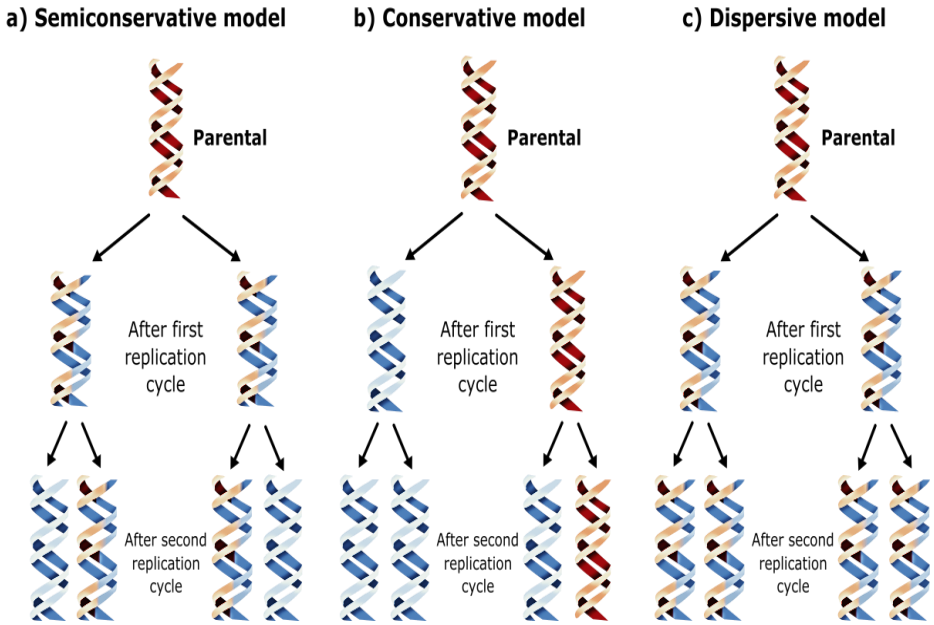


Fig. 3.7 Human DNA polymerase (Pol), a key enzyme in the base excision repair (BER) process, is depicted on the surface.

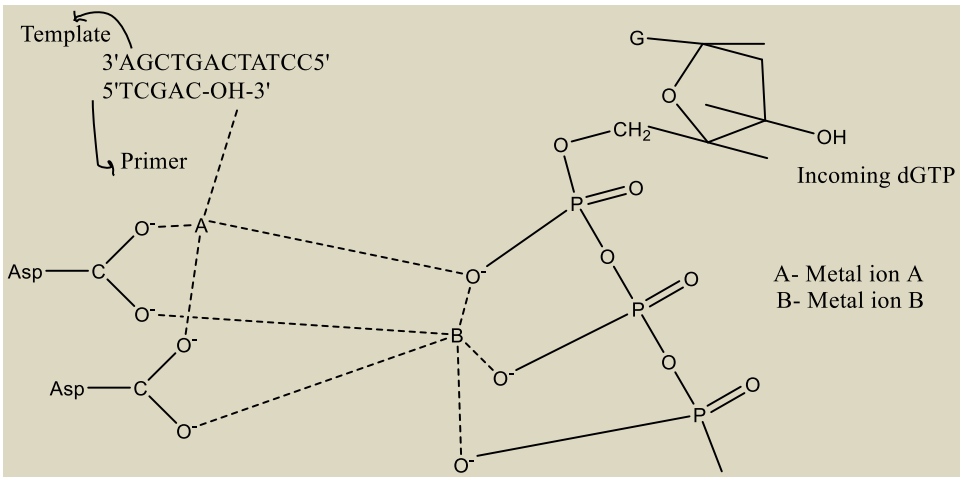


Fig. 3.8 Active sites of DNA polymerase

3.3.6 Detailed description of DNA polymerization process

The steps in the replication of DNA are as follows (Figure 7):

(1) **Initiation**: When an enzyme called helicase loosens the two strands of DNA molecule by breaking hydrogen bonds between each nucleotide, DNA replication begins. The origin of replication is the point at which a short section of DNA helix opens up, and the structure that results is known as the Replication Fork. SSB proteins (Singlestranded DNA-binding proteins) then bind to the unwound single strands of DNA, preventing them from breaking and reannealing.

(2) Elongation: However, while the helicase splits the strands, RNA primase attaches to each strand for a brief period of time and creates an RNA primer, which serves as a starting point for DNA synthesis. DNA polymerase III begins creating a new complementary strand by adding the reciprocal sequences of the DNA to a single unwinding polynucleotide strand after the primer is in place. Because DNA polymerase can only add nucleotides in a 5' (prime) to 3' (prime) direction, this process occurs in the opposite way.This means that bases are added toward the origin of replication on the leading strand, but DNA is copied in the opposite direction of fork movement on the lagging strand. As a result, the lagging strand is produced in short, Okazaki-like pieces. The primer RNA fragments are then removed from both strands by RNase enzyme, and DNA Polymerase fill in the gaps with the required nucleotides. Through the activity of DNA Ligase, a single nick on the leading strand and numerous nicks on the lagging strand are created, which are subsequently filled to produce two continuous double strands of DNA.

(3) Termination: Termination is the final phase of DNA replication, which occurs when the DNA polymerase enzyme reaches the end of the strands, where no further replication is feasible. The RNA primer is removed from the last segment of the lagging strand, which is not duplicated. Telomeres are regions of the genome that contain a repetitive non-coding sequence of nucleotides. A portion of the telomere is lost at the conclusion of each replication cycle, resulting in shorter strands after each cycle.Finally, enzymes such as nucleases "proofread" the new double helix structures, removing any reduced nucleotides that occurred during DNA replication.As a result, the removed bases leave a few gaps, which DNA Polymerase I eventually repairs.

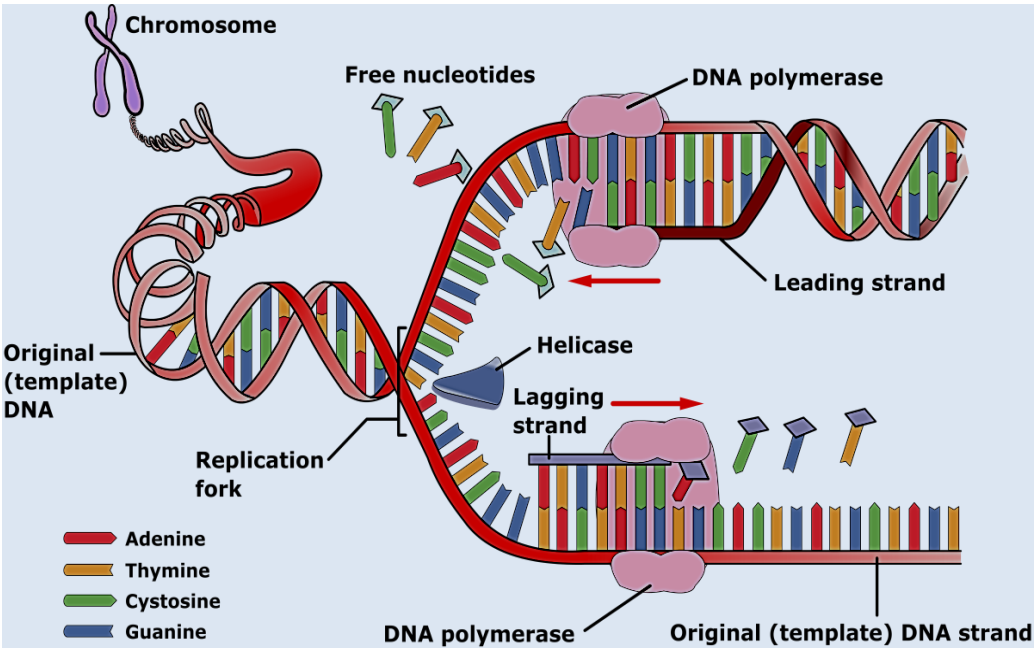
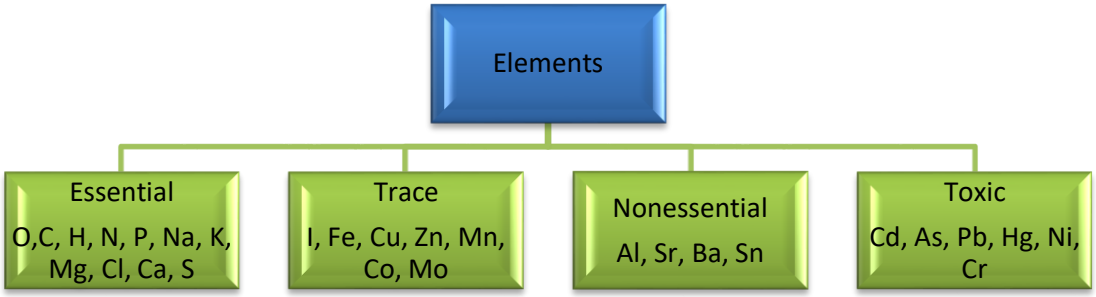


Fig. 3.9 DNA replication process

3.4 CLASSIFICATION OF ELEMENTS ACCORDING TO THEIR ACTION IN THE BIOLOGICAL SYSTEM



- Essential elements are absolutely essential or necessary for life processes.
- Trace elements are also necessary for life processes.
- Non-essential elements are not essential. If they are absent other elements may serve the same function.

- Toxic elements disturb the natural functions of the biological system.

Essential and Trace elements

Only about seven elements are known to be needed for the efficient functioning of the human body out of more than 100 identified elements. Essential elements include elements such as Na, K, Mg, Ca, P, Fe, Mn, S, Zn, Cu, Co, Cr, Mo, Cl, F, I, and Se. They are said to as crucial since the organism would not be able to thrive without them. Essential components are divided into two categories based on their absolute quantities in the body:

- Macronutrients
- Micronutrients

The rest of the elements, which are only required in small amounts by the body, are referred to as micronutrients. They are also known as trace elements because they are only needed in trace levels in the body.

3.4.1 Metal ions in biological System

i. Sodium

Sodium is the principal electrolyte found in high concentrations in extracellular fluid (140 mmol/L) Na^+ is the principal. It regulates the body's osmotic pressure. In the body, sodium is mostly found in the form of chloride and bicarbonate, as NaCl and $NaHCO_3$, respectively. Adults require between 1 and 3.5 grammes of salt each day. It enhances glucose and amino acid absorption. In conjunction with chloride and bicarbonate, it maintains acid-base balance. It is involved in the control of membrane potential.

The most frequent source of sodium in cooking is table salt (NaCl). Bread, cheese, carrots, cauliflower, egg nuts, spinach, and other foods are among the other sources. A lack of sodium causes headaches and abdominal muscle cramps. On the other hand High blood pressure is caused by a high intake of table salt.

iii. Potassium

K^+ is the principal cation of the intracellular fluid. It increases the activity of cardiac muscles. Along with Na^+ it maintains osmotic pressure of the body. It also maintains acid-base balance. It increases the activity of the enzymes like pyruvate kinase. It also plays a prominent role in blood coagulation and synthesis of ribosomes.

The good sources of potassium are chicken, beef liver, banana, orange juice, pine apple etc. deficiency of potassium leads to depression and also affects the nervous system.

Table 2 The biological roles of metal ions

Metal	Related compounds	Action
Fe	Haemoglobin (*H) Myoglobin(*H) Hemerythrin(*N) Oxygenases ((*N) Hydrogenases Cytochrome P-450 (*H) Catalase (*H) Peroxidase (*H) Cytochromes C (*H) Ferredoxins (*N) Ferritin (*N) Transferrin(*N)	Oxygen transport Oxygen storage Oxygen storage Insertion of oxygen atom into substrate Oxidation of H_2 Insertion of oxygen substrate Catalyze oxidation of substrate by H_2O_2 Catalyze oxidation of substrate by H_2O_2 Electron transport Electron Transport Iron Storage Iron Transport
Co	Coenzyme B_{12}	Methylation of organic compounds
Cu	Amine oxidase Ceruloplasmin Hemocyanin	Oxidation of amine into aldehyde Transport of Fe from transferrin to ferritin, Cu storage and transport Oxygen transport
Zn	Carbopeptidase Carbonic anhydrase	Hydrolysis of peptide bonds Catalyses the equilibrium $CO_2 + H_2O=HCO_3^-+H^+$
Mg	Chlorophyll Phosphotransferase	Photosynthesis Phosphate hydrolysis
Mg, Mn	Aminopeptide	Catalyses the cleavage of amino acid
Fe, Mo	Nitrogenase	Nitrogen fixation
Fe, Mo, Cu	Oxidase Reductase	Redox reaction involving O_2 electron acceptor Catalyses reduction reaction

	Hydroxylases	Oxidative degradation of organic compounds
Cu, Zn, Mo	Superoxide dismutase	Dismutation of O_2^- into O_2 and H_2O_2
Mg, Cu, Zn	Phosphatases	Removal of phosphate groups from substrate
Ni	Urease	Hydrolysis of urea into CO_2 and NH_3

*H= Heme iron

*N=Non-heme iron

iii. Magnesium

Magnesium is a macronutrient that the body need in substantial quantities. About 25 gms of Mg found in the human body. 70 percent of the body's mg is found in the bones and teeth. Mg2+ is a phosphatase enzyme activator and an important cation in intracellular fluid. Magnesium, calcium, and phosphorous combine to produce a complex salt of bones. It's an important part of chlorophyll. It also participates in the ATP hydrolysis process (universal source of energy). Magnesium, like calcium, helps with muscular contraction, blood coagulation, lung function, and blood pressure management. It is involved in a variety of life-sustaining processes.Role of magnesium in enzyme action, energy production, Nerve conduction, muscle protein formation nucleic acid stabilization and DNA synthesis.

Nuts, soybeans, and seafood are good sources of magnesium. Mg deficiency results in neuromuscular dysfunction.

iv. Calcium

Calcium is the most abundant mineral in the human body. It is major constituent of teeth and bones. About 90% of the body calcium is in the skeleton, when it is maintained as deposits of calcium phosphate. Calcium and phosphorus are the principal minerals of bone and teeth, where it exists as the double salt of calcium and phosphate, $CaCO_3 \cdot nCa_3(PO4)_2$ (n ranging from 2 to 3). These minerals lend hardness and strength to these tissues. A little calcium is scattered in soft tissue like muscles and organs. Calcium helps in blood coagulation. It plays a prominent role in muscle contraction. Ca acts as a cofactor of various enzymes like protein kinase, lipase, adenylate, cyclase, etc. it also helps in nerve action. The chief sources of calcium are milk, egg, nuts, beans, cabbage, cauliflower, etc. Deficiency of calcium leads to the disease rickets in children (weakness of bones) and osteoporosis in adults. However, excess of calcium adversely affects the body, giving rise to formation of stones.

THE calcium in plasma exist in three forms:

- Protein bound (35-50 % of plasma calcium)
- 5-10% in complexes with organic acids and phosphate, and
- 50-60% in ionised form.

v. Phosphorus

Phosphorous, in the form of phosphate, is present in the body. The total body phosphate content is approximately 700 g. More than 85 percent is found in bones, with only about 15 percent found in soft tissues and 1 percent found in extracellular fluid. 90% of daily dietary phosphate absorption It is found in bone and teeth in conjunction with calcium. It is also a component of DNA and RNA, which serve as the foundation for life and growth. Furthermore, it is required for the phosphorylation-mediated regulation of enzyme activity. Vitamin D boosts intestinal phosphate absorption by increasing the expression of the Na-P co-transporter in the small intestine. The parathyroid hormone (PTH) reduces phosphorous reabsorption in the kidney and thus increase its urinary excretion. The Ca:P ratio in diet affects the absorption and excretion of phosphorous. If one is in excess in diet, the excretion of the other is increased. Phospholipid is an important constituent of bone and teeth. It is also constituent of phospholipid, nucleic acids and lipoprotein. It plays important roles in biological processes and also assist various enzymatic reactions.

Phosphorus is found plentiful in most foods like milk, cheese, meat, etc. Deficiency of phosphorus leads to poor mineralisation of bones and teeth and causes osteomalacia and poor growth.

vi. Iron

Iron is a vital trace element for the human body. The average human body has 2.4 grammes of iron. It's found in the active centres of proteins involved in O2 transport (like haemoglobin and myoglobin) and electron transport (like cytochromes), as well as in the active sites of metalloenzymes like nitrogenase, reductase, and hydrogenase. Essential (or function) iron and storage iron are the two types of iron found in the human body.

- **Essential iron:** Essential iron is one which is involved in the normal metabolism of the cells. It is further divided into three groups:

- **Heme protein:** Haemoglobin and myoglobin are two heme proteins which contains an iron porphyrin prosthetic group attached to globin protein. Both of them are involved in dioxygen transport.

Other Heme proteins are catalases and peroxidases. Catalase enzyme contains four heme groups and is found in blood, bone marrow, mucous membrane, liver and kidney. It catalyses the conversion of hydrogen peroxide into molecular oxygen and water.

Peroxidase, another heme protein found in milk, erythrocytes, leucocytes and lens fibres.

Cytochrome: Cytochromes are another class of iron containing compounds. Cytochromes are chiefly found in mitochondria.

Iron containing enzymes: certain enzyme also use iron as co-factor like succinate dehydrogenase, aconitase, ribonucleotide reductase, etc.

Storage iron: Storage from iron is ferritin and hemosiderin. Free iron is toxic while the iron bound to0 ferritin is non-toxic. Ferritin, the storage protein of iron found in blood, liver, spleen, bone marrow and intestine. Hemosiderin is derived from ferritin. It contains a larger fraction of iron as compared to ferritin.

Dietary sources of iron: Foods rich in iron includes cereals, legumes, molasses, eggs, meat, fish, etc. Iron also obtained from non-food sources like foods cooked in an iron skillet. Deficiency of iron leads to anaemia. Symptoms of iron deficiency take year to develop and include fatigue, weakness, and shortness of breath.

vii. Zinc:

Zn is the second most abundant dress material average Zinc content in the body is approximately 2g.It is distributed in different part of a body like bones, teeth, skin, kidney and muscle acceptor.It is essential for normal human body growth, wood healing and tissue repairing. It regulates the function of insulin and also maintain the normal concentration of vitamin A. It is the essential component of several enzymes. Important zinc containing enzymes are superoxide dismutase, carbonic anhydrase and Carboxypeptidase. Superoxide dismutase is aCu-Zn protein complex with two Zn^{2+} per molecule of the enzyme.It is present in epithelial cells, red blood cells and brain cells. Carbonic anhydrase contains one Zn^{2+} per

molecule of enzyme. It is present in epithelial cells, red blood cells and parietal cells. Carboxypeptidase is the hydrolytic enzyme present in pancreatic juice. Another zinc containing enzymes are alcohol dehydrogenase alkaline phosphate, lactate dehydrogenase, glutamate dehydrogenase, DNA and RNA polymerase.

Fighting off of cold or flu:

- Zinc supplements to reduce the cold symptoms such as age runny nose coughing and so throat
- Zinc make skin Nails and hairy health skin hair healthy.
- Zinc helps in healing wounds.
- Zinc reduces the diabetic problems.
- Zinc increases production of testosterone and other male harmones. Therefore, it reduces the male infertility.
- Zinc helps to preserve eye sight and improves memory.
- Zinc may help teenagers with pimples.

Symptoms of Zinc Deficiency

- Reduced growth of children
- Reduced mental retardation
- Slow wound healing
- Skin irritation
- Hair loss
- Loss of sense of taste
- Frequently infections
- Zinc work best with Vitamin A, Vitamin B_6, insuline, Vitamin D, Vitamin E, glucose, Mg and Mn.

Excess of zinc in the human body causes: Dysfuntion of central nervous system, Anaemia, Diarrhoea, Dizziness, store stomach, Nausea, Vomiting, alcohol intolerance, electrolyte imbalance and increase LDS cholesterol and lower HDL cholesterol.

Chief sources of zinc are egg, seafood, milk, meat cereals, legumes and pulses, oilseeds vegetables like spinach lettuce.

viii. Copper:

Copper is vital in plants and animals, but its roles are not as well defined as those of iron. Copper is distributed 100 to 150 mg in the muscles, bones, and liver. Under normal condition, about 32% of dietary copper can be absorbed. The copper is absorbed in mucosal cells.After Fe and Zn, copper is the third most dominant element in the human body. Excessive dietary of either Zn or Mo interferes with the absorption of Cu. After absorption of Cu enter plasma where it is bound to serum proteins. It is found in a variety of proteins, metalloenzymes, and naturally occurring colours. Copper is found in cytochrome oxidase, tyrosinase, catalase, ascorbic acid oxidase, superoxide dismutase, and other enzymes. A lot of invertebrates use hemocyanin, a copper protein, as an oxygen carrier. Copper is also involved in the colouring of skin and hair, as well as the creation of a protective layer surrounding nerve fibres. Legumes and whole grains are the best suppliers of copper.

(a) Copper deficiency

The symptoms of copper deficiency include:

- Copper deficiency turns hair grey and also causes bone disorder and loss in body weight. Bone demineralisation and blood vessel fragility due to defects in collagen and elastin formation.
- Anaemia due to defect in iron metabolism.
- Hypercholesterolemia due to increase in ratio of saturated to monosaturated fatty acids of C_{18} series.

(b) Impact of Excess of Copper

Excess of copper causes: Fever, high blood pressure, diarrhoea, dizziness, depression, fatigue, irritability,joint and muscle pain, nausea, premature ageing, vomiting, wrinkling of skin,headache etc.

Wilson disease illness is brought on by an excessive amount of copper in the body. It's a genetic disorder. Wilson's disease patients have low quantities of the copper storage protein ceruloplasmin, which means that even at normal levels, copper is toxic. Wilson disease is

characterised by liver damage, neurological impairment, and brown or green rings on the cornea. To remove excess copper, many chelating ligands can be utilised, but D-penicillamine is one of the most effective. This chelating ligands forms a complex with copper ions (Cu^{+} and Cu^{2+}) that has intense purple colour and the molecular formula of the compound is $[Cu_8^{+}Cu_6^{2+}(penicillamine)_{12}Cl]$. The sulfhydryl groups of D-penicillamine effects removal of copper as Cu^{+} complex.Chelating therapy with EDTA also eliminates the symptoms.

ix. Cobalt

One of the most important trace metals is cobalt. Itis one the most ancient biocatalysts. Only around 1.5 mg of cobalt is found in the human body, and the majority of it is in the form of cobalamin, or vitamin B_{12}. Cobalt is complexed in B_{12} by a special macrocycle called corrin.a benzimidazole that is covalently linked to the corrin ring are known as cobalamins. Cobalamins are cofactors in enzymes that catalyse alkyl transfer reactions and many radical-based rearrangements. Cobalamin-containing enzymes show strong UV–visible absorption bands; EPR spectra are observed for Co(II).Vitamin B_{12} cannot be synthesised by animals or plants. In reality, only a few microorganisms are capable of producing it. Humans get 100% of their vitamin B12 from animal sources, particularly meat. Because vitamin B12 is only required in trace amounts, vitamin B12 deficiency is uncommon. However, vegans who eat no animal products have been observed to have vitamin B12 deficiency. Vitamin B12 insufficiency is caused by a failure to absorb the vitamin in the gut, which results in an increase in the excretion of methyl malonic acid, which the body cannot convert to succinic acid. Pernicious anaemia is caused by the deficiency.Cobalt is necessary for the action of various enzymes, including coenzyme A, methyl malonyl oxidoreductase, and others, in addition to being a key component of vitamin B12. Nausea, vomiting, diarrhoea, and skin rashes are all indications of too much cobalt in the body.

x. Sulphur

Sulphur is present in the body in its oxidized form sulphate as a part of some proteins. It is also present along with co-enzyme A and lipoic acid. It is the structural constituent of insulin and many more proteins. In some enzymes specific sulfhydryl groups are essential for catalytic activity. It plays an important role in the formulation of acetyl coenzyme A and S-acetyl lipoate. Protein rich food like meat, fish, egg, milk, etc. provides necessary amount of sulphates to the body.

xi. Manganese

Manganese is also an essential trace element required by the body and is found concentrated mainly in the kidneys and liver. It acts as a cofactor or as an activator of many enzymes like enolase, arginase, isocitrate dehydrogenase, cholinesterase, etc. Manganese and magnesium may replace one another in case of some of the enzymes. It assist in bone formation and plays a prominent role in fat and carbohydrate metabolism. Manganese is obtained primarily from cereals, nuts, whole grains, tea and leafy vegetables.

xii. Fluorine

Fluorine is present in human tooth in trace amounts and helps in tooth development, normal maintenance and hardening of dental enamel. Fluorine is also required for normal bone development and increases the retention of calcium and phosphate and prevent old age osteoporosis. Fluoride is mainly derived in human from drinking water. Other sources of fluoride include fish, tea, salmon, etc. Destruction of dental enamel and tooth decay are widespread among both adults and children in areas where drinking water contains less than 0.5 ppm of fluorine. But excess of fluoride in water or diet or its inhalation is harmful and is considered to be main cause of the fluorosis disease.

xiii. Chlorine

The chloride ion maintain the fluid and electrolytic balance. It also plays a prominent role in osmatic pressure regulation. In gastric juice, the chloride ion shows importance inthe production of HCl and thus helps in digestion of food. It is also maintainthe acid base equilibrium. Significance sources are table salt le maintain the acid base equilibrium. Significant sources are table salt, processed food and soya sauce. Moderate sources include meat, egg and milk.

xiv. lodine

The primary function of iodine is its role in thyroid functioning that help to regulate growth and development. In addition to iodized salt, good dietary sources of iodine are sea food, bread, dairy products. Deficiency of iodine leads to thyroid hypertrophy.

xv. Chromium

Chromium is widely distributed throughout the body. Chromium plays an important role in carbohydrate, lipid, and protein metabolism. Chromium enhances the activity of insulinand

helps to maintain insulin levels. When an individual lack chromium, a condition like diabetes can develop. Good sources of chromium include me/at, brewer's yeast, whole grains,etc.

xvi. Molybdenum

Molybdenum is principally known for its role in biological nitrogen fixation. It is the cofactor of the enzyme nitrogenase which converts atmospheric N2 into NH3 (Chapter 6 section 5). It also occurs in several flavoproteins like xanthine oxidase, NADH nitrate reductase, etc. Molybdenum of all these enzymes participates in internal transfers during oxido-reductions. Presence of small amounts of molybdenum help in the utilization of copper. On the other hand, high molybdenum intake produces copper deficiency. Significant sources of Mo are legumes, cereals and nuts.

xvii. Selenium

Selenium is a trace but an essential metal which is crucial to the heart. It is widely distributed in the body in all the tissues, high concentrations are found in liver, kidneys and fingernails. Muscles, bones, blood and adipose tissues show a low concentration of selenium. Selenium is the prosthetic group of enzyme glutathione peroxidase which is present in cell cystol and mitochondria and functions to reduce hydroperoxide. It regulate the activity of thyroid gland. It is also required for normal pancreatic function. The chief sources of selenium ar whole grains, fruits and vegetables. Deficiency of selenium leads to liver cell necrosis, pancreatic degeneration infertility, failure of growth and dilation of the heart resulting in cardiac failure.

xviii. Nickel

Nickel plays a crucial role in bacterial enzymes, particularly hydrogenases, where it exploits the 3 and 1 oxidation states, which are uncommon in traditional chemistry. Coenzyme A synthase, for example, utilises Ni to make CO, which it then combines with CH_3 (supplied by a cobalamin enzyme) to form a C-C bond in the form of an acetyl ester. Plants also have nickel as the active site of urease. Urease was the first enzyme to be crystallised (in 1926), but it wasn't identified to contain Ni until 1976

Table. 3 deficiency symptoms of trace elements

Element	Function	Main deficiency symptom
Chromium	Glucose metabolism	Impaired glucose metabolism

Cobalt	Vitamin B_{12}	Anemia
Copper	Oxidative enzymes	Anemia , skeletal defects
Manganese	Mucopolysaccharide metabolism	Growth retardation
Molybdenum	Purine metabolism, aldehyde oxidation	Joint pain
Zinc	Nucleic acid metabolism	Poor would healing

3.5 Na^+-K^+-ATPASE

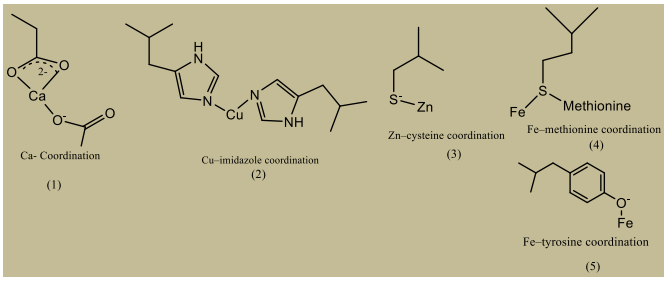
Jens Skou discovered the Na^+K^+-ATPase, a transmembrane protein, in 1957. With the hydrolysis of intracellular ATP, this enzyme is also known as the Na^+-K^+pump because it pumps three Na^+ out and two K^+ into the cell. In animal cells, the Na^+-K^+ pump keeps the cell's Na^+ concentration low while maintaining a high K^+ concentration in the extracellular medium. The transmembrane electric potential is created by ion transport. E_1 and E_2 are the two conformations of the Na^+-K^+-ATPase. E has a high affinity Na^+ binding site while E_2 has a high affinity K^+ binding site.The E_1 conformation enzyme binds three Na* from the cell's interior. The phosphate group is transferred to the aspartic acid residue of the transport protein after E_1, $3Na^+$ binds ATP, which is hydrolysed. Within the tranet protein, this aspartyl phosphate causes a conformation change from E to E. The Na^+- K^+ -ATPase E_1 conformation has a low affinity for Na^+ and a high affinity for K^+. As a result, the transporter discharges $3Na^+$ into the environment and binds $2K^+$ from the surrounding medium.The phosphate group is hydrolysed, and the enzyme returns to its E_1 shape. Because the E_1 conformation of the enzyme has a high affinity for Na but a low affinity for K^+, the transporter releases $2K^+$ to the cell's interior. A trans membrane potential of -50 to -70 mV is generated by the migration of $3Na^+$ ions out of the cell and $2K^+$ ions inside the cell. As a result, this ion transport is referred to as electrogenic, or the creation of electric potential.

3.5 BIOLOGICAL METAL-COORDINATION SITES

Compounds containing metals that are not found in biological systems appear to have a unique role. Orally given medications are preferred because they prevent the stress and risks associated

with injection. Inorganic substances, such as radioactive technetium, are also utilised to diagnose sickness or damage. Sequestration of Fe by ligands inspired by or based on siderophores is used to treat Fe overload. The term "iron overload" refers to a series of major health problems that impact a huge section of the world's population. It's important to note that, because of its importance, Fe is a potentially dangerous element, especially because of its capacity to form damaging radicals when reacting with O_2, and its amounts are usually closely regulated by regulatory systems. Many people have a breakdown in this control as a result of a genetic disease. Metal ions coordinate to proteins, nucleic acids, lipids, and a variety of other molecules. DNA is stabilized by weak coordination of K^+ and Mg^{2+} to its phosphate groups but destabilized by binding of soft metal ions such as Cu^+ to the bases. The binding of Mg^{2+} to phospholipid head groups is important for stabilizing membranes.

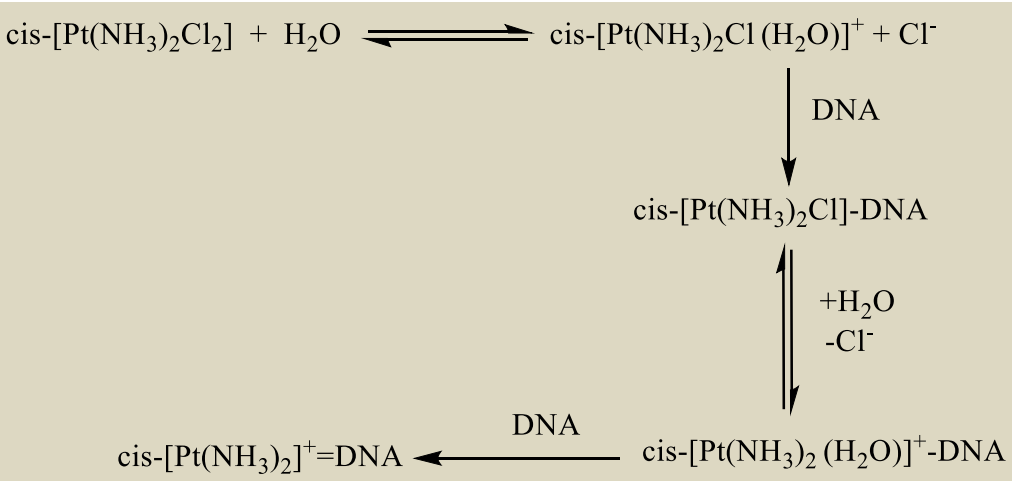
There are a number of important small ligands, apart from water and free amino acids, which include sulphide, sulphate, carbonate, cyanide, carbon monoxide, and nitrogen monoxide, as well as organic acids such as citrate that form reasonably strong polydentate complexes with Fe^{3+}. from introductory chemistry, a protein is a polymer with a specific sequence of amino acids linked by peptide bonds. Proteins are synthesized, a process called translation (of the genetic code carried by DNA), on a special assembly called a ribosome. A protein may be processed further by post-translational modification, a change made to the protein structure, which includes the binding of cofactors such as metal ions. Metalloproteins, proteins containing one or more metal ions, perform a wide range of specific functions. These functions include oxidation and reduction (for which the most important elements are Fe, Mn, Cu, and Mo), radical-based rearrangement reactions and methyl-group transfer (Co), hydrolysis (Zn, Fe, Mg, Mn, and Ni), and DNA processing (Zn). Special proteins are required for transporting and storing different metal atoms.The action of Ca^{2+} is to alter the conformation of a protein (its shape) as a step-in cell signalling (a term used to describe the transfer of information between and within cells). Such proteins are often known as metal ion-activated proteins.



3.6.1 Metal Complexes in Medicine

i. Cisplatin: Anticancer Drugs

In 1969, B. Rosenberg and co-workers discovered the antitumor activity of simple square planar Pt(II)complex, cis- diamminedichloroplatinum (II)or cisplatin , $[Pt(NH_3)_2Cl_2]$. This compound is used as chemotherapeutic agent to inhibit otherwise rapid division of tumour cells (i.e. proliferation). Chemotherapy is the use of anticancer drug designed to inhibit growth of rapid dividing cancer cell in the body. The exact action of this complex is not known. Since the *trans*-isomer is inactive, therefore chelation or atleast coordination to Donor atoms at *cis*-position is an essential part of activity. The proton NMR studies has suggested that Platinum binds to N-7 atom of a pair of adjacent quinine bases of a fast-growingtumour with the chloride ligands first being replaced by water molecules and then by a DNA base.



Cis-platin interact with N atoms of two adjacent guanine bases N-7 on DNA usually within the same strand(intrastandlinkage) or occasionally between strands (interstrand linking). The N-7position of guanine base is much more base is much more basic than that of adenine and provides, therefore, a stronger site for the attack by platinum. Recent X-ray studied on a 12-base pair fragment of double stranded DNA has suggested that the binding of Pt distorts the local DNA structure and therefore, inhibits the cells division inherent in the proliferation of cancer cells. Cisplatin has side effect in kidney and neuro-toxicity. Alternative Platinum compounds have been developed to avoid these serious side effects. The most important of these is carboplatin, which replaces the Cis Chloride right ligands with O chelate cyclobutanedicarboxylate.

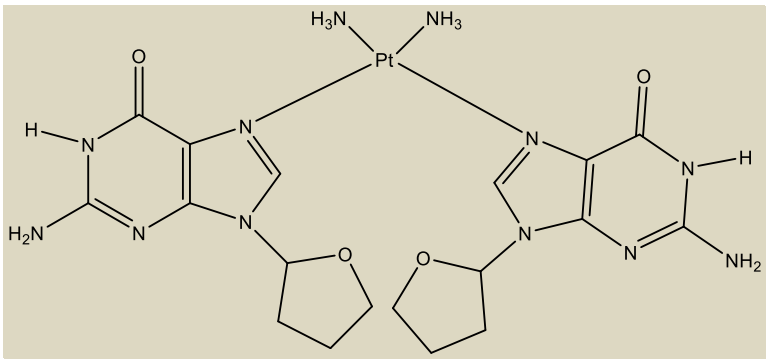


Fig. 3.10 Interaction of cis-platin with two guanine bases on a DNA strand

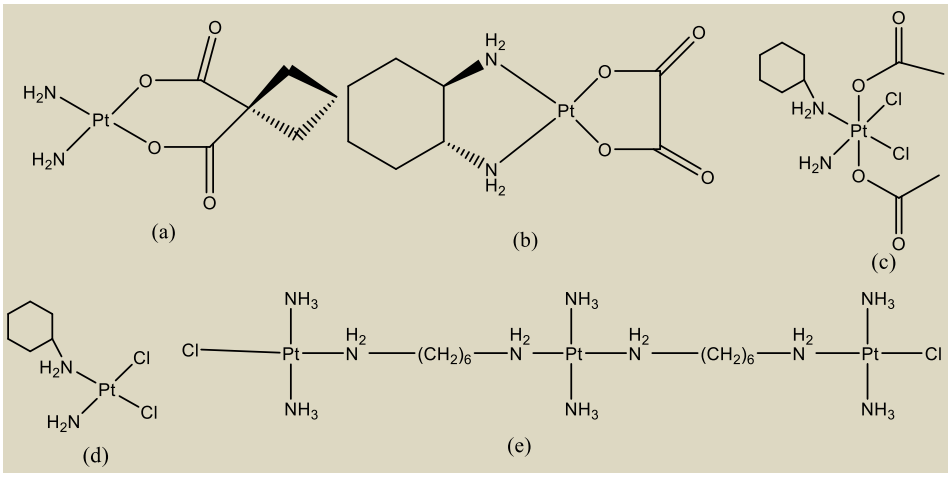


Fig. 3.10: (a) Carboplatin (b)Oxaliplatin (c) Sataraplatin (d)cis-dichloroamine (cyclohexylamine) Pt(II) (e) trinuclear Pt(II) anticancer drug

ii. Wilson disease

Wilson disease is caused by the overload of copper in the body. It is a genetic disease patients suffering from Wilson disease have low levels of the copper storage protein ceruloplasmin and therefore copper can not be tolerated even at normal levels. The Wilson disease is responsible for liver disease, neurological damage and Brown or green rings in the cornea of the eyes. Many chelating ligands can be used to remove the excess of copper but one of the best is D-penicillamine.This Chelating ligand forms a complex with copper ion (Cu^{+} and Cu^{2+}) that has intense purple colour and the molecular formula of the compound is $Cu_8^{I}Cu_6^{II}$ $(penicillamine)_{12}Cl]$. The sulfhydryl group of D-penicillamine effects removal of copper as Cu^{+}complex.

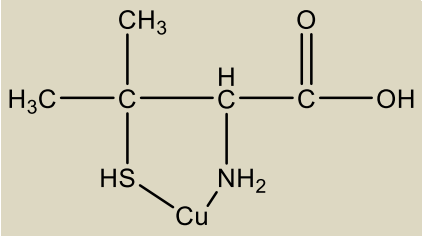


Fig. 3.11 Structure of complex of Cu with D-penicillamine chelating ligand

Chelating therapy using EDTA, MoS_4^{2-}or 2-3 dimercaptopropan-1-ol also causes the symptoms to disappear.

iii. Anti-Arthritis

Complex of Au(I) have been used most successfully for the treatment of arthritic disorders in humans. These complexes used to treat **Arthritis** where painfully administered as intramuscular injections. These complexes include $Na_3[Au(S_2O_3)_2]$ called Sanochrysin, sodium salt of thiomalate, called Myochrysin.

More recently, the compound auranofin has been developed.It has the advantage that it can be administered orally and is effective.

iv. Hypercalcemia

Hypercalcemia is a disease which causes the Rapid loss of Calcium from the bones of Cancer patients. Gallium nitrate $Ga(NO_3)_3$ has been found to be most effective for treatment of hypercalcemia.

v. Siderosis Disease

An excessive intake of iron causes various problems known as siderosis. Chelation therapy is also used to treat the excess of iron. The patients who suffer from deposit of iron in liver, kidney and heart lead to failure two of these organs. The excess of iron can be removed by using chelating ligand such as deferoxamine-B, a polypeptide having a very high affinity of Fe(III) but not a for other. The concepts of soft and hard metals and ligands can be used for the process of designing therapeutic chelating agents.

3.7 Magnetic Resonance Imaging (MRI)

Nuclear Magnetic Resonance Spectroscopy can be used to image specific tissues of biological system because of the differences in relaxation times of water Proton resonance usually brought

about by metal ions which are paramagnetic.The useful metal ions for Magnetic resonance imaging in humans are Gd(III), Fe(III) and Mn(II) ions. The paramagnetic character of these ions alters the relaxation of rate of nearby water proton and, therefore, the normal and diseased tissue can be distinguished an advantage of paramagnetic MRI over radio isotopic imaging agents is that there is no possibility of radiation damage removal of excess of metal ions from the body is called chelate therapy.

3.8 TOXICITY OF METALS: Hg,Cd, Pb, As and THE CHELATE THERAPY

The elimination of toxic metal cations or other excess cations chelating ligands is called chelate therapy.

i. Lead

Lead is a very poisonous metal it is a cumulative poison sense it keeps on accumulating in the tissues of human body and plants.The lead compound and organolead compounds such as $Pb(C_2H_5)_4$in particular, are highly toxic. Exposure to organoleadcompounds occurs via inhalation, ingestion and skin contact. Like Hg(II) the heigh toxicity of lead is due to strong affinity towards sulfhydryl groups SH of cysteine residue of enzyme and proteins.The toxicity of lead is also due to the ability of lead ions to cause oxidative stress, OH radicals and peroxides thus generated induced damage of DNA and neurones.

(a) Symptoms of lead poisoning

Symptoms of lead poisoning are anaemia loss of appetite, headache, nervous disorder, brain damage, liver damage, kidney damage, cholic and skin diseases.

(b) Treatment of lead poisoning

Lead poisoning can be treated by complexing and sequestering the lead by chelating ligands such as ethylene diamine tetra acetate $CaNa_2$(EDTA) or British anti-lewisite BAL penicillamine.

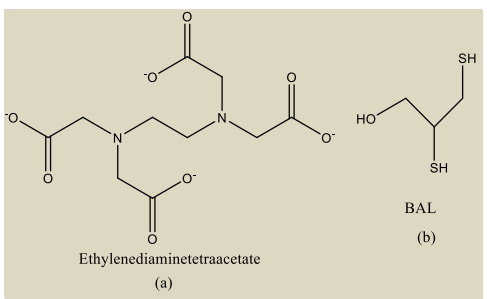


Fig. 3.12 Structure of (a) EDTA (b) BAL

ii. Mercury

Hg(II) is the congener of Zn(II) and being the congener of Hg(II) can occupy the Zn(II) binding sites in many Zn(II) enzymes and proteins. Yet Hg(II) is much larger than Zn(II) and Hg(II) proteins are formed would not function as well as corresponding Zn(II) proteins or the activity may be lost altogether. Hg(II) binds specially cysteine residue and hence inhibits enzymes whose active sites contains cysteine.

The toxicity of Hg(II) is due to the high affinity of Hg(II) and CH_3Hg^+ for sulfhydryl group (SH) of cysteine residue in proteins inhibiting the activity of enzymes and other proteins. InorganicHg(II) compounds once having entered an organism are biotically converted to CH_3Hg^+ (monomethyl mercury) being less ionic and having some affinity toward cell membrane because of methyl group, can be absorbed relatively easily through membrane.

Mercury having an appreciable vapour pressure is also extremely toxic. Mono methyl Mercury and dimethylmercury are more dangerous than metallic mercury itself and inorganic compounds such as $HgCl_2$.These organomercury compounds are more readily absorbed in gastro-intestinal tract then Hg^{2+} salts because they have greater ability to penetrate bio membranes. They concentrate in blood and have immediate and permanent effect on brain and central nervous system because they bind to the -SH group of cysteine residues in proteins.

(a) Symptoms of Mercury Toxicity

The symptoms of Mercury toxicity are: central nervous disorders, headaches, irritability, fatigue, inability to make decisions, sleeplessness, diarrhoea etc.

(b) Treatment of Mercury toxicity

More Rapid elimination of cadmium, mercury and lead requires the administration of chelating and such as 2, 3-dimercaptopropane-1-ol,$HSCH_2CH(SH)CH_2OH$ and N-acetyl penicillamine.

A very good interesting natural detoxification has been discovered in bacteria resistant to mercury. Bacteria have developed resistance to heavy metals and detoxifying process is initiated and controlled by metalloregulator proteins that are able selectively to identify metal ions. It is a small DNA binding protein that controls transcription of the mer genes.

cadmium is extremely toxic metal becauseCd(II) like Hg(II) being a congerner of Zn(II) can replace in many Zi(II) enzymes and proteins. Yet Cd(II) is large larger than Zn(II) and Cd(II) proteins formed would not function as well as the corresponding zinc enzyme or the activity

may lost altogether. Since like Hg(II), Cd(II) is also a soft acid and have strong affinity towards

Cadmium is extremely toxic. It accelerates in the Kidney and liver of human. It has a strong affinity for the -SH group of cysteine residues in protein and therefore inhibits SH enzymes.It also inhibits the action of zinc enzyme by displacing zinc.

soft base sulfhydryl (SH) of cysteine residue of protein chain, Cd(II) strongly coordinated to deprotonated Sulfhydryl groups of cysteine Residue in zinc tablet dependent proteins making the enzymes and proteins inactive and eventually denaturing the protein and forming insoluble CdS.

(c) Symptoms of cadmium toxicity

Symptoms of toxicity are:disfunctions of kidney, vomiting, irritation, hypertension, anaemia and it disease called *Hai Itai* in which the whole body feels serious pains and the bones and begins to facture very easily.

iii. Arsenic

Arsenic is a highly toxic elements for most organisms. Once inorganic compounds of arsenic enterinto the organism, they are metabolized tomethylarsenic compounds, formally derived from arsenate and arsenite by replacement of upto three OH^-/O^- functions for methyl groups.

The most commonly occurring Asis arsenate, AsO_4^{3-}which is readily reduced in organisms cells by glutathione. Hence, it reduces the availability theantioxidant, glutathione.

The toxicity of arsenic causes: chromel damage, mutagenesis, cancer and oxidative stress.

3.9 SUMMARY

In this Unit you have learnt that:

- Deoxyribonucleic acid (DNA) is a complex molecule that includes genetic instructions necessary for all known living species to develop and function.
- Watson and Crick discovered the double helical structure of DNA in 1953, making it one of the most famous discoveries of all time.

- Watson and Crick proposed a probable route for DNA replication while proposing the double helical model of DNA. DNA replication is the process by which a double-stranded DNA creates a copy of itself to produce two identical replicas.
- Since then, three distinct DNA replication models have been proposed: conservative, semi-conservative, and dispersive.
- Essential, Trace, Non-essential, and Toxic elements are divided into four groups.
- At normal quantities, no common element is hazardous, but practically everything can be dangerous at excessively high amounts.
- The concentration of metal ions in a human's system is tightly controlled.
- Metal ion deficit or excess creates disturbance, which can lead to a variety of disorders.

3.10. SAQS TYPE QUESTIONS

Objective Questions

1. Which of the following is a heme iron protein ?

   (a) Ruberodoxin

   (b) Transferrin

   (c) Hemerythrin

   (d) Cytochrome-c

2. In biological systems, the metal ions involved in electron transport are:

   (a) Na^+ and K^+

   (b) Zn^{2+} and Mg^{2+}]

   (c) a $^{2+}$ and Mg^{2+}

   (d) Cu $^{2+}$ and Fe^{2+}

3. The oxygen free form of myoglobin is a:

(a) 5-coordinate high spin Fe(ll) complex

(b) 6-coordinate low spin Fe(ll) complex

(c) 5-coordinate high spin Fe(lll) complex

(d) 5-coordinate low spin Fe(lll)

4. Iron-sulphur clusters in biological systems are involved in :

(a) proton transfer

(b) atom transfer

(c) group transfer

(d) electron transfer

5. In biological systems, the metal ion involved in the dioxygen transport besides Fe is :

(a) Co

(b) Zn

(c) Mg

(d) Cu

6. The oxidation state of iron in met-hemoglobinis :

(a) Three

(b) Two

(c) Four

(d) Zero

7. The metal ions present in the active site of nitrogenase enzyme cofactor are:

(a) Fe, Mo

(b) Fe, W

(c) Fe, Cu

(d) Fe, Ni

8. In oxyhemoglobin, the iron centre best described by which of the following ?

(a) high spin Fe(ll)

(b) high spin Fe(lll)

(c) low spin Fe(lll)

(d) low spin Fe(ll)

9. The metal atom present in the active site of carboxypeptidase enzyme is :

(a) cobalt (b) zinc

(c) iron (d) magnesium

10. The ligand system present in vitamin B12 is:

(a) porphyrin (b) corrin

(c) phthalocyanine (d) crown ether .

Answers:

1	(d)	2	(d)	3	(a)	4	(d)	5	(d)	6	(a)	7	(a)
8	(d)	9	(b)	10	(b)								

3.11 LONG ANSWER QUESTION

1. Discuss the role of metal ions in biological systems.
2. What are essential and trace metals? Give the role of iron, manganese and molybdenum in biological system.
3. Discuss DNA polymerisation in brief. Give the structure and biological significance of DNA.
4. Write toxic effect of Pb(II). Give reason of its poisonousness. How it can be treated?

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BLOCK II: BIOORGANIC CHEMISTRY

UNIT 4: INTRODUCTION

CONTENTS:

4.1 INTODUCTION

Bioorganic chemistry is a chemistry which integrates organic chemistry and biochemistry. Organic chemistry deals with structure design, synthesis and kinetics. Biochemistry deals with study of life processes by means of biochemical methodology. Organic chemistry methods are used to synthesize biological molecules and to examine their structure, to investigate biochemical reactions. Bioorganic chemistry can be defined as a branch of chemistry or broadly speaking a branch of science which utilizes the principles, tools and techniques of organic chemistry to the understanding of biochemical/biophysical process using chemical methods.

4.2 OBJECTIVE

This unit intended to provide learners with a basic understanding of the chemical nature of biomolecules. As part of this unit, learners will be introduced to biomolecules such as nucleic acids, proteins, carbohydrates and lipids. At the end of this unit, learners will be able to:

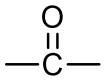
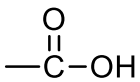
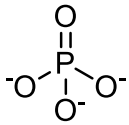
- Identify and define different types of biomolecules.
- Describe the important functional group and structural features of biomolecules.
- Identify, classify and name carbohydrates, nucleic acids, proteins and lipids on the basis of their structure and functions.
- Understand the principles of the chemistry behind biochemical molecules and their biochemical properties.
- Give the composition of nucleic acids and explain the difference between DNA and RNA.

4.3 CHEMICAL BACKGROUND OF BIOMOLECULES

Biomolecules are molecules that are involved in the maintenance and metabolic processes of all living organisms. Biomolecules made up of carbon and hydrogen, including large macromolecules such as proteins, polysaccharides, lipids and nucleic acids. These biomolecules generally known as the derivatives of hydrocarbons and some of the hydrogen atoms replaced by various types of functional groups such as hydroxyl, methyl, carbonyl, carboxyl, amino, phosphate and sulfhydryl to formed different bioorganic molecules or biological molecules (Table 4.1).

Many biomolecules are polyfunctional, containing two or more functional groups that might impact each other's reactivity. Basically functional group of biomolecules contain carbon, hydrogen, oxygen, nitrgen, phosphorus and sulphur besides these biomolecules also contain several heterocyclic and homocylic ring in their structure for example indol ring in amino acid tryptophan, phenanthrene ring in steroids. Pyrole is the basic unit of porphyrins found in several biomolecules hemoglobine, chlorophyll etc. while thiophene ring is the part of vitamine biotin. The amino acid histadine contain the ring structure of imodazole. Pyrimidines and purine are the basic constituents of the nucleic acids.

Table 4.1: Important functional group of biomolecule.

Functional group		Properties	General feature	Example of bimolecule
Name	Structure			
Hydroxyl	—OH	Polar, Hydrophilic, Capable of hydrogen bond interactions	Characterized by presence of H and O	Carbohydrates
Carbonyl		Polar, Carbonyl group of ketones and aldehydes is strongly polar group capable of acting as a hydrogen bond acceptor	Characterized by central C and O Double bond to oxygen increases the polarity	Carbohydrates
Carboxyl		Ionized to release H^+. Since carboxyl groups can release H^+ ions into a solution, they are considered acidic. Polar, Capable of hydrogen bond interactions	Characterized by central C bound to O and OH	Fatty acids
Amino	—NH_2	Capable of hydrogen bond interactions as a base, accepts H^+ to form NH_3^+. Since amino groups can remove H^+ from solution, they are considered basic.	Characterized by presence of N	Protiens
Phosphate		Charged, ionizes to release H^+. Since phosphate groups can release H^+ ions into solution, they are considered acidic.	Characterized by presence of P	Nucleic acids
Sulfhydryl	—SH	Polar	Characterized by presence of S	Coenzyme-A

Biomolecules are typically larger than organic molecules. Most biomolecules have molecular weights in the thousands, millions, or even billions, while small biomolecules have molecular weights of over 100. Because of their large size, the majority of biomolecules have specific 3-dimensional shapes in which atoms arranged in space in a precise way. The 3-dimensional shape is maintained by numerous non-covalent bonds between atoms in the molecule. The

structure of a biomolecule is flexible rather than static due to the weak nature of most noncovalent bonds and interactions between the biomolecule and the solvent. Biomolecules also exhibit stereochemistry as organic compounds. When the molecule contain stereogenic (or chiral or asymmetric) carbon then it can exist in two different isomeric enantiomers or diastereomers forms that have different configurations in space and have different properties.

4.3.1 Carbohydrates

The carbohydrates are an important class of naturally occurring organic compounds. They occur naturally in plants (where they are produced photosynthetically). During photosynthesis, plants take up water through their roots and use carbon dioxide from the air to synthesize glucose and oxygen. Because photosynthesis is the reverse of the process used by organisms to obtain energy the oxidation of glucose to carbon dioxide and water, plants require energy to carry out photosynthesis. Carbohydrate originally referred to compounds of general formula $C_n(H_2O)_n$. Where *n* is the number of carbons in the molecule represents carbohydrates. In other words, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. However, only the simple sugars or monosaccharaides fit this formula exactly. The other types of carbohydrates, oligosaccharides, and polysaccharides, are based on monosaccharaides units and have slightly different general formula. Carbohydrates are also called "saccharides" which means sugar in Greek. Carbohydrates are sugars and starches. They are the major source of energy in many organisms, serve to store energy, and are structural components in some organisms. Because of their wide distribution, carbohydrates are the most abundant type of biomolecule.

The carbohydrates are poly functional compounds and contain functional group Alcoholic hydroxy group (-OH), Aldehyde group (-CHO) and Ketone (>C=O). Thus carbohydrate can be defined as Polyhydroxyaldehydes such as D-glucose, polyhydroxy ketones such as D-fructose, and large molecules such as sucrose that produce these compounds on hydrolysis. We know that Hemiacetal or acetal is formed when a carbonyl molecule (aldehyde or ketone) reacts with an alcohol (alcoholic -OH). An aldehyde group in carbohydrates reacts with an alcoholic -OH of the same molecule to create an internal hemiacetal. We shall also see that bigger carbohydrate molecules are produced by removing H_2O molecule from between the hemiacetal OH groups of two sugar molecules. In view of the foregoing, a better definition of

carbohydrate may be: polyhydoxy molecules with an aldehyde or ketone function, either free or as hemiacetal or acetal.

4.3.1.1 Classification and nomenclature of carbohydrates

The carbohydrates are divided into three major classes monosaccharides, oligosaccharides, polysaccharides depending upon whether or not they undergo hydrolysis and if they do, on the number of products formed. Monosaccharides: The monosaccharides are polyhydroxy aldehydes (aldose) or polyhydroxy ketones (ketose) which cannot be decomposed by hydrolysis to give simpler carbohydrates. e.g. Glucose, fructose, Galactose etc. On the basis of number of carbon in main chain monosaccharides are also classified as trioses, tetroses, pentoses, hexoses and heptoses (Table 4.2).

$$\underset{\substack{\text{Glucose}\\ \text{or}\\ \text{Fructose}}}{C_6H_{12}O_6} + H_2O \xrightarrow{H^+} \text{No Reaction}$$

Table: 4.2 Clasification of monosacarides

No. of Carbon Atoms	Emprical Formula	Aldose		Ketose	
		Types	Example	Types	Example
3	$C_3H_6O_3$	Aldotriose	Gleceraldehyde	Ketotriose	Dihydroxyacetone
4	$C_4H_8O_4$	Aldotetrose	Erythrose	Ketotetrose	Erythrulose
5	$C_5H_{10}O_5$	Aldopentose	Ribose	Ketopentose	Ribulose
6	$C_6H_{12}O_6$	Aldohexose	Glucose	Ketohexose	Fructose
7	$C_7H_{14}O_7$	Aldoheptose	Glucoheptose	Ketoheptose	Sedoheptalose

Oligosaccharides (Oligo: few) consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds and upon the hydolysis gives definite number (2-10) of monosaccharide molecules. The oligosaccharides containing two monosaccharaides units are called disaccharide and those containing three units are trisaccharides. The most abundant are the disaccharides, with two monosaccharide units. For example sucrose (cane sugar), which consists of the six-carbon sugars D-glucose and D-

fructose? Thus sucrose, $C_{12}H_{22}O_{11}$ is disaccharides because on hydrolysis it gives one molecule of glucose and one molecule of fructose.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

The polysaccharides are sugar polymers containing more than 10 monosaccharide units, and some have hundreds or thousands of units. Some polysaccharides, such as cellulose, are linear chains; others, such as glycogen, are branched. Thus carbohydrates which have higher molecular weight, which yield many monosaccharide molecules on hydrolysis. E.g. Starch, glycogen, Dextrin, Cellulose etc.

$$(C_6H_{10}O_5)_n + H_2O \xrightarrow{H^+} \underset{\text{Glucose}}{n\,C_6H_{12}O_6}$$

In general monosaccharides and oligosaccharides are crystalline solids, soluble in water and sweet to taste, they are collectively known as sugars, the polysaccharides on the other hand are amorphous, insoluble in water and tasteless, they are called non-sugars (Table 4.3).

Table 4.3: Differece between monosaccharides, oligosaccharides and polysaccharides.

Character	Monosaccharides	Oligosaccharides	Polysaccharides
No. of sugar molecules	1	2-10	More than 10
Glycoside bond	Absent	Present	Present
Molecular weight	Low	Moderate	High
Taste	Sweet	Minimally sweet taste	No teste
Solubility in water	Soluble	Soluble	insoluble
Nature	Always reducing sugar	May or may not be	Always non reducing sugar
Example	Glucose, Fructose, Glactose	Sucrose, Maltose	Starch, Cellulose, Glycogen

The carbohydrates may also be classified as either reducing or non reducing sugars. All those carbohydrates which have ability to reduce Fehiling's solution and Tollen's reagents are

referred to as reducing sugars, while others are non reducing sugars. Reducing sugar bear a free aldehyde (-CHO) or ketonic [RC(=O)R'] group and have the capacity to reduce cupric ions of benedict's or Fehling solution to cuprous ion. All monosaccharides and disaccharides (like lactose, Melibiose, Cellobiose, Gentiobiose) other than sucrose are reducing sugars.

4.3.1.2 Structure and Stereochemistry of Carbohydrates

The chemical structures of carbohydrates are commonly represented by wedge-and-dash structures or by Fischer projections (Fig. 4.1). Carbohydrates come in a variety of stereocenters. Glyceraldehyde, for example, contains only one. However, as you get to more complex carbohydrates, you'll see an increase in stereocenters. There are four chiral carbons in a molecule like glucose. This indicates that there are a total of 16 stereoisomers in glucose molecule. The number of stereoisomers is equal to 2^n, where "n" is the number of chiral centres.

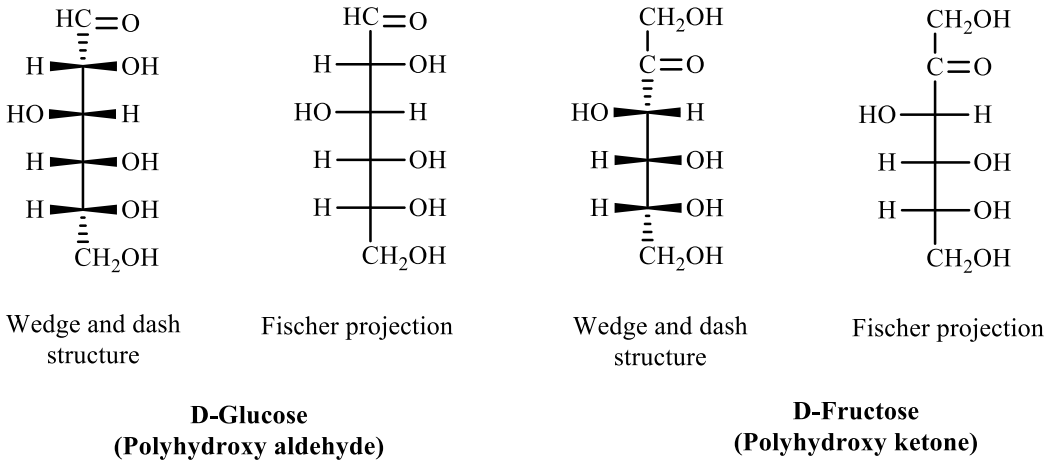


Fig. 4.1 Wedge -dash structures and Fischer projections of carbohydrates.

A. Open Chain form of carbohydrates

The stereochemistry of carbohydrates is described using D and L notation, which specifies the configuration of the last chiral carbon in the chain. It is used to give the name of molecule by relating it to glyceraldehyde. Fischer's projection is used to distinguish between D and L carbohydrates. If the hydroxyl group is positioned to the right of the last stereocenter in the Fischer's projection, the carbohydrates are given the D configuration, and if the hydroxyl group is placed to the left of the last stereocenter carbon, the carbohydrates are given the L configuration. It only gives the configuration of the carbohydrates and does not specify anything relating to the sign of the rotation of the plane-polarized light and the molecules in

which atoms have different structural arrangements are asymmetrical molecules. Absolute stereochemistry is not designated by the D and L configuration but it can be determined by the anomeric carbon center by comparing its orientation to the glyceraldehyde. D-L system is important as it gives the relative configuration of the molecules. D- and L- notation provides a quick shorthand for designating *enantiomers for example* D-Glucose is the **enantiomer** of L-Glucose (Fig. 4.2).

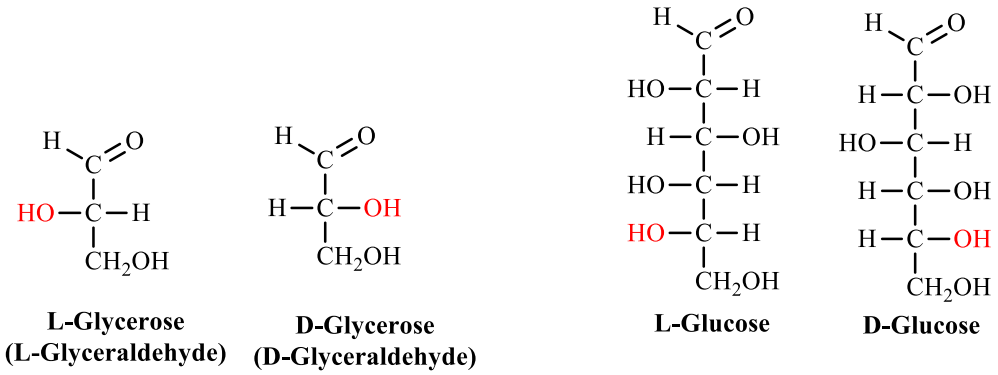


Fig. 4.2: Open Chain form of carbohydrates and D, L nomenclature.

The presence of asymmetric carbon atoms also confers optical activity on the compound. When a beam of plane-polarized light is passed through a solution of an optical isomer, it will be rotated either to the right, dextrorotatory (+); or to the left, levorotatory (-). The direction of rotation is independent of the stereochemistry of the sugar, so it may be designated D (-), D (+), L (-), or L (+). For example, the naturally occurring form of fructose is the D (-) isomer.

B. Cyclic form of carbohydrates

Carbohydrates contain a carbonyl and alcohol functional groups, they can form intramolecular (cyclic) hemiacetals. A carbohydrate must be at least a tetrose to do that, so intramolecular cyclic forms don't exist for smaller carbohydrates. When the -OH on the 4th carbon is participating in the cyclization, you get a 5-membered ring. When the 5th carbon provides the -OH, you get a 6-membered ring. Due to the analogy with the common oxygen-containing heterocyclic compounds furan and pyran, the 5-membered rings are called furanoses, and 6-membered rings are called pyranoses. Here's an example of a common sugar D-galactose forming two different cyclic forms (Fig.4.3).

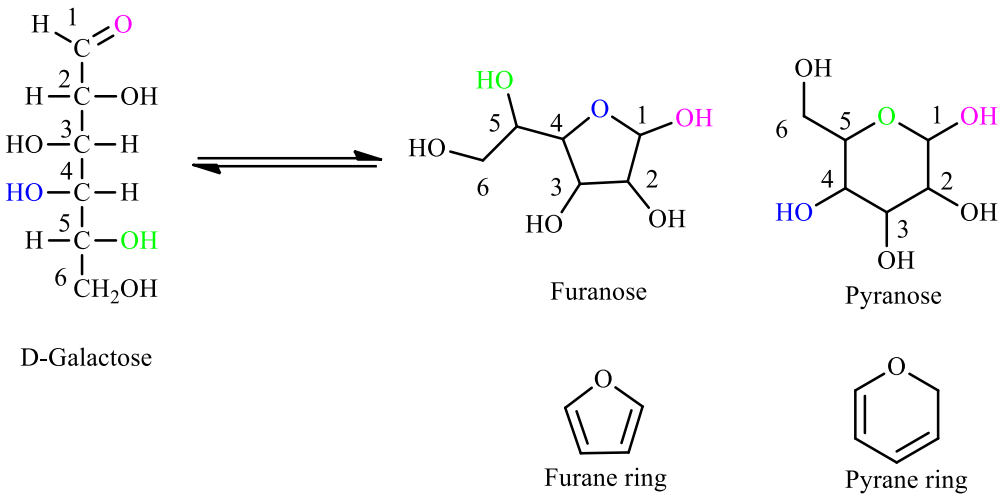


Fig.4.3 Cyclization of galactose giving two different cyclic products

C. Haworth Projections (Alpha and beta anomers or anomers)

We often use the special type of drawing to depict the cyclic forms of carbohydrates. We call them *Haworth Projections* or forms. Basically, a Haworth projection is a cyclic structure with, traditionally, carbon 1 to the right and the bottom portion of the structure oriented towards the observer.

C1 in a cyclic carbohydrate is called *anomeric* carbon. This carbon used to be a carbon of the C=O in the open-chain structure before the cyclization. Anomeric carbon is special because it doesn't have a set stereochemistry and can be in an α-form or a β-form. The α- and the β-forms are defined as trans or cis isomers of the cyclic carbohydrates where we look at the anomeric -OH and the C 5 or C6 for furanoses or pyranoses correspondingly.

Cyclization creates an anomeric carbon (the former carbonyl carbon), generating the α and β configurations of the sugar, for example, α-D-glucopyranose and β-D-glucopryanose. In a Haworth projection formula of the α-configuration, OH (C1) is trans to the CH_2OH (C5) group while in the β-configuration OH (C1) is cis to the CH_2OH (C5) group. Since the α and β forms are not mirror images, they are referred to as diastereomers. Thus monosaccharaides, differing only in this configuration around the carbon atom to which the carbonyl group is attached (the anomeric carbon), are called anomers (Fig.4.4).

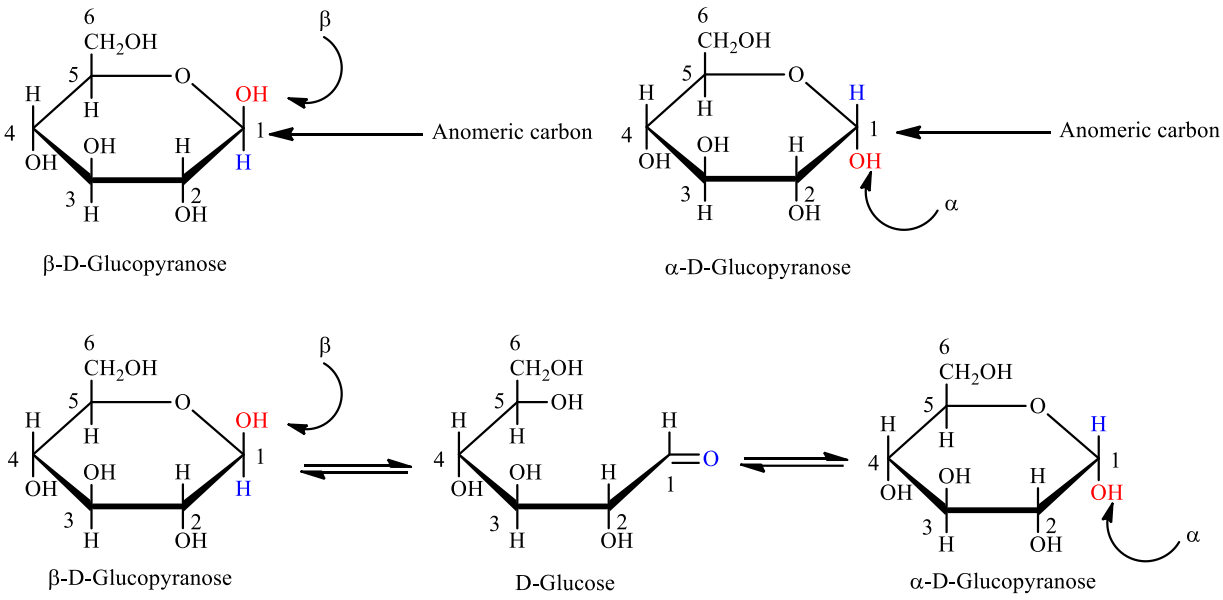


Fig.4.4 The α- and the β-forms of Glucopyranoses

D. Chair form of carbohydrates

The planar Haworth projection formulae bear little resemblance to the shape of the six membered pyranoses that actually adopt a non-planar ring conformation comparable to that of cyclohexane. The chair form is the most form of the sugar in solution cyclize into rings. The chair can have two different conformations. The principle effect of the alternative conformations is to change the axial versus equatorial orientations for the carbon functional groups. The pyranoses adopt a non-planar ring conformation and the chair form with-the highest number if equatorial rather than axial hydroxyl groups are favored. It should be noted that α- D-glucopyranose, in contrast to β -D-glucopyranose has an axial hydroxyl group. In the α-form hydroxyl group attached to C-1 is below the plane of the ring while in β –form that it is above the plane of the ring (Fig. 4.5).

E. Glycosidic bonds

- Monosaccharides can be joined to form disaccharides, oligosaccharides and polysaccharides.
- Important disaccharides include lactose (galactose + glucose), sucrose (glucose + fructose) and maltose (glucose + glucose).

- The bonds that link sugars are called glycosidic bonds. These are formed by enzymes known as *glycosyltransferases* that use nucleotide sugars such as UDP-glucose as substrates.

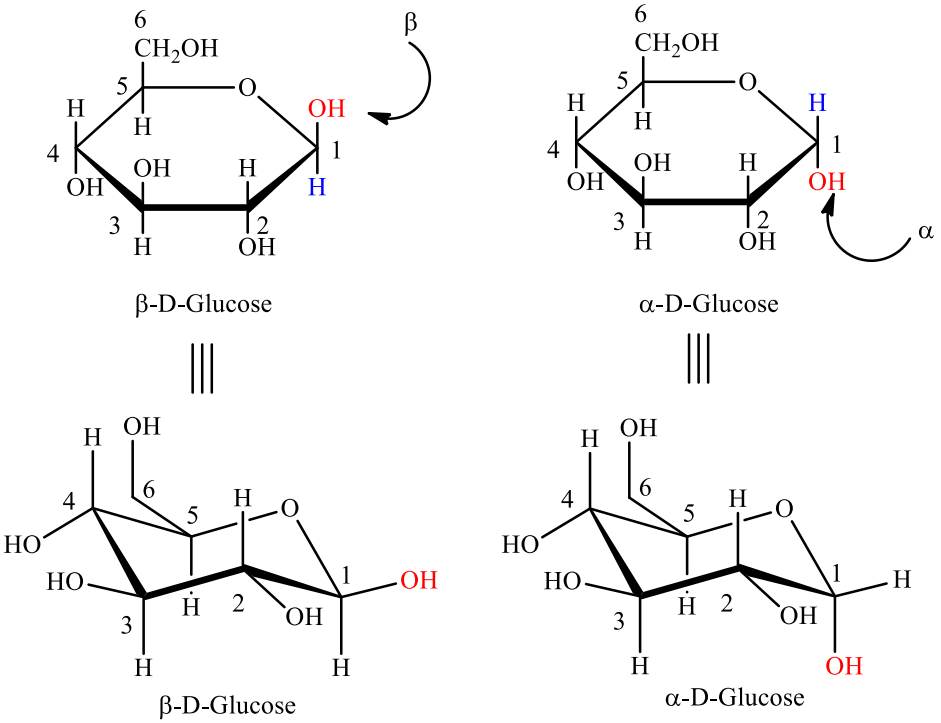
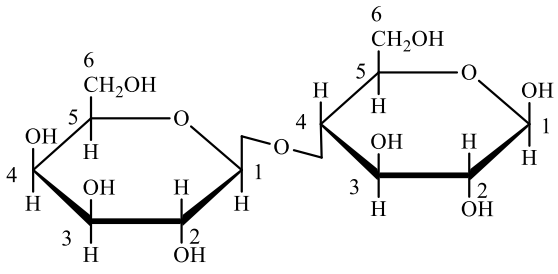


Fig. 4.5 Chair form of α and β-D-Glucose

- Glycosidic bonds between sugars are named according to the numbers of the connected carbons and also with regard to the position of the anomeric hydroxyl group of the sugar involved in the bond.
- If this anomeric hydroxyl is in the **α** configuration, the linkage is an **α-bond**. If it is in the **β** configuration, the linkage is a **β-bond**.
- Lactose, for example, forming a glycosidic bond between carbon 1 of β-galactose and carbon 4 of glucose. The linkage is, therefore, a **β (1→4)** glycosidic bond.



G. **Epimer**

Compounds that have the same chemical formula but have different structures are called isomers. For example, fructose, glucose, mannose, and galactose are all isomers of each other, having the same chemical formula, $C_6H_{12}O_6$ (Fig. 4.6).

- Carbohydrate isomers that differ in configuration around only one specific carbon atom are defined as epimers of each other. For example, glucose and galactose are C-4 epimers-their structures differ only in the position of the -OH group at carbon 4. [Note: The carbons in sugars are numbered beginning at the end that contains the carbonyl carbon-that is, the aldehyde or keto group]
- Glucose and mannose are C-2 epimers. However, galactose and mannose are **not** epimers-they differ in the position of -OH groups at two carbons (2 and 4) and are, therefore, defined only as isomers.

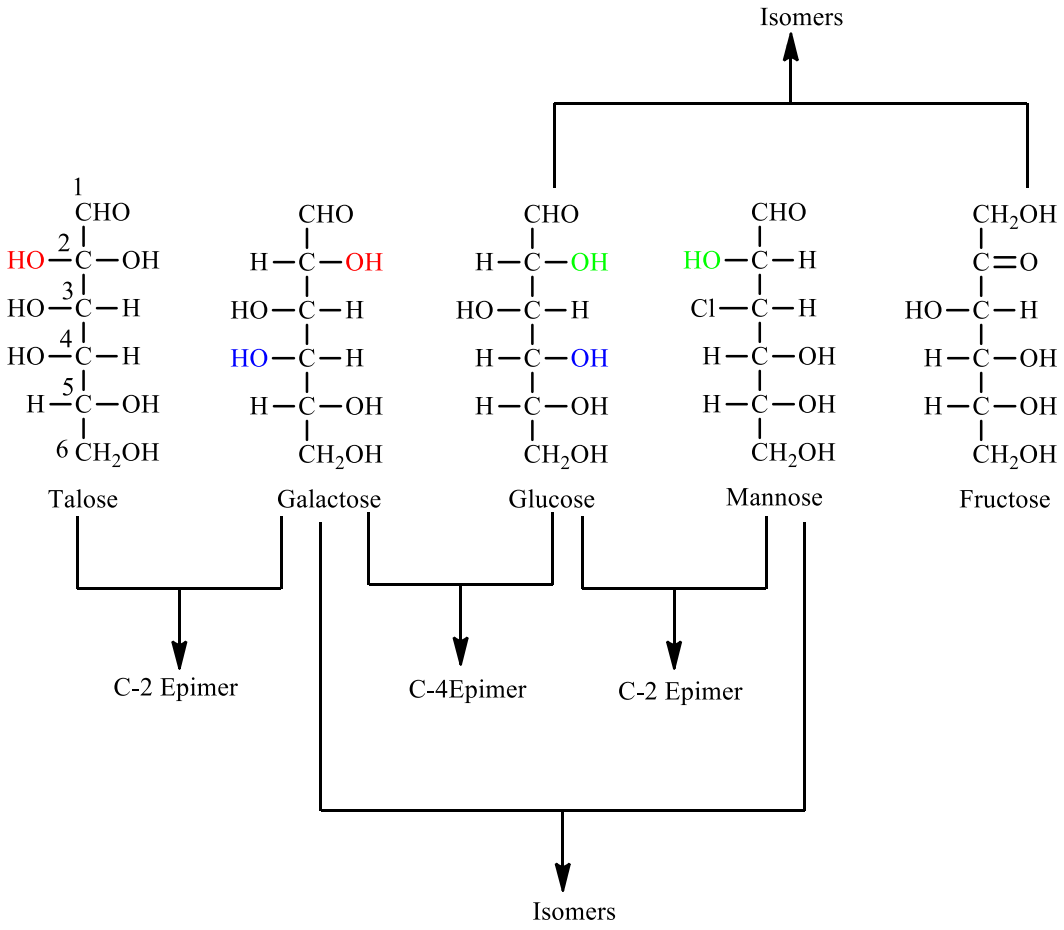
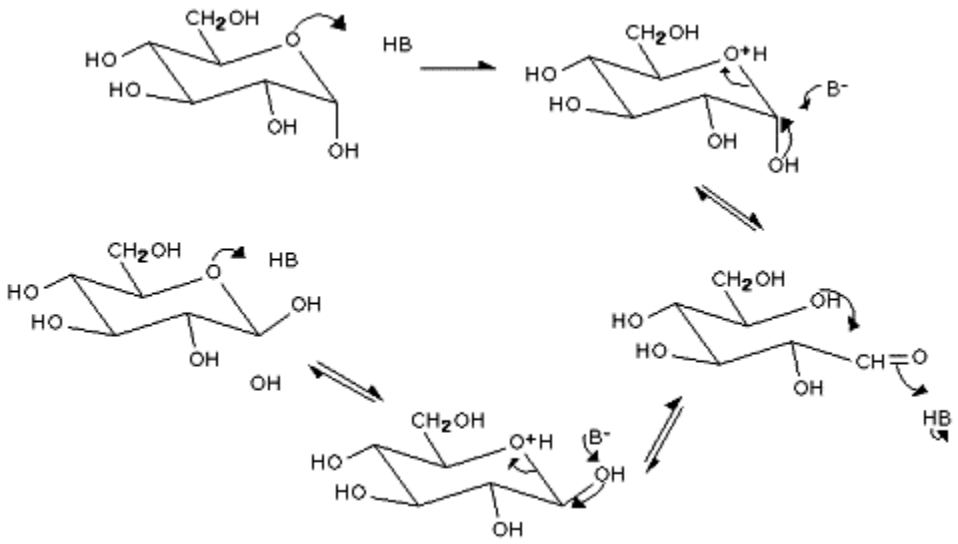


Fig. 4.6 Epimers of carbohydrate with chemical formula, $C_6H_{12}O_6$

I. Mutarotation

These two possible orientations of the hydroxyl group confer differences in optical properties when the substance is present in the crystalline anhydrous form and α-D-glucose has a specific rotation of +113° whereas that of β-D-glucose is +19.7°. However, either form in aqueous solution gives rise to an equilibrium mixture which has a specific rotation of +52.5°, with approximately 36% being in the α form and 64% in the β-form, with only a trace present as the free aldehyde. Because it takes several hours for this equilibrium to be established at room temperature, any standard glucose solution for use with a specific enzyme assay (e.g. glucose by the glucose oxidase which is specific for β-D-glucose) should be allowed to achieve equilibrium before use, so that the proportions of each isomer will be the same in the standard and test solutions. **Mutarotation** is defined as the changes in specific optical rotation by inter conversion of α and β form of D-glucose to an equilibrium mixture. Enzymes that accelerate the attainment of this equilibrium are called mutarotases and can be incorporated in assay reagents in order to speed up the equilibrium formation. Mutarotation is the change in the optical rotation because of the change in the equilibrium between two anomers, when the corresponding stereocenters interconvert. Cyclic sugars show mutarotation as α and β anomeric forms interconvert.



- The optical rotation of the solution depends on the optical rotation of each anomer and their ratio in the solution.

- Mutarotation was discovered by French chemist **Sir Dubrunfaut** in 1846, when he noticed that the specific rotation of aqueous sugar solution changes with time. [The organic fructose molecule was subsequently discovered by **Dubrunfaut** in 1847].
- Sugars in the ring form can exist in two states, one where the C-1 hydroxy group is above the plane of the ring (β) and one where it is below (α).
- In aqueous solution there is a constant interchange between the various conformations via the breaking open of the hemi acetal structure and its subsequent reforming.

4.3.1.2 Functions of Carbohydrates

Carbohydrates have wide range of functions.

- Source of energy for living beings e.g. glucose.
- Storage form of energy e.g. glycogen in animals and starch in plants.
- Serve as structural component e.g. glycosoaminoglycans in humans, cellulose in plants and chitin in insects.
- Non digestable carbohydrates like cellulose, serve as dietary fibres.
- Constituents of nucleic acid RNA and DNA. E.g. Ribose and deoxyribose sugar.
- Carbohydrates are also involved in detoxification, e.g. glucuronic acids.

4.3.2 Lipids

Lipids are fats and oils. Lipids are family of substances that are soluble in nonpolar solvents but insoluble in water that can be extracted from cells using organic solvents. Because they are grouped based on solubility properties, they are chemically more diverse than other groups of biomolecules. There are several distinct classes of lipids. Most lipids function as energy storage molecules or as structural components of membranes. Some are also harmones, vitamins and pigments.

Lipids major roles in human biochemistry are:

- They store energy within fat cells Energy is stored in the form of glycogen for quick energy when we need it. However, the burning of fats gives more than twice as much energy as the burning of the equal weight of carbohydrates.
- They are parts of membranes that separate compartments from each other. Most body constituents, for example carbohydrates and proteins are soluble in water. For

membranes that separate compartments containing aqueous solutions the body needs insoluble compounds. Lipids provide these membranes.

- They serve as chemical messengers. Primary messengers such as steroid hormones, deliver signals from one part of the body to another part. Secondary messengers, such as prostaglandins and thromboxanes, mediate the hormonal response.

4.3.2.1 Classification and Chemistry of lipid

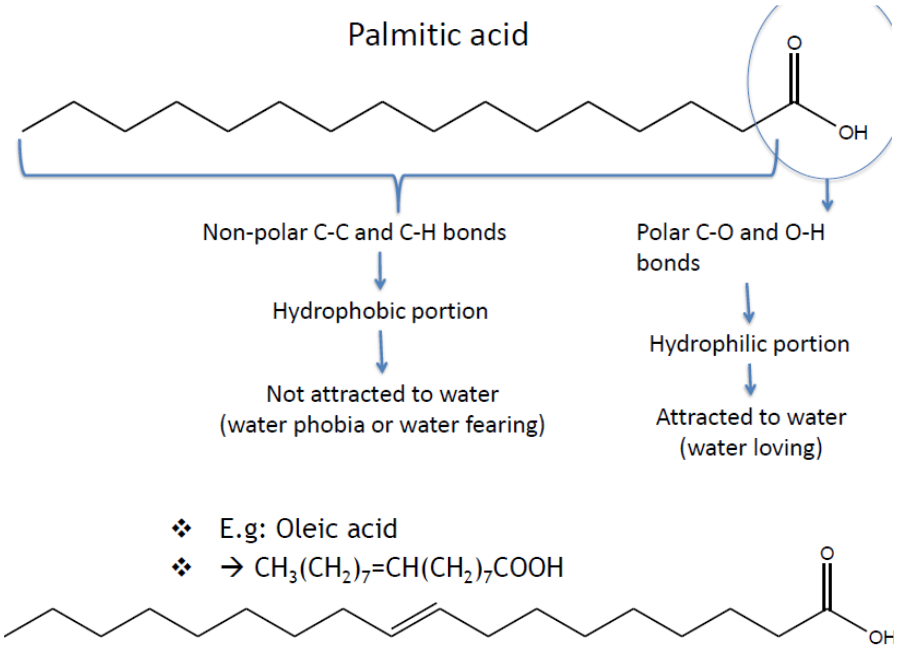
A. Fatty acids

Fatty Acids are carboxylic acids composed of long hydrocarbon chains.The long hydrocarbon chains can be either saturated or unsaturated. Most naturally occurring fatty acids are composed of even number of carbon atoms since their biosynthesis requires a concatenation of C2 units. Most commonly found fatty acids have carbon atoms between 14 and 20. In higher plants and animals, half of the fatty acids are polyunsaturated. Predominantly found saturated fatty acids include Palmitic acid (C16), Stearic Acid (C18) and Arachidic acid (C20). The notation used to represent unsaturated fatty acids is C: n where C represents the number of carbon atoms and n represents the number of double bonds in the fatty acid. Commonly found unsaturated fatty acids are Oleic acid (18:1), Linoleic Acid (18:2), Linolenic acid (18:3) and Arachidonic Acid (20:4).

In the unsaturated fatty acids, the double bonds are always found in the *cis* configuration. This *cis* configuration across the double bonds introduces a rigid 30° bend in its structure which interferes in its efficient packing due to which unsaturated fatty acids have a lower melting point than their saturated counterparts.

The Most Abundant Fatty Acids in Animal Fats, Vegetable Oils, and Biological Membranes

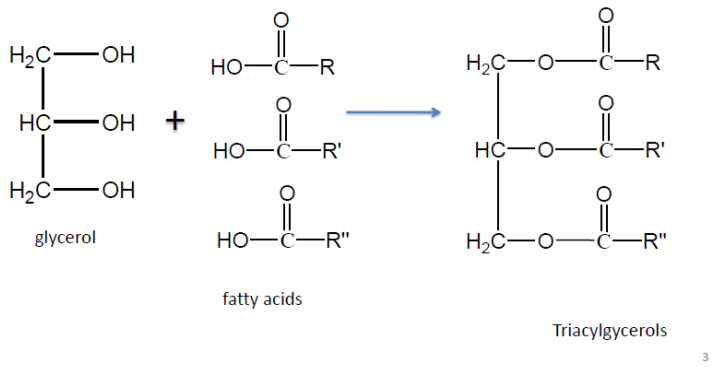
Saturated fatty acids			Unsaturated fatty acids		
Lauric	12C	$CH_3(CH_2)_{10}COOH$	Palmitoleic	16C:1	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$ *cis*
Myristic	14C	$CH_3(CH_2)_{12}COOH$	Oleic	18C:1	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ *cis*
Palmitic	16C	$CH_3(CH_2)_{14}COOH$	Linoleic	18C:2	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$ **ω-6** *cis* *cis*
Stearic	18C	$CH_3(CH_2)_{16}COOH$	Linolenic	18C:3	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$ **ω-3** all *cis*
Arachidic	20C	$CH_3(CH_2)_{18}COOH$	Arachidonic	20C:4	$CH_3(CH_2)_4(CH{=}CHCH_2)_4CH_2CH_2COOH$ **ω-6** all *cis*



B. Triacyl glycerols

Triglycerides (triacylglycerols) contain three fatty acids joined by ester bonds to a glycerol molecule. The three fatty acids may be the same or different. Without the -COOH group, they are even more non-polar than fatty acids. Triacylglycerols are water insoluble fatty acid esters of glycerol. They are characterized by the identity of the three fatty acids that are esterified to the three alcoholic groups of glycerol. They serve as energy reserves in the cell. Their utility as energy reservoirs in the cell stems from the fact that compared to carbohydrates and proteins, fats are less oxidized and hence more energy is release on oxidizing the same amount of fat. Also, since they are nonpolar, they are stored in anhydrous form unlike carbohydrate polymers like glycogen that bind twice their weight of water.

Triesters formed from glycerol and three molecules of fatty acids



Although some of the molecules contain three identical fatty acids, in most cases two or three different acids are present (Fig. 4.7).

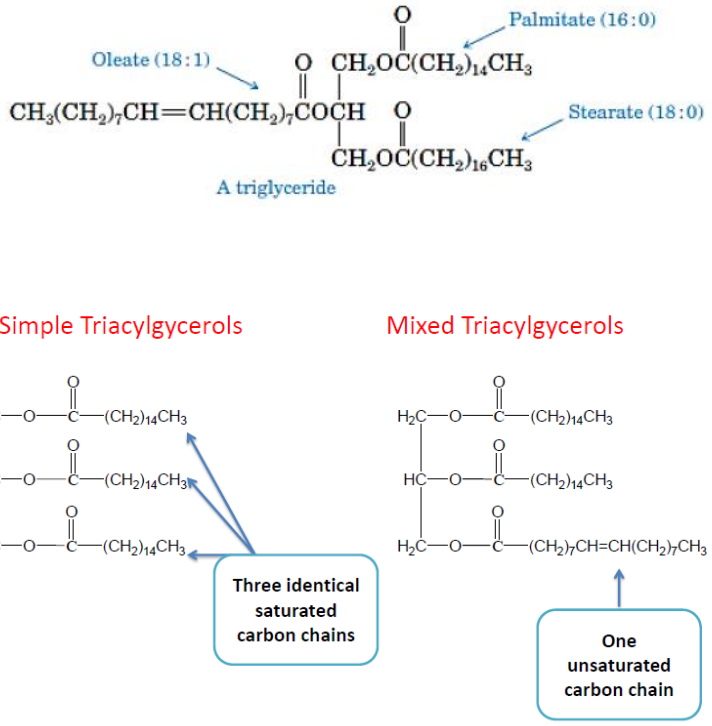
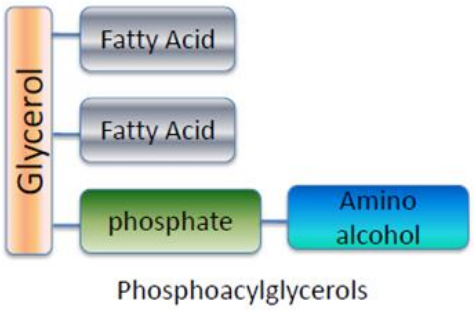


Fig. 4.7 Different types of fatty acides

C. Glycerophospholipids

Glycerophospholipids contain glycerol, two fatty acids, and a phosphate group at C-3 with a polar group attached to it. They are derivatives of phosphatidic Acid.



Glycerophospholipids, also known as phosphoglycerides are membrane lipids that are ester derivatives of L-glycerol-3-phosphate (Fig. 4.8). The C1 and C2 alcoholic groups of glycerol 3- phosphate are esterified to fatty acid residues to generate *Phosphatidic acid*. This is the structureal parent fro all glycerophospholipids. The phosphoryl group of phosphatidic acid is esterified to a highly polar and charged group via a phosphodiester linkage generating a whole class of glycerophospholipids.

All glycerophospholipids always have a negative charge on the phosphate. The polar group may have additional charges. Thus they are amphipathic with a polar head (phosphate) and non-polar tail (fatty acids).

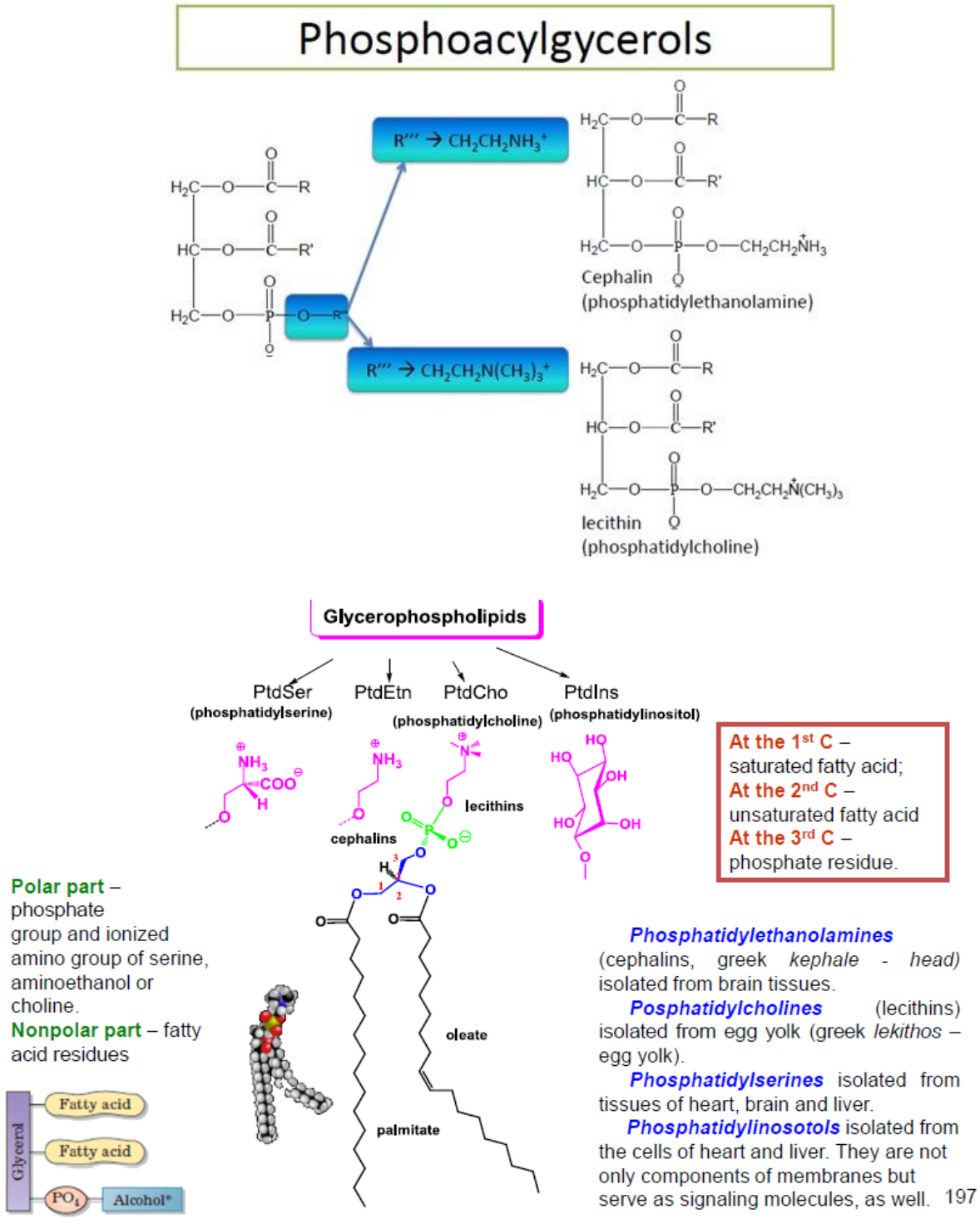


Fig. 4.8 Examples of Glycerophospholipids (phosphoglycerides)

D. Sphingolipids

Sphingolipids are major components of biological membranes. This class of lipids is derived from the C18 amino alcohol sphingosine and dihydrosphingosine. Figure 4.9 shows the structures of the sphingosine backbone, the structure ceramide and a typical Sphingolipid In a sphingolipid, the amino group at the C-2 position of sphingosine is linked via an amide bond to a fatty acid residue which can be saturated or monounsaturated with 16, 18, 22 or 24 carbon atoms. The C1 position is linked to a polar head group via either a phosphodiester or a glycosidic bond. Ceramide is the structural parent for this class of lipids.

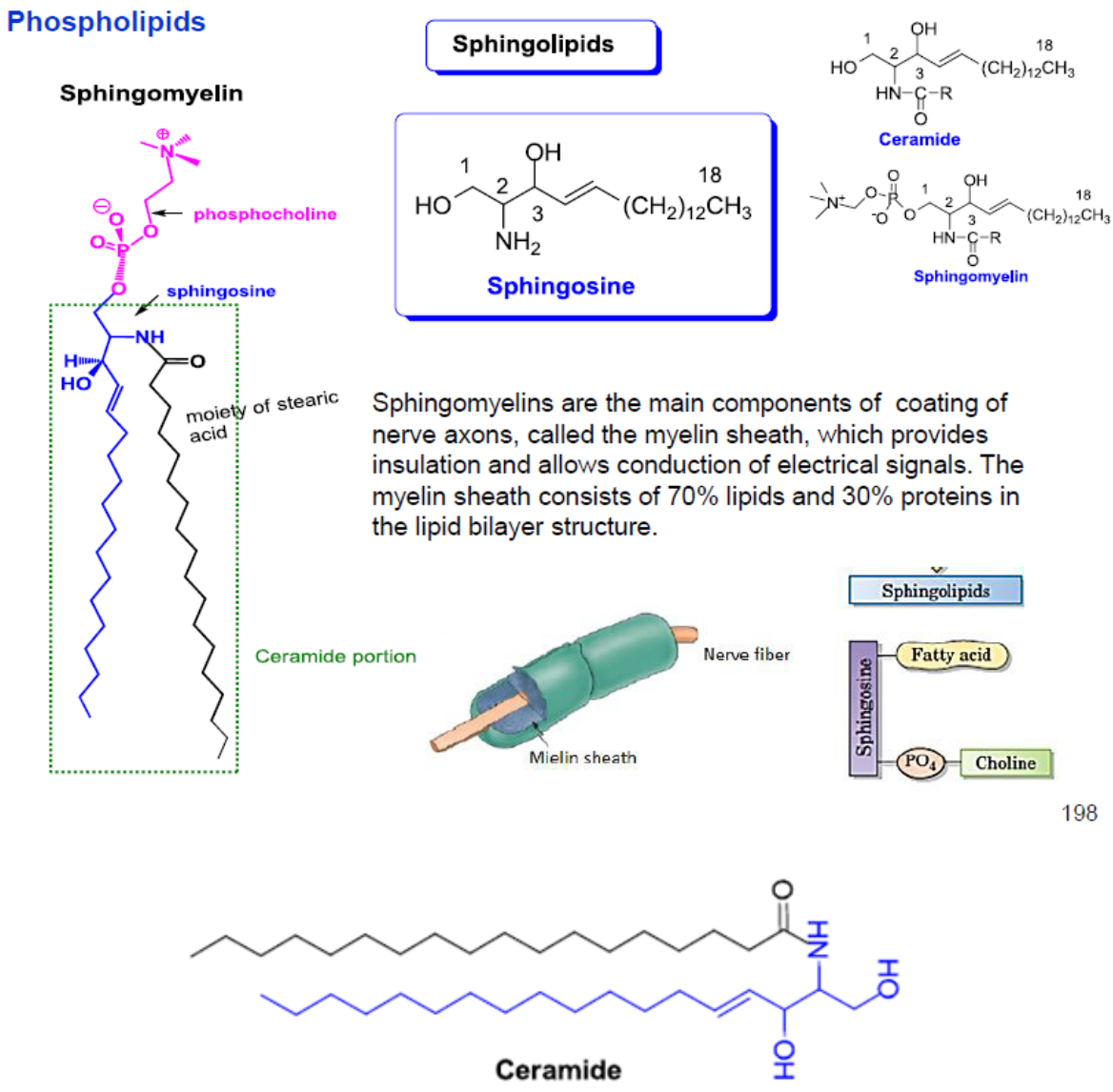


Fig. 4.9 Example of Sphingolipids

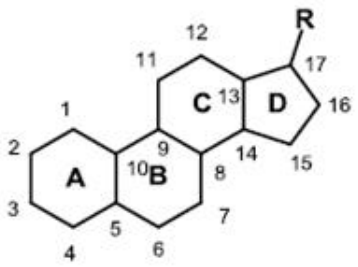
E. Sterols

Sterols are the fourth class of lipids (Fig. 4.10). In contrast to the membrane lipids discussed above, sterols are structural lipids. Their characteristic structure has a steroid nucleus composed of four fused rings. Three of these rings have 6 carbons while the fourth ring has five carbons. It's a planar ring system and relatively rigid. Any movement around the C-C bonds in this system is restricted. Cholesterol is weakly amphipathic in nature as it has a polar hydroxyl group attached to the 3rd carbon atom and a long non polar aliphatic chain attached to the 17th carbon atom. Cholesterol is the main sterol which serves as not only an important structural component of biological cell membranes but is also a precursor to major steroid hormones in the body. Steroids regulate many important physiological processes in the body as well as carbohydrate metabolism.

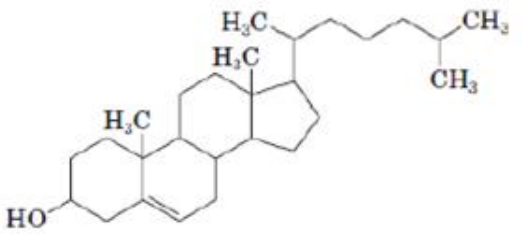
Sterols contain the steroid ring system of four fused rings, various side groups, and a hydroxyl group. Other steroids contain a carbonyl group. They do not contain a fatty acid and so are non-saponifiable. They are amphipathic with the oxygen group making a polar head for the molecule. Steroids are **non-hydrolyzable lipids**. Steroids are compounds containing four fused carbocyclic rings. Steroids are completely different in structure from the discussed lipids. They are normally not esters, although some of them are. Steroids are closely related in structure but are highly diverse in function.

This group of lipids involves:

- Cholesterol
- Sex and adrenocorticoid hormones
- Bile acids
- Vitamins D



Cholesterol



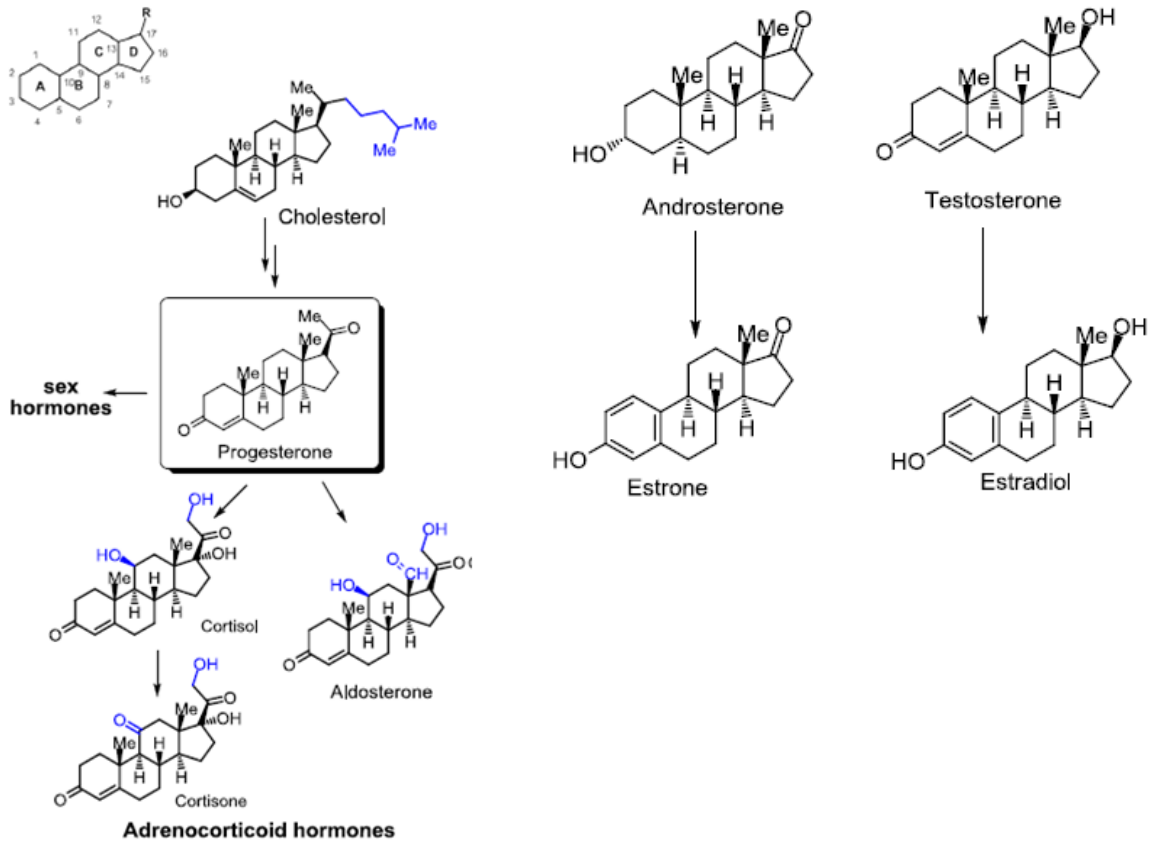


Fig. 4.10 Different types of sterols

4.3.3 Protein

A protein is a biologically functional molecule that consists of one or more polypeptides. Proteins account for more than 50% of the dry mass of most cells.Protein functions include structural support, storage, transport, cellular communications, movement, and defense against foreign substances. Protein's function is highly governed by its stable structure. This structure has four levels: Primary, secondary, tertiary and quaternary. Protein structure is stabilized by multiple weak interactions.

Primary structure is the sequence of amino acids joined to each other by peptide bonds. Secondary structure is the local folding of a part of polypeptide. Next level, the tertiary structure is mixture of α-helix and β-sheets. While quaternary structure is the subunit composition of a protein. In this module, we will have deeper insights into the protein structure and its various levels.

4.3.3.1 Amino acids

Although a wide variety of proteins exist, they all have basically the same structure: They are chains of amino acids. Amino acid is an organic compound containing an amino group and a carboxylic group (Fig. 4.11). The 20 amino acids commonly found in proteins are called alpha amino acids. Except for glycine, which is achiral, all the amino acids in all the proteins in human body are the L-isomers.

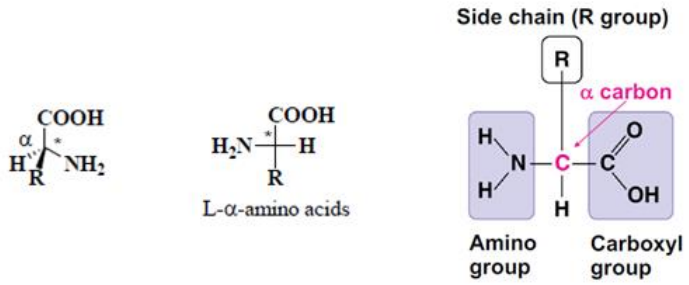
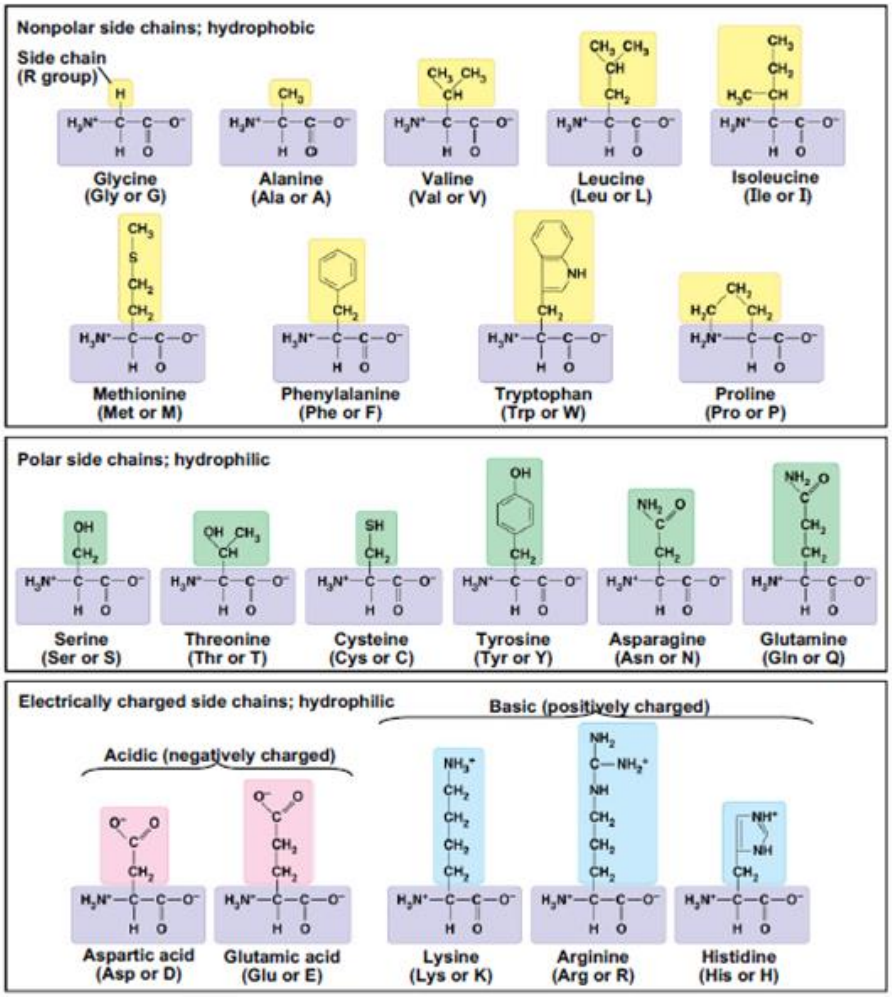


Fig. 4.11 General structure of an amino acid



The most important aspect of the R groups is their polarity. On that basis amino acids are classified into four groups: **nonpolar, polar but neutral, acidic, and basic**. The nonpolar side chains are ***hydrophobic*** (they repel water), whereas polar but neutral, acidic, and basic side chains are ***hydrophilic*** (attracted to water).

An amino acid has -COOH and -NH2 groups in the same molecule. Therefore, in water solution, the **-COOH donates a proton to the -NH2** so that an amino acid actually has the structure of the internal salt. Compounds that have a positive charge on one atom and a negative charge on another are called **zwitterions,** from the German word ***zwitter*, meaning "hybrid**." Amino acids are zwitterions, not only in water solution but also in the solid state (Fig. 4.12).

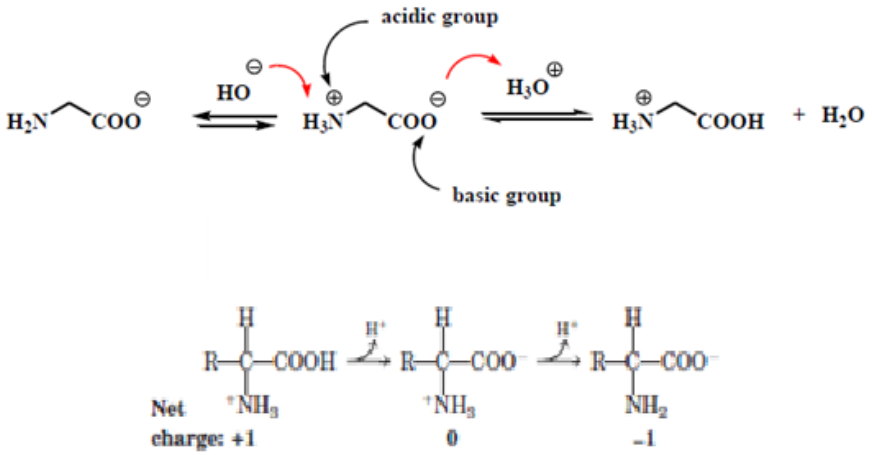
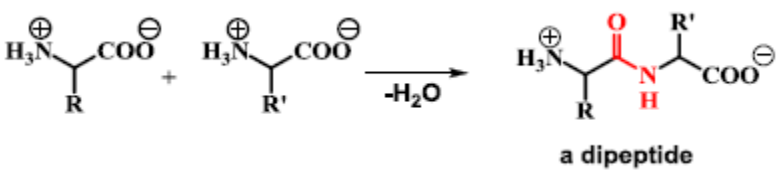


Fig. 4.12 Amino acids are zwitterions

4.3.3.2 Peptide Bond

Amino acids are polymerized in living systems by enzymes that form amide linkage from the amino group of one amino acid to the carboxyl group of another. A molecules formed by joining amino acids together are called peptides, and amide linkages are called peptide bonds or peptide linkages. Each amino acid in the peptide is called amino acid residue. Peptides that contain 2, 3, a few (3-10), or many amino acids are called dipeptides, tripeptides, oligopeptides and polypeptides, respectively.



Peptides are linear polymers. One end of a polypeptide chain terminates in an amino acid residue that has a free –NH3+ group; the other terminates in amino acid residues with a free –CO2- group. These two groups are called the N-terminal and C-terminal residues, respectively (Fig. 4.13).

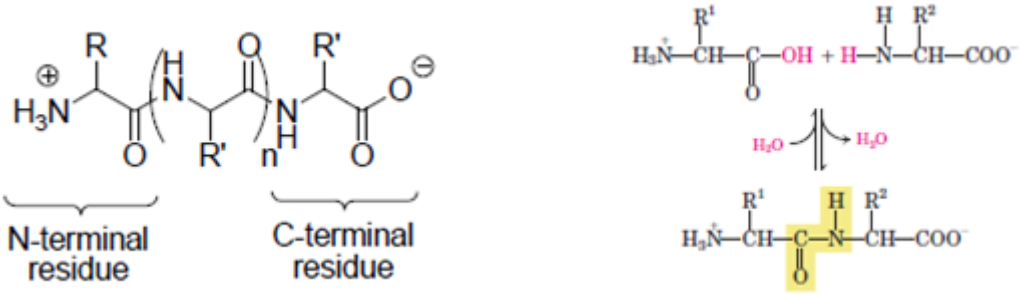


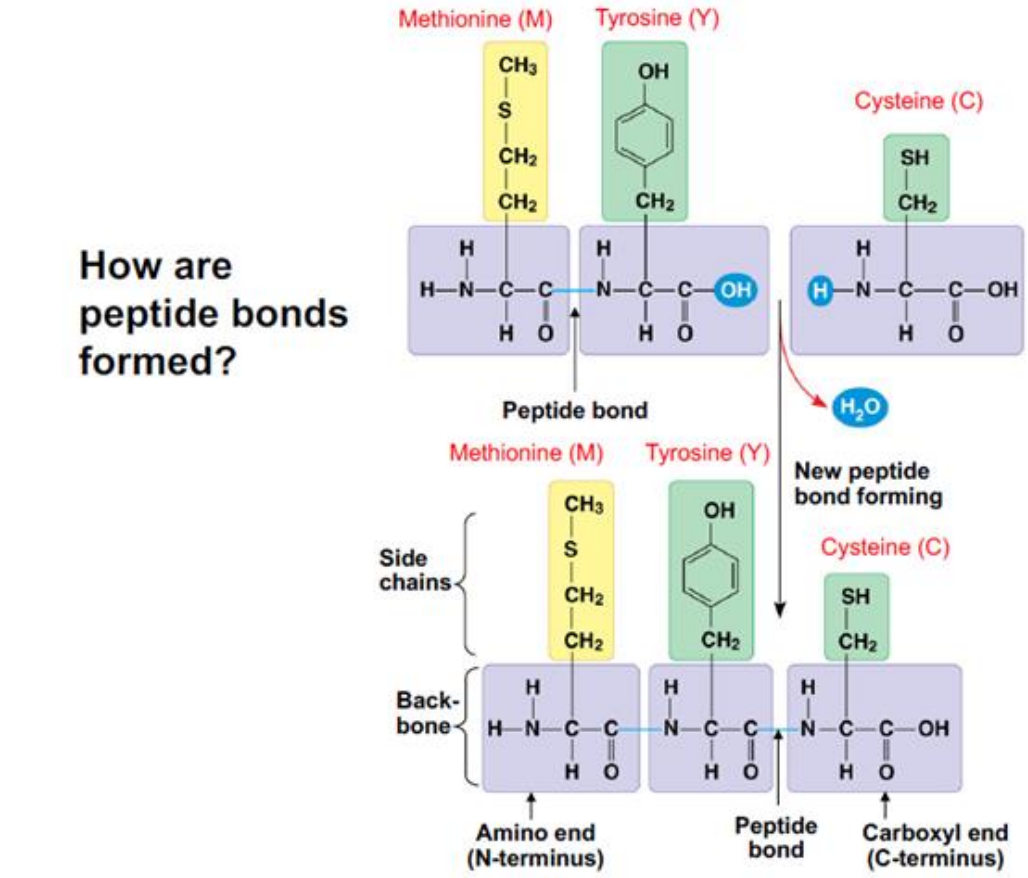
Fig.4.13 Formation of a peptide bond by condensation

Linus Pauling and Robert Corey worked on the peptide bond. They deciphered that peptide bond is planar as an outcome of C-N bond length shorter than in amine. They noticed that resonance existed between carbonyl carbon and amide nitrogen components of the peptide bond (OC-NH). The oxygen (O in OC of peptide bond) and hydrogen (H in NH of peptide bond) lie in *trans* position. No free rotation was seen around the peptide bond because of their double bond character. Hence, it is rigid and is planar. Also, the oxygen of carbonyl carbon has a partial negative charge and the nitrogen has partial positive charge. This forms the small electric dipole. The only bonds where rotation is possible are N-Cα and Cα-C bonds. The bond angles upon rotation of Cα-C bond are called the psi angle (ψ) while the bond angle upon rotation of N-Cα bond is called the phi angle (φ). Psi and phi angles should be between -180° to +180° (Fig 4.14). The permitted rotations around N-Cα bond (phi angle) and Cα-C bond (psi angle) were plotted graphically by G.N.Ramachandran (Fig. 4.15).

4.3.3.3 Structure of Protein

The structure of proteins can be described at four different levels (Fig. 4.16). They are:

- Primary Structure: Gives descriptive account of sequence of amino acids and all the covalent bonds (peptide bonds, disulphide bonds) linking the various amino acids in the polypeptide chain.



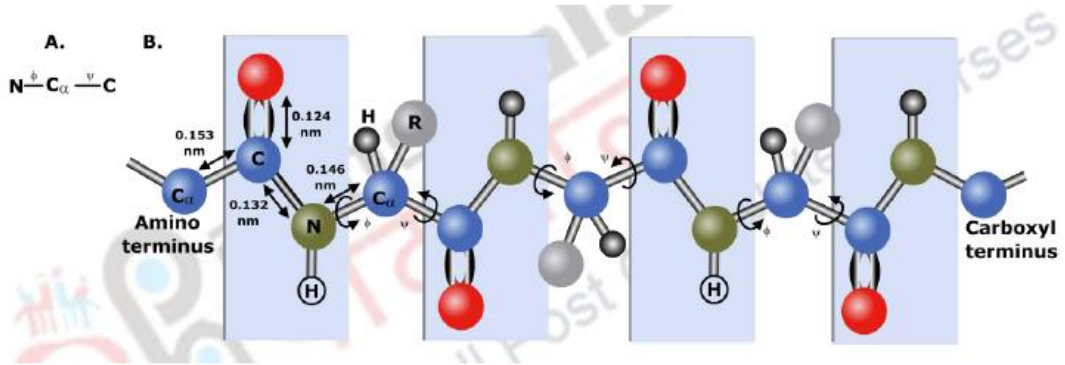
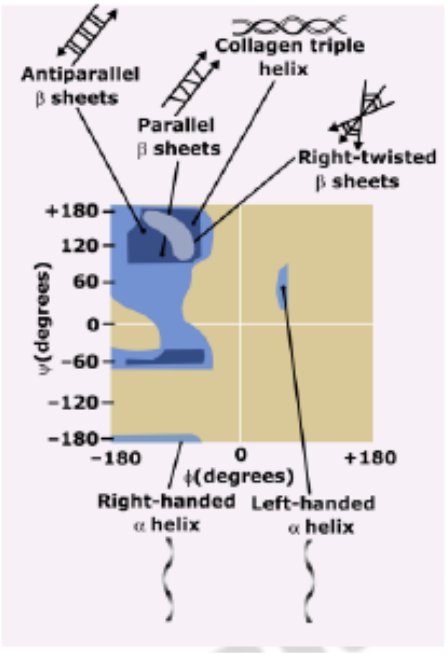
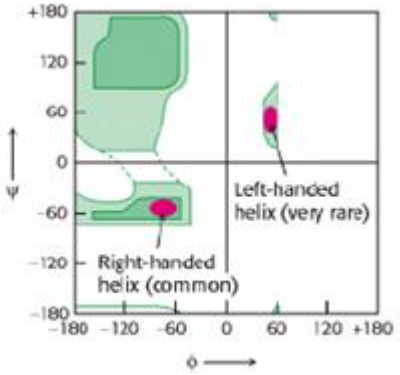
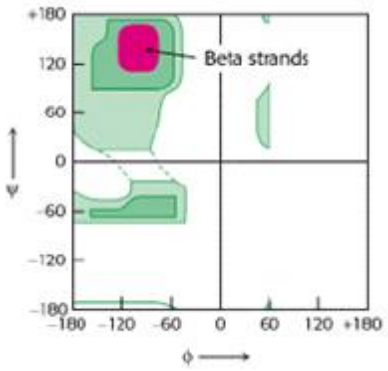


Fig. 4.14 The peptide bond is planar and has a partial double bond character that imparts rigidity to it. Rotation is allowed around the N-Cα bond (phi angle) and Cα-C bond (psi angle)





(a) Ramachandran Diagram for Helices. Both roght and left handed helices lie in regions of allowed conformations in the Ramachandran Diagram. However, essentially all α-helices in protiens are right-handed.

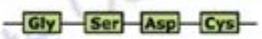
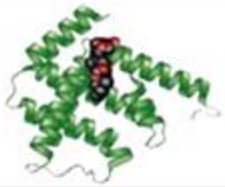
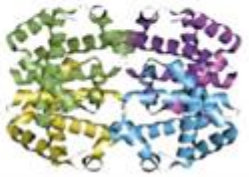


(b) Ramachandran Diagram for β strands. The red area shows the sterically allowed conformations of extended, β-strand-like structure.

Fig.4.15 Ramachandran Plot showing permissible phi and psi angles for variety of structures

- Secondary Structure: Structural patterns made from the arrangements of amino acid residues. Secondary structure, found in most proteins, consists of coils and folds in the polypeptide chain.
- Tertiary Structure: Three dimensional folding of the protein and structure is determined by interactions among various side chains (R groups).

- Quaternary Structure: This takes into account the spatial arrangement of subunits and structure results when a protein consists of multiple polypeptide chains.

A Summary of protein Structure			
Level	**Description**	**Stabilized by**	**Example:Hemoglobin**
Primary	**The sequence of amino acids in a polypeptide**	**Peptide bonds**	
Secondary	**Formation of α- helices and β-pleated sheets in a polypeptide**	**Hydrogen bonding between groups along the peptide-bonded backbone**	
Tertiary	**Overall three-dimensional shape of a polypeptide (The model on the right shows one of hemoglobin's subunits. The black and red atoms are in the heme group that carries oxygen;they are not part of the protein itself.)**	**Bonds and other interactions between R- groups,or between R-groups and the peptide-bonded backbone**	
Quaternary	**Shape produced by comibinations of polypeptides. The model on the right shows hemoglobin,which consists of four polypeptides.)**	**Bonds and other interactions between R-groups,and between peptide backbones of different polypeptides**	

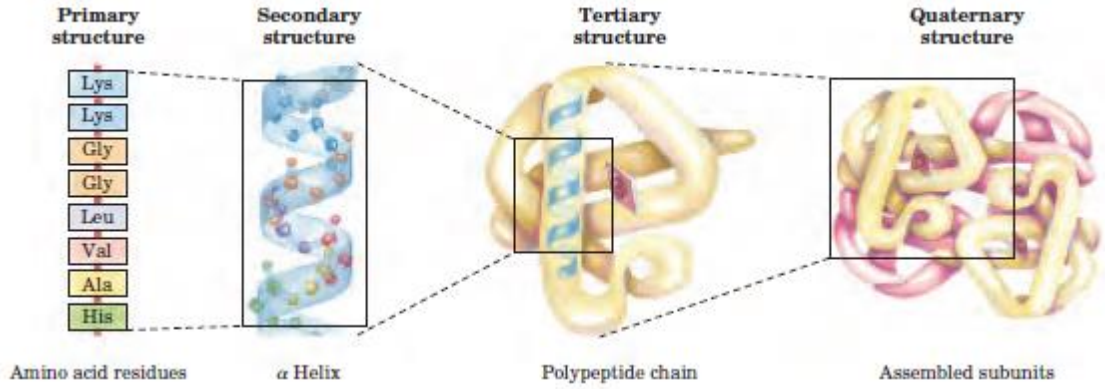


Fig. 4.16 Levels of structure in proteins

A. Primary structure

The primary structure of a protein is its unique sequence of amino acids. Each peptide or protein has its own unique sequence of amino acids. As with naming of peptides, the assignment of positions of the amino acids in the sequence starts at the N-terminal end. For example, In 1953, first amino acid sequence of a protein, bovine insulin ((Insulin is necessary for proper utilization of carbohydrates, and people with severe diabetes must take insulin

injections) was elucidated by Frederick Sanger. Bovine insulin is 51 amino acids long and is composed of two polypeptide chains: A (21 amino acids) and B (30 amino acids) joined to each other by intra- and inter-chain disulphide bonds (Fig. 4.17).

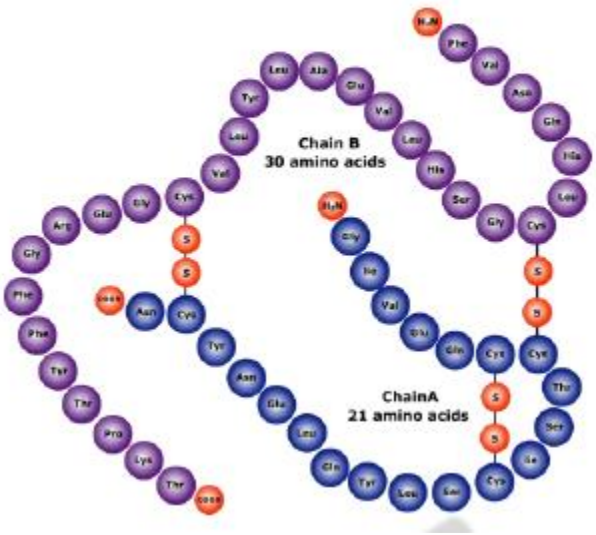


Fig.4.17 Primary Structure of bovine insulin

B. Secondary structure

Structural patterns made from the arrangements of amino acid residues are termed as the secondary structure of a protein. α-helix and β-sheets are two prominent secondary structures that occur in proteins (Fig. 4.18). Those protein conformations that do not exhibit a repeated pattern are called random coils.

i) α-Helix exists in hair protein, keratin. Various conformations can be assumed for a protein by rotation around single bonds and rigid peptide bonds. The simplest arrangement of a polypeptide chain is α-helix. The polypeptide coils around an imaginary axis with side groups protruding out from the helix. The single turn of the helix is 5.4 Angstrom which is the repeating unit of the α-helix.

   Intra-hydrogen bonding stabilizes the α-helix. This bond forms between the first amino acid and the fourth amino acid. The charge of the side chains can destabilize the helix. Adjacent Glu, Arg or Lys which are charged at neutral pH impede the formation of α-helix. Similarly, Pro with ring structure introduces a kink in the helix and destabilizes it. In the a-helix form, a single protein chain twists in such a manner

that its shape resembles a right-handed coiled spring-that is, a helix. The shape of the helix is maintained by numerous intramolecular hydrogen bonds that exist between the backbone –C=O and H-N- groups. Each –N-H points upward and each C=O points downward, roughly parallel to the axis of the helix. All the amino acid side chains point outward from the helix.

ii) The β-sheet is zig-zag extended conformation of a polypeptide. The intra molecular hydrogen bonding is formed between adjacent segments of a polypeptide. The adjacent segments can be in parallel or antiparallel orientation. Apart from β-sheets, β-turns are important components of the protein structure that connect the two adjacent segments of the antiparallel β sheet by hydrogen bonding between first and fourth amino acids.

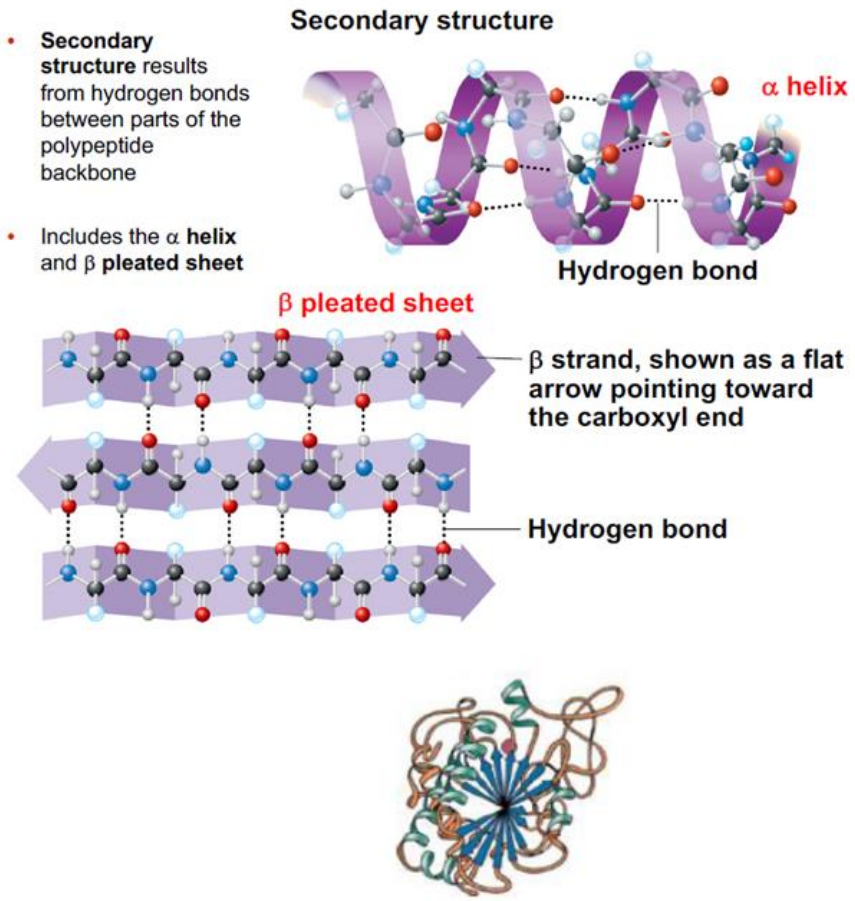


Fig. 4.18 Enzyme carboxypeptidase. The β-pleated sheet portions are shown in blue, the green structures are the a-helix portions, and the orange strings are the random coil areas

The other important orderly structure in proteins is the β-pleated sheet. In this case, the orderly alignment of protein chains is maintained by intermolecular or intramolecular hydrogen bonds. The β-sheet structure can occur between molecules when polypeptide chains run parallel (all N-terminal ends on one side) or antiparallel (neighboring N-terminal ends on opposite sides). β-Pleated sheets can also occur intramolecularly, when the polypeptide chain makes a U-turn, forming a hairpin structure, and the pleated sheet is antiparallel. The other important orderly structure in proteins is the β-pleated sheet. In this case, the orderly alignment of protein chains is maintained by intermolecular or intramolecular hydrogen bonds. In all secondary structures, the hydrogen bonding is between backbone –C=O and H-N-groups.

C. Tertiary structure

The three dimensional arrangement of all atoms in a protein is termed as the tertiary structure. When the polypeptides fold in spherical shape, they are called globular proteins while fibrous proteins have extended conformation (Fig. 4.19)

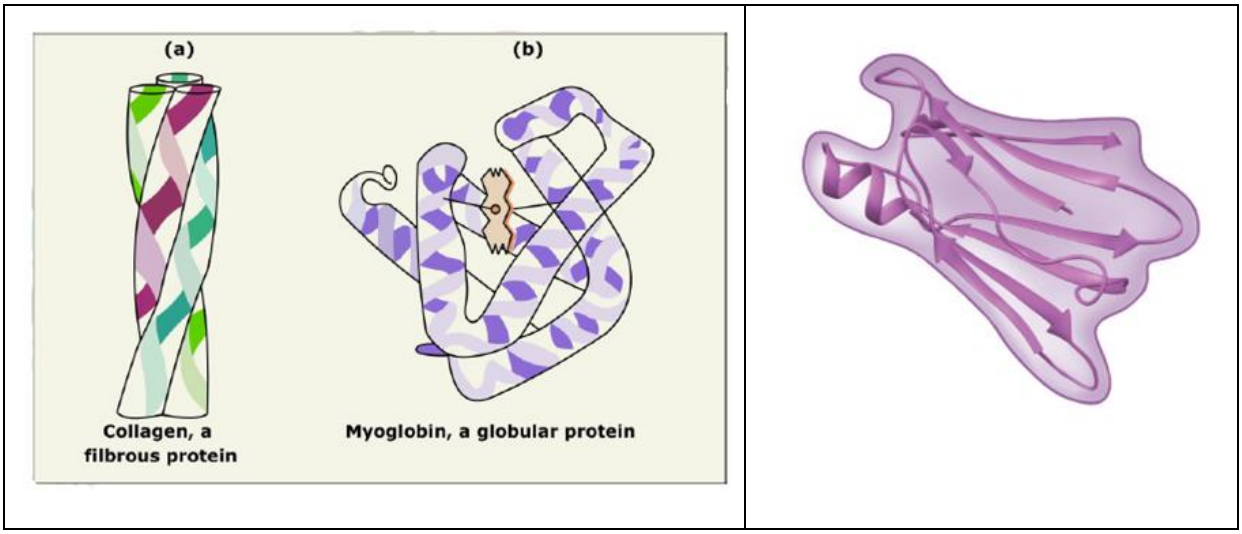


Fig. 4.19 A fibrous and a globular protein

The tertiary structure of a protein is the 3D-arrangement of every atom in the molecule. Unlike the secondary structure, it includes interactions of the side chains, and not just the peptide backbone (Fig. 4.20). In general, tertiary structures are stabilized by five ways.

i) Covalent bond:

The covalent bond most often involved in stabilization of the tertiary structure of proteins is the disulfide bond.

ii) Hydrogen bonding:

Tertiary structures are stabilized by hydrogen bonding between polar groups on side chains or between side chains and the peptide backbone.

iii) Salt bridge (electrostatic interaction):

Salt bridges, also called electrostatic attractions, occur between two amino acids with ionized side chains

iv) Hydrophobic interaction:

The nonpolar groups prefer to interact with each other, excluding water from inward regions.

v) Metal ion coordination:

Two side chains with the same charge would normally repel each other, but they can also be linked via a metal ion.

Forces that stabilize the tertiary structures of proteins. The helical structure and the sheet structure are two kinds of backbone hydrogen bonding. Although the backbone hydrogen bonding is part of the secondary structure, the conformation of the backbone puts constraints on the possible arrangement of the side chains.

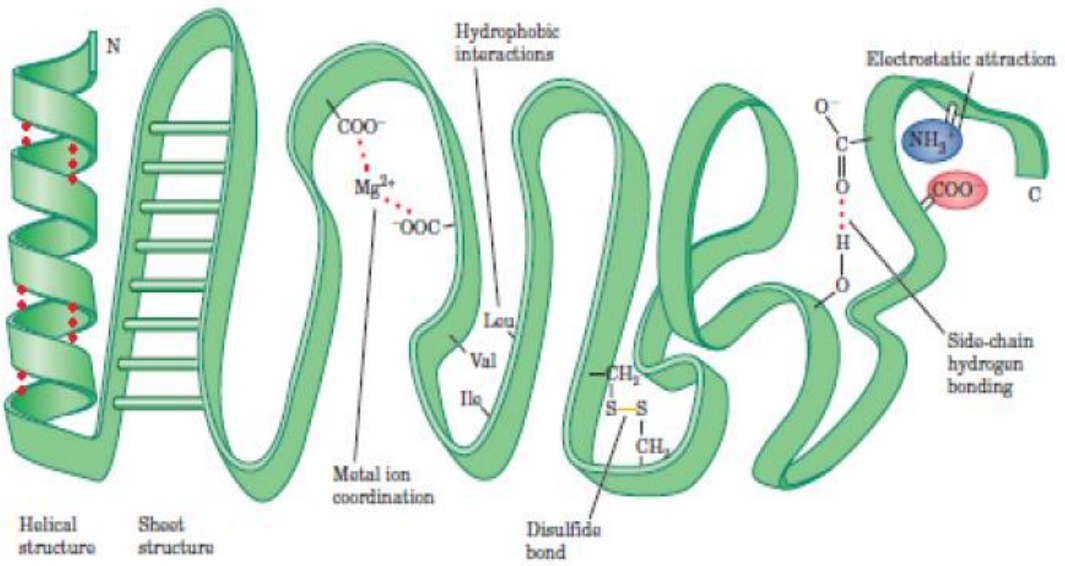


Fig.4.20 Forces that stabilize the tertiary structures of proteins

D. Quaternary structure

Quaternary structure results when two or more polypeptide chains form one macromolecule. The highest level of protein organization is the quaternary structure, which applies to proteins with more than one polypeptide chain. The subunits are packed and held together by hydrogen bonds, salt bridges, and hydrophobic interactions-the same forces that operate within tertiary structures.

Hemoglobin in adult humans is made of four chains (called globins): two identical α-chains of 141 amino acid residues each and two identical β-chains of 146 residues each. Hemoglobin, the oxygen carrier of blood, has a quaternary structure of 4 polypeptides: 2α and 2β and hence α2β2. α and β polypeptides resemble the myoglobin structure. In hemoglobin, each globin chain surrounds an iron-containing heme unit. Proteins that contain non-amino acid portions are called conjugated proteins. The non-amino acid portion of a conjugated protein is called a prosthetic group. In hemoglobin, the globins are the amino acid portions and the heme units are the prosthetic groups (Fig. 4.21).

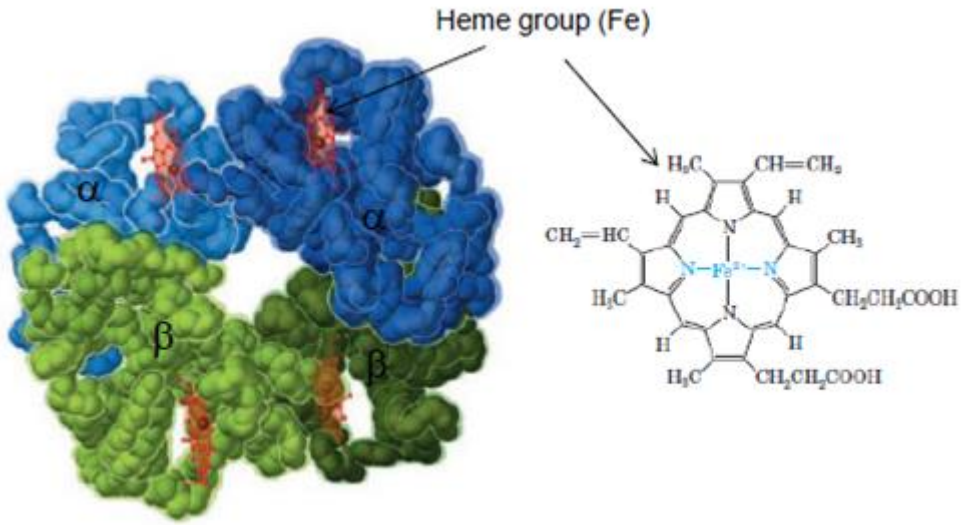


Fig. 4.21 Quaternary structure of hemoglobin

4.3.3.4. Confirmation and stability of protien

The arrangement of atoms in a protein is called its *conformation*. A conformational change is an outcome of rotation around the single bond and without breaking any covalent bonds. The most stable conformation of a protein under given set of conditions is the conformation that is

thermodynamically most stable, having lowest Gibb's free energy (G). Multiple 'most stable conformations' exist for a protein defined by the set of conditions such as binding of ligand.

- Native conformation/state of a protein is the one which is most stable form under given set of conditions.
- Stability of a protein is defined as the tendency to maintain a native state/conformation.

Different conformations assumed by the protein have different degrees of conformational entropy. The unfolded state has the maximum entropy. Disulphide bonds and multiple weak interactions such as hydrophobic interactions, ionic interactions stabilize the native conformation of a protein. Protein folds in such a way that the hydrophobic residues are buried inside the core and the polar residues are towards the exterior of the protein. Thus the protein interior has many such hydrophobic interactions. The aqueous milieu around the protein forms a solvation shell or layer around it largely defined by the hydrogen bonds that the protein makes with surrounding water. Largely, the proteins fold burying nearly all the hydrophobic interactions inside the core while the polar residues tend to stay on the surface (Fig. 4.22).

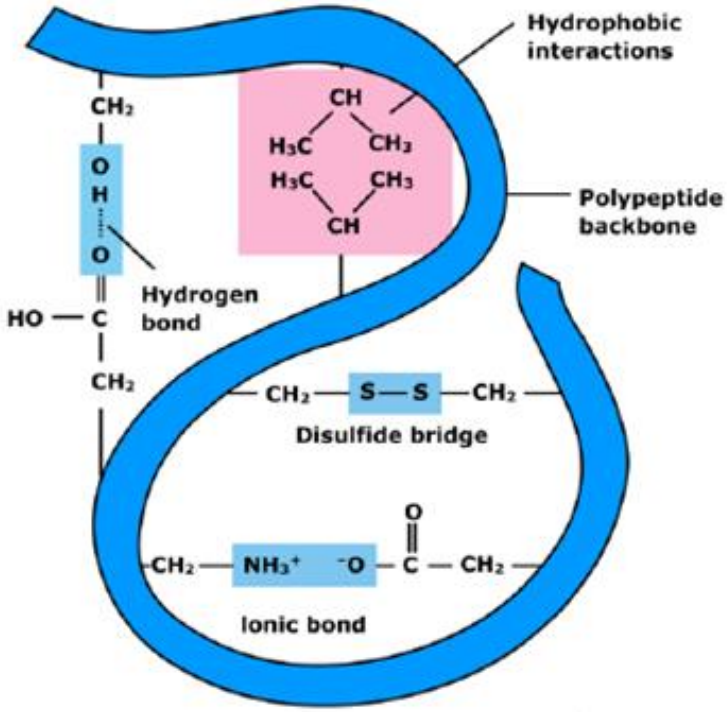


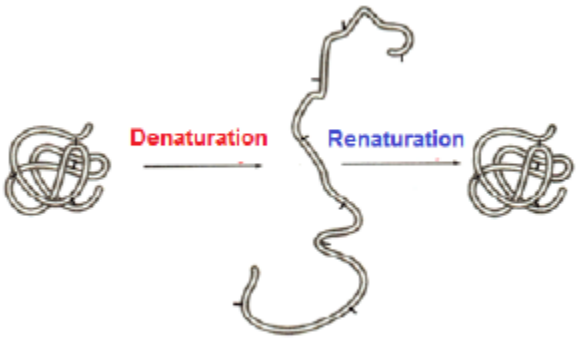
Fig. 4.22 Different interactions stabilizing the native conformation of protein

4.3.3.5. Denaturation of Proteins

Denaturation is the loss of the secondary, tertiary, and quaternary structures of a protein by a chemical or physical agent that leaves the primary structure intact. For example, heat cleaves hydrogen bonds, so boiling a protein solution destroys the a-helical and β-pleated sheet structure. In globular proteins heat causes the unfolding of the polypeptide chains; because of subsequent intermolecular protein–protein interactions, precipitation or coagulation then takes place. That is what happens when we boil an egg.

Destruction of Secondary and Higher Structures

Denaturing Agent	Affected Regions
Heat	H bonds
6 *M* urea	H bonds
Detergents	Hydrophobic regions
Acids, bases	Salt bridges, H bonds
Salts	Salt bridges
Reducing agents	Disulfide bonds
Heavy metals	Disulfide bonds
Alcohol	Hydration layers



Denaturation changes secondary, tertiary, and quaternary structures. It does not affect primary structures (that is, the sequence of amino acids that make up the chain). If these changes occur to a small extent, denaturation can be reversed. For example, when we remove a denatured protein from a urea solution and put it back into water, it often reassumes its secondary and tertiary structures. This process is called reversible denaturation. In living cells, some denaturation caused by heat can be reversed by chaperones. These proteins help a partially heat-denatured protein to regain its native secondary, tertiary, and quaternary structures. Some denaturation, however, is irreversible. We cannot unboil a hard-boiled egg.

Heavy metal ions (for example, Pb2+, Hg2+, and Cd2+) also denature protein by attacking the -SH groups. They form salt bridges, as in –S- Hg2+ -S-. This feature is taken in advance

in the antidote for heavy metal poisoning: raw egg whites and milk. The egg and milk proteins are denatured by the metal ions, forming insoluble precipitates in the stomach. These must be pumped out or removed by inducing vomiting.

Other chemical agents such as alcohol also denature proteins, coagulating them. This process is used in sterilizing the skin before injections. At a concentration of 70%, ethanol penetrates bacteria and kills them by coagulating their proteins, whereas 95% alcohol denatures only surface proteins.

4.3.4 Nucleic acid

Nucleic acids are biopolymers, macromolecules, essential to all known forms of life. **Nucleic acid**, naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines). Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing. They are composed of nucleotides, which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

4.3.4.1 Chemistry of nucleic acids:

Nucleic acids are polynucleotides-that is, long chainlike molecules composed of a series of nearly identical building blocks called nucleotides. Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn attached to a phosphate group. Each nucleic acid contains four of five possible nitrogen-containing bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). A and G are categorized as purines, and C, T, and U are collectively called pyrimidines. All nucleic acids contain the bases A, C, and G; T, however, is found only in DNA, while U is found in RNA. The pentose sugar in DNA (2′-deoxyribose) differs from the sugar in RNA (ribose) by the absence of a hydroxyl group (—OH) on the 2′ carbon of the sugar ring. Without an attached phosphate group, the sugar attached to one of the bases is known as a nucleoside. The phosphate group connects successive sugar residues by bridging the 5′-hydroxyl group

on one sugar to the 3′-hydroxyl group of the next sugar in the chain. These nucleoside linkages are called phosphodiester bonds and are the same in RNA and DNA.

A. Nitrogenous Bases

Nucleotides are comprised of single-ringed or two-ringed nitrogenous bases. One-ringed nitrogenous base is called the pyrimidine and the two-ringed nitrogenous base is called the purine. Basic structure of purine or pyrimidine is shown in (Fig. 4.23). Pyrimidines are six-membered aromatic rings containing two N-atoms. Purines' structure is a combination of pyrimidine ring and a five membered imidazole ring.

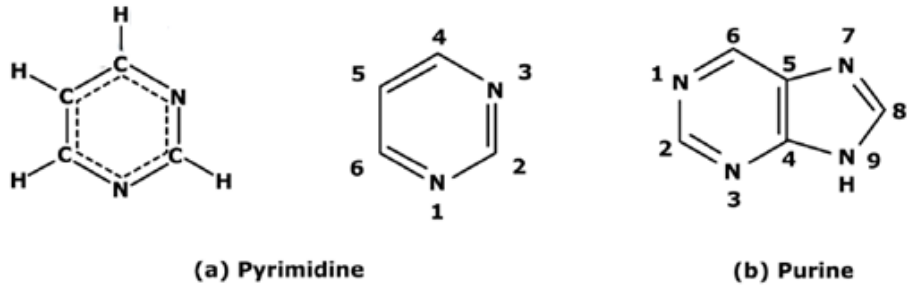


Fig. 4.23 Basic structure of purine or pyrimidine

- The Pyrimidines

  The pyrimidine bases are of three kinds: Cytosine (C), Uracil (U) and Thymine (T). Cytosine and Thymine are found in DNA while Cytosine and Uracil are found in RNA. Their structures are shown in Fig. 4.24.

- The Purines

  Two kinds of purine bases exist in nucleotides: Adenine (A) and Guanine (G). Both are found in DNA as well as RNA.

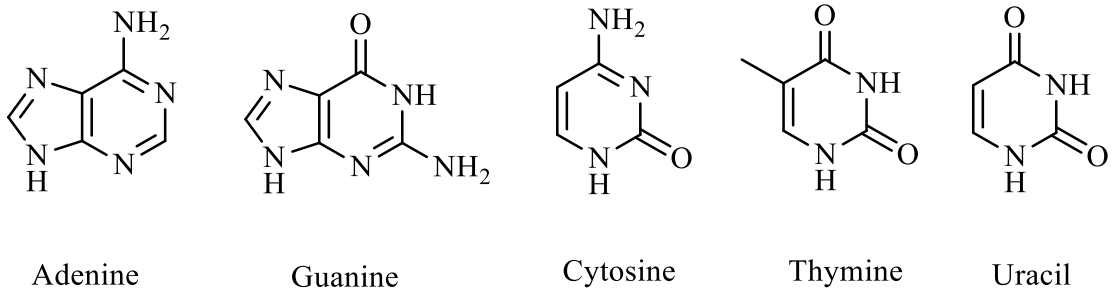


Fig. 4.24 Chemical structure of purine and pyrimidine bases

B. Pentoses

Pentoses are the five-carbon sugars present in polynucleotides. RNA contains D-Ribose while DNA contains 2'-deoxy-D-ribose (Fig. 4.25). The hydroxyl group at the 2'-position is not present in DNA, rather a H atom is present there. We shall see in later sections how this minor difference has far-reaching consequences on their properties.

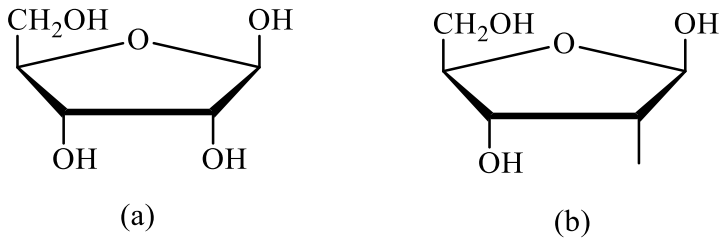


Fig.4.25 The structure of pentoses: (a) D-Ribose and (b) 2'-deoxy D-Ribose

C. Nucleoside

Nucleosides are formed by joining a sugar to a nitrogenous base via a β-N-glycosidic linkage. If the nitrogenous base is a purine, the nitrogenous base is linked to the sugar via its N-9 atom, while if it's a pyrimidine, it is linked via its N-1 atom. Nucleoside with purine as base are suffixed with 'osine'.

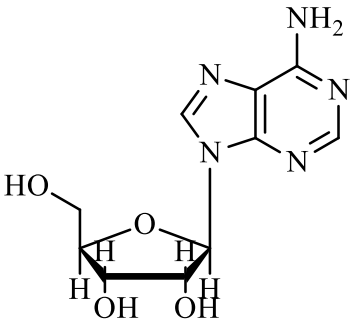
For example, **adenosine and guanosine**. While nucleoside with pyrimidine as bases are suffixed with 'idine'. For example, **cytidine, uridine, thymidine**. If the sugar of the nucleoside is 2-deoxy ribose, the nucleosides are named as deoxyribonucleosides-deoxyadenosine, deoxyguanosine etc. Fig. 4.26 shows the structures of all ribonucleosides and deoxyribonucleosides.

4.3.4.2 Secondary structure of DNA

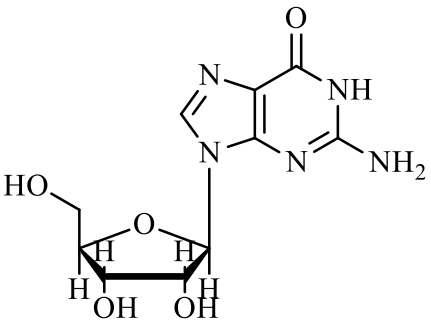
We learnt about the base composition of nucleic acids in module 5. DNA comprises of deoxyribonucleotides containing bases adenine, guanine, cytosine and thymine, while RNA comprises of ribonucleotides with uracil instead of thymine, adenine, cytosine and guanine. RNA usually exists in a single stranded form while DNA exists in double stranded form. In double stranded DNA, adenine always pairs with thymine and guanine always pairs with cytosine. DNA can exists in different forms inside a cell. They are A, B and Z- forms of DNA defined by different humidity conditions.

A. Deoxyribonucleic acid (DNA)

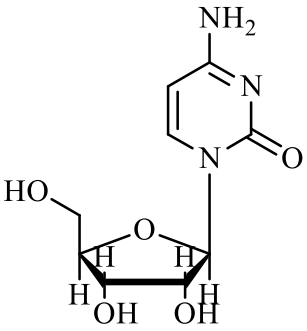
DNA is a polymer of the four nucleotides A, C, G, and T, which are joined through a backbone of alternating phosphate and deoxyribose sugar residues. These nitrogen-containing bases occur in complementary pairs as determined by their ability to form hydrogen bonds between them. A always pairs with T through two hydrogen bonds, and G always pairs with C through three hydrogen bonds. The spans of A:T and G:C hydrogen-bonded pairs are nearly identical, allowing them to bridge the sugar-phosphate chains uniformly. This structure, along with the molecule's chemical stability, makes DNA the ideal genetic material. The bonding between complementary bases also provides a mechanism for the replication of DNA and the transmission of genetic information.



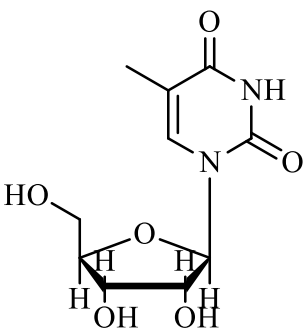
Adenosine



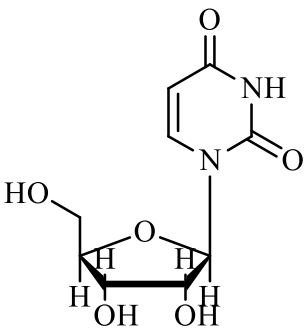
Guanosine



Cytidine



5-Methyluridine



Uridine

Ribonuleosides

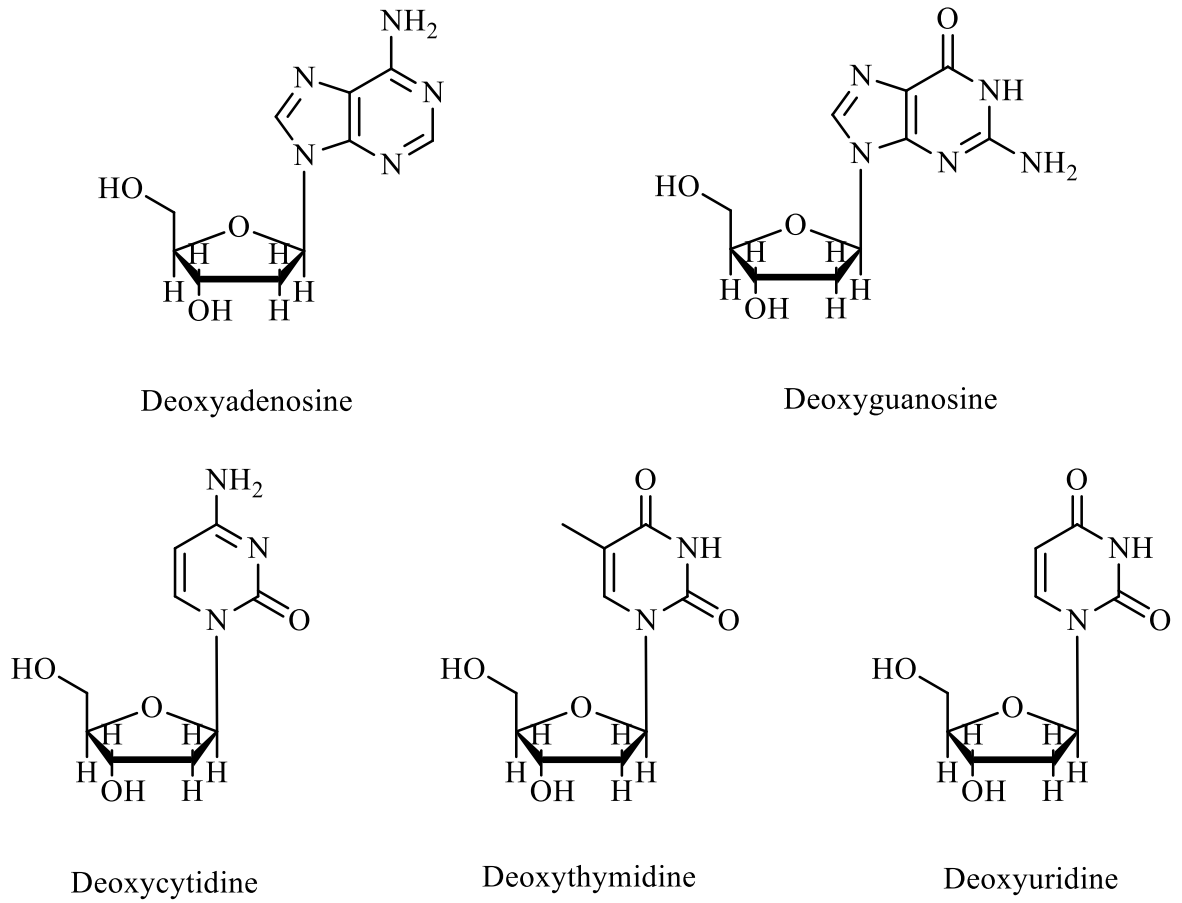


Fig. 4.26 The structure and nomenclature of ribonucleosides and deoxyribonucleosides

In 1953 James D. Watson and Francis H.C. Crick proposed a three-dimensional structure for DNA based on low-resolution X-ray crystallographic data and on Erwin Chargaff's observation that, in naturally occurring DNA, the amount of T equals the amount of A and the amount of G equals the amount of C. Watson and Crick, who shared a Nobel Prize in 1962 for their efforts, postulated that two strands of polynucleotides coil around each other, forming a double helix. The two strands, though identical, run in opposite directions as determined by the orientation of the 5′ to 3′ phosphodiester bond. The sugar-phosphate chains run along the outside of the helix, and the bases lie on the inside, where they are linked to complementary bases on the other strand through hydrogen bonds (Fig. 4.27).

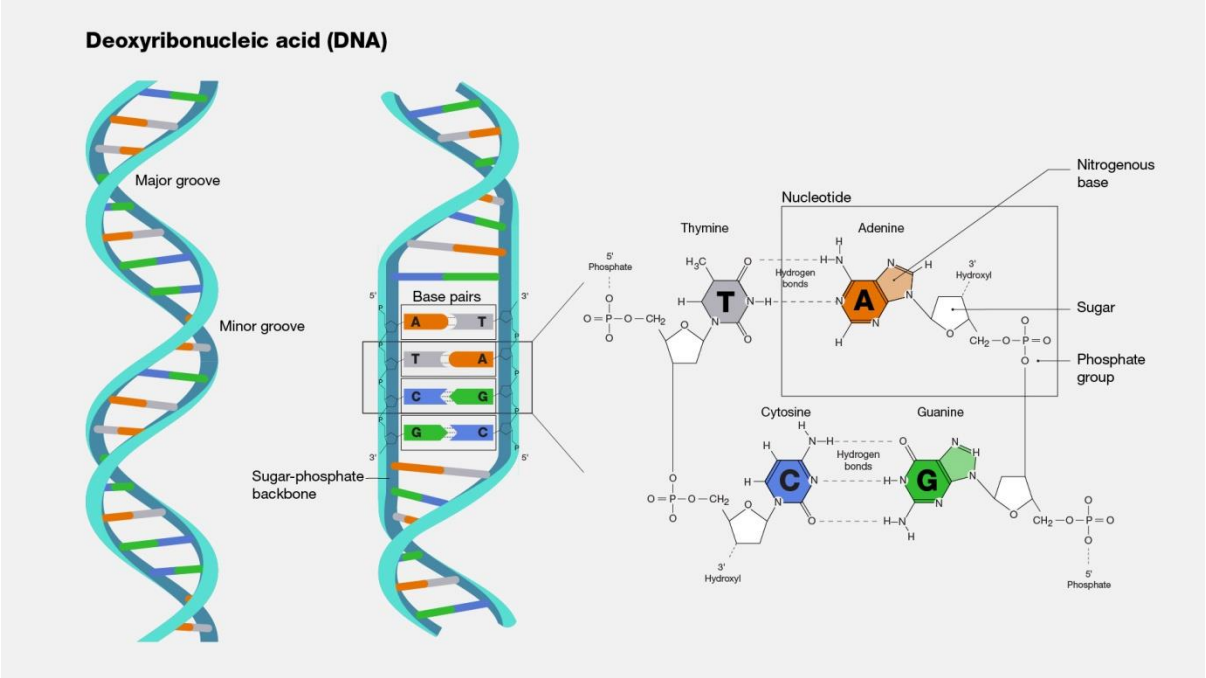


Fig. 4.27 DNA structure

B. Ribonucleic acid (RNA)

RNA is a single-stranded nucleic acid polymer of the four nucleotides A, C, G, and U joined through a backbone of alternating phosphate and ribose sugar residues (Fig. 4.28). It is the first intermediate in converting the information from DNA into proteins essential for the working of a cell. Some RNAs also serve direct roles in cellular metabolism. RNA is made by copying the base sequence of a section of double-stranded DNA, called a gene, into a piece of single-stranded nucleic acid.

Whereas DNA provides the genetic information for the cell and is inherently quite stable, RNA has many roles and is much more reactive chemically. RNA is sensitive to oxidizing agents such as periodate that lead to opening of the 3′-terminal ribose ring. The 2′-hydroxyl group on the ribose ring is a major cause of instability in RNA, because the presence of alkali leads to rapid cleavage of the phosphodiester bond linking ribose and phosphate groups. In general, this instability is not a significant problem for the cell, because RNA is constantly being synthesized and degraded.

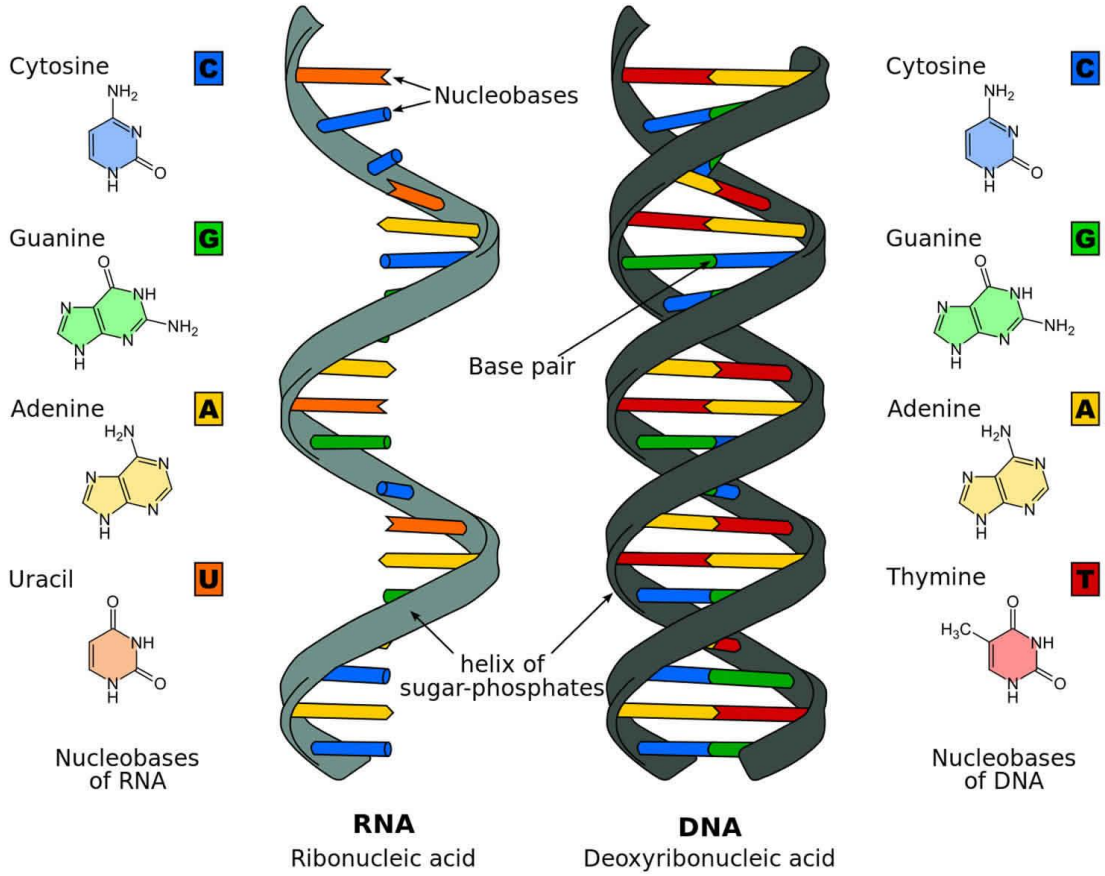


Fig. 4.28 Camporaive structure of DNA and RNA

In a cell, RNA exists in three predominant forms:

(a) messenger RNA (mRNA)

As the name suggests, this RNA is the messenger that carries the information of the DNA to protein synthesis machinery. Protein synthesis machinery translates this information into sequence of polypeptides.

(b) transfer RNA (tRNA)

It is smallest RNA in the cell (~76 amino acids). tRNA serves as a carrier of amino acids (monomers for protein synthesis). Though it is also single stranded but it forms characteristic secondary structure by virtue of intrastrand hydrogen bonding.

(c) Ribosomal RNA (rRNA)

rRNA forms the part of ribosomes. Ribosomes, the protein synthesis machinery, comprises one-third proteins and two-third rRNA. rRNA is the most abundant RNA in the cell. They

participate in the formation of peptide bonds (bonds that link any two amino acids in a protein chain), hence these are called Ribozymes or RNA enzymes.

4.3.4.3 Chemical properties of DNA and RNA

Two major differences between DNA and RNA are:

(i) Presence of Uracil in RNA versus Thymine in DNA.

(ii) Presence of 2'-deoxy ribose in DNA while RNA contains ribose.

These chemical properties render stability of DNA making it more stable storage form of genetic information. 2'-OH of ribose in RNA makes it less stable than DNA. The vicinal hydroxyl group next to 3'-OH (i.e. 2'-OH) is absent in DNA rendering it more resistant to hydrolysis by alkali. This vicinal hydroxyl group in RNA makes the phosphodiester bond susceptible to nucleophilic cleavage.

Why does DNA contain thymine instead of uracil?

Cytosine deaminates to uracil in vivo. Cytosine base pairs with G, while U base pairs with A. Thus this deamination leads to a mutation leading to conversion of CG base pair to UA base pair. Thus U in DNA is a outcome of domination and is sensed as mutation by the repair. If U would have been naturally found in the DNA, the repair system of the cell would have been unable to sense U formed from deamination of C.

4.4 PROXIMITY EFFECT

An enzyme has to form an enzyme-substrate complex with its substrate in order to catalyse a chemical reaction (Fig. 4.29). The enzyme stabilizes the reaction's transition state, making it easier for the bound substrate to form the transition state and convert to product. The resulting enzyme-product complex then dissociates, releasing free product and regenerating the free enzyme, which can carry out subsequent rounds of catalysis. The reactive chemical groups may be aligned and brought together in the ideal orientation and spatial connection for a reaction to occur through proximity in enzyme-substrate interactions. The enzymatic reaction acts kinetically like an intramolecular process once the substrates have been fixed in this manner. According to certain theories, molecules are most reactive when their orbitals are arranged in a way that reduces the electronic energy of the transition state.

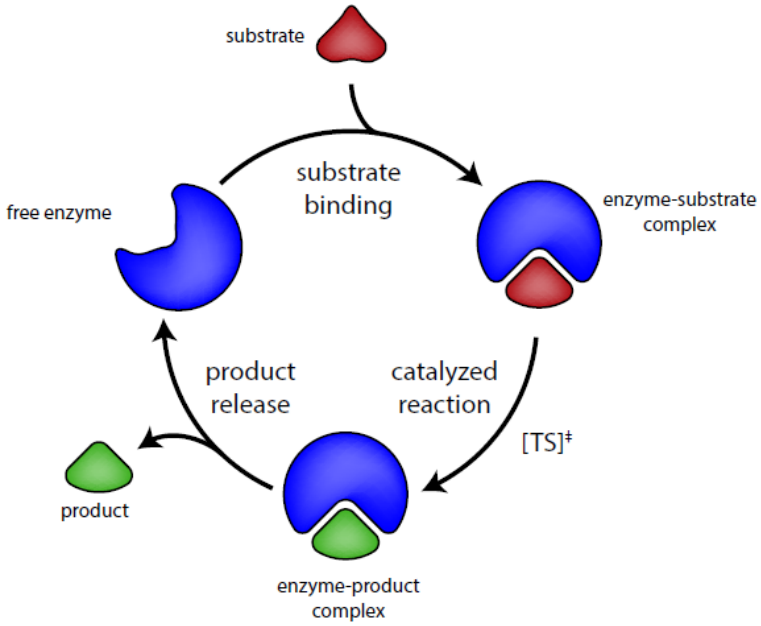


Fig.4.29 Enzymes bind to their substrates to catalyze chemical reactions

The **proximity effect**, describe the orientation and movement of the substrate molecules within the active site of the enzyme such that the reactants are much closer together than they would be in solution. Enhancing the proximity of reactants increases their collision frequency, thus causing the reaction to proceed at a faster rate. This effect of proximity and orientation is analogous to an effective increase in concentration of the reagents and endows the reaction an intramolecular character with a massive rate increase. Intramolecular reactions between groups that are tied together in a single molecule are faster than the corresponding intermolecular reactions between two independent molecules.

As an example of the proximity effect in catalysis, consider the rates of the two hypothetical reactions shown in (Fig.4.30). The reaction at the top relies on the random collision between the two substrates to bring A and B close enough to react. In contrast, it is much more likely for A and B to encounter each other in the reaction at the bottom when they are already tethered together.

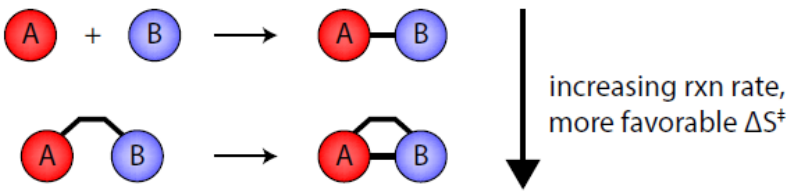


Fig.4.30 The proximity effect increase collision frequency

Proximity effect lower the entropic barrier to forming the transition state ($\Delta S^{\ddagger}$) because they pre-organize the substrates so that they lose less entropy during the formation of the transition state than the free substrates would. We can quantify the proximity effect using the effective concentration of the reactants in the reactions. The effective concentration is defined as the ratio of the rate constant for the intramolecular reaction (with units of s^{-1}) divided by the rate constant for the intermolecular reaction (with units of $s^{-1}M^{-1}$). Effective concentration has units of molarity (M) and is a measure of the concentration of a reactant you would have to have in an intermolecular reaction to achieve the same rate as that in the intramolecular reaction with a substrate concentration of 1M. In the example shown in Figure 4.31, the rate constant for the intermolecular reaction (top) is $k_{inter} = 4 \times 10^{-6}$ $s^{-1}M^{-1}$; in contrast, the rate constant of the intramolecular reaction (bottom) is $k_{intra} = 0.8$ s^{-1}. The effective concentration of A and B in the bottom case is k_{intra}/k_{inter}, or 200,000 M. In other words, A and B when tethered in close proximity and in the proper orientation by an enzyme react at the same rate as that of the untethered reaction when A or B is an impossibly high 200,000 M.

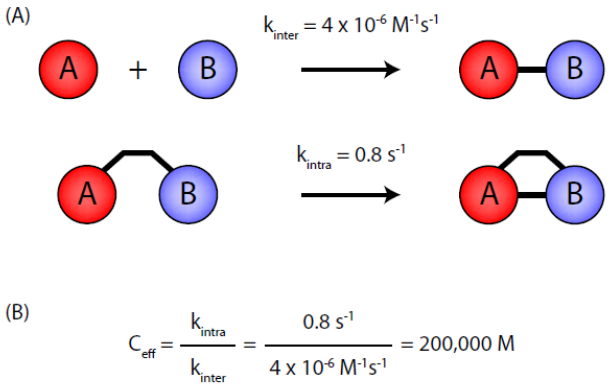
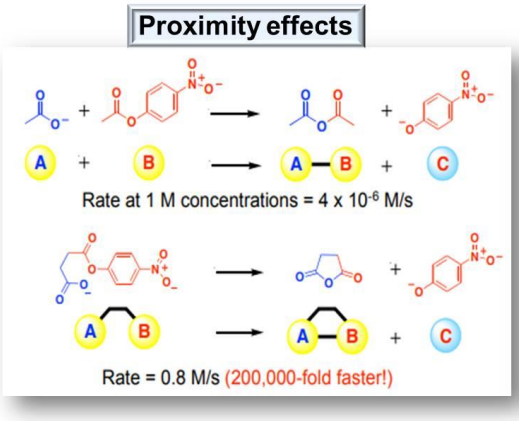


Fig.4.31 Effective concentration measures the rate enhancement of an intramolecular reaction relative to a corresponding intermolecular reaction



4.5 MOLECULAR ADAPTATION

The shape of the molecule determines whether like a bioactive molecule it can also be recognized by a receptor and thus will display the same biological activity. Compounds with similar structures may compete for the same biological target. This information can be highly useful in designing new drugs via molecular modification. Examples are presented to explain these facts.

1. All living systems use nucleic acids (DNA and RNA) to store genetic information. Thus, any compound which interferes with the synthesis of these vital materials is toxic to all life forms. These toxic compounds are called antimetabolites and 5-fluorouracil (5-Fu) which interferes with the synthesis of DNA is one antimetobolite. This derivative of the natural base uracil inhibits the enzyme which converts uracil to thymine via methylation. Notice that thymine and uracil differ only by a methyl group: thymine is 5-methyluracil (Fig. 4.32) one knows that fluorine and hydrogen are almost similar in size. Thus one can say that 5-fluorouracil being similar in shape and size with uracil is adapted by the enzyme and subsequently inhibits it, this synthesis of dTMP is inhibited and this in turn inhibts DNA synthesis.

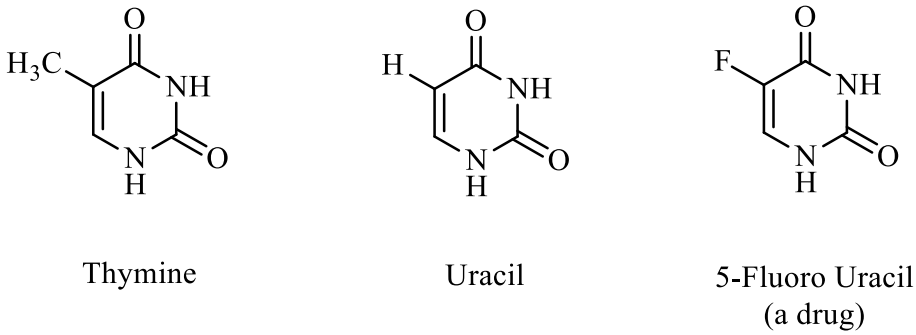


Fig. 4.32 Chemical structure of natural base and its derivatives

2. A common antibiotic used against bacterial infections is 5-fluorocytosine which is an analog of natural base cytosine (Fig. 4.33). A new approach is to disguise the drug chemically so that it can enter the bacterial cell and destroy it. This way the drug will not harm the tissues of the patient. In the modern approach, to the amino group of the drug e.g 5-fluorocytosine a small peptide is attached containing D-amino acids (thus common human enzymes cannot bring about its hydrolysis). This peptide containing drug thus can sneak into the bacterial cell, where it is metabolized to liberate the drug.

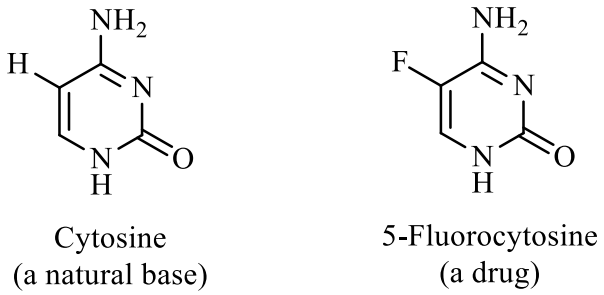


Fig. 4.33 Chemical structure of natural base and its derivatives

4.6 SUMMARY

- Carbohydrates are poly hydroxy aldehydes and ketones.
- When sugar cyclizes and anomeric carbon is created from aldehyde group of an aldose or a keto group of ketose. This carbon has two configuration α or β, if the oxygen on anomeric is not attached to any other structure.
- Nucleotides are the monomers that make the polymer DNA or RNA.
- Nucleotides are comprised of a nitrogenous base, a pentose sugar and phosphoric acid.
- Nitrogenous bases can be two ringed purines or single ringed pyrimidines.
- DNA is double stranded with interstrand hydrogen bonding between adenine and thymine pair and guanine and cytosine pair that keeps the two strands together.
- Protein structure comprises of four levels: Primary Structure, Secondary structure, tertiary structure and quaternary structure.
- α-Helix and β-sheets are two predominant secondary structures.
- Tertiary structure is the spatial arrangement of atoms in space while different polypeptides join to form the quaternary structure of a protein.

4.7 BIOBLIOGRAPHY

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- e-Pathshala, Bioorganic and Biophysical chemistry, Paper No. : 16; Module No.: 5, Nucleotides and polynucleotides
- NPTEL - Chemistry - Bio-Organic Chemistry, Joint initiative of IITs and IISc - Funded by MHRD,
- e-Pathshala , Bio-organic and Bio-physical Chemistry, Paper No. : 16; Module No.: 2: Constituents of Cells; Lipids
- e-Pathshala, Bio-organic and Bio-physical Chemistry, Paper No. : 16; Module No.: 6 : Structure of DNA
- e-Pathshala, Bio-organic and Bio-physical Chemistry, Paper No. : 16; Module No.:.3: Sugars and polysaccharides
- https://projects.iq.harvard.edu/files/lifesciences1abookv1/files/7_dna_structure_chemistry_revised_9-24-2018.pdf

4.8 SUGGESTED READINGS

- Nelson and Cox, Lehninger, Principles of Biochemistry, Fourth Edition
- Bioorganic Chemistry; H. Dugas, Springer Verlag, 1999.
- Tyagi V., Tyagi S., (2002), Bioorganic chemistry, Krishna prakashan media meerut, india,1-302

4.9 TERMINAL QUESTIONS

1. Discuss the different types of protien structure.
2. Write a note on the structure of DNA.
3. Differentiate between anomer and epimer.
4. Write a note on the mutarotation.
5. Wwrite the chemical structure of pyrimidine and purine base.
6. Write a note on the peptide bond and Ramachandran plot.

UNIT 5: ENZYME & MECHANISM OF ENZYME ACTION

CONTENTS:

5.1 INTRODUCTION

"Enzymes can be defined as biological polymers that catalyze biochemical reactions."

Perhaps oldest known to bio-chemical as well as bio-organic phenomenon is fermentation of juices to alcoholic beverages. Also, this was Ist chemical transformation catalysed by enzymes contained within living Yeast cells. It was discovered in 18th century that fermentation leads to conversion of Sugars into carbon dioxide and alcohol. The 19thcentury witnessed both the identification of fermentation as physiological act of yeast cells and introduction of forces review Pasteur's view that life and fermentation are inseparable.By now, extraction of enzymes from bio-cells was known and this Discovery like most scientific discoveries was accidental. In 1897, E. Buchner required a quantity of purified protein for therapeutic purpose. He grounded yeast and sand, filtered the broken cells, and add added a large amount of sugar to the filtrate as preservative. Enzymes are biochemical catalyst (biocatalysts) which are needed in almost all the biochemical reaction in a living system. Enzymes are proteins in nature, which can be defined as biocatalysts synthesized by living cell. Kuhne used the word enzyme, which is made up of two words Greek-en: in and zyme: yeast.Berzelius coined the term catalysis, which means to dissolve (Greek: to dissolve), in 1836, and was capable of catalysing the fermentation reaction.

5.2 OBJECTIVE

After studying this unit, you shall be able to know

- What is Enzyme? How it behaves as catalyst for biological reactions?
- Biological catalyst (Enzyme) is similar to chemical reactions wherein the rate of reaction is proportional to the concentration of reacting substrates.
- The rate of an enzyme catalysed reaction or its activity can be measured by a variety of sensitive assays.
- Classification of catalysts.
- Role of enzyme in biological reaction
- Extraction and purification of enzyme
- Different modules for biological reaction mechanism.

- Role of inhibitor in catalytic reaction.We shall be Understand about Irreversible Inhibition and Reversible Inhibition.
- Mechanism of different biological catalysts.

1.3 CHEMICAL AND BIOLOGICAL CATALYSIS-TRANSITION STATE THEORY

A catalyst age an agent which enhance the rate of the chemical reaction and it itself is not consumed. In the case of biological system, a catalyst e.g. activation energy provides a more favourable pathway for an organic reaction and acts to lowering of the activation energy and to stabilize the transition state. The difference in energy between the transition state and starting materials is termed **activation energy**. The activation energy determines the rate of reaction rather than the change in energy between the starting materials and the product.

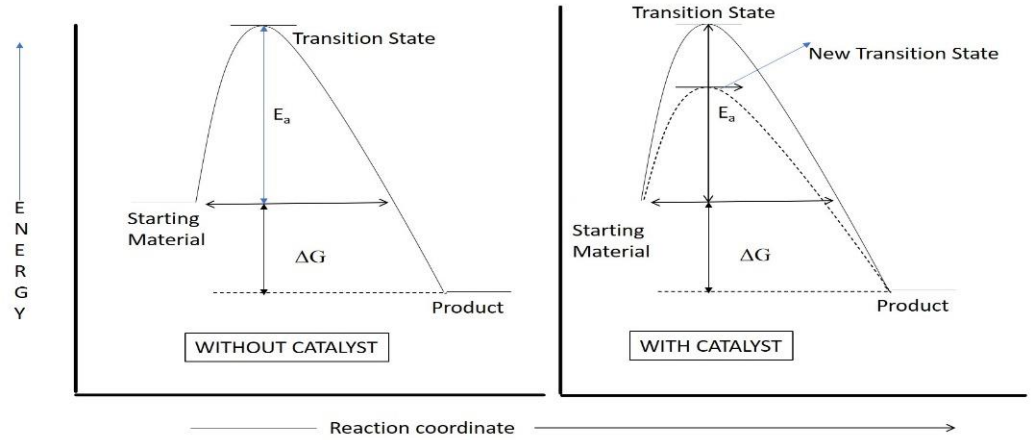


Fig 5.1 Lowering of activation energy in the presence of the catalyst

5.3.1 How catalysts lower energy of activation-transition state theory

Consider the hydrogenation of 1, 2-dimethylecyclohexene to cis-1,2-dimethylecyclohexane. This catalyst brings about this transformation and several factors are in operation:

- Catalysts deliver a reaction surface or environment
- Catalysts passage reactants together.
- Catalysts help to position reactants correctly so that transition state configurations, are attained easily.
- It stabilizes the transition state.

- Catalysts weaken bonds.
- Catalysts may contribute in the mechanism

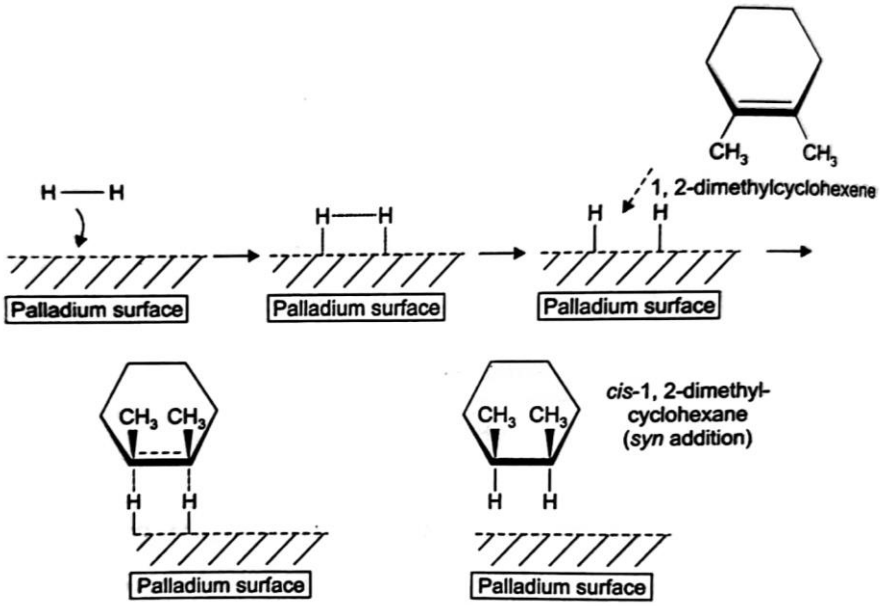


Fig. 5.2 Catalytic hydrogenation: *syn*addition with palladium catalyst

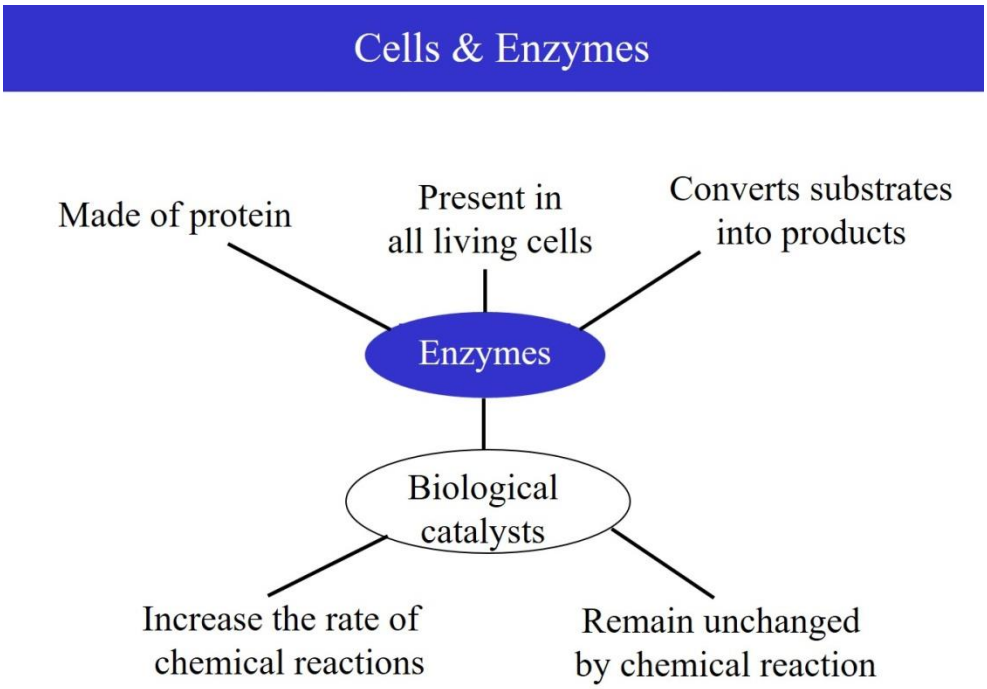


Fig. 5.3 Cells and enzymes

These factors are seen in the hydrogenation process of the alkene. We know that the metal surface (Pd) provide electron to hydrogen gas and form metal-hydrogen bond. Therefore, hydrogen gas dissociated into atoms. When H_2 gas interact with metal surface hydrogen molecule bonds are weaken.

5.3.2 Active site of an enzyme- catalytic power of enzymes

We know that enzymes display their remarkable feat of catalysis by presenting their three-dimensional environment (made out of L-amino acids) to the substrates. The active sites of an enzyme *i.e.*, the functional portion of an enzyme occupies only a very small portion of the enzyme molecule. The active sides site of enzyme may be shown in an overall fashion by either of the representations. When a substrate binds to an enzyme, the enzyme changes the substrate's structure, causing it to move toward the transition state. An enzyme's active site usually has a form that closely resembles the transition state rather than the substrate.For this region transition state analogues are potent inhibitors.

- In the tertiary structure of protein enzyme (folding of enzyme), the active sites (amino acid residues) are relatively nearer, whereas in the primary structure the active sites are far from each other. These amino acid residues are sown by the letters A, B, and C.
- Catalysis takes place in the active site of every enzyme molecule. The binding site is located within the active site, and it is here that the amino acid residues (R-groups) bind the substrate in the correct location for reaction. In an intramolecular reaction, this is analogous to the appropriate location of the reactive group. Hydrogen bonding, electrostatic interactions, and other factors all play a role in this binding. These favourable interactions with amino acid residues in the active site stabilise the transition state, lowering the active activation energy of the process and increasing the rate of reaction.
- There is also a pocket (P) in the active site which *e.g.*, in the case of carboxypeptidase can accept the side chain of terminal amino acid when this enzyme splits the terminal amino acid from a peptide chain.
- Cofactors are non-protein molecules that many enzymes require for the reaction to take place. These cofactors are either can metal ion, small organic molecule called

coenzyme (NAD^+, $NADP^+$). Most of the coenzyme are bound by ionic bond or other non-covalent bonding interactions however, others are bound covalently and are termed prosthetic groups.In carboxypeptidase A zinc ion is essential for enzymatic activity. Zinc Ion located in a groove near the surface of the enzyme and held in position via coordinate to two histidine side chains, a glutamate side chain, and water molecule.

- After the substrate is properly bound in the active site of the enzyme, the R group of other amino acids brings about the catalytic activity.

5.4 REMARKABLE PROPERTIES OF ENZYMES AS CATALYST

- Enzymes are highly effective catalysts and enhance the reaction rate by a factor of 10^5-10^{17}.
- Then substrate is tightly bound in the active site to form an ES complex.
- Like other catalysts enzyme lowering the activation energy for a reaction and enhances the reaction rate. The enzyme has no effect on the reaction's equilibrium.
- The energy used for enzymatic rate enhancement is largely derived from weak interaction like hydrogen bonding, ionic interactions and hydrophobic interactions. The active sites provide these interactions and these stabilize the transition state.
- The general acid bas4e catalysis, covalent catalysis and metal ions catalysis help to provide a lower energy path.
- The binding energy helps to lower the entropy of the substrate and can bring about a conformational change in the enzyme to bring induced fit.
- Binding energy also justify the enzyme behaves as a catalyst.

5.5 CLASSIFICATION AND NOMENCLATURE

Some enzymes were given names at random, such as trypsin (hydrolases protein) and pepsins (hydrolyse peptides), but the majority of enzymes are given names by adding the suffix 'ase' to either the function they perform or 'substrate' on which they work.For example, transferase, isomerase, hydrase, dehydrogenase, oxidase belongs to the first group

and esterase, protease, urease, and amylase belong to second group. Thus, there are two ways to classify and name the enzyme:

(A) Based upon the function they perform

(B) Based on substrate they act upon

(I) Classification into hydrolytic and oxidative enzymes:

Enzymes were earlier classified into two groups:

(a) Hydrolytic enzymes

(b) Oxidative enzymes

(a) Hydrolytic enzyme

They catalyse hydrolysis.

Sub-class of this class are:

(1) Proteases

(2) Carbohydrases

(3) Esterases

(4) Amidases

(1) Proteases

These are proteinases and peptidases. Some enzymes of sub-class are:

(i) Trypsin: this enzyme occurs in pancreas, hydrolyses, proteins and polypeptides into amino acid.

(ii) Pepsin: This occurs in cells and hydrolyses synthetic peptides into proteins and peptones.

(iii) Rennin: Rennin is found in gastric juice. It is responsible for the clotting of milk and clots ten-million times of its weight o0f milk and converts casein of milk to paracasein.

(iv) Papain: Papain is found in *Carica papaya.* It is strong proteolytic action and milk clotting power.

(2) Carbohydrases: These enzymes act upon carbohydrates. Some of them are following:

(i) Maltase: It is occurring in yeast and hydrolyses maltose into glucose.

(ii) Invertase: It is also found in yeast and hydrolyses sucrose into a mixture of glucose and fructose.

(iii) **Amylase:**Amylase found in animals and plants, particularly in saliva and pancreatic juices. It hydrolyses amylum (starch) into maltose.

(iv) Lactase: Lactase is found in yeast and gastric juices of young animals and hydrolyse lactose into a mixture of glucose and galactose.

(v) Inulase: This enzyme found in invertebrates and hydrolyse insulin into fructose.

(3) Esterase: it hydrolyses esters. Their examples are following:

(i) **Lipases:** This enzyme found in pants and pancreas. These hydrolyses fats to fatty acids and glycerol.

(ii) Phosphatases: This enzyme occurs in animal and plant tissues, it hydrolyses phosphate ester.

(4) Amidases: These enzymes act on amides.

(i) **Ureases:** It is found in jack beans, liver and few other seeds. It hydrolyses urea into other ammonia and carbon dioxide.

(ii) **Arginase:** It is found in liver cells and hydrolyses arginine into urea and ornithine.

(b) Oxidative enzyme

These enzymes bring about oxidation and reduction in biosystems.

(1) Dehydrogenase

(i) Alcohol dehydrogenase: It is found in the East and oxidizes alcohol to study height acetaldehyde.

(ii) Glutamic dehydrogenase: It is the source of beef liver. It converts glutamic acid to Alpha ketoglutaric acid.

(2) Oxidases:

(i) **Catalase:** It is also found in beef extract and oxidises hydrogen peroxide to oxygen.

(ii) **Ascorbic acid oxidase:** Plants are its sources and it oxidizes ascorbic acid to dehydroascorbic acid.

(II) INTERNATIONAL UNION OF BIOCHEMISTS (I.U.B.) CLASSIFICATION

According to the International Union of Biochemists (IUB), enzymes are classified into six functional classes based on the sort of reaction they catalyse. The six categories of enzymes are hydrolases, oxidoreductases, lyases, transferases, ligases, and isomerases.

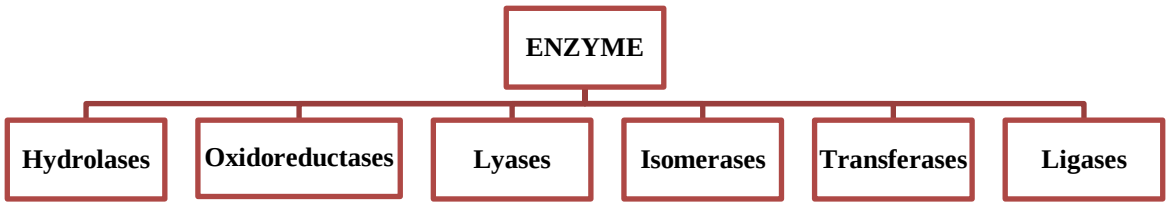


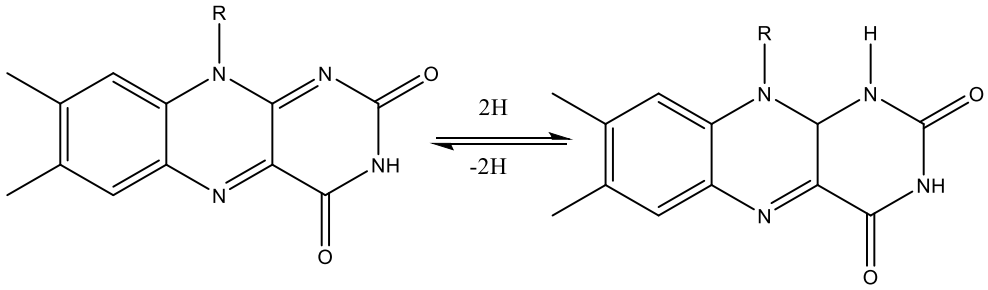
Table 5.1: Classification of enzymes and their biochemical property.

S.N.	Types	Biochemical property
1	**Hydrolases**	Hydrolases are hydrolytic enzymes that catalyse the hydrolysis reaction by cleaving and hydrolyzing bonds in the presence of water.
2	**Oxidoreductases**	The enzyme Oxidoreductase catalyses the oxidation reaction in which electrons tend to move from one form of a molecule to the other.
3	**Lyases**	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
4	**Transferases**	Transferases enzymes aid in the translocation of functional groups between acceptors and donors.
5	**Ligases**	The Ligases enzymes are known to charge the catalysis of a ligation process.
6	**Isomerases**	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.

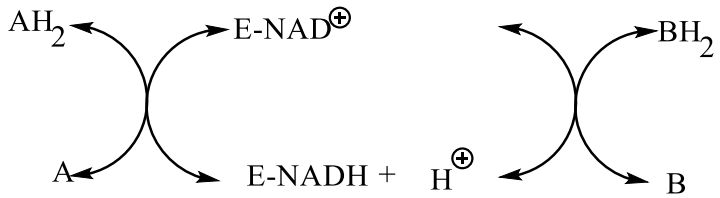
(1) Hydrolases: These enzymes are hydrolyse C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds.

(2) Oxidoreductases: These enzymes are catalyze oxidation and reduction reactions, e.g. catalysing the oxidation of pyruvate to acetyl coenzyme-A, pyruvate dehydrogenase. The substrate that is oxidized is regarded as hydrogen donor. The systematic name is based on *donor:acceptor oxidoreductase*. The common name will be *dehydrogenase*, wherever this is possible; as an alternative, *reductase* can be used. *Oxidase* is only used in cases where O_2 is the acceptor.

(i) Oxidases: It passed hydrogen directly to oxygen. In oxidases flavin moiety of coenzyme FAD acts as hydrogen carrier:



(ii) **Dehydrogenases:** It catalyse removal of hydrogen from one substrate and transfer it to other.



Here A and B stand for substrate and E for an enzyme.

Different apoenzymes have different apoenzyme but same co-enzymes.

For instance, **Nicotinamide adenine dinucleotide (NAD^+)or Nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Nicotinamide acts as hydrogen carrier or oxidising agent.

(3) Lysases: It is catalysed addition of group to double bond or elimination of groups to create double bond without oxidation, reduction or hydrolysis.

(4) Transferases: The Enzyme which catalyse to the transfer of a group of atoms, such as amine, carboxyl, carbonyl, methyl, acyl, glycosyl, and phosphoryl from a donor substrate to an acceptor compound.

5.6 EXTRACTION AND PURIFICATION OF ENZYMES

(A) Extraction

In the extraction and purification of enzymes, material availability is critical. A single enzyme's concentration can change between tissues. It is crucial to choose a tissue with a high enzyme content. As a result, yeast, bacteria, and fungi provide certain advantages as source materials. The benefit is that these cells can be cultivated in a favourable environment. However, there is one disadvantage: obtaining large quantities of microbial cells other than yeast is difficult.

After selecting the starting material, a number of techniques can be used to extract and isolate the enzyme. The following are some specific examples of various methods:

1. Sedimentation: Many mitochondrial & other particle cell bodies remain constant when liver tissue is homogenised using the Potter-Elvenhjem apparatus rather than usual blending devices. They easily sediment out of solution, taking with them a slew of enzymes. Only in the early stages of separation is physical separation by e sedimentation useful.

2. Extraction: Previously, enzymes were divided into two categories: soluble or lyoenzyme, and bound or desmoenzyme. Desmonzymes are likely enzymes for which suitable techniques have yet to be developed, hence this is a bad classification.

Because of its fat-free nature, acetone-powder (from which enzymes can be extracted using buffer) is often the easiest substance from which to extract enzymes. The initial stage, in any case, is a fine-grinding. Autolysis, lysozyme digestion, grinding, freezing and thawing, sonic disintegration, shaking with solvents, shaking with fine-glass beads, and ultimately, explosion by quick release of pressure is some of the methods for eliminating enzymes from microorganisms.

3. Salt Fractionation: In enzyme fractionation, ammonium sulphate is the most helpful salt. Its benefits include high water solubility (760 g/lt) and a nearly neutral response (pH 5 to 6) in concentrated solution. Dixon devised a monogram (chart) for preparing ammonium sulphate solutions, while Kunits devised an equation for estimating the amount of

ammonium sulphate to add to a solution in order to get the appropriate final concentration. One downside of using ammonium sulphate in a somewhat alkaline solution is that 50 percent of the ammonium ions are transformed to ammonia, even at pH 9.3. A buffer should be used to keep the pH of the ammonium sulphate solution under control. However, sodium sulphate has been widely employed to crystallise glutamic dehydrogenase from cow liver.

4. Solvent Fractionation: The isolation of enzymes is aided by water-soluble solvents such as acetone, ethanol, methanol, and dioxane.

When extracting acetone, start below 0 ^{o}C and work your way up to higher temperatures. Fractionation at the maximum temperature will not result in significant yield loss. Because acetone absorbs strongly in the ultraviolet area, it must be entirely eliminated by dialysis or distillation at a low pressure before spectral measurement.

The use of ethanol in enzyme isolation is becoming more common. It was used to extract crystalline lactic dehydrogenase from the liver of rats.

5. Solvent-Metal ion fractionation: Combining metal ions and solvents, notably Zn^{2+} and ethanol, is an important approach for separating blood proteins. Protein zinc salts are often more soluble than sodium and potassium salts and separate out of solutions more quickly. Metal ions can be extracted from these by using citrate, ethylene diamine tetraacetate, or ion-exchange resin.

6. Adsorption: Protein adsorbents have been made from a number of materials, with hydrated aluminum oxide being one of the first. The use of a calcium phosphate gel and bentonite in the isolation of lysozyme has also proven to be beneficial.

7. Adsorption chromatography: For the separation of proteins and thus enzymes, column chromatography on adsorbents is particularly successful. A calcium phosphate gel was developed by Anger. Swingle & Tiselius tested the same adsorbent for generic protein chromatography. Zechmeister has written a review on the topic of enzyme chromatography in general. Using a biochemically specific adsorbent, another method isolates enzymes based on their catalytic specialisation rather than their overall features as proteins. Various adsorbents containing p-azophenol and similar groups were produced from aromatic ethers of cellulose, for example, in the isolation of mushroom tyrosinase.

7. **Ion-exchange chromatography:** The isolation of small molecular weight molecules like amino acids has proven to be a huge success for this approach.

Cytochrome C was isolated in 1950 using an amberite IRC-50 column and the game method, which was useful in separating cytochrome from Ustilago. In a similar way, ribonuclease and lysozyme have been purified. Ion-exchange chromatography is essentially an electrophoretic separation in which the resin serves as one electrode and gravity as the other. It may yet prove to be a useful technique as the chemical industry continues to provide novel resins to enzymologists.

9. **Complex Formation:** Protamine was used as a complexing agent in this method to a lower level. To get rid of unwanted proteins, basic lead acetate has been used.

(B) Purification of Enzymes

There are various methods for isolating enzymes from tissues. Acetone powders of tissues or cells are made by blending the tissue (1 vol) with acetone (5-10 vol) at 0^{o} C in one of the techniques. To remove room moisture and lipids, the smooth slurry is filtered and rinsed with acetone many times. It is then dried after being rinsed with ether. A powdery residue is left behind, which could be a mixture of enzymes. The powdered substance is separated, and the different fractions are evaluated for catalytic activity in vitro. The fraction with the required activity is chosen for fractional crystallisation, resulting in the pure state of the desired enzyme. Chemical or physical fractionation processes are used in the purifying process.

The goal is to keep the majority of the targeted enzyme while freeing it from other proteins, nucleic acids, and other contaminants. To remove denatured protein, heat guide the cell free extract to 500C for 5 minutes. This can be accomplished by precipitating ammonium sulphate and then using ion exchange chromatographic methods, gel filtering, and so on.

Final purification of enzymes, whether in the laboratory or in commercial operations, is a time-consuming process that depends mainly on chromatographic technologies. Gel permeation chromatography & affinity chromatography hold very high promise for simplifying the purification of enzymes. The latter method entails connecting a crude enzyme preparation with a solid support to which a reversible inhibitor or other compounds that will selectively and reversibly bind to the enzyme of interest are attached.The support-

inhibitor-enzyme complex is isolated from the initial crude feed after the enzymes have been attached to immobilised inhibitors, and the purified enzyme is eluted from the support-inhibitor part. Because of the lower cost of enzyme purification as well as the bigger quantity and variety of enzymes, methods such as gel filtering and affinity chromatography, as well as other chromatographic methods, are appropriate. However, there is more work to be done in terms of scaling up these technologies. Anaphylactic or precipitation reactions are also effective in purification. The antigen-antibody reaction is carried out in a gel, such as agar, in this approach. The chemicals are evident in the gel as a precipitated zone.

5.7 ENZYME MECHANISM

5.7.1 Fisher's lock & key hypothesis

Enzymes are highly specific; therefore, the reasonable question is what is their mechanism of action. According to Arrhenius, enzymes catalyse the reaction through the formation of unstable intermediate. Simplest model to explain enzymatic action is Lock & Key model proposed by Fischer. This model assumes that enzyme is rigid three-dimensional body, the surface of which has active-sites which have slots for fitting definite substrates just as a key fit in a particular lock (Fig 5.4).

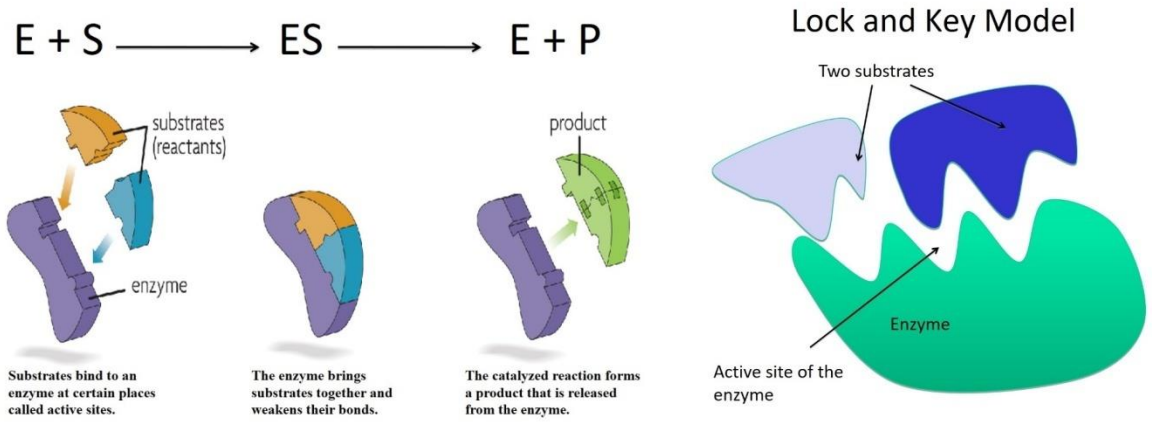


Fig 5.4 Key lock model reaction

An enzyme molecule is very large (consisting of 100 to 200 amino acid residues); but active-sites, which combine with substrate have definite shape in which substrate can fix, are comparatively small (with few amino acid residues). Amino acids of active sites are located at different places in the chain, whereas, other amino acids, which are not part of

active-sites, are located in a definite sequence. This is because of the fact that this sequence allows the whole enzyme molecule to fold in exactly required manner.

An hypothetical example of mechanism of enzyme-action is given in above fig 5.4. This is referred to as a "Lock and Key Mechanism."

An enzyme-substrate complex is formed when the enzyme reacts chemically with the substrate (Michaelis-Mention hypothesis).

$$E + S \longrightarrow ES$$

The enzyme-substrate complex then breaks down to give the products of reaction. The enzyme is released & can be used over and over again.

$$ES \longrightarrow E + \text{Product}$$

The Lock and Key model explain the action of many enzymes. But of other enzymes, there is evidence that this model is too restrictive. Enzyme molecules are in dynamic state, not in static one. There are constant motions within them, so that the active site has some flexibility.

5.7.2 Koshland's induced fit hypothesis

This model was given by Koshland the Fischer model (1966). In the Fishcher model, i.e., Lock and Key model, the active-site is presumed to be preshaped to fit the substrate. In the induce -fit theory, the substrate induces a conformational change in the enzyme. This aligns amino acid residue or the other groups on the enzyme in the correct spatial orientation for substance binding & catalysis both. At the same time, the other amino acid residues may get buried in the interior of the enzyme. This is shown in the following Fig 5.5.

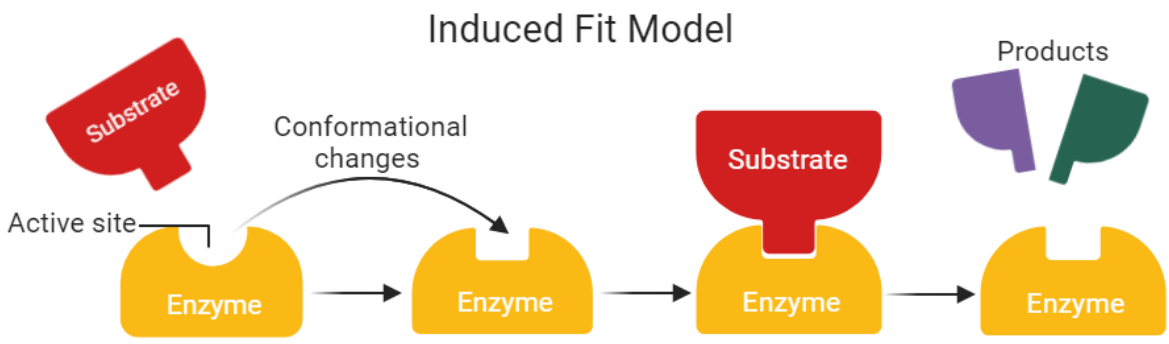


Fig. 5.5 Representation of an induced fit by conformational enzymes

In fig 5.5 in the absence of substrate, the catalytic and the substrate-binding groups are several bond distances removed from one another. When the substrate approaches there occur a conformational change in the enzyme protein, aligning the groups correctly for binding and for catalysis. At the same time there also occurs a change in the spatial orientation of the other regions.

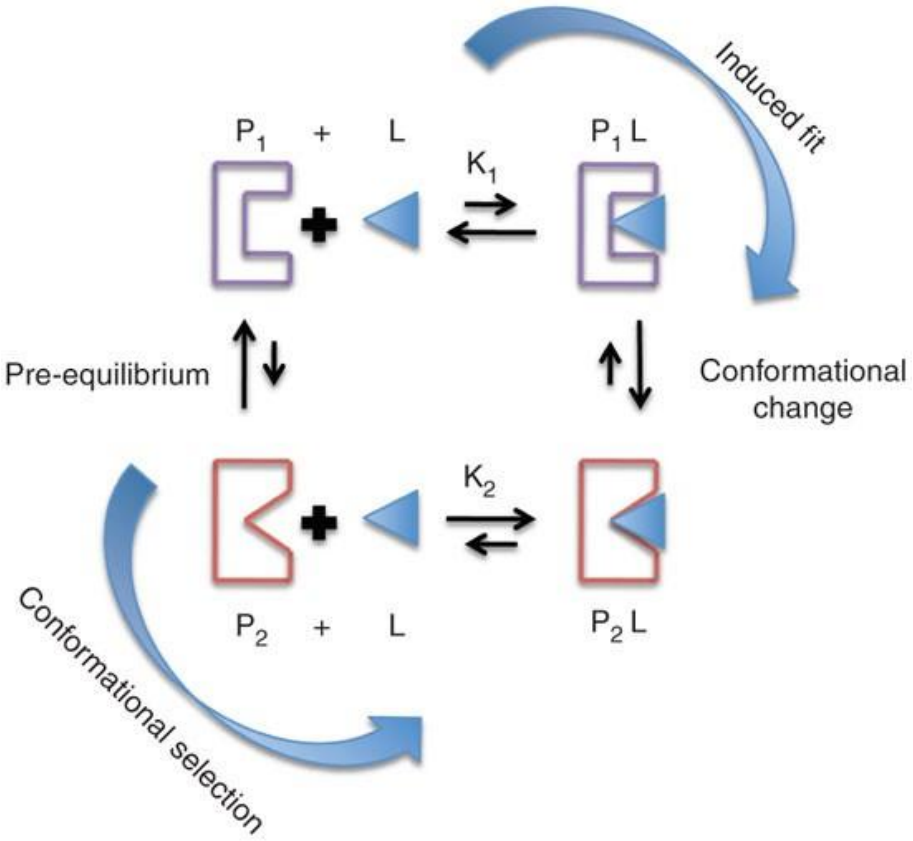


Fig. 5.6 Hypothetical representation of alternate pathways of substrate induced conformational changes.

The main evidence in favour of induced fit model comes from demonstration of conformational changes during substrate binding & catalysis with creatine kinase, phosphoglucomutase, and several other enzymes.Upto this time, the exact sequence of events in a substrate induced conformational change has not been established. There may be several possibilities as shown in Fig. 5.6. Evenif one knows the complete primary structure of enzyme, it is not very easy to decide exactly which residues exactlyconstitute

5.8 CONCEPT AND IDENTIFICATION OF ACTIVE (ENZYME INHIBITION-REVERSIBLE AND IRREVERSIBLE)

The substrate is attached to a specific cavity or site in an enzyme. The cleft has an active core, which is where the amino acids are clustered together to allow them to mix with substrate. The reactive amino acids may be separated by a long distance in the polypeptide chain. The chain, on the other hand, folds in such a way that the reactive amino acids are brought together in the active site. When the substrate molecule connects to the active site, it is thought that the Parts are held together in such a way that chemical bonds are distorted, i.e. the bonds are weakened. The reactivity of the substrate or chemical bonds is increased as a result of this distortion, which speeds up the reaction rate.The strain model of catalysis describes how the reaction products are liberated because they are less tightly bound. Inhibitors are chemicals that slow down the rate of a process catalysed by an enzyme. The following is a diagram of inhibition:

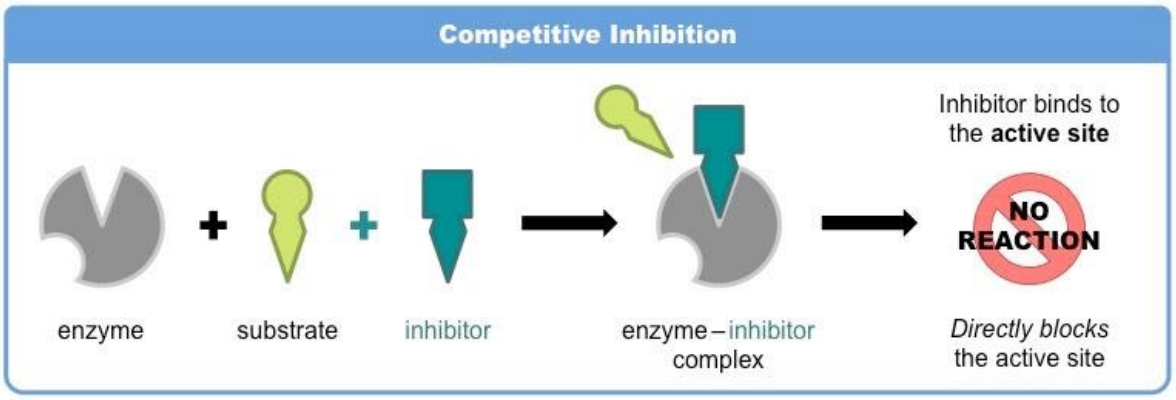


Fig. 5.7 Inhibition

Enzyme inhibitors are molecular agents, which interferes catalysis, to show or eliminate enzymatic activity. These compounds have to the ability to combine with certain enzyme but do not serve as substrate: instead these after or even block enzymatic catalysis. Poison act upon living bodies by inhibiting enzymes. For instance, Carbon monoxide poisoning by combining with haemoglobin thus making it useless fort performing its usual role as carrier of' oxygen. Cyanide poisoning due to its combination with natural substances particularly with metallic centre of Cytochrome. Poisonous effect of arsenate is due to its blocking of enzyme sites in place of phosphates. A wide range of **naturally occurring** and **synthetic**

compounds has the ability to bind **reversible** or **irreversible** to specific enzymes. These substances fall into two categories:

A) Irreversible Inhibition

B) Reversible Inhibition.

(A) Irreversible Inhibition: These substances are bind tightly to the active of the enzyme molecule alternating them, so that they permanently lose their catalytic properties. These inhibitors bind with or destroy the functional groups of the enzyme molecules that are necessary for their catalytic activities. For example, the compound diisopropylfluorophosphate (DFP) is irreversible inhibitor, which inhibits the enzyme acetylcholinesterase, important in the transmission of nerve impulses. Diisopropylfluorophosphate is a very reactive and combines with the hydroxyl group of an essential serine residue at the active site of the enzyme to form a catalytically inactive derivative. Once this derivative formed, the enzyme inhibitor is iodoacetamide, which can react with sulfhydryl (-SH) groups of essential cysteine residues or with the imidazole group of essential histidine residues.

(B) Reversible Inhibition

These substances bind less tightly to enzymes, and their inhibiting effect can be reversed. These are of three main types:

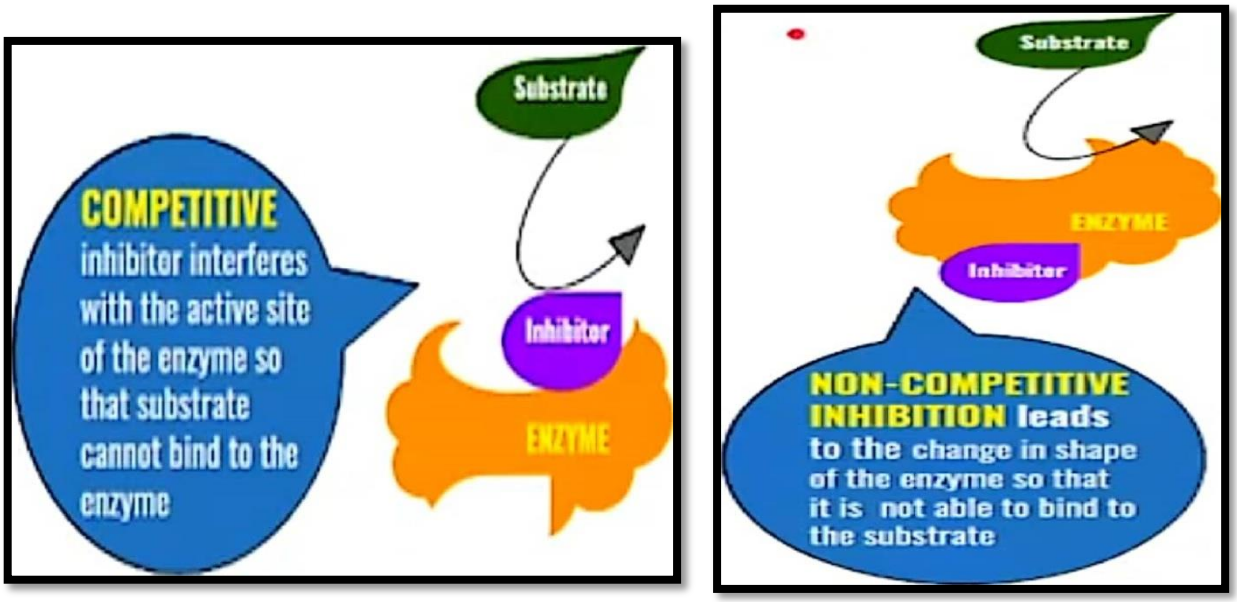


Fig. 5.8 Competitive and Non-competitive inhibitors

(i) Competitive inhibitors

(ii) Uncompetitive inhibitors

(iii) Non-competitive inhibitors

Competitive inhibition: These types of inhibitors compete with the substrate for binding to the active site of the enzyme, but once bound, cannot be transformed by the enzyme. Their effect can be reversed or relieved simply by increasing the substrate concentration.

These inhibitors generally resemble the normal substrate in three-dimensional structure. As a result, the ability of the substrate to bind to the enzyme is reduced. Because of resemblance, the competitive inhibitor tricks the enzyme into binding to the active site.

However, the inhibitor molecules can not be attacked by the enzyme molecules and since their active site is occupied.

However, if substrate concentration is increased, the inhibitor molecules are displaced from the enzyme and enzyme becomes functional. *Therefore, a higher concentration than normal is needed to achieve the same rate of reaction.* These types of inhibitors compete with the substrate for binding to the active site of enzyme, but once bound, cannot be transformed by the enzyme. Their effect can be reversed or relieved simply by increasing the substrate concentration.

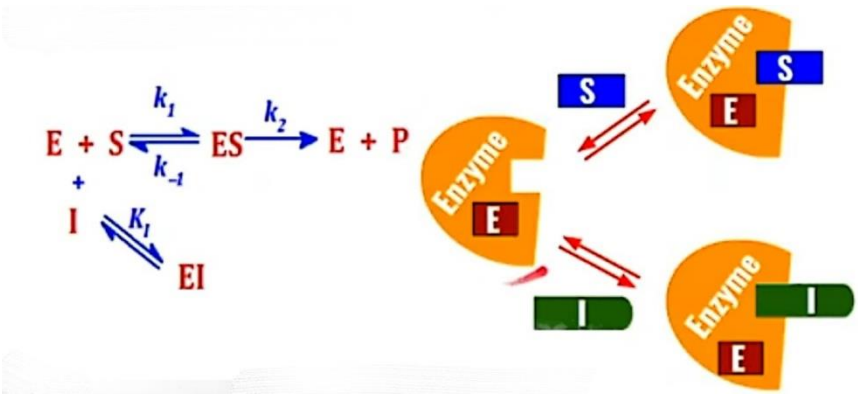
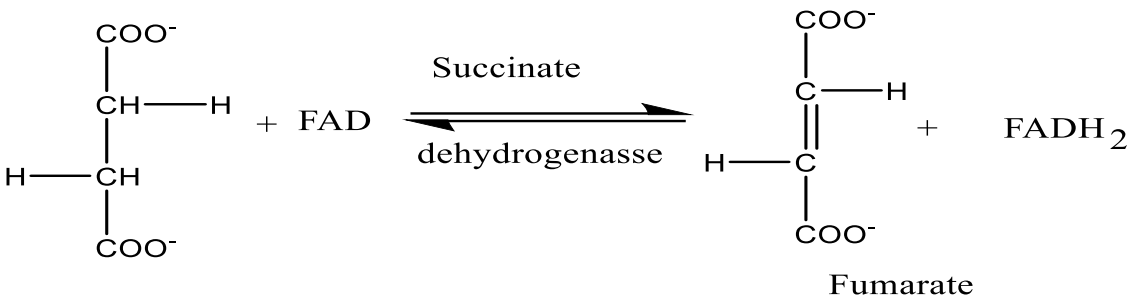
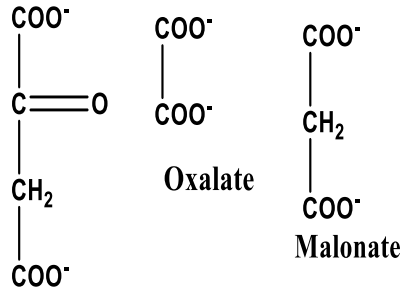


Fig 5.9 Competitive inhibition transformation

The typical example of competitive inhibition is the inhibition of succinate dehydrogenase by the malonate anion. Succinate dehydrogenase is involved in citric acid cycle, which catalyses the removal of two hydrogen atoms from succinate one from each of the two methylene groups.



Succinate ion



Oxaloacetate

Malonate resembles succinate in having two ionised carboxyl groups at pH7.0, but differs in having only three carbon atoms. However, malonate is not dehydrogenated by succinate dehydrogenase; it simply combines with the active site and prevents it from acting on its

Another common example of this type of inhibition is the inhibition of ribulose biphosphate carboxylase by oxygen molecule. Carbon dioxide (CO_2) is the normal substrate for ribulose 'biphosphate carboxylase, which is important enzyme during photosynthesis. This enzyme is competitively inhibited by O_2 and even relatively low O_2 concentrations reduce the rate at which CO_2 is incorporated into sugars. Competitive inhibitors are not always structure analogues of substrates.

Competitive inhibitors change K_m but not V_{max} of enzyme-catalysed reactions because number of active sites remains unaltered. However, larger the concentration of substrate is required for the maximum utilization of active sites, this is why K_m is increased.

Uncompetitive inhibition: These types of enzyme inhibitors are not very specific and they bind at a site on the enzyme other than the active site. This binding alters the conformation of the enzyme molecule so that reversible inactivation of the active site occurs. These inhibitors bind reversibly to both the free enzyme and the enzyme substrate complex to form the inactive complexes.

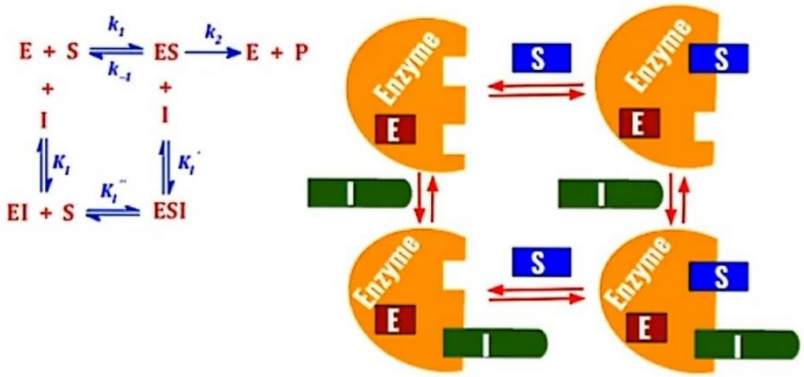


Fig 5.10 Uncompetitive inhibition reaction

The uncompetitive inhibitors are the compounds, which reversibly combine with only enzyme substrate complex but not with the free enzyme, and this type of inhibition is not overcome by high concentration of the substrate. The increase of substrate concentration increases the degree of inhibition, instead of releasing the inhibition. Uncompetitive inhibitor binds with already formed enzyme-substrate complex and has equal effects both on K_m, and V_{max}. This type of inhibition is rare in one-substrate reaction, but causes a type of product-inhibition in reactions with multiple substrates and products.

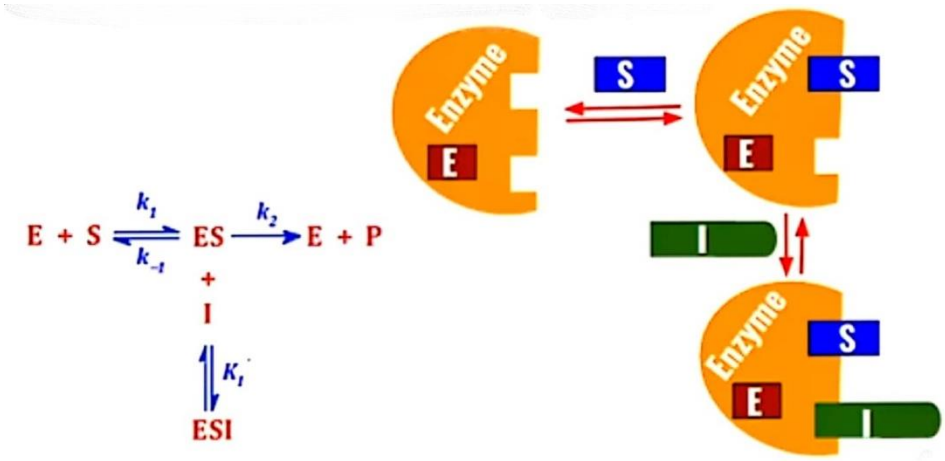


Fig 5.11 Un-competitive inhibition

Non-competitive inhibition: These inhibitors are reversible type which is not bind to the active sites of an enzyme. These are not competed with the substrate for the active site and do not prevent S from binding to the enzyme. Non-competitive inhibitors bind to the enzyme and cause a conformational change in it. The enzyme thus becomes inactive. The inhibitors reduce V_{max} for the reaction, but do not change K_m.

5.9 KINETICS OF ENZYME ACTION

Enzyme kinetics can be studied in two parts:

(A) Energy of activation

(B) Steady state enzyme kinetics

(A) Energy of activation: Enzymes catalyze the rate of reaction via an alternate pathway using low energy of activation, enabling reactions to take place under extreme conditions at suitable temperatures. The energy required to bring the reactants to a transition state in which new bonds are partially created and old bonds are partially broken. This is the state with the highest energy during the reaction, therefore it is very unstable and breaks down to generate lower energy products. Only a small fraction of the reactant molecules have enough energy to react, i.e. the threshold energy.As the temperature rises, molecules gain energy equal to the threshold energy. However, in living systems, reactions take happen in a nearly isothermal environment with very little temperature change. By activating the reactant molecules, infect enzymes aid in the development of enzyme-substrate complexes. The enzyme-substrate combination reaches high energy and creates strained bonds in the reactants, causing them to react quickly. Furthermore, the reactant molecules in the enzyme-substrate complex are arranged in such a way that their reaction becomes a certainty rather than a matter of chance, which is why the reaction rate is thousands of times faster.

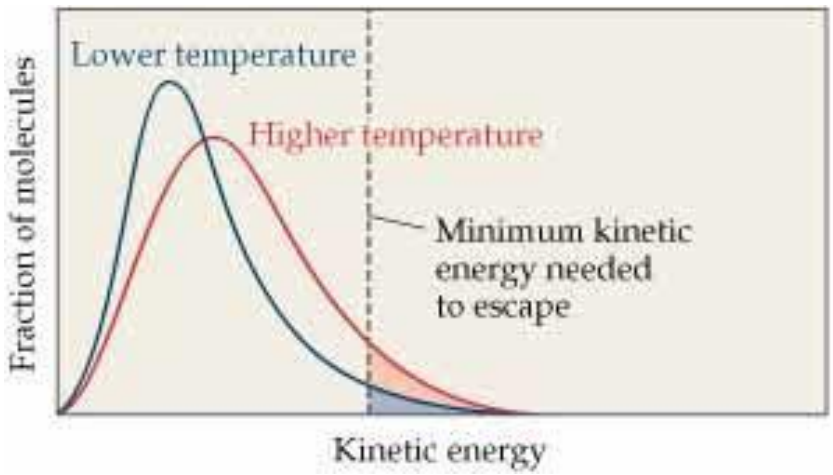


Fig. 5.12 Effect of temperature increase on fraction with threshold energy.

Alternatively, the energy of activation to reach the transition state (T.S.) is reduced in the presence of enzyme (Fig 5.12). However, given typical parameters of temperature and

pressure (ΔG^O) of reaction, the total free energy change remains constant.The enzyme catalyses both forward and reverse equilibrium reactions to the same amount. Because (ΔG^O) has not changed, the equilibrium constant has not changed either, but equilibrium is rapidly attained. The proportions of reactant and product in catalysed and uncatalyzed reactions are the same.

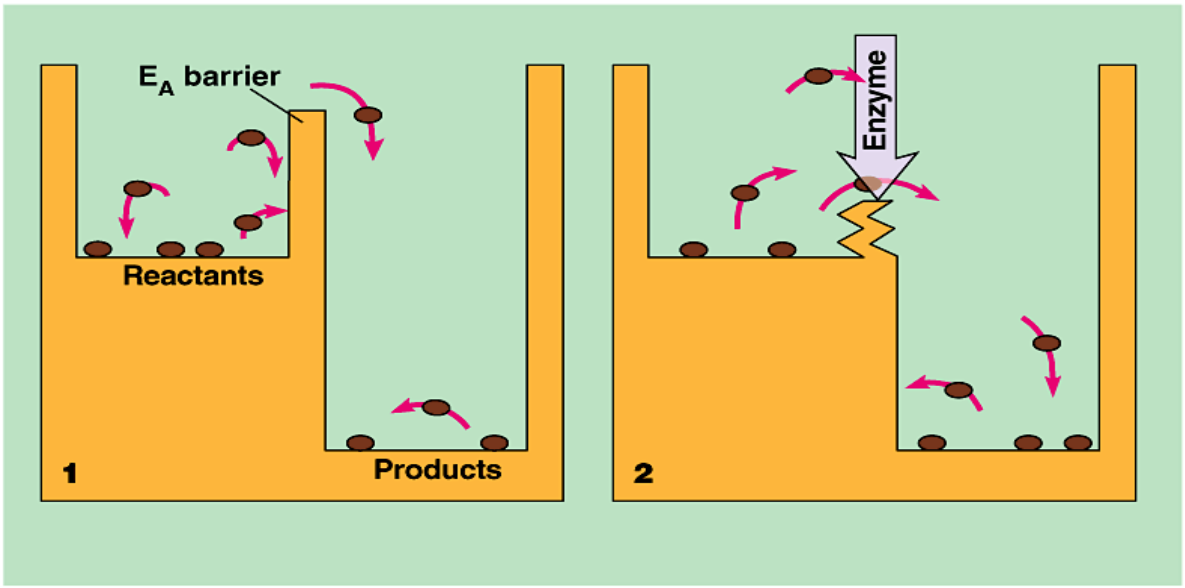


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Fig 5.13 Lowering of activation energy

(B) Steady state enzyme kinetics: This theory, proposed by Michaelis and Menten in 1913, is based upon following assumption:

(i) Enzyme-substrate complex (ES) is in equilibrium and substrate

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \quad ; \quad E=\text{Enzyme},\ S=\text{Substrate}$$

(ii) Product-formation is possible only through enzyme-substrate complex:

$$ES \xrightarrow[K_2]{\text{slow}} E + P \quad [P=\text{Product}]$$

Based on those assumptions Michaelis-Menten equation can givenbelow:

Let us consider formation of enzyme substrate complex ES.

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \quad ; \quad E=\text{Enzyme},\ S=\text{Substrate}$$

$$\frac{d[\mathrm{ES}]}{dt} = K_1[E][S] \text{.....(1)}$$

It is clear from assumptions (i) and (ii) that clear equilibrium is not in the fast process, as Enzyme-substrate complex [ESJ is constantly removed in the slow process. Concentration of enzyme is very concentration of substrates. Therefore, [E]<< [S]. Hence, [ES]<<[S]. the rate of reaction is given as:

$$r = \frac{d[\mathrm{S}]}{dt} = \frac{+d[\mathrm{P}]}{dt} = K_2[\mathrm{ES}] \text{.....(2)}$$

Using steady state approximation for the formation of ES:

$$\frac{d[\mathrm{ES}]}{dt} = K_1[E][S] - \mathrm{K}_{-1}[\mathrm{ES}] - \mathrm{K}_2[\mathrm{ES}] = 0 \text{.....(3)}$$

Concentration of free enzymes [E] is not measurable in living process. But total enzyme concentration $[\mathrm{E}]_0$ is measurable and can be given by Equation (4).

$$[\mathrm{E}]_0 = [\mathrm{E}] + [\mathrm{ES}] \text{.....(4)}$$

Here [ES] is bound enzyme concentration, therefore [E] can be given as

$$[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{ES}] \text{.....(5)}$$

Putting this value in Eq. (3), we get

$$\frac{d[\mathrm{ES}]}{dt} = K_1\{[\mathrm{E}]_0 - [\mathrm{ES}]\}[S] - \mathrm{K}_{-1}[\mathrm{ES}] - \mathrm{K}_2[\mathrm{ES}] = 0 \text{.....(6)}$$

Upon simplification of above equation & grouping the constants

$$K_1[\mathrm{E}]_0[S] = \{\mathrm{K}_{-1} + \mathrm{K}_2 + \mathrm{K}_1[\mathrm{S}]\}[\mathrm{ES}] \text{.....}(7)$$

$$[\mathrm{ES}] = \frac{K_1[\mathrm{E}]_0[S]}{\mathrm{K}_{-1} + \mathrm{K}_2 + \mathrm{K}_1[\mathrm{S}]} \text{.....(8)}$$

Upon puttting this value of [ES] in Eq. (2)

$$r = \frac{K_1 K_2[E]_0[S]}{K_{-1} + K_2 + K_1[S]} \text{.....(9)}$$

$$r = \frac{K_2[E]_0[S]}{\frac{(K_{-1} + K_2)}{K_1} + [S]} = \frac{K_2[E]_0[S]}{K_m + [S]} \text{.....(10)}$$

[upon dividing both numerator and denominator pf eq.(9) by K_1]

This equation correlates thocomponontg of cnzyrnc reaction, [S]&[E], to initial & maximum velocity through rate Constant (K_m):

$$K_m = \frac{(K_1 + K_2)}{K_1}$$

This equation correlates the components of enzyme reaction, [S] &[E], initial and maximum velocity through rate constant K_m:

$$K_m = \frac{Rate\ of\ breakdown\ of\ ES}{Rate\ of\ formation\ ES}$$

(C) **Michaelis-Menten & Lineweaver-Burk Plots**

V or ($V_{max.}$) representsmaximumvelocity of enzyme reaction, whereas, **Michaelis constant *(K_m)*** is the substrate concentration at which enzyme demonstrates 50% of its maximum velocity. Michaelis-Mentenequation shows the relation:

$$r = \frac{K_2[E]_0[S]}{K_m + [S]} \text{.....(11)}$$

This equation can be further simplified. When all the enzymes have reacted with substrate the reaction shows maximum velocity (Vmax). As at that stage no free-enzyme is left $[E]_0 = [ES]$. Therefore, Equation (2) becomes

$$r_{max} = V_{max} = K_2[E]_0 \text{.....(12)}$$

Hence, **Michaelis-Menten** equation (ll) can be written as:

$$r = \frac{V_{max}[S]}{K_m + [S]} \text{.....(13)}$$

Now, here are two cases:

(i) If Km >> (S). Then [Sl can be neglected from the denominator. Then

$$r = \frac{V_{max}[S]}{K_m} = K'[S] \qquad .....(14)$$

This is a first-order reaction,

(ii) If [S] Km. Then, K_m can be neglected from denominator;

$$r = V_{max}[S] = Constant \qquad .....(15)$$

This reaction rate follows zero-order kinetics.

If $K_m = [S]$; $r = \frac{1}{2}V_{max}$

As already stated, Michaelis Constant is equal to concentration of S_at which rate of formation of product is half of maximum rate. $K_2$of equation (12) is known as **turnover-number** of enzyme. It is the number of molecules converted in unit-time by one molecule of enzyme. It's value is in between 100-1000 per second. However, sometimes it may be as large as 10^5 to 10^6 per second.

Upon increase in concentration of substrate these active sites get occupied and cause rate enhancement.However, at high substrate concentration reaction rates become constant as all the active-sites remain occupied all the time.Michaelis -Menten plot for the kinetics of enzyme catalysed reaction is given in fig 5.14.

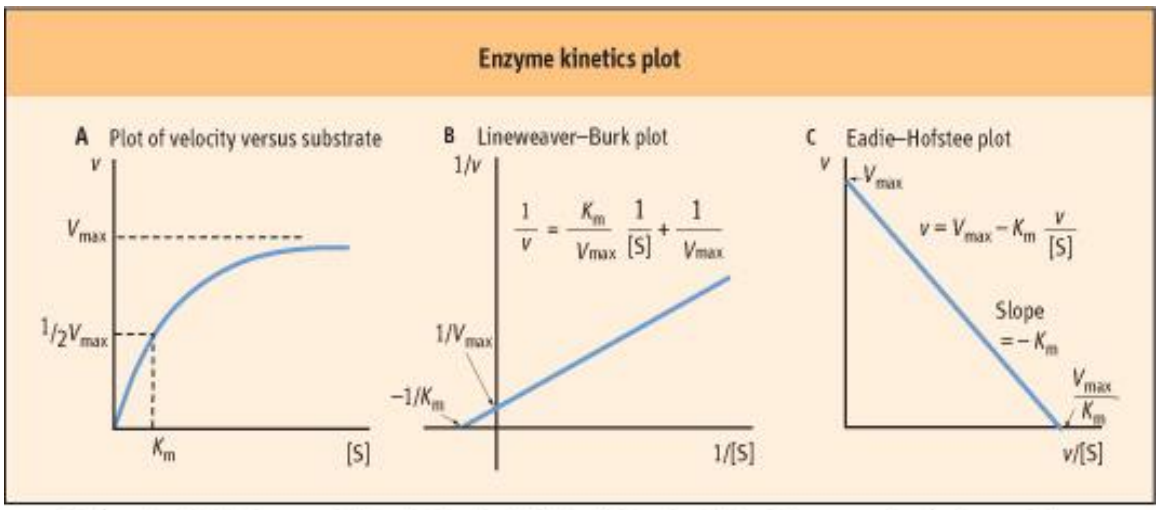


Fig. 5.14 Plot of kinetics of Enzyme-catalysed reaction

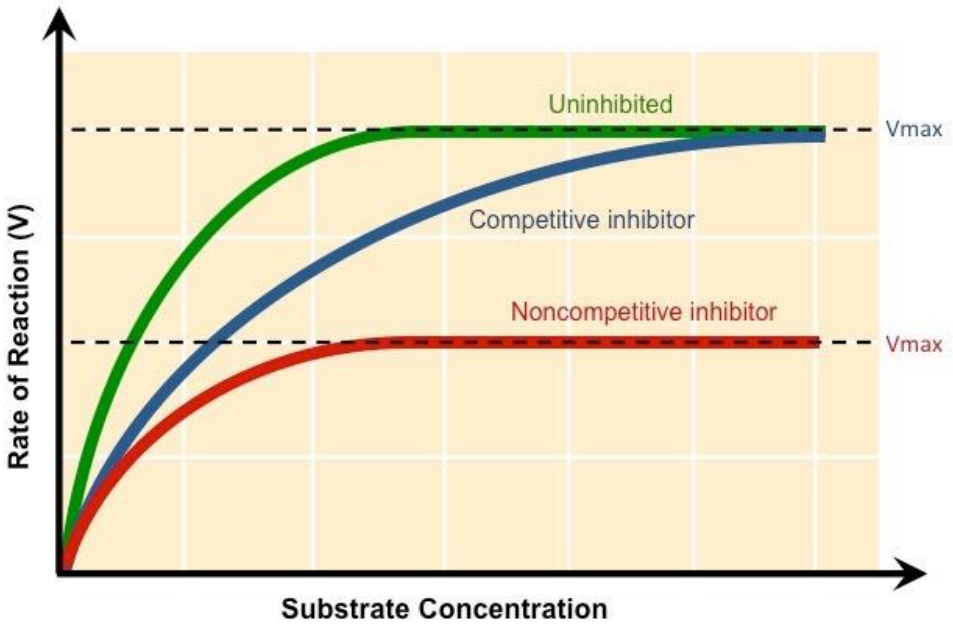


Fig. 5.15 Rate of Reaction in presence of inhibitors in Enzyme-catalysed reaction.

Thus, K_m is substrate concentration at which half of the active-sites of enzymeare involved in the formation of substrate enzyme complex. From Michaelis-Menten equation it is possible to calculate the rate of reaction at any substrate concentration if K_m&V are known. Kinetics of enzyme-action is helpful in understanding metabolic pathways. Determination of V_{max}& also K_m directly from the plot of r against [S] is rather difficult. However, Michaelis-Menten equation can be modified to get plots from which V_{max} can be easily determined. Two such plots are **Lineweaver- Burk plot and Eadic-llofstee plot.**

Lineweaver-Burk Equation & plot: Michaelis-Monten equation is

$$r = \frac{V_{max}[S]}{K_m + [S]} \qquad .....(16)$$

Taking reciprocal of both sides

$$\frac{1}{r} = \frac{K_m + [S]}{V_{max}[S]} \qquad .....(17)$$

Upon arranging it

$$\frac{1}{r} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]} \qquad .....(18)$$

$$\frac{1}{r} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \quad .....(19)$$

Equation (19) is Lineweaver-Burk equation. A plot of 1/r against 1/[S:

Note:

The Lineweaver -Burk plot of enzyme reaction rates is very useful to distinguish between some types of enzymatic reaction mechanisms and to study enzyme inhibition.

5.10 ALLOSTERIC ENZYMES

When initial velocity of enzyme catalysed reaction (V_o) is plotted as a function of concentration of substrate [S]; curve is not hyperbolic but is sigmoidal. Rate of reaction given [S] is increased or decreased by the addition of specific substance, i.e., activators or inhibitors (modulators). Such enzymes are known as allosteric enzyme. Besides substrate active sites, these enzymes possess other sites in which activators & inhibitors may bind and affect catalysis through induced conformational change; in the structure of enzyme.

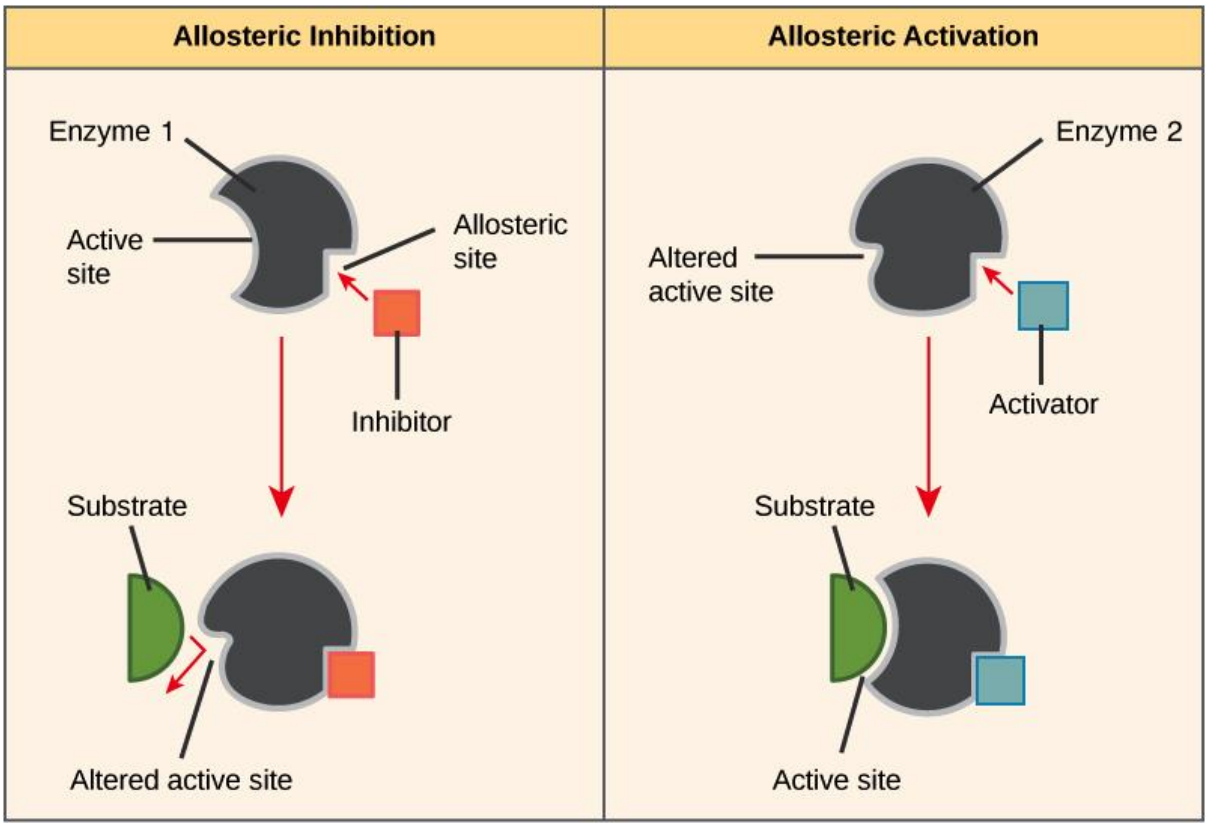
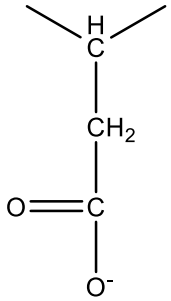


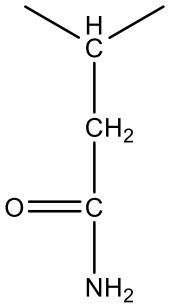
Fig 5.16 Allosteric Inhibition and activation

5.11 ENZYME MODIFICATION BY SITE-DIRECTED MUTAGENESIS

An important piece of informant between the structure and its function can be derived from mutagenesis. In this method one amino acid of a protein is replaced with another. When Asp 102 of chymotrypsin is replaced with Asp 102, although the enzyme, ability to bind the substrate remains unchanged, but its ability to catalyse the reaction decreases to less than0.05% of its value with the native enzyme. This shows that Asp 102 is involved in the catalytic process; its negative charge stabilises histidine positive charge.



side chain of Asp as present at the active site of chymotripsin

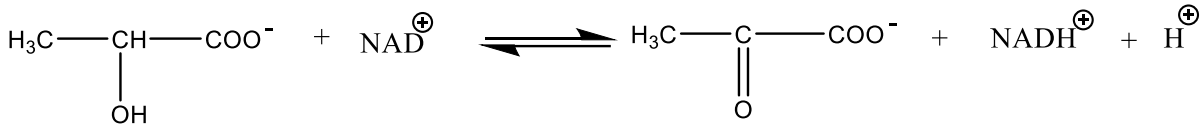


Replacement of Asp 102 of chymotripsin with Asp 102 bring aboutsite directed mutagenesis and loss of activity

5.12 ISOENZYMES

Isoenzymes (also known as isozymes) are oligomeric enzymes which catalyze same reaction but differ in their subunit composition. These differences modify the rate at which molecular species transform the substrate. Isozymes may be primary or secondary. Primary isozymes are produced by multiple gene loci which code for distinct protein molecules or are produced by multiple alleles at a single gene locus. These are also called alloenzymes. Secondary isozymes are product of post-translational modifications including glycosylation. On account of their different amino acid compositions primary isozymes may be identified on the basis of their different electrophoretic mobility. When enzymevariations are within game species they are known as intra-specific variants. But enzyme variation from different species is called interspecific or phylogeneticvariant.

Isoenzymes: Lactate dehydrogenase is an oligomeric enzyme in which each sub-unit performs same function. It catalyses following reaction:



Enzyme assay based on determination of catalytic activity can not distinguish between isoenzymes. Activity measured will be sum of contributions of active forms of enzymes being assayed. Even if only one coenzyme is present, its, molar activity, for example, in a homogenate is not the same as in pure form.

Plasma upon electrophoresis (e.g. on cellulose acetate strips) at pil of 8.6 separates all the five isoenzymes of LDH. All those isoenzymes may be located by specific stain, e.g. a mixture of lactate, NAD^+ and a chromogen which will give coloured product where LDH is present to catalyse a step of the reaction .An abnormal pattern helps in diagnosis. Separation may also be achieved by ion-exchange chromatography (for instance, on QAE-Sephadex). Relative proportions of isoenzyme in plasma can be assessed with their separation on the basis of their properties. If total LDH activity is higher than normal it can be determined if it is due to excess of (as in case of heart disease, haematologieal disorder or renal disease) or duo to excess of M_4 (as in case of skeletal muscle of liver disease).

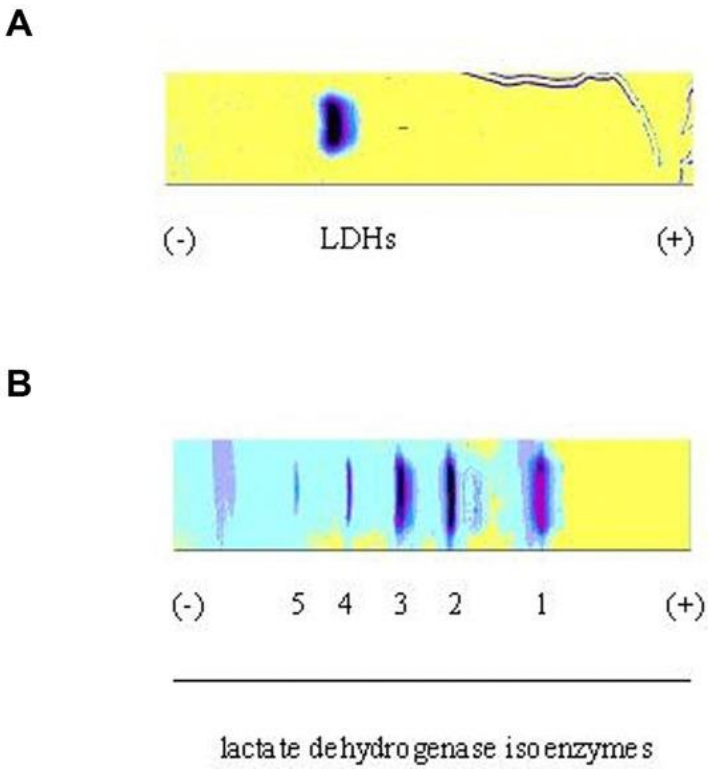


Fig. 5.17 Separation of Isoenzymes of LDII by electrophoresis at a pH of 8.6.

5.13TRANSITION-STATE THEORY

- During enzymatic reactions all reaction groups are brought together at the active site in the proper position for reaction.
- Some of the amino acid residues serve as catalytic groups, these side chains are in the proper position relative to the substrate to act as catalysts. This is analogous to the rate enhancements observed for intermolecular catalysis.
- The conformational change that an enzyme undergoes after binding a substrate can introduce strain into the substrate, to make it more reactive.
- Groups on the enzyme can stabilize an intermediate and, therefore, the transition state leading to the intermediate, by van der Waals and electrostatic interactions and by hydrogen bonding. As a direct result of stabilization of the transition state the activation energy of the reaction is lowered.

5.13.1 Catalysis

(A) Introduction when the substrate is bound to an enzyme, the catalytic functional groups which are properly positioned around the substrate in the active site lead to the cleavage and formation of bonds involving several mechanisms some of these are :

- Metal ion catalysis
- General acid-base catalysis
- Covalent catalysis

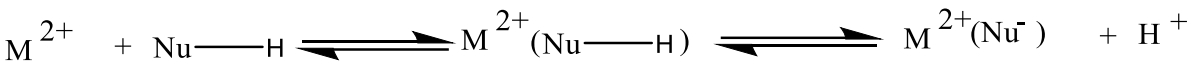
Enzymes catalyze reactions using general acid-base catalysis since at physiological pH (pH = 7.3) only a very small concentration of H+ for specific-acid catalysis or OH- for specific base catalysis is available.

A. Metal Ion Catalysis: Many enzymes require metal ions for maximum activity. *An enzyme is called a* ***metalloenzyme****, if it binds the metal very tightly or requires the metal ion to maintain its stable, native state. Enzymes which bind metal ions weakly are referred to as* ***metal activated enzymes***. One role for metals in metal activated enzymes and metalloenzymes is to act as **electrophilic** catalysts, stabilizing the increased electron density or negative charge that can develop during reactions.

The ionic interactions between a metal bound to the enzyme and a substrate can favourably orient the substrate for reaction. These metal ions can also stabilize the charged transition states.

- Metal ions complex with water and loss of a proton from such a complex gives a metal bound hydroxide ion which is a better nucleophile compared to water.
- A leaving group can become a weaker base (thus a better leaving group) in the presence of a metal ion.
- A catalyst must increase the rate of a slow step, since increasing the rate of a fast step will not increase the rate of the overall reaction

Another potential function of metal ions is to provide a powerful nucleophile at neutral pH. Coordination to a metal ion can increase the acidity of a nucleophile with an ionizable proton:



The reactivity of the coordinated, de-protonated nucleophile is typically intermediate between that of the un-ionized and ionized forms of the nucleophile. Carboxypeptidase enzyme contains an active site Zn^{2+}, which facilitates deprotonation of a water molecule in this manner.

Liver alcohol dehydrogenase: Enzyme Liver alcohol dehydrogenase catalyses the transfer of a hydride ion from NADH to acetaldehyde (CH_3CH0), forming ethanol (CH_3CH_2OH). An active-site zinc ion stabilizes negative charge development on the oxygen atom of acetaldehyde, leading to an induced partial positive charge on the carbonyl C atom. Transfer of the negatively charged hydride ion to this carbon forms ethanol.

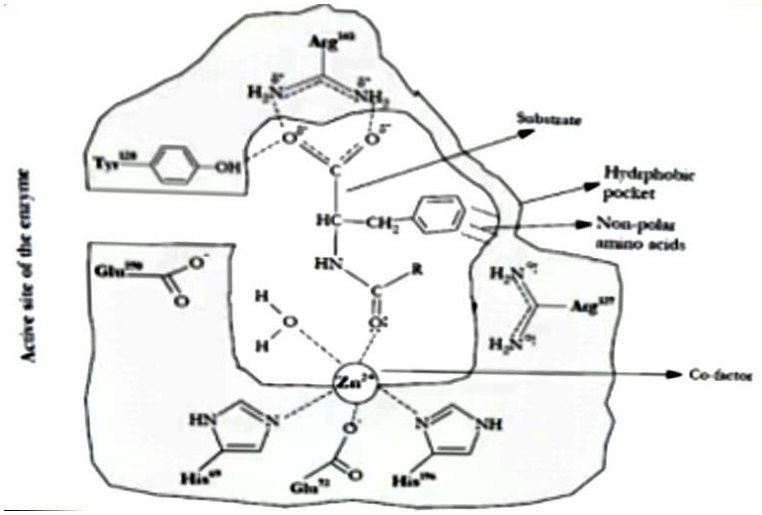
A. Acid-Base Catalysis

Almost all enzyme catalysed reactions involve some degree of acid or base catalysis. Acid-base catalysis is two types of:

- The proton must be **fully transferred to the reactant before the slow step** can occur in a **specific-acid catalysi**s. H^+ or OH^- catalysis is a type of acid-base catalysis in which the reaction is accelerated by H^+ or OH^-. As a result, a specific acid catalyst must be a strong acid in order to protonate the reactant before the slow step begins.

- The proton is transported to the reactant **during the slow step** of the reaction in **general-acid catalysis**. The use of an acid or base other than H^+ or OH^- to accelerate a process is known as general acid-base catalysis. A general acid is any material that is weakly ionizable; hence, a general-acid catalyst is a weaker acid since it only partially transfers a proton in the slow step transition state.

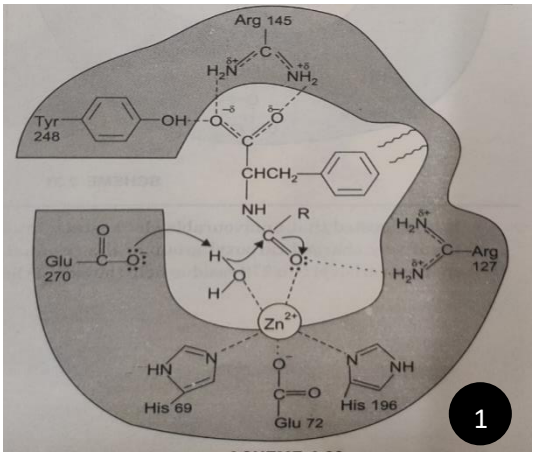
General acid or general base catalysis may increase reaction rates 10-100 times.**Specific and general acid catalysts increase the rate of the reaction** in the same way, by donating a proton to make bond formation and bond breaking easier. These differ as to the extent to which the proton is transferred in the transition state of the slow step of the reaction. In general acid catalysed reaction, the transition state has a partially transferred proton while in specific-acid catalysis the proton must be fully transferred to the reactant before the beginning of the slow step.

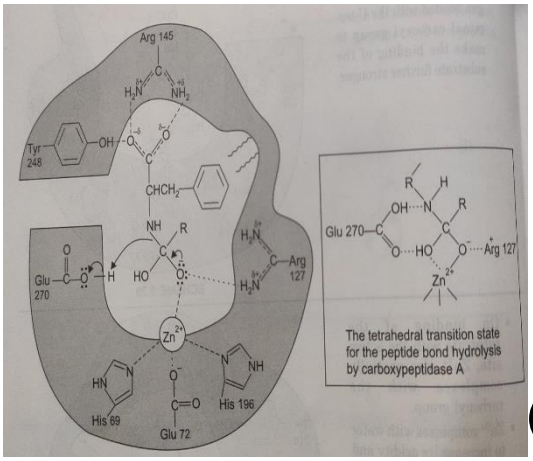


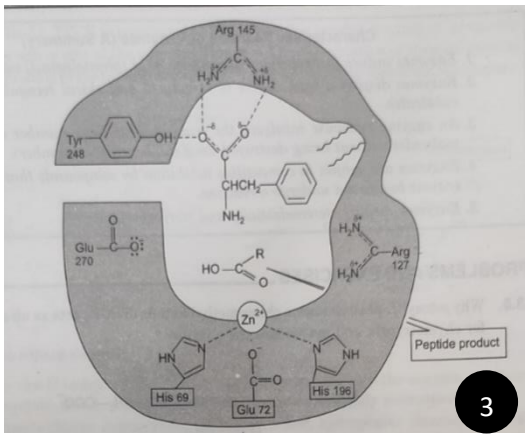
5.14 MECHENISM OF ACTION OF ENZYME CARBOXYPEPTIDASE A

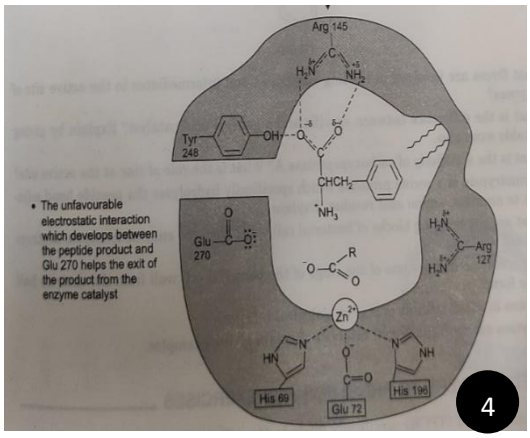
Carboxypeptidase A is a metalloenzyme in which Zn^{2+} ion is acting as cofactor for the hydrolytic cleavage of peptide bonds. A tightly bound***Zn^{2+} ion is located in a groove near the surface of the enzyme and held in its position through coordination with a molecule of water, Glu^{72}, His^{69} and His^{196}.*** The Zn^{2+} ion increases the reactivity of the bound water during the reaction as water does not react readily with peptide bonds at neutral pH in the absence of protease. But in the ***presence Zn^{2+} ion water behaves like OH^- ion***. Many groups

at the active site of the enzyme carboxypeptidase-A bind the substrate in the optimum position for the hydrolytic cleavage reaction.









Arg^{145} forms two hydrogen bonds with the ***C-terminal carboxyl group*** of the substrate. Tyr^{128} is also involved in hydrogen bonding with the substrate. The non-polar side chain of the substrate is bound in the hydrophobic pocket lined with non-polar amino acids. The hydrolytic reaction involves general acid-base mechanism. Glu^{270}acts as a general base catalyst along with Arg^{127} and Zn^{2+}.

The first step of the reaction involves the positioning of the phenyl group of the substrate in the hydrophobic pocket and binding of the C-terminal carboxylic group with Arg^{145} via electrostatic interaction as well as hydrogen bonding (1).

The binding of the substrate is made further made stronger by ***hydrogen bonding of the C-terminal carboxylic group*** with Tyr^{128}. Zn^{2+} ion also partially complexes with amidic

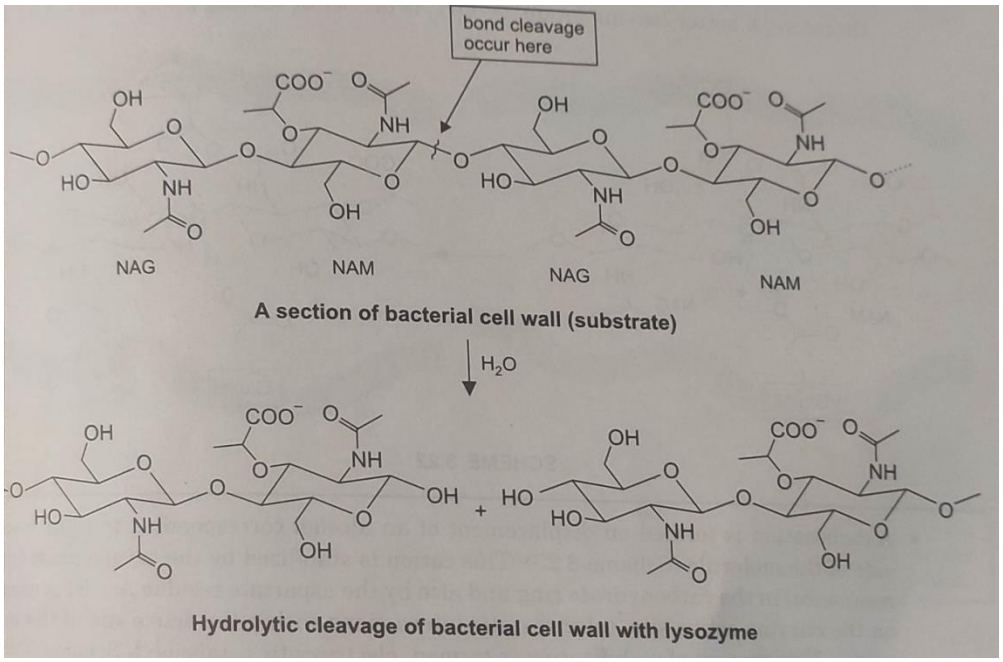
carbonyl group on binding of the substrate in the above manner. Zn^{2+} ion complexes with water to increase its acidity and thus water behave as hydroxides. Glu^{270} acts as a general base andwithdraws proton from water which attacks as hydroxide ion on amidic carbonyl group to give a ***tetrahedral intermediate*** which acquires negative charge (2).

This ***negatively charged tetrahedral intermediate*** is stabilized by Zn^{2+}ion as well as Arg^{127}. The tetrahedral intermediate undergoes collapse with Glu^{270}acting as a general acid catalyst, the leaving ability of the amino group is increased; the reaction is completed with formation of a protein with one less amino acid residue (3).

The unfavourable electrostatic interactions between the negatively charged carbonyl group of the residual peptide product and the negatively charged carboxyl of residue helps in the exit of the product from the active site of the enzyme (4).

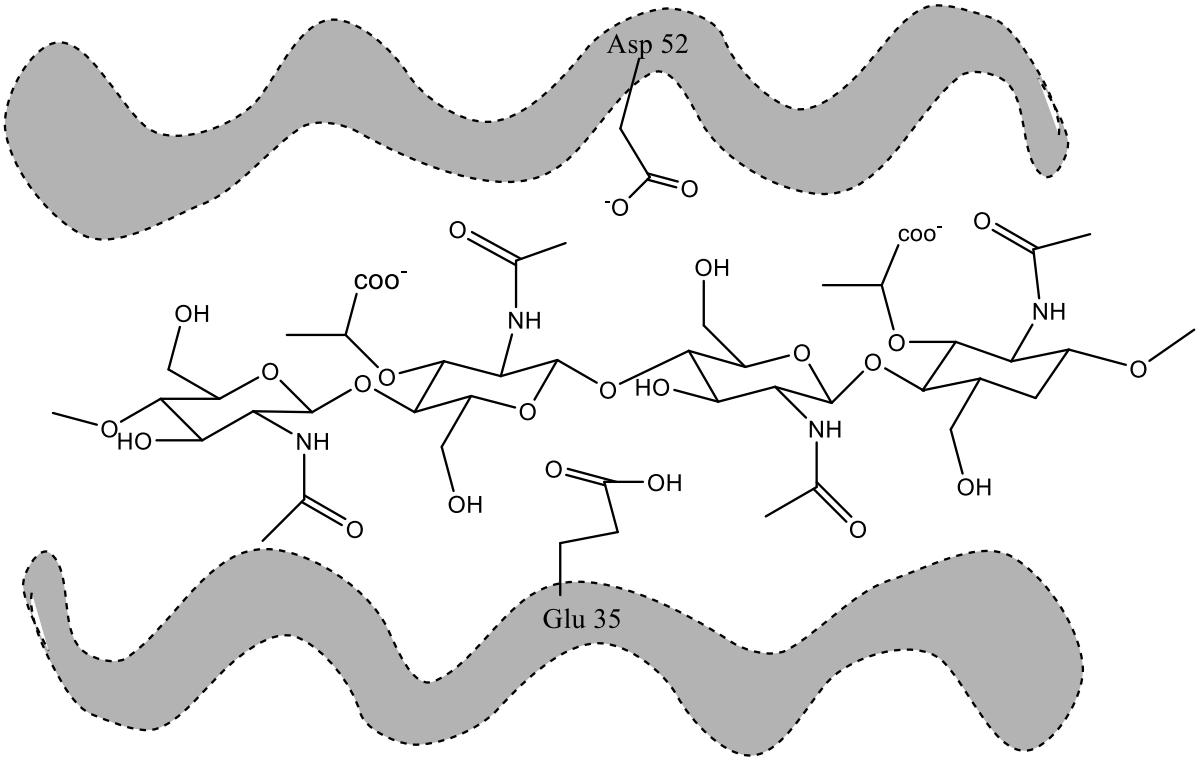
5.15 LYSOZYME

Lysozyme is an enzyme which hydrolyses polysaccharide chains. It breaks certain bacterial cells by cleaving the polysaccharide chains that build up their cell wall. The most thoroughly studied form of lysozyme is from hen egg whites. The bacteriolytic properties of hen egg white lysozyme were first described in 1909 by the Russian scientist P. Laschtchenko. In 1922 Alexander Fleming gave the name lysozyme to the agent in mucus and tears that destroyed certain bacteria, because it was an enzyme that caused bacterial lysis.



(A) Hydrolysis by lysozyme

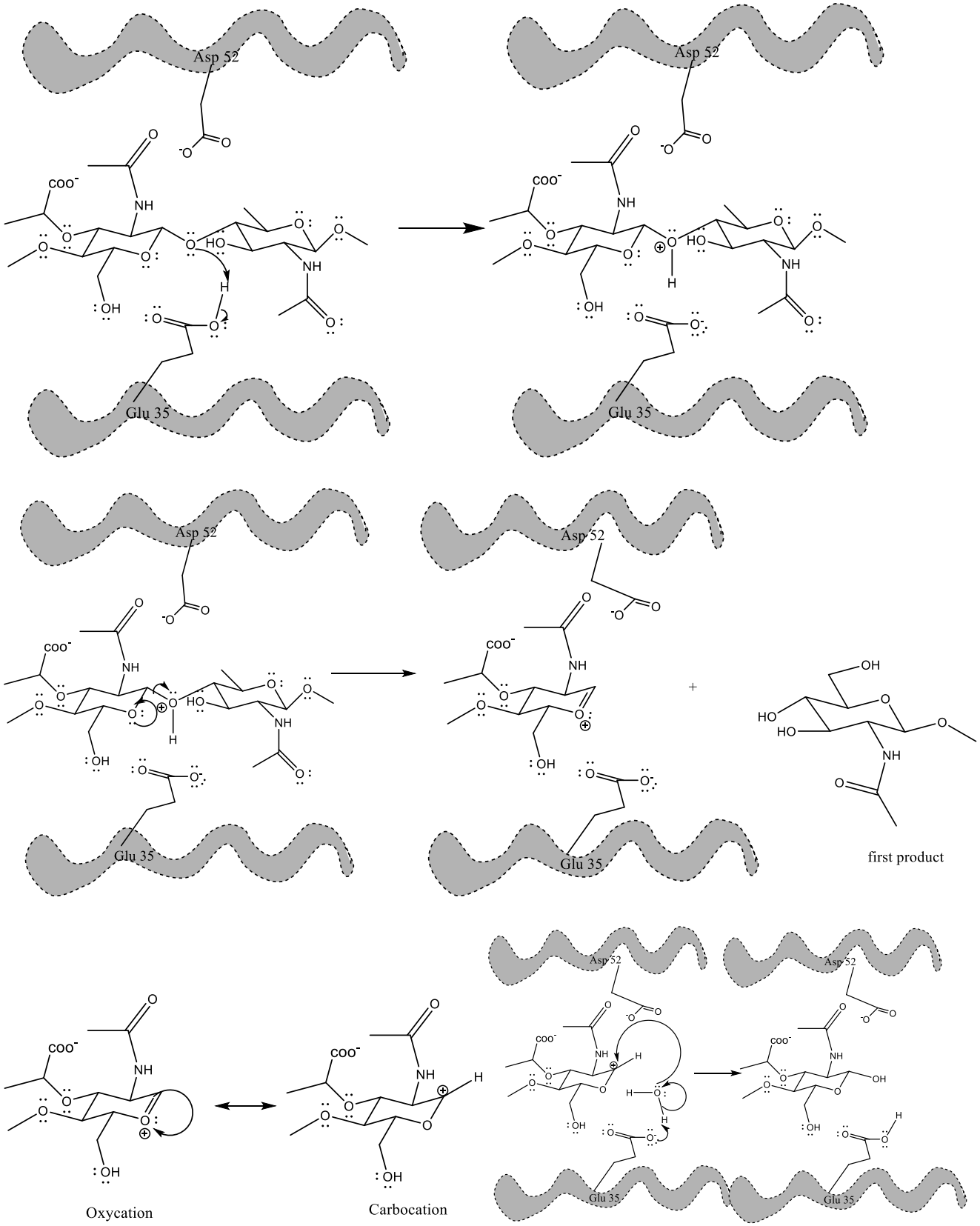
The bacterialcellsare surrounded by a rigid, strong wall of peptidoglycan,a copolymer of twosugar unit. N-acetylmuramic acid (NAM)andN-acetylglucosamine (NAG) and in bacterial cell wall polysaccharides, they are joined in ß(1-4) glycosidic linkages. Lysozymehydrolyzes glycosidic bond between C-1 of NAM and C-4 of NAG but does not act on the ß(1-4) linkages betweenNAG and NAM.



The substrate in the active site of the enzyme, the acetal linkage undergoing cleavage held near the two catalytic groups.

(i) Carbocation mechanism

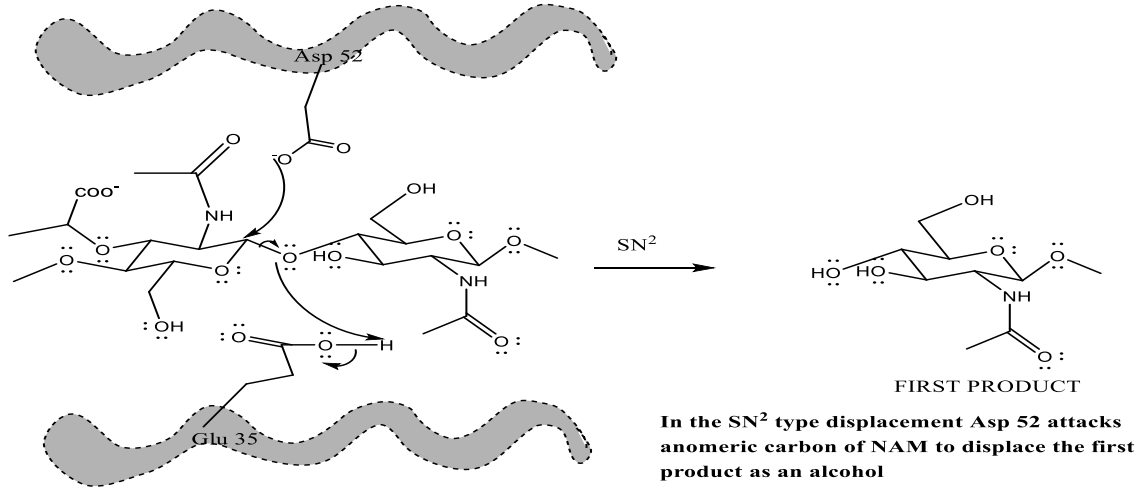
Glu^{35} is in a nonpolar or hydrophobic region of the protein, whereas $Asp^{52}$is located in a much more polar environment. Glu^{35} is protonated, but is ionized (1). In the first step, Glu^{35} act as a general acid, donating a proton to the oxygen atom of the glycosidic bond and accelerating the reaction. This protonation makes the leaving group a weaker base and hence, a better leaving group (2).


Asp 52
Glu 35
Asp 52
Glu 35
Asp 52
Glu 35
Asp 52
Glu 35
first product
Oxycation
Carbocation
Asp 52
Asp 52
Glu 35
Glu 35

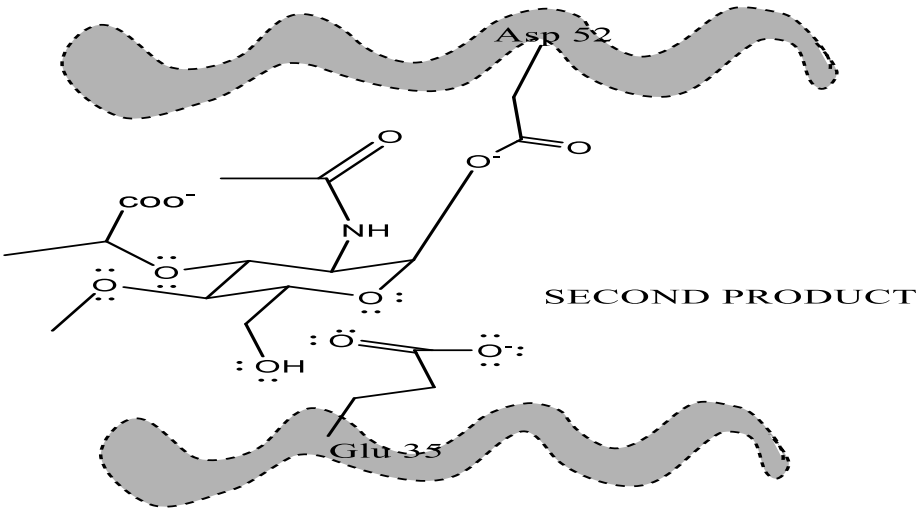
A carbocation is formed on displacement of an alcohol. Asp^{52}, on the other hand, probably stabilizes the carbonium ion generated at the site upon bond cleavage (3). Following bond cleavage, the first product formed diffuses away, and the carbonium ion intermediate can then react with H20 from the solution. can now act as a general base, accepting a proton from the attacking water (4) and formation of the second product (5).

(ii) S_N^2 mechanism

In 2001, an alternative mechanism involving two **consecutive direct bimolecular nucleophilic displacement** (SN^2) steps, with **inversion of configuration** on each displacement step at C-1 carbon of NAM residue, leading to the formation of product with **net retention of configuration**has been suggested.

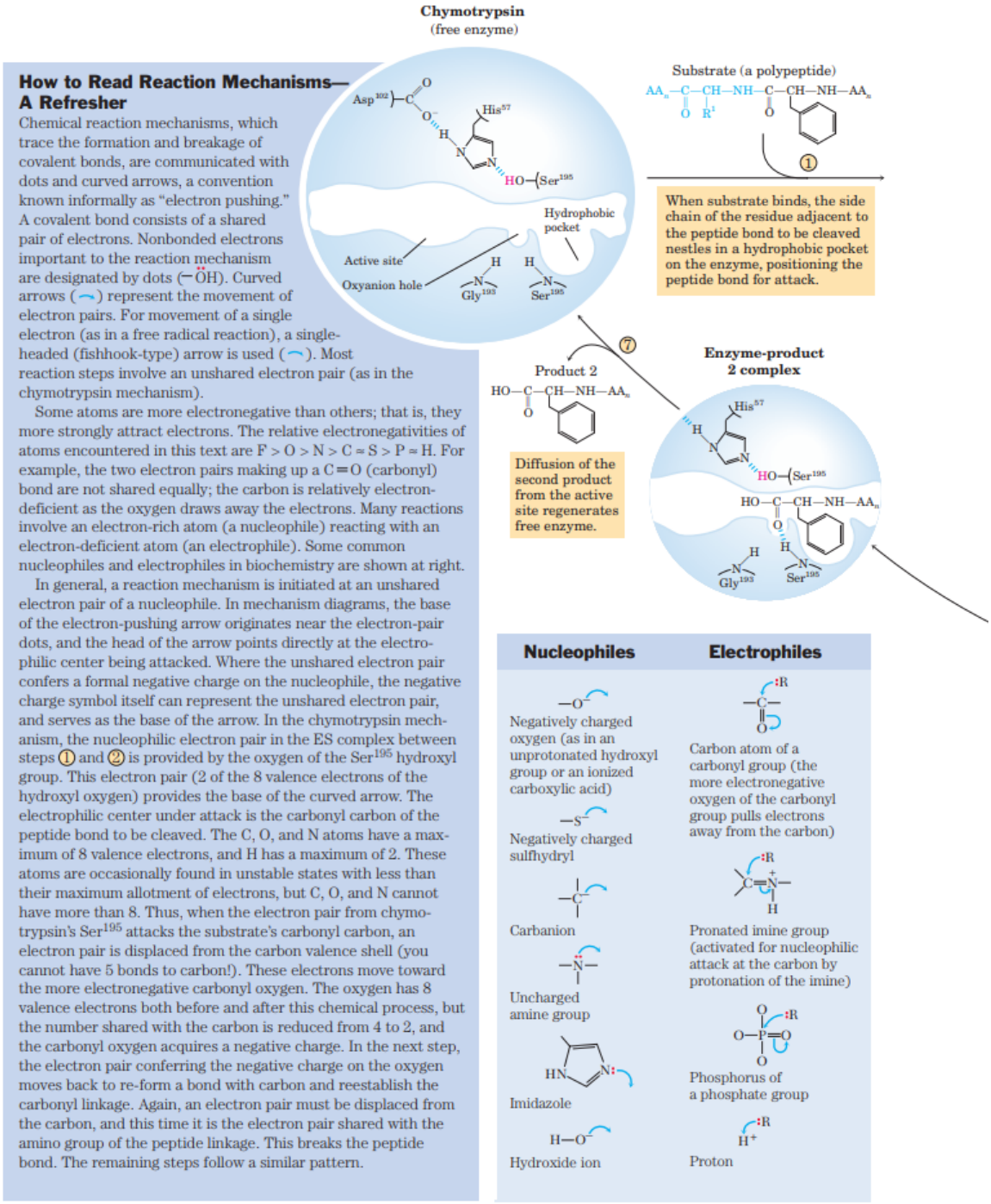


In the SN^2 type displacement Asp 52 attacks anomeric carbon of NAM to displace the first product as an alcohol

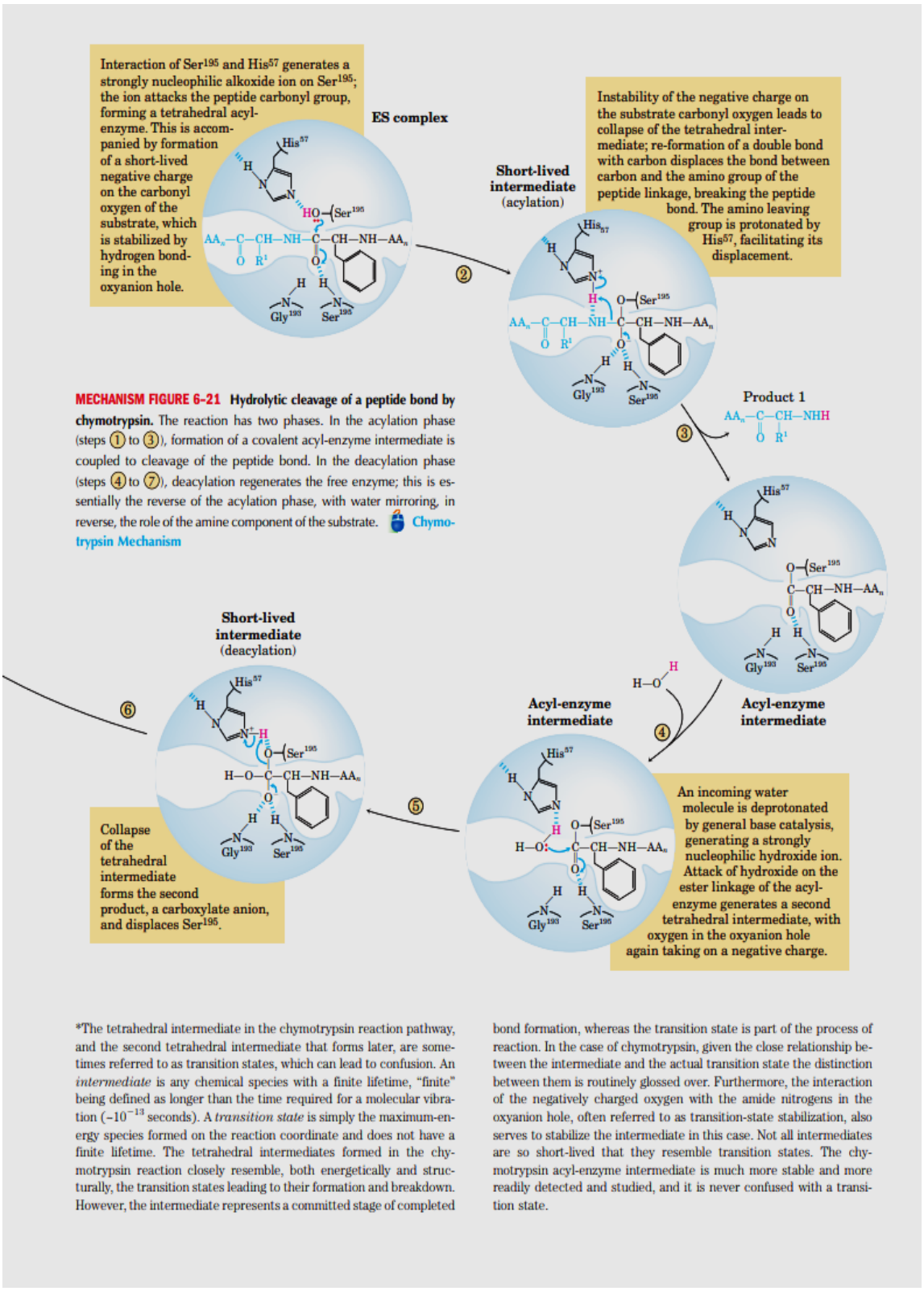


5.16 CHYMOTRIPSIN

Chymotrypsin hydrolysis to peptide bonds at body temperature and physiological pH.In Chymotrypsin is a protease (series protease since serin is at active site). The overall reaction shown below:



(Lehninger Principles of Biochemistry Fourth Edition)



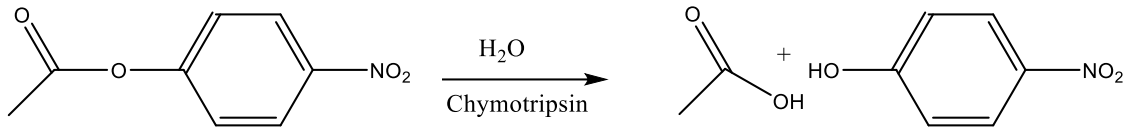
MECHANISM FIGURE 6-21 **Hydrolytic cleavage of a peptide bond by chymotrypsin.** The reaction has two phases. In the acylation phase (steps ① to ③), formation of a covalent acyl-enzyme intermediate is coupled to cleavage of the peptide bond. In the deacylation phase (steps ④ to ⑦), deacylation regenerates the free enzyme; this is essentially the reverse of the acylation phase, with water mirroring, in reverse, the role of the amine component of the substrate. Chymotrypsin Mechanism

*The tetrahedral intermediate in the chymotrypsin reaction pathway, and the second tetrahedral intermediate that forms later, are sometimes referred to as transition states, which can lead to confusion. An *intermediate* is any chemical species with a finite lifetime, "finite" being defined as longer than the time required for a molecular vibration ($\sim 10^{-13}$ seconds). A *transition state* is simply the maximum-energy species formed on the reaction coordinate and does not have a finite lifetime. The tetrahedral intermediates formed in the chymotrypsin reaction closely resemble, both energetically and structurally, the transition states leading to their formation and breakdown. However, the intermediate represents a committed stage of completed bond formation, whereas the transition state is part of the process of reaction. In the case of chymotrypsin, given the close relationship between the intermediate and the actual transition state the distinction between them is routinely glossed over. Furthermore, the interaction of the negatively charged oxygen with the amide nitrogens in the oxyanion hole, often referred to as transition-state stabilization, also serves to stabilize the intermediate in this case. Not all intermediates are so short-lived that they resemble transition states. The chymotrypsin acyl-enzyme intermediate is much more stable and more readily detected and studied, and it is never confused with a transition state.

(Lehninger Principles of Biochemistry Fourth Edition)

The following points confirm the mechanism of hydrolysis of a peptide bond (when adjacent to aromatic amino acids) by the enzyme chymotrisin. Chymotrypsin can hydrolyse

other acetate functional group for example esters amides, and anhydrides, p-niotro phenyl acetate, an ester containing an aromatic portion are react with chymotrypsin



p-Nitrophenol and acetic acid are formed at with different speed, while p-nitrophenol forms very rapidly, while acetic acid form slowly. The results indicate a two-step reaction sequence commonly observed in enzyme catalysis. In between of reactant and product the enzyme-substrate complex is found.

Enzyme+Substrate ⇌ enzyme-substrate complex → enzyme+ product 5.17 ----

5.17 SUMMARY

This unit covered following points:

- Enzyme is the biological catalyst which increases the rate of reaction and decrease the activation energy of biological reaction.
- Enzyme is classified on different basis.
- The role of inhibitor in different site of substrate. It influences the rate of reaction in the presence of enzyme.
- Learnt about Transition State theory
- Mechanism action of Carboxypeptidase A, Lysozyme and chymotrypsin enzyme in different reactions.

5.17 TERMINAL QUESTIONS

1. What are the forces involved in binding substrate and intermediates to the active sites of enzyme?
2. What is the difference between specific and general acid catalyst? Explain with suitable examples.

3. Chymotrypsinis a serine protease which specifically hydrolyses the peptide bond adjacent to aromatic amino acid residue. Explain.
4. Why lysozyme distorts one of the rings of the bacterial cell wall from the chair to half chair form?
5. Enzymes are multifunctional catalyst. Explain with example.
6. Is there a difference between the initial and the final energy levels in catalyzed and non-catalyzed reactions?
7. What are the main theoretical models that try to explain the formation of the enzyme-substrate complex?
8. What is the chemical basis of enzyme specificity?
9. What is the Michaelis-Menten equation and its Lineweaver-Burk form?
10. How does the Michaelis-Menten equation explain why the rate of an enzyme-catalyzed reaction is proportional to the amount of enzyme? How does the Michaelis-Menten equation explain why the rate of an enzyme catalyzed reaction reaches a maximum value at high substrate?
11. How does the formation of an E.S complex explain the reaching of a maximal velocity in the Vo vs So graph?
12. What is antibody and what is hapten.
13. Define the following terms: (a) Enzyme model and (b) Biomimetic Synthesis.
14. Is there a difference between the initial and the final energy levels in catalyzed and non-catalyzed reactions?
15. What are the main theoretical models that try to explain the formation of the enzyme-substrate complex?

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UNIT 6: KINDS OF REACTIONS CATALYSED BY ENZYMES

CONTENTS:

6.1 INTRODUCTION

Enzymes are biological catalysts (biocatalysts) necessary for practically all biochemical processes in a living system. Natural enzymes are proteins that may be thought of as bio-catalysts since they are created by living cells. Recently, however, it has also been shown that a small number of RNA molecules possess some catalytic activity. In a lab setting, it takes a few days for a protein to be hydrolyzed by a strong acid at pH 100. However, the digestive enzyme digests the same protein over a few hours at a considerably lower temperature (37 C, body temperature). Many laboratory reactions call for greater temperatures, as well as a number of solvents and high reagent concentrations. A live cell can't access these circumstances, but they won't kill it either. Enzymes allow the body's reactions to occur quickly and effectively at body temperature with just modest reagent concentrations in water, the cell's solvent. Kuhne used the word enzyme (Greek: in yeast) to refer to the catalysis that takes place in biological systems before Berzelius's 1836 use of the phrase catalyst (Greek: to dissolve). In 1883, Duclaux first used the word "substance." The enzyme was found in yeast (Greek: en =, and zyme = yeast) and was able to catalyse the fermentation processes. In 1883, Buchner isolated an enzyme system from a yeast extract devoid of yeast cells. Zymase was the name of the active ingredient, which was able to turn sugar into alcohol.

6.2 OBJECTIVE

You will be able to learn after this unit:

- Understand nucleophilic displacement on the phosphorus atom; numerous displacement reactions; and the connection between ATP cleavage and endergonic processes after finishing this unit.
- Sulfate transfer, addition and elimination reactions, enolic intermediates in isomerization events, cleavage and condensation, certain isomerization and rearrangement reactions, and Enzyme-catalyzed carboxylation and decarboxylation are all covered in this section.

6.3 NUCLEOPHILIC DISPLACEMENT ON PHOSPHORUS ATOM

6.3.1 Important in living things is the chemical ATP.

In most biological tissues, phosphoric esters make up more than 3% of the organic

components. They participate in practically all facets of cellular activity. Phosphorylation, which is the process by which a phosphoryl group is transferred from one group to another, is one of their most significant processes. The reaction, which involves a nucleophilic substitution on phosphorus (Fig. 6.1), results in the same kind of structural modification as that seen in acyl transfer reactions involving carboxylic acid derivatives.

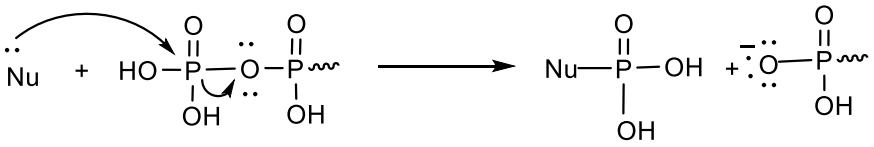


Fig 6.1 Nucleophilic substitution on phosphorus

As a "high-energy" phosphate, adenosine triphosphate (ATP) is frequently mentioned. In biological terms, this indicates that energy is released when ATP phosphorylates an acceptor (nucleophile) and transfers a phosphate group to that acceptor. The breaking of phosphate bonds is crucial for the transmission of energy in a biological system. The type of nucleophile used in the phosphorylation affects how much energy is released. With water serving as the reference (Fig. 6.2), ATP is hydrolyzed to produce adenosine diphosphate (ADP), which releases energy at a rate of around 7 kcal/mol (30 kJ/mol).

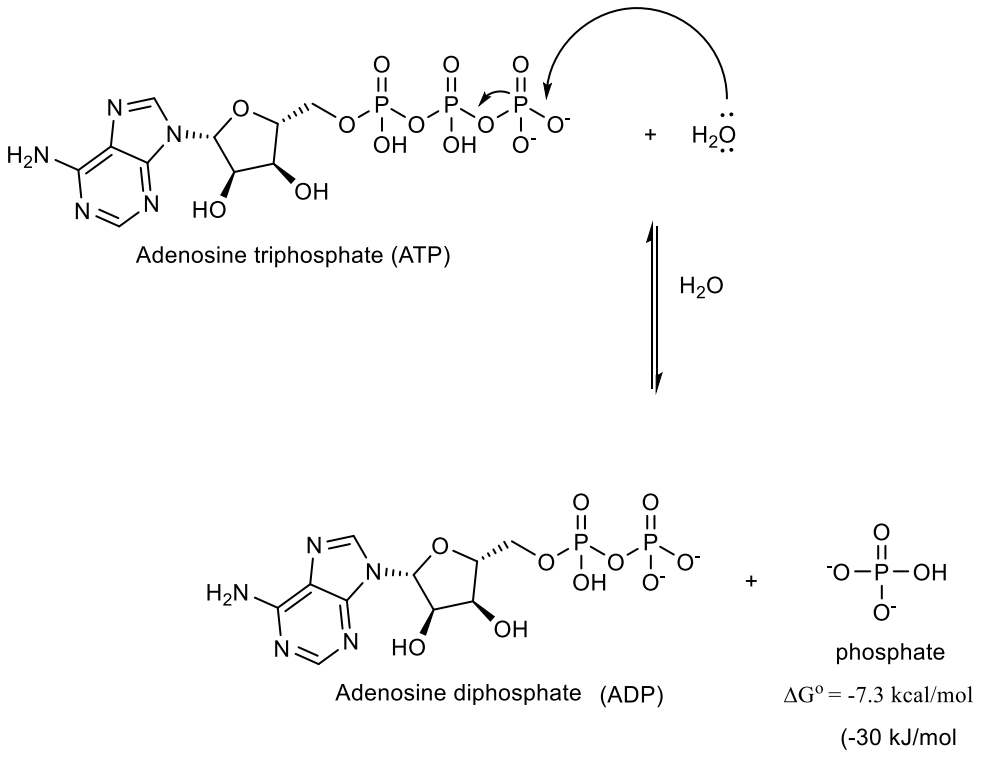


Fig. 6.2 ATP is hydrolyzed to produce adenosine diphosphate (ADP)

On a chemical level, the significant shift in free energy that occurs during ATP hydrolysis is explicable. First, the product ADP exhibits a weaker than expected electrostatic repulsion between the four negative charges in ATP (Fig.6.3). Resonance serves to stabilise the inorganic phosphate (Pi), the other byproduct of hydrolysis.

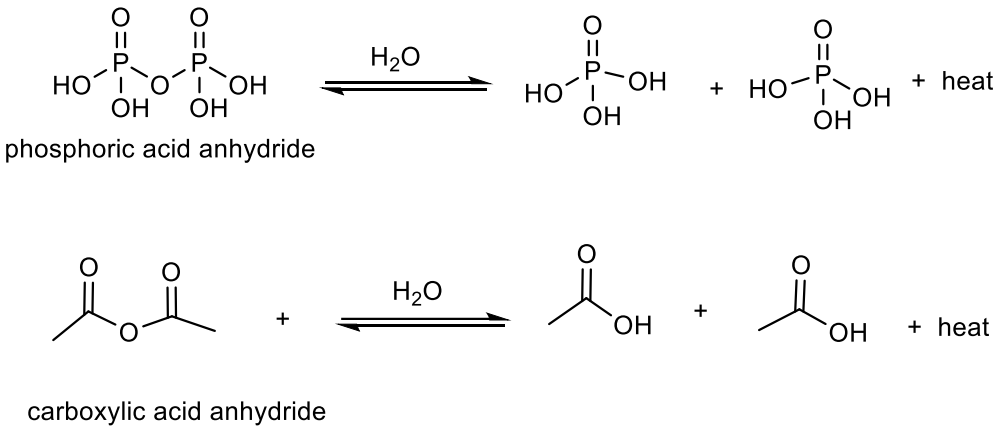
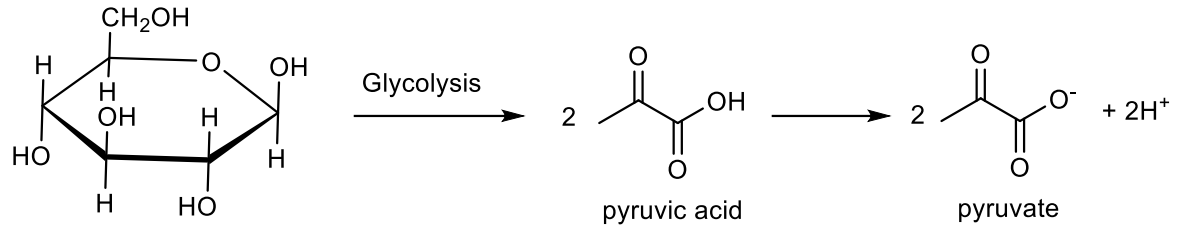


Fig.6.3 Electrostatic repulsion between the four negative charges in ATP

Due to its two anhydride connections, adenosine triphosphate (ATP) is an energetically dense molecule. The free energy required to hydrolyze ATP into the corresponding monoanhydride (ADP) is -7.3 kcal/mol (fig. 6.2). This significant decrease in free energy demonstrates how favourable the reaction is. With Go =-7.3 kcal/mol, one may conclude that ATP hydrolysis is a strongly exergonic process.

6.3.2 Phosphorylation of glucose

The ATP hydrolysis-induced favourable free energy change is employed to shift the equilibrium of unfavourable biological reactions in the desired direction. Think about how glycolysis, which is derived from the Greek words for "sweet" and "splitting," releases energy from glucose in biological systems. In contrast to glycolysis, which begins with the phosphorylation of glucose with ATP to produce glucose-6-phosphate, the breakdown of glucose involves a sequence of enzyme-catalyzed processes to produce two molecules of pyruvate (Fig. 6.4). If one considers the reaction of D-glocose with hydrogen phosphate (Fig.6.5), the formation of a phosphate ester.



$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD}^+ \xrightarrow{\text{Glycolysis}} 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\text{NADH}$$

Fig. 6.4 Enzyme-catalyzed processes

Glucose 6-phosphate will be produced at the main hydroxy group. The free energy of this reaction, however, is +3.3 kcal/mol (13.8 kJ/mol), making it an endergonic reaction (fig.6.6). Due to the equilibrium favouring the reactions, there is a positive free energy. Thus, this phase in the metabolism of glucose is undesirable.

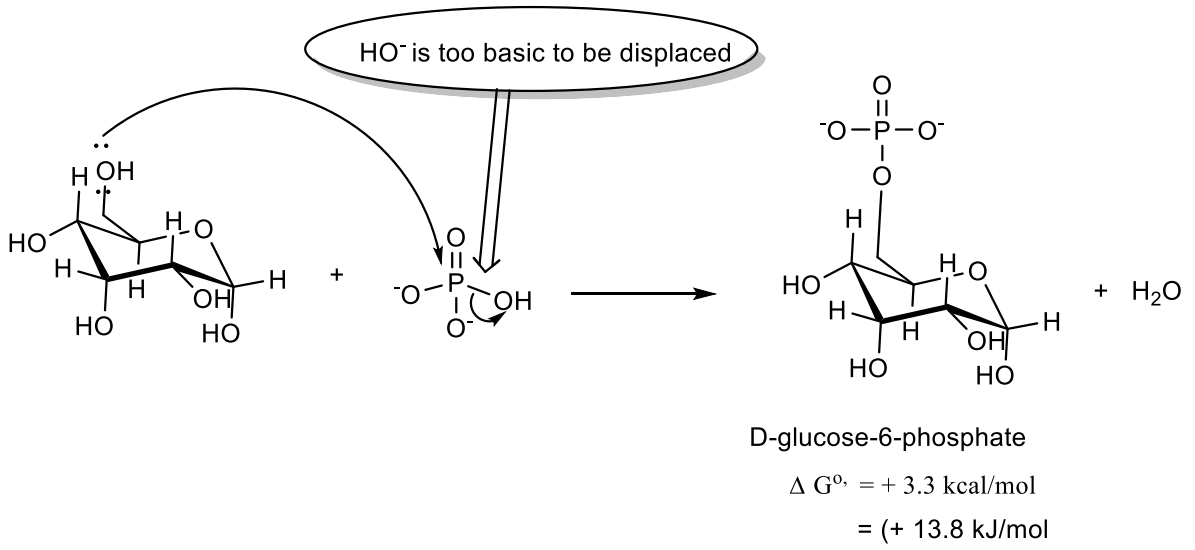


Fig.6.5 Reaction of glucose with hydrogen phosphate-an unfavourable reaction

However, glucose and ATP easily combine to form glucose 6-phosphate and ADP in a straightforward one-step nucleophilic substitution process (Fig. 6.6). Without the need for an intermediary, a phosphoanhydride bond is broken when the 6-OH group of glucose engages the terminal phosphate of ATP as a nucleophile. In order to phosphorylate glucose, ATP is needed because the 6-OH group of D-glucose acts as a nucleophile and displaces weakly basic ADP. Without ATP, the 6-OH of glucose would have to displace a very basic OH group (Fig 6.5).Instead of cleaving the link, glucose's nucleophilic attack on ATP cleaves a

phosphoanhydride bond. This is comparable to a carbonyl group being attacked by a nucleophile when the bond is the first bond to break.

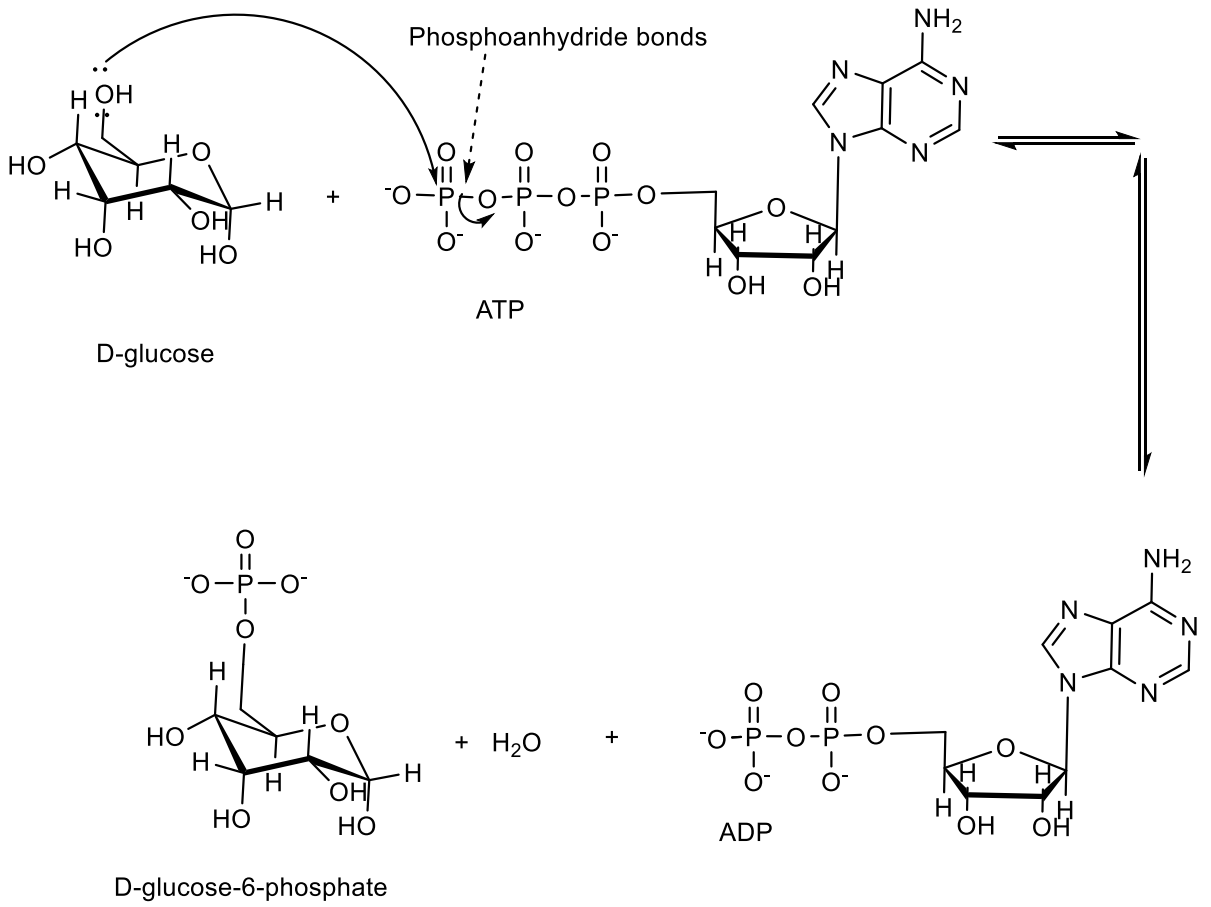


Fig. 6.6 Nucleophilic substitution process

The free energy change of this reaction is computed by combining the free energies for the phosphorylation of glucose and the hydrolysis of ATP (Fig. 6.6), where the species present on either side of the reaction arrow cancel. This reaction has a free energy change of -4.0 kcal/mol. Coupled reactions are two reactions in which the energy of one reaction is used to drive the other, such as the favourable hydrolysis of ATP and the unfavourable phosphorylation of glucose (with hydrogen phosphate).

A phosphoanhydride bond is broken during the beneficial reaction of phosphorylation, which transfers the electrophilic phosphate group from one nucleophile to another. The primary role of ATP is phosphorylation because it offers good leaving for reactions that would not otherwise take place due to insufficient leaving groups.

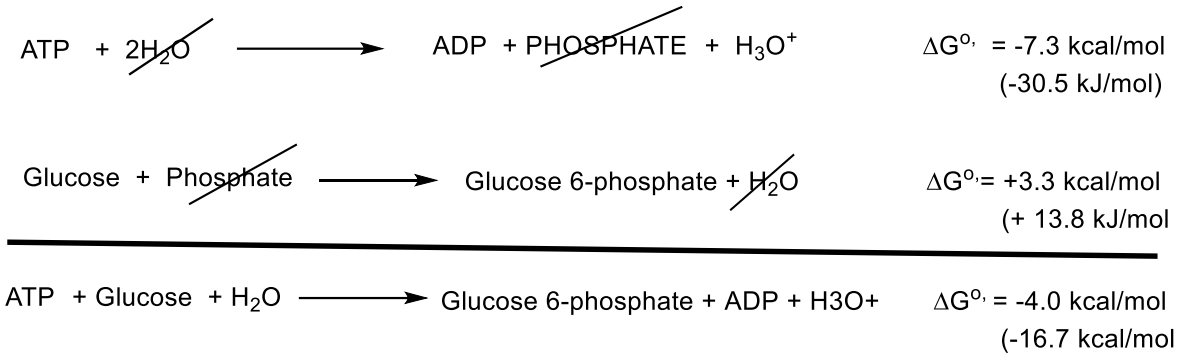
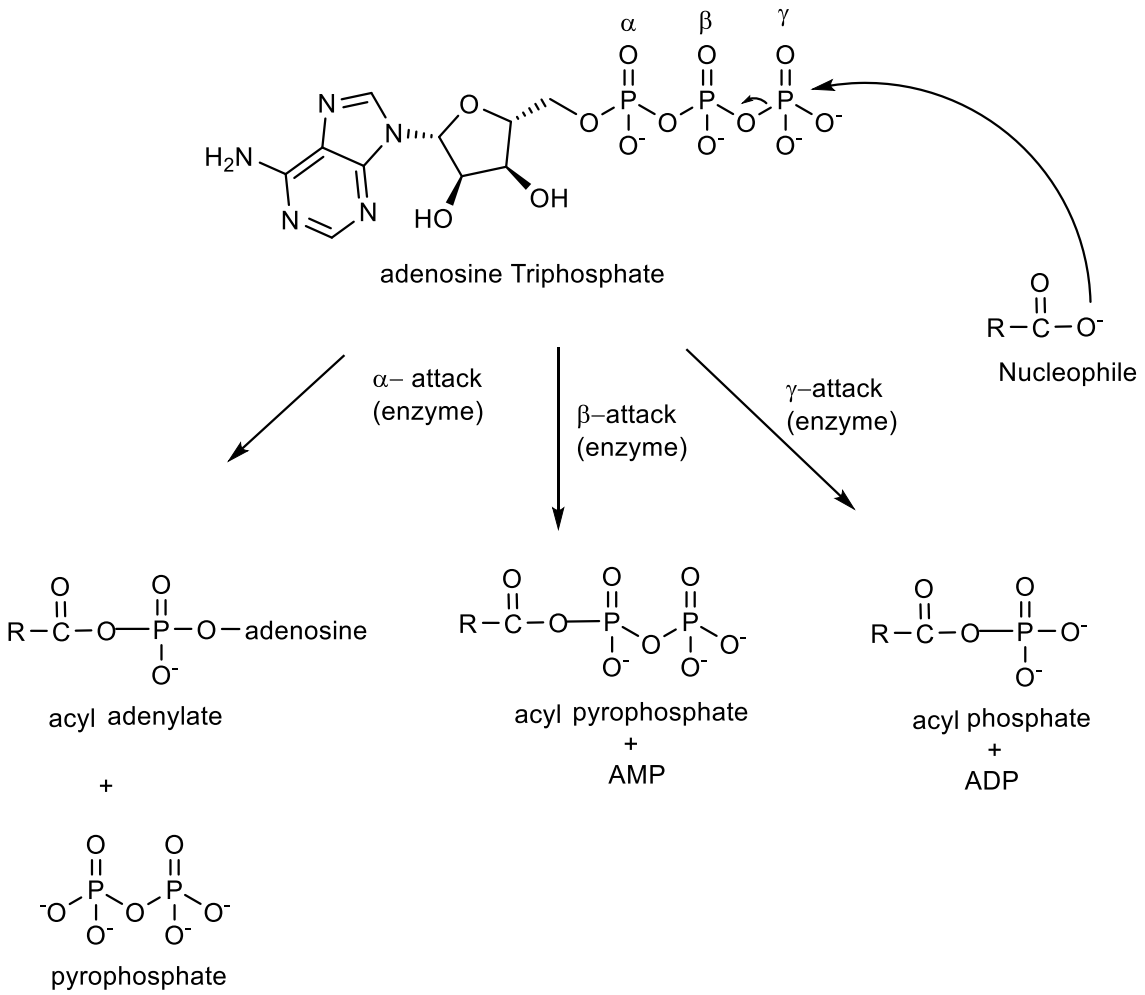


Fig. 6.7 Total enegry change from ATP to ADP conversion process

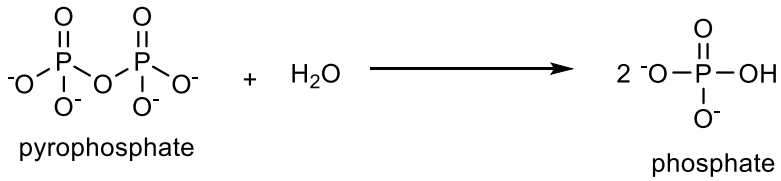
6.4 MULTIPLE DISPLACEMENT REACTIONS OF PHOSPHORUS



In biological systems, ATP reacts with a variety of nucleophiles. These are oxygen from an alcohol or carboxylate, nitrogen from creatine, oxygen from the side chain of arginine, or

oxygen from the side chain of histidine. Depending on the enzyme initiating the reaction, each of the three phosphates of ATPα-, β-, orγ-is susceptible to nucleophilic displacement and gives a unique kind of product.

When a pyrophosphate is hydrolyzed, it yields two equivalents of phosphate. So, when a pyrophosphate is produced as a result of nucleophilic displacement on phosphorus, its subsequent hydrolysis moves the reaction to the right, guaranteeing its irreversibility. When such irreversibility is necessary, biochemical processes take place by nucleophilic displacement on α or β phosphorus of ATP.



6.5 COUPLING OF ATP CLEAVAGE TO ENDERGONIC PROCESSES

As long as the overall path is exergonic, high energy compound exergonic reactions can be connected to endergonic processes to drive them to completion. The exergonic hydrolysis of ATP to produce ADP and Pi supplies free energy for a number of bodily functions. Instead of using the conventional chemistry terms "exothermic" and "endothermic," exergonic and endergonic are used to indicate that a process is accompanied by a loss or gain of free energy in any form, not just heat.A connected exergonic-endergonic reaction system with an overall net change that is exergonic is the only way that an endergonic reaction can exist independently. Exergonic processes refer to catabolism, the disintegration of fuel molecules. Molecules are assembled synthetically during anabolism. Catabolic and anabolic processes both occur during metabolism, and a number of organophosphates, including ATP, ADP, and phosphoenolpyruvate, are important for energy storage and transfer.

This ATP cleavage is frequently connected to an endergonic metabolic process that is thermodynamically forced upward. Such reactions require the sequential displacement of two different types of reactions. The sequential displacement of an atom of phosphorus followed by an atom of carbon, or vice versa, is used to couple two processes. The initial stage in the coupling process involves the displacement of one of the three phosphorus atoms on which

the phospho, pyrophospho, and adenyly groups from the ATP molecule are transferred to a nucleophile. A second nucleophile attacks a carbon atom in the following step, dislodging the transferred group.The creation of acetyl CoA is a typical illustration of a multiple displacement reaction. In bacteria, acetyl-CoA is produced from acetate in two separate processes that are each mediated by an S-acetyl transferase and an acetate kinase. The oxygen atom in the carboxylate group acts as a nucleophile in the first step, dislodging the phosphorus in ATP to create acetyl phosphate. In the second reaction, Pi is displaced by the S atom of the coenzyme A SH group, acting as a second nucleophile and attacking the carbon atom of acetyl phosphate.

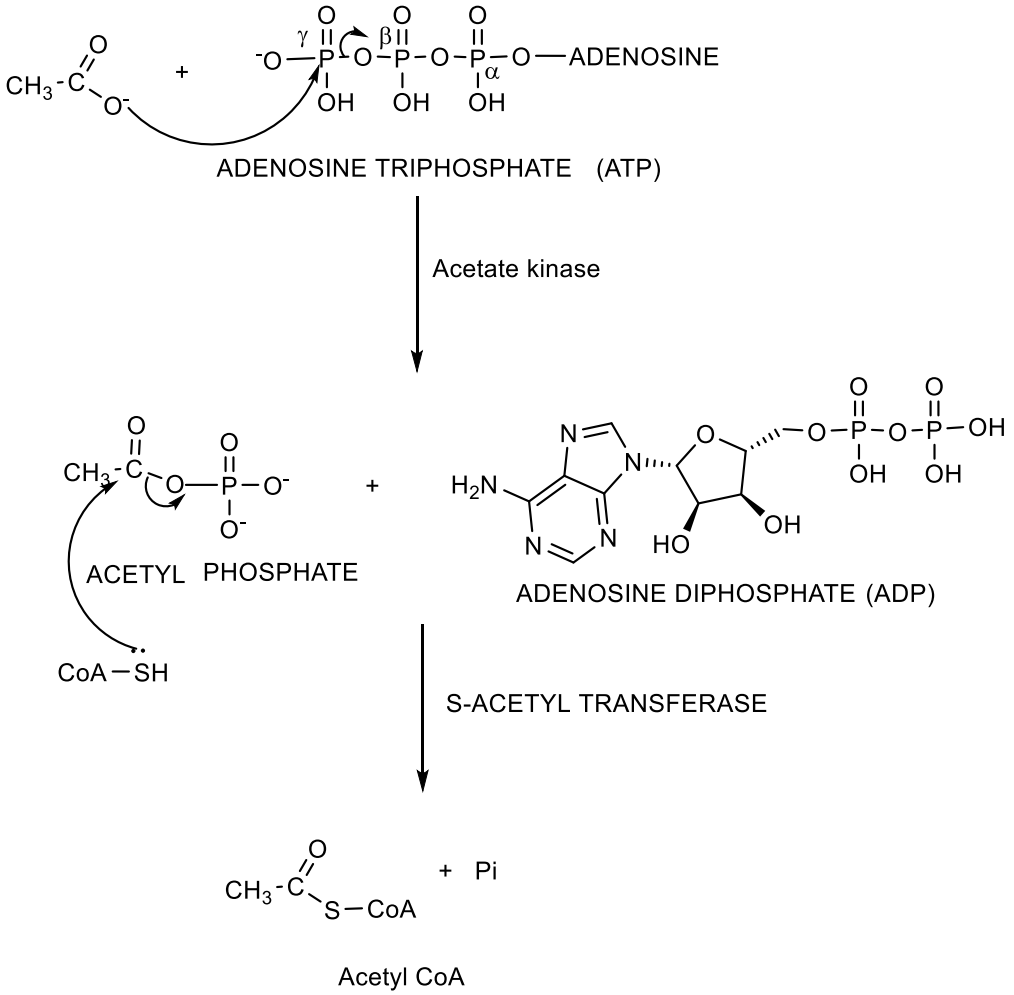


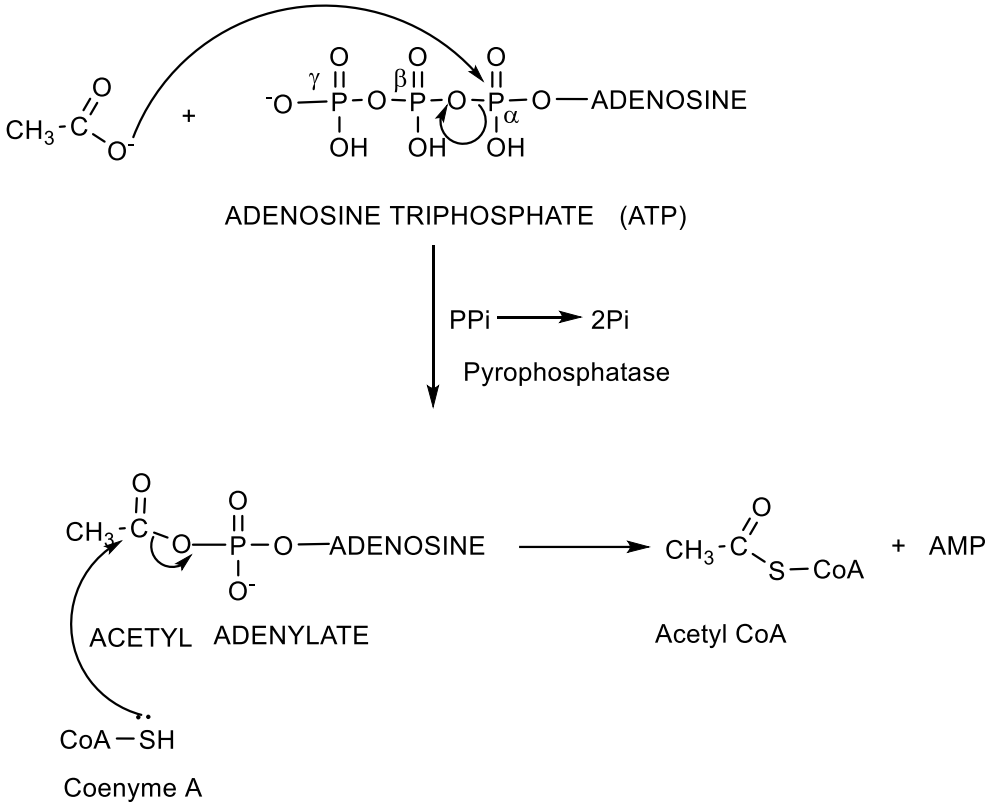
Fig. 6.8 Synthesis of acetyl-CoA in bacteria

The cleavage of ATP provides the energy needed for the synthesis of acetyl-CoA, which has a high group transfer potential. In this manner, acetyl-CoA production and the energy of ATP hydrolysis are linked.

Acetate + Coenzyme A □→ Acetyl-CoA ΔG = + 35.1 kJ/mol

ATP + H2O → ADP + Pi ΔG = − 34 .5 kJ/mol

The two processes required for the synthesis of acetyl-CoA from acetate are catalysed by the same enzyme, acetyl-CoA synthetase, also referred to as acetate thiokinase, in the majority of eukaryotic cells. The displacement of the adenylyl group from the ATP's phosphorus atom by the nucleophilic attack of the carboxylate's O atom to produce acetyl adenylate, followed by the displacement of the AMP group from the carbon atom, is how two processes are coupled.



Fatty acyl CoA synthetase is an enzyme that catalyses a process similar to acetyl CoA synthetase. With the help of the cleavage of ATP into AMP + PPi and the production of fatty acyl-CoA, this enzyme catalyses the activation of fatty acids. Two stages are required to complete the reaction:

1. To produce a fatty acyl-adenylate and PPi, which are then quickly hydrolyzed into two molecules of Pi, the oxygen atom of the carboxylate group of the fatty acid displaces the adenylyl group on the phosphate atom of ATP.

2. To generate the thioester fatty acyl-CoA, the coenzyme A thiol group displaces AMP and causes the second carbon atom to move.

6.6 TRANSFER OF SULPHATE

Natural product sulfation is a common occurrence. The enzyme ATP sulphurylase, which has been investigated using kinetic and stereochemical techniques, is responsible for bringing inorganic sulphate to cells and activating it there. It has been demonstrated that the enzyme catalyses the direct "in line" displacement of inorganic pyrophosphate by inorganic sulphate from P of ATP. The resulting adenosine 5'-phosphosulphate is then phosphorylated by APS kinase at the 3' site to produce 3'-phosphoadenosine 5'-phosphosulphate, the most prevalent sulphating species in biology.

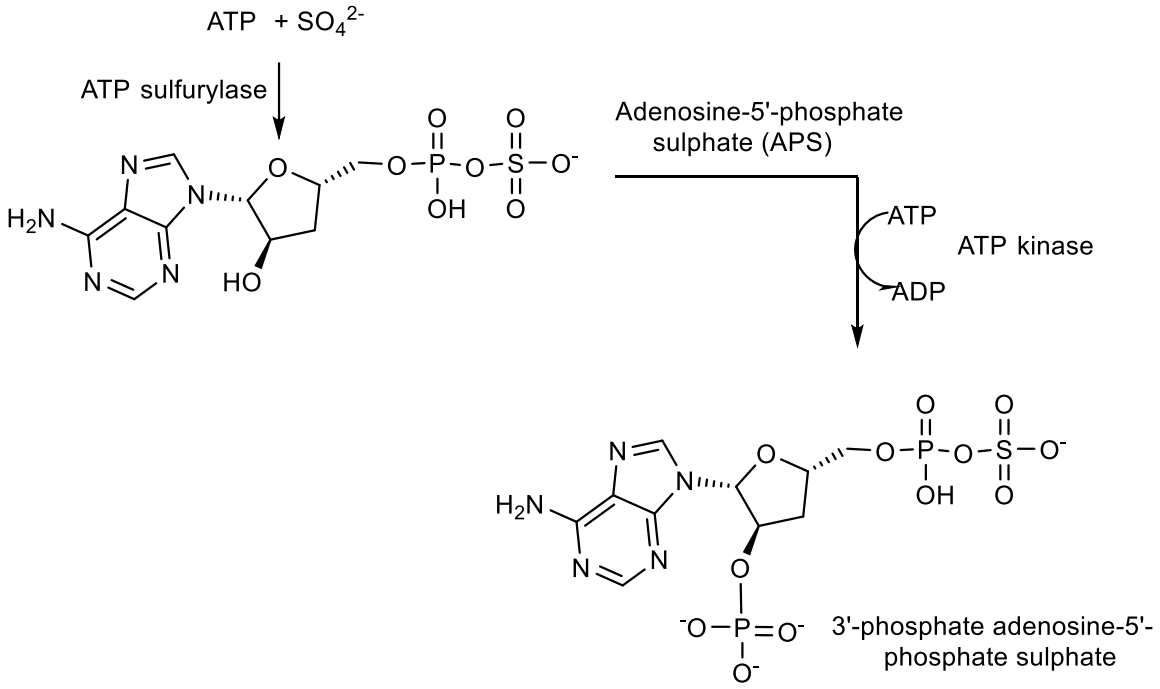
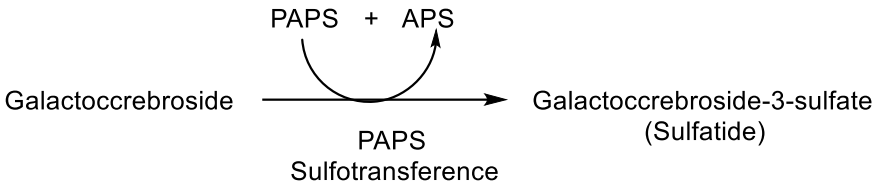


Fig. 6.9 Activation of sulphate

Fourier Transform Infrared Spectroscopy has been used to build a method for the stereochemical analysis of chiral [$^{16}O^{17}O^{18}O$]-sulphate esters. A generic strategy for their synthesis has also been proposed. It has been demonstrated that an Aspergillus oryzae aryl sulphotransferase's stereochemical course proceeds with the retention of configuration at sulphur, supporting a ping-pong-type mechanism with a sulpho-enzyme intermediate on the reaction pathway.During enzymatic reactions mediated by sulfotransferases, nucleophilic displacements of the sulphur atom also take place. These enzymes transfer the sulphate group from 3′-phospho-adenosine-5′-phospho sulphate (PAPS) to the oxygen and nitrogen atoms of

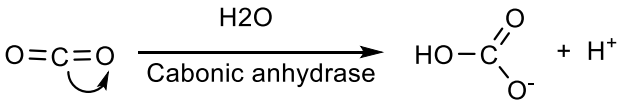
an acceptor molecule. PAPS, also known as activated sulphate, is created when the enzymes ATP sulfurylase and APS kinase work together to create sulphate.

Sulfatides (15 percent of white matter lipids in the brain) are formed, for example, by transferring the sulphate group from PAPS to the C3-OH group of galactose in cerebroside (a component of brain lipids).



6.7 ADDITION REACTIONS

In an addition reaction, a nucleophile and a proton are added to a polarised double bond, such as C=O or C=N. If the C=C bond has been polarised by conjugation with C=O or C=N, the nucleophile may also attack it. Alcohols, amines, and thiols are the most common nucleophiles used because they quickly attack the electrophilic carbon atom of the carbonyl group. The water molecule can add to the carbonyl group by acting as a nucleophile. The transformation of CO2 into bicarbonate ion, which is catalysed by the enzyme carbonic anhydrase, is an illustration of an addition process.



The Zn^{2+} ion that is coupled to the protein is tetrahedrally connected to three histidine residues, and a water molecule occupies the fourth co-ordination site. As a base, His 64 pulls a proton from the water molecule and binds it to Zn^{2+} to create an OH ion. However, the distance between His 64 and the water-bound Zn^{2+} prevents it from directly abstracting a proton; as a result, a hydrogen bonding network between these two molecules connects them. The hydrogen-bonded network functions as a proton shuttle. As a result, the Zn^{2+}-bound OH attacks the CO_2 substrate to change it into HCO_3^- by acting as a nucleophile. The production of imine intermediates is a common step in many enzymatic processes. These imine intermediates are called Schiff base. The formation of amine intermediates involves the amine engaging in a nucleophilic assault on the carbonyl group, followed by the removal of the OH ion.

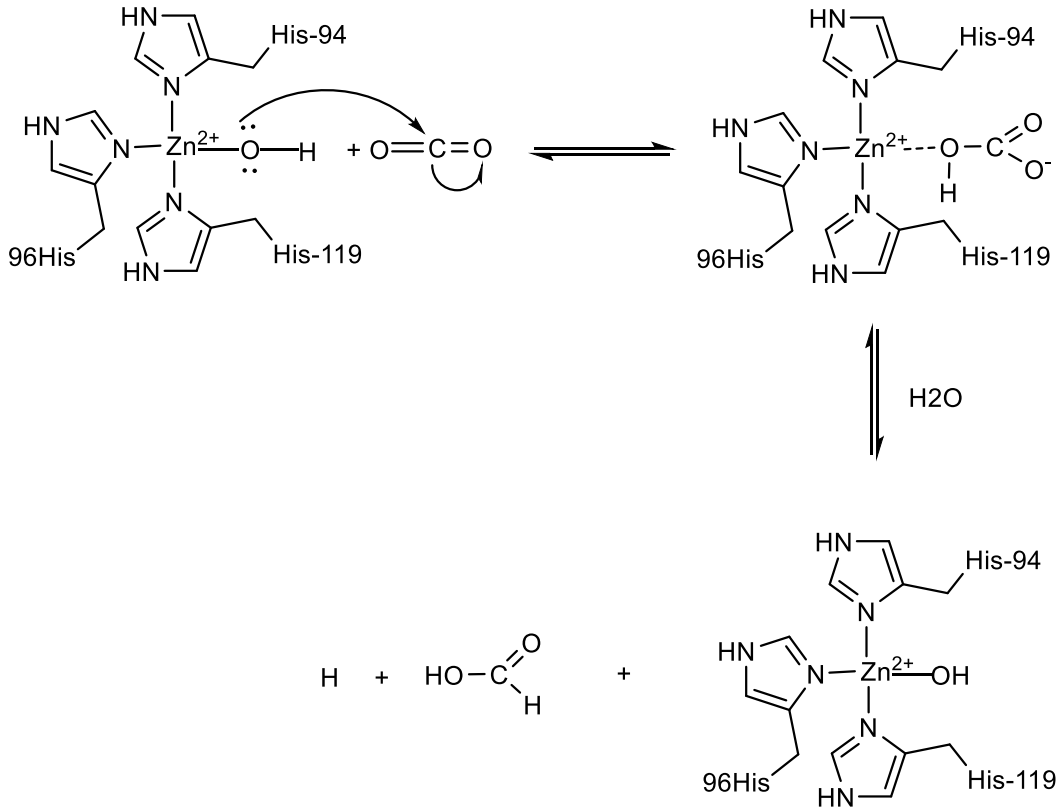
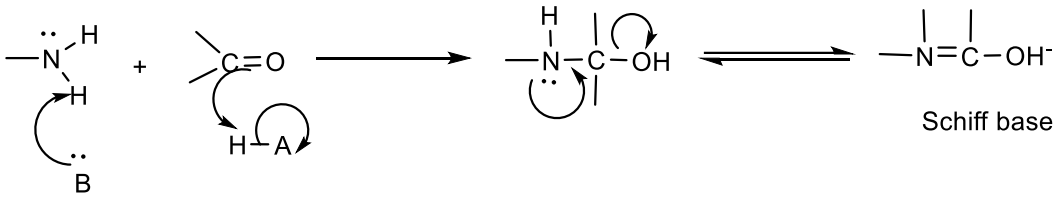
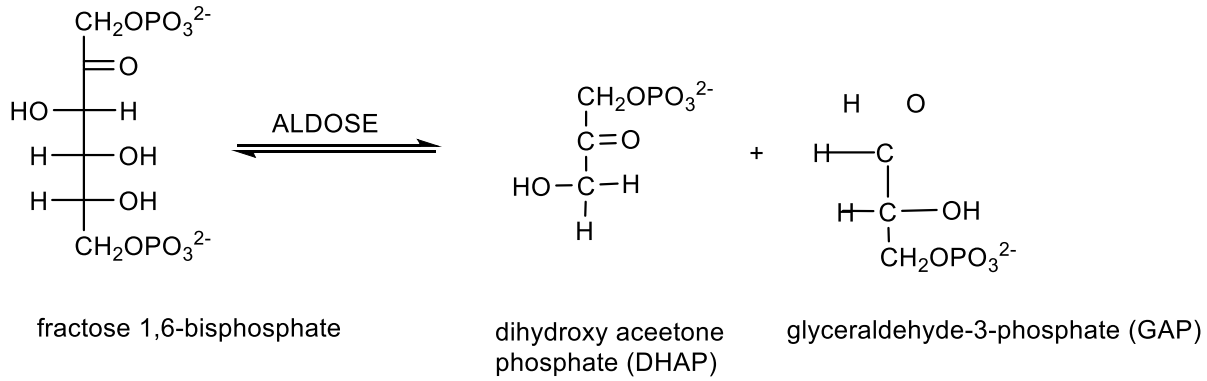


Fig 6.10 Mechanism of action of carbonic anhydrase

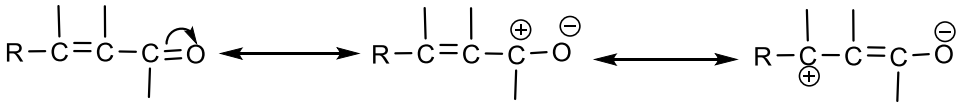


At its active site, the lysine side chain of the enzyme aldolase forms Schiff bases with the substrate's ketonic group. Fructose-1,6-bisphosphate is transformed into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate by the enzyme aldolase.

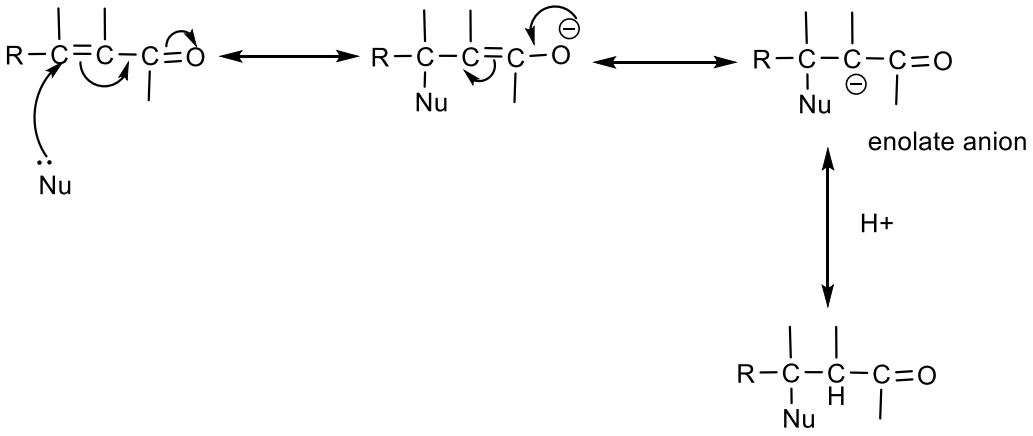


The protonated Schiff base, an iminium cation, is created when the substrate fructose 1,6-bisphosphate binds to the lysine residue in the aldolase enzyme's active site. The release of glyceraldehyde-3-phosphate, the initial reaction product, causes the cleavage of the C—C link, which causes the creation of the intermediate enamine. Dihydroxyacetone phosphate is the second result of the reaction with the regeneration of free enzyme after protonation of the enamine to iminium cation and hydrolysis.

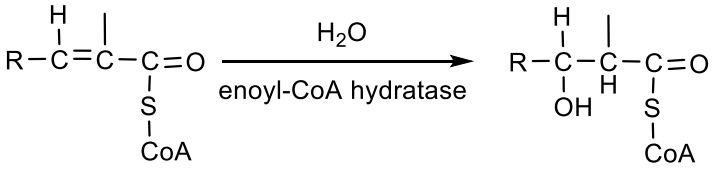
There are other metabolic processes in which the nucleophile is also added to the C=C bond if the C=C bond is conjugated with the C=O bond and the polarisation from the C=O is transferred to the C=C bond.



At the carbon of the carbonyl group, the nucleophile adds to the carbon double bond. By dispersing negative charge, the added product stabilises itself. The reaction is finished by adding a proton to the enolate anion.

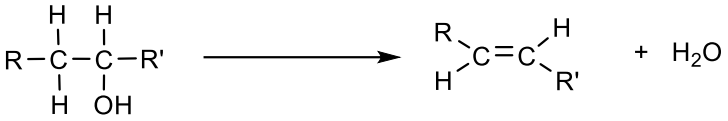


A typical example of this reaction is the addition of water molecule to α,β-unsaturated CoA derivative catalyzed by the enzyme enoyl-CoA hydrase.



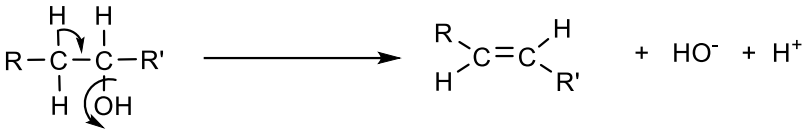
6.8 ELIMINATION REACTIONS

Small molecules like H_2O, NH_3, and other similar molecules are eliminated in elimination reactions by creating a double bond between the carbon atoms from which the atoms or groups are separated.

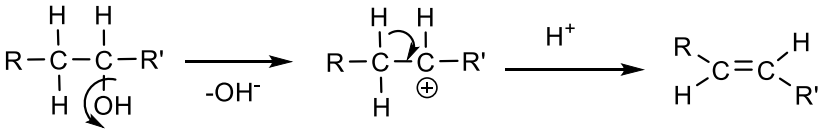


Reaction proceeds buy any of tree mechanism

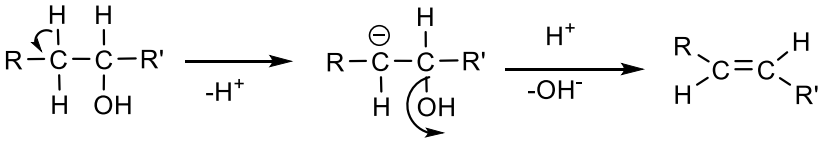
1. By concerted reaction



2. By the formation of a carbocation resulted by the breaking of C-O bond



3. By the formation of a carbanion resulted by the breaking of C-H bond



However, enzymes either use one of two mechanisms to catalyse the dehydration reaction:

(i) Acid catalysis, or protonation of the OH group by an acidic group.

(ii) Proton extraction by a basic group, also known as base catalysis.

The dehydration of 2-phosphoglycerate to phosphoenol pyruvate, which is catalysed by the enzyme enolase, is a classic illustration of an elimination reaction.

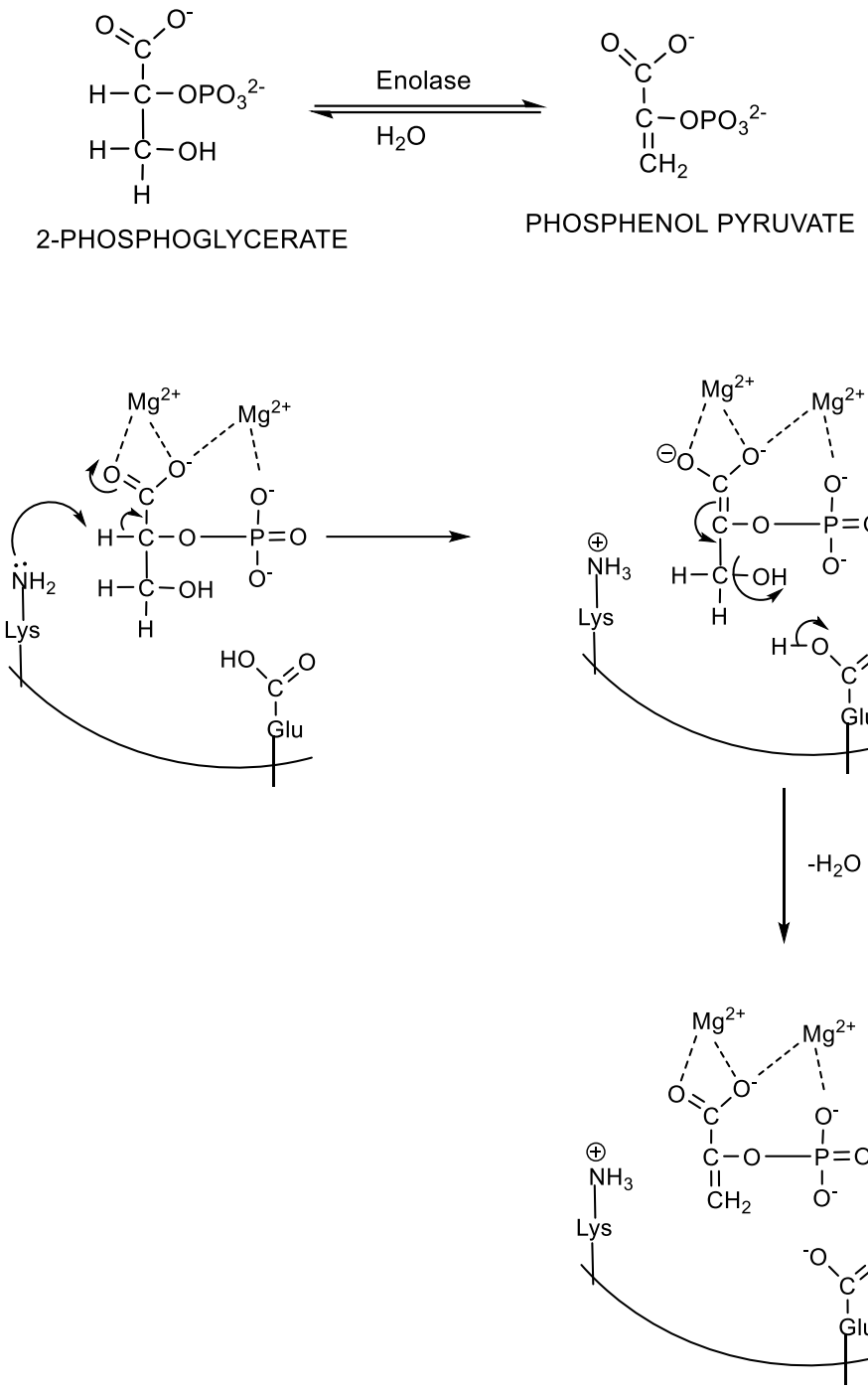


Fig.6.11 Mechanism of action of the enzyme enolase

The two bound Mg^{2+} ions in the enzyme's active site interact strongly with the substrate 2-phosphoglycerate, increasing the acidity of the C—2 proton. Before the substrate binds to the enzyme, it forms a combination with Mg^{2+}. In the first phase of the reaction, the amino acid Lys-345 functions as a base to remove a proton from the 2-phosphoglycerate's C—2, and in the

second step, the amino acid Glu-211 functions as an acid to provide a proton to the leaving group (—OH). As a result, this is a straightforward instance of acid-base catalysis.

6.9 ENOLIC INTERMEDIATES IN ISOMERIZATION REACTIONS

Isomerization reactions are those in which an H-atom is moved intramolecularly to alter the location of a double bond. A proton is moved from one C-atom to another in these reactions. The process proceeds through an intermediate called enediol.

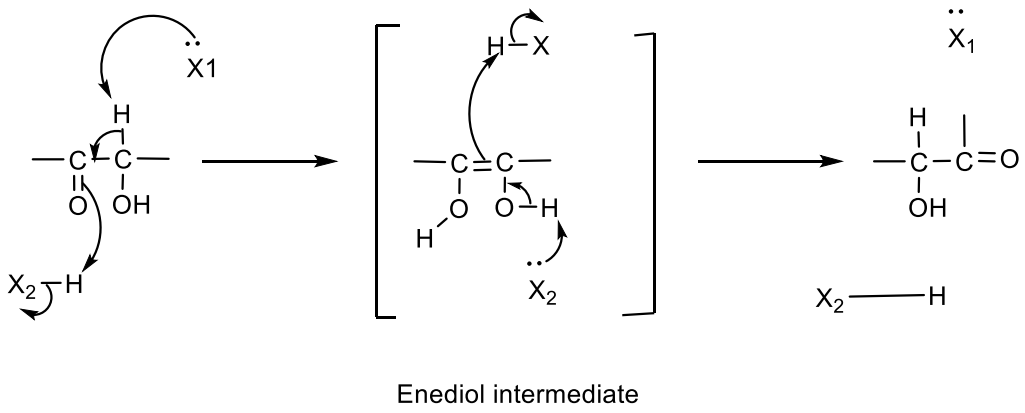
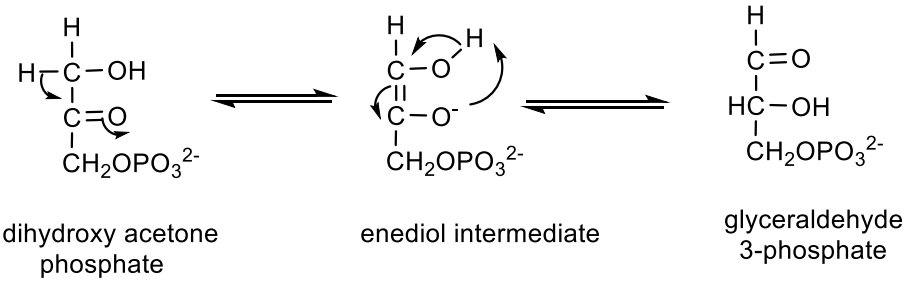


Fig.6.12 Formation of enediol intermediate

The two ionizable groups on the enzyme are X1 and X2. In the first step, X1 provides a proton and X2 donates an electron to allow the production of a C=C bond and the intermediate enediol. The second phase involves the donation of a proton by X1 and the abstraction of a proton by X2, which allows the C=O bond to form. The C—H bond is created as a result of the displacement of an electron pair from the C=C bond. The enzyme triose phosphate isomerase catalyses the conversion of dihydroxy acetone phosphate to glyceraldehyde-3-phosphate, which is an illustration of an isomerization reaction. The enediol intermediate is formed as the reaction progresses.



The catalytic mechanism of the enzyme triose phosphate isomerase involves the abstraction of a proton from the substrate by glutamate residue and the donation of a proton by lysine residue, resulting in the creation of the intermediate enediol. Glyceraldehyde-3-phosphate is produced in the second stage by proton donation by glutamate and proton abstraction by lysine.

The transformation of glucose-6-phosphate into fructose-6-phosphate, which is catalysed by the enzyme phosphoglucose isomerase, is another illustration of an isomerization reaction. In order to create a cis-enediol intermediate, a base from the imidazole component of the His-Glu dyad abstracts a proton from the C-2 atom. In a whole proton transfer, a proton is transferred onto the C-1 atom.

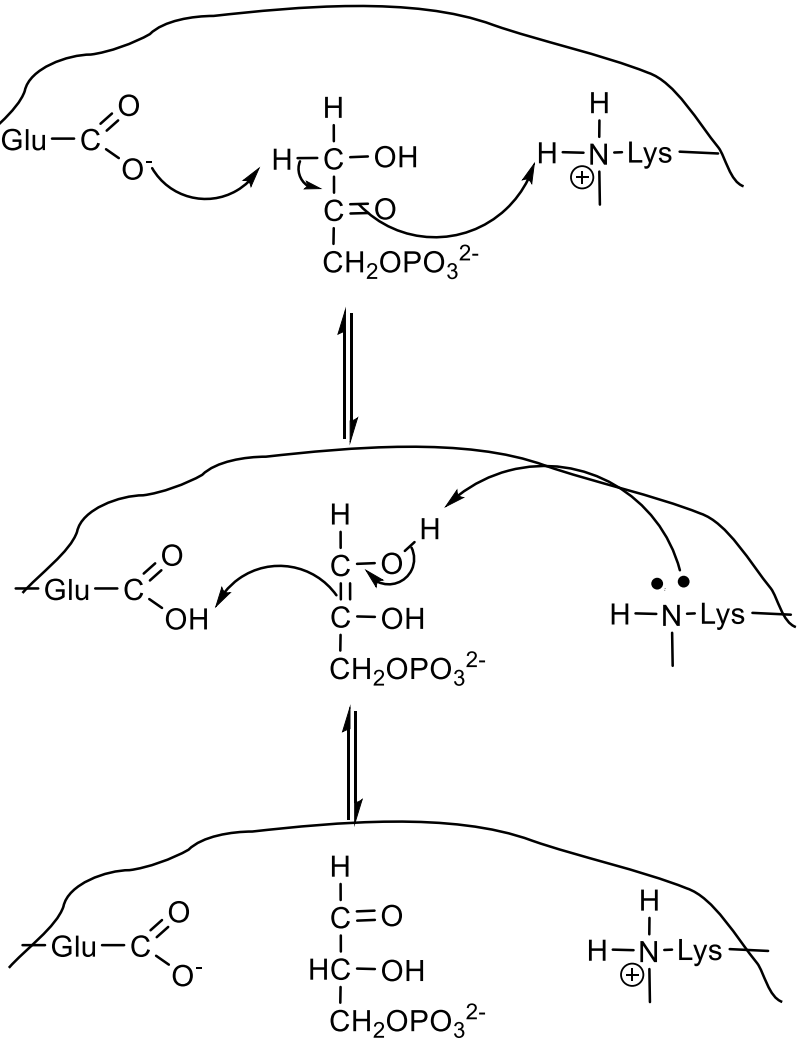


Fig. 6.13 Mechanism oF action of triose phosphate isomerase

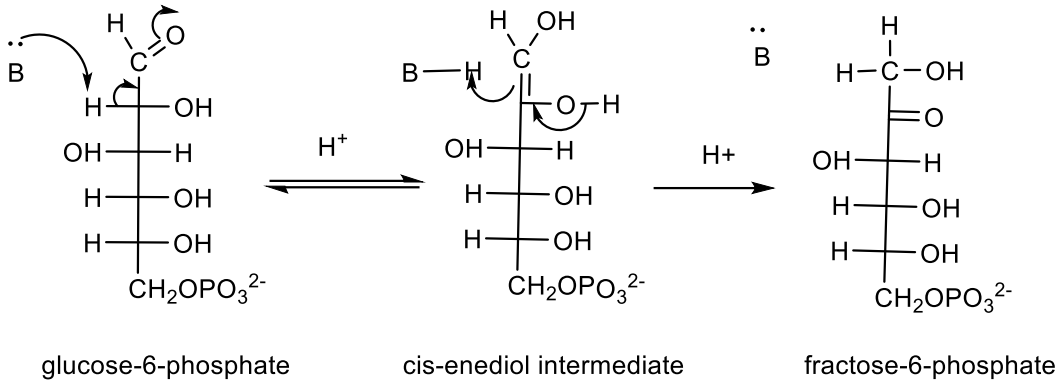
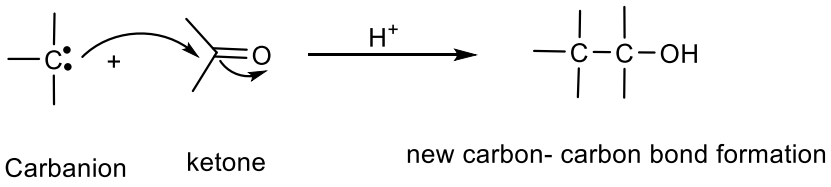


Fig. 6.14 Conversion of glucose-6-phosphateto fructose-6-phosphate

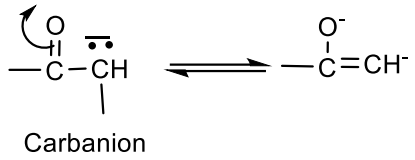
Isopentenyl pyrophosphate isomerase, an enzyme, catalyses the conversion of isopentenyl pyrophosphate to dimethyl allyl pyrophosphate in the cholesterol biosynthesis process, which is another example of an isomerization reaction. An intermediate carbocation is created when the enzyme's Cys residue acts as a proton donor by donating a proton to the substrate. As the product is formed, the Glu-residue simultaneously removes a proton from the intermediate carbocation.

6.10 CLEAVASE AND CONDENSATION

The processes that form or dissolve carbon-carbon (C—C) bonds are a part of the catabolic and anabolic pathways of metabolism. Organic molecules' tightly bonded carbon skeleton makes it difficult to create or break them. A nucleophilic carbanion must be joined to an electrophilic carbon atom in order to form a C—C bond. Due to its ability to extract electrons, the carbonyl atom in aldehydes, ketone esters, and CO2 is the most frequently electrophilic carbon atom in these processes.



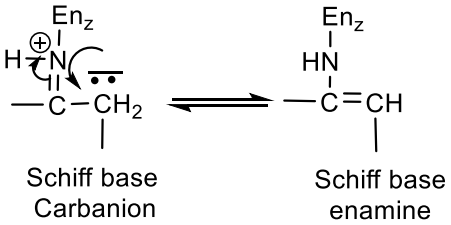
To add to an electrophilic centre, one needs a stabilised nucleophilic carbanion. A resonance stabilised carbanion, or enolate, is produced by the carbon atom of the carboxylate group on an adjacent carbon. This enolate anion acts as a nucleophile.. The cleavage and production of the C—C bond are thus made easier by the carbonyl group.

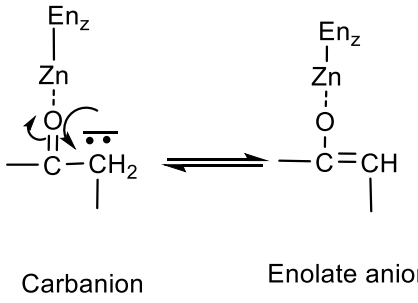


Aldol cleavage, Claisen condensation, and decarboxylation of β-keto acids are examples of processes of this sort. These reactions involve the formation and breaking of bonds between α and β carbon atoms of a carbonyl compound and will be referred to as β-condensation and β-cleavage.

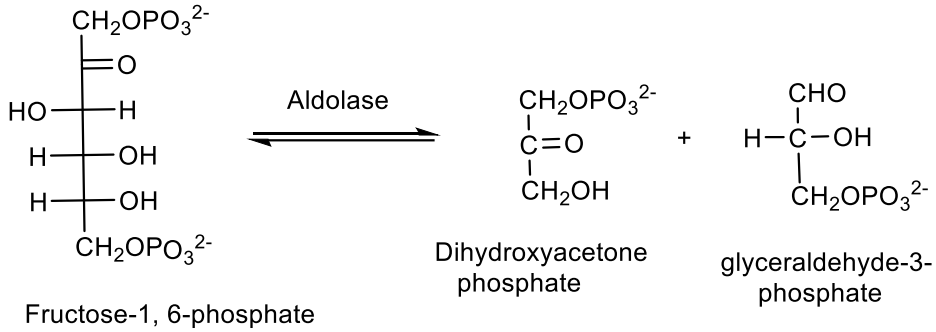
6.10.1 Aldol Cleavage

A typical example of aldol cleavage, which is a common reaction of C—C bond formation, is the reaction that fructose bisphosphate aldolase catalyses during glycolysis. By stabilising its enolate intermediate, which functions as a nucleophile, aldol cleavage is catalysed. Aldolases come in type I and type II varieties. The intermediate enolate ion is stabilised by type I aldolases, which are found in both plants and mammals. They achieve this by converting the carbonyl group into a protonated Schiff base. The enolate ion is stabilised by the type-II aldolases, which are found in fungi, algae, and some bacteria. They achieve this by coordinating the enolate ion with a metal ion, usually Zn^{2+} or Fe^{2+}.



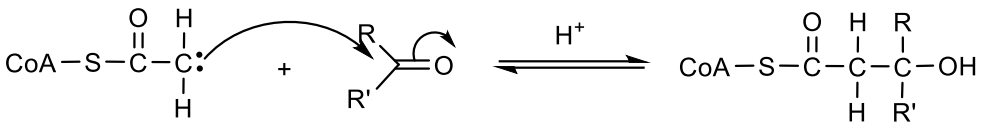


A type I aldolase called fructose bisphosphate aldolase is responsible for converting fructose-1,6 bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate during glycolysis.



6.10.2 Claisen Condensation

One instance of claisen ester condensation in which the carbanion is stabilised by the carboxyl of a neighbouring thioester is the reaction catalysed by citrate synthesis during the citric acid cycle.



Acetyl CoA and oxaloacetate are condensed in the first phase of the citric acid cycle, which is catalysed by citrate synthase (ketone). Due to the fact that the substrate (acetyl CoA) is a thioester, the reaction is known as Claisen ester condensation. There are three steps involved in the reaction those citrate synthase catalyses.

- An enolate of acetyl CoA is produced when the enzyme Asp-375 removes a proton from the methyl group. The H bonds to His 274 stabilise the enolate intermediate.
- Citryl-CoA is created when the acetyl CoA enolate nucleophilically attacks the carbonyl carbon of oxaloacetate by donating a proton from His-320 to the carboxyl group of oxaloacetate.
- After that, citryl-CoA is hydrolyzed to produce citrate and coenzyme A.

The formation of acetoacetyl CoA, a precursor to cholesterol, from the condensation of two acetyl CoA molecules by thiolase is sometimes referred to as a claisen condensation.

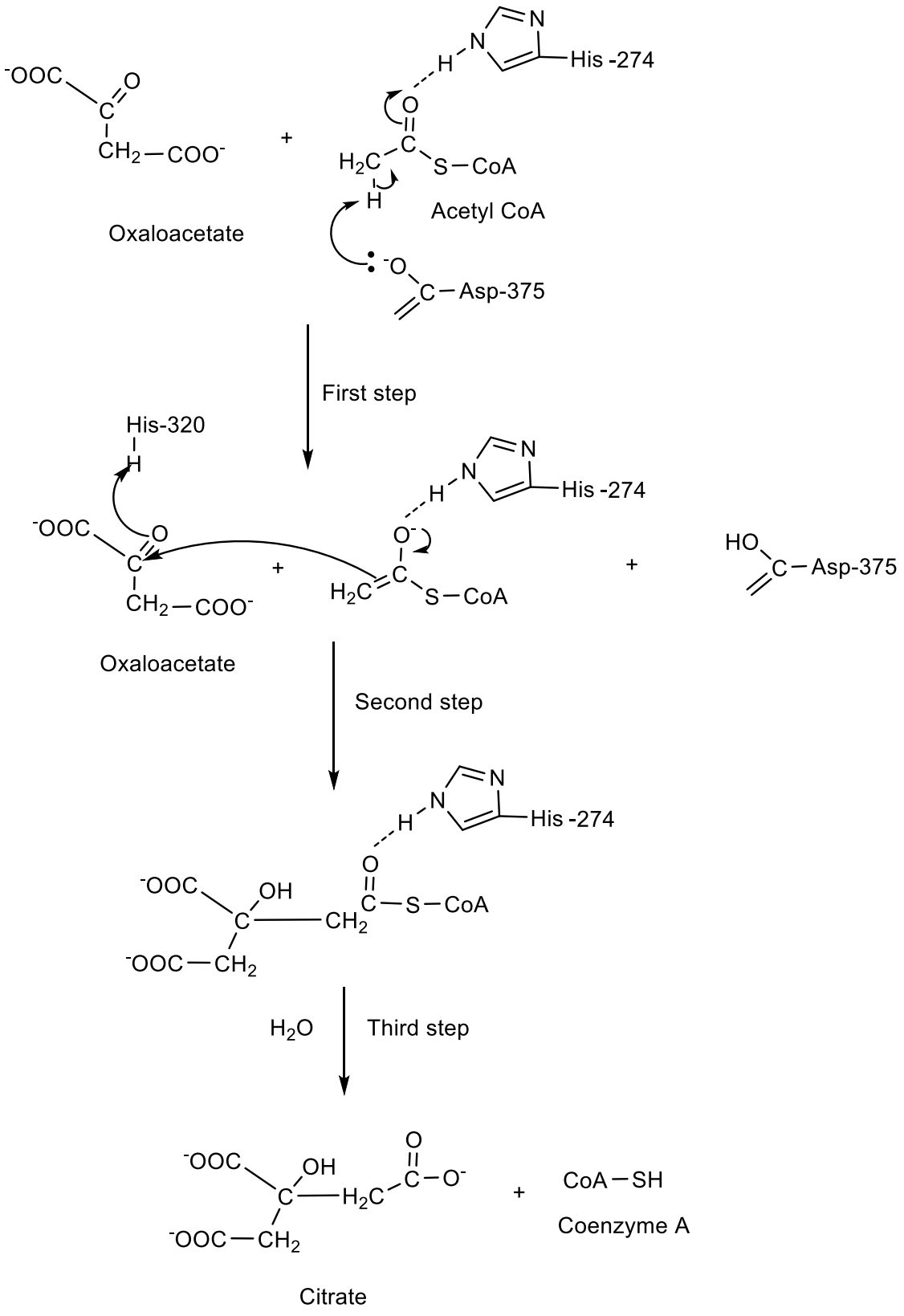
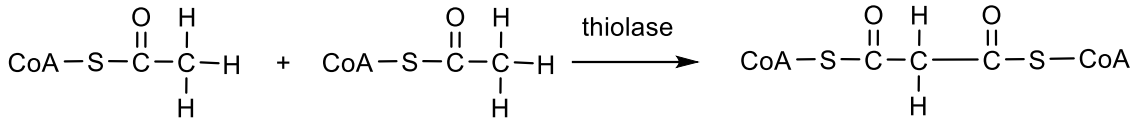
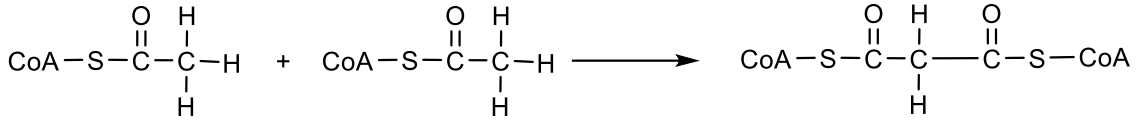


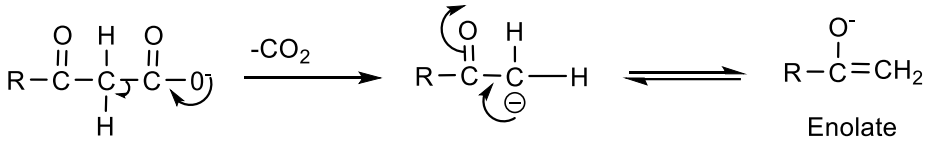
Fig. 6.15 Claisen Condensation





6.10.3 Decarboxylation of β-keto acids

A resonance stabilised carbanion is produced after the decarboxylation of β-keto acids by the removal of CO_2. An illustration of this kind of reaction is the acetoacetate decarboxylase catalysed process.

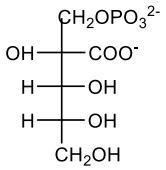


6.11 ENZYME CATALYZED CARBOXYLATION ANI DECARBOXYLATION

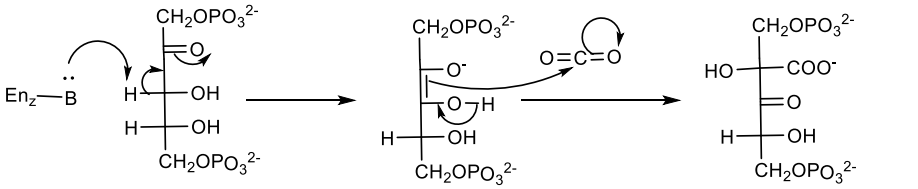
The most important C—C bond building and bond breaking reactions in biological processes result in the gain or loss of one carbon, in the form of CO_2. Carboxylation refers to the addition of a CO_2 unit to a substrate molecule, whereas decarboxylation refers to the removal of carbon in the form of CO_2. The bulk of carboxylation reactions that occur in metabolic pathways are catalysed by RuBisCO and biotin-dependent carboxylases. The enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO) catalyses the synthesis of 3-phosphoglycerate from ribulose-1, 5-bisphosphate (RuBP).

The most prevalent protein on the planet, this enzyme contains up to 50% of leaf proteins. The 500–560 KD protein known as RuBisCO from higher plants is made up of eight large (L) subunits that are encoded by chloroplast DNA and eight tiny (S) subunits that are encoded by a series of nuclear genes. At the top and bottom of the protein, eight small subunits are

grouped as two caps (tetramer), while eight big subunits are present in the space between the two caps. The L-subunit contains the catalytic site of the enzyme. The removal of a proton from RuBP's C-3, which results in the production of an enediolate, initiates the carboxylation process of RuBisCO.The intermediate enediolate then engages in nucleophilic attack on CO2 to produce a β-keto acid. This β-keto acid then reacts with water to produce an adduct, which splits to produce a molecule of 3-phosphoglycerate and an intermediate carbanion. In order to create a second molecule of 3-phosphoglycerate, the carbanion is protonated. The finding that the homolog of the -keto acid intermediate, 2-carboxyarabinitol-1-phosphate (CA1P), binds closely to the active site of the enzyme from spinach supports this enzyme's mechanism.

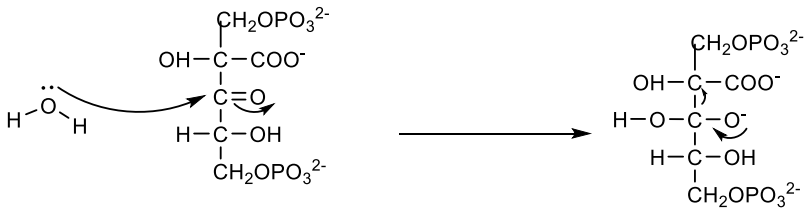


2-carboxyarabinitol-1-phosphate



Ribulose-1, 5-bisphosphate (RuBP) | Enediolate intermediate | 2-carboxy-3-keto arabinitol-1,5-bisphosphate (keto Acid)

(A) Production of an enediolate intermediate that attacks CO_2 nucleophilically to produce a keto acid.



2- carboxy-3-keto arabinitol 1,5-bisphosphate (β–keto acid)

Cleavage

Carboanion | 3-phosphoglycerate

(B) Water and β-keto acid combine to generate an adduct that breaks into 3-phosphoglycerate and carbanion.

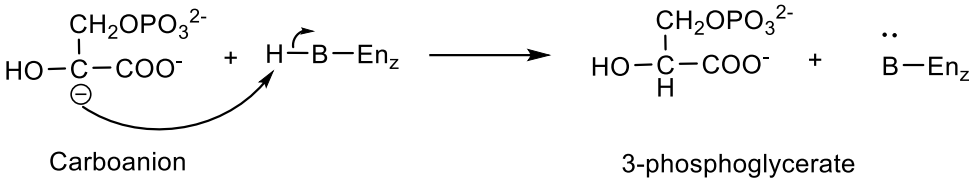
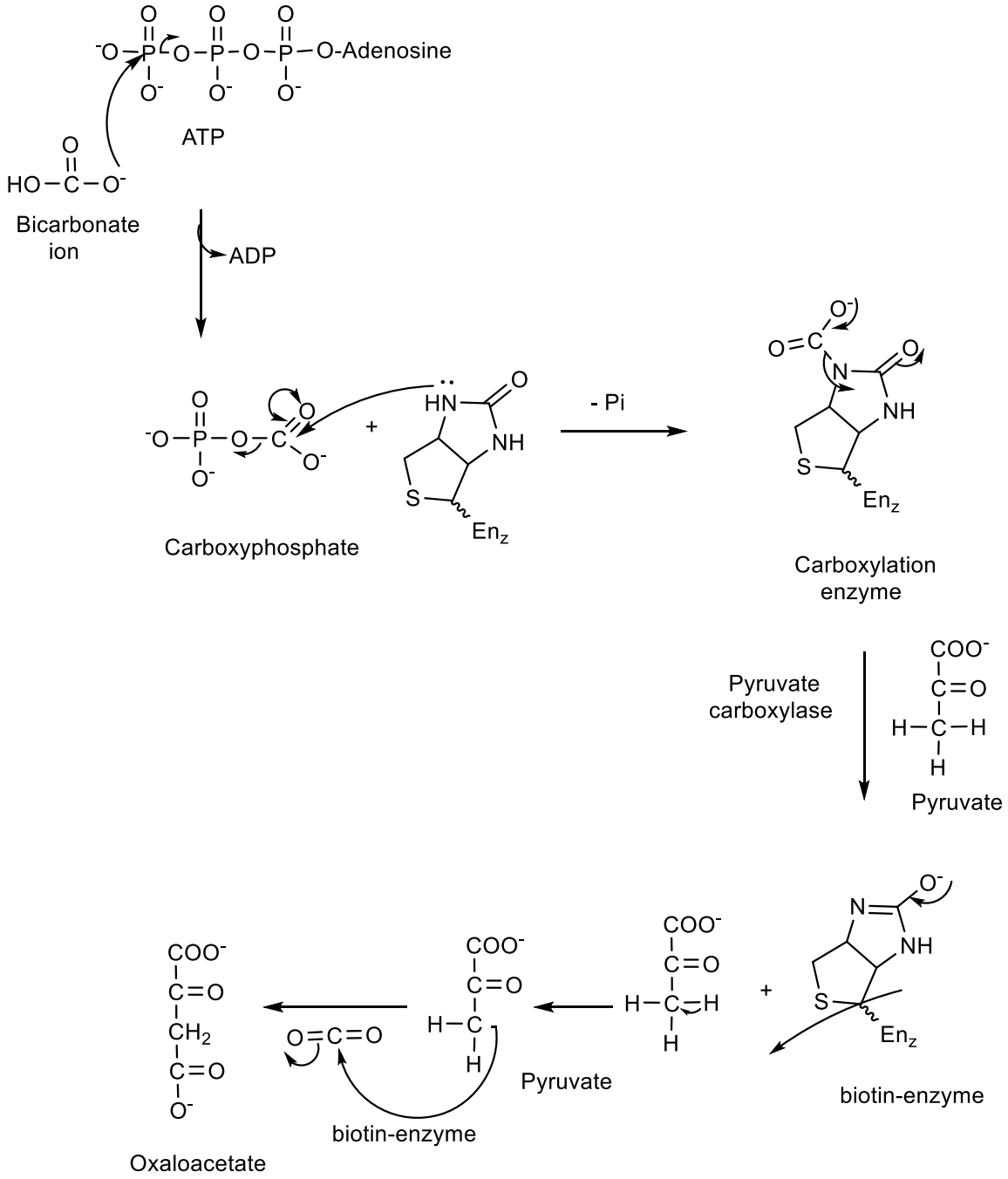


Fig. 6.16 The carboxylation reaction catalyzed by RuBisCO

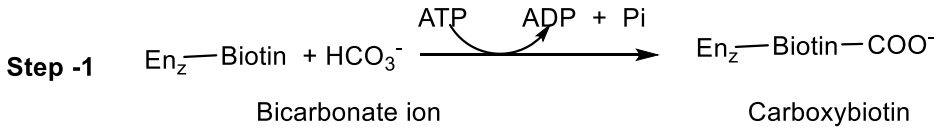


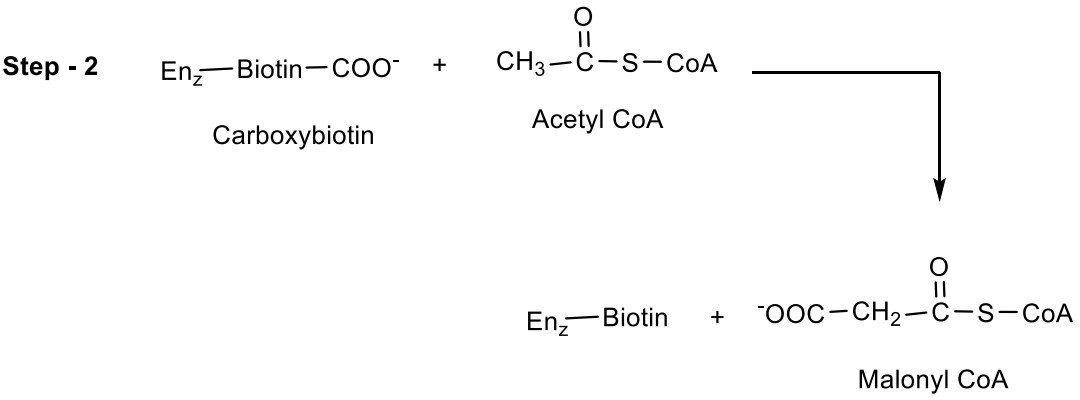
Bacteria use phosphoenol pyruvate (PEP) carboxylase for biosynthetic purposes, and C4 plants like sugarcane and corn use it as part of a CO2 concentrating mechanism. In the leaves of C4 plants, mesophyll cells encircle a single layer of bundle sheath cells in the leaves. The PEP carboxylase but not the RuBisCO enzyme is present in the mesophyll cells. In the mesophyll cells, the PEP carboxylase combines HCO_3^- with PEP to produce oxaloacetate. Malate, which is transferred to the bundle sheath cells, is created by the reduction of oxaloacetate.In animals, the pyruvate carboxylase catalyses the carboxylation of pyruvate with ATP cleavage to produce oxaloacetate (OAA). A biotin prosthetic group on pyruvate carboxylase serves as a CO_2 carrier during carboxylation. Two stages make up the pyruvate carboxylase reaction:

(i) An enzyme-catalyzed transfer of a phosphoryl group from ATP to bicarbonate results in the formation of a carboxyphosphate. Biotin is carboxylated when its N1 atom hits the C-atomof carboxy phosphate.

(ii) Oxaloacetate is produced when the activated carboxyl group from carboxybiotin is transferred to the pyruvate's carbon.

Acetyl-CoA carboxylase, which also facilitated the first committed step of fatty acid biosynthesis, catalyses a similar process. Two stages are needed to complete the reaction.





The enolate ions generated during the carboxylation reaction are used in carbon-carbon (C—C) bond formation. Decarboxylation in metabolism can be either non-oxidative or oxidative. The reaction catalysed by PEP carboxykinase is a good example of non-oxidative decarboxylation.

Phosphoenol pyruvate (PEP), a metabolic intermediate, is the phosphate ester of pyruvate which is formed by GTP-driven decarboxylation of oxaloacetate (OAA). The reaction is catalysed by PEP carboxykinase (PEPCK). The decarboxylation of OAA generates an enolate anion whose oxygen atom attacks the phosphate group of GTP, forming PEP.

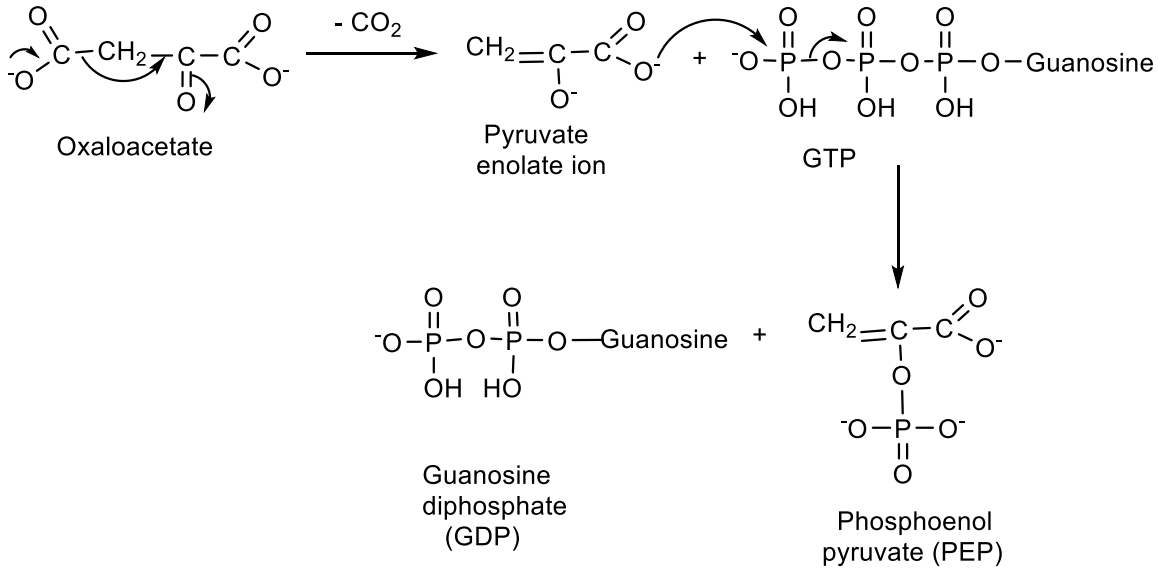


Fig. 6.17 The catalytic mechanism of reaction catalyzed by PEP carboxykinase

A β-hydroxy acid is converted to a β-keto acid through the process of oxidative decarboxylation, which is followed by its decarboxylation. Isocitrate dehydrogenase, malic enzyme, and -ketoglutarate dehydrogenase all catalyse this kind of reaction.

6.12 SUMMARY

The present chapter is summarised as follows:

- "Metabolic reactions catalysed by enzymes are categorised into 5 classes: substitution, addition, elimination, isomerization, and rearrangement reactions.
- In a substitution reaction, one atom or group is swapped out for another. In an electrophilic substitution reaction, the atom or group is replaced by an electrophile, and in a nucleophilic substitution reaction, a nucleophile.
- On saturated C-atoms, carbonyl C-atoms, phosphorus atoms, and sulphur atoms, a nucleophilic substitution process can occur. The endergonic process and ATP hydrolysis are coupled through a number of displacement processes.

- The reactions known as addition reactions occur when the attacking reagent adds to the substrate. Small molecules are removed during the product generation process in elimination reactions.
- The intramolecular displacement of the H atom to modify the location of the double bond occurs during the isomerization reaction.
- The production of the intermediate enediol is the first step in isomerization reactions.
- In rearrangement processes, substituents are moved around within the molecule to create the molecule's structural isomer.
- Condensation and -cleavage are the terms used to describe the reactions that result in the production and dissolution of a C—C bond between a carbonyl group's carbon atoms, respectively. While CO2 is removed during a decarboxylation reaction, it is added to the substrate during a carboxylation reaction.

6.13 SAQs TYPE QUESTIONS

A. **Multiple choice questions**

1. The conversion of 2-phosphoglycerate into phosphoenol pyruvate:

   (a) Enolase (b) Aldolase

   (d) Mutase. (c) Isomerase

2. The following reactions transform geranyldiphosphate into geraniol:

   (a) Isomerization reaction (b) Elimination reaction

   (c) Addition reaction (d) Nucleophilic displacement

3. From dimethyl allyl pyrophosphate produced isopentenyl pyrophosphate is through the following reactions:

   (a) Schiff base (b)Tertiary carbocation

   (c) Enediol intermediate (d) Carbanion intermediate

4. Triose phosphate isomerase catalyzes the conversion of dihydroxy acetone phosphate toglyceraldehyde-3-phosphate via the formation of:

   (a) Enediol intermediate.(b) Carbocation intermediate

(c)Carbanion intermediate (d) Schiff base

5. Which of the following enzyme can catalyze the thiol disulfide exchange reaction?

(a) RNase A (b) PEP carboxylase

(c) RuBisCO(d) Thiol transferase

6. The enzyme that converts fructose-1, 6-bisphosphate into dihydroxy acetone phosphate and glyceraldehyde-3-phosphate:

(a) Enolase (b) Isomerase

(c) Mutase (d) Aldolase

7. When dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate, the reaction is catalyzed by?

(a) Pyruvate kinase (b) Phosphoglycerate mutase

(c) Triose phosphate isomerase (d) Enolase

8. Conversion of glucose-6-phosphate to fructose-6-phosphate is catalyzed by the enzyme:

(a) Aldolase (b) Phosphoglucose isomerase

(c)Enolase(d) Triose phosphate isomerase.

9. What is the name for the following reaction intermediate? $H_2N^+=CH_2$ □

(a) Nucleophilic (b) Carbanion

(c) Schiff base (d) Carbocation

10. The reaction catalyzed by acety-CoA synthetase is an example of:

(a) Carboxylation reaction.(b) Multiple displacement reaction

(c) Elimination reaction(d)Addition reaction

11. $Mg^{2+}$is an inorganic activator for the enzyme:

(a) Aldolase. (b) Carbonic anhydrase

(b) Chymotrypsin (d) Enolase

12. The reaction catalyzed by RuBisCO is:

(a) Decarboxylation of RuBP (b) Carboxylation of RuBP

(c) Carboxylation of PEP(d) Decarboxylation of β-keto acid.

13. The cleavage of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate is an example of:

(a) Aldol condensation(b) Claisen condensation

(c) Aldol cleavage (d) None of the above.

14. The enzyme which incorporates $HCO3^-$ into phosphoenol pyruvate to form oxaloacetate:

(a) PEP carboxylase(b) Aldolase

(c)RuBisCO (d) None of the above.

15. Zn^{2+} is an inorganic activator for enzyme :

(a) Chymotrypsin (b) Carbonic anhydrase

(c) Phosphate (d) Mutase

16. In nucelophilic displacement reaction on sulfur atom, which of the following act as a nucleophile?

(a) Enolate anion (b) Carboxyl oxygen

(c) Hydroxyl group(d) Thiolate anion

17. When a nucleophile attacks the α-phosphorus atom of ATP, what kind of transfer occurs?

(a) Pyrophosphoryl transfer (b) Phosphoryl transfer

(c)Adenosine transfer(d) Adenylyl transfer

18. Which of the following protein can reduce the disulfide bridges in proteins?

(a) Reduced form of thioredoxin (b) Oxidized form of thioredoxin

(c) Glutathione reductase (d) All of the above

19. Which of the following has a thioester bond?

(a) ATP(b) Phosphoenolpyruvate

(c) Phosphocreatine (d) Acetyl-CoA.

20. Which of the following is not a nucleophile that commonly participates in biochemical

reactions?

(a) Imidazole(b) Hydroxide ion

(c) Phosphorus of a phosphate group (d) Carbanion

B. Fill in the blank

(i) The exergonic hydrolysis of ATP to produce ADP and Pi supplies……. for a number of bodily functions.

(ii) A phosphoanhydride bond is broken during the beneficial reaction of phosphorylation, which transfers the ……. group from one nucleophile to another.

(i) Isomerization reactions are those in which an H-atom is moved …….to alter the location of a double bond.

(ii) First, the product ADP exhibits a weaker than expected electrostatic repulsion between the ……. charges in ATP.

(iii) A resonance stabilised carbanion is produced after the decarboxylation of β-keto acids by the removal of ……..

C. True/ False

1. A nucleophilic substitution process occurs when one nucleophile is replaced by another on a saturated carbon atom. **True/False**

2. The disulfide exchange reaction is catalyzed in its oxidized state by the protein disulfide isomerase. **True/False**

3. Dihydroxyacetone phosphate is transformed into glyceraldehyde-3-phosphate by an isomerization reaction catalyzed by the triose phosphate isomerase. **True/False**

4. The phosphorus atom of ATP nucleophilically attacks the carboxylate oxygen of acetate in the reaction mediated by acetyl-CoA synthetase. **True/False**

5. One instance of a multiple displacement reaction is the production of fatty acyl CoA from fatty acid and coenzyme A. **True/False**

6. The Claisen ester condensation reaction is the condensing of acetyl CoA with oxaloacetate.

True/False

D. Match the following

A. Berzelius's **i**.Zn^{2+}

B. Carbonic anhydrase **ii**.Schiff base

C. imine intermediates **iii**.Sugar

D. Glucose **iv**. Enzyme

Answer key

A. 1 a 2 d 3 b 4 a 5 d 6 d 7 c 8 b 9 c 10 b

11 d 12 b 13 c 14 15 d 16 d 17 d 18 a 19 d 20 c

B. i free energy, ii electrophilic phosphate, iii intramolecularly, iv four negative, v CO_2

C. 1 True 2 False 3 True 4 False 5 True 6 False

D. A vi B i C ii D iii

6.14 GLOSSARY

PEP = Phosphoenol pyruvate

RNA = Ribonucleic acid

DNA = Deoxyribonucleic acid

ATP = Adenosine triphosphate

ADP = Adenosine diphosphate

AMP = Adenosine diphosphate

GDP = Guanosine diphosphate

GTP = Guanosine diphosphate

RuBP = RIbulose-1, 5-bisphosphate

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6.17 TERMINAL QUESTIONS

1. What are processes involving nucleophilic displacement? Describe their mechanism using an appropriate example.
2. Describe the addition reaction's mechanism using an appropriate example.
3. The transformation of 2-phosphoglycerate into phosphoenol pyruvate is catalysed by the enzyme enolase. Describe the reaction's mechanics.
4. The enzyme triose phosphate isomerase catalyses the transformation of dihydroxy acetone phosphate into glyceraldehyde 3-phosphate.
5. Using an appropriate substrate, how would you explain the enolic intermediate in bio-organic isomerization? or Through enolic intermediates, discuss the involvement of enzymes in the isomerization step.
6. Describe how SN 1 and SN 2 reactions work.
7. Talk about the phosphorus atom's nucleophilic displacement process. Explain the sulfotransferase-catalyzed sulphate group transfer mechanism.

UNIT 7: CO-ENZYME CHEMISTRY

CONTENTS:

7.1 INTRODUCTION

Enzyme is the most efficient catalyst known in nature. They have the ability to enhance reaction rate by lowering the activation energy of reaction and by stabilizing the reacting molecules at their activated complex states. Enzyme catalyse a wide variety of chemical reactions, but to bring about some biochemical reaction**,** the amino acid side chain of the protein is not sufficient e.g they are less suitable for catalyzing oxidation reduction reaction and many type of group transfer reaction. Although, enzyme catalyze these reaction, in association with some other non-enzymatic substance. Such substance are called **co-factor**.

In the presence of these co-factors the catalytic activity of enzyme is greatly enhanced. Enzymes are the biological catalyst as well as act as receptor by acting with the substrate.

7.2 OBJECTIVE

After going through this unit you will be able to:

- Define Coenzyme
- Know the important Co-enzyme i.e Co-enzyme A, Nicotinamide nucleotide, Flavin nucleotides and lipoic acid
- Know the mechanism of oxido-reduction by NAD^+/ $NADP^+$
- Understand NADH and NADPH dependent reaction
- Learn about the metabolic role of Co-enzyme

7.3 CO-FACTORS

Co-factors assist the enzymes in their catalytic action. A catalytically active enzyme co-factor complex is called haloenzyme. The enzymatically inactive protein resulting from the removal of co-factor is called an apoenzyme.

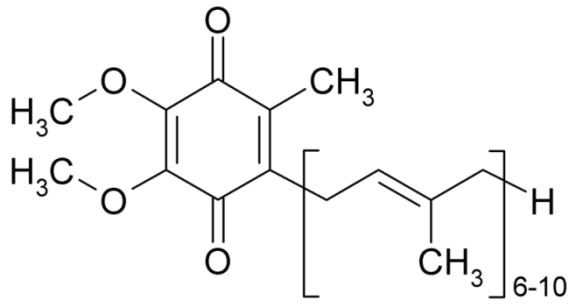
Apoenzyme + Co-factor → Haloenzyme

Co-factor may be metal ions, such as Zn^{+2} required for the catalytic activity of carboxypeptidase-A. Such metal ion bound enzyme is called metallo enzymes. If the co-factors are organic molecules, these are called coenzyme. Some factor like NAD^+ is transiently associated with a given enzyme. Other co-factors, which are permanently associated with the protein by covalent linkage are called prosthetic group e.g., heme, the prosthetic group of haemoglobin is tightly bound to its protein through covalent bond along with extensive hydrogen bonding and hydrophobic interaction.

7.4 CO-ENZYME

Coenzyme as derived from vitamins are organic molecules required by many enzymes for catalytic activity.Co-enzymes are transiently associated with a given enzyme and function as co-substrates,e.g.,an enzyme alcohol dehydrogenase utilize NAD^+ as coenzyme in the catalytic oxidation of primary or secondary alcohols.Coenzyme plays vital roles in

biochemical reactions,e.g.,they transfer atoms or groups from substrate to other molecules,they participate in redox reactions and thus help in electron transfer,they function as hydrogen acceptors like NAD^+ or NADP,and they provide nucleophilic sites for the biochemical reactions.



7.5 COENZYME A

Coenzyme A has a complex structure consisting of an adenosine triphosphate, a pantothenic acid which is a vitamin and cysteamine. The coenzyme is involved in transfer of acyl-groups. The sulfhydryl (-SH) group of cysteamine moiety of this coenzyme forms a thioester with the carboxyl (-COOH) group of the acyl-compound, such as acetic acid to produce acetyl-CoA which is one of the most important CoA derivatives. The thioester bond is energy-rich and can easily transfer the acetyl-group to an acceptor.Coenzyme A is an acyl activating enzyme derived from vitamin pantothenic acid. Panthotheric acid is pentoic acid and β alanine joined together in a amide linkageCoenzyme A can bedivided into two components, adenosine 3, 5 diphosphate and pantotheine, which is formed the combination of pantothenic acid and βmercaptoethylamine.

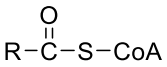
The SH group of thioethanol amine moeity is an active group acting as carrier and coenzyme A is abbreviated as CoA or CoASH. The rest of molecule provides enzyme binding site. In acylated derivatives, such as acetyl-coenzyme A, the acyl group is linked to thiol group of form an energy rich thio ester.

CoA reacts with many compounds to form important derivatives such as:

- Acetyl CoA
- Succinyl CoA

- HMG CoA
- Fatty acyl CoA

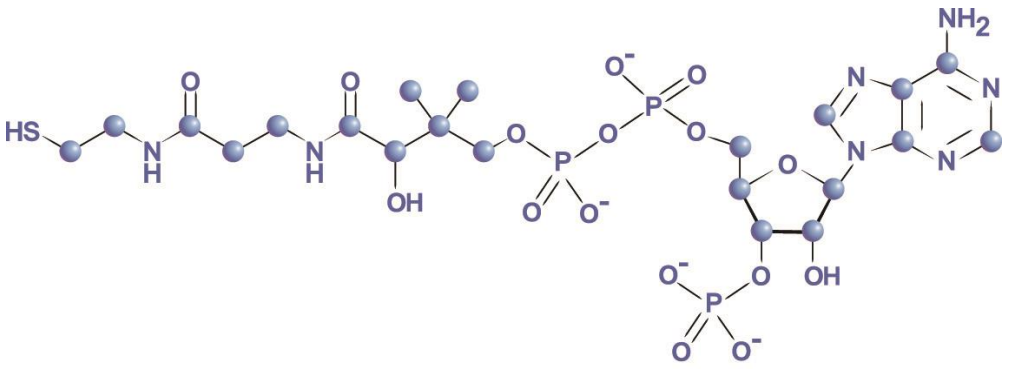
Acetyl Co-A and succinyl Co-A are important intermediates at cross roads of many metabolic pathways. Acyl Co-A is formed as an intermediate both in fatty acid biosynthesis and oxidation. HMG Co-A is an important intermediate involved in ketogenesis as well ascholestrol biosynthesis we designate the acylated forms of coenzyme A as



or acyl Co-A, and unacylated as CoA-SH. While Co-A wasdiscovered as the "acetylation coenzyme". It has far more general function. It is required in the form of acetyl Co-A, to catalyze the synthesis of citrate in citric acid cycle.

It is essential to the β oxidation of fatty acids and carries propionyl and other acyl groups in a great variety of other metabolic reactions. The acyl group [such as acetyl or aceto acetyl group] is attached to the Co-A through a thio ester linkage to the β- mercaptoethylamine moiety. Acyl groups are covalently linked to thiol group, forming thioesters. Because of their relatively high standard free energies of hydrolysis, thioesters have high acyl group transfer potential and cars donate their acyl group of variety of acceptor molecules. The acyl group attached to coenzymeA may thus be thought of as "activated" for group transfer.

The vitamin precursorof coenzyme A is pantothenate. Panthotheric acid is pentoic acid and β-alanine joined together in a amide linkage.



Coenzyme A

Pyruvate is oxidized to acetyl CoA by pyruvate dehydrogenase complex by an oxidative decarboxylation reaction.

The **pyruvate dehydrogenase** complex is a group of three enzymes responsible for the conversionof pyruvate to acetyl-CoA, viz. pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. The pyruvate dehydrogenase complex also requires TPP along with four other coenzymes (lipoate, coenzymeA, FAD and NAD^+).

The first enzyme pyruvate dehydrogenase, a TPP requiring enzyme, decarboxylates pyruvate, with the intermediate formation of hydroxyethyl thiamine pyrophosphate. This reaction is same as catalyzed by pyruvate decarboxylase, however, unlike pyruvate decarboxylase, pyruvate dehydrogenase does not convert the intermediate hydroxyethyl thiamine pyrophosphate into TPP and acetaldehyde but transfer the intermediate to the second enzyme dihydrolipoyl transterase . Second enzyme requires lipoate, a coenzyme that is attached to its enzyme by an amide linkage to a lysine residue.

The hydroxyethyl thiamine pyrophosphate carbanion attacks the disulphide linkage of lipoate followed by the eliminationof TPP carbanionfrom the intermediate adduct to form acetyl-dihydrolipoamide and regenerate active pyruvate dehydrogenase. Dihydrolipoyl transacetylasecatalyzesthe transfer of the acetyl group to CoA forming acetyl CoA and dihydrolipoamide-dihydrolipoyl transacetylase. The third enzyme dihydrolipoyl dehydrogenasealso calledlipoamidedehydrogenasereoxidizesdihydrolipoamide utilizing the coenzyme FAD. Oxidation of dihydrolipoate by FAD forms enzyme bound $FADH_2$. NAD^+ then oxidizes $FADH_2$ back to FAD.

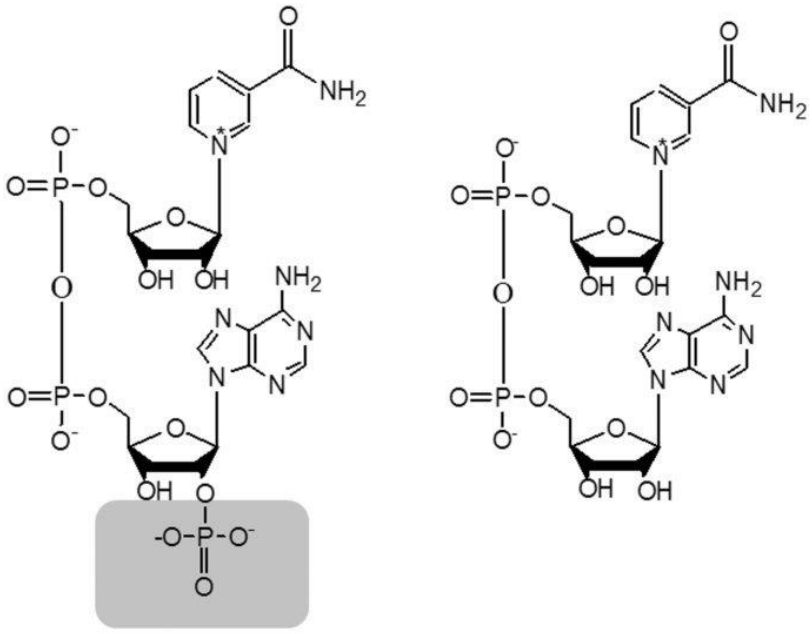
7.6 NAD^+ AND $NADP^+$

The dinucleotide coenzymes, nicotinamide adenine dicucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) play a vital role in biochemical reaction. These are derived from the vitamin niacin (nicotinic acid). These are also known as pyridine nucleotide due to the presence of pyridine ring.

Nicotinamideadenine dinucleotide NAD^+ is two-electron oxidizing agent, and is reduced to NADH. NADH is in turna two-electron reducing agent and is oxidized to NAD^+.

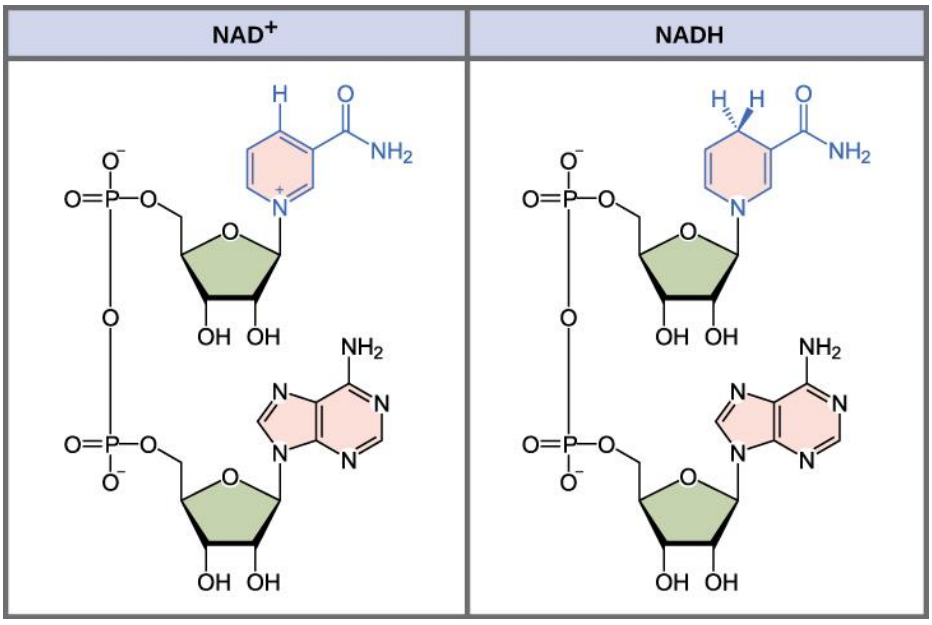
NAD in derived from vitamin commonly called niacin and niacinamide is the equivalent form of the vitamin. NAD^+ is made of two nucleotideunits joined to one another via their phosphategroups. The heterocycliccomponentof the nucleotidesof NAD^+ is nicotinamide, and the heterocyclic component of the other is adenine. This explains the coenzyme's name

(nicotinamideadenine dinucleotide). The positive charge in the NAD abbreviation refers to the positivelycharged nitrogen of thepyridine ring.



NADP⁺ **NAD⁺**

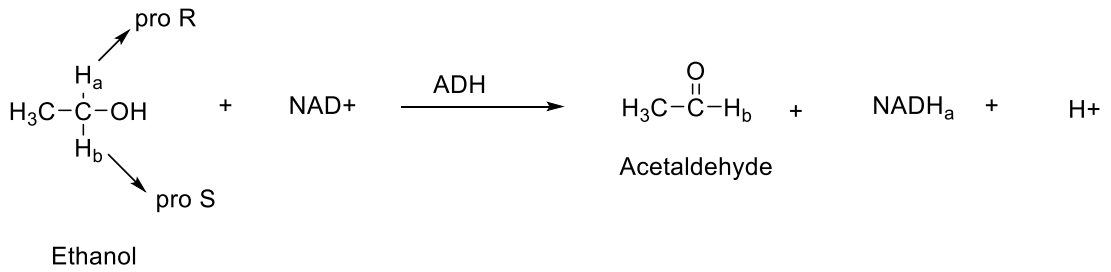
The only difference between $NADP^+$and NAD^+ is the phosphate group bonded to the 2'-OH group of the ribose of the adenine nucleotide; this explains the addition of "P'' to its name. NAD^+ and NADH are generally used as coenzymes in catabolic reactions and the phosphorylated derivatives, $NADP^+$ and NADPH, are generally used as coenzymes in anabolic reaction.



An enzyme alcohol dehydrogenase (ADH) involves NAD^+ as coenzyme in the catalytic oxidation of primary or secondary alcohol to aldehyde or ketone respectivly.

$$RCH2OH + NAD+ \underset{}{\overset{ADH}{\rightleftharpoons}} RCHO + NADH + H+$$

The coenzyme NAD^+ binds to the Alcohol dehydrogenase enzyme at the specific site and accepts a hydride anion from the substrate at C-4 position ring to form the carbonyl compound. The enzyme alcohol dehydrogenase in the presence of NAD^+ has the ability to differentiate between enantiotopic hydrogen. For example, Alcohol dehydogenase remove only the pro R hydrogen of ethanol.



The mechanism for the reduction of NADH is the reverse of the mechanism for oxidation of NAD^+. If a substrate is to be reduced, a hydride ion from the 4-position of the dihydropyridine ring is donated to the substrate. An acidic group of the enzyme aids the reaction by donating a proton t the substrate.

Another enzyme which uses NAD^+ and $NADP^+$ as coenzyme are as follows:

Malate dehydrogenase, Lactate dehydogenase, Homoserine dehydrogenase, Glyceraldehyde 3-phosphate dehydrogenase.

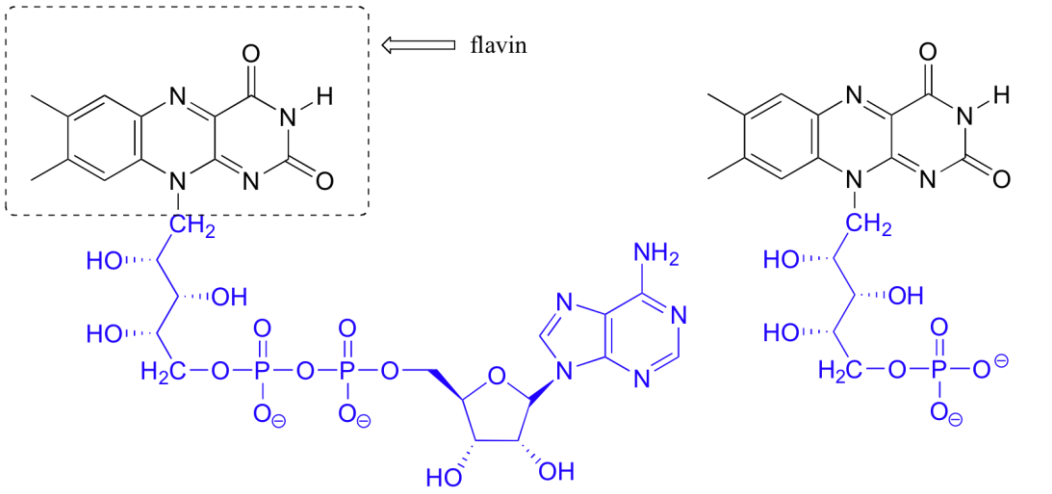
7.7 FLAVIN ADENINE DINUCLEOTIDE (FAD) AND FLAVIN MONONUCLEOTIDE (FMN)

Flavin nucleotides are derived from riboflavin (vit. B_2). Vit B_2 deficiency causes inflamation of the skin. Like nicotinamide nucleotide, Flavin Adenine Mononucleotide (FMN) and Flavin Adenine Dinucleotide (FAD), also participate in oxidation- reduction reaction.

Flavin nucleotides are derived from vitamin B_{12} [Riboflavin] and are actively involved in hydrogen transfer reactions.Riboflavin is 6, 7 dimethyl-iso-alloxan bound to ribitol [alcohol

of ribose]. The intense yellow color is due to iso-alloxan ring. Active co-enzyme forms are FMN and FAD.

FMN [Flavin Mono-Nucleotide] and FAD [Flavin Adenine Dinucleotide] are the active or coenzyme form of riboflavin.FMN is formed by phosphorylation of riboflavin by kinase using ATP in intestine. Flavin nucleotides are derived from vitamin B_{12} [riboflavin] and are actively involved in hydrogen transfer reactions.



FAD and FMN are co-enzymes of proteins known as flavoproteins.Those coenzymes which are the flavin nucleotides are derived from vitamin riboflavin. As the names indicate FAD is a dinucleotidein which one of the heterocycliccomponentsis flavin and the other is adenine. FMN contains flavin but not adenine and therefore, it is a mononucleotide. Riboflavin (vitamin B_2) is thus composed of flavin and ribitol. FAD and FMN participate in several types of enzyme-catalyzedoxidation/reduction reactions.Oxidationof a carbon-carbonsingle bond in the hydrocarbon chain to a carbon-carbondouble bond is brought about by FAD or FMN. As seen from balanced half-reactions, the two-electron oxidation of the hydrocarbon chain is coupled with the two-electron reduction of FAD.

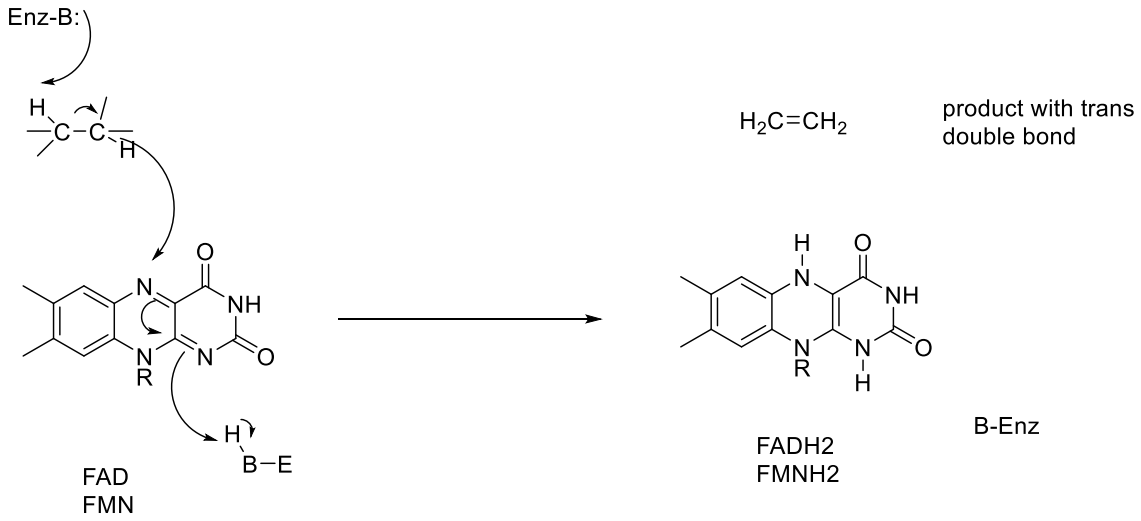
Balanced Half Reaction:

oxidation of the hydrocarbon chain: -CH2-CH2- ⟶ -CH=CH- +2H+ + 2e-

Reduction of FAD: FAD + 2H+ + 2e- ⟶ FADH2

After oxidation of the substrate the coenzyme is reduced to ($FADH_2$) or ($FMNH_2$). Significantlyall the oxidation/reduction reactionsoccur on the flavin ring. An example is the dehydrogenation of succinic acid into fumaric acid by succinate dehydrogenase. The active

site of the enzyme consists of FAD covalently bonded to the enzyme. Suchtightlybound coenzymes are correctly called prosthetic groups. In the conversion of succinic acid intofumaric acid not a trace of the other geometrical isomer (maleic acid) is formed, thus the reaction is like stereoselective trans-elimination. The mechanism of conversion of -CH2CH2-to-CH=CH- with these co-enzymes may be presented as:



The reaction involves a transfer of a hydride ion from the carbon of the hydrocarbon chain (a substrate)to FAD (or FMN). A basic group on the surface of the enzyme removes a hydrogen from the adjacent carbon and ultimately a new basic group is created on the surface of the enzyme.

Thus when e.g., FAD is convertedto $FADH_2$, one of the hydrogen atoms comes as a hydride from the hydrocarbon chain, while the other comes as a proton from an acidic group on the surface of the enzyme catalyzing this oxidation. Moreover, one may note that one group of the enzyme functions as a protonacceptorwhile the other acts as a protondonor.

7.7.1 Metabolic role as Co-enzyme

Riboflavin containing proteins are called flavoproteins. Many oxido-reductase enzymes are flavoproteins containing FMN and FAD as prosthetic group. Example L-amino oxidase [FMN dependent], succinate dehydrogenase in citric acid cycle is FAD^+ dependent.

These enzymes participate in reversible oxidation reduction reactions. N-atoms at position 1 and 10 in isoalloxan ring of riboflavin undergo oxidation reduction. Example of enzymes requiring FMN or FAD as a coenzyme and reaction where they are involved.

7.7.2 FMN dependent enzymes

L- amino acid oxidases

They catalyze oxidative deamination of amino acid producing H_2O_2 which is split by catalase.

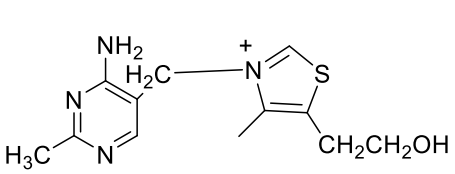
NADH dehydrogenase complex

This enzyme is a part of electron transport chain and contains FMN. The electrons are transferred from NADHto FMN and then to CoQ in the electron transport chain.

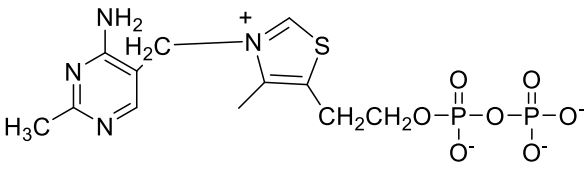
7.8 THIAMINE PHOSPHATE (TPP)

TPP is a coenzyme which has a thiazole ring containing an acidic hydrogen (the hydrogen attached to imine carbon) and is thus capable of giving a carbanion. Thus carbanion act as a nucleophilic towards carbonyl groups.

Thiamine was the first of B vitamins to be identified. It is also called as vitamin B_1. The vitamin is structurally complex, made up of 2,5 dimethyl 6-amino pyrimidine joined to 4 methyl, 5 hydroxythyl thiazole by a methylene linkage. But its conversion to the coenzyme form, thiamine pyrophosphate, or TPP involves simply and ATP dependent pyrophosphorylation.Thiazole ring of TPP is the functional part of coenzyme.



Thiamine (Vitamin B1)



Thiamine pyrophosphate (TPP)

7.8.1 Role of thiamine phosphate as a Coenzyme

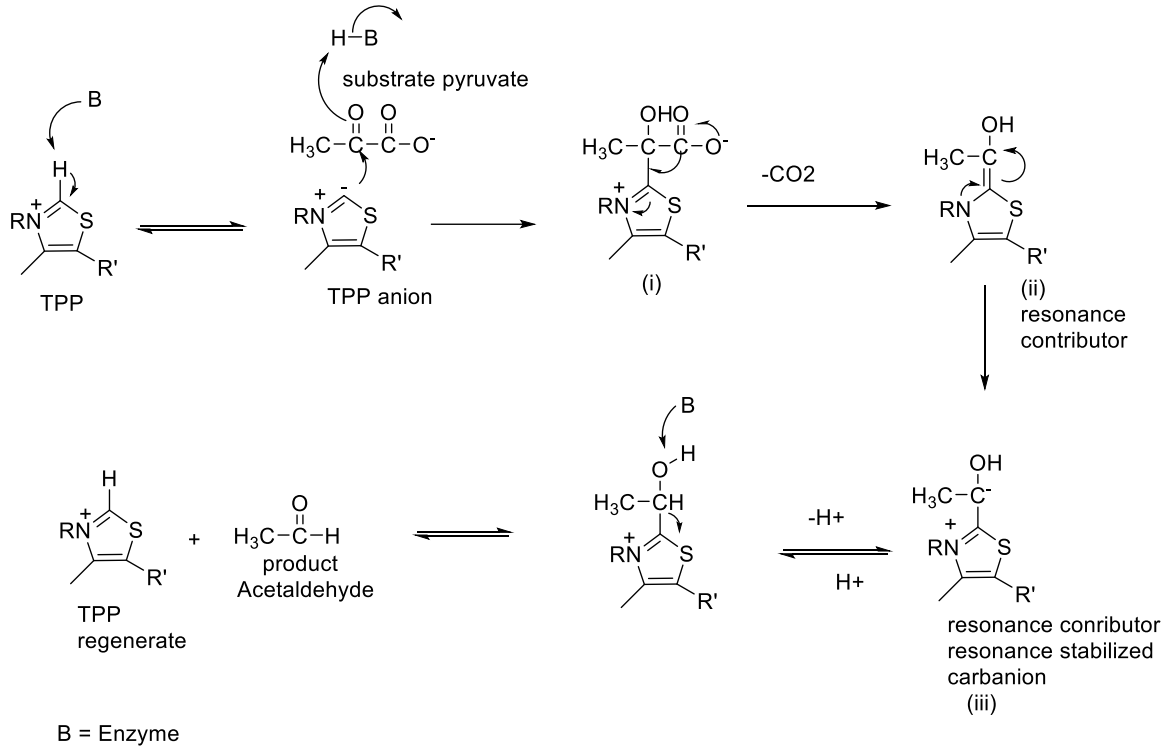
Thiamine pyrophosphate participate as a coenzyme in following reactions:

Decarboxylation of an α-keto acid (pyruvate to acetaldehyde)

Pyruvate decarboxylase is the enzyme which needs the coenzyme TPP to catalyze the decarboxylation of pyruvate. Thiamine pyrophosphate is the coenzyme for all decarboxylations of α-keto acids. The mechanism shown below for pyruvate decarboxylation is involved in all of these reactions. Note that TPP contains two heterocyclic rings a

substituted pyrimidine and a thiazole. Recent NMR studies have shown that both rings participate in the formation of a reactive carbanion at C-2 of the thiazole ring-the carbon atom between the nitrogen and the sulfur. As shown in the following diagram, a glutamate carboxyl group in the enzyme attracts a proton. Linked to N-1 of the pyrimidine, which in turn increases the basicity of the amino group, facilitating the deprotonation of C-2 of the thiazole ring.

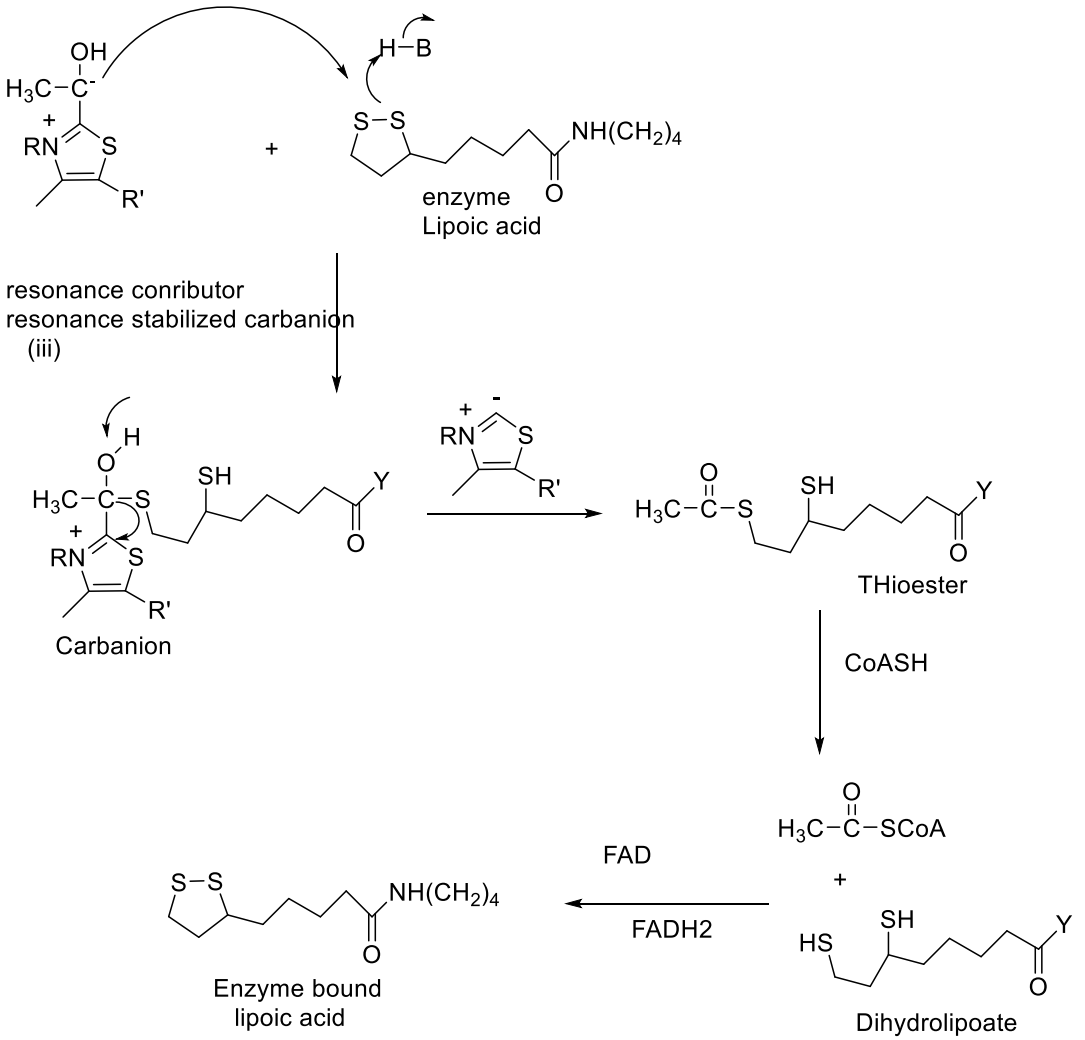
This carbon forms a carbanionwhich in turn can attack the carbonyl carbon of α-keto acids, such as pyruvate, giving an addition compound. The addition compound undergoes nonoxidative decarboxylation with the thiazole ring acting as an elect stabilized eneamine. Protonationgive hydride or more accurately, hydroxyethyl-TPP.



7.8.2 Thiamine pyrophosphate in the pyruvate dehydrogenase reaction

Thiamine pyrohphosphate (TPP) is the coenzyme for the pyruvate dehydrogenase reaction and other nonoxidative de-carboxylations of α-keto acids. The key reaction is attack by the carbanion of TPP on the carbonyl carbon of pyruvate and is followed by nonoxidative decarboxylation of the coenzyme - bound pyruvate the electron pair remains with the ring nitrogen. In next stepthe two-carbon fragment bound to TPP extracpts a proton from pyruvatedecarboxylase, generating a hydroxyethyl group. This fragments remains at the

aldehyde oxidation level. The pyridoxal phosphate is a phosphate ester of aldehyde form of vitamin B_6. Pyridoxal phosphate [or PLP]. Vitamin B_6, which is also known as anti-dermatitis factor includes three closely related forms, pyridoxanine, pyridoxal and pyridoxine. Active forms are phosphate ester such as pyridoxal phosphate and pyridoxamine phosphate or coenzyme form are formed by phosphorylation formed by enzyme pyridoxal kinase using ATP. Pyridoxal phosphate is required by many enzymes catalysing reactions of amino acid and amines.

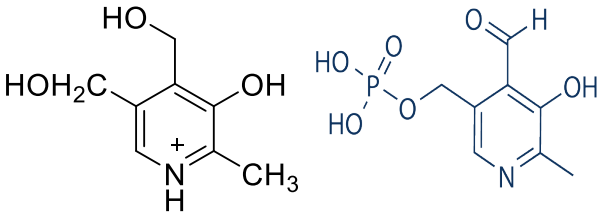


The reactions are numerous and pyridoxal phosphate is surely on of nature's most versatile catalyst. The story begins with biochemical transamination. In 1937, Alexander Braunstein and Maria Kritzmann, in Moscow, described the transamination reaction by which amino groups can be transferred from one carbon skeleton to another. The transamination reaction is a widespread process of importance in many aspects of nitrogen metabolism of organisms. For large number of transaminases glutamate is one of the reactant.In 1944, Esmond snell

reported the non-enzymatic conversion of pyridoxal into pyridoxamine by heating with glutamate. He recognized that this was also trannsamination and proposed that pyridoxal might be a part of coenzyme needed for amino transterases. The hypothesis was soon verified and coenzyme was identified as pyridoxal 5'-phosphate or pyridoxamine 5'- phosphate.

7.9. PYRIDOXAL PHOSPHATE

Pyridoxal PhosphatePuridoxal phosphate (PLP) is derived from pyridoxine (vit. B_6). Deficiency of vit. B_6 causes anemia. This coenyzme is required by the enzymes that catalyze certain transformations of amino acids. It possessreactivealdehydegroup throughwhich it can bind itself to a number of enzymes.

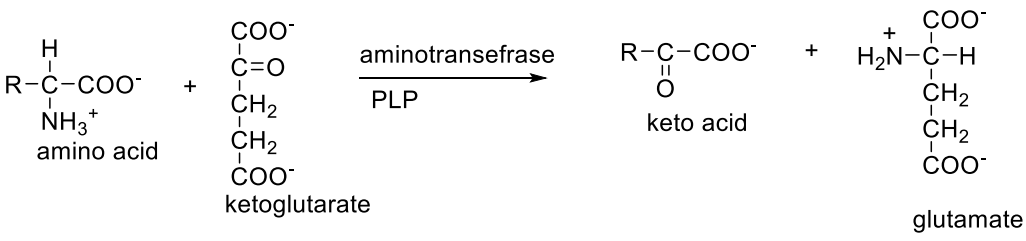


Pyridoxin (Vit. B_6) Pyridoxal-5'phosphate (PLP)

PLP is covalently attached to the enzyme via a Schift base (imine) linkage by the condensation of its transimination reaction, the substrate (amino acid) reacts with the enzymePLP Schiff base, forming a tetrahedral intermediate. A new imine is formedbetweenPLP and the amino acid by the expulsion of lysine residue. PLP catalyzes several different amino acid transformations as given below.

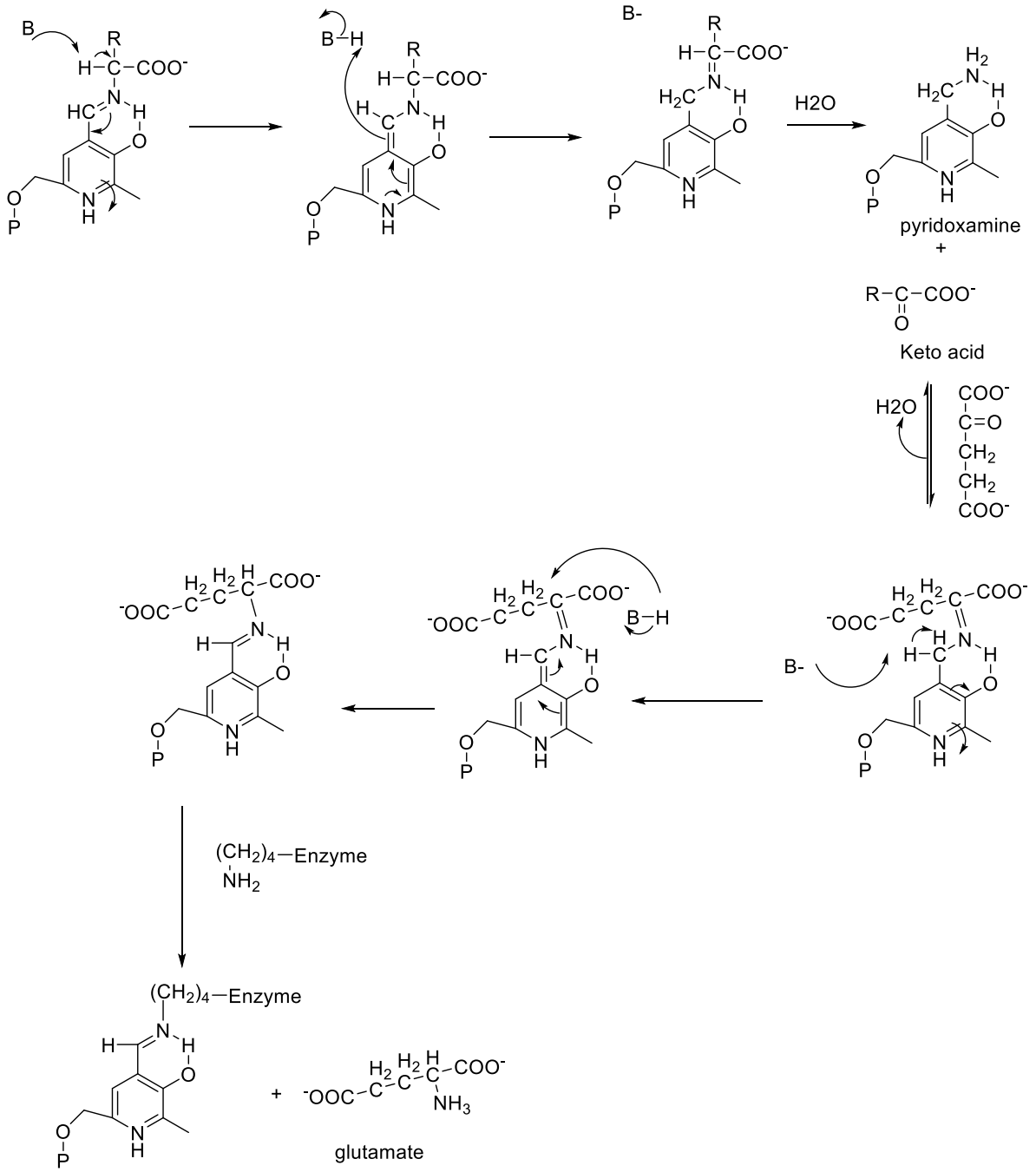
A. Decarboxylation

If the PLP catalyzes the decarboxylation reaction, the carboxyl group of the α-carbon of the amino acid is removed. Decarboxylatedintermediateattains its aromaticity from lysine residue or some other acid group. Transimination with a lysine side chain regenerate enzyme bound PLP by releasing the decarboxylated substrate.



B. Mechanism of amino acid metabolism/ transformation- transamination

The enzymes that catalyze transamination reaction are called aminotransterase (transaminase). In the first step of the reaction, a proton is removed from the α-carbon of the amino acid bound PLP.

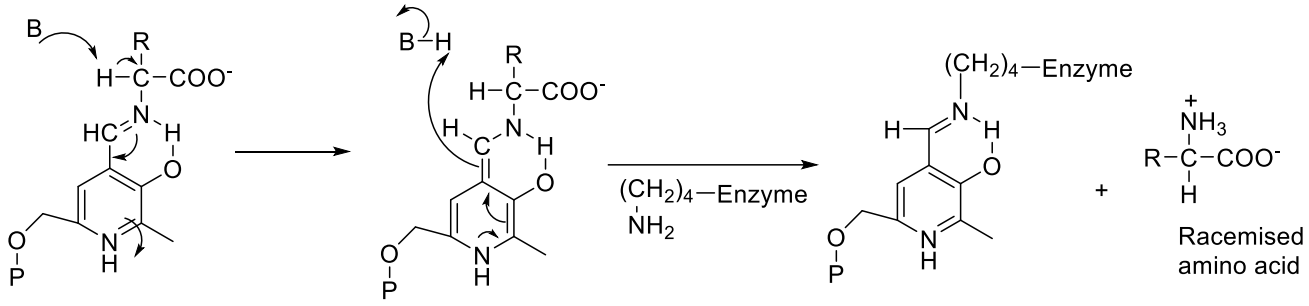


Protonation of the α-carbon attached to the pyridine ring followed by hydrolysis of imine, forms the α-keto acid and pyridoxamine. In the second step, pyridoxamine foms an imine with α-ketoglutarate, the second substrate of the reaction. Removal of the proton from the

carbon attached to the pyridine ring and donation of a proton to the α-carbon of the sulosuate forms an imine, which on transimination with lysine residue releases glutamate and regenerate the enzyme bound PLP-Schiff base.

C. Racemization

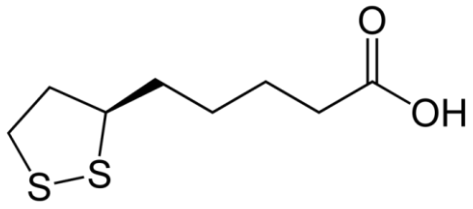
The first step of racemization is same as that of transamination of amino acid i.e., removal of proton from the carbon of the amino acid bound PLP. In the second step, reprotonationoccurs. The proton can attack the sp hybridized carbon from either side of the plane forming the racemic mixture of both D- and L-amino acid.



Mechanism for Racemisation of L-amino acid catalyzed by PLP

7.10. LIPOIC ACID

Lipoic acid (often called lipoic acid) is a naturally occuring compounds that is also synthesized by human.



Lipoic acid

Lipoic acid acts in the transfer of hydrogen during oxidative decarboxylation reactions. In the structure of lipoamide, where lipoic acid is bound in the amide linkage to the ε amino group of lysine residue of dehydrolipoamide acyl transferases. The complex reactions of the carbohydrate metabolism catalyzed by pyruvate dehydrogenase system and α-ketoglutarate

dehydrogenase require participation of lipoic acid. It acts as a carrier and undergoes inter conversion between reduced and oxidized form as. The two thiol groups that can undergo reversible oxidation to a disulfide (-s-s-), similar to that between two Cys residues in a protein. Because of its capacity to undergo oxidation- reduction reactions, lipoate can serve both as an electron hydrogen carrier and as an acyl carrier.

Lipoic acid is also known as α-Lipoic acid or ALA and thioctic acid. Lipoic acid is cofactor for atleast five enzymes systems, two of these are in the citric acid cycle through which many organisms turn nutrients into energy e.g. The pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase.

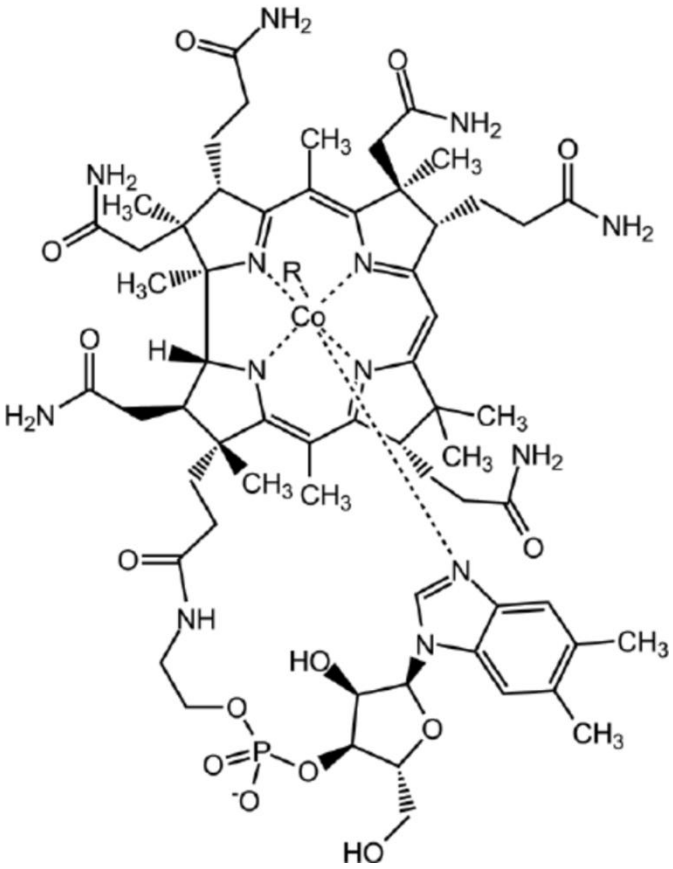
7.11. VITAMINE B_{12}

Coenzyme B_{12} as derived from vit. B_{12} catalyzes certain rearrangement reactions. In the structure of B_{12} the cobalt metal is co-ordinated with a tetra pyrole ring system, called a corrin ring which is as that porphyrin ring of heme compounds. The metal ion is linked to the four nitrogen atoms of the ring by an covalent and three co-ordinate bonds but the four bonds are almost equivalent due to resonance. The fifth which is as that the ring by one orientation site on the cobalt is filled by a nitrogen atom from an imidazole ring [dimethyl benzimidazole (DMB)]. The sixth co-ordination site is occupied by OH group or cyano group. In coenzyme B_{12}, the cyano group is replaced by a 5-deoxyadenosyl group. Vit. B_{12} is the only vitamin which contain a metal ion (the metal ion has an oxidation state of +3). Deficiency of vit. B_{12} causes pernicious anemia.

The coenzyme form of vit. B_{12} is obtained after activation of NADH linked reducing systems. Reaction with ATP, mediated by an adenosyltransferase, resultin the formation of 5'-deoxyadenosyl cobalamin coenzyme. Coenzyme $B_{12\ is}$ the co-factor form of vitamin B_{12}. This vitamin is unique among all the vitamins in that is not only consist complex organic molecule but an essential trace element Cobalt.

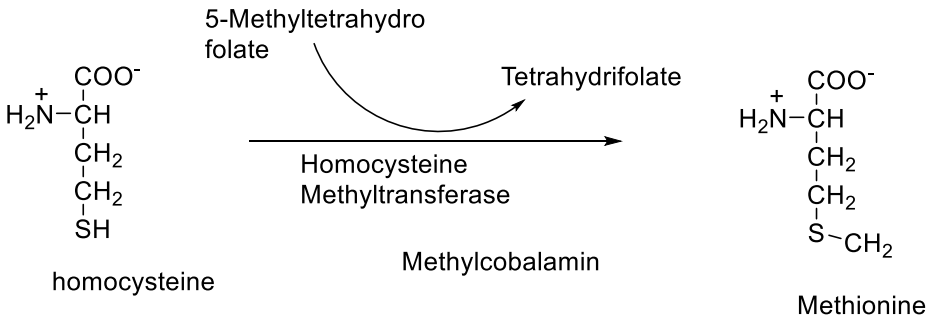
Methyl malonyl Co-A mutase convert methyl malonyl CoA to succinyl-CoA utilizing coenzyme B_{12}. The cleavage of C-Cobond forms 5-deoxyadenosyl radical and cobalamin in its +2 oxidation state. Removal of a proton from methyl malonyl CoA by the deoxyadenosyl radical generates method Co-Aradical. Rearrangement of the intermediate form succinyl CoA radical. Abstraction of a succinyl Co-A radical from 5-deoxyadenosine form succinyl Co-A

and again generates the 5-deoxuad radical 5-deoxyadenosyl radical and Co(II) combinesto generate the coenzyme.



Coenzyme B_{12}

Methyl cobalamin is known to participate during the transfer of methyl groups e.g., in the regeneration of methionine from homocysteine in mammals. In this reaction, the methyl group of 5-methyl tetrahydrofolate is passed to a reactive, reduced form of cobalamin to form methylcobalamin, which can transfer the methyl group to the thiol side chain of homocysteine.



7.11.1. Co-enzyme form of B_{12}

Two active forms of vitamin B_{12} are involved in metabolism. These are (a) methyl cobalamin and (b) 5'- deoxy adenosyl cobalamine also called cobamide coenzyme only two reactions use vitaminB_{12} as coenzymes. Conversion of homo cysteine to Methionine, Methyl cobalamine is the coenzyme in this reaction. Conversion of methyl malonyl CoA to succinyl CoA and 5'- deoxy adenosyl cobalamine is used as coenzyme.

Function of 5'-deoxyadenosylcobalamin involves isomerizations involving exchange of carbon bound hydrogen with another carbon bound functional group as methyl malonyl Co-A mutase catalyzes reaction of this type given.

7.12 SUMMARY

- Four enzymes which are involved in transfer of group are Vitamin B_{12}, Biotin, TPP, and Coenzyme A.
- Biotin participates in the transfer of Carboxylic groups.
- Thiamin pyrophosphate or thiamine TDP is the coenzyme responsible for the transfer of aldehyde and glyoxal group, and it is derived from vitamin B, by phosphorylation.
- Coenzyme A is derived from vitamin pantothenic acid. This is abbreviated as CoA. It is also called as acetylation coenzyme
- Coenzyme B_{12} is the cofactor form of vit B_{12} and involved in isomerisation reaction and methyl group transfer.

7.13 SAQs

Multiple Choice Questions

1. When metal ions are loosely attached with the enzymes, they become

(a) Catalysts

(b) Coenzymes

(c) Cofactors

(d) Substrates

2. Which of the following participates in enzyme-catalysed reactions

(a) Cofactors

(b) Coenzymes

(c) Meatl ions

(d) None

3. In which form coenzymes transfer energy from one enzyme to another

(a) Oxygen atoms

(b) Nitrogen atoms

(c) Carbon atoms

(d) Hydrogen atoms

4. Nicotinamide adenine dinucleotide (NAD) is made from which vitamin

(a) Vitamin A

(b) Vitamin B

(c) Vitamin C

(d) Vitamin D

5. The non-protein organic molecules are

(a) Coenzymes

(b) Enzymes

(c) Cofactors

(d) All the above

7.14 TERMINAL QUESTIONS

1. Expain the β-oxidation of fatty acids.

2. What are the co-enzymes.

3. Why are co-enzymes necessary. Explain

4. Explain NAD^+ and $NADP^+$

5. What are the cofactors? Discuss role of cofactors in coenzyme chemistry.

7.15 REFRENCES

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2. H.K.Chopra, A.Parmar and P.S.Panesar, Bioorganic Chemistry, Narosa Pub.

3. D.V.Vranken and G.A.Weiss, Introduction of Bioorganic Chemistry.

Answers of MCQ

1.	c	2.	b	3.	d	4.	b	5.	a

UNIT 8: BIOTECHNOLOGICAL APPLICATIONS OF ENZYMES

CONTENTS:

8.1 INTRODUCTION

Enzymes are high molecular weight proteins that act as biological catalysts. Enzymes have the tendency to participate in reaction and accelerate it via lowering the activation energy. Due to such properties enzymes have wide applications industrial sectors such as paper and food industry, starch industry, textile industry, baking and brewing industry etc. For industrial applications enzymes can be isolated from various sources including animals, plants and microorganisms. Enzymes play an essential role in manufacturing of various industrial food products also. Cheese, beer, wine and vinegar are such few examples. Enzymes are helpful in controlling various processes such as process time, enrich flavour, improve texture, increase shelf life etc.

In this unit, we find the commercial methods for production of enzymes, different immobilization techniques of enzymes, its applications in different sectors i.e. food and drink industries, cheese making etc., enzymes used as target for drug designing, enzyme therapy and recombinant DNA technology for developing recombinant enzymes. Enzyme immobilization is a widespread technology to achieve more stable, active and reusable enzymes. In this technique, the confinement of enzyme molecules is done onto/within a support or matrix via physically or chemically or both for retaining full activity. Enzymes not only energies the industrial sectors but also clinical sectors. Currently, various kind of enzyme associated diseases are there which is cured via the enzyme therapy or enzyme targeted drugs. Such diseases or disorders may include lysosomal storage disorders, cancer, Alzheimer's disease, irritable bowel syndrome, exocrine pancreatic insufficiency, and hyperuricemia etc. Enzymes may act as markers in various disease such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. Thus, enzymes can provide insight into the disease process by diagnosis, prognosis and assessment of response therapy.

8.2 OBJECTIVES

In this unit you will be able to learn the

- The commercial methods for enzyme production including the downstream and upstream processes.
- Techniques and methods of immobilization of enzymes
- Enzyme applications in clinical and industrial sectors i.e. food and drink industry, brewing and cheese making industry etc.
- Enzyme therapy and recombinant DNA technology for developing recombinant enzymes

8.3 LARGE-SCALE PRODUCTION AND PURIFICATION OF ENZYMES

Enzymes are specific, versatile, and efficient biocatalysts which participate in reaction and cause lowering of activation energy. Activation energy is the minimal amount of extra energy required by a reacting molecule to convert into product. The sources of various industrial enzymes are based on animals (pepsin, trypsin, pancratin, chimosin), plants (papain,

bromelain, ficin), and microorganisms (amylase, proteases, isomerases, glucose oxidases, pectinases, lactase, cellulase, xylanase, lipase, phytase, invertase, catalase, etc.). Industrial fermentation is one of the important step in enzyme production in which the substrates are converted into products. The factors influence this process may depend on temperature, substrate, pH, aeration and agitation, inhibitors etc.

The essential steps for industrial enzyme production can be categories into two parts including (1) upstream and (2) downstream processes.

8.3.1 Upstream process

Upstream process refers to all those activities which needed to gather the materials required to create a particular desired industrial product including inoculum development, media preparation, cell culture, cell Separation and harvest. When the cells have reached the desired density, they are harvested and moved to the downstream section of the bioprocess.

8.3.1.1 Sources

Enzymes can be obtained from three different sources including plants, animals and microorganisms. Some commercial enzymes such as papain, bromelain, ficin, and malt diastase are obtained from plants. While, pepsin, trypsin, alpha-chymotrypsin, lipase, catalase, rennin, and pancreatic enzymes are derived from animal sources. The microorganisms i.e. fungi, bacteria and yeast are chiefly used for the production of industrial enzymes. A brief list of industrial enzymes, their source and applications are mentioned in Table 8.1 & 8.2.

Table 8.1 Commercial enzymes from plant sources and their applications

Enzyme	**Plant source**	**Industrial applications**
β-Amylase	Barley, Soybean	Baking, preparation of maltose syrup
Bromelain	From Bromeliaceae family members i.e. pineapple	Pharmaceuticals industry
Esterase	Wheat	Ester hydrolysis
Ficin	Fig	Meat tenderiser

Malt diastase	Malted barley	Food supplements
Papain	Papaya	In baking industries, meat tenderiser, tanning
Peroxidase	Horse Radish	Diagnostic purposes
Urease	Jack Bean	Diagnostic purposes

Table 8.2 Industrial microbial enzymes, source and their applications

Enzyme	**Microbial source**	**Industrial applications**
Amylase	Bacteria (*Bacillusamyloliquifaciens, B. licheniformis, B. coagulans, Geobacillus* sp.); Fungi (*Aspergillusoryzae, A.niger, Rhizopus* sp.)	Starch industry, paper and food industry, in preparation of glucose and maltose syrups, high fructose corn syrups, clarified fruit juices etc.
Catalase	*Aspergillus* sp.	Used in food industry and also in egg processing, in textile industry removing hydrogen peroxide from fabrics
Cellulose	Fungi (*Trichodermareesei, T. viride, Penicillium* sp., *Humicolagrisea, Aspergillus* sp., *Chrysosporiumlucknowense, Acremonium* sp.)	Textile industry, pulp and paper industry, and food industry, as well as an additive in detergents and in improvement of digestibility of animal feeds
Dextrinase	*Gluconobacteroxydans*	Used in preparation of corn syrup in starch and syrup making
Glucose oxidase	*Aspergillusniger, Penicillin* sp.	Bakery and food industry
Hemicellulase	Fungi (*A. niger, T. reesei, Penicillium* sp.)	Food, feed, bioethanol, pulp and paper, chemical, and beverage producing industries as well as in biorefineries and environmental biotechnology
Invertase	*Saccharomyces* spp.	Food industry, baking and brewing industries

Keratinase	Bacteria (*Bacillussubtilis*), Fungi (*Aspergillusstelliformis*)	Textile industry, detergent preparation, leather industry, Animal feed production
Lactase	Yeast (*Kluceromyces*)	Dairy and food industries
Laccases and peroxidase	*Aspergillusnidulans, Aspergillus* sp., Basidiomycetes	Textile and biofuel production
Lipase and proteinase	*A. oryzae,A. terreus, Pseudomonas* sp., *Alcaligena* spp., *Staphylococcus* sp., *Candidaalbicans, Rhizopus* sp., *Mucor* sp.	In dairy, baking, fruit juice, beer and wine industries
Pectinases	*Aspergillusniger*, *Penicillin* sp.	In food industry useful for fruit juice extraction and wine clarification; tea, cocoa, and coffee concentration and fermentation
Phytase	*Aspergillus* sp., *A. ficuum*, *P. funiculosum*, *Bacillus* sp., *Pseudomonas*, *Xanthomonasoryzae*	Paper and pulp industries
Protease	Bacteria (*Bacillusamyloliquifaciens, Pesudomonas, Clostridium*); Fungi (*Aspergillusoryzae, A. niger*, *Penicilliumchrysosporium*, *Rhizopusoligosporus*, *Actinomycetes* sp.)	Leather processing, food industry, pharmaceuticals, Washing powders
Xylanase	Fungi (*Myeciliophthorathermophila*, *Bacillus* sp., *A. oryzae*, *Trichoderma* sp.)	Wood pulp bioleaching, papermaking, the manufacture of food and beverages, animal nutrition, and bioethanol production

8.3.1.2 Screening of microorganisms

The microbial screening is theprocedure of isolation, detection and separation of microorganisms of interest from a particular population. In term of enzyme production, screening procedure helps to detect the potential microorganisms with higher production or yield of certain enzymes. For examples, fungi (*Trichodermareesei*, *T. viride*, *Penicillium* sp., *Humicolagrisea*, *Aspergillus* sp., *Chrysosporiumlucknowense*, *Acremonium* sp.) yeast (*Saccharomycescerevisiae*), bacteria (*Bacillussubtilis*, *Pseudomonas*, *Xanthomonasoryzae*,

Clostridium) are few examples of potential microorganisms used in various industrial production including leather processing, food industry, pharmaceuticals etc.

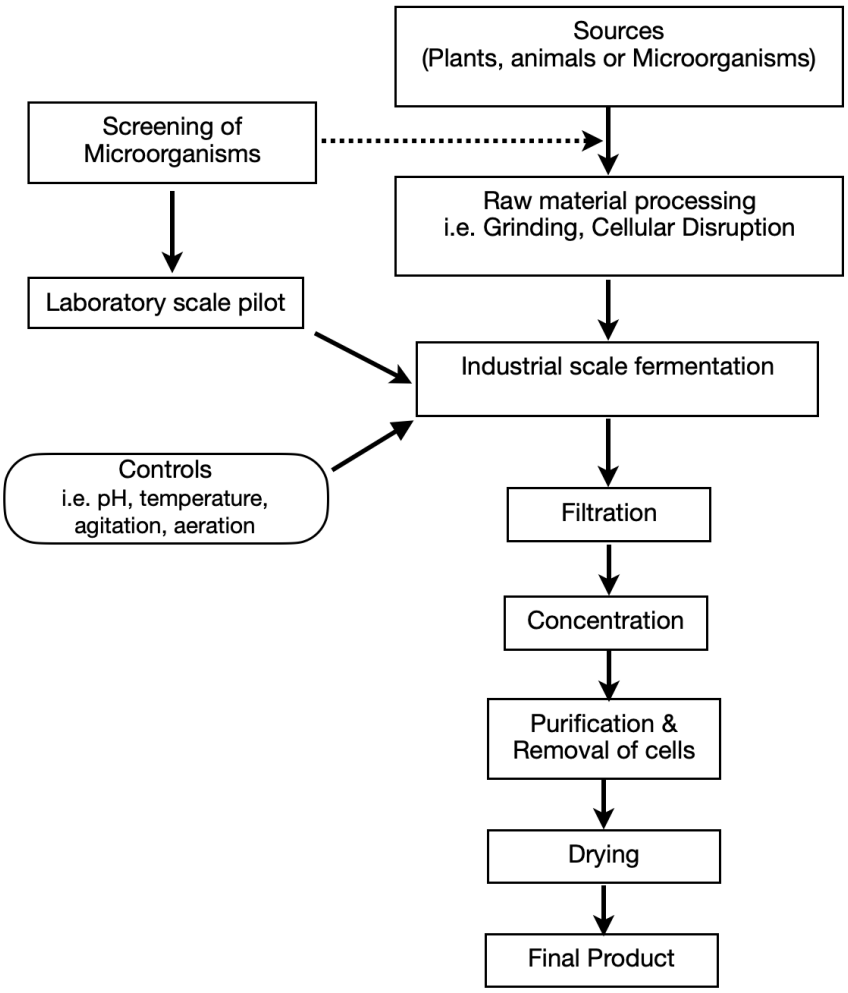

Fig. 8.1 Steps of Industrial enzyme production

For commercial production the selected microorganisms should fulfil the following criteria.

1. The microorganisms should be capable to grow on an inexpensive medium at a rapid and constant rate.
2. The microorganisms should produce the enzyme with high yields at a higher concentration.
3. The selected microorganisms should be genetically stable during the entire production process.

4. The microorganisms should be able to grow on a concentrated medium for better/higher enzyme yield.
5. Undesirable enzyme contaminants and the content of metabolites in the fermentation broth should be minimal.
6. Recovery of the enzyme should be technically feasible and inexpensive.
7. In terms of biohazards, the production process and its product must be safe to the personnel involved and to the consumer.

8.3.1.3 Raw materials, media formulation and processing

Microorganisms constitute the major source of enzymes, but several enzymes are obtained from animals and plants sources. The traditional enzyme production relied on the natural hosts as raw materials. However, selection of wild or genetically engineered microorganisms may yield sufficiently higher quantities of enzymes. The formulation of culture media is an essential step. It should provide all nutrients supporting for enzyme production in high amount but not for good microbial growth. For this, an ideal medium must have a cheap source of carbon, nitrogen, amino acids, growth promoters, trace elements and little amount of salts. Some parameters should be regulated including pH, temperature etc. of the culture media. In media preparation, the source of carbohydrates including molasses, barley, corn, wheat and starch hydrolysate and for proteins meals of soybean, cotton seed, peanut and whey, corn steep liquor and yeast hydrolysate are generally used.

8.3.1.4 Industrial scale fermentation process

Medium is sterilized batch-wise in a large size fermenter. For this purpose, continuous sterilization method is generally used. After medium sterilization, sufficient amount of inoculum is inoculated to start fermentation process. In traditional method, enzymes production are done by surface culture technique where inoculum remains on upper surface of broth. This technique is useful for production of some of the fungal enzymes i.e. amylase (from *Aspergillus* sp.), protease (from *Mucor* sp. and *Aspergillus* sp.) and pectinase (from *Penicillium* sp. and *Aspergillus* sp.) **(table 8.2)**. Currently, submerged culture method is widely used due to less chance for contamination and higher yield of enzymes. Growth conditions e.g. pH, temperature and oxygen are maintained in fermenter at optimum level. These factors may vary with particular group of microorganisms. Antifoaming agents may

add to fermenter to control foaming during fermentation process. After 30-150 h incubation, extracellular enzymes are produced by the inoculated microbe in culture medium. Most of enzymes are produced when exponential phase of growth completes but in a few cases, they are produced during exponential phase. Besides extracellular enzymes, other metabolites (10-15 %) are also produced in the fermented broth. These metabolites are removed after enzyme purification. When fermentation is over broth is kept at 5°C to avoid contamination.

8.3.2 Downstream process

8.3.2.1 Recovery of enzymes

Enzyme purification is a complex process. Recovery of enzymes from the fermented broth (fluid) of bacteria is quite difficult in comparison to filamentous fungi. Fungal broth is directly filtered or centrifuged after pH adjustment. Therefore, the bacterial broth is treated with calcium salts to precipitate calcium phosphate which help in separation of bacterial cells and colloids. Then the liquid is filtered and centrifuged to remove cell debris.

Thus, the essential steps of purification are mentioned below:

(i) Preparation of concentrated solution by vacuum evaporation at low temperature or by ultrafiltration

(ii) Clarification of concentrated enzyme by a polishing filtration to remove other microbes

(iii) Addition of preservatives or stabilizers i.e. calcium salts, proteins, starch, sugar, alcohols, sodium chloride (18-20 %), sodium benzoate etc.

(iv) Precipitation of enzymes with acetone, alcohols or organic salts, e.g. ammonium sulfate or sodium sulfate,

(v) Drying the precipitate by free drying, vacuum drying or spray drying, and Packaging for commercial supply.

8.4 TECHNIQUES AND METHODS OF IMMOBILIZATION OF ENZYMES

Enzyme immobilization is a widespread technology to achieve more stable, active and reusable enzymes. Enzyme immobilization can be defined as the confinement of enzyme

molecules onto/within a support or matrix via physically or chemically or both for retaining full activity. The properties of an ideal support or matrix used should be inert, physically strong and stable, cost effective, regenerable, biocompatibility, ease of derivatization, mean particle diameter and swelling behaviour, reduction in product inhibition, enhance specificity of enzymes, and made up of insoluble material e.g. calcium alginate. The first immobilized enzyme was amino acylase isolated from *Aspergillusoryzae* for the production of L-amino acids in Japan.

8.4.1 Advantages of enzyme immobilization

1. Protection from degradation and deactivation of enzymes
2. Retention of enzymes, enzyme-free products
3. Recycling or repetitive use of enzymes
4. Cost efficiency
5. Enhanced stability and efficiency of enzymes
6. Use as controlled release agents
7. Ability to stop the reaction rapidly by removing the enzyme from the reaction
8. Development of multi-enzyme system
9. Minimum reaction rate and high enzyme substrate ratio
10. Less chance of contamination of products

8.4.2 Methods of Immobilization

Based on support or matrix and types of bonds involved, there are six major types of principal techniques for immobilization of enzymes mentioned below.

1. Adsorption
2. Covalent binding
3. Entrapment
4. Cross-linking or copolymerization
5. Metal linked Immobilization
6. Encapsulation

8.4.2.1 Adsorption

Adsorption is the simplest, oldest and reversible method. In this method, enzyme is adsorbed to external surface of the support. The support may be different types (1) mineral support (e.g. aluminium oxide, clay), (2) organic support (e.g. starch), and (3) modified sepharose and ion exchange resins. No parmanent bond formation happens between the carrier and enzyme in this method. Adsorption of enzymes onto the carriers can proceed via different types of interactions i.e. hydrogen bonds, hydrophobic bonds and vander Waals forces.

Fig. 8.2 Diagrammatic representation of Adsorption method of enzyme immobilization

This method depend on following four processes.

A) Static process

Immobilization to carrier by allowing the solution containing enzyme to contact the carrier without stirring. It usually uses saturated salt solutions kept inside desiccators with still air.

B) Dynamic batch process

This method uses air with a low flow velocity and this low velocity air will reduce the equilibrium time. The carrier is placed in the enzyme solution and mixed by stirring or agitation.

C) Reactor loading process

The carrier is placed in the reactor and the enzyme solution is transferred to the reactor with continuous agitation.

D) Electrode position process

The carrier is place near to an electrode in an enzyme bath and then the current is put on. Under the electric field the enzyme migrates to the carrier and deposited on its surface.

Advantages of Adsorption

1. Easy to perform and cheap
2. No reagents are required
3. Enzymes can be mobilized in native states, no activation is required
4. Less disruption of enzyme than chemical methods, and
5. Wide applicability and capable of high enzymes loading.

Disadvantages

1. Desorption of the enzyme resulting from changes in pH, temperature and ionic strength
2. Slow process, and
3. Low efficiency.

8.4.2.2 Covalent binding

Covalent binding is a classical method. It involves direct attachment of the enzyme onto the support matrix through the covalent linkage. It provides a strong and stable attachments as these are formed through reactions between functional group in the support matrix and the enzyme surface that contains the amino acid residues. It can be used on unmodified proteins since they relay only on naturally present functional group. Use of spacer arm may provide greater degree of the mobility to the enzymes to show higher activity. Common support or carriers are polyacrylamide porous glass, cellulose, collagen, gelatin, DEAE cellulose, and porous silica.

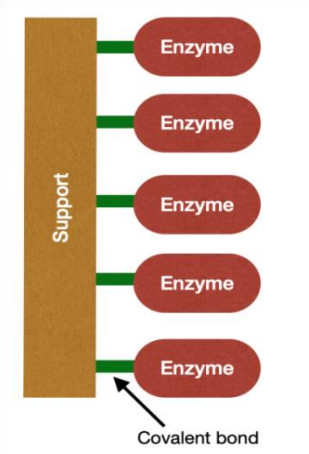

Fig. 8.3 Diagrammatic representation of Covalent bonding method of enzyme immobilization

Advantages

1. Tight binding of enzyme to support matrix
2. Comparatively simple and mild method
3. Minimized enzyme leaching in aqueous media
4. Wide applicability.

Disadvantages

1. Enzymes may be chemically modified, can be denatured.
2. Only small amounts can be immobilised (about 0.02 g/ gram of Matrix).

8.4.2.3 Entrapment

In this method, enzymes are physically entrapped inside a porous matrix. This method is the best immobilization strategy to avoid any negative influence on enzyme structure. Enzymes are immobilized by occlusion in the synthetic or natural polymeric networks. Entrapment can be achieved through gel or fibre entrapping and micro-encapsulation. Sol-gels widely used or highly porous silica materials, extensively used for protein immobilization particularly for the development of the biosensors.

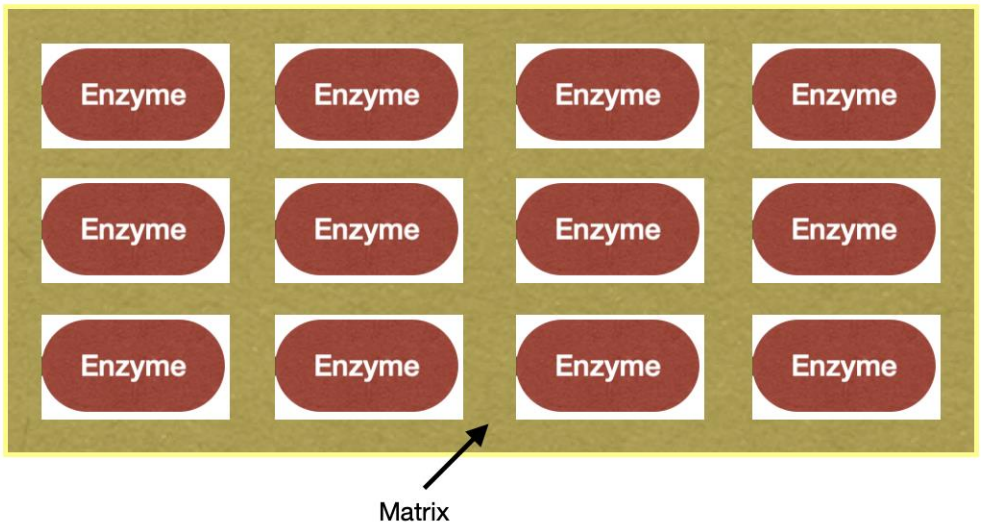

Fig. 8.4 Diagrammatic representation of Entrapment method

Entrapment can be further classified into lattice type and microcapsule types.

A) Lattice-type entrapment involves entrapping enzymes within the interstitial spaces of a cross-linked water insoluble polymer.

B) Microcapsuletype

It involves enclosing the enzymes within semipermeable polymer membranes.

Advantages

1. Fast cheap and mild conditions required for reaction process.
2. Easy to practice at small scale.
3. Large surface area between polymer and enzyme.
4. Loss of enzyme activity upon immobilization is minimized.
5. Used in sensing application.
6. Very less chance for conformational changes in enzyme.

Disadvantages

1. Limitation in mass transfer.
2. Enzyme inactivation during encapsulation.
3. Leakage of enzyme.
4. Pore diffusion limitation.
5. Chance of microbial contamination.

8.4.2.4 Cross linking or copolymerization

This method is an irreversible carrier-free enzyme immobilization. The enzyme acts as its own carrier.Formation of intermolecular cross linkage between the enzyme molecules by meaning of bi or multifunctional reagents i.e. glutaraldehyde. Nanodiametric supports such as electrospun.

Cross-linked enzyme aggregates is prepared by first aggregating the enzymes in precipitance such as acetone, ammonium sulphate, ethanol or 1,2-dimethoxyethane followed by addition of a cross-linker like glutaraldehyde.

Advantages

1. Very little enzyme desorption.
2. Best used in conjunction with other methods.

Disadvantages

Cross-linking may cause significant changes in the enzyme's active site.

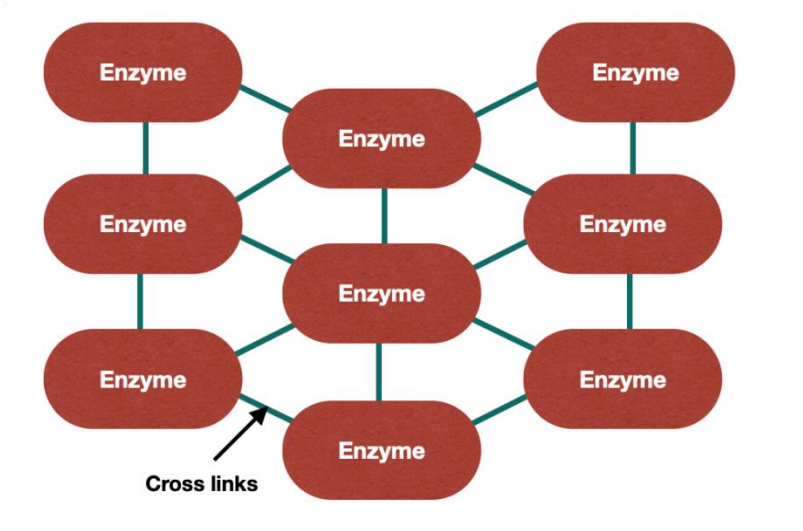

Fig. 8.5 Diagrammatic representation of Cross linking method

8.4.2.5 Metal linked Immobilization

In this method, transition metal salts or hydroxides are precipitated onto the support by heating or neutralization. The deposited matrix cannot occupy all coordination sites of metal, some are free to bind with enzymes. The metals i.e. titanium and zirconium salts and for supports i.e. cellulose, chitin, algenic acid, and silica – based carriers are used. The immobilized metal – ion affinity (IMA) absorbents - Chelator ligands viz. EDTA can immobilized first onto the solid supports by means of a stable covalent bonds, metal irons are then bound by coordination.

Advantages

Simple and the immobilized specific activities can be obtained with enzymes.

Disadvantages

Operational stabilities are highly variable and the results are not easily reproducible.

8.4.2.6 Encapsulation

This type of immobilization is done by enclosing the enzymes in a membrane capsule. The capsule made up of semipermeable membrane like nitrocelulose or nylon. In this method, the effectiveness depends upon the stability of enzymes inside the capsule.

Advantages

1. This method is cheap and simple method.
2. Large quantity of enzymes can be immobilized by encapsulation.

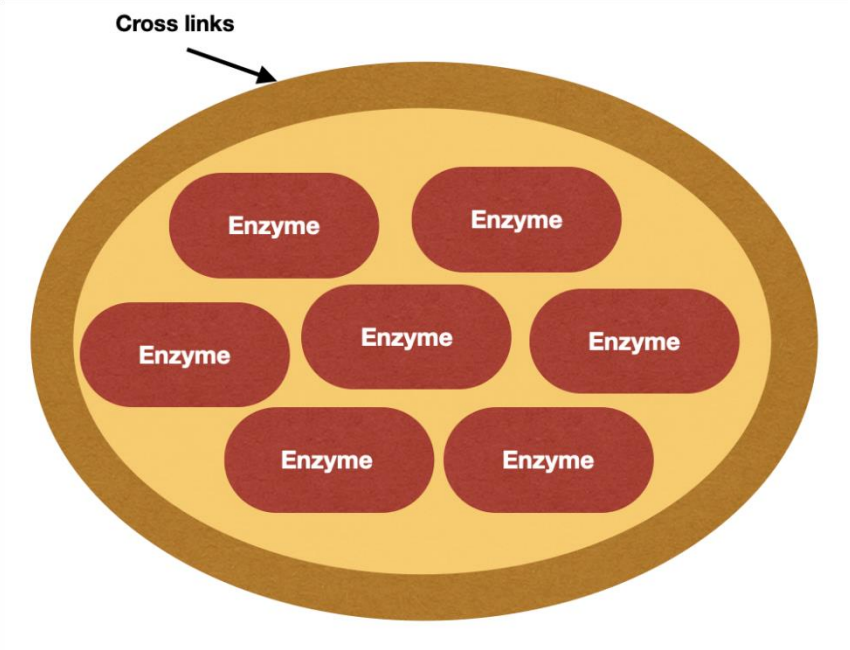

Fig. 8.6 Diagrammatic representation of encapsulation method

Disadvantages

1. Pore size limitation.
2. Only small substrate molecule is able to cross the membrane.

8.5 USE OF ENZYMES IN FOOD AND DRINK INDUSTRY-BREWING AND CHEESE-MAKING

Enzymes play an essential role in manufacturing of various industrial food products. Cheese, beer, wine and vinegar are such few examples. Enzymes are helpful in controlling various processes such as process time, enrich flavour, improve texture, increase shelf life etc. Enzymes breakdown complex molecules into smaller units such as carbohydrates into sugar. They are natural substances involved in all biochemical reactions. Enzymes are produced by all living cells and act as catalysts for specific chemical reactions. Enzymes are highly efficient which help in cut off effective cost of its respective processes. The application of enzymes as used in various sectors of food, drink, brewing and other industries are mentioned below.

8.5.1 Enzymes in food industry

Enzymes are very essential component of life processes such as digestion, respiration and metabolism in organisms. Due to its impressive catalytic efficiency, it has wide application in food industry and processing, especially in the preparation of beer, wine, cheese and bread. Enzymes are extracted from edible plants/plant parts and animal tissues or from microorganisms like bacteria, yeasts, and fungi. Rennet is a natural enzyme from the stomach of calves and other domestic animals which used in the preparation of cheese. Other industrial revolutionary growth of the enzyme industry may include the baking, beverages, brewing and dairy products. Additionally, enzymes also find applications in detergents, leather, textile pulp, and paper industries. Some of the enzymes as used in food industry are mentioned following:

1. ***Alpha-amylase***is used to solubilize the carbohydrates found in barley and other cereals used in brewing.
2. ***Beta-glucanase***cause breakdown of glucans in malt and other materials.
3. ***Lipase***used to shorten the time for cheese ripening. It is employed in the production of enzyme-modified cheese/butter from cheese curd or butterfat.
4. ***Papain*** is widely used as a meat tenderizer.
5. ***Chymosin*** helps in the curdling of milk by breaking down kappa-caseins in cheese making.
6. ***Microbialproteases***used in the production of fish meals, meat extracts, texturized proteins, etc.
7. ***Pectinase***used in treatment of fruit pulp to facilitate juice extraction. It also helps in the clarification and filtration of fruit juice.
8. ***Lactase***used as additive for dairy products for individuals lacking lactase.
9. ***Glucose oxidase***causeconversion of glucose to gluconic acid to prevent Maillard reaction (reaction that gives browned food a particular flavor) in products caused by high heat used in dehydration.
10. ***Cellulase*** cause conversion of cellulose waste to fermentable feedstock for ethanol or single-cell protein production.

8.5.2 Enzymes in drink and brewing industry

Enzymes are novel alternatives to chemical or mechanical methods for improving yield and quality in the beverage industry. Some enzyme i.e. pectinases, amylases, cellulases, and xylanases are used in the extraction and clarification of fruit juices. In beer production, sugar is converted into alcohol. During mashing, enzymes from malt, which is germinated barley, act on the starch of different grains to form sugars. The malting requires grains and heat for drying, by passing this process will save energy as well as agricultural land. *B. subtilis* proteases and used to solubilize proteins from barley adjuncts for production of wort. Haze formation due to proteinaceous substances in beer is also hydrolysed by microbial proteases. Following are some of the enzymes as used in drink and brewing industry.

1. ***Alpha amylases*** are used for the production of ethanol.
2. ***Carbohydrase*** enzymes are used as a wine enzyme that helps to reduce grape-juice viscosity and fouling of ultrafilter membranes.
3. ***Cellulases*** used in the beer industry to hydrolyzed cellulose and barley β-glycans during wheat are added as an adjunct.
4. ***Esterases*** play a prominent role in various food industries and alcoholic beverage industries. It is widely used to produce fragrances and flavours and in modification of oil and fat of fruit juices.
5. ***Papain*** is used in post-fermentation stages for beer production.
6. ***Tannase*** is extensively used as a clarification of beer and fruit juices. It is used in the manufacturing of coffee-flavored soft drinks and manufacturing for instant tea.
7. ***Xylanases*** enzyme employed for clarifying the wine and to reduce the viscosity in beverage industries.
8. P***ectinase*** enzyme is used in the beverage industry for making wine. This enzyme influences the viscosity of wine, the colour, and the flavour of the finished products.

8.5.3 Enzymes in cheese making

Cheese is way of preserving milk for long periods of time. Cheese-making is a long and involved process that makes use of bacteria, enzymes and naturally formed acids to solidify milk proteins and fat and preserve them. Once turned into cheese, milk can be stored for

many months or years. Acids and salts play role as main preservatives that give cheese its longevity. There are four types of enzymes used to produce cheese including -

1. Rennet

During cheese making, milk is first inoculated with lactic acid bacteria and rennet. Rennet contains a enzyme named as rennin helps in modifying proteins in milk. Specifically, rennin converts a common protein in milk called caseinogen into casein, which does not dissolve in water. The casein precipitates out as a gel-like substance that we see it as curd. The casein gel also captures most of the fat and calcium from the milk. So the lactic acid and the rennet cause the milk to curdle, separating into curds and whey.

2. ***Proteases***

In cow milk, whey protein is found which is breakdown by certain enzyme known as proteases. Thus, proposes help in breakdown of these proteins and peptide bonds. During the production of the soft cheese, the whey protein is separated from the milk after curdling.

3. Catalase

This enzyme helps in breakdown hydrogen peroxide into hydrogen and water. This is used in making cheese in order to preserve the natural milk enzymes that are beneficial to the flavour development of the cheese product.

4. Lipases

Lipases help in breakdown of milk fatty acids during cheese making process. For stronger flavoured cheeses they are prepared using lipases. These flavours come from the fatty acids that were produced when the milk fats are hydrolysed.

8.6 SYRUPS FROM CORN STARCH

Corn syrup is one of the food corn made from starch of corn. This is a generic name for a whole spectrum of nutritive sweeteners prepared from corn starch. Corn syrup is a purified and concentrated mixture of the saccharides obtained from the hydrolysis of corn starch. The corn starch contains very rich amount of sugar glucose, maltose and some higher oligosaccharides. Practically, corn syrup is the liquid hydrolysate of mono-, di, and higher saccharides can be prepared from starch, wheat, tapioca, and potatoes etc. It is also known as the glucose syrup and generally used to soften texture volume, prevent crystallisation of sugar

and enhance flavour. High fructose corn syrup (HFCS) is a liquid alternative sweetener to sucrose. According to fructose content, the fructose – glucose syrup is divided into three specifications $HFCS_{42}$, $HFCS_{55}$ and $HFCS_{90}$.

8.6.1 Production of corn syrups

For corn syrup production, corn starch is chiefly used as row material. In some industries, corn powder is directly converted into corn syrup. But in case of high quality corn syrup, corn starch is best for producing corn syrup. In brief, the process of corn syrup production is mentioned below:

8.6.1.1 Raw material preparation

The corn variety popularly known as "yellow dent corn" is majorly used as source of corn syrup preparation. It is one of the common variety cultivated in mid western portion of the United States and other part of world. Other materials used during the process of converting corn to corn syrup including sulphur dioxide, hydrochloric acid or various enzymes and water. For raw material preparation, the corn starch should have high amount of corn starch. Ideally, the specification should be as starch content (85.4%), moisture (14%), fat (0.1%), protein (0.4%), and ash 0.1%.

8.6.1.2 Mixing process

The mixing process helps corn starch or starch milk to adjust condition for liquefaction. This process should be done under controlled temperature and pH (5.3 - 5.6). A suitable amount of water should be added to get desirable concentration of medium.

8.6.1.3 Liquefaction

The starch milk is fully contacted with steam through the injection port, rapidly heats up, the starch is fully liquefied which inactivated by a high temperature and high pressure jet.

8.6.1.4 Saccharification

After liquefaction, the pH value is reduced to between 4.2 and 4.5, after the solution is cooled up to 60 ℃. The liquefied material keeps reaction for a certain time under the action of enzyme. The required dextrose equivalent (DE) value of the glucose should be 15-20%. The reaction time for saccharification is usually between 24-48 hour depending on enzyme reaction speed. In this step, Glucoamylase enzyme help in releasing single glucose units from

the ends of dextrin molecule. Syrups of 95% glucose or higher are manufactured by this process.

8.6.1.5 Filtration and decolourization

Glucose gets through the filter to remove protein and other impurities, then at the appropriate temperature passes by active carbon to decolouring, finally send to filters to remove activated carbon and send to the next section.

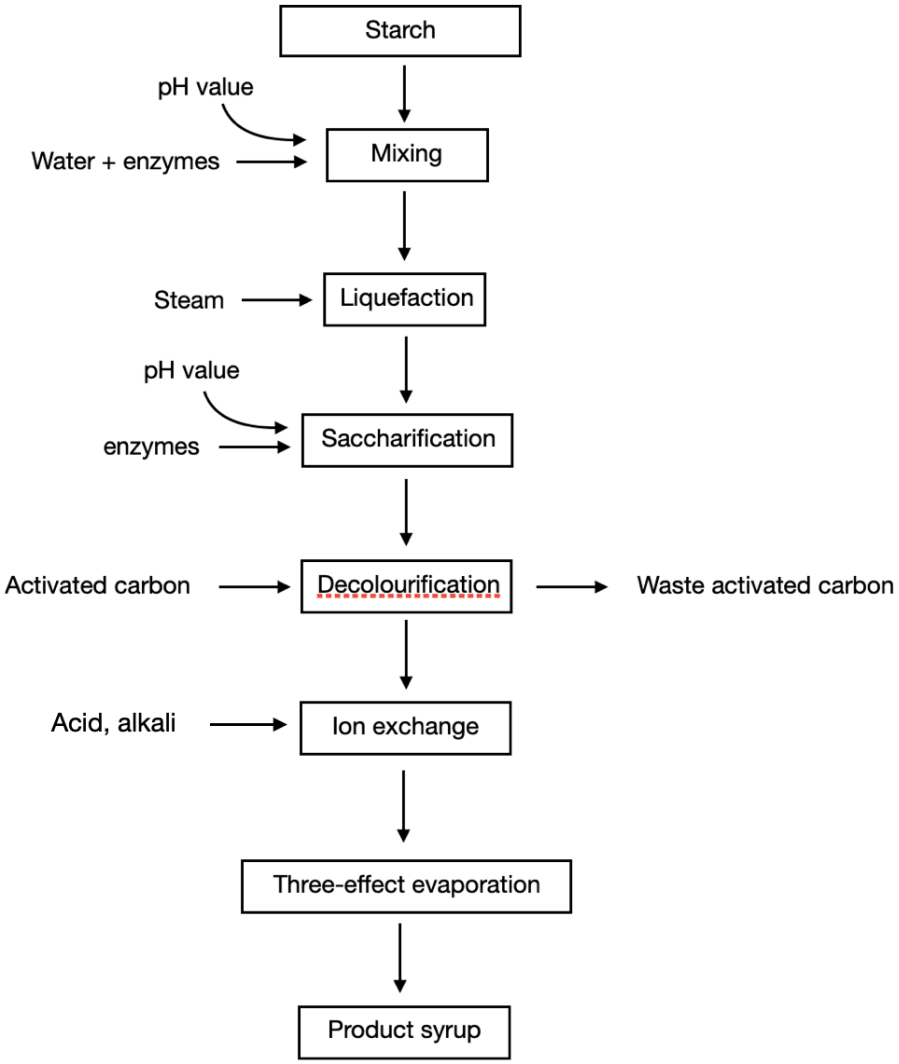

Fig. 8.7 Process of making corn starch syrup

8.6.1.6 Evaporation

The glucose is totally cleaned through safety filter machine then sent to evaporator for concentrating to reach the required DS as final product. The corn syrup powder, also called

corn syrup solids, is prepared by a drum or spray dryer to remove 97% of the water. This produces a crystalline corn syrup powder.

8.6.2 Applications of corn starch

Corn syrups have wide commercial applications in preparation of food stuffs as mentioned below:

1. It is widely used in food, canned foods, jam, dairy products, beverages, tobacco, cold drinks, fruit juices, preserve foods and wines etc.
2. It act as thicker, sweetener and humectant of commercial foods such as breads, cereals, yogurts, soups and condiments etc.
3. It retains moisture and maintain freshness of food products.
4. Corn syrup is used in the production of HFCS which include baked foods, sodas, yogurts and condiments. HFCS enhances sweetness, texture and flavour, stabilise colour and cause cost reduction of products.
5. HFCS is effective in enhancing texture and sparsity of ice cream and other dairy products i.e. chocolate milk.
6. It can replace cane sugar with similar concentration. While, its flavour is similar to natural juice.
7. The application in other sectors such as pharmaceutical, agriculture, animal feed, poultry feed is yet not reported.

8.7 ENZYMES AS TARGET FOR DRUG DESIGN

Enzymes are known to catalyse thousands of biochemical reaction types. They act as a target for drugs for the desired therapeutic effect, which called as biological targets. Enzymes offer unique opportunities for drug designing that are not available to cell surface receptors, nuclear hormone receptors, ion channels, transporters. Drugs acting on enzymes by two ways, first inhibition of enzymes (inhibitors) and second inactivation of enzyme (inactivators). In this respect, first one is more common. The enzyme-inhibitor or inactivator drugs have do so either via covalent reaction with nucleophilic enzymatic residues or by noncovalent binding. Such drugs prevent the formation of the product by inhibiting the enzyme - substrate interaction via competitive, non-competitive or uncompetitive interactions. Few examples of

such targeted enzymes are microsomal enzymes in neonatal jaundice and cushing's disease, transmembrane receptors such as receptor tyrosine kinases, JAK-STAT receptors, receptor Serine-Threonine Kinases, Toll-like receptors and TNF-α receptors etc.

8.7.1 Mechanism of inhibition of enzymes by drugs

The enzyme-substrate reaction is well known. In this, substrate molecule reacts with the enzyme to form an enzyme-substrate (ES) complex which at the end results in the formation of the final products. Drugs inhibiting enzymes prevent the formation of the product by inhibiting the enzyme-substrate interaction competitively, non-competitively or uncompetitively. The type of inhibition by the drug is hence classified accordingly.

8.7.1.1 Competitive Enzyme Inhibition by Drugs

Competitive inhibition is caused by compounds which resemble the chemical structure and molecular geometry of substrate molecule. In this type of inhibition, the inhibitor binds only to the enzyme and not to the enzyme-substrate complex. The inhibitor gets strongly stuck on the enzyme which prevents any substrate molecules from further reacting with the enzyme.However, competitive inhibition is usually known to be surmountable if sufficient substrate molecules are available to ultimately.

8.7.1.2 Non-competitive inhibition by drugs

Non-competitive inhibition is caused by a substance that reacts with the enzyme at the allosteric site. It binds to the enzyme as well as the ES complex with equal affinity. Drugs need not have a similar structure as that of the substrate of the enzyme. This type of inhibition is unsurmountable. Due to change in shape of active site, the substrate can no longer react with the enzyme to give the response. Non-competitive inhibition is usually irreversible but in few cases reversible inhibition have been observed e.g. carbonic anhydrase inhibition by acetazolamide.

Disulfiram is a non-competitive inhibitor of the enzyme aldehyde dehydrogenase. It forms an active metabolite diethylthiomethyl carbamate (DETMC). Conversion of acetaldehyde to acetic acid is stopped. Acetaldehyde accumulation causes nausea, vomiting, flushing, etc. which is used as a favouring factor in process of alcohol de-addiction. Disulfiram like reaction is also seen with metronidazole, schlorpropamide, glibenclamide, tolbutamide,

griseofluvin, cefotetan, cefoperazone, etacrynic acid and urinary antiseptics such as nitrofurantoin because of inhibition of Aldehyde Dehydrogenase by them.

8.7.1.3 Uncompetitive Inhibition by drugs

Uncompetitive inhibition occurs when a drug binds reversibly to the enzyme when the substrate is already bound to the active site. The inhibitor binds to the enzyme-substrate complex. Increasing the concentration of substrate will not overcome the inhibition in this case. The level of inhibition depends on the sufficient substrate being present at the active site to make E-S complex. Initially, reaction rate is speeded up to the formation of the E-S complex till a point after which the rate slows down and never reaches Vmax. Hence, the enzyme will not reach Vmax. The Lineweaver-Burk double reciprocal plot for this set of data doesn't show inhibitor line converging on the same point on the X (1/S) axis or the Y (1/V) axis i.e. Km and Vmax is also reduced. Following are few examples of targeted enzymes at various sites i.e. suicide inhibitor drugs, microsomal enzymes, transmembrane receptors linked to intracellular enzymes etc.

8.7.1.4 Suicide Inhibitor drugs

Suicide inhibition, also termed as suicide inactivation or mechanism-based inhibition.It causes an irreversible form of enzyme inhibition wherein an enzyme binds an inhibitor and forms an irreversible complex with the enzyme. For example β-lactamase inhibitors are given with β-Lactams to inhibit β-Lactamase enzyme and hence prevent hydrolysis of β-Lactam drugs like penicillins. Clavulanic acid covalently bonds to a serine reside present in the active site of the β- lactamase, which results in restructuring of the clavulanic acid molecule.

8.7.1.5 Microsomal enzymes as drug targets

Microsomal enzymes are enzymes which are typically found in the endoplasmic reticulum of hepatocytes. They play a very important role in the metabolism of drugs and some endogenous substrates. These enzymes can be induced or inhibited by certain drugs in order to affect the metabolism of certain endogenous substrates or drugs.

8.7.1.6 Transmembrane receptors linked to intracellular enzymes

Transmembrane receptors act in cell signaling by binding to extracellular molecules. Signal transduction is a process through membrane receptors which involves some external reactions, in which the ligands binds to a membrane receptor, and the corresponding internal

reactions, in which intracellular response is triggered. Majority of the enzyme-linked receptors are protein kinases or are associated with it.There are various types of Transmembrane receptors linked to intracellular enzymes i.e. receptor tyrosine kinases, JAK-STAT receptor, receptor serine-threonine kinases, toll-like receptors (TLR), TNF-α receptors (Tumor necrosis factor alpha receptors) etc.

8.7.2 Applications

Pharmaceutical industries use enzymes as drug targets to manufacture drugs e.g. dihydrofolate reductase inhibitors such as methotrexate, phosphodiesterase inhibitors such as theophylline, etc. Role of enzymes is also extended to therapeutics for example serratiopeptidases as antii-inflammatory, β-lactamases for penicillin allergy, tissue plasminogen activators as fibrinolytics. Various anti-cancer drugs have been designed to resolve cancerous diseases.

8.8 CLINICAL USES OF ENZYMES

Enzymes act as markers in various disease such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. They can provide insight into the disease process by diagnosis, prognosis and assessment of response therapy. The major applications and uses of such enzymes in clinical sectors are mentioned below.

8.8.1 In medical device cleaning

Enzyme based detergents is used for cleaning of various reusable medical devices. In which two enzymes are chiefly used named as protease and lipase. Proteases breakdown the protein rich soils like blood etc. While, lipases used for fatty soils like adipose tissues. Some other examples i.e. amylases and cellulases help in breakdown of starch and cellulose polymers.

8.8.2 In medicine

Enzymes are typically used as medicines to interchange enzyme deficiencies in patients such like blood coagulation factors to treat bleeder’s disease, cleaning of wounds, healing, improving metabolism, in diagnosis of diseases etc. Some popular enzymes i.e. proteases, carbohydrases, and lipases are widely used in pharmaceutical and healthcare sectors.

8.8.3 In treatment of disorders

In terms of disorders, enzymes are used in three cases including 1) to break the internal blood clots, 2) to dissolve the hardening of walls of blood vessels, and 3) to dissolve the wound swelling to promote healing. In some disorders like low blood pressure, or head or spinal injuries, there are chances of formation of blood clots. Thus, only way is to dissolve the clots. These clots are usually removed by dissolution by enzymes that can break them. Similarly, when there is atherosclerosis, hardening and thickening of blood vessel walls. The best way out at this junction is to decrease the fat intake and also dissolve the formed thickenings. Enzymes like serratiopeptidase work well at this place. For wound healing, the swelling formed might be painful and tend to form pus. Enzymes such as trypsin, chymotrypsin, serratiopeptidase are used to dissolve the swelling.

8.8.4 Used to assist metabolism

In geriatric patients, the digestive capacity is low due to insufficient secretion of digestive enzymes. In such cases, the patients can be experienced malnutrition, constipation, bloating etc. To improve such digestive condition, enzymes like papain are administered orally after food for better digestion.

8.8.5 To assist drug delivery

Some drugs have higher penetration capability inside the tissues. For this, some enzymes i.e. hyaluronidase are used with such drugs in intra-muscular injection forms. Hyaluronidase is one of kind of natural enzyme to assist sperms in penetration of female internal reproductive organ and fertilise with ova.

8.8.6 To diagnose disorders

Enzymes of the liver, kidney, skeletal muscle, heart, etc. leak into blood during related disorders. Measuring the amount of the corresponding protein in high or low levels in blood indicates the particular disorder. For example, creatine kinase is for muscle weakness and injury. Similarly, polymerase chain reaction (PCR) help to diagnose genetic diseases in the prenatal stage for disorders like sickle cell anaemia, Huntington’s disease, beta-thalassemia etc.

8.8.7 In preparation of toothpaste

Enzymes of papaya and pineapple are used in the dentifrice. They are found to remove the stain on teeth to give white and sparkling teeth.

8.9 ENZYMES THERAPY

Enzyme therapy is a therapeutic tool for improvement of body's ability to maintain certain disorders or diseases related to any enzymatic biological processes. The biological processes may relate to metabolism deficiencies, immune system, cancer, cardiovascular disease, microbial system etc. The enzymes act as a target for drug designing for the desired therapeutic effects, known as biological targets. Currently, the enzyme therapy is being studied in treatment of the virus SARC-CoV2 and its associative other viruses, the causative agents of CoVID-19 disease.

8.9.1 How the enzyme therapy works?

The enzyme-based therapy may be systemic or non-systemic. They have multiple administration routes including oral, topic, respiratory or intravenous. Diseases caused by absence or dysfunction of enzymes are the main target of enzyme replacement therapy (ERT). The use of enzymes for therapy is highly disease specific and varies with each and every diseased condition especially while considering ERT. These medical conditions are tried to restore the lost or altered enzyme activities via administration of enzymes intravenously. Such diseases or disorders may include lysosomal storage disorders, cancer, Alzheimer's disease, irritable bowel syndrome, exocrine pancreatic insufficiency, and hyperuricemia.

8.9.2 Benefits behind enzyme therapy

Enzymes have been associated with multiple futuristic therapies including cancer, microbial infection, wound healing and gene therapies, lysosomal storage disorders, Alzheimer's disease, irritable bowel syndrome, exocrine pancreatic insufficiency, hyperuricemia,

8.9.3 Limitations of enzyme therapy

The limitations of enzyme therapy are mentioned below:

1. The modification of targeting lysosomes

2. Low availability of enzymes in some tissues
3. Short half-life of the enzyme
4. Lack of ability to permeate the blood-brain barrier
5. Side effects that arise from immunogenicity of the enzyme, and
6. An inefficient mode of delivery and a high cost of enzymes etc.

8.10 ENZYMES AND RECOMBINANT DNA TECHNOLOGY

The recombinant DNA technology (RDT) provides a molecular tools to produce recombinant DNA (rDNA) by using a set of enzymes known as recombinant enzymes e.g. DNA ligase, restriction enzymes, DNA polymerases, exonuclease etc. The functionality of these enzymes are quite diverse and may used as powerful tools in various areas i.e. genetic engineering, molecular biology, proteomics, bioinformatics etc. The multiple functions of these enzymes may include host-controlled restriction, chain elongation, ligation, protection of their own DNA from cleavage, methylation etc. A detailed description of enzymes used in RDT with their functionality has been mentioned in given table 8.3.

Table 8.3 Enzymes used in recombinant DNA technology

Enzyme(s)	**Function**
Type I Restriction endonucleases	It has both methylation and endonuclease activity. It cut DNA about 1000 bp away from the restriction site e.g. EcoKI
Type II Restriction endonucleases	It cuts DNA at restriction site itself e.g. EcoRI, Hind III
Type III Restriction endonucleases	It cuts DNA about 25 bp away from the restriction site e.g. EcoPI
DNA polymerases	It helps in elongation of DNA strand via addition of nucleotide to free 3'OH end. DNA polymerase I isolated from *E. coli* as used in gene cloning and Taq polymerase isolated from *Thermusaquatics* used in PCR.

Reverse transcriptase	It is used to synthesise complementary strand (cDNA) from mRNA. It is isolated from retrovirus.
Exonuclease III	It removes nucleotide residues from the 3' end of a DNA strand
DNA ligase	Join two DNA molecules or fragments
Polynucleotide kinase	It add a phosphate group to the 5' OH end of DNA strand or permit ligation
Terminal transferase	Add homopolymer tails to the 3'OH ends of linear duplex
Alkaline phosphatase	It removes terminal phosphates from either the 5' or 3' end or both.
Ribonuclease H	It removes mRNA from DNA-RNA heteroduplex which used by mRNA to synthesise cDNA

8.11 SUMMARY

Enzymes are specific, versatile, and efficient biocatalysts which participate in reaction and cause lowering of activation energy. Industrial fermentation is one of the important step in enzyme production in which the substrates are converted into products. The factors influence this process may depend on temperature, substrate, pH, aeration and agitation, inhibitors etc. The essential steps for industrial enzyme production can be categories into two parts including (1) upstream and (2) downstream processes. Upstream process includes inoculum development, media preparation, cell culture, cell Separation and harvest. While, the downstream process includes the recovery of enzymes from the fermented broth.

Enzyme immobilization is a widespread technology to achieve more stable, active and reusable enzymes. It can be defined as the confinement of enzyme molecules onto/within a support or matrix via physically or chemically or both for retaining full activity. There are six major types of principal techniques for immobilization of enzymes include adsorption, covalent binding, entrapment, cross-linking or copolymerization, metal linked Immobilization, and encapsulation. The advantages of enzyme immobilization includes the protection from degradation and deactivation of enzymes, retention of enzymes, enzyme-free

products, recycling or repetitive use of enzymes, enhanced stability and efficiency of enzymes, use as controlled release agents, ability to stop the reaction rapidly by removing the enzyme from the reaction, development of multi-enzyme system and minimum reaction rate and high enzyme substrate ratio.

In industrial application, enzymes are widely used in various products including baking, beverages, brewing and dairy products. Additionally, enzymes also find applications in detergents, leather, textile pulp, and paper industries. The enzymes used in such processes include alpha-amylase, beta-glucanase, lipase, papain, chymosin, pectinase, lactase, glucose oxidase, cellulase etc. The enzymes used in cheese making are rennet, proteases, catalase, lipases etc. respectively. Enzymes are also useful in drug designing. They act as a target for drugs for the desired therapeutic effect, which called as biological targets. Enzymes offer unique opportunities for drug designing that are not available to cell surface receptors, nuclear hormone receptors, ion channels, transporters. The drug inhibitions of enzymes are classified into three major types including Competitive inhibition, non-competitive inhibition, and Uncompetitive inhibition. Enzymes act as markers in various disease such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. They can provide insight into the disease process by diagnosis, prognosis and assessment of response therapy. Currently, recombinant DNA technology (RDT) provides a molecular tools to produce recombinant DNA (rDNA) by using a set of enzymes known as recombinant enzymes e.g. DNA ligase, restriction enzymes, DNA polymerases, exonuclease etc. The functionality of these enzymes are quite diverse and may used as powerful tools in various areas i.e. genetic engineering, molecular biology, proteomics, bioinformatics etc. The multiple functions of these enzymes may include host-controlled restriction, chain elongation, ligation, protection of their own DNA from cleavage, methylation etc.

8.12 SAQs TYPE QUESTION

A. Multiple choice questions

1. Which enzyme cause conversion of cellulose waste to fermentable feedstock for ethanol or single-cell protein production -

A) Pectinase B) Cellulase C) Lactase D) Glucose oxidase

2. Which enzyme is used as a clarification of beer and fruit juices?

A) Papain B) Cellulase C) Tannase D) Carbohydrase

3. The enzyme used to synthesise complementary strand (cDNA) from mRNA named as -

A) Type I restriction enzyme B) DNA ligase C) Reverse transcriptase D) DNA gyrase

4. DNA molecules or fragments can be ligated by -

A) DNA ligase B) Alkaline phosphatase C) Reverse transcriptase D) None of these.

5. The first immobilized enzyme was amino acylase isolated from -

A). *Aspergillusoryzae* B) *Clostridium* spp. C) Asergillus niger D) Penicillium spp.

6. Enzymes are specific, versatile, and efficient biocatalysts which participate in reaction.

A) Amylases B) papain C) cellulases D) Pectinases and pectinesterases

7. Phytase, the enzyme used in -

A) Paper and pulp industry B) meat industry C) dairy industry D) None of these

8. It removes nucleotide residues from the 3' end of a DNA strand -

A) DNA ligase B) Type III Restriction endonucleases

C) Exonuclease III D) None of these

9. Pepsin, trypsin and pancratin isolated from -

A) Animal B) Plant C) Fungi D) Bacteria

10. For commercial production of enzymes the selected microorganisms should be -

A) genetically stable B) genetically unstable C) Morphologically stable D) Morphologically unstable

B. Fill in the blanks

1. …….. is widely used as a meat tenderizer.
2. Taq polymerase isolated from a bacterium named as …….. used in PCR.
3. Adsorption is one of the simplest, ……. and …… method.
4. Calcium alginate is an insoluble material chiefly used in …… of enzymes.

5. Rennet contains a enzyme named as …… helps in modifying proteins in milk.
6. HFCS stands for…….
7. The enzyme known as …… converts a common protein in milk called caseinogen into casein.
8. The first immobilized enzyme was amino acylase isolated from …… for the production of L-amino acids.
9. Invertase, the enzyme used in food industry isolated from ……
10. In ……. method, enzymes are physically entrapped inside a porous matrix.

C. True/False

1. Enzymes are non-specific and inefficient biocatalysts which cannot participate in reaction. (True/False)
2. Disulfiram is a non-competitive inhibitor of the enzyme aldehyde dehydrogenase. (True/False)
3. High fructose corn syrup (HFCS) is a liquid alternative sweetener to sucrose. (True/False)
4. Corn syrup is one of the food corn made from starch of wheat. (True/False)
5. Rennet contains a enzyme named as rennin helps in modifying proteins in milk. (True/ False)
6. Catalase helps in breakdown of hydrogen peroxide into hydrogen and water. (True/False)
7. The preparation of cheese is not a way of preserving milk for long periods of time. (True/False)
8. Lipases help in breakdown of milk fatty acids during cheese making process. (True/False)
9. *Carbohydrase*enzymes are used as a wine enzyme that helps to reduce grape-juice viscosity. (True/False)
10. *Papain*is used in post-fermentation stages for beer production. (True/False)

D. Match the following

1. PCR	-	(A) Severe Acute Respiratory Syndrome Coronavirus 2
2. cDNA	-	(B) *Gluconobacter oxydans*
3. HFCS	-	(C) Join two DNA molecules or fragments
4. Encapsulation	-	(D) Jack Bean
5. Papain	-	(E) High fructose corn syrup
6. Urease	-	(F) enzyme replacement therapy
7. Dextrinase	-	(G) Reverse transcriptase
8. ERT	-	(H) A method of immobilization
9. DNA ligase	-	(I) Papaya
10. SARC-CoV2	-	(J) Polymerase chain reaction

Answer Key

A. Multiple choice questions

1. B 2. C 3. C 4. A 5. A 6. D 7. A 8. C 9. A 10. A

B. Fill in the blanks

1. Cellulose 2. T*hermusaquatics* 3. oldest, reversible 4. immobilization

5. rennin 6. High fructose corn syrup 7. Renin 8. *Aspergillusoryzae*

9. *Saccharomyces* spp.

10. entrapment

C. True and False -

1. False 2. True 3. True 4. False 5. True 6. True

7. False 8. True 9. True 10. True

D. Match the following -

1. J 2. G 3. E 4. H 5. I 6. D 7. B 8. F 9. C 10. A

8.13 GLOSSARY

ERT	-	Enzyme Replacement Therapy
HFCS	-	High fructose corn syrup
TLR	-	Toll-like receptor
PCR	-	Polymerase chain reaction
SARC-CoV2	-	Severe Acute Respiratory Syndrome Coronavirus 2
RDT	-	Recombinant DNA technology
cDNA	-	Complementary DNA
rDNA	-	Recombinant DNA
DETMC	-	Diethylthiomethyl carbamate

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8.16 TERMINAL QUESTIONS

1. For commercial production what types of criteria should be followed for screening of microorganisms?
2. Explain the essential steps of purification of enzymes.
3. Describe about clinical uses of enzymes.
4. How the enzyme therapy works? Explain in detail with suitable examples of diseases or disorders cured by enzyme therapy.
5. Give a detail note on use of enzymes in recombinant DNA technology.
6. What do you understand of enzymes? Describe the various steps of large scale production of enzymes.
7. What is immobilization of enzyme? Explain various methods of immobilization with help of suitable examples.
8. Give detailed notes on “use of enzymes in food industry”.
9. Explain the role of enzymes in process of cheese making with suitable examples.
10. How the enzymes act as target for drug designing? Explain the mechanism of inhibition of enzymes by drugs.

BLOCK III: BIOPHYSICAL CHEMISTRY

UNIT 9: BIOENERGETICS

CONTENTS:

9.1 INTRODUCTION

The branch of biochemistry dealing with the energy needed to create and break chemical connections in living molecules is called bioenergetics. Energy exchanges, as well as energy transformations and transductions, are all being explored in biological organisms. Through a variety of metabolic processes, all living things in the universe may acquire energy. Because the function of energy is fundamental to such biological activities, growth, development, anabolism, and catabolism are among the most important processes in the study of living entities.

Energy is exchanged between living tissues and cells and the environment to keep living organisms alive. Autotrophic organisms, for example, may be able to get energy from sunshine without eating or breaking down nutrients (photosynthesis). Heterotrophs, for example, rely on food for nutrients in order to maintain their energy levels via metabolic processes like glycolysis and the citric acid cycle, which break down chemical bonds in food. As a result of the first law of thermodynamics, autotrophic and heterotrophic organisms interact in a global metabolic network. Heterotrophs derive their energy by eating autotrophs (plants). As energy is transmitted and transformed, chemical connections in a living creature are destroyed and new ones are established.

Energy becomes available for labour (such as mechanical activity) or other activities when weak connections are broken and stronger ones are established (such as chemical synthesis and anabolic processes in development). When our connections are stronger, we may be able to discharge usable energy. The goal of metabolic and catabolic processes is to make ATP from readily available starting materials (in the environment) and then break them down (into ADP and inorganic phosphate) for use in biological activities. The ratio of ATP and ADP concentrations determines the "energy charge" of a cell. The cell can use ATP to conduct work if there is more ATP than ADP available, but if there is more ADP than ATP, the cell must synthesise ATP by oxidative phosphorylation.In living organisms, oxidative phosphorylation from energy sources such as sunlight or oxygen is the primary source of ATP. When ATP is hydrolyzed (broken down) into adenosine diphosphate and inorganic phosphate, the terminal phosphate connections become weak. The energy released here is not part of the phosphoanhydride link between the terminal phosphate group and the remainder of the ATP molecule, but rather comes from hydrolysis's thermodynamically favourable free

energy. Similar to a battery, an organism's ATP stockpile is used to store energy in cells. All living species need the chemical energy generated by such molecular bond rearrangement to fuel their biological activities.

9.2 OBJECTIVE

The objective of this unit, you will be able to-

- After completing this unit, you will be able to understand how standard free energy changes in biological reactions.
- A process in which ATP is hydrolyzed
- How do you make ATP from ADP?

9.3 BIOLOGICAL ENERGY TRANSFORMATIONS OBEY THE LAWS OF THERMODYNAMIC

Many quantitative findings by physicists and chemists on the interconversion of various forms of energy led to the creation of two key thermodynamic ideas in the nineteenth century. Any physical or chemical change requires energy conversion, according to thermodynamics' first law. The overall quantity of energy in the cosmos remains constant; it may be transferred from one location to another, but it cannot be generated or destroyed. The second rule of thermodynamics states that the universe's entropy is rising in all natural processes, implying that the cosmos is always heading toward greater chaos.

Living things, despite the second rule of thermodynamics, are made up of a collection of molecules that are substantially more ordered than the material from which they are built, and they preserve and develop order. The second rule, on the other hand, is scrupulously followed by all living beings. To see whether the biological systems' second rule applies, we must first identify the systems and the environment in which they function. Three thermodynamic variables indicate the energy changes that take place during a chemical process.

9.3.1 Gibbs free energy, G: Gibbs free energy, also known as Gibbs function, Gibbs energy, or free enthalpy, is a phrase used to measure the amount of work done in a thermodynamic system at constant temperature and pressure. Gibbs free energy is represented by the symbol"G." Its energy is often expressed in joules or kilojoules. Gibbs free energy is

the most work that can be extracted from a closed system.While conducting studies to anticipate the behaviour of systems when they are connected or if a process may occur concurrently and spontaneously in 1876, an American scientist called Josiah Willard Gibbs discovered this feature. "Available energy" was a term used by Gibbs to describe his free energy. It's the quantity of energy that can be put to use in a thermodynamic system. Gibbs free energy is calculated by subtracting the system's enthalpy from the temperature and entropy product. Here's how to figure it out:

$$G = H - TS$$

Where, G = Gibbs free energy, H = enthalpy, T = temperature, S = entropy

Gibbs free energy is a state function hence it doesn't depend on the path. So change in Gibbs free energy is equal to the change in enthalpy minus the product of temperature and entropy change of the system.

$$\Delta G = \Delta H - \Delta(TS)$$

If the reaction is carried out under constant temperature $\{\Delta T=O\}$

$$\Delta G = \Delta H - T\Delta S$$

This equation is called the Gibbs Helmholtz equation.

$\Delta G > 0$; the reaction is non-spontaneous and endergonic

$\Delta G < 0$; the reaction is spontaneous and exergonic

$\Delta G = 0$; reaction is at equilibrium

9.3.2 Enthalpy, H: The enthalpy, or H, is the heat content of a reacting system. This number reflects the quantity and kinds of chemical bonds in the reactants and products. The term "exothermic" refers to a chemical reaction that generates heat. Because the heat content of the products is lower than that of the reactants, ΔH is usually negative. Endotners are heat-absorbing devices with positive ΔH values.

9.3.3 Entropy, S: Entropy, S, is a numerical representation of the randomness or disorder in a system. The reaction is considered to occur with a gain in entropy when the products are less complicated and disordered than the reactants.

ΔG and ΔH are measured in joule per mole or calorie per mole (recall that 1 cal = 4.184 J) and entropy is measured in joule/mole Kelvin (j/mole. K).

Table 9.1: Some physical constant and used in thermodynamics

Boltzmann constant, k = 1.381×10^{-23}J/K
Avogadro's number, N = 6.022×10^{23}mol
Faraday constant, J = 96,680 J/V. mol
Gas constant, R = 8.315 Jmol. K (= 1.987 cal/mol. K)
Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol. K (or cal/mol.K)
1 cal = 4.185 J
Units of absolute temperature, T, are Kelvin, K
25'C = 298K
At 25°C, RT = 2.479 kJ/mol (= 0.592 kcal/mol)

Changes in free energy, enthalpy, and entropy are quantitatively related in biological systems (including constant temperature and pressure) by the equation

$$\Delta G=\Delta H-T\Delta S$$

where ΔG is the change in Gibbs free energy of the reacting system, ΔH is the change in enthalpy of the system, T is the absolute temperature, and as is the change in entropy of the system. When entropy grows, S has a positive sign, while ΔH, as previously stated, has a negative value when heat is emitted by the system to its surroundings. ΔG tends to be negative in any of these situations, which are indicative of a favourable process. In reality, ΔG is always negative in a spontaneously operating system (Table 9.2).

The second law of thermodynamics implies that all chemical and physical activities increase the entropy of the universe, but it does not specify that this entropy growth must occur inside the responding system. As cells expand and divide, the order they generate in their surroundings far surpasses the order they create inside themselves. Living beings maintain

their internal order by absorbing free energy from their surroundings in the form of nutrients or sunshine and returning it as heat and entropy.

Table 9.2: Variation of response spontaneity (sign of ∆G) in comparison to the signs of ∆H and ∆S

∆H	∆S	∆G= ∆H-T∆S
-	+	The reaction is both enthalphically favoured (exothermic) and entropically favoured. It is spontaneous (exergonic) at all termperatures.
-	-	The reaction is enthalpically favoured but entropically opposed. It is spontaneous only at temperatures below T= ∆H/∆S.
+	+	The reaction is enthalpically opposed (endothermic) but entropically favoured is spontaneous only at temperatures above T= ∆H/∆S.
+	-	The reaction is both enthalpically and entropically opposed. It is unspontaneous (endergonic) at all temperatures.

9.4 CHEMICAL EQUILIBRIA AND THE STANDARD STAT

If entropy changes with concentration, then free energy must change as well. As a consequence, the concentrations of both reactants and products have an impact on the free energy change of a chemical process. Because many biological processes function in either direction depending on the relative concentrations of their reactants and products, this phenomenon is very important.

9.5 RELATION BETWEEN EQUILIBRIUM CNSTANT AND GIBBS FREE ENERGY (∆G)

The relationship between the concentration and the free energy of a substance A is approximately

$$\overline{GA} - \overline{GA}^0 = RT \ln [A] \qquad .....1$$

GA bar denotes A's partial molar free energy or chemical potential (the bar denotes (2) the quantity per mole), GA^0 denotes A^0 partial molar free energy in its standard state, R the gas constant, and [A] the molar concentration. As a consequence, the general reaction has been positive.

$$aA + bB \rightleftharpoons cC + dD$$

The free energy change is

$$\Delta G = c\overline{G}C + d\overline{G}D - a\overline{G}A - b\overline{G}B \qquad .....2$$

and $$\Delta G^0 = c\overline{G^0}C + d\overline{G^0}D - a\overline{G^0}A - b\overline{G^0}B \qquad .....3$$

Due to the associative nature of free energies, the overall energy change of a reaction is equal to the sum of the free energies of the products minus the free energies of the teactants. We obtain the following when we put these connections into equation (1):

$$\Delta G = \Delta G^0 + RT \ln \left[\frac{[C]^c [D]^d}{[A]^a [B]^b} \right] \qquad .....4$$

When all of the reactants and products are in their traditional states, ΔG is the free energy change of the process. As a result, the expression for the free energy change of a reaction has two parts: (i) a constant term whose value is solely determined by the reaction taking place, and (ii) a variable term whose value is determined by the concentration of the reactants and products, the reaction's stoichiometry, and the temperature. Because the forward reaction's free energy completely balances the reverse reaction's, there is no net change in a reaction at equilibrium. As a result, $\Delta G = 0$, and equation

$$\Delta G = - RT \ln Keq \qquad ……..5$$

Keq is the reaction's well-known equilibrium constant.

$$Keq = \left[\frac{[C]_{eq}^{c} [D]_{eq}^{d}}{[A]_{eq}^{a} [B]_{eq}^{b}} \right] = e^{-\Delta G^*/RT} \qquad .....6$$

The subscript "eq" denotes the equilibrium concentration of reactant and product (Because the equilibrium condition is generally obvious from the context of the event, equilibrium

concentrations are often presented without this subscript.). As a consequence, conventional free energy data may be used to determine the reaction's equilibrium constant and vice versa. By replacing equation for equation, it is possible to observe how the equilibrium constant fluctuates with temperature (1). i.e. in equation (5), $\Delta G = \Delta H - T\Delta S$

$$\ln Keq = \frac{-\Delta H^0}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^0}{R} \qquad .....7$$

In the standard state, H^0 and S^0 are the enthalpy and entropy, respectively. $y = mx + b$, the equation for a straight line, is the form of equation (7). From observations of Keq at two (or more) different temperatures, a van't Hoff plot of In Keq versus 1/T may be used to derive the values of ΔH^0 and ΔS^0 (and consequently ΔG). This method is usually more practical than directly measuring H and S using calorimetry.

9.6 STANDARD STATE CONVERSIONS IN BIOCHEMISTRY

It is required to establish ΔG values relative to some reference state that is arbitrarily assigned the height of zero, which we refer to as the heights of geographic areas, in order to compare free energy changes for distinct processes. When the temperature is 25°C, the pressure is 1 atm, and the solute's activity is 1, the solute is said to be in its standard state, according to physical chemistry norms (A substance's activity is its concentration adjusted for non-ideal behaviour at greater concentrations than infinite dilution).

In most biological processes, the concentrations of reactants and products are so low (millimolar or less) that their activities are roughly approximated by their molar concentrations. Biochemists have developed a slightly modified standard state convention because biological processes occur around neutral pH.

(i) Even though pure water has a concentration of 55.5 M, its activity is given a value of 1. Because the $[H_2O]$ component may be disregarded, the free energy equations for reactions in dilute solutions containing water as a reactant are simplified.

(ii) At the physiologically appropriate pH of the biochemical standard state, pH 70 (neutral pH, where $[H^+] = 10^{-7}$ M), rather than pH O ([H] = 1 M), the physical chemical standard state, where many biological compounds are unstable, the hydrogen ion activity is given a value of 1.

9.7 CELLS REQUIRE SOURCES OF FREE ENERGY

Isothermal systems, which include cells, operate at a generally constant temperature (they also function at constant pressure). Because heat can only accomplish work when it moves to a zone or object with a lower temperature, heat flow is not a source of energy for cells. The Gibbs free energy function is a mathematical function that determines the amount of energy that cells may and must utilise, allowing for prediction of chemical process direction, accurate equilibrium position, and theoretical work output under constant temperature and pressure. Heterotrophic cells derive their free energy from food molecules, whereas photosynthetic cells get it from solar rays absorbed. At constant temperatures, both types of cells convert this free energy into ATP and other energy-rich molecules capable of powering biological functions.

9.8 THERMODYNAMICALLY UNFAVOUARABLE REACTION CAN BE DRIVEN BY A FAVOURABLE ONE

As previously stated, the most relevant thermodynamic notion in biology is free energy. Only if ΔG is presentIt is a negative shift in free energy. Remember that ΔG is the formula for forming products C and D from substrates A and B.

$$\Delta G = \Delta G^0 + RT \log_e \frac{[C]\ [D]}{[A]\ [B]}$$

As a result, the kind of reactant (as described by the ΔG^0 term, the standard free energy change) and their concentrations have an impact on the process' ΔG (expressed by the logarithmic term). At pH 7, ΔG^0 represents the change in free energy. For a chemically connected chain of events, the total of the free energy changes at various stages equals the overall free energy change, which is a crucial thermodynamic component. Take a look at the process in the presence of standard ions.

$$A \rightleftharpoons B + C \qquad \Delta G^{0,} = +5 \text{ kcal/mol}$$

$$A \rightleftharpoons D \qquad \Delta G^{0,} = -8 \text{kcal/mol}$$

$$A \rightleftharpoons C + D \qquad \Delta G^{0,} = -3 \text{ kcal/mol}$$

Because ΔG is positive, A cannot change into both B and C.However, under normal condition, it is conceivable to transform B to D thermodynamically.Because free energy changes add up, the conversion of A to C and D has a ΔG^0 of -3 kcal/mol, indicating that it might occur spontaneously under normal conditions. As a consequence, a thermodynamically favourable reaction linked to it might cause a thermodynamically unfavourable reaction. A common chemical intermediary known as B connects the processes in this example. To link an uphill and downhill reaction, there are two major ways. By storing free energy in an active protein structure, a thermodynamically unfavourable process may be pushed.

In a number of methods, proteins have been exploited as energy conversion devices. The phosphoryl potential of ATP is converted into mechanical energy by molecular motors such as myosin, kinesin, and dynein. ATP phosphorylates and subsequently dephosphorylates the sodium-potassium pump, which causes active Na^+ and K^+ transport across membranes. The pump's affinity for the transported ions changes as a result of this reaction cycle, as does the orientation of its ion-binding sites with reference to the cell's inside and outside walls. Bacteriorhodopsin's capacity to capture photons and employ them to pump protons across the cell membrane highlights the diversity and strength of proteins in energy transduction.

Ionic gradients may also be employed to link uphill and downstream activities. For example, the electrochemical potential of Na^+ might be employed to transport nutrients like carbohydrates and amino acids into cells or to pump Ca^{2+} out of cells. Proton gradients generated by the oxidation of fuel molecules or photosynthesis power most of the ATP production in cells.Membrane-bound proteinos control all of these energy conversion processes by cycling through conformational modifications.

9.9 ROLE OF ATP IN BIOLOGICAL SYSTEMS (HYDROLYSIS OF ATP)

It takes a lot of energy to keep the human body working well because it is such a complex thing. The energy source for usage and storage at the cellular level is adenosine triphosphate (ATP). Adenosine triphosphate (ATP) is a nucleoside triphosphate that consists of three serially connected phosphate groups, a nitrogenous base (adenine), and ribose sugar. The connection between the second and third phosphate groups in ATP is regarded as the "energy currency" of the cell because it produces swiftly releasable energy. In addition to generating

energy, ATP hydrolysis performs a variety of activities in the cell, including signalling and DNA/RNA synthesis. Catabolic pathways such as cellular respiration, beta-oxidation, and ketosis provide energy for ATP production.

The mitochondrial matrix produces the bulk of the ATP during cellular respiration, with each molecule of glucose oxidised yielding around 32 ATP molecules. Ion transport, muscular contraction, nerve impulse transmission, substrate phosphorylation, and chemical synthesis are just a few of the processes that ATP is responsible for. A large amount of ATP is required for these and other actions. As a result, 100 to 150 moles of ATP must be hydrolyzed each day in the human body for cells to operate properly. The relevance of ATP as a critical molecule in cell activity will be discussed in the following sections.

Because of the phosphate groups that interact through phosphodiester connections, ATP is an effective energy storage molecule that may be used as "money." Electronegative charges resist the phosphate groups, resulting in high-energy bonds. The phosphate-phosphate bonds still have a lot of energy stored in them. As a result, metabolic activities hydrolyze ADP, AMP, and free inorganic phosphate groups. ATP hydrolysis into ADP has a Gibbs-free energy of -7.3 cal/mol. ATP must be replenished on a regular basis to keep the cell operating. The intracellular concentration of ATP ranges from 1 to 10 uM on a regular basis. The cell's ATP level is kept constant by a number of feedback systems. A frequent regulation approach is to stimulate or inhibit the ATP synthase enzyme. When cellular ATP levels are high enough, it inhibits two essential enzymes in glycolysis: phosphofructokinase-1 (PFK1) and pyruvate kinase, functioning as a negative feedback loop to restrict glucose breakdown. Autonomic process control, brain glia interactions, pain management, and vascular tone modulation are only some of the purinergic responses that ATP may cause.

Fritz Lipmann and Herman Kalckar first recognised the importance of ATP in energy exchange in biological systems in 1941. ATP is a nucleotide consisting of a purine base adenine, a pentose-sugar ribose, and a triphosphate unit (Fig. 9.1). It contains two oxygen to phospnorous bonds between two phosphate units which are represented by wavy lines. These phosphate bonds are called nigh energy phosphate bonds, The active form of ATP is usually a complex of ATP with Mg^{2+} Or Mn^{2+}. In considering the role of ATP as an energy carrier, we can focus on its triphosphate moiety. ATP is an energy rich molecule becouse of the presence

of four negatively charged oxygen atoms very close to each other. These four negatively charged O-atoms experience very high repulsive energy.

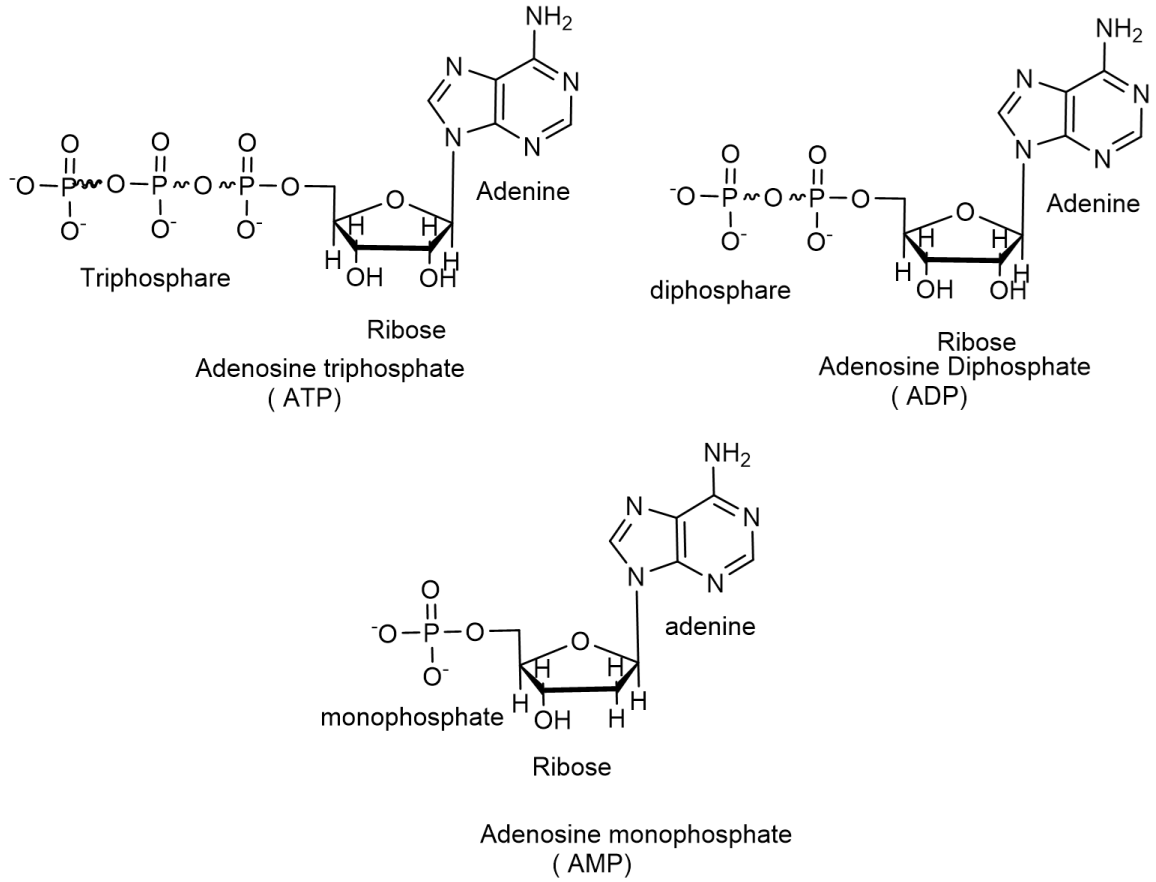


Fig.9.1 AMP, ADP and ATP molecules

When ATP is hydrolyzed to adenosine diphosphale (ADP) and orthophosphate (Pi) or adenosine monophosphate (AMP) and pyrophosphate, a large quantity of free energy is released owing to the decrease in repulsive forces (PPi).

$ATP+H_20 ADP + Pi+H^+ \rightleftharpoons \Delta G^{o'}= -7.3kcal/mol$

$ATP+H_20 \rightleftharpoons AMP+PPi+H^+\Delta G^{o'}= -7.3$ kca/mol

The ΔG^{o}' for these reactions is determined by the medium's ionic strength as well as the quantities of Mg^{2+} and Ca^{2+}. We'll choose -7.3 kcal/mol as a starting point. Under typical cellular conditions, the actual ΔG for these hydrolyses is about -12 kcal/mol.. The enzyme enzvme adenvlate kinase converts ATP, AMP, and ADP to ADP (also known as myokinase).

ATP + AMP $\leftrightarrow$ ADP + ADP

Free energy released during ATP hydrolysis is used to fuel activities that need it, such as muscle contraction. When chemotrophs oxidise fuel molecules or phototrophs catch light, ADP and Pi are converted to ATP. The ATP-ADP cycle is the most basic energy exchange mechanism in biological systems. Many cell processes, including muscle movement, the export of molecules from all cell activity, nutrient intake, nerve impulse transmission, and so on, may be carried out using the energy supplied by ATP hydrolysis. As a consequence, ATP is the focal point for all cell processes.

Anaiogous nucleoside triphosphates including guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP) are responsible for a variety of metabolic functions (CTP). GDP, UDP, and CDP are diphosphated nucleotides, whereas GMP, UMP, and CMP are monophosphated. The transfer of the terminal phosphoryl group from one nucleotide to the next can be catalysed by enzymes. Nucleoside diphosphate kinase is a multifunctional enzyme that catalyses the phosphorylation of nucleoside diphosphates.

$$NMP + ATP \underset{\text{Monophosphate}}{\overset{\text{Nucleoside}}{\rightleftharpoons}} NDP + ADP$$

$$NDP + ATP \underset{\text{Kinase}}{\overset{\text{Nucleoside diphosphate}}{\rightleftharpoons}} NTP + ADP$$

It's worth emphasising that ATP is the main cellular energy transporter, despite the fact that all nucleotide triphoaphaets have the same energetic value. NAD^+ and FAD, two essential electron carriers, are also ATP derivatives. The significance of ATP in energy metabolism cannot be overstated.

9.10 ATP IS CONTINUOUSLY FORMED AND CONSUMED

ATP is the primary immediate donor of free energy in biological systems, rather than functioning as a long-term storage form of free energy. One ATP molecule is used up in a normal cell within a minute after its creation. The ATP has an extremely high turnover rate. In a 24-hour period, a resting adult consumes roughly 40 kg of ATP. During high-intensity exercise, ATP consumption might reach 0.5 kg per minute. Constant ATP production from ADP allows for motion, active transport, signal amplification, and biosynthesis (Fig.9.2).

Chemotrophs manufacture ATP by oxidising fuel molecules, whereas phototrophs gather the free energy in light to make it. Pumping protons over a membrane to generate a proton-

motive force is the energy-saving event in both systems. ATP generation is aided by the proton gradient.

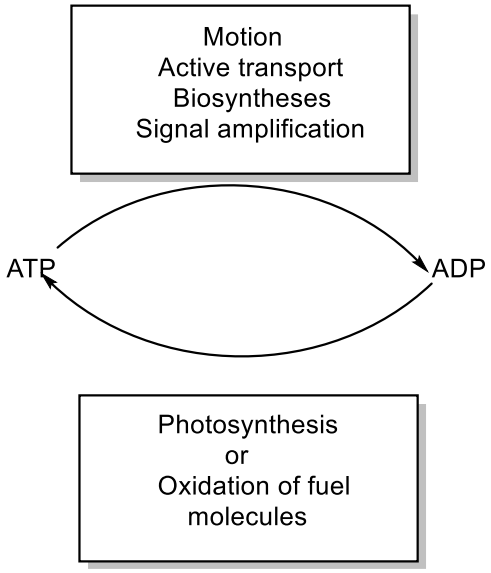
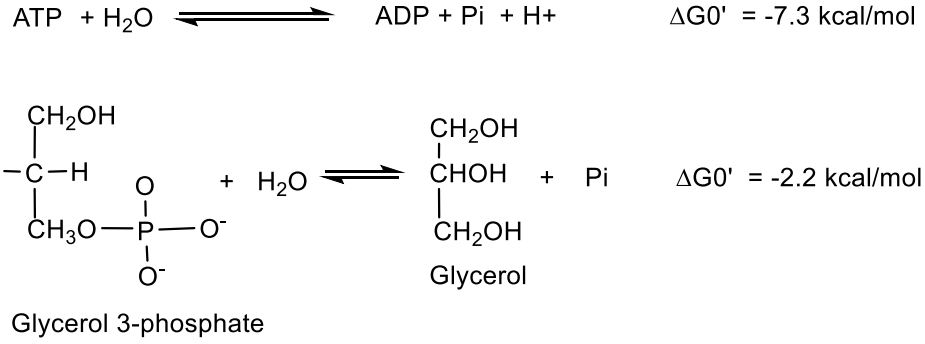


Fig. 9.2 In biological systems, the ATP-ADP cycle is the most basic method of energy exchange.

9.11 STRUCTUAL BASIS OF THE HIGH PHOSPHORYL TRANSFER POTENTIAL OF ATP

Let's compare the energy of ATP hydrolysis to that of a phosphate ester like glycerol 3-phosphate:



The quantity of glycerol 3-phosphate hydrolysis $\Delta G^{o'}$ is substantially less than that of ATP hydrolysis. This demonstrates that ATP has a higher tendency than glycerol 3-phosphate to transfer its terminal phosphoryl group to water. Glycerol 3-phosphate's phosphoryl potential (phosphoryl group-transfer potential) is lower than that of ATP.

What is the structural basis behind ATP's greater phosphoryl potential? Because $\Delta G^{o'}$ is determined by the difference in free energy between the products and reactants, it is crucial to examine the structures of both ATP and its hydrolysis products, ADP and Pi. Electrostatic repulsion and resonance stablization are the two most important qualities. The ATP triphosphate unit contains four negative charges at pH 7. Due to their proximity, these charges are aggressively hostile to one another. When ATP is hydrolyzed, the repulsion between them is lessened. Furthermore, ADP and Pi have a higher level of resonance stability than ATP.

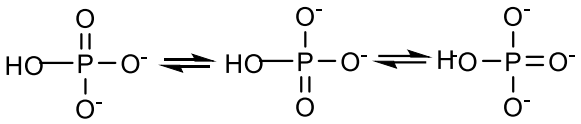


fig 3. Significant resonance forms of orthophosphate

The γ-phosphoryl group of ATP has just a few resonance forms, while orthophosphate has a lot of resonance forms of comparable energy (Fig. 9.3). Because the two phosphorus atoms fight for electron pairs on oxygen, forms like those seen in fig. 9.4 are uncommon. Also, an oxygen atom next to a positively charged phosphorus atom has a positive charge, which is electrostatically unfavourable.

9.12 SYNTHESIS OF ATP

The proton-motive force may now be used to produce ATP. The use of submitochondrial particles formed by ionic rupture of the inner mitochondrial membrane has benefited oxidative phosphorylation studies.ATP synthase is shown as spherical projections from the outer surface of these inside-oul vesicles in electron micrographs. In undamaged mitochondria, these 85-diameter projections may be detected on the matrix side of the inner mitochondrial membrane. In 1960, Efrain Racker found that mechanical agitation might be used to remove these knobs. The stripped submitochondrial particles can still transport electrons along their electron transport chain, but they can no longer synthesise ATP. On the other hand, the separated 85 spheres catalyse ATP hydrolysis. According to Racker, the insertion of these ATPase spheres into the stripped submitochondrial particles restored their capacity to produce ATP. These spheres are dubbed F_1. The fundamental function of the F_1 unit is to catalyse the synthesis of ATP. The ATPase activity of solubilized F_1 is the reversal

of its typical action when there is no proton gradient. F_1 is made up of five polypeptide chains with a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$. This assembly has a mass of 378 kD, as shown in table 9.4.

Table 9.4: Fundamental function

Subunit	Mass (kd)	Role	Location
F_1	378	Contains catalytic site for ATP synthesis	Spherical headpiece On matrix side
α	56		
β	52		
γ	34		
δ	14		
ε	6		
F_0	25	Contains proton channel	Transmembrane
	21		
	12		
	8		
F_1 Inhibitor	10	Regulates proton flow And ATP synthesis	Stalk between F_0 and F_1
Oligomycin-sensitivity-Conferring proton (OSCP)	23		
Fc_2 (F_6)	8		

The other key component of ATP synthase is F_0, a hydrophobic segment that penetrates the inner mitochondrial membrane. The proton channel of the complex is designated F0. It's made up of four polypeptide chains of varying lengths. The 8-kd chain, of which there are six per F_1, is believed to create the transmembrane pore for protons. The stalk between Fo and F_1 contains a number of other proteins (Table 9.4). One of them exposes the complex to oligomycin, an antibiotic that limits ATP generation by interfering with the proton gradient's function. The enzyme ATP synthase is known as FoF_1. ATPases ADP and orthophosphate are converted to ATP by ATP synthase, a catalytic enzyme.

$$ADP^{3-} + P^{2-}{}_1 + H^+ \rightleftharpoons ATP^{4+} + H_20$$

The real substrates are Mg^{2+} ADP and ATP complexes, as in earlier phosphoryl transfer reactions with these nucleotides. ADP's terminal oxygen atom collides with Pi phosphorus

atom to form a pentacovalent intermediate that degrades to ATP and H_2O [Figure (4)]. The apices of a trigonal bipyramid are occupied by the attackin oxygen atom of ADP and the departing oxygen atom of Pi.

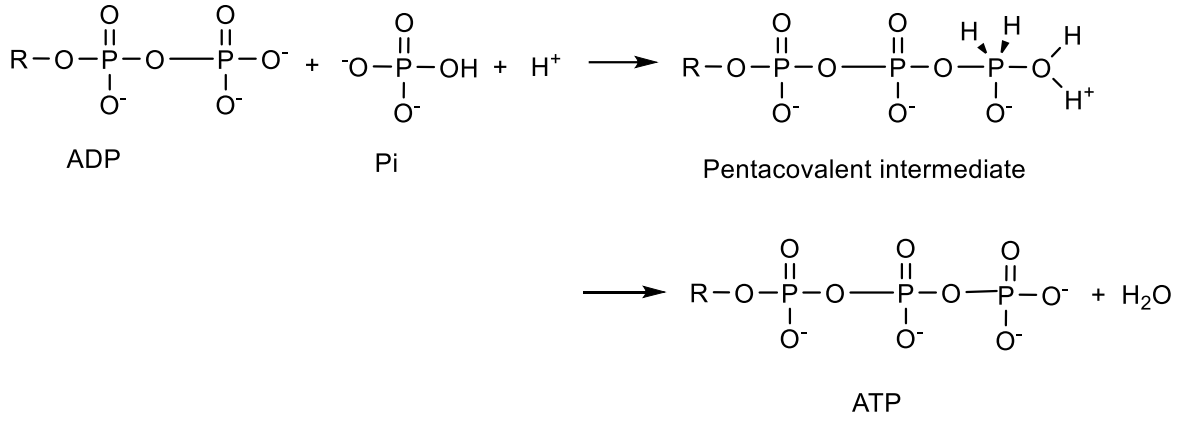


Fig. 9.4 A pentacovalent phosphoryl intermediate is formed during ATP synthesis. The opposing apices of the trigonal bipyramid contain ADP's attacking oxygen atom and Pi's leaving oxygen atom.

The attacking and leaving groups in the catalytic mechanism of ribonuclease A are also in-line. What effect does the intake of protons have on the production of ATP? One theory is that supercharged protons travelling via Fo are channelled to F_1 catalytic site, where they remove oxygen from Pi, tipping the balance toward ATP generation. In the absence of a proton motive force, however, isotopic exchange studies demonstrated that enzyme-bound ATP forms quickly. In H_2O^{18}, ADP and Pi were delivered to ATP synthase, which created ATP and subsequently hydrolyzed it, allowing H_2O^{18} to be integrated into Pi (Fig. 9.5).

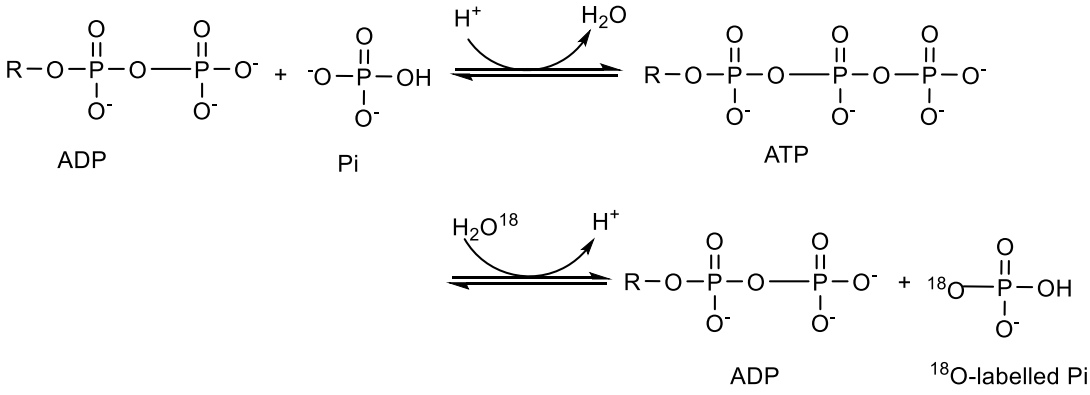


Fig. 9.5. Enzyme-bound ATP is produced from ADP and Pi in the absence of a proton-motive force.

The rate of incorporation of ^{18}O into Pi implies that roughly equal quantities of bound ATP and ADP are in equilibrium at the catalytic site, even in the absence of a proton gradient. In contrast, adenosine triphosphate does not exit the catalytic site until protons have gone through. The proton gradient, according to Paul Boyer, is responsible for releasing rather than producing ATP from the synthase. In addition, he discovered that this enzyme's nucleotide-binding sites interact. ADP and Pi are transported from one site to another, causing ATP to be released from the other. ATP synthase, to put it another way, participates in the catalytic process alongside other enzymes.

9.13 SUMMARY

The summary of this chapter is:

- Energy exchange between living species' tissues and cells, as well as their environment, keeps them alive.
- Every living entity in the cosmos has the ability to extract energy through a number of metabolic processes. Every physical or chemical change requires energy conversion, according to the basic rule of thermodynamics.
- According to the second rule of thermodynamics, the cosmos is moving toward greater disorder. Living creatures, on the other hand, faithfully follow the second rule. Gibbs free energy is the amount of work that can be done in a thermodynamic system while the temperature and pressure remain constant.
- The Gibbs free energy is the difference between the change in enthalpy and the product of the temperature and entropy changes in the system. Living things maintain internal order by receiving free energy from their surroundings in the form of nutrients or sunshine and returning an equivalent amount of energy to them.
- The Gibbs free energy function is a thermodynamic concept that describes how much energy cells can and must use. It allows for the prediction of chemical processes' directions, as well as their exact equilibrium positions and the amount of work they can accomplish under constant temperature and pressure.
- The molecule ATP is essential to the cell's everyday functions. When ATP is hydrolyzed, it produces ADP or AMP as well as free inorganic phosphate groups. The intracellular concentration of ATP ranges between 1 and 10 uM on a regular basis.

The nucleotide adenosine triphosphate (ATP) is made up of adenine and the sugar pentose ribose. Two oxygen-to-phospnorous linkages exist between two phosphate units.

- The active form of ATP is usually a combination of ATP with Mg2+ or Mn2+. There are two main techniques for linking uphill and downhill reactions. A thermodynamically unfavourable process can be pushed forward by storing free energy in an active protein structure. The ability of bacteriorhodopsin to collect photons and use them to pump protons across the cell membrane exemplifies the variety and power of proteins in energy transduction.

9.14 SAQs TYPE QUESTION

A. Fill in the blings

i. ATP is produced primarily through……..from energy sources such as sunlight or oxygen in living organisms.

ii. Gibbs free energy is the most work that can be extracted from a ……..

iii. The second law of thermodynamics implies that all chemical and physical activities increase the…… but it does not specify that this entropy growth must occur inside the responding system.

B. Match thev following

a. ATP	i. Oligomycin-sensitivity-Conferring proton
b. OSCP	ii. Adenosine Diphosphate
c. ADP	iii. Adenosine Monophosphate
d. AMP	iv. Adenosine Triphosphate

C. True and false

i. The proton-motive force may now be used to produce ATP. True/ False

ii. The ATP triphosphate unit contains Two negative charges at pH 7. True/ False

iii. When ATP is hydrolyzed to adenosine diphosphale (ADP) and orthophosphate (Pi) or adenosine monophosphate (AMP). True/ False

iv. Fritz Lipmann and Herman Kalckar first recognised the importance of ATP in energy exchange in biological systems in 1941. True/ False

D. Multiple choise quations

i. Catabolic pathways such as cellular respiration, beta-oxidation, and ketosis provide energy for

A. ATP production

B. ATM production

C. DNA production

D. RNA production

ii. The reaction is both enthalphically favoured (exothermic) and entropically favoured. It is spontaneous (exergonic) at all termperatures if,

A. H positive and S negative

B. H negative and S positive

C. Both Hand S negative

D. Both Hand S positive

iii. Value of the Boltzmann constant, k

A. 1.381×10^{-23} J/K

B. 2.381×10^{-23} J/K

C. 1.381×10^{23} J/K

D. 3.181×10^{-20} J/K

iv. The disorder or randomness movement of molecule in a system is called

A. Enthalpy

B. Gibbs free energy

C. Entropy

D. Internal energy

v. Heat contant of a system is known as

A. Enthalpy

B. Gibbs free energy

C. Entropy

D. Internal energy

vi. The ATP triphosphate unit contains four negative charges at pH

A. 7

B. 8

C. 6

D. 5

vii. $\Delta G > 0$; the reaction is non-spontaneous and,

A. Equlibrium

B. Exothermic

C. Endothermic

D. All of these

viii. NAD stand for,

A. Nicotinamide Adenine Dinucleotide

B. Nicotinamide Adonosine Diphosphate

C. Nicotinamide Adenine Disulphate

D. Nicotinamide acid Dineturation

Answer

A. i. oxidative phosphorylation, ii closed system, iii entropy of the universe

B. a. iv b. i c. ii d. iii

C. i True ii False iii True iv True

D. i A ii B iii A iv C v A vi A vii C viii A

9.15 GLOSSARY

ATP = Adenosine Triphosphate

ADP = Adenosine Diphosphate

AMP = Adenosine Monophosphate

OSCP = Oligomycin-Sensitivity-Conferring Proton

GTP = Guanosine Triphosphate

UTP = Uridine Triphosphate

CTP = Cytidine Triphosphate

NAD =Nicotinamide Adenine Dinucleotide

FAD = Flavin Adenine Dinucleotide

NMP = N Methyl Pyrrolidone

NDP = nucleoside diphosphate

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9.18 TERMINAL QUESTIONS

1. Explain how biochemical processes change their standard free energy.
2. In biochemistry, mention the standard state conventions.
3. Discuss the process of ATP hydrolysis.
4. How is ADP converted into ATP?
5. Determine the relationship between the equilibrium constant and the change in free energy in the system.
6. Demonstrate that biological energy change is governed by thermodynamic rule.
7. How do cells demand free energy sources?
8. Explain how ATP functions in biological systems.
9. Explain how biological processes use the ATP-ADP cycle.

UNIT 10: BIOPOLYMER INTERACTIONS, THERMODYNAMICS OF BIOPOLYMER SOLUTIONS

CONTENTS:

10.1 INTRODUCTION

Properties of biopolymer depend upon intermolecular forces like van der Walls forces and hydrogen bonds existing in the macromolecules. Although these intermolecular forces are present in simple molecules also, their effect is less significant in them as compared to that in macromolecules. This is due to the accumulative effect of these forces all along the long chains of the polymers. Apparently, longer the chain, more intense is the effect of intermolecular forces.

Several different-types of bonds are involved in maintaining the four levels of protein structure. The covalent bond in proteins is of two main types. The first is the peptide bond uniting amino acid monomers in the primary sequence. The second is the disulfide bond (S-S bridge) which, as we have just seen, is established between the -SH groups of two cysteine residues and is responsible for some aspects of secondary and tertiary structure.

The solution of a polymer is a function of molecular structure, composition, and molecular weight. Polar polymers are usually more soluble in polar solvents, e.g., polyvinyl alcohol, in water, where as non-polar polymers are more soluble in non-polar solvents, e.g., polystyrene in toluene.

In crystalline polymer the inter-molecular crystalline forces must be overcome by the solvent. Cross -linked polymers swell in a compatible solvent rather than dissolve. The rate of solution decreases with increasing molecular weight and increasing length of side chain launching.

$$\Delta G_{sol.} = \Delta H_{soln.} - T\Delta S_{soln.}$$

The dissolution of a polymer is favored if H < TDS.

Thus for solubility to occur ΔH must be negative or if positive must be very close to zero.

$$\Delta H - \Delta E = \emptyset_1 \emptyset_2 (\delta_1 \delta_2)^2$$

where ΔE is the change in internal energy on forming a solution, $\emptyset_1$ and $\emptyset_2$ are volume fractions of polymer and solvent and δ_1 and δ_2 are solubility parameters of the polymer and the solvent. Thus for solubility for solubility.

$$\delta = \left(\delta_d + \delta_p + \delta_n\right)^{1/2}$$

where δ_d ,is due to dispersion or Vander Waals forces, δ_p, is permanent dipole interaction and δ_n is due to hydrogen bonds.

This is more helpful where two non-solvents can form a solution mixture. This approach is important in the coating fields. Thus the viscosity of a polymer solution or the stability of a suspension of polymer particles are affected by the solvent. Crystallization and morphological changes are induced by solvent/Various solution methods are used for determination of molecular weights of macromolecules.

10.2 OBJECTIVE

After going through this unit you will be able to:

- Understand the Forces involved in biopolymer interactions
- Understand the electrostatic charge and molecular expansion

- Understand the concept of Osmotic pressure.
- Understand the meaning of Membrane equilibrium.
- Molecular mass, size and shape of biopolymers.

10.3 FORCES INVOLVED IN BIOPOLYMER INTERACTION

Various kinds of weak interactions are important in the establishment of secondary and tertiary structure. These weak bonds are all non-covalent; the main types are as follows:

Ionic or electrostatic bonds result from the attractive force between ionized groups having opposite charges. Hydrogen bonds result when a H^+ (proton) is shared between two neighboring electronegative atoms. The H^+ can be shared between nitrogen or oxygen atoms that are close to each other. Hydrogen bonds have many important biochemical functions. They are essential for the specific pairing between nucleic acid bases, thus providing the main force that holds the two DNA strands together as well as allowing the specific copying of DNA into RNA. Hydrogen bonds in DNA and protein play important part. Thus the forces that stabilise biopolymer structures are as follows:

(a) Hydrogen bonding: Weak force of attraction between partially positive hydrogen and a partially negative atom such as oxygen, fluorine or nitrogen on the same or another molecule.

(b) Ionic bonding: Side chain cross-linking can occur as a result of bonding between anionic and cationic side chains.

(c) Covalent bonding: The most common form of inter-chain bonding is the disulfide bond formed between the sulfur atoms of two cysteine residues. The insulin consists of two polypeptide chains linked together by this type of bridges.

(d) Hydrophobic bonding: Several amino acid residues have hydrophobic (water-hating) side chains. Proteins in aqueous solutions fold so that most of the hydrophobic chains become clustered inside the folds. The polar side chains which are hydrophilic (water-loving) lie on the outside or the surface of the protein.

10.4 ELECTROSTATIC CHARGES AND MOLECULAR EXPANSION

Hydrophobic interactions involve the clustering of molecular groups, which associate with each other in such a way that they are not in contact with water. In globular proteins the side chains of the most hydrophobic amino acid tend to aggregate inside the molecule, and the hydrophilic groups protrude from the surface of the structure. The hydrophobic residues tend to repel the water molecules that surround the protein, thereby causing the globular structure to be more compact.Van der Waals interactions occur only when two atoms come very close together. The closeness of two molecules can induce charge fluctuations, which may produce mutual attraction at very short range.The essential difference between a covalent and a non-covalent bond in the amount of energy needed to break the bond. For example, breaking a hydrogen bond requires only 4.5 kcal $mole^{-1}$, as compared with 110 kcal $mole^{-1}$, for the covalent O-H bond present in water. Although each individual bond is weak, larger numbers of them can produce stable structures, as in the case of double-stranded DNA. Covalent bonds are generally broken by the intervention of enzymes whereas non-covalent bonds are easily dissociated by physicochemical forces.

10.5 HYDROPHOBIC INTERACTIONS

When a nonpolar substance is added to an aqueous solution, it does not dissolve but instead is excluded by water. The tendency of water to minimize its contact with hydrophobic molecule is called the hydrophobic effect. Many large molecules such as proteins and cellular membranes, assume their shapes in response to the hydrophobic effect.

Consider the thermodynamics of transferring a nonpolar molecule from an aqueous solution to a nonpolar solvent. In all the cases, the free energy change is negative indicates such transfers are spontaneous processes (Table 10.1).

Table 10.1: Thermodynamic changes for transferring hydrocarbons from water to nonpolar solvents at 25º C

Process	$\Delta H(kJ.mol^{-1})$	$-T\Delta S\ (kJ.mol^{-1})$	$\Delta G\ (kJ.mol^{-1})$
CH_4 in $H_2O \rightleftharpoons CH_4$ in C_2H_6	11.7	-22.6	-10.9
CH_4 in $H_2O \rightleftharpoons CH_4$ in CCl_4	10.5	-22.6	-12.1
C_2H_6 in $H_2O \rightleftharpoons C_2H_6$ in Benzene	0.2	-25.1	-15.9
C_2H_4 in $H_2O \rightleftharpoons C_2H_4$ in Benzene	0.7	-18.8	-12.1

C_2H_2 in H_2O $\rightleftharpoons$ C_2H_2 in Benzene	0.8	-8.8	-17.2
Benzene in H_2O $\rightleftharpoons$ Liquid Benzene	0.0	-17.2	-8.0
Toluene in H_2O $\rightleftharpoons$ Liquid Toluene	0.0	-20.2	-20.2

Entropy or randomness is a measure of the order of a system. If entropy increases when a nonpolar molecule leaves an aqueous solution, entropy must decrease when the molecule enters water. This decrease in entropy when a nonpolar molecule is solvated by water is an experimental observation, not a theoretical conclusion. Yet, the entropy changes are too large to reflect only the changes in the conformations of the hydrocarbons. Thus, the entropy changes must arise mainly from some sort of ordering of the water itself. What is the nature of ordering?

The extensive hydrogen bonding network of liquid water molecule is disrupted when a nonpolar group intrudes. A nonpolar group can neither accept nor donate hydrogen bonds, so the water molecules at the surface of the cavity occupied by the nonpolar group cannot hydrogen bond tp other molecules in their usual fashion. In order to recover their lost hydrogen-bonding energy, these surface water molecules orient themselves to form a hydrogen bonded network enclosing the cavity (Fig.10.1).

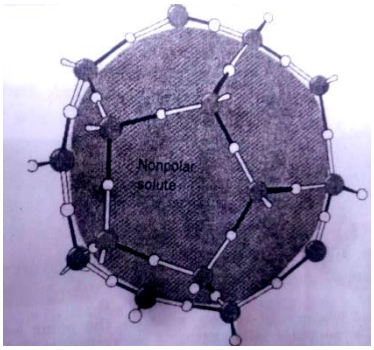


Fig. 10.1 Orientation of water molecules around a nonpolar solute. In order to maximize their number of hydrogen bonds, water molecules form a 'cage' around the solute.

The unfavourable free energy of hydration of a nonpolar substance caused by its ordering of the surrounding water molecules has the net result that the nonpolar substance tends to be excluded from the aqueous phase. This is because the surface area of a cavity containing an aggregate of nonpolar molecule is less than the sum of the surface areas of the cavities that each of these molecules would individually occupy. The aggregation of the nonpolar groups

thereby minimizes the surface area of the cavity and, therefore, the entropy loss of the entire system. In a sense, the nonpolar groups are squeezed out of the aqueous phase.

10.6 OSMOTIC PRESSURE

The properties of solution that depend on the number of particles without any consideration of their kind are called colligative properties (Colligates-collected together). Thus most of the thermodynamic properties like lowering of vapour pressure, elevation of bonding point, depression of freezing point, osmotic pressure etc., and come under colligative properties. Various colligative properties are used for determining molecular weight of substances in solution particularly osmotic pressure.

Osmotic is defined as the process of net diffusion of water molecules from a dilute solution or pure water (solvent) itself to a more concentrated solution, when both are separated by a semipermeable membrane. This membrane allows the water to diffuse but not the solute. Thus, separated from water by semipermeable the membrane. Water molecules diffuse in both directions across the semipermeable membrane, but a net diffusion or osmosis of water from the dilute to the concentrated solution results from a larger number of water molecules diffusing in that direction than in the reverse direction. Water continues to flow into the more concentrated solution across the membrane in this way until the hydrostatic pressure rises so high on the concentrated side of the membrane to cause a transmembrane diffusion of water in the opposite direction at the rate as the osmotic inflow. This hydrostatic pressure which exactly balances the osmotic influx of water from pure water to concentrated solution is called the osmotic pressure of that solution.

Thus, osmotic pressure (g) can also be defined as the pressure which has to be exerted on the concentrated solution which has to be exerted on the concentrated solution, separated from pure water by a semipemeable membrane. In order to counteract and stop the osmotic inflow into the solution. It equals the difference between the hydrostatic pressures on the two sides of membrane. Osmotic pressure is a colligative property of a solution. A rise in the number of solute particles in the solution increases the number of solvent particles bound by solute particles in complexes of solvent particles bound by solute particles in complexes called solvates. Osmotic pressure may be considered to be caused by the higher partial pressure of solvent molecules on that side of the semipermeable membrane as has higher concentration of the solvent.

- Osmotic pressure of a dilute solution is directly proportional to other colligative properties like this fall in freezing point, lowering of vapour pressure, etc.
- Osmotic pressure is inversely proportional to the MW of the solute.

Colloid osmotic pressure of plasma proteins partly counteracts the filtering effect of blood pressure and retains water in the plasma. In Kwashorkor, hepatic cinnboses and nephrosia a fall in plasma concentration of albumin, refuses the colloid osmotic pressure of blood and lowers the relation of water in circulation leading to oedema.

Methods of Measuring Osmotic Pressure: There are three methods for measuring the osmotic pressure viz. static method, dynamic method and half sum method.

(I) Static Method

In the static method, the solvent is allowed to diffuse through the membrane until there is no further interchange in the internal head h. The osmometer measures the equilibrium difference of level. Corrections for the effect of surface tension and the resultant equilibrium concentration of the solution due to passage of solvent through the membrane has to be applied. Sometimes during the establishment of equilibrium, which usually is a long period, the concentration of the solution near the membrane may increase due to adsorption of solvent. This can be avoided by the proper production and storage of the membrane in the solvent. This is a simple method but takes unusually long period for the attainment of the equilibrium.

(II) Dynamic Method

In the dynamic method, the solvent flows through the membrane. The rate of penetration is measured by applying an external gas pressure to the solution. The interpolated pressure for zero rate will be equal to osmotic pressure. By establishing an equilibrium pressure which remains constant for quite a good period give more reliable results. This is a quick method but requires a leak tight complex cell.

(III) Half Sum Method

The internal head h is adjusted initially to be close to the expected equilibrium, say slightly above the equilibrium value. Frequent readings of the depression in volume with time are taken. After sufficient time the cure between h and time is plotted as represented by x. The experiment is repeated by adjusting the head slightly below the equilibrium value and the

increase in volume with time is noted till the g curve represented by Y becomes asymptotic. By calculating the one half the sum of the ordinates of x & y at several values, a new curve A is obtained which converges to a constant value. Since in both the cases, the change in volume involved is small, the equilibrium concentration is assumed equal to the critical concentration.

10.7 MEMBRANE EQUILIBRIUM

It was Pfeiffer who first deposited cupric ferrocyanide on the pores of earthenware pot and used it as a semipermeable membrane. Since then a large number of substances ranging from animal bladder to cellulose have been used as semipermeable membrane. The semipermeable membrane is critically important in osmometry. The reliability of osmotic measurements depends to a considerable extent on proper choice of' membranes. Selection of a membrane involves reconciliation of high permeability towards the solvent with virtual impermeability to the smallest solute molecules present in the solution. The membrane should not swell to any great extent in the solvent and it should have sufficient fine pores to allow the solvent molecules to pass through freely.

The most convenient material used is cellulose in the form of a non-water proofed cellophane sheet or specially treated films of denitrated cellulose nitrate. Generally cellophane membrane is used because treatment of cellophane with ammonia solution or other reagents increases the pore size of the membrane very little. It is possible to prepare membranes of varying porosity depending on the swelling and solvent transfer treatment. Hookway has reported some fast membranes permeable to solutes of molecular weight 50000, while nitrocellulose membrane can be used up to molecular weights of 2000. To condition the membrane in water for use with organic solvents, it is essential at first to wash it with 25, 50, 75 and 100 per cent alcohol or acetone solutions and then displace the alcohol or acetone by similar washings with the desired organic solvent. Precaution should be taken that the membranes is not allowed to dry out. It should always be stored in the solvent.

Theories of Semipermeable Membranes: There are three theories of semipermeable membranes, viz. sieve theory, solution theory and adsorption theory.

(I) Sieve theory

Traube considered the semipermeable membranes as atomic or molecular sieves or bundles of capillaries through which larger molecules find it difficult to diffuse. According to him, the

only difference between various membranes (copper ferrocyanide, parchment, etc.) is in the size of their pores. This theory fails to explain as to why a rubber membrane is impermeable to water but permeable to a large molecule like benzene and pyridine.

(II) Solution theory

Liebig and Hermite postulated that a membrane will be permeable to substances that dissolve in it and impermeable to those that do not dissolve which explains why rubber is permeable to benzene, toluene, pyridine, etc., which are soluble in rubber and diffuse through it, but water which is insoluble does not pass through it. It can be concluded from the above that substances that diffuse through the membrane, first dissolve in the membrane. Although this proved to be a necessary criterion, but it was not all. Bigelow and Bartell observed osmotic effects with inert substance where neither solution nor chemical reaction was possible: Porus cups, with very fine pores or pores clogged with substances, acted as semipermeable membranes. Silica, carbon, metallic copper, silver, gold can be compressed into disks with very fine pores also acted as semi-permeable membrane. Bartell concluded that copper limit of the pores should be 9.0×10^{-3} cm.

(III) The adsorption theory

Wieser and other workers have shown that inert membranes sometimes take up relatively more of the solvent than the solute. This is known as negative adsorption and the solution gets more concentrated. Mathieu observed similar phenomenon with a number of solutions using porus plates as membranes. He concluded that with sufficiently fine capillaries only water would be adsorbed. Similarly sugar was negatively adsorbed by copper ferrocyanide membrane. Thin palladium foil is permeable to hydrogen but impermeable to nitrogen. Thus a semipemeable membrane acts like a solvent than like a sieve. Irrespective of the mechanism by which the semipermeable membrane operates, the chemical potential of the diffusing component is the same on both sides, of the membrane.

10.8 SIZE OF BIOPOLYMERS

We know that under an ultramicroscope, the ture image of the particle does not appear, but only a ‘halo’ of light surrounding the particle, only indicating its position appears. Lobry de Bruyn considered that biopolymer particles would not have a diameter less than 5-10

mμ(1mμ= 10^{-7}cm), since smaller particles do not polarize light. There are several methods by which the size of particles may be determined indirectly.

(I) From Electromagnetic Theory

According to electromagnetic theory of light, the wavelength (λ) which suffers maximum absorption on passing through a solution of biopolymer, e.g.,protein, is connected with the radius of the particle by the expression:

$$r = \frac{\sqrt{3}.\lambda}{4\pi n}$$

Where n = refractive index of the dispersion medium.

(II) From Tyndall Effect

The volume of a particle in a sol of a biopolymer may be approximately determined by means of Tyndall light, the intensity of which may be measured by 'Tyndall meter' or photographically. Rayleigh (1871) showed that the intensity of scattered light (I_s) by a suspension from a beam of known intensity (I) is given by the realtion

$$I_s = I.\frac{V^2}{d^2\lambda^4}$$

Where V is the volume of particles, d is the distance between the particle and the observer and λ is the wavelength of the scattered light.

As the expression involves the volume term, the radius of particle (r) can be calculated by the relation, $V = 4/3\ \pi r^3$.

(III) From Brownian Motion

Protein or gelatin particles suspened in a liquid medium exhibit Brownian motion. They tend to settle down due to gravity. Due to the influence of both these effects, the colloidal particles distribute themselves in a vertical column according to the equation

$$\frac{2.303\ RT}{N}\log_{10}\frac{n_1}{n_2} = \frac{4}{3}\pi r^3 g(h_2 - h_1)(\rho - \rho')$$

Where N is the Avogadro's number, n1 and n2 are the number of particles at heights h1 and h2 of the vertical column, ρ and ρ' are the density of dispersed phase and dispersed medium and r is the radius of the particles. Thus, the value of r,i.e.,the size of particle can be calculated.

(IV) From Scattering of Light

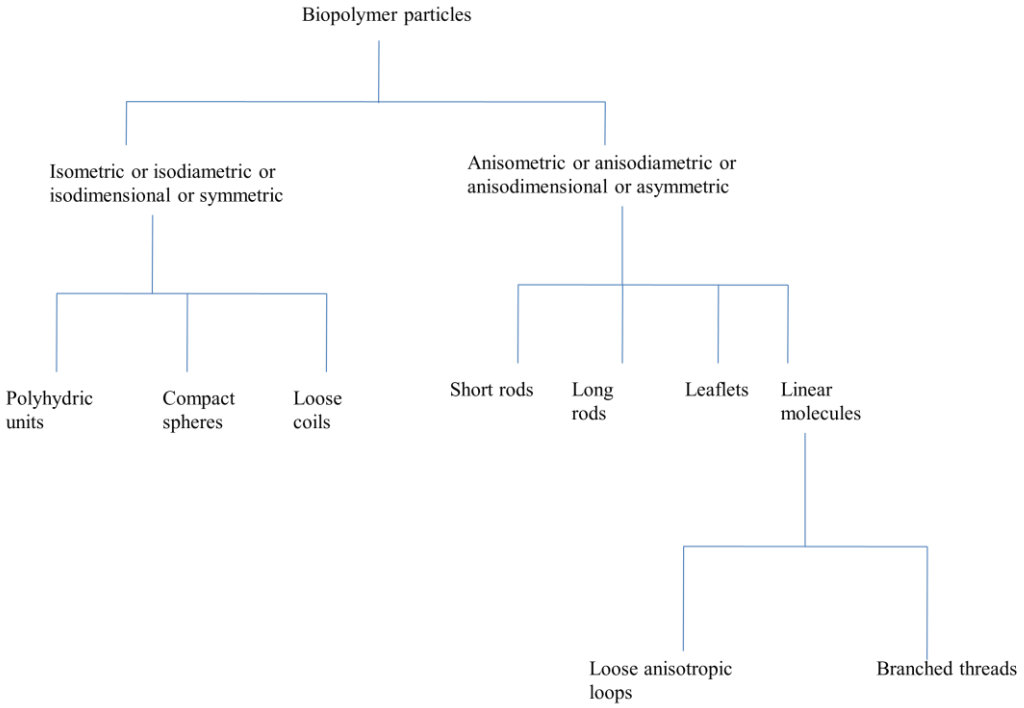
Zsigmondy used ultamicroscope for determining the size of particles. Each spot of light seen in an ultramicroscope corresponds to a particle. So the number of particles in a given volume of a solution can be counted. The obseravation is made a number of times and an average is taken. The length and breadth of the field of vision are measured by an eye piece micrometer. The depth is measured by rotating the slit through 90°. from this data the exact volume of the solution containing the observed number of particles can be measured. Thus, the number of particles per unit volume of the solution can be calculated. Let the number be n.

Next, a known volume of polymer solution is evaporated to dryness. From the weight of the residue, the mass of the particles per unit volume is calculated. Let it be m gm. Now, assuming the particles to the spherical and density (d) of the particles to be the same as that in the bulk state, the volume of solid phase is m/d.

$$\frac{m}{d} = \frac{4}{3}\pi r^3 . n \text{ or } r = \left(3\frac{m}{4\pi dn}\right)^{1/n}$$

10.9 METHODS FOR DETERMINING PARTICLE SHAPE

Biopolymer particles may have several shapes. The most common types of shapes are shown in figure. Although it becomes very difficult to give a classification of biopolymers based on particles shape, yet the following type of classification has been found to be of great value.



There are several methods for the determination of biopolymer shapes and sizes. The most important methods are given as follows:

(I) X-ray method

As most lyophilic biopolymers have crystalline properties, it is possible to use X-ray methods to find the size and shape of the particles. The X-ray method allows particle size determinations down to approximately 10^{-7} cm of edge length, assuming the particle to be cubic in shape.

The size of the biopolymers can also be determined by small angle scattering but it is found that the latter is advantageous over the broadnening of diffraction lines in the respect that small angle of scattering depends upon the density fluctuations in the sample. The small angle diffraction patterns are due to the action of monochromatic X-rays scattered by the separate particles of the sample. The scattering depends solely upon the electron distribution within the sample, which in turn is measured by the boundries of grains, their size and shape. In other words, scattering pattern is formed by separate electron packets which have the size of the grains of the sample. This type of scattering has been observed by Hosemann (1939), Guinier (1930) and Kratky (1938).

According to Yodwitch (1951), it is possible to determine the size of the particles from peak analysis and from stop analysis of the experimental curves.

(II) Electron diffraction method

The size of the smallest particles which can be determined by X-ray method is about 10 A^0 the method cannot be improved even by using short wavelengths. Electron diffraction method has been found to be most suitable for particle size determination in the range of 100-10 A^0, and even below 10 A^0 i.e., 1 mμ. so, for the determination of the size of slightly smaller particles, electrons can be successfully used, and it has been found to be more accurate than X-ray method.

According to de Broglie (1924), the material particles also possess the properties of waves. The wavelength of diffracted electron is given as follows:

$$\gamma = \sqrt{\left(\frac{150}{V}\right)} \times 10^{-8}\text{cm}$$

Where V is the applied voltage

From this equation, it is clear that the wavelength of the beam of electrons is shorter if voltage is higher. This property of shorter wavelength is of great importance in the determination of size of very small particles.

The diffraction methods are used for calculation of grain sizes and determination of shapes. An electron microscope as the name implies, is a device to magnify minute objects which can not be seen by naked eye, where a beam of electrons is employed instead of light rays as in an ordinary microscope. The electron microscope therefore depends upon the following two principles:

(i) A beam of electrons under a constant voltage is having the properties of a beam of light and at the voltage used; the wavelength corresponds to 0.05 A^0

(ii) Electrons can be focused by suitable electric and magnetic fields, very much like light rays get focused by glass lenses.

Electron microscope ia an apparatus in which the glass or quartz lenses of light microscope are replaced by electrostatic or electromagnetic lenses and in which an electron beam instead of a beam light is used. The voltage and current are closely controlled. The biopolymer to be studied is kept on a very thin collodion film and a focused beam of electrons is passed through. The image can be seen on a fluorescent screen or recorded on a photographic plate.

(III) From radius of gyration:

The particles in a solution which scatter light by diffraction are small as compared to the wavelength of the incident light. In a solution of macromolecules, the dimensions of the solute molecules are of an appreciable magnitude and so they cannot behave as point sources. Individual centres with in a particle scatter light in their own way resulting in interference of the radiation scattered. The scattering envelope in such a case is asymmetric. If the macromolecule has a linear dimension greater than about $1/10^{th}$ the wavelength of the incident light, the scattering becomes progressively more pronounced in the forward direction. The ration of the scattered light in the forward direction to that in the backward direction is a function of the size and shape of the particles.

The radius of gyration, R_g, gives an approximate measure of the effective radius of the macromolecule. The value of R_g is obtained from the scattering of incident light at low angles by X-ray scattering.

10.10 Molecular Weight Determination of Biopolymers

It was Avogadro who developed the concept of a mole. According to him a mole consists of amount of substance containing the same number of atoms as are present in 12 gm of C-12. This has a value 6.0229×10^{23}. The weight of a mole in grams is numerically the same as the weight of a single molecule in atomic mass units (amu). The weight of one gram atom of an element is called gram-atomic weight and of one mole of molecules is referred to as gram molecular weight. They are simplified as atomic and molecular weight. One amu is equal to 1.66×10^{-24} gm. The molecular weight is the sum of all the atoms present in the molecule.

10.10.1 Molecular Mass of Polymers

Natural polymers such as proteins contain chains of identical length. Therefore, their molecular masses are singular in nature. On the other hand, during the process of synthesis of polymers, the growth of a polymer chain depends upon the availability of the monomer units in its neighbourhood which differs from one place to another in the reaction mixture. Hence all the polymers are heterogeneous with respect to molecular weight. The values of molecular weight of the same polymer will differ with the solvent and method of determination. A series of polymeric compounds having the same chemical structure but differing only in molecular weight is known as polymeric homologous series. The molecular weight so determined will give the value of average molecular weight. Depending on the method of determination, different average molecular weights are obtained. They are given as follows:

(a)Number average molecular weight $\overline{M_n}$

(b)Weight average molecular weight $\overline{M_w}$

(c)Viscosity average molecular weight $\overline{M_v}$

(d)Z − average molecular weight $\overline{M_z}$

In the case of mono-dispersed systems:

$$\overline{M_n} = \overline{M_w} = \overline{M_v} = \overline{M_z}$$

while they differ very much in polydisperesed system.

(a)Number Average Molecular Mass ($\overline{M_n}$)

It is obtained by dividing the sum of masses of all the molecules of different monomer units of different masses by the total number of molecules. We can understand it by considering a polymer made up of three monomeric units of masses M_1, M_2 and M_3.If N_1 molecules of monomer of mass M_1, N_2 molecules of mass M_2 and N_3 molecules of mass M3 constitute the polymer then,

the total mass of N_1, molecules = N_1M_1 (i)

the total mass of N_2 molecules = N_2M_2 (ii)

the total mass of N_3 molecules = N_3M_3 (iii)

Adding (i), (ii), and (iii), we will get the total mass.

Total molecular mass = $N_1M_1 + N_2M_2 + N_3M_3$ (iv)

and total molecules = $N_1 + N_2 + N_3$ (v)

Number Average Molecular mass=$N_1M_1+N_2M_2+N_3M_3 \div (N_1+N_2+N_3)$

$$(\overline{M_n}) = \frac{\sum N_i M_i}{\sqrt{N_i}}$$

$$\text{where } \sum N_i = N_1 + N_2 + N_3 \ldots\ldots$$

$$\text{where } \sum N_i M_i = N_1 M_1 + N_2 M_2 + N_3 M_3 \ldots\ldots$$

$(\overline{M_n})$ is generally determined by osmotic pressure measurement, depression in freezing point and elevation in boiling point.

(b)Weight average molecular weight ($\overline{M_w}$)

It is obtained by multiplying the sum of total molecular masses of different monomeric units by their respective molecular masses, adding all the molecular masses and then dividing by the total mass of all the molecules.

If a polymer consists of N_1 molecules of monomeric unit of molecular mass M_1,N_2 molecules of another monomeric unit of molecular mass M_2 and N_3, molecules of the third monomeric unit of molecular mass of M_3 then

$$\text{Weight average molecular weight } (\overline{M_w}) = \frac{N_1M_1 \times M_1 + N_2M_2 \times M_2 + N_3M_3 \times M_3}{N_1M_1 + N_2M_2 + N_3M_3} \ldots\ldots (vi)$$

$$\text{or } (\overline{M_w}) = \frac{\sum N_iM_i^2}{\sum N_iM_i} \ldots\ldots (vii)$$

$$\text{where, } \sum N_iM_i^2 = N_1M_1^2 + N_2M_2^2 + N_3M_3^2 \ldots\ldots$$

$$\text{and , } \sum N_iM_i = N_1M_1 + N_2M_2 + N_3M_3 \ldots\ldots$$

$(\overline{M_w})$ is generally determined by ultracentrifugation or sedimentatione.g. the number average molecular mass and the weight average molecular mass of a polymer sample containing 30 molecules of molecular mass 10,000, 30% of molecular mass 20,000 and the remaining 40% of molecular mass 30,000, will be be

$$\overline{M_n} = \frac{30 \times 10000 + 30 \times 20000 + 40 \times 30000}{30 + 30 + 40} = 21000$$

$$\overline{M_w} = \frac{30 \times 10000 \times 10000 + 30 \times 20000 \times 20000 + 40 \times 30000 \times 30000}{30 \times 10000 + 30 \times 20000 + 40 \times 30000} = 24286$$

Ratio of $\overline{M_w}$ and $\overline{M_w}$ is called polydispersion index (PDI)

$$\text{PDI} = \frac{\overline{M_w}}{\overline{M_n}}$$

For natural polymers, PDI = 1 whereas for synthetic fibres, PDI >1.

10.10.2 Molecular Weight Determination

The molecular weights can be determination by ebiometry, cryscopy, osmometry, end group determination, light scattering, ultra centrifuge and dilute viscometry (Table 10.2.)

Molecular weight size of	Symbol	Method of Determining	Molecule
Number average	$\overline{M_n} = \frac{n_1 M_1}{\sum n_i} + \frac{n_2 M_2}{\sum n_i} + \frac{n_3 M_3}{\sum n_i} + \cdots$	Colligative method	Small
Weightaverage	$\overline{M_w} = \frac{n_1 M_1^2}{\sum n_i M_i} + \frac{n_2 M_2^2}{\sum n_i M_i} + \frac{n_3 M_3^2}{\sum n_i M_i} + \cdots$	Light scattering	Large
Average	$\overline{M_z} = \frac{n_1 M_1^3}{\sum n_i M_i^2} + \frac{n_2 M_2^3}{\sum n_i M_i^2} + \frac{n_3 M_3^3}{\sum n_i M_i^2} + \cdots$	Sedimentatio n method	Large
Viscosity	$\overline{M_v} = [\eta] K^{-1} \overline{M_v}^a$	Viscometer method	

Table 10.2 Methods of molecular weight determination

Where n = number of molecules in sample.

n_1, n_2, n_3 = number of molecules of molecular weight M_1, M_2, M_3

The molecular weights can be determined directly or in solution.

(I) Direct measurement

The method for direct determination of molecular weights can only be applied to gases or volatile liquids and solids. They are known as:

1. Vapour density method
2. Low pressure effusion of gases
3. Mass spectrometry

These methods are useful for gaseous or volatile substances. Hence, molecular weights of biopolymers are generally determined by solution methods.

(II) Solution Methods

There are two types of important methods of determining the molecular weight of substances in solution, viz. chemical method and physical method.

(III) Chemical Method

Chemical methods are important for the molecular weight determination of macromolecular compounds. Application to this method requires that the structure of polymer should contain a known number of the chemically determinable functional groups, radicals or elements per molecule. In polymers these functional groups occur as end groups (δ) except branched polymers. In linear polymers the quantitative determination of all end groups, e.g., a polyester where-OH groups is at one end and- COOH group at the other end, can be estimated by acidimetric titrations. Therefore determination of one of the functional groups suffices for the evaluation of number average molecular weight. Katchalski determined the molecular weight of polyamino acids by the chemical, as well as by other, methods and found them in reasonable agreements.

In case the polymer is formed by chain transfer the number of transfer agents molecules in the polymer can be determined by chemical analysis. From these the molecular weight of the polymer can be computed.

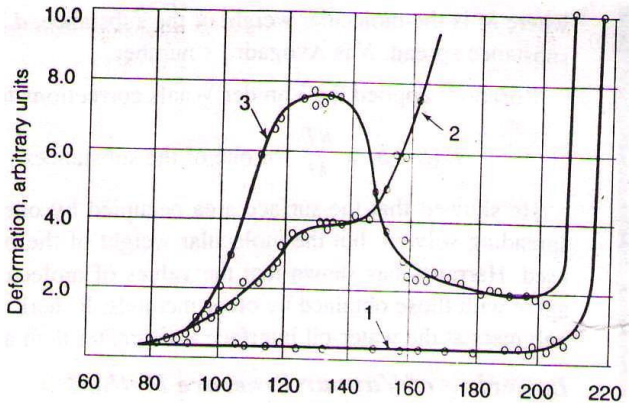
$$\frac{1}{DP_n} = \left(\frac{1}{(DP_n)_0} + C_1\frac{(S)}{(M)}\right)$$

where DP_n is the number average of polymerization, $(1/(DP_n)_0)$ is the reciprocal of DP_n without chain transfer agent or solvent, and (S) & (M) are the concentrations of chain transfer agent and monomer respectively and C_1 is the chain transfer constant.

$$C_m = \frac{K_m}{K_{b,}}, C_b = \frac{K_s}{K_b} \text{ and } C_1 = \frac{K_i}{K_{b,}}$$

Where K_m, K_s and K_i are the velocity constants of chain transfer with monomer, solvent and initiator respectively.

In the case of branched chain polymer, the end group determination establishes the branching of chains. Kern[19] polymerized styrene in presence of bromobenzoyl-peroxide and determined the amount of bromine in the polymer. He found that each molecule contains three to four atoms of bromine. In another sample polystyrene containing hydroxyl as end groups and the presence of end groups was ascertained by infrared spectroscopy. The molecular weight was found to be 17,300.



Temperature (^{0}C)

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$M = 100A_a/x$

Hemoglobin from mammalian blood contain 0.335 per cent ion

$$M = \frac{100 \times 55.85}{0.335} = 16700$$

Similarly small amounts of amino acids, sulphur, and other specific constituent can be used to determine the molecular weight of the polymer.

Tablet 10.3 gives the molecular weight determined by chemical method.

S. No	Biopolymer	Molecular weight
01	Egg Albumin	44,000
02	Hemoglobin	16,700
03	Insulin	12,000, 6000
04	Edestin	46, 000

In the case of copolymerization of two monomers of M_1 and M_2 the copolymer will be formed on the basis of their respective reactivities r_1 and r_2.

$r_1 = K_{11}/K_{12}$ and $r_2 = K_{22}/K_{21}$

where K_{11}, K_{12} are the rate constants for the reactions M_1, free radical and monomers M_1, M_2 and K_{22}, K_{21} are the rate constant for the reactions M_2 free radical with monomer M_2, & M_1.

Generally $r_1 \times r_2 < 1$ but sometimes $r_1 = r_2 = 1$, then random copolymers will result. If r_1 & r_2 are low and $r_1 r_2$ is approaching zero, alternating copolymers will result.

If M_0 = M_1 charged/ M_2 charged and P_0 = mole of initial monomer of M_1/ mole of initial monomer of M_1

then it can be shown that,

$$M_0 = \frac{(P_0 - 1) + [(1 - P_0)^2 + 4P_0 r_1 r_2]^{1/2}}{2r_1}$$

Therefore if the composition M_o is allowed to copolymerize, a copolymer of desired composition can be obtained and the molecular weight can be computed.

The chemical methods only find use in condensation polymers which have average molecular weight of the order of 25,000. This method is not sensitive to large molecular weights sometimes some of end groups not considered for computing the molecular weight becomes consequential, especially in the case of cross-linked and highly branch chained structures.

(IV) Physical Methods

Various physical methods depend on either the evaluation of thermodynamic properties or the kinetic behavior or the combination of the two, in dilute solutions. Polymer solutions exhibit large deviation from their limiting infinite dilution behavior. Hence, not only, all the experiments are carried out at low concentrations but they are invariably extrapolated to infinite dilution. In the case of chain molecules, a polymer molecule is assumed to have a symmetrical statistical distribution of chain elements about a centre of gravity and the volume occupied by this distribution is many times the actual molecular volume. Thus the volume over which an individual polymer molecule exerts its influence depends on the chain length and on the interaction between the polymer and the solvent. Hence the solution taken for

physical measurements should be so dilute that each of the molecule couples separate portion of the volume. This will not only require very sensitive equipment to measure small physical changes, but the polymer should also be very properly fractionated, otherwise the molecular weight obtained by different methods will vary in wide range.

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(V) Colligative Properties

The dilute solutions show more or less ideal behavior as the heat and volume changes, accompanying the mixing of solute and solvent are negligible for all practical purposes. Dilute solution obey Raoult's law.

Dilute solutions containing non volatile solute exhibit some special properties which depend only on the number of particles in the solution irrespective of their nature. These properties are termed as:

Colligative properties and these include:

(i) Lowering in the vapour pressure

(ii) Elevation in the boiling point

(iii) Depression in the freezing point

(iv) Osmotic pressure

The important of these properties lies in the fact that they provide methods for the determination of molecular weights of dissolve solute.

A. Lowering in Vapour pressure

When a nonvolatile solute is dissolved in a solvent, its vapour pressure decreases, Von Babo showed that although both vapour pressure of pure solvent (p_o) and vapour pressure of solution (p_s) increase with increase of temperature yet the ration, $p_o - p_s/p_o$ remains the same at all temperature. While $p - p_s$ is the lowering in vapour pressure, $p_o - p_s/p_o$ is termed relative lowering of vapour pressure. According to Raoult's law, the relative lowering of vapour pressure of a dilute solution is equal to the mole fraction of the solute present in the dilute solution.

Now, if 'n' moles of solute be dissolved in N moles of the solvent, the

mole fraction of the solute will be = n / n+N

$$\therefore \text{According to Raoult's Law}, \frac{p_0 - p_s}{p_0} = \frac{n}{n+N} \ldots\ldots (i)$$

If a solution is made by dissolving w.gm. of the solute (molecular weight m) in W gm of the solvent (molecular weight M) the mole fraction of the solute will be,

$$= \frac{w/M}{w/m + W/M}$$

As in dilute solution the amount of solute is very small, w/m can be neglected in the denominator as compared to W/M, the equation (i) becomes,

$$\frac{p_0 - p_s}{p_0} = \frac{w}{m}\frac{M}{w}$$

So, measuring relative lowering of vapour pressure, the molecular-weight of the solute can be determined.

Relative lowering of vapour pressure is determined experimentally by Ostwald and Walker method.

B. Elevation in Boiling Point (Ebullioscopic method) and Depression of Freezing Point (Cryoscopic method)

We know, the boiling point of a liquid is the temperature at which its vapour pressure is equal to the atmospheric pressure.

Similarly, the freezing point is defined as the temperature at which the vapour pressure of its liquid is equal to the vapour pressure of the corresponding solid.

As the vapour pressure of a pure solvent is higher than that of the solution hence the elevation in boiling point and depression in freezing point will be proportional to the mole fraction of the solute.

If ΔT_b = Elevation in Boiling Point and ΔT_f = Depression in Freezing point

Then,

$$\Delta T_b \propto \frac{p_o - p_s}{p_0} \times \frac{\Delta P}{p_o} \text{ where } \Delta P = p_o - p_s$$

$$\text{and } \Delta T_f \propto \frac{\Delta P}{p_o}$$

Now, according to Roult's Law

$$\frac{\Delta P}{p_o} = \frac{w}{m} \times \frac{M}{W}$$

$$\text{So,} \qquad \Delta T_b \times p_o \left(\frac{w}{m} \times \frac{M}{W}\right) \text{ and } T_b \times p_o \left(\frac{w}{m} \times \frac{M}{W}\right)$$

As for the pure solvent po and M are constant, therefore

$$\Delta T_b \propto \frac{w}{m}\frac{i}{w} \times \frac{w}{mw} \text{ or } \Delta T_b = K_b \left(\frac{w}{m.W}\right) \ldots\ldots \qquad \text{(ii)}$$

$$\Delta T_f \propto \frac{w}{mW} \text{ or } \Delta T_f = K_f \left(\frac{w}{m.W}\right) \ldots\ldots \qquad \text{(iii)}$$

where, K_b is pulsation constant, and K_f is depression constant

When, w/m =1 (i.e. one mole of nonvolatile solute is dissolved in 1 gm of the solvent) $\Delta T_b = K_b$ (i.e. K_b is equal to the elevation in B.P. when 1 mole of solute is dissolved in 1 gm of the solvent)

and, $\Delta T_f = K_f$ (i.e. K_f is equal to the depression in F.P. when 1 mole of solute is dissolved in 1 gm of the solvent)

For practical purpose, in place of k_b or k_f, k_b and k_f are used, where.

k_b = Modal elevation constant = The elevation in B.P. when 1 mole of solute is dissolves in 1000 gm of the solvent.

Thus, 1000 $k_b = k_b$

Similarly.

k_f = Model depression constant = The depression in F.P. when 1 mole of solute is dissolved = 100 gm of the solvent.

Thus, 100 $k_f = k_f$

So (ii) and (iii) relation will be-

$$\Delta T_b = \text{molality} \times k_b \text{ or } = \frac{1000 k_b w}{m.W}$$

$$\Delta T_f = \text{molality} \times k_f \text{ or } = \frac{1000 k_f w}{m.W}$$

Elevation in B.P. is experimentally determined by Landsberger method and depression in F.P. is experimentally determined by Beekmann's method. In both of these methods Beckmann thermometer is used to record small change in temperature. Knowing the value of ΔT_b and ΔT_f and the strength of the solution molecular weight of the solute (m) can be determined.

A. Osmotic Pressure

For dilute solutions, according to Van't Hoff theory, the equation PV = nST holds good.

If w gm of solute (molecular weight m) be present in V liters of solution, then

n = w/m and $V = V^1$

Thus, the equation PV = nST becomes,

$$PV^1 = \frac{w}{m} ST \quad \text{or } m = \frac{w \times S \times T}{PV^1}$$

where, P = Osmotic pressure, T = absolute temperature

S = Molar Solution Constant = 0.082 lit atm. K^{-1} mol^{-1}

Knowing the value of P experimentally, the value of m (molecular weight of the solute) can be determined.

Osmotic pressure is determined using Berkeley and Hartley method.

Extent of Hydration: Solubility

As has been pointed out earlier the solubility of a polymer is a function of molecular structure, composition, and molecular weight.

Polar polymers are usually more soluble in polar solvents, e.g. poly vinyl alcohol, water, whereas non-polar polymers are more soluble in non-polar solvents, e.g. polystyrene in toluene.

In crystalline polymers the intermolecular crystalline forces must be overcome by the solvent. Cross-linked polymers swell in a compatible solvent rather than dissolve. The rate of solution decreases with increasing molecular weight and increasing length of side chain launching.

As a matter of fact polymer solutions exhibit large deviations from their limiting infinite dilution behavior. Hence, not only, all the experiments are carried out at low concentrations but they are invariable extrapolated to infinite dilutions. In the case of chain molecules, a polymer molecule is assumed to have a symmetrical statistical distribution of chain elements about a centre of gravity and the volume occupied by this distribution is many times the actual molecular volume. Thus the volume over which an individual polymer molecular exerts its influence depends of the chain length and on the interaction between the polymer and the solvent. Hence, the solution taken for physical measurements should be so dilute that each of the molecule occupies separate portion on the volume.

Sedimentation Equilibria

On ultracentrifuging the polymer solution, several boundaries are observed revealing the presence of different components in the polymer.

This method is useful only where there is sufficient difference in the molecular weights. This fact is used in Sedimehtaism equilibrium method for molecular weight determination.

In this method at the equilibrium stage rate at which the solute is driven outwards by the centrifugal force is equal to the rate at which it diffuses inwards due to concentration gradient.

$$\text{The sedimentation rate} = Cw^2 \times M\left(1 - v_p\right)\left(\frac{I}{f}\right) \ldots\ldots \quad \text{(i)}$$

$$\text{The diffusion rate} = -\frac{KRT}{f}\frac{dc}{dx} \ldots\ldots \quad \text{(ii)}$$

$$\frac{dc}{C} = -\frac{(1-v_p)w^2 dx}{RT} \quad \ldots\ldots \qquad (iii)$$

Integrating (ii)

$$\overline{M_w} = \frac{CRT\ln(c_2 c_1)}{(1-v_p)w^2(x_2^2 - x_1^2)} \ldots\ldots \qquad (iv)$$

This method requires the time for equilibrium which was found to be too long with substances with molecular weight greater than 500. Shortly after the centrifuge is brought to speed a determination of concentrations at the top meniscus and bottom of the cell, gives the equilibrium values.

(VI) Hydrodynamic Methods

A. Diffusion

The transport of molecule in the absence of bulk flow is called diffusion. It is the directed thermal motion of molecules or fine particles from places of high concentrations. This random movement was observed by Brown and is known as Brownian movement. This is brought about from the bombardment of the dispersed particles by the molecules of dispersion medium.

Ficks has shown that if an amount d_w i.e., the number of grams of macromolecules is transferred across the boundary of area A in the direction X in time dt, it is proportional to the concentration gradient dc/dx

$$\frac{dw}{dt} = -DA\frac{dc}{dx}$$

where D is known as diffusion coefficient.

The force that derives the molecules to more dilute region is given by

$$f = \frac{RT}{N}.\frac{1}{C}\frac{dc}{dx}$$

This is balanced by the frictional force exerted by the viscous solution. Stokes found that for a spherical molecule of radius r the force for a viscous flurid of viscosity η is given by

$$f = 6\pi r\eta\frac{dx}{dt} = -\frac{RT}{N}.\frac{1}{C}\frac{dc}{dx}$$

$$\text{or } C\frac{dx}{dt} = -\frac{RT}{N}.\frac{1}{6\pi r\eta}\frac{dc}{dx}$$

$$\frac{dw}{dt} = -\frac{RT}{N}.\frac{1}{6\pi r\eta}\frac{dc}{dx}$$

$$\therefore D = \frac{RT}{N}.\frac{1}{6\pi r\eta N}$$

The volume of the spherical molecules is 4/3 $\pi r^3 Nd$. Therefore the molecular weight is given by 4/3 $\pi r^3 Nd$ where d is the density,

$M = 4/3\ \pi r^3 Nd$

If the is molecule is non-spherical, then

$$\frac{D_{spherical}}{D_{non-spherical}} = \frac{f_{D_{non-spherical}}}{f_{spherical}} = \text{ asymmetric factor}$$

Fick's second law state that

$$\frac{dc}{dx} = D\frac{d^2c}{d_x^2} \quad \ldots\ldots \qquad (v)$$

The rate at which the boundary between the solution of the polymer and the solvent get blurred is measured and then D can be calculated. Integrating Eq. (v) we obtain Wiener's equation

$$\frac{dc}{dx} = \frac{C_0}{\sqrt{4\pi DT}}\exp\frac{-x^2}{4\pi Dt} \quad \ldots\ldots \qquad (vi)$$

where C_o is the concentration of the solution in glcm3, t is the time for the beginning of diffusion and x is the distance of the gradient from the boundary (Figure 10.4)

There are two methods of determining D, namely

(1) Free boundary spreading, and (2) Diffusion through porous plate.

Rectangle cells (Figure 10.3) are used to study free boundary diffusion. A sharp boundary can be easily formed by using a sliding joint to superimpose the solvent on the solution. The boundary spreading is observed by refraction changes in a polarization diffusion meter at certain levels x at infinite time interval (Figure 10.4).

$$\frac{d_n}{dx_{max}} - \frac{d_n}{dx_{max}} = \frac{A}{\sqrt{4\pi Dt}}$$

where n is the refractive index and A is the area under curve.

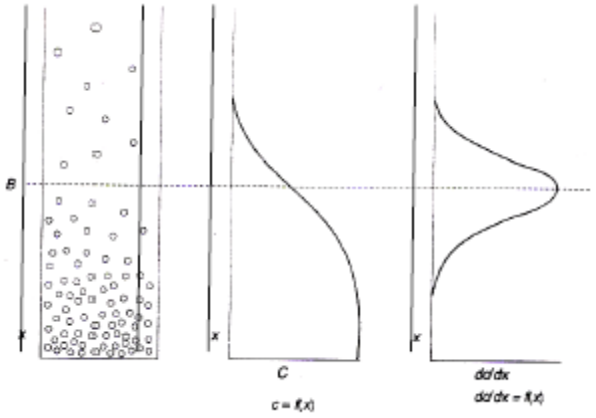

Figure 10.3(a)

From equation (vi) can be seen that D depends on concentration, Extrapolating to zero concentration, D_o can be obtained.

For a wide range of polymers

$$D_0 = K_\theta M^{-b}$$

where K_θ is a constant for the given polymer solvent system and b is a parameter.

Lamm has designed micro apparatus to measure D which requires only I cc of solution.

A. **Sedimentation Velocity Method**

The macromolecules having a large size and heavy mass, settle out of dispersion under the gravitational force. The force F causing sedimentation of spherical particles is given by

$$F = \frac{4}{3}\pi r^3(p - p_\theta)g = 6\pi\eta r\frac{dx}{dt}$$

where r is the radius of the particle, p and p_o are the densities of the particle and suspension medium respectively, η is the viscosity of the solution and dx/dt is the velocity of sedimentation.

$$\frac{dx}{dt} = 2r^3\frac{(p - p_\theta)g}{9\mu}$$

Since the retarding force is equal to the sedimentation velocity

$$r = \frac{\sqrt{9\frac{dx}{dt}}}{2(p - p_\theta)g}$$

The radius of the particle can be determined by the path it traverses in a definite time. It was observed that a particle of radius 10^{-7} mm with a density of 2.5 gm per cm^3 will take about 100 years to settle down. Wiegner, Kelly and Stamm have designed equipment to measure the sedimentation velocity of colloidal particles.

Svedberg and others developed analytical ultracentrifuges to determine the velocity of sedimentation. A particle of mass m at a distance x from the centre of rotation will experience a centrifugal at force, fc. given by

$$f_c = m \times w^2$$

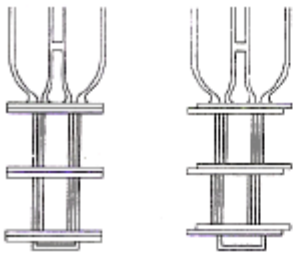

Figure 10.3 (b)

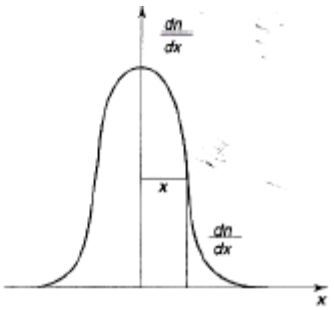

Figure 10.4

where w is the angular velocity in radian per second, i.e. 2π times the number of revolutions per second

$$f\frac{dx}{dt} = v(p - p_\theta)w^2x$$

according to Stokes law

$$v(p - p_\theta)w^2x = 6\pi\eta r\frac{dx}{dt}$$

where r is the radius of a given macromolecule in a given solution is its sedimentation coefficients

$$S\frac{dx/dt}{w^2x} = \frac{m(1 - v_p)g}{6\pi\eta r}$$

on integration –

$$S = \frac{\ln x_2 \ln x_1}{w^2(t_2 - t_1}$$

The value of w^2x comes out to be 2.36×10^8 cm sec^{-2} in a centrifuge with 60,000 rpm and at a distance of 6 cm which is 240,000 times greater than the acceleration in the earth's field.

If one mole of the substance is sedimenting, then

$M = vpN$

$$\text{or } M(1 - \frac{p}{p_0})w^2x = 6\pi\eta r\frac{dx}{dt}$$

$$\therefore\ M = \frac{dx/dt}{w^2x}.\frac{\eta}{\eta_0}\frac{1 - vp_0}{1 - p}$$

Where V is the partial specific volume of the dispersed phase η and p is the viscosity and density of the polymer solution andη_0,p_o is the density of the medium.

$$\therefore\ M = \frac{RTS}{D(1 - vp)}$$

Where D is the diffusion coefficient.

For precise measurements S, D and V are extrapolated to infinite dilution. This method takes very long time. The solution to be studied is placed in a cell with thick quartz windows. A beam of light is passed through the solution placed in the cell of ultracentrifuge.This beam of light falls on a photographic plate placed beyond the cell when the cell is rotated at velocity of 50,000 rpm, the interface between the solution and the solvent gradually shifts with the sedimentation of the particles and the light is absorbed to different extent at different heights of the cell. By measuring the optical densities at different time intervals, the sedimentation velocity can be measured. The curve of distribution of concentration gradient along the height of the cell at different time integrals is plotted. Then the curve between lnx and t is plotted which comes out to be a straight.

The slope of the curve gives the sedimentation constant 4.5 extra polating lasting it to infinite dilution S_o is obtained

$$S_0 = K_3M^{1-b}$$

Where K_s is a constant for a given polymer solvent and b has the same interpretation as in diffusion. The values of $b+K_3$ are given in the literature. Thus M can be calculated.

C. Viscosity

A shear stress when applied to a body displaces a plane in the body parallel to itself relative to other parallel planes in the body. The fluids begin to flow as soon as the stress is applied. Even solids somewhat flow when the stress is maintained for a long time. This slow flow of solids is called creep. Under high stress creep passes over into plastic deformation of solids. Silicone polymer gives bouncing putty which is a hybrid of solid and liquid in regard to its flow properties. Viscosity is a measure of resistance that a body offers to flow. Maxwell showed that the relaxation time t is given by

$$T = \eta/K$$

Where η is the shear viscosity and K is shear elasticity, liquid of complex structures display considerable elasticity due to coiling and uncoiling of molecular chains.

Streamline Flow: If the speed of the flow is not very fast, and the liquid moves under a pure shearing motion it is called the laminar or streamline flow. When the liquid moves with high velocities, the flow becomes turbulent. The Reynolds number R_N attains a value 10^3 to 10^4

$$R_N = \frac{Pva}{\eta}. \text{where a gives the dimensions of the flowing object.}$$

$$R_N = r\frac{\tilde{\upsilon}a}{\eta}. \text{where r is tube radius and } \tilde{\upsilon} \text{ the average velocity of the fluid..}$$

Restricting to isotropic bodies, let us consider the plane X having planes X_1 & X_{11} above and below at a distance l, known as mean free path. Let n be the number of molecules of mass m per unit volume. Let dv be the difference in velocities of two layers as a distance dx. Therefore the net rate of upward flow of momentum through any given plane is given by

$$= -\frac{1}{3}nm\tilde{\upsilon}1\frac{dv}{dt} = \eta\frac{dv}{dx}$$

Where $\tilde{\upsilon}$ is the root men square velocity, The rate of down flow and transfer of momentum per unit area of the plane x from above is

$$+\frac{1}{3}nm\tilde{\upsilon}1\frac{dv}{dt} = \eta\frac{dv}{dx}$$

This change of momentum is balanced by a force f acting on area A of the moving plane.

$$\frac{f}{A} = \frac{1}{3} nm\tilde{\upsilon} 1 \frac{dv}{dt} = \eta \frac{dv}{dx}$$

Where η is called the coefficient of viscosity

$$\eta = \frac{1}{3} nm\tilde{\upsilon} l$$

In the case of gases

$$\tilde{\upsilon} = \left(\frac{3RT}{M}\right)^{1/2} \text{ and } mn = pM/RT$$

$$\therefore \eta = \frac{1}{3} \frac{pM}{RT} \left(\frac{3RT}{M}\right) lgmsec^{-1}cm^{-1} = 2.96 \times 10^{-25} \sqrt{MRT}\, ld^2$$

Where d is the molecular diameter and p is the pressure and N is Avogadro's number. Poise is the unit of viscosity. If a force of 1 dyne cm^{-2} causes a plane to slide past a parallel surface 1 cm^{-2} apart with a velocity of 1 cm sec^{-1}, then the viscosity will be 1 poise.

Electrophoresis and Rotational Motions

Each protein has a characteristic isoelecric point, and this property can be used in the separation of proteins. In the technique called isoelectric focusing, proteins are subjected to electrophoresis on a pH gradient. Each protein moves until it reaches a pH equal to its individual isoelectric point. At that moment, migration in the electrical field stops because the net charge of the protein is zero.

The techniques of isoelectric focusing and SDS polyacrylamide gel electrophoresis have been combined to produce two-dimensional separation of proteins. Several hundred cellular proteins can be resolved from one another. This technique is increasingly used in cell biology, and its great resolving power is due to the use of two independent properties of proteins. The proteins are first separated by isoelectric focussing (This is the dimension) which separates proteins according to their charge (isoelectric point). The proteins are subsequently separated by electrophoresis (this is the second dimension) in polyacrylamide gels containing SDS, which separates proteins according to their size (molecular weight). This technique results in a series of spots distributed throughout the polyacrylamide gel (if the same property of proteins had been used in both dimensions, the spots would be distributed along a diagonal).

When the detergent sodium dodecyl sulfate (SDS) is used in electrophoresis, the proteins are separated mainly according to their molecular weight. This is because SDS binds to the proteins, giving them large numbers of negative charges due to the sulfate. Thus, most of the proteins charges will come from the SDS, minimizing the role of charge differences between individual proteins (differences which would otherwise affect electrophoretic mobility), and all the proteins migrate according to their size. The larger proteins move more slowly than the smaller ones because they encounter more resistance when traversing the molecular proess within the polyacrylamide gel used for electrophoresis. SDS electrophoresis is widely used as a method for determining molecular weights of proteins.

In summary, the vapous pressure methods are applicable to vapours that follow perfect gas equation. In this case also Victor Meyer's method is of higher precision. These can be applied to oligomers only whose critical temperature and critical pressure are known.

Among the ebulliometric and cryoscopic methods, the cryoscopic methods are more precise. Let us suppose a solution of concentration o 1 gm / 100 ml. of solvent which at M = 100 correspond to a number of moles n = 0.01. If cryoscopic constant is 5, then the depression in freezing point will be 0.05^0C. If molar mass is M = 10^6, the value of n=10^{-6}. Hence T_f = 5 x 10^{-6}. No existing method can measure such insignificant changes in temperature. The conventional cryoscopic method can determine the molecular mass from 15,000 to 30,000. The osmometry can be used for determining molecular masses from 10^4 to 10^6.

Viscometric methods are not recommended for determining absolute molecular masses but only changes in molecular masses during various processes (polymerization, degradation, etc.)

The method of sedimentation in the ultra-centrifuge is an absolute method for measuring the molecular mass of a polymer.

The light scattering method is more precise and makes possible to calculate the value of the m_w molecular mass of polymers without assumption of the shape of macromolecules in a solution.

10.11 SUMMARY

By going through this unit you must have achieved the objectives laid down at the start of the unit. Let us sum up what we have discussed so far:

- Out of the various forces involve in biopolymers hydrophobic interactions involve the clustering of non-polar groups, which associate with each other in such a way that they are not in contact with water.
- It is possible for the polymers to have the average molecular weight but different molecular weight distribution (MWD).
- Statistically, we can express the average in terms of number or weight. Consequently, the molecular mass of a polymer is expressed as number average mass ($\overline{M_n}$) or weight average molecular mass ($\overline{M_w}$).
- The ratio of the weight and number average molecular masses ($\overline{M_n}/\overline{M_w}$) is called 'Poly-dispersity index' (PDI). In natural polymers which are generally mono dispersed, the PDI is unity.
- $\overline{M_n}$ is determined by employing methods which depend upon the number of molecules present in the polymer sample, viz colligative properties like osmotic pressure, depression in freezing point and elevation in boiling points. On the other hand methods such as light scattering and ultra-centrifuging depend on the mass of the individual molecules and yield weight average molecular mass.
- Osmosis is defined as the forces of net diffusion of water molecule from a dilute solution or pure water (solvent) itself to a more concentrated solution, when both are separated by a semi permeable membrane.The membrane allows the water to diffuse but not the solute.
- The hydrostatic pressure which exactly balances the osmotic influx of water from pure water to concentrated solution is called the osmotic pressure of that solution.
- Molecular weights are determined either directly or in solution.
- The method for direct determination of molecular weights can only be applied to gases or volatile liquids and solids.
- However, molecular weights of biopolymers are generally determined by solution methods. The important methods involve use of colligative properties viz (i) lowering in the vapour pressure (ii) elevation in the boiling point (iii) depression

in the freezing point and (iv) osmotic pressure for the determination of molecular weight.

- The hydrodynamic methods for determination of biopolymer molecular weight, include (i) diffusion method,(ii) sedimentation method and (iii) viscosity method.
- However SDS electrophoresis is widely used as a method for determination of molecular weights of proteins.

10.12 SAQs

Multiple Choice Questions (MCQ)

1. Molecular mass of biopolymers are expressed as a/an

(a) Average

(b) Median

(c) Mode

(d) Percentage

2. The polydispercity index (PDI) of natural polymers is

(a) 0

(b) 2

(c) 1

(d) 1.5

3. What are colligative properties useful for

(a) Determining boiling and melting temperature

(b) Determining molar mass

(c) Determining equivalent weight

(d) Determining van't Hoff factor

4. What is the necessary condition for osmosis to takes place

(a) Semi permeable membrane

(b) Same concentration of solvent

(c) High temperature

(d) Pressure greater than osmotic pressure

5. Which of the following is not a biopolymer?

(a) Proteins

(b) Cellulose

(c) Rubber

(d) RNA

10.13 TERMINAL QUESTIONS

1. Explain thermodynamics of biopolymer solutions.

2. Explain the forces involved in biopolymer interactions.

3. Define osmosis and membrane equilibrium

4. What role do electrostatic charges play in molecular expansion.

5. How will you determine the size of biopolymers? Mention any two methods.

6. What are the different methods to determine the shape of particles of biopolymers?

7. What are the different methods to determine the molecular mass of biopolymers?

10.14 REFERENCES

1. Gurtu-Gurtu, Biophysical Chemistry, A Pragati Edition

2. J.P Allen, Biophysical Chemistry

3. N.Mahanta and P.S.Kalsi, Biophysical Chemistry

Answers of MCQ

1.	a	2.	c	3.	b	4.	a	5.	c