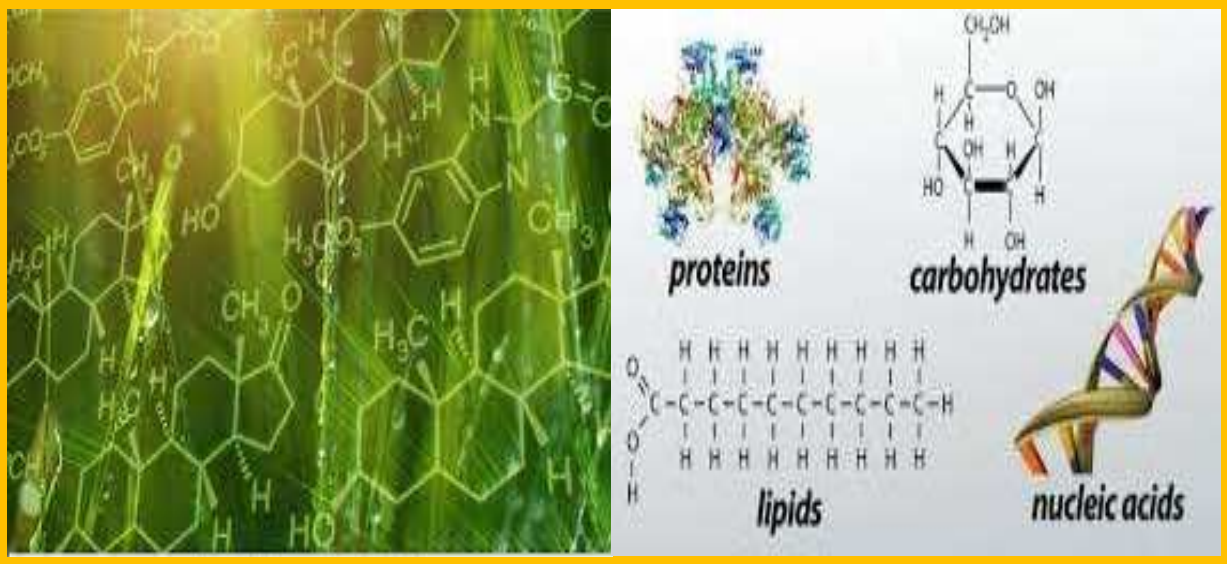
MSCZO-504

M.Sc. I Semester

BIO-CHEMISTRY



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BIOCHEMISTRY MSCZO-504

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Course Title and Code : Biochemistry (MSCZO504)
ISBN : 978-93-90845-51-4
Copyright : Uttarakhand Open University
Edition : 2021
Published By : Uttarakhand Open University, Haldwani, Nainital- 263139
Printed By :

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Course 1: BIO-CHEMISTRY
Course code: MSCZO504 **Credit: 3**

UNIT 1: CHEMICAL EQUILIBRIUM

Contents

1.1 OBJECTIVES

After reading this unit you will be able to-

- Explain laws of thermodynamics,
- Know relationship between forms of energy and biological work,
- Discuss high energy compounds, their functioning and role,
- Define the concept of biological oxidation and reduction,
- Determine the role of buffers and their functioning.

1.2 INTRODUCTION

All living beings perform work to stay alive, to grow, and to reproduce. The capability to channelize energy to work is a principal property of all living organisms. Life depends on constant energy transduction mechanisms of one form of energy to another. In most organisms, from prokaryotes to eukaryotes the obtained energy whether from light, inorganic or organic compounds is transuded into a transmembrane difference of electrochemical potential across the membranes to bring about the synthesis of complex, highly ordered macromolecules from simple precursors.

The chemical processes that underlie biological energy transductions have charmed and challenged biologists for eras. Till now further most of the chemistry behind the biological processes has been explored, however a lot is yet to be decoded. Since, biological energy transductions obey the same physical laws as all other natural processes. It is therefore crucial to understand these laws and their applicability in the biosphere.

1.3 CONCEPTS OF BIOENERGETICS AND THERMODYNAMICS

1.3.1 BIOENERGETICS

Bioenergetics is a field in biochemistry and cell biology that involves the quantitative analysis of the energy transductions occurring in the living system. It also studies the nature and function of the chemical processes underlying these transductions.

1.3.2 THERMODYNAMICS

Thermodynamics is a branch of science concerned with heat and temperature and their relation to energy and work.

1.3.3 LAWS OF THERMODYNAMICS

In the nineteenth century, many quantitative observations made by physicists and chemists on the interconversion of different forms of energy led, to the formulation of two fundamental laws of thermodynamics.

1.3.3.1 THE FIRST LAW

It involves the principle of the conservation of energy. It states that for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed.

1.3.3.2 THE SECOND LAW

It states that the universe always tends toward increasing disorder: in all processes, the total entropy always increases. Spontaneous entropy is a measure of randomness within a closed system. To discuss the application of the second law to biological systems, we must know some of the facts as below-

- **The reacting system**– is that portion of the *universe* with which we are concerned, whereas the **surroundings** include everything else in the universe. It is the collection of matter that is undergoing a particular chemical or physical process; it may be an organism, a cell, or two reacting compounds.
- The reacting system and its surroundings together constitute the universe.
- An **isolated system** cannot exchange matter or energy with its surroundings.
- A **closed system** may exchange energy, but not matter, with the surroundings.
- Living cells and organisms are **open systems**, exchanging both material (nutrients and waste products) and energy/heat (from metabolism, for example) with their surroundings
- Living systems are never at equilibrium with their surroundings.
- The continual transactions between system and surroundings justify how organisms can create order within themselves while operating within the second law of thermodynamics.

1.3.4 GIBBS FREE ENERGY (G)

It expresses the amount of energy capable of doing work during a reaction at constant

temperature and pressure. When a reaction progresses with the release of free energy (when the system changes to possess less free energy), the free-energy change**,** Gibbs has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and Gibbs is positive.

1.3.5 ENTHALPY (H)

It is a measurement of energy in a thermodynamic system. It is equivalent to the total heat content of a system. It is equal to the internal energy of the system plus the product of pressure and volume. It reflects the number and kinds of chemical bonds in the reactants and products.

When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and H shape has, by convention, has a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of heat

1.3.6 ENTROPY (S)

It is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

The units of G and H are joules/mole or calories/mole (1 calculated = 4.184 J); units of entropy are joules/mole Kelvin (J/mol K)

Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

$\Delta G = \Delta H - T\Delta S$

Where, ΔG = change in Gibbs free energy of the reacting system

ΔH= change in enthalpy of the system

T = absolute temperature and S = change in entropy of the system.

S has a positive sign when entropy increases and H, as noted above, has a negative sign when heat is released by the system to its surroundings. Both conditions are characteristic of favorable processes, and tend to make G negative.

G of a spontaneously reacting system is always negative.

Now you have understood that, as per second law of thermodynamics, the entropy of the universe within a closed or isolated system increases during all chemical and physical processes.

The order produced within cells as they grow and divide is more than the disorder they create in their surroundings during growth and division. In other way we can say, living organisms preserve their internal order by taking free energy from the surroundings in the form of nutrients or sunlight, and return to their surroundings an equal amount of energy as heat and entropy.

1.3.7 STANDARD FREE ENERGY CHANGE ΔG°

A reacting system tends to change continually until an equilibrium is reached where the concentration of reactants and products, the rates of the forward and reverse reactions are equal and no further net change occurs in the system. The concentrations of reactants and products at equilibrium define the equilibrium constant, K_{eq}.

In the general reaction aA+ bB ⇌cC+ dD

a, b, c, and dare the number of molecules of A, B, C, and D respectively

the equilibrium constant is given by

$K_{eq} = [A]^a [B]^b/[C]^c [D]^d$ (eq-1)

[A], [B], [C], and [D] are the molar concentrations of the reaction components at equilibrium.

Interestingly, when a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force, the magnitude of which can be expressed as the free-energy change for the reaction, ΔG.

Under standard conditions (298 K =25°C), where reactants and products are initially present at 1 M concentrations or, for gases, at partial pressures of 101.3 kilopascals (kPa), or 1 atm, the force driving the system toward equilibrium is defined as the standard free-energy change, ΔG°. By this definition, the standard state for reactions that involve hydrogen ions is $[H^+]$= 1 M, or pH 0.

Most biochemical reactions, however, occur in well-buffered aqueous solutions near pH 7; both the pH and the concentration of water (55.5 M) are essentially constant. For calculative convenience, biochemists therefore define a different standard state, in which the concentration of $H^+$is 10^{-7}M (pH 7) and that of water is 55.5 M; for reactions that involve Mg^{2+} (including most in which ATP is a reactant), its concentration in solution is commonly taken to be constant at 1mM. Physical constants based on this biochemical standard state are called **standard transformed constants** and are written with a prime(suchas ΔG'°andK'_{eq})to distinguish them from the untransformed constants used by chemists and physicists.

There is a simple relationship between ΔG'° and K'eq:

$\Delta G'^{\circ} = - RT \ln K'_{eq}$

If K'_{eq} of a reaction is greater than 1.0, its $\Delta G'^{\circ}$ is negative. If K'_{eq} is less than 1.0, $\Delta G'^{\circ}$ is positive. Because the relationship between $\Delta G'^{\circ}$ and K'_{eq} is exponential, relatively small changes in $\Delta G'^{\circ}$ correspond to large changes in $K'_{eq.}$

In other way, when $\Delta G'^{\circ}$ is negative, the products contain less free energy than the reactants and the reaction will proceed spontaneously under standard conditions; all chemical reactions tend to go in the direction that results in a decrease in the free energy of the system. A positive value of $\Delta G'^{\circ}$ means that the products of the reaction contain more free energy than the reactants, and this reaction will tend to go in the reverse direction.

1.3.8 RELATION BETWEEN STANDARD FREE ENERGY CHANGE (ΔG°) AND ACTUAL FREE ENERGY CHANGE (ΔG)

$\Delta G'^{\circ}$ is constant, for a given reaction. But the actual free-energy change, ΔG, is a function of reactant and product concentrations and of the temperature prevailing throughout the reaction, which will not essentially match the standard conditions as defined for $\Delta G'^{\circ}$ (initial concentration of each component=1.0 M, pH= 7.0, temperature=25°C, pressure =101.3 kPa). Moreover, the ΔG of any reaction proceeding spontaneously toward its equilibrium is always negative, becomes less negative as the reaction proceeds, and is zero at the point of equilibrium, indicating that no more work can be done by the reaction.

ΔG and $\Delta G'^{\circ}$ for any reaction A +B ⇌ C +D are related by the equation

$\Delta G = \Delta G'^{\circ} + RT \ln [C][D]/[A][B]$ (eq—2)

The term [C] [D]/ [A] [B] is mass-action ratio. When concentrations of A, B, C, and Dare unequal and none of the components is present at the standard concentration of 1.0 M, then to determine the actual free-energy change, G, we simply enter the actual concentrations of A, B, C, and D in eq—2 the values of R, T, and $\Delta G'^{\circ}$ are the standard values. ΔG is negative and approaches zero as the reaction proceeds because the actual concentrations of A and B decrease and the concentrations of C and D increase. Interestingly, when a reaction is at

equilibrium—when there is no force driving the reaction in either direction and ΔG is zero—then (eq—2) reduces to

$$0 = \Delta G = \Delta G'^{\circ} + RT \text{ in } [C]_{eq} [D]_{eq} / [A]_{eq} [B]_{eq}$$

or

$$\Delta G'^{\circ} = - RT \ln K'_{eq}$$

which is the equation relating the standard free-energy change and equilibrium constant given earlier.

The criterion for spontaneity of a reaction is the value of ΔG, not $\Delta G'^{\circ}$.

1.3.8.1 Standard Free-Energy changes are additive

In the case of two sequential chemical reactions, A⇌B and B ⇌C, each reaction has its own equilibrium constant and each has its characteristic standard free-energy change, $\Delta G'^{\circ}_1$. and $\Delta G'^{\circ}_2$. As the two reactions are sequential, the overall reaction A ⇌C, has its own equilibrium constant and thus its own standard free-energy change, $\Delta G'^{\circ}_{total}$. The $\Delta G'^{\circ}$value for the two sequential reactions would be additive.

For the overall reaction A ⇌ C, $\Delta G'^{\circ}_{total}$ is the sum of the individual standard free-energy changes, $\Delta G'^{\circ}_1$and $\Delta G'^{\circ}_2$ of the two reactions thus

$$\Delta G'^{\circ}_{total} = \Delta G'^{\circ}_1 + \Delta G'^{\circ}_2$$

Bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction proceeds in the forward direction by coupling it to a highly exergonic reaction through a common intermediate.

For eg.

$$X + P_i \longrightarrow XPO_4 + H_2O \qquad \Delta G'^{\circ} = 13.8 \text{ kJ/mol}$$

The positive value of G indicates that under standard conditions the reaction will tend not to proceed feasibly. Another cellular reaction, the hydrolysis of ATP to ADP and P_i , is highly exergonic:

$ATP + H_2O \longrightarrow ADP + P_i \quad \Delta G'^\circ = -30.5$ kJ/mol

These two reactions share the common intermediates P_i and H_2O and may be expressed as sequential reactions:

. (1) $X + P_i \longrightarrow XPO_4 + H_2O$

. (2) $ATP + H_2O \longrightarrow ADP + P_i$

. *Sum:* $ATP + X \longrightarrow ADP + XPO_4$

The overall standard free-energy change is obtained by adding the G values for individual reactions:

$\Delta G'^\circ = 13.8$ kJ/mol + (-30.5 kJ/mol) = -16.7 kJ/mol

The overall reaction is exergonic.

1.4 HIGH ENERGY COMPOUNDS

A small family of universal biomolecules mediates the flow of energy from exergonic reactions to the energy requiring processes of life. These molecules are the *reduced* coenzymes and the high-energy phosphate compounds (Table 1). Phosphate compounds are considered high energy compounds if they exhibit large negative free energies of hydrolysis (that is, if G° is more negative than 25 kJ/mol).

The most important members of the high-energy phosphate compounds include *phosphoric anhydrides* (ATP, ADP), an *enol phosphate* (PEP), an *acyl phosphate* (acetyl phosphate), and a *guanidino phosphate* (creatine phosphate). The family also includes thioesters, such as acetyl-CoA, that do not have phosphorus, but have high free energy of hydrolysis.

The exact amount of chemical free energy available from the hydrolysis of such compounds depends on concentration, pH and temperature, but the ΔG° values for hydrolysis of these substances are substantially more negative than for most other metabolic species. Two important points that explains this fact are:

1) High-energy phosphate compounds are not long-term energy storage substances. They are transient forms of stored energy, meant to carry energy from point to point, from

one enzyme system to another, in the minute-to-minute existence of the cell.

2) The term *high-energy compound* should not be interpreted to infer that these molecules are unstable and hydrolyze or decompose unpredictably. ATP, for example, is a stable molecule. Substantial activation energy must be delivered to ATP to hydrolyze the terminal, orγ, phosphate group. The *activation energy* that must be absorbed by the molecule to break the O-Pγ bond is normally 200 to 400 kJ/mol (provided by certain enzymes), which is substantially larger than the net 30.5 kJ/mol released in the hydrolysis reaction. The net release of large quantities of free energy distinguishes the high-energy phosphoric anhydrides from their "low-energy" ester companions, such as glycerol-3-phosphate.
3) **Entropy Factors Arising from Hydrolysis and Ionization:**For the most of the high-energy compounds, there is an additional "entropic" contribution to the free energy of hydrolysis. Most of the hydrolysis reactions result in an increase in the number of molecules in the solution. The entropy of the solution increases as the disorder increases, more disordered is the system.(This effect is ionization-dependent because, at low pH, the hydrogen ion formed in many of these reactions simply protonates one of the phosphate oxygens, and fewer "particle" results from the hydrolysis.)

COMPOUND	$\Delta G'^{\circ}$ (kJ/mol)
Phosphoenolpyruvate	-61.9
1,3-bisphosphoglycerate ($\rightarrow$3-bisphosphoglycerate + P_i)	-49.3
Phosphocreatine	-43.0
ADP ($\rightarrow$AMP +P_i)	-32.8
ATP ($\rightarrow$ADP +P_i)	-30.5
ATP ($\rightarrow$AMP +PP_i)	-45.6
AMP ($\rightarrow$adenosine +P_i)	-14.2
PP_i ($\rightarrow 2P_i$)	-19.2
Glucose 1-phosphate	-20.9
Fructose 1-phosphate	-15.9
Glucose 6-phosphate	-13.8
Glycerol 1-phosphate	-9.2
Acetyl-CoA	-31.4

Table 1- Standard Free Energies of hydrolysis of some phosphorylated compounds and Acetyl-CoA*

Source: Jencks, W.P. (1976) in Handbook of Biochemistry and Molecular Biology, 3rd edn (Fasman, G.D., ed.), Physical and Chemical Data, Vol. I, pp. 296–304, CRC Press, Boca Raton, FL. The value for the free energy of hydrolysis of PP_i is from Frey, P.A. & Arabshahi, A. (1995) Standard free-energy change for the hydrolysis of the phosphoanhydride bridge in ATP. Biochemistry* ***34,* *11,307–11,310*

1.4.1 ATP –AN INTERMEDIARY ENERGY-SHUTTLE MOLECULE

Interestingly ATP holds an intermediate place in the rank of high-energy phosphates. Phosphoenolpyruvate, cyclic AMP, 1, 3-BPG, phosphocreatine and many other moleculesexhibit higher values of $\Delta G°$. This is not a biological incongruity. ATP is uniquely situated between the very high-energy phosphates. It is synthesized during the breakdown of metabolic fuel molecules and the numerous lower-energy acceptor molecules that are phosphorylated in the course of further metabolic processes can donate both phosphates and energy to the lower-energy molecules of metabolism. The ATP/ADP pair is an intermediately placed acceptor/donor system among high-energy phosphates. In this perspective, ATP functions as a very versatile but intermediate energy-shuttle device that interacts with many different energy-coupling enzymes of metabolism.

1.4.1.1 The Free-Energy Change for ATP Hydrolysis Is Large and Negative

Figure 1. Illustrates the chemical basis for the relatively large, negative, standard free energy of hydrolysis of ATP.

Step 1 The hydrolytic cleavage of the terminal phosphoric acid anhydride bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP

Step 2 The Pi (HPO_4^{2-}) released is stabilized by the formation of several resonance forms not possible in ATP; and ADP^{2-}, (each of the four phosphorus–oxygen bonds have the same degree of double-bond character and the hydrogen ion is not permanently associated with any one of the oxygens).

Step 3 The other direct product of hydrolysis (ADP^{2-}), immediately ionizes, releasing H^+ into a medium of very low $[H^+]$ ($\sim 10^{-7}$ M). Because the concentrations of the direct products of ATP hydrolysis are, in the cell, far below the concentrations at equilibrium, mass action favors the hydrolysis reaction in the cell.

A fourth factor that favors ATP hydrolysis is the greater degree of solvation (hydration) of the products Pi and ADP relative to ATP, which further stabilizes the products relative to the reactants

Although the hydrolysis of ATP is highly exergonic ($\Delta G'° = -30.5$ kJ/mol), the molecule is kinetically stable at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphor anhydride bonds occurs only when catalyzed by an enzyme.

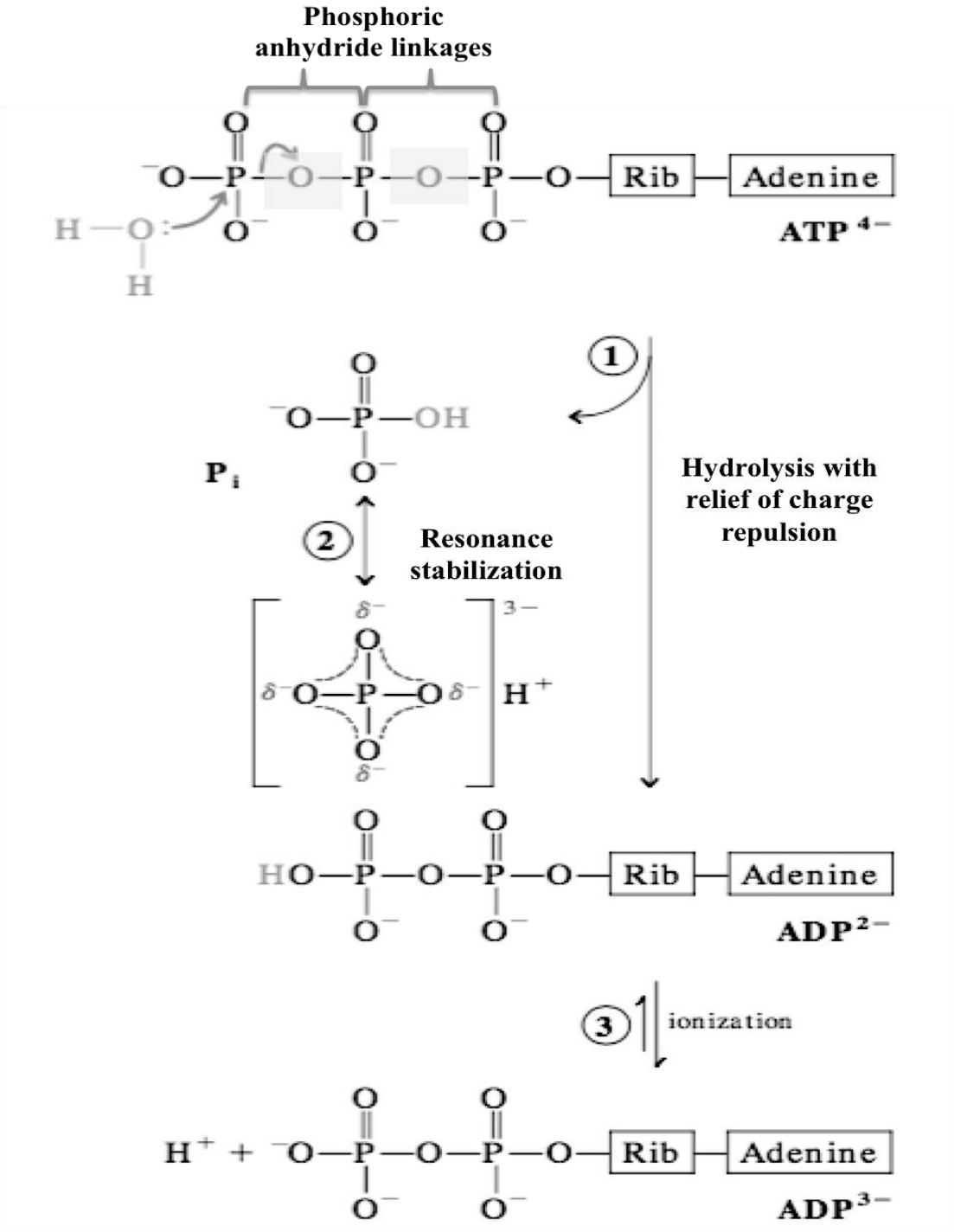


Figure 1 Chemical depiction of the large free-energy change associated with ATP hydrolysis.

The standard free-energy change for ATP hydrolysis and the actual free energy of hydrolysis (ΔG) of ATP in living cells differs due to the fact that the cellular concentrations of ATP, ADP, and Pi are not identical and are much lower than standard conditions. Moreover, Mg^{2+} in the cytosol binds to ATP and ADP (Figure 2), and for most enzymatic reactions that

involve ATP as phosphoryl group donor, the true substrate is $MgATP^{2-}$. The relevant ***ΔG'°***is therefore that for $MgATP^{2}$ hydrolysis. In fact formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP. In intact cells, ***Δ***G for ATP hydrolysis, usually designated ***Δ***Gp, is much more negative than ***ΔG'°***, ranging from -50 to -65 kJ/mol. ***Δ***Gpis often called the phosphorylation potential.

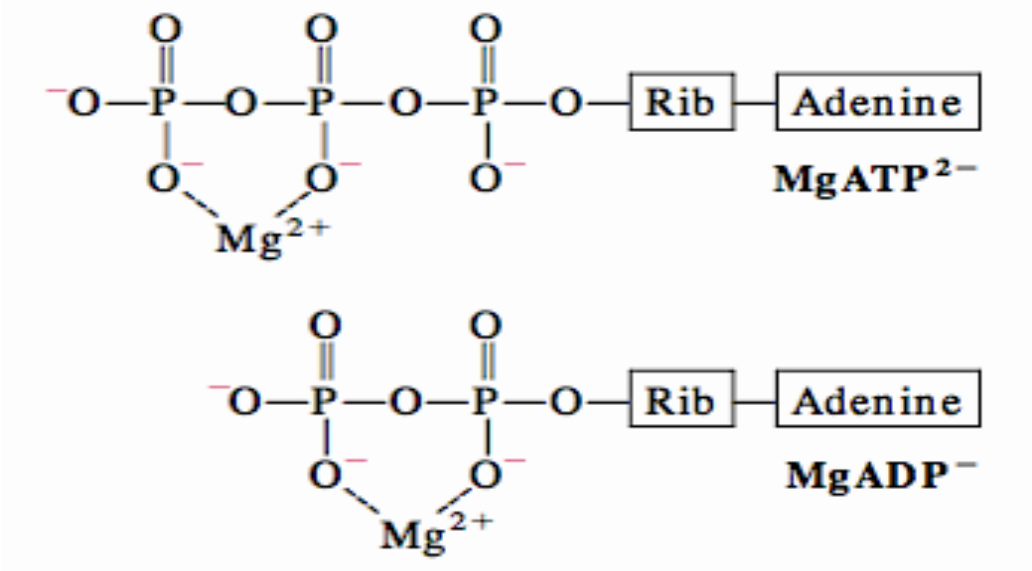


Figure 2 $Mg^{2+}$and ATP

1.4.1.2 A Comparison of the Free Energy of Hydrolysis of ATP, ADP, and AMP

ATP and ADP are destabilized relative to the hydrolysis products by electrostatic repulsion, competing resonance, and entropy. AMP, on the other hand, is a phosphate ester (not an anhydride) possessing only a single phosphoryl group and is not markedly different from the product inorganic phosphate in terms of electrostatic repulsion and resonance stabilization. Thus, the ***ΔG'°***for hydrolysis of AMP is much smaller than the corresponding values for ATP and ADP.

1.4.2 OTHER HIGH ENERGY COMPOUNDS OF BIOLOGICAL IMPORTANCE

1.4.2.1 Enol Phosphates

Phosphoenolpyruvate (PEP) an enolic phosphate, involved in glycolysis, has a larger negative ***ΔG'°*** of phosphate hydrolysis than ATP. PEP is formed via dehydration of 2-phosphoglycerate by enolase during fermentation and glycolysis. PEP is subsequently

transformed into pyruvate upon transfer of its phosphate to ADP by pyruvate kinase. The very large negative value of $\Delta G'^{\circ}$ for the latter reaction is to a large extent is the result of a secondary reaction of the *enol* form of pyruvate. On hydrolysis, the comparatively unstable enolic form of pyruvate immediately converts to the keto form with a resulting large negative $\Delta G'^{\circ}$ (Figure 3). Together, the hydrolysis and subsequent *tautomerization* result in an overall $\Delta G'^{\circ}$ of -61.9 kJ/mol.

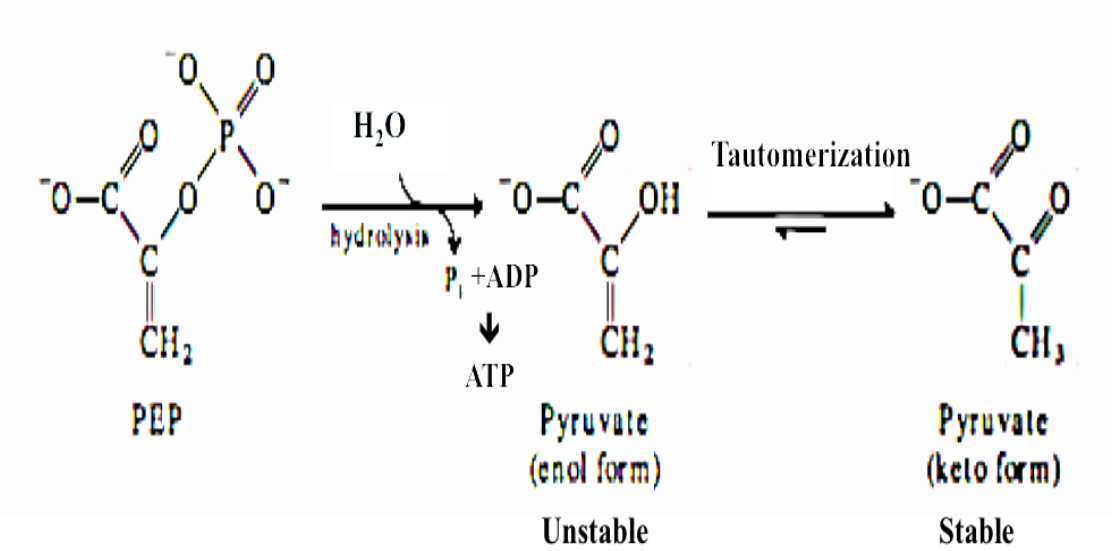
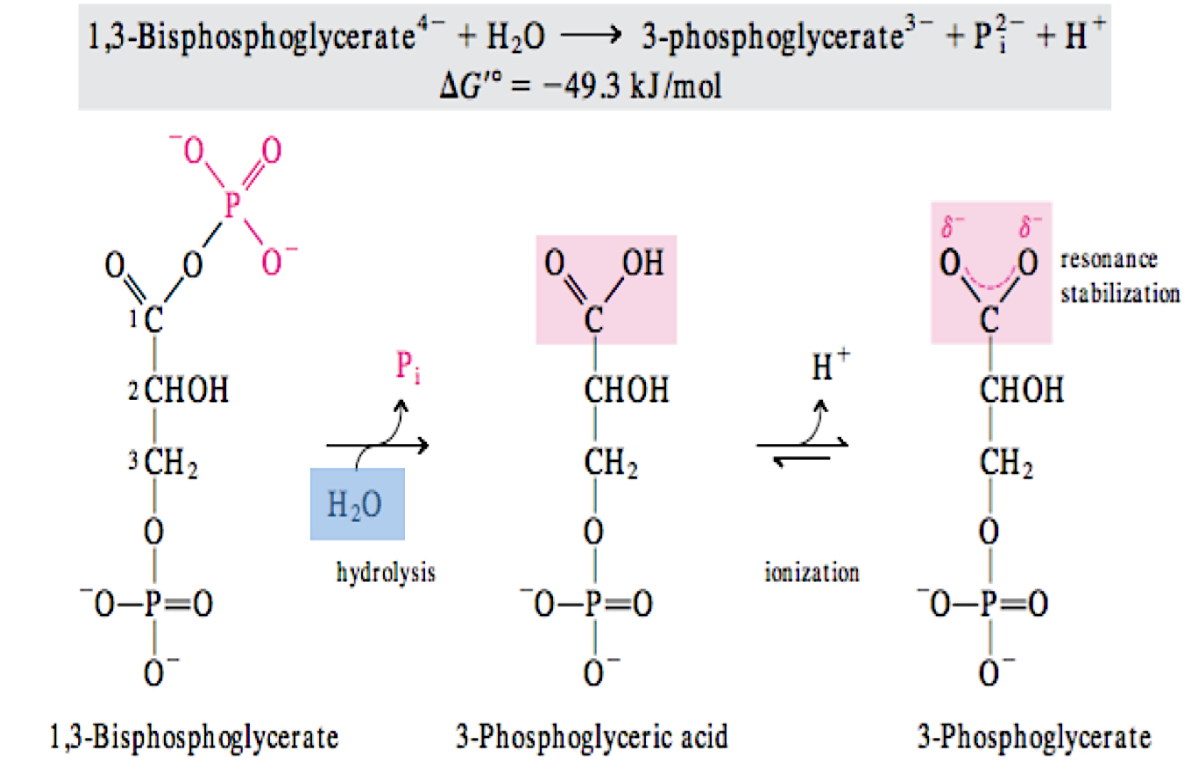


Figure 3: Hydrolysis of Phosphoenol Pyruvate (PEP)

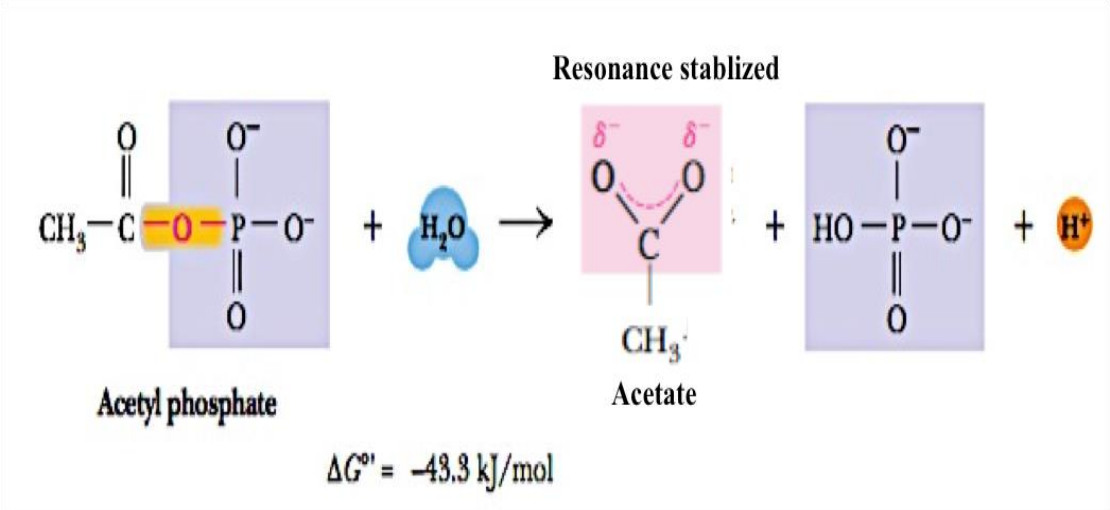
1.4.2.2 Phosphoric–Carboxylic Anhydrides

The mixed anhydrides of phosphoric and carboxylic acids, frequently called acyl phosphates, are also energy-rich. Two biologically important acyl phosphates are acetyl phosphate and 1,3-bisphosphoglycerate. Hydrolysis of these molecules(Figure 4 (A) & (B) yields acetate and 3-phosphoglycerate, respectively (which has two equally probable resonance forms), in addition to inorganic phosphate Once again, the large $\Delta G'^{\circ}$values indicate that the reactants are destabilized relative to products. This arises from bond strain, which can be traced to the partial positive charges on the carbonyl carbon and phosphorus atoms of these structures. The energy stored in the mixed anhydride bond (which is required to overcome the charge–charge repulsion) is released upon hydrolysis. Increased resonance possibilities in the products relative to the reactants also contribute to the large negative $\Delta G'^{\circ}$values. The value of $\Delta G'^{\circ}$depends on the p*K*a values of the starting anhydride and the

product phosphoric and carboxylic acids, and of course also on the pH of the medium.



(A)



(B)

Figure 4 Hydrolysis of (A) 1,3- diphosphoglycerate and (B) acetyl phosphate

1.4.2.3 Phosphocreatine and Thioesters

In phosphocreatine, the P-N bond gets hydrolyzed to generate free creatine and P_i(Figure

5a). The release of P_i and the further resonance stabilization of creatine favor the forward reaction. The standard free-energy change of phosphocreatine hydrolysis is again large, 43.0 kJ/mol.

Thioesters, in which a sulfur atom replaces the usual oxygen in the ester bond do not release Pi on hydrolysis but nevertheless have large, negative, standard free energies of hydrolysis. Acetyl-coenzyme A, or acetyl-CoA (Fig. 5b), is one of many thioesters important in metabolism. There is no resonance stabilization in thioesters comparable to that in oxygen esters, consequently, the difference in free energy between the thioester and its hydrolysis products, which are resonance-stabilized, is greater than that for comparable oxygen esters. In both cases, hydrolysis of the ester generates a carboxylic acid, which can ionize and assume two resonance forms as described above for acyl phosphates. The free energy of hydrolysis for acetyl-CoA is large and negative, about -31 kJ/mol.

To summarize, for hydrolysis reactions with large, negative, standard free-energy changes, the products are more stable than the reactants for one or more of the following reasons:

(1) The bond strain in reactants due to electrostatic repulsion is relieved by the charge separation, as for ATP

(2) The products are stabilized by ionization, as for ATP, acyl phosphates, and thioesters

(3) The products are stabilized by isomerization (tautomerization), as for phosphoenolpyruvate

(4) The products are stabilized by resonance, as for creatine released from phosphocreatine, carboxylate ion released from acyl phosphates and thioesters, and phosphate (P_i) released from anhydride or ester linkages.

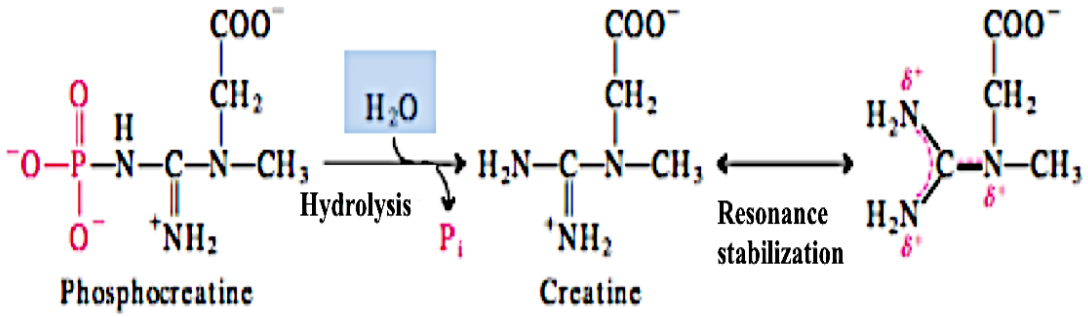


Figure 5a- Hydrolysis of P-N bond for generation of creatine and Pi

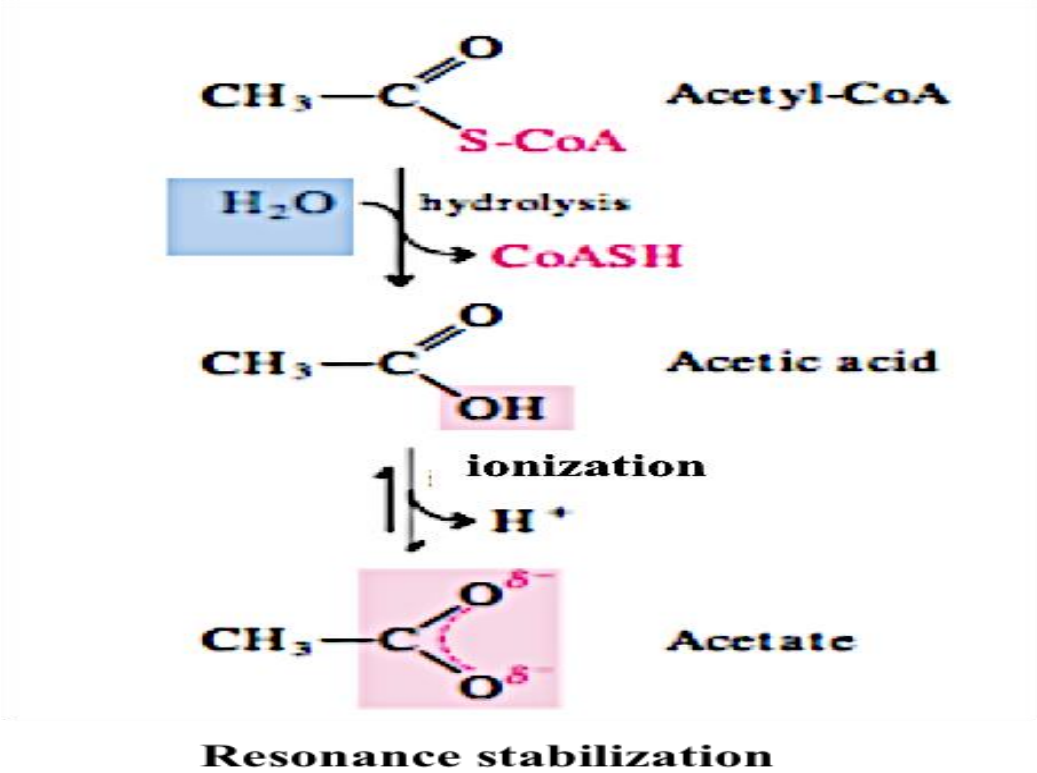


Figure 6- Hydrolysis of Acetyl-coenzyme A

1.5 BIOLOGICAL OXIDATION REDUCTION

The transfer of electrons in oxidation-reduction reactions is a crucial feature of metabolism like the transfer of phosphoryl groups. These reactions involve the loss of electrons by one chemical species, which is thereby oxidized, and the gain of electrons by another, which is reduced. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems. The flow of electrons in oxidation-reduction reactions is responsible, directly or indirectly, for all work done by living organisms. In no photosynthetic organisms, the sources of electrons are reduced compounds (foods) whereas, in photosynthetic organisms, the initial electron donor is a chemical species excited by the absorption of light. The path of electron flow in metabolism is complex. Electrons move from various metabolic intermediates to specialized electron carriers in enzyme-catalyzed reactions. The carriers in turn donate electrons to acceptors with higher electron affinities, with the release of energy. Cells contain a variety of molecular energy transducers, which convert the energy of electron flow into useful work.

1.5.1 Oxidation-Reductions can be described as half-reactions

Although oxidation and reduction must occur together, it is convenient while describing electron transfers to consider the two halves of an oxidation-reduction reaction separately. For example, the oxidation of ferrous ion by cupric ion,

$\mathbf{Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}}$

The above reaction can be described in terms of two half-reactions:

(1) $\mathbf{Fe^{2+} \rightleftharpoons Fe^{3+} + e^{-}}$

(2) $\mathbf{Cu^{2+} + e^{-} \rightleftharpoons Cu^{+}}$

The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant whereas the electron-accepting molecule is the oxidizing agent or oxidant. In the above equation an iron cation exists in the ferrous ($\mathbf{Fe^{2+}}$) or ferric ($\mathbf{Fe^{3+}}$) state, functions as a conjugate reductant-oxidant pair (redox pair)where $\mathbf{Fe^{2+}}$ is the electron donor and $\mathbf{Fe^{3+}}$is the electron acceptor and together, $\mathbf{Fe^{2+}}$ and $\mathbf{Fe^{3+}}$constitute a **conjugate redox pair.**

In biological systems, oxidation is often synonymous with **dehydrogenation,** and many enzymes that catalyze oxidation reactions are **dehydrogenases.**

Electrons are transferred from one molecule (electron donor) to another (electron acceptor) in one of four different ways:

1. Directly as electrons. For example, the Fe^{2+}/Fe^{3+} redox pair can transfer an electron to the Cu^{+}/Cu^{2+} redox pair:

   $Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$

2. As hydrogen atoms. [A hydrogen atom consists of a proton (H^{+}) and a single electron (e^{-})]. In this case we can write the general equation

   $XH_2 \rightleftharpoons X + 2e^{-} + 2H^{+}$

3. Where XH_2 is the hydrogen/electron donor.XH_2 and X together constitute a conjugate redox pair (X/XH_2), which can reduce another compound Y (or redox pair, Y/YH_2) by transfer of hydrogen atoms:

   $XH_2 + Y \rightleftharpoons X + YH_2$

4. As a hydride ion (:H), which has two electrons. e.g., case of NAD-linked

dehydrogenases,

5. Through direct combination with oxygen. In this case, oxygen combines with an organic reductant and is covalently incorporated in the product.

1.5.2 Standard Reduction Potential, $E°$

The **standard reduction potential, $E°$,** a measure (in volts) of the affinity of the electron transfer from the electron donor of one pair to the electron acceptor of the other, when two conjugate redox pairs are together in solution.

The reduction potential of a half-cell depends not only on the chemical species present but also on their activities, approximated by their concentrations. About a century ago, Walther Nernst derived an equation that relates standard reduction potential ($E°$) to the reduction potential (E) at any concentration of oxidized and reduced species in the cell:

$E = E°+RT/nF \ln$ [electron acceptor]/[electron donor]

where R and T have their usual meanings, n is the num- bar of electrons transferred per molecule, and F is the Faraday constant (96.5 kJ /V mol). At 298 K (25 C), above expression reduces to

$E = E°+0.026V/n \ln$ [electron acceptor]/[electron donor]

Same as for $\Delta G'°$, Biochemists define the standard state for oxidation-reduction reactions at pH 7 and express reduction potential as $E°$, the standard reduction potential at pH 7 that represents the potential difference when the conjugate redox pair, at 1 M concentrations and pH 7, is connected with the standard (pH 0) hydrogen electrode.

1.5.3 Standard Reduction Potentials Can Be Used to Calculate the Free-Energy Change

The energy available by the spontaneous electron flow (the free-energy change for the oxidation- reduction reaction) is proportional to E:

$\Delta G = - nF\Delta E$ or $\Delta G'° = - nF\Delta E'°$,

Here n represents the number of electrons transferred in the reaction. With this equation we can calculate the free-energy change for any oxidation-reduction reaction from the values of $E'°$ and the concentrations of the species participating in the reaction.

1.5.4 A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

The multitude of enzymes that catalyze cellular oxidations channel electrons from their hundreds of different substrates into just a few types of universal electron carriers. The

reduction of these carriers in catabolic processes results in the conservation of free energy released by substrate oxidation.

Enzymes involved in oxidation and reduction are called **oxidoreductases** and are classified into four groups: **oxidases, dehydrogenases, hydroperoxides,** and **oxygenase.**

1.5.4.1 OXIDASES

Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. They form water or hydrogen peroxide as products. Some oxidases contain copper e.g., cytochrome oxidase while others are flavoproteins that contain **flavin mononucleotide (FMN)** or **flavin adenine dinucleotide (FAD)** as prosthetic groups. FMN and FAD are usually tightly—but not covalently—bound to their respective apoenzyme proteins.

1.5.4.2 DEHYDROGENASES

They can't use oxygen as a hydrogen acceptor. A number of enzymes belong in this class. These enzymes perform two main functions:

(1) Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction. These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+. Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell.

(2) As components in the **respiratory chain** of electron transport from substrate to oxygen

These dehydrogenases use coenzymes such as **nicotinamide adenine di- nucleotide (NAD+**) or **nicotinamide adenine dinucleotide phosphate (NADP+**)—or both. The coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor. They may freely and reversibly dissociate from their respective apoenzymes.

Other dehydrogenase use riboflavin as coenzymes. The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases. They are generally more tightly bound to their apoenzymes than are the nicotinamide coenzymes. Most of the riboflavin-linked dehydrogenases are concerned with electron transport in (or to) the respiratory chain

1.5.4.3 HYDROPEROXIDASES

Two types of enzymes found both in animals and plants fall in this category: **peroxidases** and **catalase.** In the reaction catalyzed by peroxidase, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate,

quinones, and cytochrome *c.* The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows:

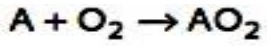
Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor.

1.5.4.4 OXYGENASES

Oxygenaseis concerned with the synthesis or degradation of different types of metabolites. They catalyze incorporation of oxygen into a substrate molecule in two steps: (1) oxygen is bound to the enzyme at the active site, and (2) the bound oxygen is reduced or transferred to the substrate. Oxygenase may be divided into two subgroups, as follows.

Dioxygenases incorporate both atoms of molecular oxygen into the substrate

The basic reaction is shown below:



Monooxygenases (Mixed-Function Oxidases, Hydroxylases)

They incorporate Only One Atom of Molecular Oxygen into the Substrate, the other oxygen atom is reduced to water, an additional electron donor or cosubstrate (Z) being necessary for this purpose. e.g., cytochromes P450

1.6 pH AND BUFFERS

1.6.1 pH

Hydrogen ion concentrations are typically quite small numbers, which is of the greatest importance in chemistry. Therefore, the hydrogen ion concentration of a solution is reported

in terms of pH. It is defined as ***the negative of the base-10 logarithm (log) of the H^+ ionconcentration***. Mathematically it may be expressed as **pH = – log $[H^+]$** where $[H^+]$ is the concentration of hydrogen ions in moles per liter. Alternative and more useful forms of pH definition are:

$pH = \log 1/[H^+]$

$[H^+]=10^{-pH}$

Sorensen introduced the pH concept in 1909 and it is very convenient way for expressing hydrogen ion concentration and expressing other quantities also, for example,

(a) Concentration of OH^- ions in aqueous solution of a base is expressed as

$p[OH^-]= -\log [OH^-]$

(b) Equilibrium constant for water is written as

$pK_W= -\log [K_W]$

"p" in these expression stands for **"negative logarithm of".**

Note that the pH scale is logarithmic, not arithmetic. To say that two solutions differ in pH by 1 pH unit means that one solution has ten times the H concentration of the other, but it does not tell us the absolute magnitude of the difference

pH Scale

In order to express the hydrogen ion concentration or acidity of a solution, pH scale was evolved. The pH is defined as

$pH= \log 1/[H^+]$ or $[H^+]=10^{-pH}$

The hydrogen ion concentrations of different acidic solutions were determined experimentally. These were converted to pH values using the above relations. Then these pH values were computed on a scale taking water as the reference substance. ***The scale on which pH values are computed is called the pH scale***.

Water dissociates to $H^+$and OH^- ions to a very small degree so that we have the equilibrium.

$H_2O \rightleftharpoons H^+ + OH^-$

We can write the equilibrium expression as

$K= [H^+]\,[OH^-]/[H_2O]$

Since water is so little dissociated, the concentration of undissociated molecules, $[H_2O]$, is presumed to be constant. Therefore, we can write

$[H^+]\,[OH^-] = K[H_2O] = K_W$

where Kw= ***water dissociation constant or the water ionization constant***. When the concentrations of H^+ and OH^- ions in water are expressed in mole per litre, the value of Kw found experimentally is 1.0×10^{-14}. From the equation it is obvious that one molecule of water dissociates to give one H+ ion and one OH– ion. This means that the concentration of H^+ and OH^- ions in pure water is equal. Using the expression, we have

$[H^+]\,[OH^-] = \sqrt{K_W} = \sqrt{1.0 \times 10^{-14}}$

$[H^+] = [OH^-] = 1.0 \times 10^{-7}$ mol/l

The ion product of water, Kw, is the basis for the pH scale. It is a convenient means of determining the concentration of H (and thus of OH) in any aqueous solution in the range between 1.0 M H and 1.0 M OH.

Thus, the H^+ion and OH^- ion concentrations in pure water are both 10^{-7}mol/l at 25°C and it is said to be neutral. In acidic solution, however, the concentration of H^+ions must be greater than 10^{-7}mol/l. Similarly in a basic solution; the concentration of OH^- ions must be greater than 10^{-7}mol/l. Thus, we can say:

Neutral solution $[H^+] = [OH^-]$

Acidic solution $[H^+] > [OH^-]$

Basic solution $[H^+] < [OH^-]$

1.6.2 BUFFERS

A buffer is a solution that resists changes in pH when small amounts of acid (H^+) or base (OH^-) are added to it. A buffer consists of a solution of a weak acid (the proton donor) and the corresponding conjugate base (the proton acceptor).

So by definition "***A buffer solution is one which maintains its pH fairly constant even on the addition of small amounts of acid or base***. "

In other words, a buffer solution resists (or buffers) a change in its pH. That is, we can add a small amount of an acid or base to a buffer solution and the pH will change very little. Two common types of buffer solutions are:

[1] A weak acid together with a salt of the same acid with a strong base. These are called ***Acid buffers*** *e.g.*, $CH_3COOH + CH_3COONa$.

[2] A weak base and its salt with a strong acid. These are called ***Basic buffers***. *e.g.*, $NH_4OH + NH_4Cl$.

1.6.3 MECHANISM OF BUFFER ACTION

Let us illustrate buffer action by taking example of a common buffer system consisting of solution of acetic acid and sodium acetate (CH_3COOH/CH_3COONa).

$CH_3COOH \rightleftharpoons CH_3COOH^- + H^+$

$CH_3COONa \rightarrow CH_3COO^- + Na^+$

Since the salt is completely ionized, it provides the common ions CH_3COO^- in excess. The common ion effect suppresses the ionization of acetic acid. This reduces the concentration of H^+ ions which means that pH of the solution is raised. The buffer solution maintains fairly constant pH and the changes in pH could be described as marginal.

How does a Buffer Operates?

Now we proceed to discuss how the addition of a small amount of HCl or NaOH to the buffer solution affects its pH. The pH of the buffer is governed by the equilibrium

$CH_3COOH \rightleftharpoons CH_3COOH^- + H^+$

The buffer solution has a large excess of CH_3COO^- ions produced by complete ionization of sodium acetate,

$CH_3COONa \rightarrow CH_3COO^- + Na^+$

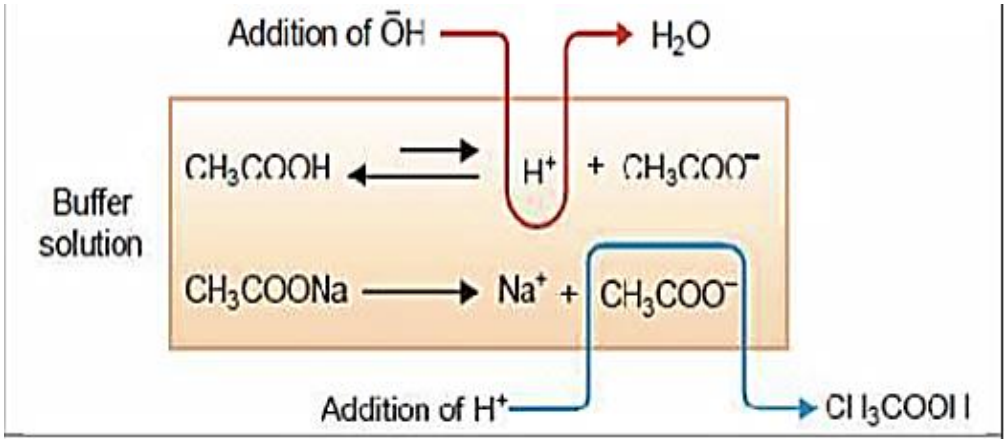
[1] ***Addition of HCl*****:** On the addition of HCl, the increase of H^+ ions is counteracted by association with the excess of acetate ions to form unionized CH_3COOH. Thus, the added H^+ ions are neutralised and the pH of the buffer solution remains virtually unchanged. However, owing to the increased concentration of CH_3COOH, the equilibrium shifts slightly

to the right to increase H^+ ions. This explains the marginal decrease of pH of the buffer solution on addition of HCl.

Mechanism of buffering of an acid buffer

[2] **Addition of NaOH.** When NaOH is added to the buffer solution, the additional OH^- ions

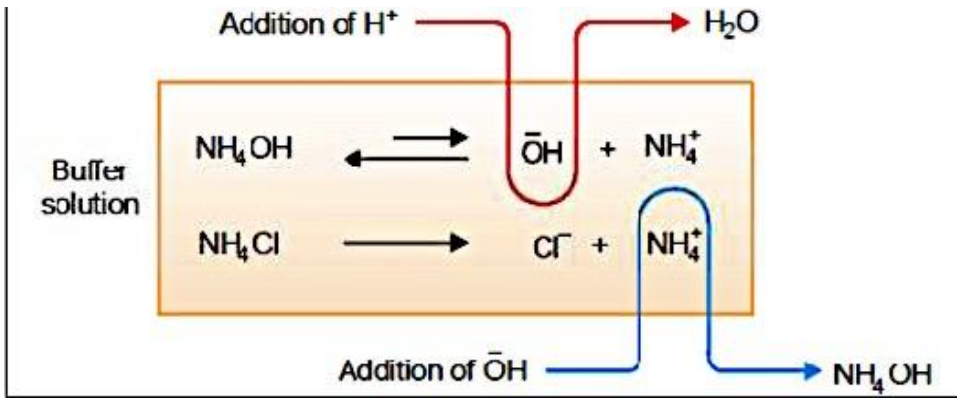
combine with H^+ ions of the buffer to form water molecules. As a result, the equilibrium shifts to the right to produce more and more H^+ ions till practically all the excess OH^- ions are neutralized and the original buffer pH restored. However, a new equilibrium system is set up in which [CH_3COOH] is lower than it was in the original buffer. Consequently [H+] is also slightly less and pH slightly higher than the buffer pH values.



Operation of a Basic buffer as NH_4OH/NH_4Cl can also be explained on the same lines as of an acid buffer upon addition of HCl the H^+ ions combine with OH– ions of the buffer to form water molecules. The equilibrium,

$NH_4OH \rightleftharpoons NH_4^+ + OH^-$

is shifted to the right till all the additional H^+ ions are neutralized and the original buffer pH restored. When NaOH is added to the buffer solution, OH^- ions associate with excess of NH_4^+ ions to form unassociated NH_4OH. Thus, the pH of the buffer is maintained approximately constant.



Mechanism of Buffer action of a Basic buffer

1.6.4 Calculation of the pH of Buffer Solutions

The pH of an acid buffer can be calculated from the dissociation constant, K_a, of the weak acid and the concentrations of the acid and the salt used. The dissociation expression of the weak acid, HA, may be represented as

$HA \rightleftharpoons H^+ + A^-$

$K_a = [H^+][A^-]/[HA]$

$[H^+] = K_a [HA]/[A^-]$

The weak acid is only slightly dissociated and its dissociation is further depressed by the addition of the salt (Na^+A^-), which provides A^-ions (Common ion effect). As a result, the equilibrium concentration of the unionized acid is nearly equal to the initial concentration of the acid. The equilibrium concentration $[A^-]$ is presumed to be equal to the initial concentration of the salt added since it is completely dissociated. Thus, we can write the equation as

$[H^+] = K_a \times [acid]/[salt]$

Where [acid] is the initial concentration of the added acid and [salt] that of the salt used. Taking negative logs of both sides of the above equation, we have

$-\log[H^+] = -\log K_a - \log [acid]/[salt]$

$-\log[H^+] = pH$ and $\log K_a = p K_a$

therefore,

$pH = pK_a - \log [acid]/[salt] = pK_a + \log [salt]/[acid]$

$pH = pK_a + \log [salt]/[acid]$ **$pOH = pK_b + \log [salt]/[base]$**

This relationship is called the Henderson equation. In a similar way, the Henderson equation for a basic buffer (pOH) can also be derived.

1.6.5 Significance of the Henderson equation:

1. The pH of a buffer solution can be calculated from the initial concentrations of the weak acid and the salt provided K_a is given.
2. The Henderson equation for a basic buffer will give pOH and its pH can be calculated as (14 – pOH).

3. The dissociation constant of a weak acid (or weak base) can be determined by measuring the pH of a buffer solution containing equimolar concentrations of the acid (or base) and the salt.

   pH = pK_a + log [salt]/[acid]

   [salt]=[acid] then

   log [salt]/[acid] = log 1= 0

   pK_a = pH

4. The measured pH, therefore, gives the value of **pK_a** of the weak acid. Likewise, we can find the **pK_b** of a weak base by determining the pH of equimolar basic buffer.
5. A buffer solution of desired pH can be prepared by adjusting the concentrations of the salt and the acid added for the buffer. It is noteworthy that buffer solution is most effective when the concentrations of the weak acid (or weak base) and the salt are about equal. This means that pH is close to the value of **pK_a**of the acid (or**pK_b**of the base).

1.6.6 Buffer capacity

It can be seen from the Henderson–Hasselback equation that when the concentration (or more strictly the activity) of the weak acid and base is equal, their ratio is one and their logarithm zero so that pH=$pK_a$the ability of a buffer solution to resist a change in pH on the addition of strong acid or alkali is expressed by its buffer capacity (β). This is defined as the amount (moles) of acid or base required to change the pH by one unit i.e.

β = db/dpH = -da/dpH

Where db and da are the amount of base and acid respectively and dpH is the resulting change in pH. In practice, β is largest within the pH range pKa ±1.

1.6.7 Selection of a buffer

When selecting a buffer for a particular experimental study, several factors should be taken into account:

Select the one with a pK_a as near as possible to the required experimental pH and within the range pK_a±1, as outside this range there will be too little weak acid or weak base present to maintain an effective buffer capacity;

- Select an appropriate concentration of buffer to have adequate buffer capacity for the particular experiment. Buffers are most commonly used in the range 0.05–0.5 M;
- Ensure that the selected buffer does not form insoluble complexes with any anions or cations essential to the reaction being studied (phosphate buffers tend to precipitate polyvalent cations, for example, and may be a metabolite or inhibitor of the reaction);
- Ensure that the proposed buffer has other desirable properties such as being non-toxic, able to penetrate membranes, and does not absorb in the visible or ultraviolet region.

1.6.8 Biological buffers

Biochemical reactions are especially sensitive to pH. Most biological molecules contain groups of atoms that may be charged or neutral depending on pH, and whether these groups are charged or neutral has a significant effect on the biological activity of the molecule. In all multicellular organisms, the fluid within the cell and the fluids surrounding the cells have a characteristic and nearly constant ph. This pH is maintained in a number of ways, and one of the most important is through buffer systems. Two important biological buffer systems are the dihydrogen phosphate system and the carbonic acid system.

1.6.8.1 The phosphate buffer system operates in the internal fluid of all cells. This buffer system consists of dihydrogen phosphate ions ($H_2PO_4^-$) as hydrogen-ion donor (acid) and hydrogen phosphate ions (HPO_4^{2-}) as hydrogen-ion acceptor (base). These two ions are in equilibrium with each other as indicated by the chemical equation below.

$$H_2PO_4^-(aq) \rightleftharpoons H^+(aq) + HPO_4^{2-}(aq)$$

If additional hydrogen ions enter the cellular fluid, they are consumed in the reaction with HPO_4^{2-}, and the equilibrium shifts to the left. If additional hydroxide ions enter the cellular fluid, they react with $H_2PO_4^-$, producing HPO_4^{2-}, and shifting the equilibrium to the right.

Buffer solutions are most effective at maintaining a pH near the value of the pK_a. In mammals, cellular fluid has a pH in the range 6.9 to 7.4, and the phosphate buffer is effective in maintaining this pH range.

1.6.8.2 Blood **plasma**in blood plasma, the carbonic acid and hydrogen carbonate ion equilibrium buffers the pH. In this buffer, carbonic acid (H_2CO_3) is the hydrogen-ion donor (acid) and hydrogen carbonate ion (HCO_3^-) is the hydrogen-ion acceptor (base).

$$H_2CO_3(aq) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

This buffer functions in exactly the same way as the phosphate buffer. Additional H^+ is consumed by HCO_3^- and additional OH^- is consumed by H_2CO_3. The value of K_a for this equilibrium is 7.9×10^{-7}, and the pK_a is 6.1 at body temperature. In blood plasma, the concentration of hydrogen carbonate ion is about twenty times the concentration of carbonic acid. The pH of arterial blood plasma is 7.40. If the pH falls below this normal value, a condition called *acidosis* is produced. If the pH rises above the normal value, the condition is called *alkalosis*.

The concentrations of hydrogen carbonate ions and of carbonic acid are controlled by two independent physiological systems. Carbonic acid concentration is controlled by respiration, that is through the lungs. Carbonic acid is in equilibrium with dissolved carbon dioxide gas.

$$H_2CO_3(aq) \rightleftharpoons CO_2(aq) + H_2O(l)$$

An enzyme called carbonic anhydrase catalyzes the conversion of carbonic acid to dissolved carbon dioxide. In the lungs, excess dissolved carbon dioxide is exhaled as carbon dioxide gas.

$$CO_2(aq) \rightleftharpoons CO_2(g)$$

The concentration of hydrogen carbonate ions is controlled through the kidneys. Excess hydrogen carbonate ions are excreted in the urine.

The much higher concentration of hydrogen carbonate ion over that of carbonic acid in blood plasma allows the buffer to respond effectively to the most common materials that are released into the blood. Normal metabolism releases mainly acidic materials: carboxylic acids such as lactic acid (HLac). These acids react with hydrogen carbonate ion and form carbonic acid.

$$HLac(aq) + HCO_3^-(aq) \rightleftharpoons Lac^-(aq) + H_2CO_3(aq)$$

The carbonic acid is converted through the action of the enzyme carbonic anhydrase into aqueous carbon dioxide.

$$H_2CO_3(aq) \rightleftharpoons CO_2(aq) + H_2O(l)$$

An increase in CO_2 (aq) concentration stimulates increased breathing, and the excess carbon dioxide is released into the air in the lungs.

The condition called *respiratory acidosis* occurs when blood pH falls as a result of decreased respiration. When respiration is restricted, the concentration of dissolved carbon

dioxide in the blood increases, making the blood too acidic. Such a condition can be produced by asthma, pneumonia, emphysema, or inhaling smoke.

The carbonic acid-hydrogen carbonate ion buffer works throughout the body to maintain the pH of blood plasma close to 7.40. The body maintains the buffer by eliminating either the acid (carbonic acid) or the base (hydrogen carbonate ions). Changes in carbonic acid concentration can be affected within seconds through increased or decreased respiration. Changes in hydrogen carbonate ion concentration, however, require hours through the relatively slow elimination through the kidneys

1.6.8.3 Protein buffer (Buffering in Cells and Tissues)

Proteins are mainly composed of amino acids. These amino acids contain functional groups that act as weak acid and bases when there are sharp changes in pH in order to stabilize the pH within the body cells. In short it can be said that proteins act as buffers themselves. Protein is a significant buffer the main buffering site for protein is cells and tissues but even in blood it acts as a buffer consuming hydrogen ion producing due to the dissociation of the carbonic acid into hydrogen bicarbonate. To understand the proteins as a buffer we have to look into the structure of amino acids which consists of

- Carboxyl group (COOH)
- Amino group (NH_2)
- Hydrogen atom
- R group

From the above four groups COOH and NH_2acts as buffer systems for acidic and basic conditions.

At a near neutral pH, like the pH of blood, the carboxyl group is actually COO^- instead of COOH. Then, if a protein finds itself in a more acidic solution, the carboxyl group will be able to take on the extra hydrogen ions and return to the COOH configuration.

At a near neutral pH, like in blood, the amino group is actually NH_3^+ rather than just NH_2. It actually tends to carry an extra hydrogen ion on it at a normal pH. Then, if a protein finds itself in a more basic environment, its amino groups on its amino acids can actually release their hydrogen ions and return to NH_2. As all cells and tissues are composed of proteins mainly so in the absence of protein buffer the sharp changes in pH may cause cell death or tissue damage of a living organisms.

1.7 SUMMARY

- Living cells constantly perform work. They require energy for maintaining their highly organized structures, synthesizing cellular components, generating electric currents, and many other processes.
- Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics.
- Gibbs free energy (G) expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.
- Enthalpy (H) is equivalent to the total heat content of a system whereas Entropy (S) is a quantitative expression for the randomness or disorder in a system.
- Free energy, enthalpy, and entropy are related to each other quantitatively by the equation

  $\Delta G = \Delta H - T\Delta S$
- The standard transformed free-energy change, $\Delta G'^{\circ}$, is a physical constant that is characteristic for a given reaction and can be calculated from the equilibrium constant for the reaction:

  $\Delta G'^{\circ} = - RT \ln K'_{eq}$
- The actual free-energy change, ΔG, is a variable that depends on $\Delta G'^{\circ}$ and on the concentrations of reactants and products:

  $\Delta G = \Delta G'^{\circ} + RT \ln ([products]/[reactants])$

- When ΔG is large and negative, the reaction tends to go in the forward direction; when ΔG is large and positive, the reaction tends to go in the reverse direction; and when $\Delta G = 0$, the system is at equilibrium.
- ATP, the energy currency of the living cell is a chemical link between catabolism and anabolism.
- It is not ATP hydrolysis but the transfer of a phosphoryl, pyrophosphoryl, or adenylyl group from ATP to a substrate or enzyme molecule that couples the energy of ATP breakdown to endergonic transformations of substrates.
- Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine.

These high-energy compounds, like ATP, have a high phosphoryl group transfer potential; they are good donors of the phosphoryl group.

- Biological oxidation-reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, E'°.
- The standard free-energy change for an oxidation-reduction reaction is directly proportional to the difference in standard reduction potentials of the two half-cells: $\Delta G'^{\circ} = -nF\Delta E'^{\circ}$
- The multitude of enzymes that catalyze cellular oxidations channel electrons from their hundreds of different substrates into just a few types of universal electron carriers.
- pH is defined as ***the negative of the base-10 logarithm (log) of the H^+ concentration***.
- A buffer is a solution that resists changes in pH on the addition of the small amounts of acid (H^+) or base (OH^-).
- Buffer consists of a solution of a weak acid (the proton donor) and the corresponding conjugate base (the proton acceptor).
- The pH of a solution of a weak acid (or base) and its salt is given by the Henderson- Hasselbalch equation: **$pH = pK_a + \log [salt]/[acid]$.**
- Dihydrogen phosphate system and the carbonic acid system are the two important biological buffer systems.

1.8 GLOSSARY

Acid- a molecule or other species that can donate a proton or accept an electron pair in reactions.

Acidosis- is caused by an overproduction of acid in the blood or an excessive loss of bicarbonate from the blood (metabolic acidosis), or by a buildup of carbon dioxide in the blood that results from poor lung function or depressed breathing (respiratory acidosis).

Alkalosis- is excessive blood alkalinity caused by an overabundance of bicarbonate in the blood or a loss of acid from the blood (metabolic alkalosis), or by a low level of carbon dioxide in the blood that results from rapid or deep breathing (respiratory alkalosis).

Anabolism– is the synthesis of complex molecules in living organisms from simpler ones together with the storage of energy; constructive metabolism.

ATP- Adenosine triphosphate (ATP) is a nucleotide also called as nucleoside triphosphate often referred to as the "molecular unit of currency" of intracellular energy transfer.

Base-accepts protons from any proton donor, and/or contains completely or partially displaceable OH^- ions.

Bioenergetics- is a field in biochemistry and cell biology that involves the quantitative analysis of the energy transductions occurring in living system. It also studies the nature and function of the chemical processes underlying these transductions.

Buffers- A buffer solution is one, which maintains its pH fairly constant even upon the addition of small amounts of acid or base

Catabolism-the breakdown of complex molecules in living organisms to form simpler ones, together with the release of energy; destructive metabolism.

Common ion effect- The common ion effect is responsible for the reduction in the solubility of an ionic precipitate when a soluble compound containing one of the ions of the precipitate is added to the solution in equilibrium with the precipitate.

Enthalpy-a thermodynamic quantity equivalent to the total heat content of a system. It is equal to the internal energy of the system plus the product of pressure and volume.

Entropy- a thermodynamic quantity often interpreted as the degree of disorder or randomness in the system.

Endergonic reaction-In chemical thermodynamics, an **endergonic reaction** (also called a nonspontaneous **reaction** or an unfavorable **reaction**) is a chemical **reaction** in which the standard change in free energy is positive, and energy is absorbed

Exergonic reaction-An exergonic reaction refers to a reaction where energy is released. Because the reactants lose energy (G decreases), Gibbs free energy (ΔG) is negative under constant temperature and pressure. These reactions usually do not require energy to proceed, and therefore occur spontaneously.

Free energy –is the energy in a physical system that can be converted to do work.

High-energy compounds- A small family of universal biomolecules mediates the flow of energy from exergonic reactions to the energy requiring processes of life. These molecules are the *reduced* coenzymes and the high-energy phosphate compounds

K_a-An acid dissociation constant, K_a, (also known as acidity constant, or acid-ionization constant) is a quantitative measure of the strength of an acid in solution. It is the equilibrium constant for a chemical reaction known as dissociation in the context of acid–base reactions.

Oxidation-It is the loss of electrons or an increase in oxidation state by a molecule, atom, or ion.

pH- is defined as the negative of the base-10 logarithm (log) of the H+ concentration.

Reduction- is the gain of electrons or a decrease in oxidation state by a molecule, atom, or ion.

Thermodynamics-Thermodynamics is a branch of science concerned with heat and temperature and their relation to energy and work.

1.9 SELF ASSESSMENT QUESTIONS

1.9.1 Long answer type questions: -

Q 1. What do you understand by bioenergetics? Describe the laws of thermodynamics and their biological applicability.

Q 2. Discuss the reasons behind the high free energy of hydrolysis of ATP.

Q 3. What do you understand by biological oxidation-reduction reaction? Describe it in detail. How can we calculate the Free-Energy Change using Standard Reduction Potentials?

Q 4. What are buffers? Describe their mechanism of action using a suitable example.

1.9.2 Short answer type questions: -

Q 1. Write a short note on High-energy compounds

Q 2. Define enthalpy, entropy and Gibss free energy.

Q 3. Discuss the role of biological buffers.

Q 4. Write a short note on pH and application of Henderson Hasselbalch equation

Q 5. Write a short note on biological oxidation-reduction reaction

1.9.3 Fill in the blanks.

Q 1. NAD and NADP are the freely diffusible coenzymes of many _____.

Q 2. _____is the degradative phase of metabolism in which organic nutrient molecules are converted into smaller, simpler end products.

Q 3. A proton donor and its corresponding proton acceptor make up a _____acid-base pair.

Q 4. _____is the loss of electrons or an increase in oxidation state by a molecule, atom, or ion.

Q 5. An _____ refers to a reaction where energy is released. Because the reactants lose energy (G decreases), Gibbs free energy (ΔG) is negative under constant temperature and pressure.

Q 6.A buffer is a solution that resists changes in ____when small amounts of acid (H^+) or base (OH^-) are added to it.

Q 7. _____is the quantitative study of energy relationships and energy conversions in biological systems.

Q 8. _____catalyzes the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor.

Q 9. Living cells and organisms are _____.

REFERENCES AND SUGGESTED READINGS

- Lehninger Principles of Biochemistry (5th Edition, W. H. Freeman) by David L. Nelson (Author), Michael M. Cox (Author)
- Biochemistry (5th edition, Wadsworth Publishing Co Inc) by Reginald H Garrett (Author), Charles M Grisham (Author)
- Harper's Biochemistry – Murray, Granner, Mayes, Rodwell – Prentice Hall International Inc.

ANSWERS

1.9.3 Fill in the blanks

1. Dehydrogenases, 2. Catabolism, 3. Conjugate, 4. Oxidation, 5. Exergonic reaction, 6. pH, 7. Bioenergetics, 8. Oxidases, 9. Open systems

UNIT 2: ENZYMES

Contents

2.1 OBJECTIVES

After reading this unit you will be able to understand-

- What are enzymes.
- Basic concepts, classification and their properties.
- Details of their mechanism of action and factors affecting enzyme activity
- Coenzymes and their significance

2.2 INTRODUCTION

A myriad of chemical reactions proceeds very rapidly in all living cells. The large majority of these biochemical reactions do not take place spontaneously. Most of the chemical reactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. Catalysis is defined as the acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change. The phenomenon of catalysis makes possible biochemical reactions necessary for all life processes. The catalysts of biochemical reactions are enzymes. Enzymes act sequentially to catalyze individual steps in metabolic pathways. The existence of enzymes has been known for well over a century. French chemist Anselme Payen was the first to discover an enzyme, diastase, in 1833. A few decades later Louis Pasteur recognized in 1860 that enzymes were essential to fermentation but assumed that their catalytic action was intricately linked with the structure and life of the yeast cell. In 1877, German physiologist Wilhelm Kühne (1837–1900) first used the term *enzyme*, which comes from Greek word "leavened" (Leavening makes bread rise), to describe this process. Not until 1897 was it shown by German chemist Edward Buchner that cell-free extracts of yeast could ferment sugars to alcohol and carbon dioxide; Buchner denoted his preparation *zymase.* This important achievement was the first indication that enzymes could function independently of the cell.

The first enzyme molecule to be isolated in pure crystalline form was urease, prepared from the jack bean in 1926 by American biochemist J. B. Sumner, who suggested, contrary to prevailing opinion, that the molecule was a protein. In the period from 1930 to 1936, pepsin, chymotrypsin, and trypsin were successfully crystallized; it was confirmed that the crystals were protein, and the protein nature of enzymes was thereby firmly established.

2.2.1 DEFINITION

Enzymes are macromolecularbiologicalorganic catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis and proceeds without itself being altered in the process.

2.2.2 BASIC PROPERTIES

1. Enzymes differ from ordinary chemical catalysts by:
   - Higher reaction rates, 10^6-10^{12}
   - Milder reaction conditions (temp, pH)
   - Greater reaction specificity (no side products) - capacity for regulation
2. Enzyme reactions are always reversible.
3. Being highly efficient, enzymes are also extremely selective catalysts.
4. Enzymes speed up the rates at which the equilibrium positions of reversible reactions are attained.
5. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily.
6. The reactants at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products.
7. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life.
8. The set of enzymes made in a cell determines which metabolic pathways occur in that cell.
9. The study of enzymes is called *enzymology*.
10. Enzymes are known to catalyze more than 5,000 biochemical reaction types.
11. Most enzymes are proteins, although a few are catalytic RNA molecules.
12. Enzyme's specificity comes from their unique three-dimensional structures, each enzyme catalyzes the reaction of a single type of molecules or a group of closely related molecules.

2.2.3 CHEMICAL NATURE AND STRUCTURE

All enzymes were once thought to be proteins, but since the 1980s the catalytic ability of certain nucleic acids, called ribozymes (or catalytic RNAs), has also been demonstrated.

Enzyme specificity comes from unique three dimentional structure. As you know enzymes

are proteins, the amino acid sequence determines the characteristic folding patterns of the protein's structure, which is essential to enzyme specificity. If the enzyme is subjected to changes, such as variations in temperature or pH, the protein structure may lose its integrity (denature) and its enzymatic ability. Denaturation is sometimes, but not always, reversible. The key to enzyme activity lies in its active site. The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes.

Moreover, unrelated to its active site, there is an allosteric site on an enzyme, which can bind an effector molecule. This interaction is another mechanism of enzyme regulation. Allosteric modification usually happens in proteins with more than one subunit. Allosteric interactions are often present in metabolic pathways and are beneficial in that they allow one step of a reaction to regulate another step. They allow an enzyme to have a range of molecular interactions, other than the highly specific active site.

An additional chemical non-protein component called a cofactor, is a direct participant in the catalytic event binds to enzyme and makes it functionally active. A cofactor may be either a coenzyme—an organic molecule (which is dialyzable, thermostable and loosely attached to the protein part), such as a vitamin—or an inorganic metal ion; some enzymes require both. A cofactor may be either tightly or loosely bound to the enzyme. If tightly connected, the cofactor is referred to as a prosthetic group (- an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.

This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion- activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

ZYMOGEN: A zymogen is an enzymatically inactive precursor of an enzyme, but not always a proteolytic enzyme (or proteinase). Some zymogens are named by adding the suffix -ogen to the name of the enzyme itself, as in trypsinogen or pepsinogen, whereas others are indicated by the prefix pro-, as in pro-collagenase or pro-carboxypeptidase.

2.3 NOMENCLATURE AND CLASSIFICATION

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase" to the name of their substrate. Sometimes the same enzyme has two or more names, or two different enzymes have the same name. Such ambiguities, and the ever- increasing number of newly discovered enzymes led biochemists, by international agreement, to adopt a system nomenclature and classification of enzymes. This system divides enzymes into six classes, each with sub- classes, based on the type of reaction catalyzed. Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes. The first Enzyme Commission, in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. These code numbers, precede by EC (enzyme commission), which are now widely in use, contain four elements separated by points, with the following meaning:

(i) The first number shows to which of the six main divisions (classes) the enzyme belongs,

(ii) The second figure indicates the subclass,

(iii) The third figure gives the sub-subclass,

(iv) The fourth figure is the serial number of the enzyme in its sub-subclass.

The main divisions are:

1. OXIDOREDUCTASES

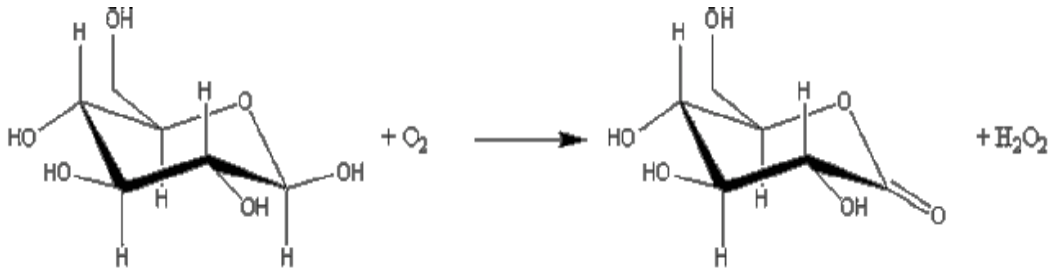
Oxidoreductases are a class of enzymes that catalyze oxidation-reduction reactions. Oxidoreductases catalyze the transfer of electrons from one molecule (the oxidant) to another molecule (the reductant). Oxidoreductases catalyze reactions similar to the reaction, $A^- + B \rightarrow A + B^-$ where A is the oxidant and B is the reductant. Trivial names of Oxidoreductases include oxidases and dehydrogenases. Oxidases are enzymes involved when molecular oxygen acts as an acceptor of hydrogen or electrons. Whereas, dehydrogenases are enzymes that oxidize a substrate by transferring hydrogen to an acceptor that is either $NAD^+/NADP^+$ or a flavin enzyme. Other oxidoreductases include peroxidases, hydroxylases, oxygenases, and reductases. Oxidoreductases are classified as

EC 1 in the EC number classification of enzymes. Oxidoreductases can be further classified into 22 subclasses

An example, would be:

$$A^- + B \rightarrow A + B^-$$

where A = reductant (electron donor) and B = oxidant (electron acceptor).



β-D-glucose + oxygen ⟶ D-glucono-1,5-lactone + hydrogen peroxide

Peroxidases are localized in peroxisomes, and catalyzes the reduction of hydrogen peroxide. Hydroxylases add hydroxyl groups to its substrates. Oxygenase incorporate oxygen from molecular oxygen into organic substrates. Reductases catalyze reductions, in most cases, reductases act like oxidases.

Oxidoreductase enzymes play an important role in both aerobic and anaerobic metabolism. They can be found in glycolysis, TCA cycle, oxidative phosphorylation, and in amino acid metabolism.

2. TRANSFERASES

Transferases are the class of the enzymes that catalyze transfer of specific functional groups (e.g. a methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor). Transaminases, for example, catalyze the transfer of an amino group (NH2) from an amino acid to a keto acid. They are involved in hundreds of different biochemical pathways, and are integral to some of life's most important processes.

Transferases include acetyltransferase, methylase, protein kinase and polymerase enzymes. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.

Transferases are involved in innumerable reactions in the cell. For example, the activity of coenzyme A (CoA) transferase, which transfers thiol esters, the action of N-acetyltransferase is part of the pathway that metabolizes tryptophan, and also includes the regulation of pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA. Transferases are also utilized during translation. In this case, an amino acid chain is the functional group transferred by a peptidyl transferase. The transfer involves the removal of the growing amino acid chain from the tRNA molecule in the A-site of the ribosome and its subsequent addition to the amino acid attached to the tRNA in the P-site.

Based on the type of biochemical group transferred, transferases can be divided into ten categories (based on the EC Number classification). In the EC numbering system, transferases have been given a classification of EC 2.

Systematically, such reaction would be:

where, X= donor, and Y = acceptor. "Group" would be the functional group transferred as a result of transferase activity. The donor is often a coenzyme.

EXAMPLE: Carbamoyl phosphate + L-aspartate→ L-carbamyl aspartate + phosphate

3. HYDROLASES

Hydrolases are hydrolytic enzymes that catalyze the hydrolysis of a chemical bond, usually dividing a large molecule into two smaller molecules. Examples of common hydrolases include esterase's, proteases, glycosidases, nucleosidases, and lipases.

Hydrolases carry out important degradative reactions in the body. During digestion, lipases hydrolyze lipids and proteases convert protein to amino acids. Hydrolases cleave large molecules into fragments used for synthesis, the excretion of waste materials, or as sources of carbon for the production of energy. In these reactions, many biopolymers are converted to monomers. Hydrolytic reactions are accompanied with the release of energy. Hydrolases are classified as EC 3 in the EC number classification of enzymes. Hydrolases can be further classified into several subclasses, based upon the bonds they act.

Systematically, such type of reaction would be:

$A–B + H_2O \rightarrow A–OH + B–H$

EXAMPLE: Nucleases split nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may further be sub-divided into exonuclease and endonuclease. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

4. LYASES

Lyaseenzyme catalyzes the breaking (an "elimination" reaction) of various chemical bonds (C-C, C-O, C-N) by means other than hydrolysis (a "substitution" reaction) and oxidation. These bonds are cleaved by the process of elimination and the resulting product is left with a double bond or a new ring. The reverse reaction is also possible (called a "Michael addition"). Lyases differ from other enzymes in that they require only one substrate for the reaction in one direction, but two substrates for the reverse reaction. This class of enzymes differs from other enzymes in that two substrates are involved in one reaction direction, but only one substrate is involved in the other direction. Lyases are classified as EC 4 in the EC number classification of enzymes.

5. ISOMERASES

Isomerases are a class of enzymes which convert a molecule from one isomer to another, meaning that the end product has the same molecular formula but a different physical structure. Isomers themselves exist in many varieties but can generally be classified as structural isomers or stereoisomers. They can either facilitate it by intramolecular rearrangements in which bonds are broken and formed or they can catalyze conformational changes. The general form of such a reaction is as follows:

$A–B \rightarrow B–A$

Where A and B = Isomers.

There is only one substrate yielding one product. This product has the same molecular formula as the substrate but differs in bond connectivity or spatial arrangements. Isomerases catalyze reactions across many biochemical pathways, such as in glycolysis and carbohydrate metabolism. All isomerases have Enzyme Commission numbers

beginning in EC 5. A variety of isomerization's can be carried out, including racemization, cis-trans isomerization, enolization, and many others. Examples of isomerases include triose phosphate isomerase, and diphosphoglycerate mutase.

Isomerases can help to prepare a molecule for subsequent reactions such as oxidation-reduction reactions. Additionally, isomerases can catalyze phosphorylation reaction pathways throughout the Kreb Cycle by preparing the molecule for oxidation states. The change in position is facilitated through isomerases without affecting the overall chemical composition of the substrate or product.

Example: In the phosphoglucose isomerase reaction, glucose-6-phosphate (an aldehyde sugar) and fructose-6-phosphate (a ketone sugar) are interconverted.

glucose-6-phosphate fructose-6-phosphate

6. LIGASES

Ligases also called synthetases these enzymes catalyze reactions which make bonds to join together (ligate) smaller molecules to make larger ones. Ligase enzymes tend to raise the energy of a system, but the hydrolysis of ATP is often coupled with these reactions to make the reaction spontaneous. All enzymes tend to have the same basic catalytic effect in that they lower the overall activation energy often by moving the two substituents into close proximity. In general, a ligase catalyzes the following reaction:

$Ab + C \rightarrow A–C + b$

or sometimes

$Ab + cD \rightarrow A–D + b + c + d + e + f$

where the lowercase letters denote the small, dependent groups. Ligases are classified as EC 6 in the EC number classification of enzymes. Ligase can join two complementary fragments of nucleic acid and repair single stranded breaks that arise in double stranded DNA during replication.

Example: Tyrosine-tRNA ligase is an enzyme that catalyzes the following reaction. The substrates for this enzyme are ATP, L-tyrosine, and RNA, whereas its products are AMP, diphosphate, and L-tyrosyl-tRNA(Tyr).

Tyrosine t-RNA Synthetase

ATP+ L-Tyrosine+ t-RNA ⟹ L-tyrosyl-tRNA + AMP + PPi

2.3.1 SPECIFICITY OF ENZYME ACTION:

A characteristic feature of enzymes that makes them important as diagnostic and research tools is the specificity, they exhibit relation to the reactions they catalyze. Some enzymes are specific for a particular type of chemical bond or functional group whereas few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. In general, there are four distinct types of specificity:

1. Absolute, High or substrate specificity – Such enzymes catalyze only one particular reaction.

. EXAMPLE:

a) Uricase, which acts only on uric acid.
b) Arginase, which acts only on arginine.
c) Carbonic anhydrase, which acts only on carbonic acid.
d) Lactase, which acts on lactose.

. **2. Structural or Group specificity** - these enzymes act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

. EXAMPLE:

a) Trypsin is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine, lysine and histidine.
b) Chymotrypsin is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.
c) Aminopeptidase is an exopeptidase that hydrolyzes peripheral peptide bond at the amino terminal (end) of polypeptide chain.

. **3. Linkage specificity**– these enzyme acts on a particular type of chemical bond regardless of the rest of the molecular structure.

. EXAMPLE:

a) Amylase, which acts on α 1-4 glycosidic, bonds in starch, dextrin and glycogen.
b) Lipase that hydrolyzes ester bonds in different triglycerides

. **4. Stereochemical specificity** - these enzymes act on a particular steric or optical isomer.

. EXAMPLE

a) L amino acid oxidase acts only on L amino acids.
b) D amino acid oxidase acts only on D amino acids.
c) α- glycosidase acts only on α- glycosidic bonds, which are present in starch, dextrin and glycogen.

2.4 MECHANISM OF ENZYME ACTION

2.4.1 ENZYME CATALYSIS

The mechanism of enzyme catalysis is similar in principle to other types of chemical catalysis. By providing an alternative reaction route the enzyme reduces the energy required to reach the highest energy transition state of the reaction. The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a sufficient level of energy, such that they reach the activation energy and form the product. As with other catalysts, the enzyme is not consumed during the reaction (as a substrate is) but is recycled such that a single enzyme performs many rounds of catalysis.

An enzyme provides a specific environment within which a given reaction can occur more rapidly. An enzyme-catalyzed reaction takes place within the restricted pocket on the enzyme called the active site. The substrate molecule that binds in the active site acted by the enzyme is called the substrate. The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation. The enzyme-substrate complex, whose existence was first proposed by Charles-Adolphe Wurtz in 1880, is fundamental to the action of enzymes.

2.4.2 MECHANISM OF CATALYSIS

A simple enzymatic reaction is as follows

E+ S ⇔ES ⇔EP ⇔E P

where E=enzyme, S= substrate and P = product ES and EP are transient complexes of the enzyme with the substrate and with the product.

The function of a catalyst is to increase the rate of a reaction. Catalysts do not affect reaction equilibrium. The bidirectional arrows put in the equation on page make the point clear: any enzyme that catalyzes the reaction, S → P also catalyzes the reverse reaction; P → S. The

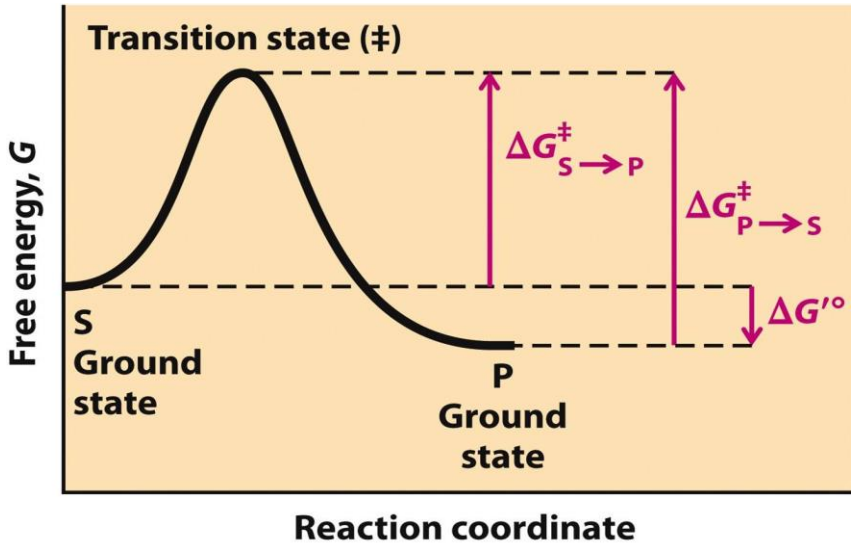
role of enzyme is to accelerate the interconversion of S and P. It is not consumed in the process, and the equilibrium point remains unaffected. However, the reaction reaches equilibrium much faster in the presence of an enzyme because the rate of the reaction is increased.

Energy in biological systems is described in terms of free energy, G. In the **Fig no. 1-A**, Enzyme has no overall thermodynamic effect. The starting point for both the reaction (forward or reverse) is called the ground state. The free energy change for under standard set of conditions (temperature, 298 K; partial pressure of gases, each 1 atm or 101.3 kPa; pH = 0; concentration of solutes, each 1M) is known as standard free-energy change, $\Delta G°$.

The equilibrium between S and P reflects the difference in the free energies of their ground states. the free energy of the ground state of P is lower than that of S, so G for the reaction is negative and the equilibrium favors P. The position and direction of equilibrium are not affected by any catalyst.

A favorable equilibrium does not mean that the S → P conversion will occur at a noticeable rate. There is an energy barrier between S and P: the energy required for arrangement of reacting groups, formation of transient unstable charges, bond rearrangements, and other alterations required for the reaction to proceed in both directions. To undergo reaction, the molecules must overcome this “energetic hill” or barrier and therefore must be raised to a higher energy level. At the top of the energy hill is a point at which deterioration to the S or P state is equally possible. This is called the transition state. The term should not be confused with a reaction intermediate (such as ES or EP). It is basically a transient molecular moment in which events such as bond breakage, bond formation, and charge development proceed to the point at which decay to either substrate or product is equally possible. The difference between the energy levels of the ground state and the transition state is the activation energy, G‡. The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction. Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst **(Fig. no. 1-B).** Catalysts enhance reaction rates by lowering activation energies.

(A)



(B)

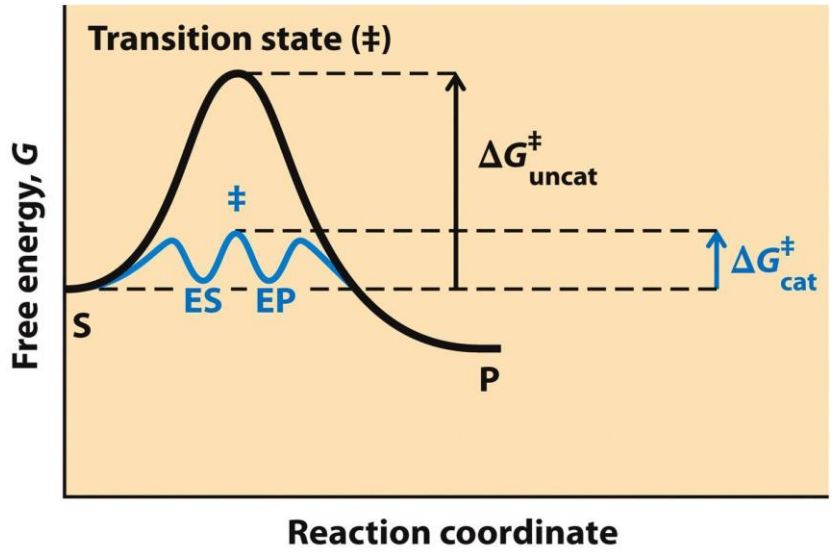


Figure No. 1 (A) Reaction coordinate diagram for a chemical reaction. (B) Reaction coordinate diagram comparing enzyme- catalyzed and uncatalyzed reactions.

LOCK AND KEY MODEL

In order to explain why enzymes, have such a high level of specificity, Emil Fischer in 1894 suggested that both substrate and enzyme have specific complementary shapes that fit exactly into each other **(Fig No. 2).** This enzyme-substrate complex is highly unstable and breaks down to release end products of the reaction as well as regenerates the free enzyme. The enzyme-substrate union results in the release of energy. It is this energy, which in fact,

raises the energy level of the substrate molecule, thus inducing the *activated state*, in which certain bonds of the substrate molecule become more susceptible to cleavage.

This idea of both substrates and enzymes having a natural structural fit has been called the lock and key hypothesis. However, this hypothesis fails to explain the stabilization of the enzyme. When an enzyme has a substrate enter into its active site, the enzyme will change its shape slightly to match the substrate. If the enzymes were to be specifically designed to fit a substrate, then there would be no need for it to adjust its shape.

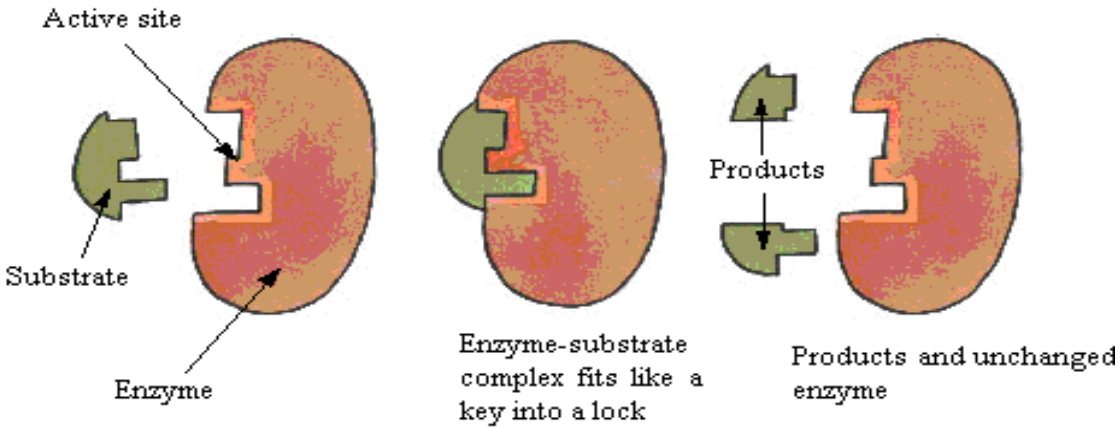


Figure No. 2. Lock and Key Model

INDUCED FIT MODEL

In 1958, another scientist Daniel Koshland suggested a slight modification to the lock and key hypothesis to explain the enzyme properties more efficiently.

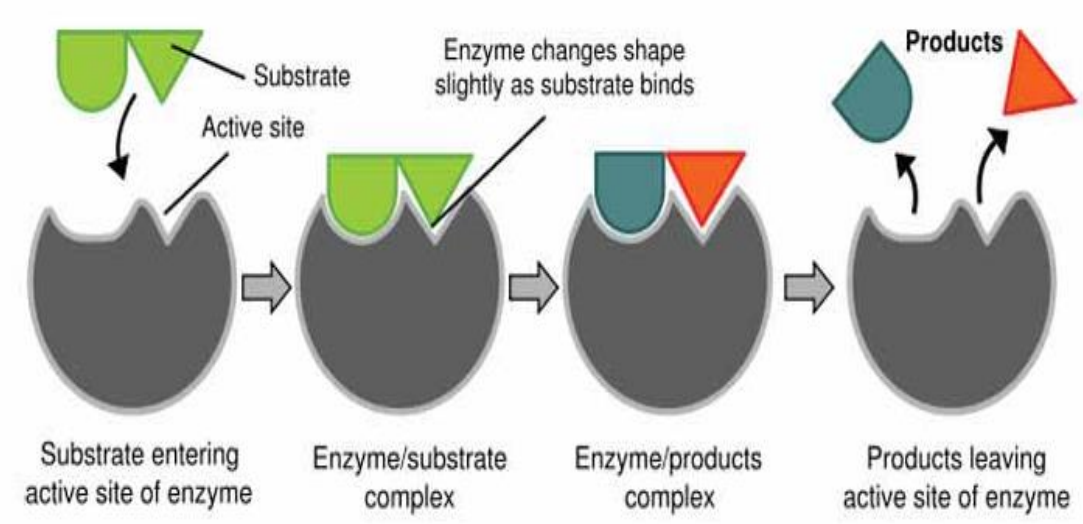


Figure No. 3. Induced fit Model

Koshland in his induced fit hypothesis suggested that substrate structure may be complementary to the active site of enzyme but its binding to enzyme brings change in conformation in the enzyme with required matching of structures. Such changes bring catalytic site and reacting groups together. This type of mechanism assists in bringing specificity to the enzyme-substrate complex. Koshland's modified suggestions have been called the induced fit theory.

2.4.3 TYPES OF CATALYTIC MECHANISMS

Enzyme catalyzed reactions are typically 10^7 to 10^{14} times faster than the uncatalyzed reaction. After binding takes place, one or more mechanisms of catalysis lowers the energy of the reaction's transition state, by providing an alternative chemical pathway for the reaction. There are seven possible mechanisms of "over the barrier" catalysis as well as a "through the barrier" mechanism:

1. **Acid-base catalysis**
2. **Covalent catalysis**
3. **Metal ion catalysis**
4. **Proximity and orientation effects**
5. **Electrostatic catalysis**
6. **Bond strain**
7. **Quantum tunneling**

These mechanisms are not mutually exclusive, and a given enzyme might incorporate several types in its overall mechanism of action. For most enzymes, it is challenging to compute the role of any one catalytic mechanism to the rate or specificity of a particular enzyme-catalyzed reaction.

1. Acid-Base Catalysis

The mechanism of acid- and base-catalyzed reactions is explained in terms of the Brønsted–Lowry concept of acids and bases as one in which there is an initial transfer of protons from an acidic catalyst to the reactant or from the reactant to a basic catalyst. In terms of the Lewis's theory of acids and bases, the reaction involves sharing of an electron pair donated by a base catalyst or accepted by an acid catalyst.

There are two types of acid-base catalysis:

- General acid-base catalysis

- Specific acid-base catalysis

The term "general" refers to the fact that any acid or base we add to the solution will affect the rate of the reaction, and hence the catalysis is quite general. The term" specific" refers to the fact that just one acid or base, that from the solvent, affects the rate. The catalysis is therefore very specific.

2. Covalent Catalysis

It involves formation of a temporary covalent bond between the enzyme (residues in the enzyme's active site or with a cofactor usually a nucleophile) and the substrate. This adds an additional covalent intermediate to the reaction; more reactive than the substrate itself originally was and helps to reduce the energy of later transition states of the reaction. The covalent bond must, at a later stage in the reaction, be broken to regenerate the enzyme. This mechanism is utilized by the enzymes such as proteases like chymotrypsin and trypsin.

Assume the hydrolysis of a bond between groups A and B:

A-----B H_20 → A + B

In the presence of a covalent catalyst (an enzyme with a nucleophilic group Z:) the reaction becomes

A-----B+ Z: → A-----Z: + B H_20 → A + Z: + B

This alters the pathway of the reaction, and it results in catalysis only when the alternative pathway has a lower activation energy than the uncatalyzed pathway. The covalent bond formed between the enzyme and the substrate can activate a substrate for further reaction in a manner that is usually specific to the particular group or coenzyme.

3. Metal Ion Catalysis

Enzymes that bind metal ions tightly are referred to as metalloenzymes. Enzymes that bind metal ions weeklies, perhaps only during the catalytic cycle, are said to be metal activated. One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts stabilizing the increased electron density that can develop during reactions.

The metal ion acts as a bridge between the substrate and the enzyme increasing the binding energy. Alternatively, the metal ion may bridge the substrate to a nucleophilic group. The metal ion may stabilize negative charges on a leaving group to make it a better leaving group, or shield negative charges on the molecule to allow for nucleophilic attack which

otherwise may be repelled. It may also participate in oxidation-reduction reactions by changing their oxidation state. An example is liver alcohol dehydrogenase where a zinc ion stabilizes negative charge development on the oxygen atom of acetaldehyde. Another potential function of metal ions is to provide a powerful nucleophile at neutral pH.

4. Proximity and orientation effects

Enzyme catalytic efficiency arises from the specific physical conditions at enzyme catalytic sites. Enzymes bring reacting species close together; It can accelerate a reaction between two species simply by holding the two reactants close together in an appropriate orientation. Enzymes, which have specific binding sites for particular reacting molecules, essentially take the reactants out of dilute solution and hold them close to each other. This proximity of reactants is said to raise the *effective* concentration over that of substrates in solution, and leads to an increased reaction rate. Enzymes not only bring substrates and catalytic groups together, they orient (specific geometric alignment) them in a manner suitable for catalysis as well. Clearly, proximity and orientation play a role in enzyme catalysis, but there is a problem with each for comparisons since we cannot separate true proximity and orientation effects from the effects of entropy loss when molecules are brought together.

By simply binding their substrates, enzymes facilitate their catalyzed reactions in three ways (+ electrostatic catalysis):

1. Enzymes bring substrates in contact with their catalytic groups and, in reactions with more than one substrate, with each other.

2. Enzymes bind their substrates in the proper orientations for reaction. Molecules are not equally reactive in all directions. Rather, they react most readily if they have the proper relative orientation.
3. Enzymes freeze out the relative translational and rotational motions of their substrates and catalytic groups.

In short, this mechanism increases the rate of the reaction as enzyme- substrate interactions by aligning reactive chemical groups and holding them close together. This reduces the entropy of the reactants and thus makes reactions such as ligations or addition reactions more favorable, there is a reduction in the overall loss of entropy when two reactants become a single product.

5 .Electrostatic catalysis

An electrostatic effect gives the largest contribution to catalysis. The enzyme provides an environment that is more polar than water, and the ionic transition states are stabilized by fixed dipoles. This is very different from transition state stabilization in water, where the water molecules must pay with "reorganization energy", in order to stabilize ionic and charged states. Thus, catalysis is associated with the fact that the enzyme polar groups are preorganized.

The magnitude of the electrostatic field exerted by an enzyme's active site is highly correlated with the enzyme's catalytic rate enhancement in catalytic rate of enzyme.

Binding of substrate usually excludes water from the active site, thereby lowering the local dielectric constant to that of an organic solvent where electrostatic interactions are much stronger than they are in aqueous solutions. This strengthens the electrostatic interactions between the charged/polar substrates and the active sites. Furthermore, the charge distributions about the active sites are arranged so as to stabilize the transition states of the catalyzed reactions. In several enzymes, these charge distributions serve to guide polar substrates toward their binding sites so that the rates of these enzymatic reactions are greater than their apparent diffusion-controlled limits.

6.Bond strain

This is the principal effect of induced fit binding. The binding induces structural rearrangements by which strained substrate bonds into a position closer to the conformation of the transition state, thereby lowering the energy difference between the substrate and transition state and helps to catalyze the reaction. During binding process, if the active site of enzyme possesses a loose structure and block out around the substrate, as the substrate is in undistorted form, enzyme-substrate complex interactions distort the substrate (precise conformational changes takes places) and the strain assist subsequent reaction by transition state stabilization.

However, the strain effect is, in fact, a ground state destabilization effect, rather than transition state stabilization effect. Furthermore, enzymes are very flexible and they cannot apply large strain effect. In addition to bond strain in the substrate, bond strain may also be induced within the enzyme itself to activate residues in the active site.

2.5 FACTORS AFFECTING ENZYME ACTIVITY

The enzyme activity is affected by several environmental conditions, enzymes which are adapted to function well in extreme conditions where they live. Several factors affect the rate at which enzymatic reactions - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

1.Temperature

Molecules will react only if they come in contact with each other. An increase in temperature increases the kinetic energy that molecules possess, leading to more random collisions between molecules per unit time. So, rate of reaction also increases.

However, increase in the temperature also increases the vibrational energy of molecules, which puts strain on the bonds that hold them together. As temperature increases, more bonds, especially the weaker hydrogen and ionic bonds. Breaking of bonds within the enzyme will cause the active site to change in the shape.

Since enzymes are proteins, rise in temperature will cause their denaturation and they will lose their characteristic shape. This will lead to decrease in the rate of reaction.

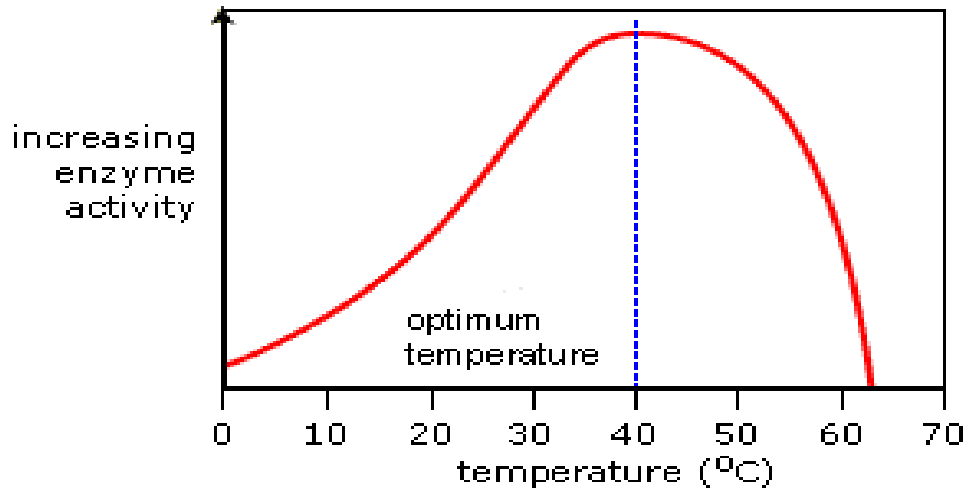


Figure No. 4 Effect of Temperature on enzyme catalyzed reaction

As shown in Figure 4, initially the rate of reaction will increase; however, the effect of bond breaking will become apparent and henceforth rate of reaction raising the temperature

further increases heat energy of the enzyme more than the energy barrier. This will lead to disruption of non-covalent interactions and therefore enzymes will lose their structural shape with loss of catalytic activity.

Generally, enzymes exhibit stable catalytic conformation within the normal temperature of cell in which it resides. Enzymes in the human body are stable up to temperatures 45-55°C. The rate of enzyme catalyzed reactions double for a 10°C rise in temperature. *Most enzymes in the human body have an Optimum Temperature of around 37.0 °C*

2.pH - Acidity and Basicity

Each enzyme has its own range of pH in which it will work. H^+ and OH^- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges. This interference causes a change in shape of the enzyme significantly at the active site

As shown in Figure 5, majority of the enzymes within cell exhibit optimal activity at pH values 5-9. Enzymes get denatured at extreme pH especially in acid-base catalysis of enzymes. The residues at a particular pH must be in protonated form for the reaction to proceed further. The carboxylate anions and protonated amines are commonly found charged groups. Any change will disrupt the substrate binding and loss of catalytic activity.

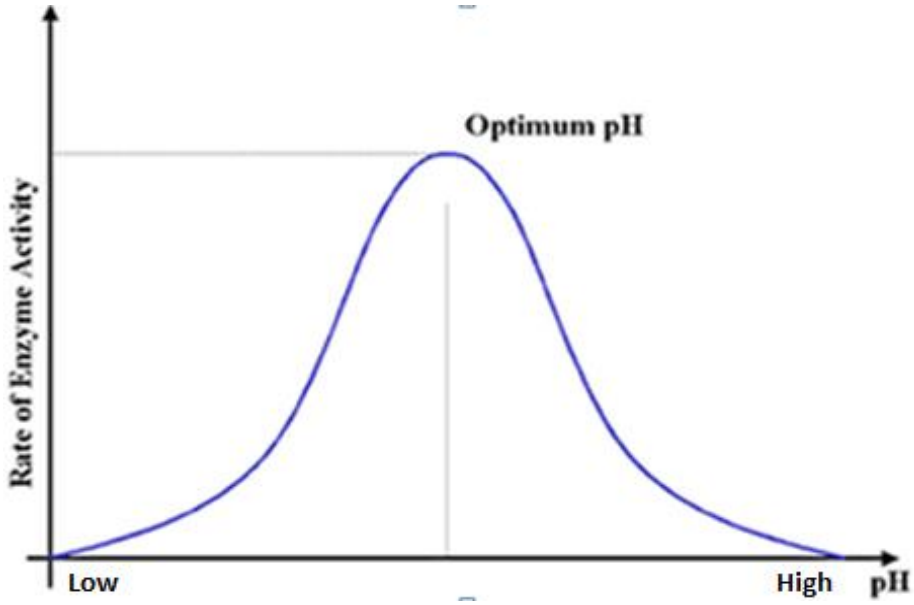


Figure No. 5 Effect of pH on enzyme catalyzed reaction

3. Concentration

A. Substrate Concentration

As shown in Figure 6, if we keep the concentration of the enzyme constant and increase the concentration of the substrate, it leads to increase in the rate of reaction. However, after certain

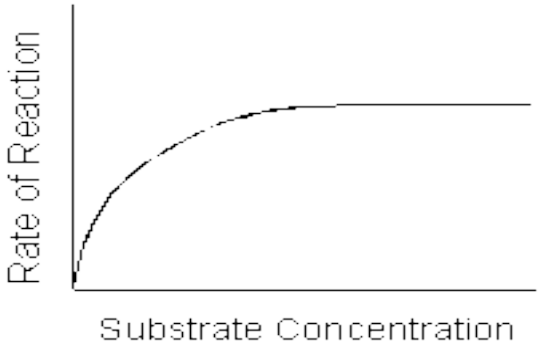


Figure No. 6 Effect of varying Substrate concentration on enzyme catalyzed reaction

concentration, any increase will have no effect on the rate of reaction. The enzymes will effectively become saturated.

B. Enzyme Concentration

If we keep the concentration of the substrate constant and increase the concentration of the enzyme, the rate of reaction increases linearly as more enzymes will be colliding with substratemolecules. Moreover, this is because rationally in all enzyme reactions the molar concentration of the enzyme is almost always lower than that of the substrate.

However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

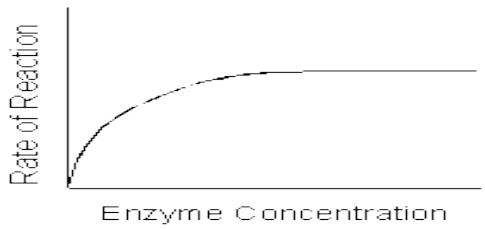


Figure No. 7 Effect of varying enzyme concentration on enzyme catalyzed reaction

4. Cofactors

Several enzymes require cofactors to function properly. There are three main types of cofactors; co-enzymes, inorganic ions and prosthetic groups.

1. Coenzymes are organic molecules, which often contain a vitamin molecule as part of their structure. Coenzymes are loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g., NAD^+ may react with several enzymes in different types of reaction. NAD^+ transfers hydrogen in reactions involving dehydrogenase enzymes.
2. Inorganic metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g., Fe^{2+} in catalase. Others accelerate the binding between the enzyme and the substrate, e.g., Mg^{2+} in phosphotransferases.
3. Prosthetic groups are coenzymes that bind permanently to the enzyme molecule e.g., FAD (flavin adenine dinucleotide). Like NAD^+ it carries hydrogen atoms, with oxidase enzymes.

5. Inhibitors

Enzyme inhibition: Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction. Enzyme inhibitors are classified in several ways. The inhibitor may interact either reversibly or irreversibly with the enzyme.

(a) Reversible Inhibitors: These inhibitors bind the enzyme in a reversible fashion. They can be removed from the enzyme. Reversible inhibitors are of following types:

- Competitive reversible inhibitor
- Non-competitive reversible inhibitor
- Un-competitive inhibitor

Enzyme inhibition can be represented as-

$$E + I \rightleftharpoons EI \quad \text{and/or} \quad I + ES \rightleftharpoons IES$$

E = Enzyme, S = Substrate, I = Inhibitor, EI = Enzyme bound with Inhibitor

Competitive reversible inhibitors are structurally similar to the normal substrate and compete with the substrate for the active sites of enzyme. Thus, increasing the concentration

of substrate (S) favors the likelihood of S binding to the enzyme instead of the inhibitor, I. That is, high [S] can overcome the effects of I.

Non-competitive reversible inhibitors bind with the enzyme but not at the active site, thus inhibition cannot be overcome by increasing the substrate concentration [S]. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those enzymes. There are two types of noncompetitive inhibition: pure and mixed.

- ***Pure Noncompetitive Inhibition***

  In this situation, the binding of inhibitor [I] by enzyme [E] has no effect on the binding of substrate [S] by E. That is, S and I bind at different sites on E, and binding of I does not affect binding of S.

- ***Mixed Noncompetitive Inhibition***

  In this situation, the binding of inhibitor [I] on enzyme [E] influences the binding of substrate [S] by [E]. Either the binding sites for inhibitor [I] and substrate [S] are near one another or conformational changes in enzyme [E] caused by inhibitor [I] affect the substrate [S] binding.

(B) IRREVERSIBLE INHIBITORS

Irreversible inhibitors bind covalently and permanently to the enzyme, preventing enzyme to function. Usually, this type of inhibition can be distinguished from the noncompetitive, reversible inhibition case since the reaction of I with E (and/or ES) is not instantaneous. Instead, there is a *time-dependent decrease in enzymatic activity* as $E + I \rightarrow EI$ proceeds, and the rate of this inactivation can be followed. Also, unlike reversible inhibitions, dilution or dialysis of the enzyme: inhibitor does not dissociate the EI complex and restore enzyme activity. Aspirin is an irreversible inhibitor of cyclooxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH group on enzymes.

2.6 ENZYME KINETICS

Enzyme Kinetics deals with the quantitative measurement of the rates of enzyme-catalyzed reactions. In enzyme kinetics, we seek to determine the maximum reaction velocity that the enzyme can attain and its binding affinities for substrates and inhibitors. Analysis of the

enzymatic rate under different reaction conditions yields insights regarding the enzyme's mechanism of catalytic action.

2.6.1 Significance of Enzyme kinetics

- Kinetic analyses permit to reconstruct the **number** and **order** of the individual steps by which enzymes transform substrates into products.
- It also represents the principal way to identify potential therapeutic agents that selectively enhance or inhibit the rates of specific enzyme-catalyzed processes.
- It can also reveal details of the catalytic mechanism.
- Enzyme kinetics is important for understanding how physiologic stresses such as hypoxia, metabolic acidosis or alkalosis, toxins, and pharmacologic agents affect homeostatic balance.
- **Pharmaceuticals,** or **drugs,** are often special inhibitors specifically targeted to a particular enzyme in order to overcome infection or to alleviate illness. A detailed knowledge of the enzyme's kinetics is indispensable to rational drug design and successful pharmacological intervention.

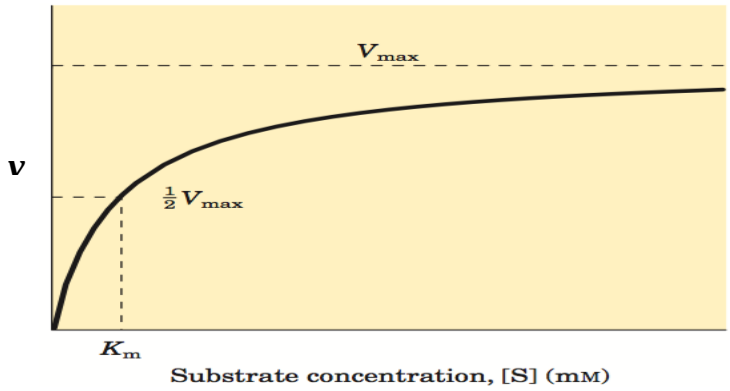


Figure No. 8 *Substrate saturation curve for an enzyme-catalyzed reaction.*

2.6.2 Enzyme Kinetics

Examination of the change in reaction velocity as the reactant concentration is varied is one of the primary measurements in kinetic analysis. In enzyme-catalyzed reactions involving only a single substrate, at low concentrations of the substrate S, *v* is proportional to [S], as expected for a first-order reaction. However, *v* does not increase proportionally as [S] increases, but instead begins to level off (Figure 8). At high [S], *v* becomes virtually independent of [S] and approaches a maximal limit. The value of *v* at this limit is written

*V*max. Because rate is no longer dependent on [S] at these high concentrations, the enzyme-catalyzed reaction is now obeying **zero-order kinetics;** that is, the rate is independent of the reactant (substrate) concentration. This behavior is a saturation effect: when *v* shows no increase even though [S] is increased, the system is saturated with substrate. The physical interpretation is that every enzyme molecule in the reaction mixture has its substrate-binding site occupied by S.

The Michaelis–Menten Equation

The theory was based on the assumption that the enzyme, E, and its substrate, S, associate reversibly to form an enzyme-substrate complex, ES:

$$\mathbf{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{ES} \qquad \text{----------(1)}$$

K_s is the *enzyme: substrate dissociation constant.* At equilibrium,

$$k_{-1}[\mathrm{ES}] = k_1[\mathrm{E}][\mathrm{S}] \qquad \text{----------(2)}$$

and

$$K_S = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} = \frac{k_{-1}}{k_1} \qquad \text{----------(3)}$$

Product, P, is formed in a second step when ES breaks down to yield E+ P.

$$\mathbf{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{ES} \xrightarrow{k_2} \mathbf{E + P} \qquad \text{----------(4)}$$

Michaelis-Menten equation was based on assumption that an equilibrium is reached instantly between enzyme, substrate and enzyme-substrate complex. The breakdown of enzyme-substrate complex to products is so slow that it does not cause any change in the equilibrium.

Steady-State Assumption

The interpretations of Michaelis and Menten were refined and extended in 1925 by Briggs and Haldane. Briggs and Haldane introduced a more valid steady state assumption since concentration of enzyme and enzyme-substrate is small in comparison to the substrate concentration that the rate of change of ES is very small in comparison to rate of change of

product [P]. So [ES] once formed remains constant or in steady state, this assumption is termed the **steady-state assumption** and is expressed as

d[ES]/dt =0----------[5]

That is, the change in concentration of ES with time, *t,* is 0

Initial Velocity Assumption

One other simplification will be advantageous. Because enzymes accelerate the rate of the reverse reaction as well as the forward reaction, it would be helpful to ignore any back reaction by which E + P might form ES. The velocity of this back reaction would be given by $v = k_{-2}[E][P]$. However, if we observe only the *initial velocity* for the reaction immediately after E and S are mixed in the absence of *P*, the rate of any back reaction is negligible because its rate will be proportional to [P], and [P] is essentially 0. Given such simplification, we now analyze the system described by Equation (4) in order to describe the initial velocity *v* as a function of [S] and amount of enzyme.

The total amount of enzyme is fixed and is given by the formula

Total enzyme, $[E_T]=[E]+[ES]$----------[6]

where [E] free enzyme and [ES] =the amount of enzyme in the enzyme– substrate complex.

From Equation (4), the rate of [ES] formation is

$$v_f = k_1([E_T] - [ES])[S]$$

were

$$[E_T] - [ES] = [E]\text{----------[7]}$$

From Equation (4), the rate of [ES] disappearance is

$$v_d = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES \quad \text{----------(8)}$$

At steady state, d[ES]/dt=0, and therefore, $v_f = v_d$

So,

$$k_1([E_T] - [ES])[S] = (k_{-1} + k_2)[ES] \quad \text{----------(9)}$$

rearranging gives

$$\frac{([E_T] - [ES])[S]}{[ES]} = \frac{(k_{-1} + k_2)}{k_1} \quad \text{----------(10)}$$

The Michaelis Constant, K_m

The ratio of constants $(k_{-1} + k_2)/k_1$ is itself a constant and is defined as the **Michaelis constant, K_m**

$$K_m = \frac{(k_{-1} + k_2)}{k_1}$$ ----------(11)

K_m is given by the ratio of two concentrations (([E_T] [ES]) and [S]) to one ([ES]), so K_m has the units of *molarity.* From Equation (10), we can write

$$\frac{([E_T] - [ES])[S]}{[ES]} = K_m$$ ----------(12)

Which rearranges to

$$[ES] = \frac{[E_T][S]}{K_m + [S]}$$ ----------(13)

Now, the most important parameter in the kinetics of any reaction is the **rate of product formation.** This rate is given by

$$v = \frac{d[P]}{dt}$$ ----------(14)

and for this reaction

$v = k_2[ES]$ ----------[15]

Substituting the expression for [ES] from equation [13] into [15] gives

$$v = \frac{k_2[E_T][S]}{K_m + [S]}$$ ----------(16)

The product $k_2[E_T]$ has special meaning. When [S] is high enough to saturate all of the enzyme, the velocity of the reaction, *v*, is maximal. At saturation, the amount of [ES] complex is equal to the total enzyme concentration, E*T*, its maximum possible value. From Equation (15), the initial velocity *v* then equals $k_2[E_T] = V_{max}$. Written symbolically, when [S] >>[E_T] (and K_m), [E_T] = [ES] and $v = V_{max}$. Therefore,

$V_{max} = k_2[E_T]$----------[17]

Substituting this relationship into the expression for *v* gives the Michaelis–Menten equation

$$v = \frac{V_{max}[S]}{K_m + [S]}$$ ----------(18)

This equation says that the rate of an enzyme-catalyzed reaction, *v*, at any moment is determined by two constants, K_m and V_{max}, *and* the concentration of substrate at that moment.

When [S]=K_m, $v = V_{max}/2$

Or K_m is defined by the substrate concentration that gives a velocity equal to one-half the maximal velocity

2.6.3 Linear Plots Can Be Derived from the Michaelis–Menten Equation

Because of the hyperbolic shape of *v* versus [S] plots, *V*max can only be determined from an extrapolation of the asymptotic approach of *v* to some limiting value as [S] increases indefinitely

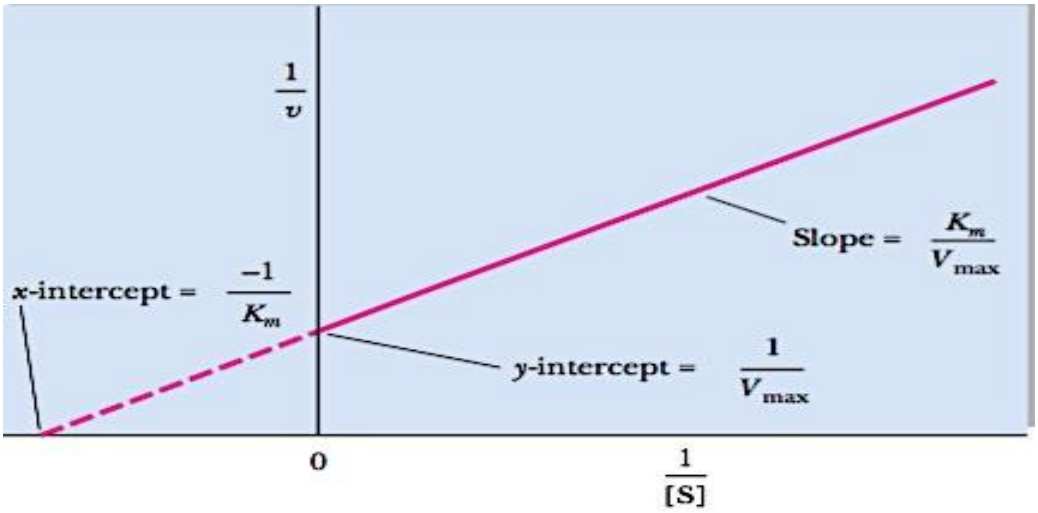


Figure 9 The Lineweaver–Burk double-reciprocal plot

(Figure 3); and $K_m$is derived from that value of [S] giving $v = V_{max}/2$. However, several rearrangements of the Michaelis – Menten equation transform it into a straight-line equation. The best known of these is the Lineweaver–Burk double-reciprocal plot:

Taking the reciprocal of both sides of the Michaelis–Menten equation, Equation (18), yields the equality

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}} \qquad \text{----------(19)}$$

This conforms to *y mx b* (the equation for a straight line), where $y = 1/v$; *m*, the slope, is K_m/V_{max}; $x = 1/[S]$; and $b = 1/V_{max}$. Plotting $1/v$ versus $1/[S]$ gives a straight line whose *x* -intercept is $-1/K_m$ whose *y* -intercept is $1/V_{max}$, and whose slope is K_m/V_{max} (Figure 9).

Departures from Linearity:

If the kinetics of the reaction does not follow the Michaelis – Menten equation shows a departure from linearity in these straight-line graphs. Such deviations from linearity are characteristic of the kinetics of regulatory enzymes known as **allosteric enzymes.** Such regulatory enzymes are very important in the overall control of metabolic pathways.

2.6.4 ENZYME UNITS

Enzyme activity is measured as the rate of substrate consumed or product formed per unit time. The enzyme commission of the International Union of Biochemistry (IUB) defined enzyme unit (U), later known as (IU), as the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute under specified conditions.

Later the term "**Katal"** was introduced by the Commission on Biochemical Nomenclature as the SI (Systems International) unit of enzyme activity as follow:

However, there is a direct relationship between the number of units of activity and the amount of sample assayed. Therefore, to minimize these problems, units of enzyme activity may be related to the total protein content of the sample assayed, termed as "**Specific activity",** expressed as International Units per mg protein or Katals per kg protein.

Turn-Over Number (k_{cat}): The number of substrate molecules converted into product in an enzyme-catalyzed reaction under saturating conditions in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate. For the simple Michaelis–Menten reaction (18) under conditions of initial velocity measurements, $k_2=k_{cat}$. Provided the concentration of enzyme, $[E_T]$, in the reaction mixture is known, k_{cat} can be determined from

V_{max}. At saturating [S], $v = V_{max} = k_2[E_T]$. Thus,

$k_2 = V_{max}/[E_T] = k_{cat}$----------[20]

The term k_{cat} represents the kinetic efficiency of the enzyme.

k_{cat}/K_m

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

and $V_{max}=k_{cat}[E_T]$, then

$$v = \frac{k_{cat}[E_T][S]}{K_m + [S]}$$

When [S] <<K_m *the* concentration of free enzyme, [E], is approximately equal to [E_T], so that

$$v = \left(\frac{k_{cat}}{K_m}\right)[E][S]$$

That is, k_{cat}/K_m is an *apparent second-order rate constant* for the reaction of E and S to form product. Because K_m is inversely proportional to the affinity of the enzyme for its substrate and k_{cat} is directly proportional to the kinetic efficiency of the enzyme, k_{cat}/K_m provides an index of the catalytic efficiency of an enzyme operating at substrate concentrations substantially below saturation amounts.

2.7 CHEMICAL STRUCTURE AND SIGNIFICANCE OF COENZYMES

As we have discussed earlier cofactor is any non-protein component in enzyme. It is an organic molecule or metal ion which the enzymes require in order to catalyze a reaction. Cofactors can be categorized into two groups- organic cofactors and inorganic cofactors. Coenzymes are organic cofactors which are again divided into two groups- cosubstrates and prosthetic groups. Cofactors which bound loosely to an enzyme are termed as coenzymes and cofactors which bound tightly to an enzyme are termed as prosthetic groups. Coenzymes are heat stable, low molecular weight organic compounds required for the activity of enzymes. Coenzymes act as group transfer reagents. Most coenzymes are linked to enzymes by noncovalent forces. Coenzymes can be separated from the enzyme by dialysis. Essential ions are inorganic cofactors that can be classified as- activator ions (loosely bound) and metalloenzymes (tightly bound).

Coenzyme classification:

There are two types of coenzymes- (1) Metabolite coenzyme (2) Vitamin derived coenzyme

Metabolite coenzymes- These are generally derived from common metabolites usually from nucleotides. The most commonly used metabolite coenzyme is adenosine triphosphate

(ATP).

Vitamin derived coenzyme- These are synthesized by microorganisms and plants, generally obtained from nutrients. Most vitamins must be enzymatically transformed to the coenzyme. Some vitamins directly act as coenzymes but some vitamins help the body to produce coenzymes.

Vitamin derived coenzymes:

1. Vitamin B1 in the form of thiamin diphosphate
2. Vitamin B2 in the form of riboflavin 5'-phosphate sometimes called Flavin mononucleotide (FMN)
3. Vitamin B3 in the forms of forms of niacinamide (partial coenzyme), nicotinamide diphosphate (NAD), and nicotinamide diphosphate hydrate (NADH)
4. Vitamin B5 in the form of pantetheine
5. Vitamin B6 in the form of pyridoxal 5'-phosphate
6. Folate in the forms of folic acid (5-formyl tetrahydrofolate) and methyl tetrahydro folate
7. Vitamin B12 – cyanocobalamin
8. Biotin (vitamin H)
9. Vitamin K

THIAMIN

Thiamine, **thiamin**, or **vitamin B_1**, named as the "thio-vitamine" ("sulfur-containing vitamin") is one of the eight vitamin of the B complex.

STRUCTURE & SIGNIFICANCE

Thiamine (Figure 10) is a colorless organosulfur compound with a chemical formula$C_{12}H_{17}N_4OS$. Its structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and insoluble in less polar organic solvents.

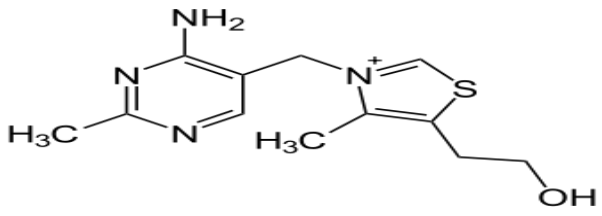


Figure 10- Structure of Vitamin B_1 (thiamine)

Thiamin pyrophosphate (TPP) is the coenzyme for three multi-enzyme complexes that catalyze oxidative decarboxylation reactions: pyruvate dehydrogenase in carbohydrate metabolism; α-ketoglutarate dehydrogenase in the citric acid cycle; and the branched-chain keto-acid dehydrogenase involved in the metabolism of leucine, isoleucine, and valine. It is also the coenzyme for transketolase, in the pentose phosphate pathway. In each case, the thiamin pyrophosphate provides a reactive carbon on the thiazole moiety that forms a carbanion, which then adds to the carbonyl group of, for instance, pyruvate.

RIBOFLAVIN (Vitamin B_2)

Riboflavin fulfills its role in metabolism as the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

STRUCTURE & SIGNIFICANCE

Riboflavin's (Figure 11) chemical formula is $\mathbf{C_{17}H_{20}N_4O_6}$. It is polar and water-soluble compound. Flavin coenzymes (FMN and FAD) are derived from riboflavin (Figure 11). These coenzymes are prosthetic groups and cannot be separated from the proteins so the term flavoproteins is also used.

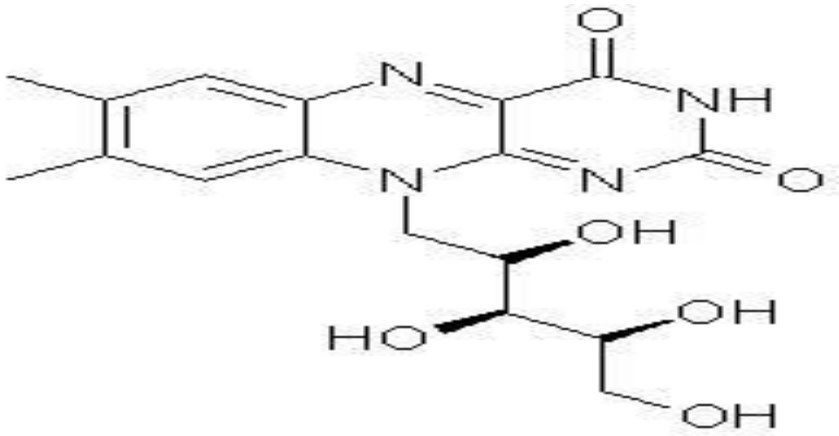


Figure 11- The Chemical Structure of Riboflavin (Vitamin B2)

Flavin Coenzymes Are Electron Carriers in Oxidoreduction Reactions. These include the mitochondrial respiratory chain, key enzymes in fatty acid and amino acid oxidation, and the citric acid cycle. Reoxidation of the reduced flavin in oxygenases and mixed-function oxidases proceeds by way of formation of the flavin radical and flavin hydroperoxide, with the intermediate generation of superoxide and perhydroxyl radicals and hydrogen peroxide.

NIACIN

It is not strictly a vitamin since it can be synthesized in the body from the essential amino acid tryptophan. Two compounds, **nicotinic acid** (Figure 12a)and **nicotinamide** (Figure 12b),have the biologic activity of niacin; its metabolic function is as the nicotinamide ring of the coenzymes **NAD** and **NADP** in oxidation-reduction reactions.

STRUCTURE & SIGNIFICANCE

This colorless, water-soluble solid is a derivative of pyridine, with a carboxyl group (COOH) at the 3-position. Other forms of vitamin B_3 include the corresponding amide and nicotinamide ("niacinamide"), where the carboxyl group has been replaced by a carboxamide group ($CONH_2$), as well as more complex amides and a variety of esters.

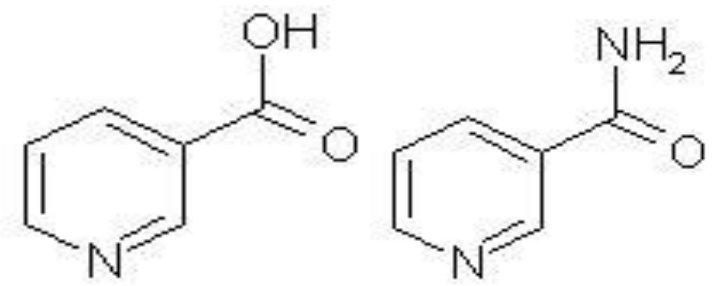


Figure 12 (a) Structure of Nicotinic acid (b) Structure of Nicotinamide

PANTOTHENIC ACID

Pantothenic acid (Figure 13) is used in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group carrier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell.

STRUCTURE & SIGNIFICANCE

The molecular formula is butyryl-beta-alanine ($C_9H_{17}NO_5$) and it is the amide between D-pantoate and beta-alanine; its IUPAC name is 3-[(2,4-dihydroxy-3,3-dimethylbutanoyl)amino] propanoic acid.

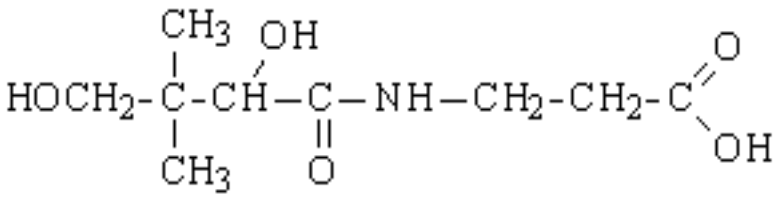


Figure 13- Pantothenic acid

Pantothenic acid has a central role in acyl group metabolism when acting as the pantetheine functional moiety of coenzyme A or acyl carrier protein (ACP). The pantetheine moiety is formed after combination of pantothenate with cysteine, which provides the SH prosthetic group of CoA and ACP. CoA takes part in reactions of the citric acid cycle, fatty acid synthesis and oxidation, acetylation's, and cholesterol synthesis. ACP also participates in fatty acid synthesis.

PYRIDOXAL PHOSPHATE (PLP)

STRUCTURE & SIGNIFICANCE

It has the structure, of 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine. The trivial name is "pyridoxine". Pyridoxal is the aldehyde, or 4-formyl analogue of pyridoxine and a phosphoric derivative of pyridoxal, later identified as pyridoxal 5'-phosphate (Figure 14), is the coenzyme of a large group of specific enzymes catalyzing reactions of amino-group transfer, decarboxylation and other metabolic transformations. It is a required coenzyme of glycogen phosphorylase, the enzyme necessary for glycogenolysis to occur. PLP is an essential component of enzymes that facilitate the biosynthesis of sphingolipids. PLP aids in the synthesis of hemoglobin, by serving as a coenzyme

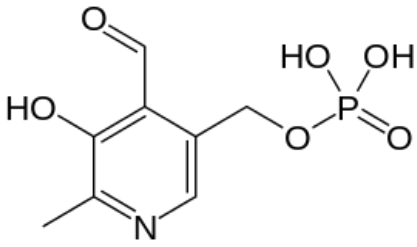


Figure 14- Pyridoxal phosphate

FOLIC ACID

Folic acid (Figure 15), also known as **folate**, is one of the B vitamins that stimulate the hematopoietic system. The active form of folic acid (pteroyl glutamate) is tetrahydrofolate.

STRUCTURE & SIGNIFICANCE

The folates are a group of heterocyclic compounds based on the 4-[(pteridin-6-ylmethyl) amino] benzoic acid skeleton conjugated with one or more L-glutamate units. Folic acid is a

collective term for pteroylglutamic acids and their oligoglutamic acid conjugates. Folate and folic acid are the preferred synonyms for pteroylglutamate and pteroylglutamic acid, respectively.

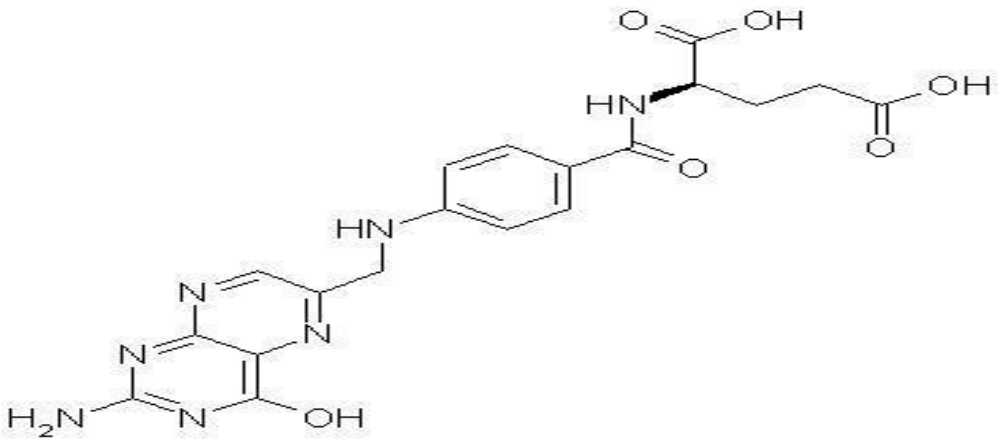


Figure 15- Chemical Structure of Folic Acid

Tetrahydrofolic acid, or tetrahydrofolate, is a folic acid derivative. The only folate coenzymes in the body assists in the transfer of one-carbon units. Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids. Folate coenzymes play a vital role in DNAmetabolism. The synthesis of DNA from its precursors (thymidine and purines) is dependent on folate coenzymes. Folate coenzymes are required for the metabolism of several important amino acids, namely methionine, cysteine, serine, glycine, and histidine.

CYANOCOBALMINE

Vitamin B_{12} is the precursor to two B_{12} coenzymes, which, while possessing similar chemical structures, play quite different biochemical roles. The key biological function of both coenzymes is their ability to form a unique, stable, covalent bond between the central cobalt atom of the coenzyme and carbon. One form, methyl cobalamin (MeCbl), is involved in methylation reactions in which the methyl group is transferred to and from cobalt. The other form, adenosyl cobalamin (AdoCbl), serves as a source of carbon-based free radicals that are ‘unmasked’ by homolysis of the bond between cobalt and the 5 carbons of adenosine.

STRUCTURE & SIGNIFICANCE

Coenzyme B_{12}, or cobalamin, is derived from the same porphyrin precursor as haem and chlorophyll, but further elaborate tailoring. At the heart of the coenzyme is the cobalt atom that is chelated by a macrocyclic ring called a corrin

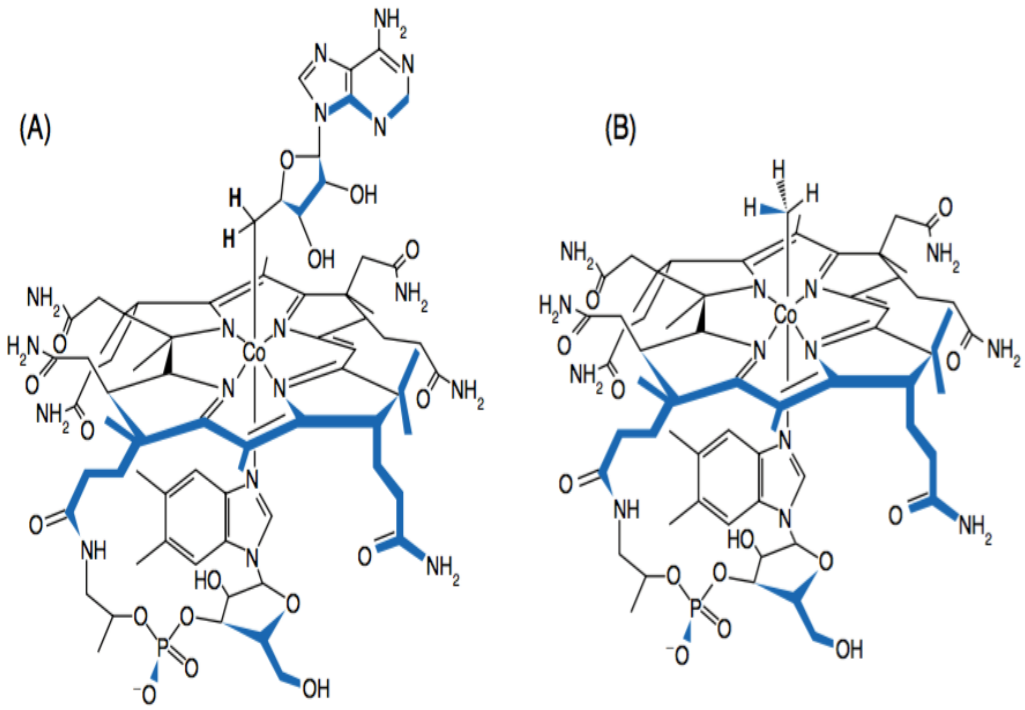


Figure 16- The structures of the B12 coenzymes (A) *AdoCbl;* ***(B)*** *MeCbl.*

Both AdoCbl (Figure 16a) and MeCbl (Figure 16b) play essential roles in the metabolism of higher eukaryotes. In humans, lack of B_{12} in the diet, or an inability to absorb it, is the cause of pernicious anaemia. MeCbl is involved in the methylation of homocysteine to form methionine by methionine synthase as part of the methionine salvage pathway; homocysteine is toxic in high concentrations and may be responsible for many of the symptoms of pernicious anemia. AdoCbl is the coenzyme for methyl malonyl-CoA mutase, an enzyme that converts methyl malonyl-CoA to succinyl-CoA, an essential step in the metabolism of odd-chain fatty acids.

BIOTIN

Biotin is a water-soluble B-vitamin (vitamin B_7), formerly known as vitamin H or coenzyme R. Biotin is an important component of enzymes in the body that break down certain substances like fats, carbohydrates, and others.

STRUCTURE & SIGNIFICANCE

Biotin (Figure 17) has the chemical formula $C_{10}H_{16}N_2O_3S$. Biotin is a heterocyclic, S-containing monocarboxylic acid. Biotin is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring, which is an organic compound consisting of a five-membered ring containing four carbonatoms and a sulfur atom. A valeric acid substituent—straight chain alkyl carboxylic acid with the chemical formula $CH_3(CH_2)_3COOH$)—is attached to one of the carbon atoms of the tetrahydrothiophene ring.

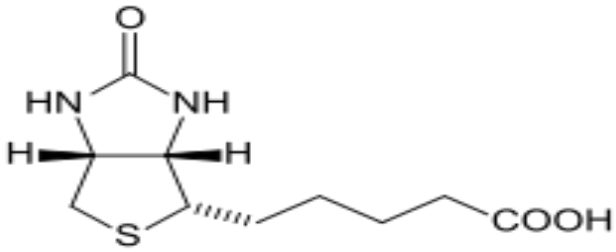


Figure 17- Structure of biotin

Biotin functions to transfer carbon dioxide in a carboxylation reaction. The attachment of biotin to another molecule, such as a protein (e.g.,histone), is known as 'biotinylation'. Carboxylases catalyze many essential metabolic reactions required for the synthesis of fatty acids, gluconeogenesis, the catabolism of leucine (an essential amino acid), and the metabolism of certain amino acids, cholesterol, and certain fatty acids.

VITAMIN K

Vitamin K is known as the "blood-clotting vitamin" for its important role in healing wounds. The "K" is derived from the German word koagulation.

STRUCTURE & SIGNIFICANCE: Chemically, the vitamin K family comprises 2-methyl-1,4-naphthoquinone (3-) derivatives. Vitamin K includes two natural vitamers: vitamin K_1 (Figure 18) and vitamin K_2. Vitamin K_1, also known as **phylloquinone** or **phytomenadione** is synthesized by plants. It may be thought of as the "plant" form of vitamin K. It is active as a vitamin in animals and performs the classic functions of vitamin K, including its activity in the production of blood-clotting proteins.

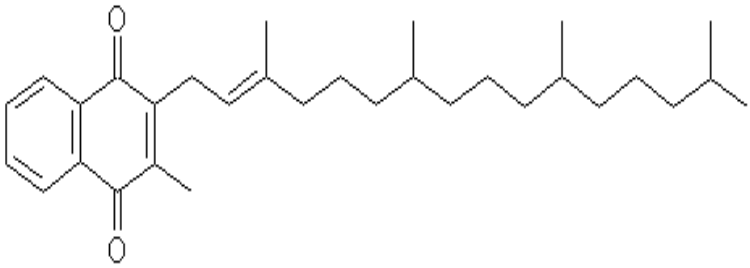


Figure 18- Structure of Vitamin K1 (phylloquinone)

Vitamin K is the coenzyme for carboxylation of glutamate in the postsynthetic modification of calcium-binding proteins. Vitamin K-dependent factors form enzymatic complexes with calcium and membrane phospholipids. The insufficiency of gamma glutamic carboxylation impairs the hemostatic function. Vitamin K is also important in the synthesis of bone calcium-binding proteins.

VITAMIN C OR ASCORBIC ACID

Ascorbic acid is a naturally occurring organic six carbon compounds related to glucose with antioxidant properties.

STRUCTURE & SIGNIFICANCE

Vitamin C, Ascorbic acid or ascorbate (Figure 19) is a weekorganic acid that appears as a white, crystalline compound. Structurally, it is related to the six-carbon sugar glucose, from which most animals are able to derive the molecule in a four-step process. The ionized form of ascorbic acid is known as *ascorbate.* The ascorbate ion represents the *pharmacophore* of vitamin C; that is, the structural feature (or set of features) responsible for the molecule's biological activity. Presence of the ascorbate ion contributes to vitamin C's role as a strong reducing agent *(antioxidant).*

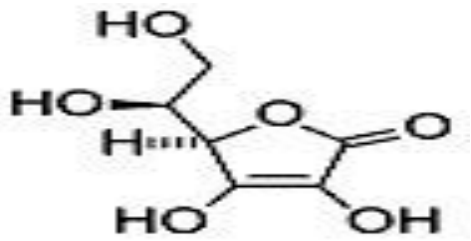


Figure 19- Structure of Ascorbic acid (Vitamin C)

Vitamin C is the coenzyme for the copper-containing hydroxylases and the α-ketoglutarate-linked iron-containing hydroxylases. It is involved in hydroxylation of proline in collagen, therefore important for wound healing. It also increases the activity of a number of other enzymes in vitro, though this is a non- specific reducing action.

NON-VITAMIN COENZYMES

1. Adenosine triphosphate
2. Glutathione
3. Lipoic acid
4. Coenzyme Q

ATP

Adenosine triphosphate (ATP) (Figure 20) is a coenzyme of vast importance in the transfer of chemical energy derived from biochemical oxidations. Adenosine triphosphate (ATP) is an example of an essential non-vitamin coenzyme. In fact, it is the most widely distributed coenzyme in the human body. It transports substances and supplies energy needed for necessary chemical reactions and muscle contraction. ATP carries both a phosphate and energy to various locations within a cell. When the phosphate is removed, the energy is also released. Adenosine phosphates, like the nicotinamide nucleotides, are loosely bound by enzymes and may be regarded both as coenzymes and as co-substrate/co-products. Other nucleotides (formed from uracil, cytosine, guanine, and inosine) have also been found to act as coenzymes. For example, uridine triphosphate—a derivative of uracil—has been demonstrated to be of great importance in the metabolism of carbohydrates, as in the biosynthesis of glycogen and sucrose.

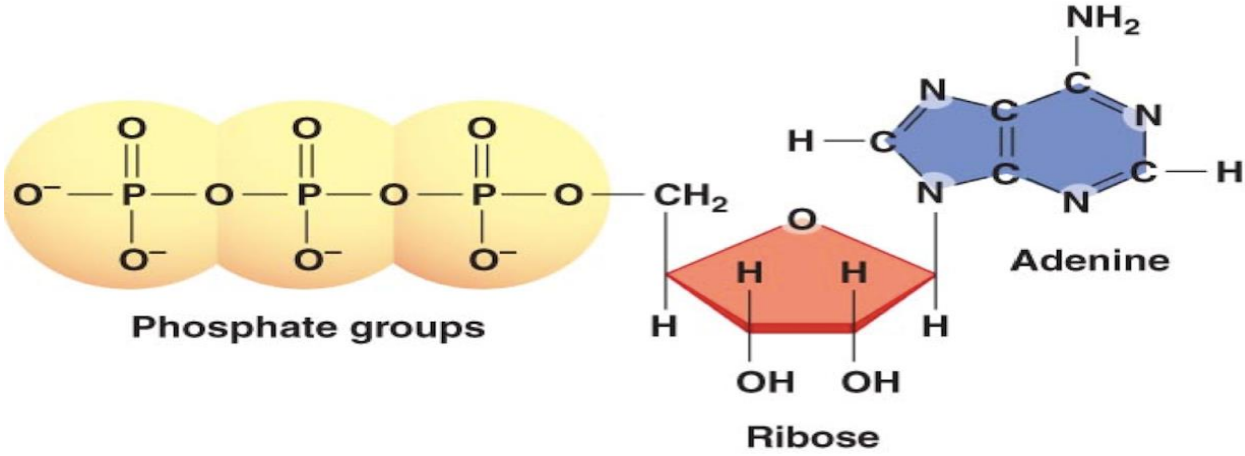


Figure 20- Structure of ATP

Glutathione (Figure 21), a tripeptide consisting of residues of glutamic acid, cysteine, and glycine, is known to act as a coenzyme in a few enzymatic reactions, but its importance may lie in its role as a nonspecific reducing agent within the cell. It is hypothesized that glutathione serves to maintain the biological activity of certain proteins by keeping selected cysteine sidechains in the reduced thiol form, thereby not allowing these residues to oxidize and cross-link with one another to form cystine residues. (Unnecessary cross-links often result in distortions of protein structure.)

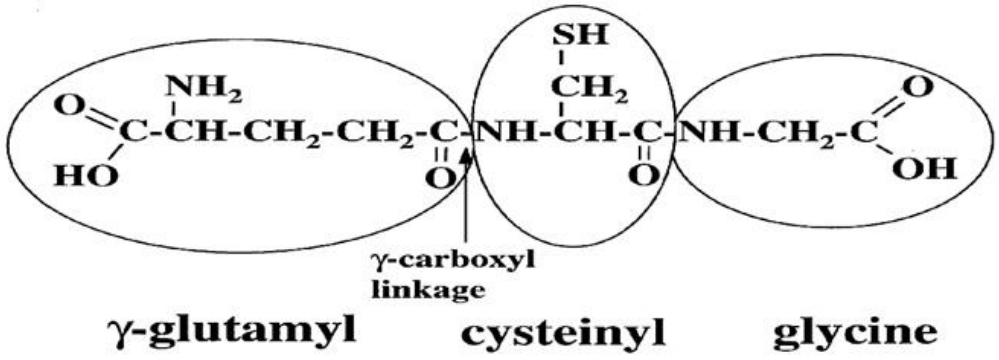


Figure 21- Structure of Glutathione

Lipoic acid

Lipoic acid (Figure 22)is involved in the removal of carboxyl groups from α-keto acids and in the transfer of the remaining acyl groups to various acceptors. Lipoic acid in fact transfers the acetyl group of pyruvic acid to coenzyme A. Like biotin, lipoic acid is commonly found attached to lysine residues within several enzymes.

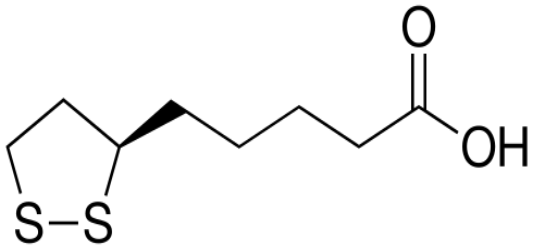


Figure 22- Structure of Lipoic acid

Coenzyme Q: Coenzyme Q (Figure 23) or Coenzyme Q_{10}, is ubiquitous in the bodies of most animals. It is a 1,4-benzoquinone, where Q refers to the quinone chemical group and 10 refers to the number of isoprenyl chemical subunits in its tail.

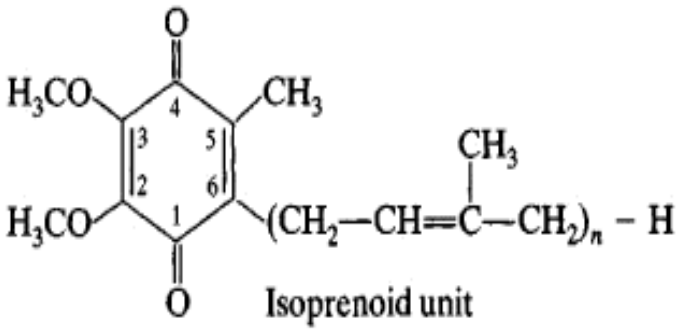


Figure 23- Structure of Coenzyme Q

2.8 SUMMARY

- Enzymes are highly specific biological catalysts.
- They catalyze almost every biochemical reaction.
- All known enzymes are proteins except a few catalytic RNAs. Enzymes require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names.
- The region which contains the binding and catalytic site is called as active site. The rest of the enzyme is much larger and is involved in maintaining the specific shape of the Enzyme.
- Enzymes increase the rate of a reaction by lowering its activation energy.
- The Lock-and-key Hypothesis is a model of how enzymes catalyze reactions. The theory states that the shape of the active sites of enzymes is exactly complementary to the shape of the substrate.
- Several factors affect the rate of enzymatic reactions - temperature, pH, substrate concentration, and the presence of any inhibitors or activators.
- Coenzymes are heat stable, low molecular weight organic compounds required for the activity of enzymes.

2.8.1 GLOSSARY

Activation energy: Energy a substrate molecule must have before it can undergo a chemical change.

Active site: Region of enzyme molecule where substrate molecule binds.

Coenzymes:A complex organic (or metalloorganic) molecule required by a an enzyme to carry out catalysis of a chemical reaction.

Competitive inhibitor: An inhibitor, which binds to the active site of an enzyme.

Denaturation: A structural change in a protein that results in a loss (usually permanent) of its biological properties.

Inhibitor: A substance which slows or blocks enzyme action.

Non-competitive inhibitor: An inhibitor, which binds at a site other than the active site of an enzyme.

Optimum temperature: Temperature at which enzyme functions most effectively.

Optimum pH: pH at which enzyme functions most effectively.

Reversible reaction: A reaction where the reactants form products, which react together to give the reactants back.

Specific Activity: This is the activity of an enzyme per milligram of total protein (expressed in $\mu mol\ min^{-1}mg^{-1}$).

Substrate: A molecule that is the starting point for a biochemical reaction and that forms a complex with a specific enzyme.

Turnover number: Defined as the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate.

Vitamin: A vitamin is an organic compound and a vital nutrient that an organism requires in limited amounts.

2.9 SELF ASSESSMENT QUESTIONS

2.9.1 LONG ANSWER TYPE QUESTIONS

Q 1. Give a detailed account of the classification of enzymes with example.

Q 2. Describe the mechanism of catalysis and the factors affecting the rate of reactions catalyzed by enzyme.

Q 3. Derive the Michaelis-Menten equation.

Q 4. Describe the significance of LineweaverBurk plot.

2.9.2 SHORT ANSWER TYPE QUESTIONS

Q 1. What are enzymes? Name all the classes of the enzyme

Q 2. What do you mean by enzyme kinetics?

Q 3. What are Coenzymes?

Q 4. What is K_m? Describe its significance.

2.9.3 Fill in the blanks:

1. Some enzymes require an additional chemical non-protein component called _____.
2. Enzyme reactions are always (Irreversible/Reversible) _____.
3. Prior to 1984, ligases were also called _____.
4. Oxidoreductases are a class of enzymes that catalyze_____ reactions.
5. Enzymes that bind metal ions tightly are referred to as _____.
6. Apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion- activator) is called the _____.
7. Enzymes are usually named by adding "_____" to the name of the substrate.
8. An _____ reaction requires more activation energy than a _____ reaction.
9. A _____ is a non-protein component imperative to catalytic activity (organic, usually made from vitamins)
10. The amount of energy required in order to activate a reaction_____.

Answer: Fill in the blanks:

1. Cofactors, 2. Reversible 3. Synthetases, 4. Oxidation-reduction, 5. Metalloenzymes, 6. Holoenzyme, 7. Ase, 8. Uncatalysed , catalyzed, 9. Coenzyme, 10.Activation energy.

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UNIT 3: VITAMINS

Contents

3.1 OBJECTIVES

After reading this unit you will be able to understand-

1. **Vitamins as micronutrient.**
2. **The basis of their classification.**
3. **The structure, occurrence and functions of vitamins.**
4. **The source, significance and deficiency related diseases of vitamins.**

3.2 INTRODUCTION

Vitamins are the distinct group of organic vital nutrients required in controlled quantities for a number of metabolic functions. Generally, they cannot be synthesized by the body and must therefore be supplied in the diet to maintain the normal functions of cells and organs, and to promote growth and development. ***They generally act as cofactors; combine with apoenzymes to generate functionally active enzymes (holoenzymes) that in turn catalyzes hundreds of important chemical reactions throughout the body. Without vitamins, most of these reactions would slow down or cease).***

The term vitamin is derived from the words vital and amine, because vitamins are required for life and were originally thought to be amines. Although not all vitamins are amines, they are organic compounds required by humans in small amounts from the diet.

Conventionally the term *vitamin* does not includes essential nutrients, such as dietary minerals, essential fatty acids, or essential amino acids that are required in large amounts than vitamins) often to maintain the health of the organism.

Moreover, we can also say that vitamins are organic nutrients which are not synthesized by the body, and so must be obtained through the diet; thus, the term "vitamin" is conditional upon the circumstances and the particular organism. You can understand by the example that, ascorbic acid (one form of vitamin C) is a vitamin for humans, but not for several other animal organisms. Interestingly its supplementation is important for several biochemical functions, thirteen vitamins are known universally at present, required to maintain our health.

3.3 CLASSIFICATION

Before their detailed chemical structures were known, vitamins were named by being given a letter. They are generally still referred to by that letter, as well as by their chemical name;

for example, vitamin C or ascorbic acid. Vitamins are classified not by their structure but on the basis of biological and chemical activity. Therefore, It is interesting to note that each "vitamin" includes a number of *vitamer* (a number of chemical compounds, mostly having a similar molecular structure) compounds that all show the vitamin related biological activity associated with that particular vitamin in a vitamin-deficient biological system. Such a set of chemicals is grouped under an alphabetized vitamin title, for eg. "Vitamin A", has at least six vitamer that all qualify as "vitamin A", each with marginally different properties which includes the compounds retinal, retinol, and four known carotenoids. Vitamers by definition are convertible to the active form of the vitamin in the body, and are sometimes inter-convertible to one another, as well.

The main classification for vitamins is based on the ability to be absorbed in fat or water.

Fat Soluble

These are oily and hydrophobic compounds. These are stored in the liver and adipose tissues thus are not excreted out of the body. Bile salts and fats are required for their absorption. Vitamin A, D, E and K are fat soluble. Fat-soluble vitamins except for vitamin K are stored for long periods at a time and then excreted. Their excessive intake can be toxic and result in **Hypervitaminosis**.

Water Soluble

Vitamins in B-group and vitamin C are water soluble and cannot be stored in our body as they excreted out of the body via urine. These vitamins must be supplied to our body with regular diets. The daily requirement of certain vitamins is much less than 1 mg (1mg is one-thousandth of a gram), and so is measured in micrograms per day, written as μg per day, where 1 μg is one-thousandth of a milligram. The values for the daily requirements of vitamins are regularly updated as more information becomes available.

3.4 STRUCTURE OCCURRENCE AND FUNCTIONS OF FAT SOULBLE VITAMINS

VITAMIN A

Vitamin A is a generic term that refers to fat-soluble compounds found as preformed vitamin A. The three active forms of vitamin A in the body are retinol, retinal, and retinoic acid (found only in food of animal origin), and carotenoids (carotenes and related compounds, most particularly beta-carotene found in plants) known as provitamin A.

STRUCTURE

Vitamin A is a pale-yellow primary alcohol derived from carotene. Retinol (Figure 1 a) is the immediate precursor to two important active metabolites: **retinal**, which plays a critical role in vision, and **retinoic acid**, which serves as an intracellular messenger that affects transcription of a number of genes. Vitamin A does not occur in plants, but many plants contain **carotenoids such as beta-carotene** (Figure 1 b) that can be transformed to vitamin *A within the intestine and other tissues. It has following structure; ring shown is called β-* ionone ring. There are several isomers of vitamin A; the most important of these are 13-cis retinol found in many fish liver oils and 11-cis retinol which occurs in the retina. Vitamin A is quite heat stable but it gets destroyed at high temperature in the presence of O_2 or air.

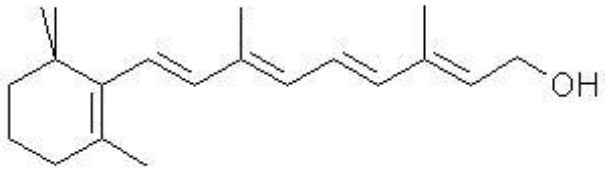


Figure 1a: Structure of Retinol

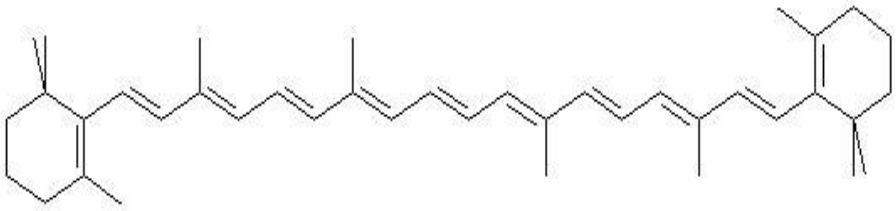


Figure 1b: Structure of Beta Carotene

Occurrence & sources

Preformed Vitamin A occurs in nature exclusively in the animal kingdom. Major sources of vitamin A are liver oils of certain species of fish, e.g. halibut (richest source), shark and cod; it is also found in the livers of other animals, egg yolk, whole milk and dairy products. In plants it occurs as its precursors, pro-vitamins A or carotenes which are yellow-red pigments found especially in carrots, yellow corn, sweet potato, peaches and green leafy vegetable such as spinach. The carotenes do not have any vitamin A activity but are converted to vitamin A in the liver. The conversion of carotenes to vitamin A decreases in diseases such as diabetes mellitus and hypothyroidism. B-carotene is the most efficient precursor of vitamin A. However, carotenes, are poor sources of vitamin A because they are not absorbed completely and their conversion to the active vitamin A is not absolute.

Good sources of vitamin A include: cheese, eggs, oily fish, fortified low-fat spreads, milk and yoghurt. Liver is a rich source of vitamin A. You can also contribute to your vitamin A intake by including good sources of beta-carotene in your diet, as this can yield vitamin A inside the body. The main food sources of beta-carotene are: yellow, red and green (leafy) vegetables, such as spinach, carrots, sweet potatoes and red peppers, yellow fruit such as mango, papaya and apricots.

FUNCTIONS AND SIGNIFICANCE

Vitamin A is required for several vital functions in the body. Some of the most important functions of Vitamin A are described below.

Vision

Vitamin A is required for the maintenance of normal vision. A deficiency in vitamin A can lead to visual disturbances. In the eyes, a form of vitamin A retinal combines with a protein called opsin to give rhodopsin (rod cells) and iodopsin (cone cells).

Immune system

Vitamin A was initially coined “the anti-infective vitamin” because of its importance in the normal functioning of the immune system. The skin and mucosal cells, lining the airways, digestive tract, and urinary tract function as a barrier and form the body's first line of defense against infection. Retinoic acid (RA) is produced by antigen-presenting cells (APCs), including macrophages and dendritic cells, found in these mucosal interfaces and associated lymph nodes. RA acts on dendritic cells themselves to regulate their differentiation, migration, and antigen-presenting capacity.

Cell growth

Retinoic acid is a crucial hormone-like growth factor for epithelial cells and other cell types in the body. It plays an important role in reproduction, cell division, and cell differentiation.

Gene transcription and protein formation

Vitamin A in the form of retinoic acid is essential for gene transcription. Retinol is taken up by the cell where it is oxidized to retinaldehyde (by retinol dehydrogenases), which is then oxidized to give retinoic acid. The conversion of retinal to retinoic acid is irreversible and the process is therefore tightly regulated because retinoic acid functions as a ligand for nuclear receptors. Retinoic acid binds to these nuclear receptors called in order to regulate gene transcription.

Skin health

Vitamin A, and more specifically, retinoic acid, appears to maintain normal skin health by activating genes and differentiating keratinocytes (immature skin cells) into mature

epidermal cells. The exact mechanism is still not known. The retinoic drug isotretinoin is the most commonly prescribed agent in the treatment of acne. It acts by decreasing the size of sebaceous glands and reduces their secretions.

Antioxidant

Beta-carotene is an antioxidant. Antioxidants protect cells from damage caused by free radicals. Free radicals are believed to contribute to several chronic diseases and play a significant role in the aging processes. It is also reported to reduce the risk of cancer.

Bone growth

Vitamin A plays an important role in bone growth. However, excess of vitamin A has been linked to bone loss and an increase in the risk of hip fracture. Large amounts of vitamin A trigger an increase in osteoclasts. Moreover, excess of vitamin A may also interfere with vitamin D, which plays an important role in preserving bone.

VITAMIN A RELATED DISEASES

1. Vitamin A deficiency can occur as either a primary or a secondary deficiency.
2. A primary vitamin A deficiency occurs among children and adults who do not consume an adequate intake of provitamin A, carotenoids from fruits and vegetables or preformed vitamin A from animal and dairy products.
3. Secondary vitamin A deficiency is associated with chronic malabsorption of lipids, impaired bile production and release, and chronic exposure to oxidants, such as cigarette smoke, and chronic alcoholism.
4. One of the earliest and specific signs of vitamin A deficiency is impaired vision, particularly in reduced light – night blindness. Vitamin A deficiency is the leading cause of xerophthalmia in childrens.
5. It also increases the mortality rate from common childhood diseases such as diarrhea.
6. Mild deficiency of Vitamin A leads to increased susceptibility to infectious diseases.
7. Due to limited capacity to metabolize vitamin A, higher intakes may lead to tissue damage. Excessive vitamin A consumption can lead to nausea, irritability, anorexia (reduced appetite), vomiting, blurry vision, headaches, hair loss, muscle and abdominal pain and weakness, drowsiness, and altered mental status.

VITAMIN D

Vitamin D comprises a group of fat-soluble secosteroids/secosterols that are not strictly a vitamin since they can be synthesized in the skin. In humans, the most important compounds

in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from diet and the supplements.Synthesis of vitamin D (specifically cholecalciferol) in the skin is the major natural source of the vitamin that depends on the sun exposure (specifically ultraviolet (UV) radiation). Vitamin D_2 is produced by UV irradiation of plant sterol ergosterol, which occurs in molds, yeast, and higher-order plants. Under conditions of regular sun exposure, dietary vitamin D intake is of minor importance. However, latitude, season, aging, sunscreen use, and skin pigmentation influence the production of vitamin D_3 by the skin.

STRUCTURE

Several vitamers of vitamin D exist. The two major forms are vitamin D_2 (Figure 2) or ergocalciferol, and vitamin D_3 (Figure 3) or cholecalciferol; vitamin D without a subscript refers to either D_2 or D_3 or both. These are known collectively as calciferol. Chemically, the various forms of vitamin D are secosteroids, i.e., steroids in which one of the bonds in the steroid rings is broken. The structural difference between vitamin D_2 and vitamin D_3 is the side chain of D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24.7-Dehydrocholesterol (an intermediate in the synthesis of cholesterol), undergoes a nonenzymic reaction on exposure to ultraviolet light, yielding previtamin D (Cholecalciferol Vitamin D_3)

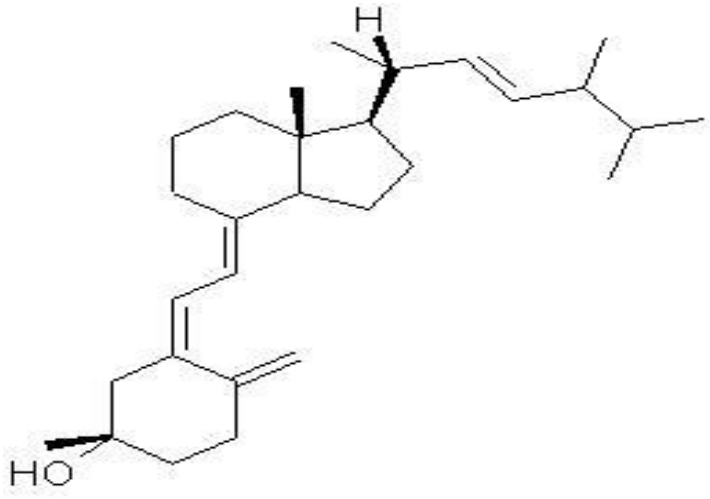


Figure 2: Structure of Vitamin D_2 (Ergocalciferol)

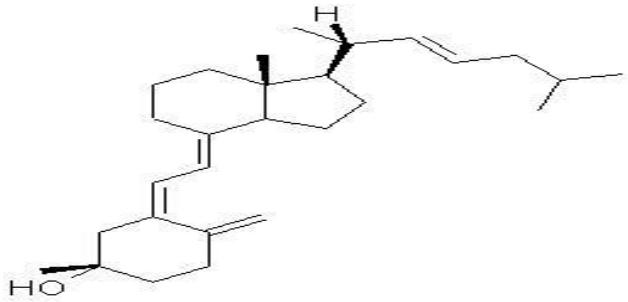


Figure 3: Vitamin D_3

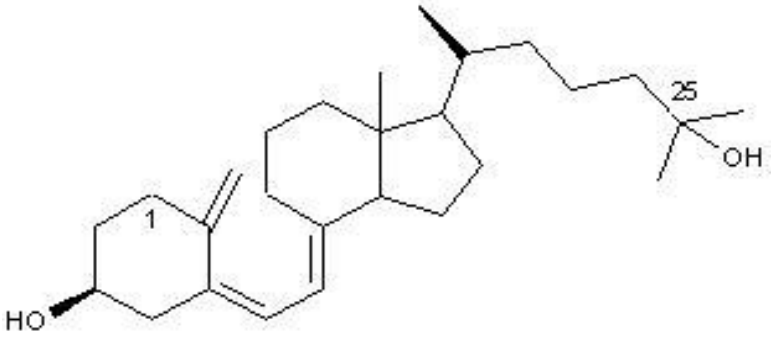


Figure 4: Calcidiol

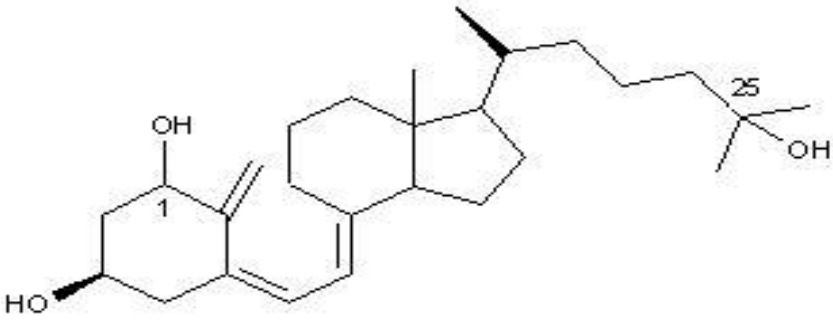


Figure 5: Calcitriol

Vitamin D, as either D_3 or D_2, does not have significant biological activity. Rather, it can be metabolized within the body to the hormonally active form known as 1,25-dihydroxycholecalciferol. This transformation occurs in two steps, 1. **In the liver,** Cholecalciferol (vitamin D_3) is hydroxylated to *25-hydroxycholecalciferol* (calcidiol [figure 4]) by the enzyme 25-hydroxylase. Whereas Ergocalciferol (vitamin D_2) is converted in the liver to 25-hydroxyergocalciferol

2. **In the kidney,** part of calcidiol is converted by the kidneys to calcitriol(Figure 5), the

biologically active form of vitamin D by 1-hydoxylation or inactive metabolite 24-hydroxycalcidiol by 24-hydroxylation. Ergocalciferol from fortified foods undergoes similar hydroxylations to yield ercalcitriol.

All the forms of vitamin D are hydrophobic, and are transported in blood bound to carrier proteins.

Occurrence and sources

There are two main sources of vitamin D: dietary consumption and endogenous production. Vitamin D is found in few dietary sources. Vitamin D may be consumed in the diet as either ergocalciferol (D_2) from plant sources or cholecalciferol (D_3) from animal sources. Sunlight exposure is the primary source of vitamin D for the majority of people, other than supplements. Mushroom, alfa alfa, lichen, fish liver oils, such as cod liver oil, fatty fish species, such as salmon, tunaare other sources of vitamin D.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin D is essential for maintenance of bone mineralization through the regulation of calcium and phosphorus homeostasis. Vitamin D also exhibits many non-skeletal effects, particularly on the immune, endocrine, and cardiovascular systems. It behaves like a **steroid hormone,** binding to a nuclear receptor protein. It regulates the expression of hundreds of genes involved in skeletal and other biological functions.
2. In addition, calcitriol is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones.
3. Vitamin D functions to activate the innate and dampen the adaptive immune systems. Its deficiency has been linked to an increased risk of viral infections.
4. There are reports on probable association between vitamin D status and the susceptibility or severity of autoimmune diseases, including type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.
5. Vitamin D supplementation can limit cognitive deterioration and disease progression in subjects with neurodegenerative diseases.

VITAMIN D RELATED DISEASES

1. In the vitamin D deficiency, due to poor calcium absorption**,** the bones of children are under mineralized resulting in diseases such as **rickets (the bowing of the leg**

bones in children). Similar problems occur in adolescents who are deficient during their growth spurt.

2. **Osteomalacia** in adults results from demineralization of bone in women who have little exposure to sunlight.
3. Vitamin D toxicity is rare, however too much vitamin D can make the intestines absorb too much calcium resulting in hypercalcemia, noted with an increase in urination and thirst. If hypercalcemia is not treated, it results in excess deposits of calcium in soft tissues and organs such as the kidneys, liver, and heart, resulting in pain and organ damage, confusion and disorientation, Nausea, vomiting, constipation, poor appetite, weakness, and weight loss are some of other symptoms of vitamin D toxicity.

VITAMIN E: Naturally occurring Vitamin E is not a single compound, but is a generic term denoting group of eight closely related molecules, yet, the body preferentially uses α-tocopherol, whose supplementation can reverse vitamin E deficiency symptoms. It acts as a lipid-soluble antioxidant in cell membranes.

STRUCTURE: Vitamin E comprises of eight fat soluble compounds that split into two families the **tocopherols** (Figure 6) and the **tocotrienols** (Figure 7), identified by the prefix's alpha- (α-), beta- (β-), gamma- (γ-), and delta- (δ-). The different vitamers (compounds having similar vitamin activity) have different biologic potencies; the most active is D-α-tocopherol, and it is usual to express vitamin E intake in milligrams of D-α-tocopherol equivalents.

OCCURENCE AND SOURCES

γ-Tocopherol can be found in corn oil, soybean oil, margarine, and dressings. α-tocopherol, the most biologically active form of vitamin E, is the second-most common form of vitamin E in the diet. Good dietary sources of vitamin E include nuts such as almonds, peanuts and hazelnuts, and vegetable oils such as sunflower, wheat germ, safflower, corn and soybean oils. Sunflower seeds and green leafy vegetables such as spinach and broccoli also contain vitamin E. Other sources include tomato, avocado, spinach, asparagus, Swiss chard, and broccoli.

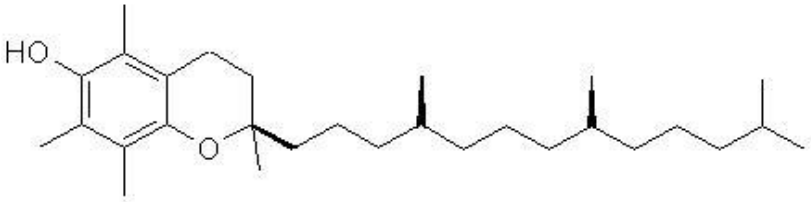


Figure 6: α-tocopherol

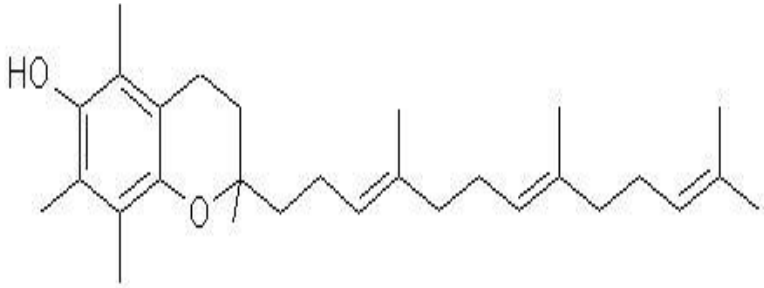


Figure 7: alpha-Tocotrienol

FUNCTIONS AND SIGNIFICANCE

1. Vitamin E does not have precisely defined metabolic function. The main function of vitamin E is as a free radical trapping antioxidant in cell membranes and plasma lipoproteins. Due to the potent antioxidant properties of tocopherols, the influence of α-tocopherol is believed to be associated with oxidative stress. It protects body tissue from damage caused by free radicals. Free radicals can harm cells, tissues, and organs.
2. α-Tocopherol is also likely involved in strengthening several aspects of cell-mediated immunity. The body also needs vitamin E to help keep the immune system strong against viruses and bacteria.
3. As an enzymatic activity regulator, for instance α-tocopherolinhibits protein kinase C.
4. Vitamin E also plays a role in neurological functions and inhibit ion of platelet coagulation.
5. Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids.
6. Vitamin E thickens endometrium.
7. Vitamin E protects red blood cells and helps prevent destruction of vitamin A and C.

VITAMIN E RELATED DISEASES

Vitamin E deficiency is rare. Instead, there are three specific situations when a vitamin E deficiency is likely to occur

1. Premature, very low birth weight infants

2. Rare disorders of fat metabolism
3. Fat malabsorption

Severe deficiency symptoms include vitamin E deficiency-induced ataxia, peripheral neuropathy, muscle weakness, and damage to the retina of the eye. Clinical evidence suggests that vitamin E supplementation may be beneficial for managing age-related macular degeneration and fatty liver diseases secondary to type 2 diabetes mellitus.

VITAMIN K

Naturally occurring forms of vitamin K include phylloquinone (vitamin K1) and a family of molecules called menaquinones (MKs or vitamin K2). Vitamin K is known as the “blood-clotting vitamin” for its important role in healing wounds. The "K" is derived from the German word koagulation. Vitamin K was discovered as a result of investigations to determine the cause of a bleeding disorder.It is a group of structurally similar, fat-soluble vitamins the human body requires for the complete of certain proteins that are prerequisites for blood coagulation.

STRUCTURE: Chemically, the vitamin K family comprises of 2-methyl-1, 4-naphthoquinone (3-) derivatives. Vitamin K includes two natural vitamers: vitamin K_1 and vitamin K_2. Vitamin K_2, in turn, consists of a number of related chemical subtypes, with differing lengths of carbon side chains made of isoprenoid groups of atoms.Vitamin K_1, (Figure 8) also known as **phylloquinone** or **phytomenadione** is synthesized by plants. It may be thought of as the "plant" form of vitamin K. It is active as a vitamin in animals and performs the classic functions of vitamin K, including its activity in the production of blood-clotting proteins. Animals may also convert it to vitamin K_2 (Figure 9).Bacteria in the gut flora can also convert K_1 into vitamin K_2 **(menaquinone),** with differing lengths of side-chain. Moreover, **menadione, menadiol (Figure 10),** and **menadioldiacetate, are** synthetic compounds that can be metabolized to phylloquinone.

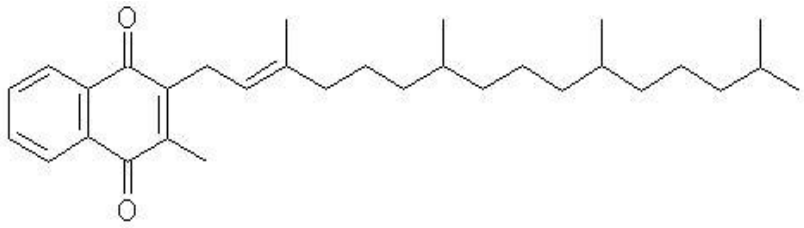


Figure 8: Vitamin K1 (phylloquinone)

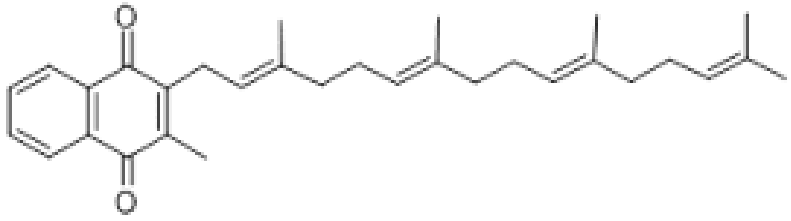


Figure 9: Vitamin K2 ***(menaquinone)***

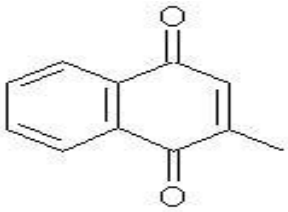


Figure 10: Vitamin K3 (Menadione)

OCCURRENCE AND SOURCES

Vitamin K is widely distributed in the diet and it is absorbed from the small intestine with the assistance of bile acids. Vitamin K_1, functions as an electron acceptor in photosystem I during photosynthesis. For this reason, vitamin K_1 is found in large quantities in the photosynthetic tissues of plants (green leaves, and dark green leafy vegetables such as Brussels sprouts, parsley, bokchoy, asparagus, cabbage, turnip, broccoli, lettuceand spinach), but it occurs in far smaller quantities in other plant tissues (roots, fruits, etc.). It is also found in avocados, plums, raspberry, soyabeans, dairy products and eggs, while most menaquinones are usually found in animal livers and fermented foods. Interestingly,

Vitamin K is also manufactured by the bacteria that inhabited the human large intestine and appears to be absorbed there too.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin K is the essential cofactor for the carboxylation of glutamic acid residues in many vitamins K-dependent proteins (VKDPs) that are involved in blood coagulation, bone metabolism, prevention of vessel mineralization, and regulation of various cellular functions.
2. Vitamin K plays an important role in bone formation. It is also involved in the prevention of bone loss. Vitamin K modifies the protein osteocalcin and makes it able to bind to calcium. Calcium can then aid to form the bone matrix.
3. Recent research has shown that vitamin K also works with vitamin D to facilitate the function of osteoblasts, the bone building cells. On the other hand, it works to inhibit the production of osteoclasts.
4. Protein Gas6 is an important regulator for cell growth, proliferation and for preventing cell death.

VITAMIN K RELATED DISEASES

1. **Haemorrhagic disease of the newborn**, also known as **vitamin K deficiency bleeding** (**VKDB**), is a coagulation disturbance in newborn infants due to vitamin K deficiency.
2. Vitamin K_1 deficiency can result in coagulopathy, a bleeding disorder. Symptoms of K_1 deficiency include anemia, bruising, and bleeding of the gums or nose in both sexes, and heavy menstrual bleeding in women.
3. Vitamin K deficiency may impair the activity of VKDPs and increase the risk of osteoporosis and fractures. Vitamin K_2 deficiency is associated with the inhibition of calcification and arterial stiffening of arteries.
4. Abnormal mineralization of blood vessels increases with age and is a major risk factor for cardiovascular disease. Vitamin K inadequacy may inactivate several VKDPs that inhibit the formation of calcium precipitates in vessels.

3.5 STRUCTURE, OCCURRENCE AND FUNCTIONS OF WATER SOULBLE VITAMINS

3.5.1 VITAMIN B COMPLEX

Vitamin B, frequently called the vitamin B complex, consists of a whole range of different compounds, some of which have similar functions and work together. However, unlike groups of compounds forming vitamins E and K, the B vitamins are sufficiently different from one another to be given individual names or numbers. Except for vitamin B_{12}, the body can only store limited amounts of B vitamins and because they are all water-soluble, excess of vitamin can be easily excreted in the urine. Their water-solubility also accounts for the fact that they are easily lost in cooking. They are described as follows:

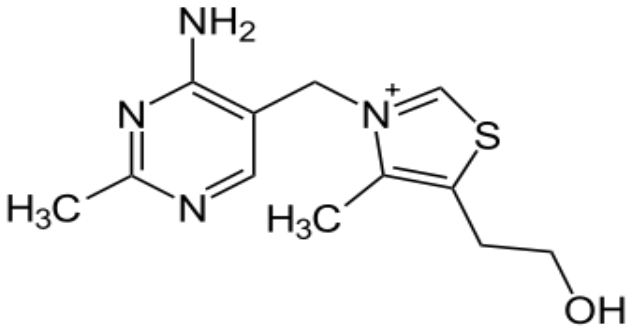
VITAMIN B_1 **(THIAMIN):** All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which the body uses to produce energy. These B vitamins, often referred to as B-complex vitamins, also help the body to metabolize fats and protein. These vitamins are water soluble, meaning that the body does not store them.

Thiamine, **thiamin**, or **vitamin** B_1, named as the "thio-vitamine" ("sulfur-containing vitamin") is one of the 8 vitamin of the B complex. Its phosphate derivatives are involved in many cellular processes. The best-characterized form is thiamine pyrophosphate (TPP), a coenzyme. Vitamin B_1, also called thiamine or thiamin, is one of eight forms of vitamin B.

Thiamine is usually considered as the transport form of the vitamin. There are five known natural thiamine phosphate derivatives: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), also sometimes called thiamine pyrophosphate (TPP), thiamine triphosphate (ThTP), and the recently discovered adenosine thiamine triphosphate (AThTP), and adenosine thiamine diphosphate (AThDP). The coenzyme role of thiamine diphosphate is well-known and extensively characterized.

STRUCTURE

Thiamine is a colorless organosulfur compound with a chemical formula$C_{12}H_{17}N_4OS$ (Figure 11). Its structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and insoluble in less polar organic solvents.



Structure of Vitamin B_1 (thiamine)

OCCURRENCE AND SOURCES

Thiamine occurs widely in foods, but generally in small amounts. In general, cereal grains are the most important dietary sources of thiamine, by virtue of their wide use. Moreover, whole grains contain more thiamine than refined grains, as thiamine is found mostly in the outer layers of the grain and processing alters thiamin levels. However, the most highly concentrated sources of thiamine are yeast, yeast extract, and pork. Some other foods naturally rich in thiamine are oatmeal, flax, and sunflower seeds, brown rice, whole grain rye, asparagus, kale, cauliflower, potatoes, oranges, liver (beef, pork, and chicken), and eggs.

FUNCTIONS AND SIGNIFICANCE

1. The main functions of vitamin B_1 (thiamin pyrophosphate) are linked to its role as a coenzyme – which activates enzymes during the biochemical processes occurring in the body. **Thiamin** has a crucial role in energy-yielding metabolism, and especially of carbohydrate, branched-chain amino acids, and fatty acids in which **Thiamin diphosphate** acts as the coenzyme.
2. Thiamin triphosphate helps in conduction of nerve impulses. Additionally, in *E. coli*, it seems to play a role in response to amino acid starvation.

VITAMIN B1 RELATED DISEASES

1. Thiamin deficiency can result from poor dietary intake, inadequate provision in parenteral nutrition, reduced gastrointestinal absorption, increased metabolic requirements, or excessive loss of thiamin. Chronic alcohol consumption is the primary cause of thiamin deficiency in industrialized countries.
2. Thiamine deficiency would seem to adversely affect all of the organ systems; however, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism. Well-known syndromes caused by

thiamine deficiency include beriberi, Wernicke-Korsakoff syndrome, and optic neuropathy.

3. Vitamin B_1 (thiamin) deficiency is rare, but can occur in people who get most of their calories from sugar or alcohol. People with thiamin deficiency have difficulty digesting carbohydrates, causing a loss of mental alertness, difficulty breathing, and heart damage.
4. Thiamin deficiency and decreased thiamin-dependent enzyme activity are associated with Alzheimer's disease.

VITAMIN B_2 (RIBOFLAVIN)

Riboflavin (**vitamin B_2**) formerly knowm as vitamin G is part of the vitamin B group. It is the precursor of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) and as such required for a variety of flavoprotein enzyme reactions. The word comes from "ribose" (the sugar whose reduced form, ribitol, forms part of its structure) and “flavin” comes from the Latin “flavus,” meaning yellow. So, vitamin **B_2** gets its name from its color. This can be seen when high supplementation occurs, as the urine becomes bright yellow as the excess riboflavin is excreted.

STRUCTURE

Riboflavin's chemical formula is $C_{17}H_{20}N_4O_6$ **(Figure 12)**. It is a polar and therefore water-soluble compound. The polar nature of molecules arises from the fact that it includes its two polar bonds between carbon and oxygen and between hydrogen and nitrogen Riboflavin's chemical structure includes four ‘H-Bonds' between hydrogen and oxygen. The structure of Vitamin **B_2** features three benzene rings, an abundance of six tetrahedron bond shapes,

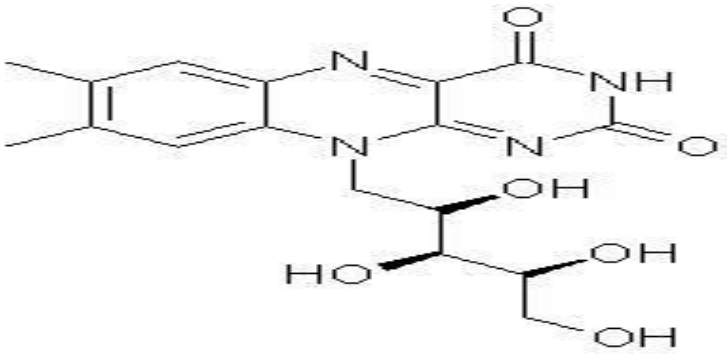


*Figure 12: The chemical structure of Riboflavin (Vitamin **B_2**)*

OCCURRENCE AND SOURCES

The best sources of riboflavin that provide riboflavin without fortification are milk, cheese, eggs, leaf vegetables, liver, kidneys, legumes, mushrooms, almonds, brewer's yeast, organ meats, mushrooms, soybeans, yogurt, eggs, broccoli, brussels sprouts, and spinach. Flours and cereals are often fortified with riboflavin.

Riboflavin is destroyed by light, so food should be stored away from light to protect its riboflavin content. While riboflavin is not destroyed by heat, it can be lost in water when foods are boiled or soaked. Riboflavin is also best absorbed when taken between meals.

Synergistic NutrientsNutrients that can help with absorption of vitamin **B_2** are vitamins A, **B_1**, **B_3**, **B_5**, **B_6**, and **B_{12}** as well as biotin, chromium, copper, cysteine, folate, glutathione, iron, magnesium, phosphate, potassium, and zinc.

FUNCTIONS AND SIGNIFICANCE

The active forms of riboflavin flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) plays its vital role in metabolism as the coenzymes in series of biochemical reactions:

1. The primary coenzyme form of vitamin B_6 (pyridoxal phosphate) is FMN dependent.
2. They act as electron carriers in a number of oxidation-reduction (redox) reactions involved in energy production and in numerous metabolic pathways. Oxidation of pyruvate, α-ketoglutarate, and branched-chain amino acids requires FAD.
3. Fatty acyl CoA dehydrogenase requires FAD in fatty acid oxidation.
4. FAD is required to convert retinol (vitamin A) to retinoic acid.
5. Synthesis of an active form of folate (5-methyltetrahydrofolate) is $FADH_2$ dependent.
6. FAD is required to convert tryptophan to niacin (vitamin B_3).
7. Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) by glutathione reductase is FAD dependent
8. Riboflavin acts as an antioxidant working to rid the body of free radicals. Other important functions include body growth and red blood cell production.
9. Riboflavin (in the form, FAD) is required as a cofactor for the key folate-metabolizing enzyme, tetrahydrofolatereductase. A low status of riboflavin status

may interfere with the metabolism of folate.

VITAMIN B_2 RELATED DISEASES

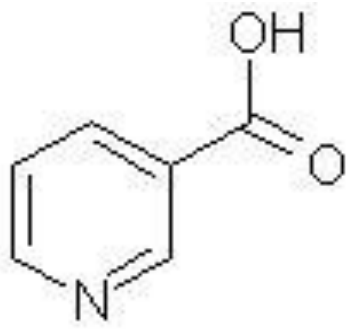
1. Riboflavin deficiency can affect multiple pathways involved in the metabolism of vitamin **B_6**, folate, niacin, and iron.
2. Riboflavin deficiency (also known as ariboflavinosis) results in stomatitis symptoms include painful red tongue with fissured lips (cheilosis) and sore throat involving in Flammarion of the corners of the mouth (angular stomatitis).
3. Oily scaly skin rashes appears on the scrotum, vulva, lip, or the nasolabial folds. The eyes can become itchy, watery and sensitive to light.
4. Mild to moderate riboflavin deficiency results in an anemia with normal cell size and normal hemoglobin content (i.e. normochromic normocytic anemia), due to interference with iron absorption.
5. Riboflavin deficiency has been linked to pre-eclampsia in pregnant women. This condition may progress to eclampsia and cause severe bleeding and death. It can result in birth defects including limb deformities and congenital heart defects.
6. In other animals, riboflavin deficiency results in lack of growth, weakness, ataxia, and inability to stand.

VITAMIN B_3 (NIACIN)

Niacin also known as **vitamin B_3** and **nicotinic acid** was discovered as a nutrient and is not strictly a vitamin since it can be synthesized in the body from the essential amino acid tryptophan. This vitamin is the generic descriptor for two vitamers, **nicotinic acid** and **nicotinamide**. The vitamin is obtained from the diet in the form of nicotinic acid, nicotinamide and tryptophan, and its metabolic function is as nicotinamide adenine dinucleotides, NAD and NADP (serves as coenzymes). NAD converts to NADP by phosphorylation in the presence of the enzyme NAD+ kinase.

STRUCTURE

This vitamin is colorless, water-soluble solid and a derivative of pyridine, with a carboxyl group (COOH) at the 3-position (Figure 13). Other forms of vitamin B_3 include the corresponding amide and nicotinamide ("niacinamide") (Figure 14), where the carboxyl group has been replaced by a carboxamide group ($CONH_2$), as well as more complex amides and a variety of esters.



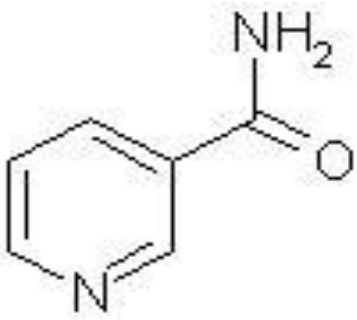


Figure 13: NICOTINIC ACID *Figure 14: NICOTINAMIDE*

OCCURRENCE AND SOURCES

Both forms of niacin occur widely in nature in a variety of foods, including liver, chicken, beef, fish, cereal, peanuts, and legumes. It is also synthesized from tryptophan, an essential amino acid found in most forms of protein.

Some of the major sources of niacin are:

Animal products: liver, chicken, chicken, breast, beef, fish (tuna, salmon), eggs
Fruits and vegetables: avocados, dates, tomatoes, leaf vegetables, broccoli, carrots, sweet potatoes, asparagus
Seeds: nuts, whole grain products, legumes
Fungi: mushrooms, brewer's yeast
Other: beer, Peanut butter , Tofu

FUNCTIONS AND SIGNIFICANCE

1. Niacin and nicotinamide are dietary precursors of the coenzymesnicotinamide adenine dinucleotide (NAD), which can be phosphorylated (NADP) and reduced (NADH and NADPH). NAD participates actively in oxidation-reduction (redox) reactions and non-redox reactions.
2. NAD is the source of ADP-ribose for the **ADP-ribosylation** of proteins and poly ADP-ribosylation of nucleoproteins involved in the DNA repair mechanism thus, NAD is critical for genome stability. Several studies, suggest a possible role for niacin in cancer prevention. It facilitates stress responses, reduction of tiredness and fatigue.
3. It is also involved in energy-yielding metabolism.

4. Niacin plays significant role in the functioning of the nervous system as well as in the maintenance of normal skin and mucous membranes.
5. At pharmacologic doses, niacin, but not nicotinamide, improves the lipid profile and reduces coronary events and total mortality in patients at high risk for coronary heart disease. Several clinical trials have explored the cardiovascular benefit of niacin in combination with other lipid-lowering medications.

VITAMIN B_3 RELATED DISEASES

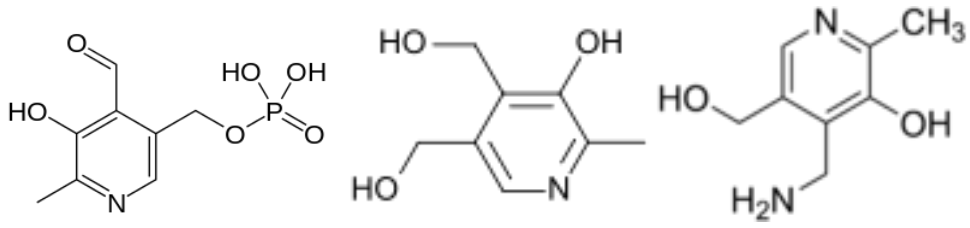
1. Niacin deficiency arises due to low oral intake, poor bioavailability from unlined grains, defective tryptophan absorption, metabolic disorders, and the long-term use of chemotherapeutic treatments.
2. Symptoms of mild niacin deficiency include: indigestion due to slow metabolism, fatigue, canker sores, vomiting, depression and intolerance to cold.
3. Severe deficiency, called pellagra is characterized by a photosensitive dermatitis, hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue, digestive disturbances. As the condition progresses, there is dementia, possibly diarrhea, and, if untreated, death.
4. Hartnup disease is a hereditary nutritional disorder resulting in niacin deficiency.
5. Niacin synthesis is also deficient in carcinoid syndrome, because of metabolic diversion of its precursor tryptophan to form serotonin.

VITAMIN B_6 **:** Vitamin B_6 is a water-soluble vitamin(Figure 15). Six common forms of vitamin B_6 are known, namely pyridoxal, pyridoxine (pyridoxol), pyridoxamine, and their phosphorylated forms. The phosphate ester derivative pyridoxal 5'-phosphate (PLP) is the bioactive coenzyme form in over 4% of all enzymatic reactions mostly involved in amino acid metabolism. Several medications, including anti-tuberculosis drugs, anti-parkinsonians, nonsteroidal anti-inflammatory drugs, and oral contraceptives, may interfere with vitamin B6 metabolism.

STRUCTURE

Vitamin B_6has the structure, of 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine. The trivial name "pyridoxine", proposed for this compound by P. György, came into general use as a synonym for "vitamin B-6". Two other natural compounds possessing vitamin B-6 *activity was recognized as the aldehyde, or 4-formyl analogue of pyridoxine, and the*

corresponding amine, or 4-aminomethyl analogue were designated "pyridoxal" and "pyridoxamine" respectively.It was demonstrated that a phosphoric derivative of pyridoxal, later identified as pyridoxal 5'-phosphate, is the coenzyme of a large group of specific enzymes catalysing reactions of amino-group transfer, decarboxylation and other metabolic transformations.



A) Pyridoxal phosphate B) Pyridoxine c) Pyridoxamine

Figure 15: Forms of Vitamin B6

OCCURRENCE AND SOURCES

Vitamin B_6 is widely distributed in foods in both its free and bound forms. Cooking, storage, and processing leads to losses of vitamin B_6 depending on the form of vitamin present in the food. Plant foods contain mostly pyridoxine, which is far more stable than the pyridoxal or pyridoxamine found in animal foods. Vitamin B_6 is found in the germ and aleurone layer of grains, and milling results in the reduction of vitamin B_6 in white flour.

Foods that contain large amounts of vitamin B_6 include pork, turkey, beef, bananas, chickpeas, potatoes, pistachios, Fortified ready-to-eat cereal, Chicken, Tuna, Salmon, shrimp, beef liver, milk, cheese, lentils, beans, spinach, carrots, brown rice, bran, sunflower seeds, whole-grain flour etc.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin B6 and its derivative pyridoxal 5'-phosphate (PLP) are essential to over 100 enzymes mostly involved in the protein metabolism.
2. PLP, the metabolically active form of vitamin B_6, is involved in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and function, and gene expression.

3. PLP is a required coenzyme of glycogen phosphorylase, the enzyme necessary for glycogenolysis to occur.
4. PLP is an essential component of enzymes that facilitate the biosynthesis of sphingolipids.
5. PLP aids in the synthesis of hemoglobin, by serving as a coenzyme.

VITAMIN B_6 RELATED DISEASES: Although Vitamin B_6 deficiency is not very usual, studies have linked vitamin B_6 deficiency with an increased risk for a range of different disorders and symptoms.

1. Several observations have suggested that low dietary intake of vitamin B_6 is associated with higher risk of heart disease.

2. An adequate vitamin B6 intake is especially important in the elderly, as this group often suffers from impaired immune function.

3. Clinical deficiency disease is rare, however, moderate deficiency results in abnormalities of tryptophan and methionine metabolism
4. Excess of vitamin $B_{6\ can}$ cause sensory neuropathy.
5. Vitamin B_6 deficiency is linked most commonly with neuropsychiatric disorders, including seizures, migraines, chronic pain and mood disorders like depression and **confusion** with inflammation of the tongue, sores or ulcers of the mouth, and ulcers of the skin at the corners of the mouth.
6. Its deficiency can worsen the symptoms of premenstrual syndrome and anemia.

VITAMIN B_{12} : The term "vitamin B_{12}" is used as a generic descriptor for the **cobalamins**—those **corrinoids** (cobalt containing compounds possessing the corrin ring) having the biologic activity of the vitamin. It is the most complex chemically of all the vitamins and the only known essential biomolecule with a stable metal-carbon bond, that is, it is an organometallic compound. VitaminB_{12} is involved in cellular metabolism in two active coenzyme forms—methylcobalamin and 5-deoxyadenosylcobalamin.

STRUCTURE : Cyanocobalamin's structure (Figure 16) is based on a corrin ring, which, although similar to the porphyrin ring found in heme, chlorophyll, and cytochrome, has two of the pyrrole rings directly bonded. The central metal ion is Co (cobalt)

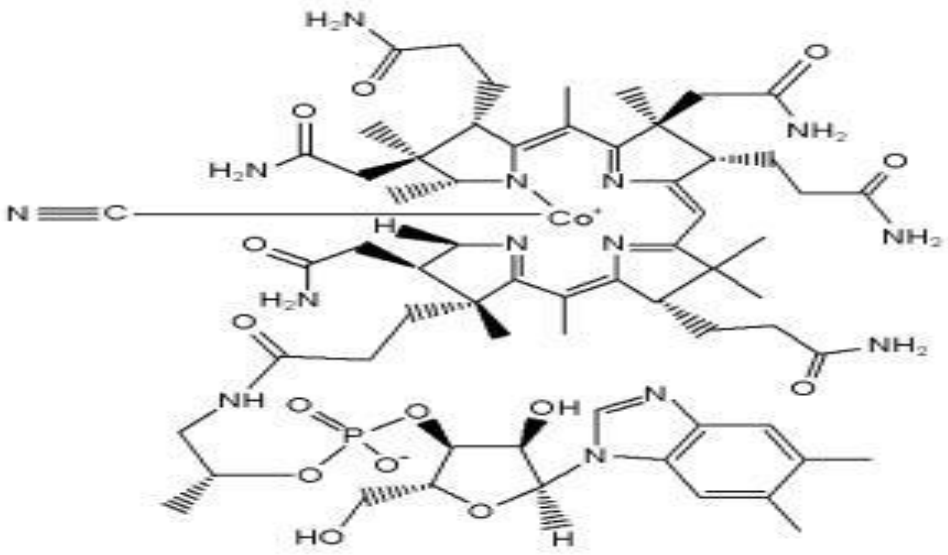


Figure 16: Structure of Vitamin B_{12}

OCCURRENCE AND SOURCES

The vitamer is produced by bacteria as hydroxocobalamin, but conversion between different forms of the vitamin occurs in the body after consumption. A common synthetic form of the vitamin is cyanocobalamin, produced by chemically modifying bacterial hydroxocobalamin. Because of superior stability and low cost this form is used in many pharmaceuticals and supplements as well as for fortification of foods. In the body, it is converted into the human physiological forms methyl cobalamin and 5'-deoxyadenosylcobalamin. Animals must obtain vitamin B_{12} directly or indirectly from bacteria will.

Vitamin B_{12} is found in most animal-derived foods, including fish and shellfish, meat (especially liver), poultry, eggs, milk, and milk products. Besides this, foods fortified with B_{12} are also dietary sources of the vitamin. Foods for which B_{12}-fortified versions are widely available include breakfast cereals, soy products, energy bars, and nutritional yeast.

FUNCTIONS AND SIGNIFICANCE

1. B_{12} is required for key biochemical reactions in the body. The products of these reactions are needed to make DNA, and many proteins, hormones, and fats.
2. Vitamin B12 or cobalamin plays essential roles in folate metabolism and in the synthesis of the citric acid cycle intermediate, succinyl-CoA.

3. B_{12} works together with folate, in many biochemical pathways. B_{12} helps to convert homocysteine into methionine, a major route for lowering homocysteine levels. This reaction also transforms folate into the active form needed to make DNA.
4. Vitamin B_{12} is an essential vitamin that's crucial for addressing **adrenal fatigue**, multiple metabolic functions including enzyme production, DNA synthesis and **hormonal balance** and maintaining healthy nervous and cardiovascular systems.
5. Vitamin B_{12} benefits the central nervous system by maintaining the health of nerve cells including those needed for neurotransmitter signaling — and helps to form the protective covering of nerves, known as the cell's myelin sheath.
6. Vitamin B_{12}, sometimes is also called as cyanocobalamins. It helps in digestion and heart health. Therefore, its deficiency can lead to both digestive disorders and an increased risk for **heart disease**.

VITAMIN B_{12} RELATED DISEASES

1. Low maternal vitamin B12 status has been associated with an increased risk of neural tube defects (NTD). Vitamin B_{12} deficiency can cause irreversible damage, specifically to the brain and nervous system. Even at levels only marginally lower than normal, symptoms such as fatigue, depression, and poor memory may appear.
2. Vitamin B_{12} deficiency can also cause symptoms of mania and psychosis.
3. Vitamin B_{12} deficiency is commonly associated with chronic stomach inflammation, which may contribute to an autoimmune vitamin B_{12} malabsorption syndrome called pernicious anemia and to a food-bound vitamin B_{12} malabsorption syndrome. Impairment of vitamin B_{12} absorption can cause megaloblastic anemia and neurologic disorders in deficient subjects.
4. Other symptoms of vitamin deficiency are:
   Yellowing of the skin, sore, red tongue, mouth ulcers, changes or loss of some sense of touch, feeling less pain, walking and visionary problems, mood changes, dementia.

VITAMIN B9 (FOLIC ACID)

Folic acid, also known as **folate**, generic term referring to both natural folates in food and folic acid, the synthetic form used in supplements and fortified food. It is one of the B vitamins that stimulates the hematopoietic system, used as a supplement during pregnancy to

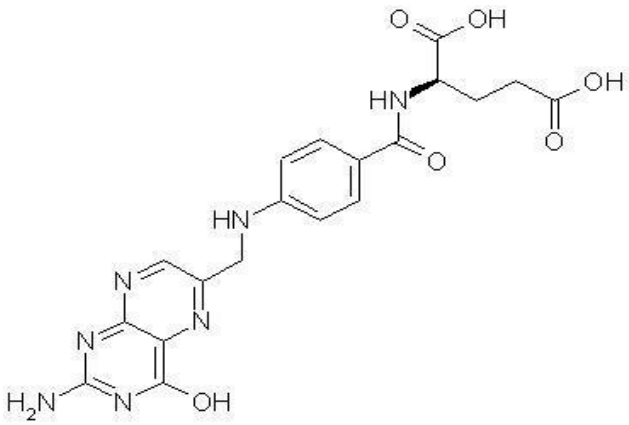
prevent neural tube defects (NTDs). The active form of folic acid (pteroyl glutamate) is tetrahydrofolate. The folates in foods may have up to seven additional glutamate residues linked by γ-peptide bonds. It is the most important medications needed in a basic health system.

STRUCTURE

The folates are a group of heterocyclic compounds based on the 4-[(pteridin-6-ylmethyl) amino] benzoic acid skeleton conjugated with one or more L-glutamate units. Folic acid (Figure 17) is a collective term for pteroylglutamic acids and their polyglutamic acid conjugates. Folate and folic acid are the preferred synonyms for pteroyl glutamate and pteroylglutamic acid, respectively.

OCCURRENCE AND SOURCES

Folic acid is pteroylmonoglutamic acid. Naturally occurring folates exist in many chemical forms; folates are found in food, as well as in metabolically active forms in the human body. Folic acid is the major synthetic form found in fortified foods and vitamin supplements. Reduced polyglutamates are found in animal and plant foods. There are many food sources containing folic acid, the most important being:Green leafy vegetables, Beans, Liver, mushrooms, spinach and grasses (POACEAE), yeast extract, whole grains, egg yolk, milk and milk products, oranges, beets, whole meal bread, beer.



17: Folic Figure Acid

FUNCTIONS AND SIGNIFICANCE

1. **Tetrahydro folic acid**, or **tetrahydrofolate**, is a folic acid derivative. The only function of folate coenzymes in the body appears to be mediating transfer of one-carbon units. Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids.
2. Folate coenzymes play a vital role in DNAmetabolism . The synthesis of DNA from its precursors (thymidine and purines) is dependent on folate coenzymes.
3. Folate coenzymes are required for the metabolism of several important amino acids, namely methionine, cysteine, serine, glycine, and histidine.
4. Folic acid intake during pregnancy has been linked to a lessened risk of neural tube defects and some other specific kinds of birth defects.

VITAMIN B9 RELATED DISEASES

1. Folate deficiency is most often caused by a dietary insufficiency; however, folate deficiency can also occur in a number of other situations for example chronic and heavy alcohol consumption.
2. Folate deficiency leads to megaloblastic anemia, which is reversible on folic acid treatment. Symptoms include headache, fatigue, weight loss, anemia, nausea, anorexia, diarrhea, insomnia, irritability; Signs are macrocytic red cells and megaloblasts in the bone marrow.
3. Vitamin deficiency may result in neural tube defects. The importance of adequate folate intake at conception and for the first 3 weeks when the neural tube closes is essential to few mothers.
4. Vitamin deficiency may result in elevated homocysteine (HCS) which is associated with increased risk for coronary disease.
5. Folate deficiencies are seen under conditions of poor nutrition, heavy alcohol.
6. B_{12} deficiency results in a folate deficiency because folates are not recycled. This is because 5-methyl tetrahydro folic acid is not converted back to tetrahydro folic acid in the absence of B_{12}. Megaloblastic anemia therefore results from a deficiency of B_{12}.
7. Folate deficiency may lead to glossitis, diarrhea, depression, confusion, fatigue, gray hair, mouth sores, poor growth and swollen tongue.

VITAMIN B_7BIOTIN: Biotin is a water-soluble B-vitamin (vitamin B_7), formerly known as vitamin H or coenzyme R. Biotin is an important component of enzymes in the body that break down certain substances like fats, carbohydrates, and others. It is also used orally for hair loss, brittle nails, skin rash in infants (seborrheic dermatitis), diabetes, and mild depression.

STRUCTURE

Biotin has the chemical formula $C_{10}H_{16}N_2O_3S$ (Figure 18). Itis a heterocyclic, S-containing monocarboxylic acid. Biotin is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring, which is an organic compound consisting of a five-membered ring containing four carbonatoms and a sulfur atom. A valeric acid substituent—straight chain alkyl carboxylic acid with the chemical formula $CH_3(CH_2)_3COOH$)—is attached to one of the carbon atoms of the tetrahydrothiophene ring.

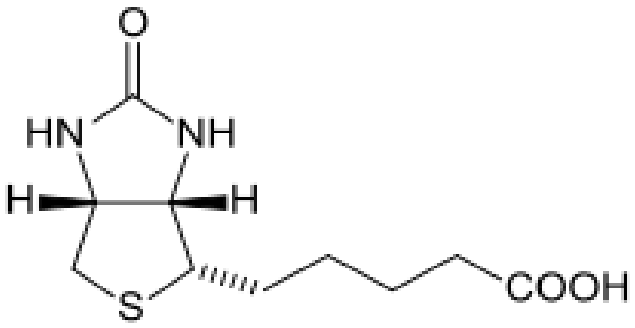


Figure 18: STRUCTURE OF BIOTIN

OCCURRENCE AND SOURCES

Biotin is consumed from a wide range of food sources in the diet. It is found in foods, either as the free form that is directly taken up by enterocytes or as biotin bound to dietary proteins. The richest sources of vitamin B_7 (biotin) are yeast, liver, egg yolk, soybeans, nuts, cereals, peanuts, swiss chard, leafy green vegetables and egg yolk.

Almonds, sunflower seeds, walnuts, sweet potatoes, strawberries, mushrooms, broccoli, avocado, spinach and carrots are other good sources rich in biotin.
Interestingly, normal intestinal bacteria synthesize biotin, but it is not known if it is absorbed.

FUNCTIONS AND SIGNIFICANCE

1. humans, vitamin B_7 (biotin) is the coenzyme attached at the active site of several 'carboxylases' enzymes. The attachment of biotin to another molecule, such as a protein (e.g. histone), is known In as 'biotinylating'.
2. Carboxylases catalyze metabolic reactions required for the synthesis of fatty acids, gluconeogenesis, the catabolism of leucine (an essential amino acid) metabolism ofamino acids and cholesterol. Biotinylating of histones plays a role in regulating DNA replication and gene expression as well as cell division and other cellular responses.
3. Biotin serves as a key element in maintaining healthy hair and skin.

VITAMIN B_7 RELATED DISEASES

Biotin deficiency is rare. Bacteria in the intestine make biotin, or vitamin B_7, and the human body actually recycles unused biotin through the intestines too, making biotin deficiency unlikely. Certain medications and dietary practices (consumption of raw eggs), however, can predispose individuals to deficiency of B_7 vitamin.

1. Biotin deficiency can arise due to various inborn genetic errors that affect the activity of biotin-related enzymes. Since endogenous biotin production occurs in the gut, dysbiosis could also upset the metabolic processes that allow the body to generate biotin on its own.
2. Inherited metabolic disorders characterized by deficient activities of biotin-dependent carboxylases are termed multiple carboxylase deficiency. It results in inhibition of the usage of biotin from the body's cells. Biochemical and clinical manifestations include: ketolactic acidosis, organic aciduria, hyperammonemia, skin rash, feeding problems, hypotonia, seizures, delay, alopecia and coma.developmental

Other deficiency symptoms include:

- Brittle and thin fingernails,
- Conjunctivitis,
- Dermatitis in the form of a scaly, red rash around the eyes, nose, mouth, and genital area,
- Neurological symptoms in adults, such as depression, lethargy, hallucination, and numbness and tingling of the extremities.

VITAMIN B_5

Vitamin B_5, otherwise known as pantothenic acid or pantothenate is also commonly called the anti-stress nutrient because it plays a role in the production of stress hormones by the adrenal glands. Pantothenic acid is an essential nutrient.

STRUCTURE

The molecular formula is butyryl-beta-alanine ($C_9H_{17}NO_5$) and it is the **amide** between D-pantoate and beta-alanine; its **IUPAC** name is 3-[(2,4-dihydroxy-3,3-dimethylbutanoyl)amino]propanoic acid (Figure 19).

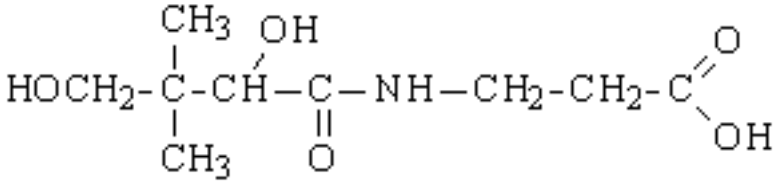


Figure 19: Vitamin B_5, pantothenic acid

OCCURRENCE AND SOURCES

The word 'pantothenic' is derived from the Greek word pantos, meaning everywhere. This implies that it is ubiquitously found in foods of plant and animal origin, and its dietary deficiency is rare. Small quantities of pantothenic acid are found in most foods. The major food source of pantothenic acid is meat. The concentration of vitamin B_5 found in human muscle is about double that in other animals' muscle. Whole grains are another good source of the vitamin, but milling removes much of the pantothenic acid, as it is found in the outer layers of whole grains. Vegetables, such as avocados kale and other vegetables in the cabbage family and broccoli, also have vitamin in abundance. In animal feeds, the most important sources are alfalfa, cereal, fishmeal, peanut meal, molasses, milk, mushrooms, rice, wheat bran and yeasts.

FUNCTIONS AND SIGNIFICANCE

1. Pantothenic acid is a precursor in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group carrier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell.

2. Coenzyme A is essential to many biochemical reactions that sustain life. Also, the phosphopantetheinyl moiety of coenzyme A is required for the biological activity of several proteins, including the acyl-carrier protein involved in fatty acid synthesis.
3. Vitamin B_5 plays a pivotal role in the breakdown of fats, carbohydrates and proteins for providing energy to the cells.
4. It is also required for the production of red blood cells, steroids, neurotransmitters and stress related hormones.
5. It helps in maintaining a healthy digestive tract and also assists the body in making an optimal use of vitamin B_2.
6. Pantothenic acid is used in the synthesis of cholesterol.CoA is important in the energy metabolism and in the biosynthesis of many important compounds such as fatty acids, cholesterol, and acetylcholine.
7. Pantothenic acid in the form of CoA is also required for acylation and acetylation, involved in signal transduction and enzyme activation and deactivation, respectively.

VITAMIN B_5 RELATED DISEASES

1. Pantothenic acid deficiency is exceptionally rare the symptoms are similar to other B vitamin deficiencies including:
2. Impaired energy production, due to low CoA levels, causing symptoms of irritability, fatigue, and apathy
3. Acetylcholine synthesis is impaired resulting in neurological symptoms, like numbness, paresthesia, and muscle cramps.
4. Deficiency in pantothenic acid can also cause hypoglycemia, or an increased sensitivity to insulin.
5. Additional symptoms could include restlessness, malaise, sleep disturbances, nausea, vomiting, and abdominal cramps.

3.5.2 VITAMIN C or ASCORBIC ACID

Ascorbic acid is a naturally occurring organic six carbon compounds related to glucose with antioxidant properties. It is an essential nutrient in human diets. The name, ascorbic acid, is derived from *a-* (meaning "no") and *scorbutus* (scurvy), the disease caused by deficiency of vitamin C. It is derived from glucose, hence many animals are able to produce it, but humans require it as part of their nutrition. Other vertebrates which lack the ability to

produce ascorbic acid include some primates, guinea pigs, bats, some fishes and birds, all of which require it as a dietary micronutrient. Vitamin C comprises several vitamers that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid.

STRUCTUR

Vitamin C, Ascorbic acid or ascorbate is a weak organic acid that appears as a white, crystalline compound (Figure 20). Structurally, it is related to the six-carbon sugarglucose, from which most animals are able to derive the molecule in a four-step process. The ionized form of ascorbic acid is known as *ascorbate.* The ascorbate ion represents the *pharmacophore* of vitamin C; that is, the structural feature (or set of features) responsible for the molecule's biological activity. Presence of the ascorbate ion contributes to vitamin C's role as a strong reducing agent *(antioxidant).*

Ascorbate occurs in two forms, both of which are mirror images of the same molecular structure *(enantiomers).* Vitamin C is specifically the L-enantiomer of ascorbate; the D-enantiomer has no physiological significance. L-ascorbate naturally occurs either attached to a hydrogen ion, forming *ascorbic acid*, or joined to a metal ion, forming a mineral ascorbate.

When L-ascorbate carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate, which can then be converted back to the active form in the body by specialized enzymes and the peptide glutathione.

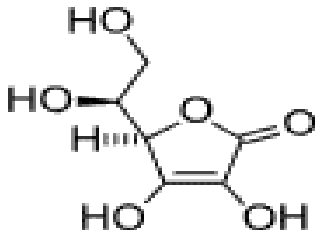


Figure 20: Ascorbic acid (Vitamin C)

OCCURRENCE AND SOURCES

The richest natural sources of vitamin C are fruits and vegetables. Interestingly richest sources are not citrus juices, but broccoli, Brussel sprouts and peppers. The other sources

high in ascorbate are citrus products, potatoes, and tomatoes. It is also present in meat, especially liver. Vitamin C is the most widely taken nutritional supplement and is available in a variety of forms, including tablets, drink mixes, crystals in capsules or naked crystals. Cereal products, grains, and meats contain very little amount of vitamin C.The majority of species of animals (but *not* humans, guinea pigs or fruit bats) and plants synthesize their own vitamin C. Therefore, some animal products can be used as sources of dietary vitamin Vitamin C is most present in the liver and least present in the muscle. It is present in human breast milk and only in limited quantity in raw cow's milk. All excess vitamin C is disposed of through the urinary system.

FUNCTIONS AND SIGNIFICANCE

1. The most prominent role of vitamin C is its immune-stimulating effect, e.g., important for defenses against infections such as common colds.
2. It also acts as an inhibitor of histamine, a compound that is released during allergic reactions.
3. As a powerful antioxidant it can neutralize harmful free radicals and it aids in neutralizing pollutants and toxins.
4. It acts as a prooxidant, reduces metals to their prooxidant form.
5. Vitamin C is helps to regenerate other antioxidants such as vitamin E.
6. Inside our bodies, vitamin C functions as an essential cofactor in numerous enzymatic reactions, e.g., in the biosynthesis of collagen, carnitine, and catecholamines, and as a potent antioxidant.
7. Vitamin C – especially in combination with zinc – is also important for the healing of wounds.
8. Vitamin C contributes to the health of teeth and gums, preventing hemorrhaging and bleeding.
9. It also improves the absorption of iron from the diet.
10. Vitamin C is also needed for the metabolism of bile acids which may have implications for blood cholesterol levels and gallstones.
11. Vitamin C plays an important role in the synthesis of several important peptide hormones, neurotransmitters and carnitine.
12. Vitamin C is also a crucial factor in the eye's ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration (AMD) and

vision-loss.

VITAMIN C RELATED DISEASES

Vitamin C deficiency results in scurvy that includes skin changes, fragility of blood capillaries, gum decay, tooth loss, and bone fracture, many of which can be attributed to deficient collagen synthesis. The first symptoms of vitamin C deficiency tend to be:Tiredness and weakness, muscle and joint pains, easy bruising, spots that look like tiny red-blue bruises on skin.

Other symptoms include:

Dry skin, splitting hair, swelling, discoloration, sudden and unexpected bleeding of gums, nosebleeds, poor healing of wounds, bleeding and severe joint pains, tooth and weight loss.

3.6 SUMMARY

- A **vitamin** is an organic compound and a vital nutrient that an organism requires in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and it must be obtained through the diet.
- Vitamin A, D, E and K are fat-soluble. These are stored in liver and adipose tissues and hence are called fat-soluble vitamins.
- Vitamin A is found in the body in three forms: retinol, retinal, and retinoic acid. Together, they are essential for vision, healthy epithelial tissues, and growth.
- Vitamin D is a steroid prohormone yielding the active derivative calcitriol, which regulates calcium and phosphate metabolism. Vitamin D deficiency leads to rickets.
- Vitamin E acts as an antioxidant, defending lipids and other components of the cells against oxidative damage and also required for the synthesis of blood clotting proteins.
- Vitamin K functions as cofactor to a carboxylase that acts on glutamate residues of clotting factor precursor proteins to enable them to chelate calcium.
- Vitamins in B-group and vitamin C are water-soluble and cannot be stored in our body as they excreted out of the body via urine and acts as enzyme cofactors.
- Thiamin has a crucial role in energy-yielding metabolism, and especially of carbohydrate in which thiamin diphosphate acts as the coenzyme.

- Riboflavin (vitamin B2) works with the other B vitamins. It is important for body growth and the production of red blood cells.
- Niacin is a B vitamin that helps to maintain healthy skin and nerves. It is also has cholesterol-lowering effects. Niacin and nicotinamide are precursors of the coenzymes nicotinamide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).
- Pyridoxal phosphate is a coenzyme for many enzymes involved in amino acid metabolism Vitamin B_{12} is required for key biochemical reactions in most of the cells in body. The products of these reactions are needed to make DNA, and many proteins, hormones, and fats. Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions of the metabolism of nucleic acids and amino acids and plays a vital role in DNA metabolism. Vitamin B_7 (biotin) is the coenzyme attached at the active site of several 'carboxylases' enzymes and catalyzes an essential metabolic reaction required for the synthesis of fatty acids, gluconeogenesis and metabolism ofamino acids.
- Pantothenic acid is used in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group car. rier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell.

- Vitamin C is a water-soluble antioxidant that maintains vitamin E and many metal cofactors in the reduced state

3.7 GLOSSARY

Acidosis: an excessively acid condition of the body fluids or tissues.

Antioxidant: An **antioxidant** is a molecule that inhibits the oxidation of other molecules.

Carotenes: an organic, strongly colored red-orange pigment abundant in plants and fruits.

Cholesterol: a sterol lipid molecule, biosynthesized by all animal cells.

Coenzyme: **coenzymes** are organic molecules that are required by enzymes to carry out catalysis.

Cofactor: A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's biological activity.

Diarrhoea:a condition in which faeces are discharged from the bowels frequently and in a liquid form.

Enzyme: **enzymes** are biological molecules (proteins) that act as catalysts.

Free radicals: an uncharged molecule (typically highly reactive and short-lived) having an unpaired valency electron.

Hormones: member of a class of signaling molecules produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behaviour.

I**mmune system:** is a network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders.

Metabolism: refer to the sum of all chemical reactions that occur in living organisms.

Nervous System: the network of nerve cells and fibres which transmits nerve impulses between parts of the body.

Pre-eclampsia: a disorder of pregnancy characterized by the onset of high blood pressure and often a significant amount of protein in the urine.

Prooxidant: A species that causes or promotes oxidation.

Retinol: **Retinol** (Vitamin A1) is one of the animal forms of vitamin A.

Steroid: An organic compound with four rings arranged in a specific configuration.

Vitamer: one of multiple related chemical compounds possessing a given vitamin activity.

3.8 SELF ASSESSMENT QUESTIONS

3.8.1 Long answer type questions

Q.1 Give a detailed account of fat-soluble vitamins.

Q.2 What are water soluble vitamins? Describe in detail.

Q.3 Discuss the diseases caused by the deficiency of vitamin B complex.

3.8.2 Short answer type questions

Q.1 Write short notes on Vitamin A deficiency?

Q.2 What are the sources of Vitamin E?

Q. 3 Write the functions of Vitamin C.

Q. 4 Discuss the role of folic acid in metabolism.

3.8.3 **Fill in the blanks:**

1. Vitamin _____are fat soluble.
2. Vitamin _____are water soluble
3. Scurvy is caused by the deficiency of _____.
4. _____are vitamers of Vitamin D.
5. Vitamin B5 is also known as_____.
6. _____ is known as the “blood-clotting vitamin” for its important role in healing wounds.
7. _____is a coenzyme for many enzymes involved in amino acid metabolism.
8. Pantothenic acid is used in the synthesis of _____.
9. _____are precursors of the coenzymesnicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).
10. _____act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids.

Answer: Fill in the blanks:

1. A,D.E.K 2. B&C 3. Vitamin C 4**.** Cholecalciferol and ergocalciferol 5. Pantothenic acid 6. Vitamin K 7. Pyridoxal phosphate 8. Coenzyme A (CoA) 9. Niacin and nicotinamide 10. Folate coenzymes

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UNIT 4: CARBOHYDRATES I

Contents

4.1 OBJECTIVES

After reading this unit you will be able to understand-

- What are carbohydrates?
- Structure, sources and the basis of their classification
- Metabolism of carbohydrates and regulation of metabolism

4.2 INTRODUCTION

Carbohydrates are the most abundant and of importance class of organic matter found on Earth because of their extensive roles in all forms of life.They comprise one of the four major classes of biomolecules along with proteins, nucleic acids, and lipids.

Carbohydrates constitute a versatile class of molecules. Energy from the sun netted by green plants, algae, and some bacteria during photosynthesis is stored in the form of carbohydrates. They are the metabolic pioneers of virtually all other biomolecules and their oxidation is the central energy-yielding pathway in most nonphotosynthetic cellsthat sustainslife. They play broad roles such as:

- Carbohydrates serve as energy stores, fuels, and metabolic intermediates.
- Ribose and deoxyribose sugars form part of the *structural framework of RNA and DNA.*
- Insoluble carbohydrate polymers are *structural elements in the cell walls of bacteria, plants and* in the connective tissues of animals. Interestingly, cellulose, the main constituent of plant cell walls, is one of the most abundant organic compounds in the biosphere.
- Complex carbohydrate polymers are covalently *linked to many proteins and lipids* [Carbohydrates linked to lipid molecules, or glycolipids, are common components of biological membranes. Proteins that have covalently linked carbohydrates are called glycoproteins. These two classes of biomolecules, together called glycoconjugates]. These glycoconjugates play key roles in mediating interactions among cells by acting as signals that determine the intracellular location or metabolic fate of these hybrid molecules and maintains the interactions between cells and other elements in the cellular environment. Recognition events are important in normal cell growth, fertilization, transformation of cells, and other processes.

All of these functions are made possible by the tremendous *structural diversity* possible within this class of molecules and other chemical characteristics as follows:

(1) The existence of at least one and often two or more asymmetric centers
(2) The ability to exist either in linear or ring structures
(3) The capacity to form polymeric structures via *glycosidic* bonds
(4) The potential to form multiple hydrogen bonds with water or other molecules in their environment.

4.3 STRUCTURE

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical formula$(CH_2O)_n$, some also contain nitrogen, phosphorus, or sulfur.

4.3.2 CLASSIFICATION

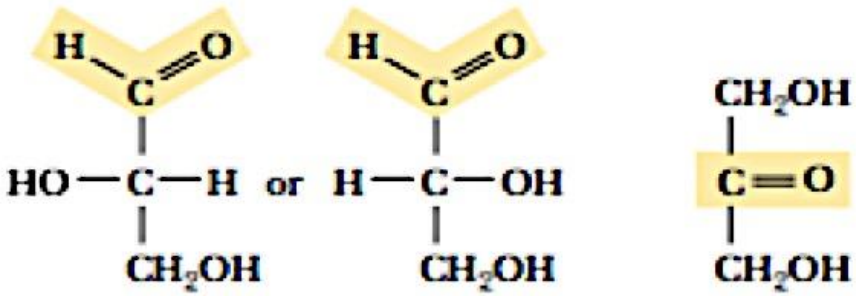
Carbohydrates are generally classified into three major classes: monosaccharides (and their derivatives), oligosaccharides, and polysaccharides (the word "saccharide" is derived from the Greek sakcharon, meaning "sugar"). The monosaccharides are also called simple sugars and have the formula $(CH_2O)_n$, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose. Monosaccharides of more than four carbons tend to have cyclic structures.Monosaccharides cannot be broken down into smaller sugars under mild conditions.

Oligosaccharides originate their name from the Greek word *oligo*, meaning "few," and consist of from two to ten simple sugar molecules joined by characteristic linkages called glycosidic bonds. Disaccharides are common in nature, and trisaccharides also occur frequently. In cells, most oligosaccharides consisting of three or more units do not occur as free entities but are joined to nonsugar molecules (lipids or proteins) in glycoconjugates.

As name suggests, polysaccharides are polymers of the simple sugars and their derivatives containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units. They may be either linear such as cellulose or branched polymers such as glycogen and may contain hundreds or even thousands of monosaccharide units. Their molecular weights range up to 1 million or more.

4.3.3 MONOSACCHARIDES: STRUCTURE AND NOMENCLATURE

Monosaccharides consist typically of three to seven carbon atoms and are described either as **aldoses** or **ketoses,** depending on whether the molecule contains an aldehyde function or a ketone group. They are important fuel molecules as well as building blocks for nucleic acids. The smallest monosaccharides, for which n = 3 is, glyceraldehyde (aldose), and dihydroxyacetone (ketose) (Figure 1). Monosaccharides with four, five, six, and seven carbon atoms in their backbones are called, respectively, tetroses, pentoses, hexoses, and heptoses. There are aldoses and ketoses of each of these chain lengths: aldotetroses and ketotetroses, aldopentoses and ketopentoses, and so on.

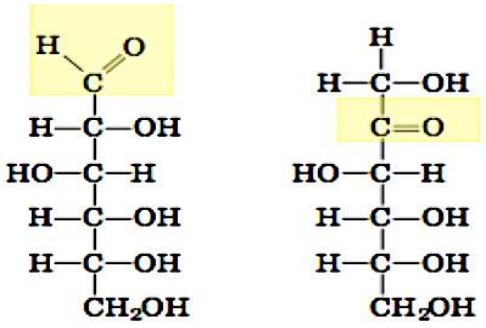


L-Isomer **D-Isomer**

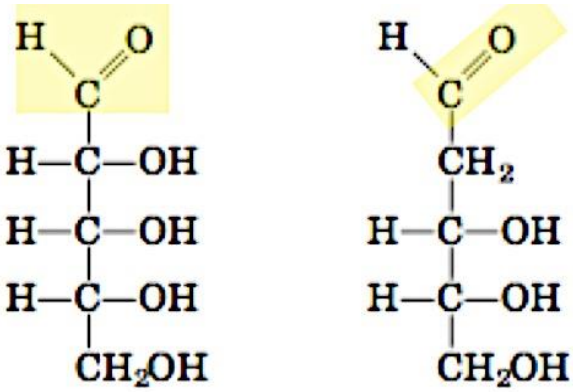
GLYCERALDEHYDE *DIHYDROXYACETONE*

Figure 1 Structure of a simple aldose (glycer aldehyde) and a simple ketose (dihy- droxyacetone)

The hexoses, which include the aldohexose D-glucose and the ketohexose D-fructose (Fig. 2A), are the most common monosaccharides in nature. The aldopentoses D-ribose and 2-deoxy-D-ribose are components of nucleotides and nucleic acids (Fig. 2B). Nevertheless, sugars from all these classes are important in metabolism.



(A) **D-Glucose (aldohexose) D-Fructose (ketohexose)**



(B) **D-Ribose (aldopentose) 2-Deoxy-D-ribose (aldopentose)**

Figure 2 (A)*Two common hexoses.****(B)*** *The pentose components of nucleic acids*

4.3.3.1Monosaccharides Have Asymmetric Centers

All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms. The simplest aldose, glyceraldehyde, contains one chiral center (the middle carbon atom) and therefore has two different optical isomers (D-Glyceraldehyde and L-Glyceraldehyde), or enantiomers (Fig. 1). By convention, one of these two forms is designated theDisomer, the other the L isomer. To represent three-dimensional sugar structures on paper, we often use Fischer projection formulas (Fig. 7–2)in which a molecule, atoms joined to an asymmetric carbon atom by horizontal bonds project out of the plane of the paper, toward the reader (you); vertical bonds project behind the plane of the paper, away from the reader (you).

Generally, a molecule with n chiral centers can have 2^nstereoisomers. Glyceraldehyde has 2^1= 2stereoisomers.

For monosaccharides with two or more asymmetric carbons, the prefix D or L refers to the configuration of the highest numbered asymmetric carbon (the asymmetric carbon most distant from the carbonyl carbon). A monosaccharide is designated D if the hydroxyl group on the highest numbered asymmetric carbon is drawn to the right in a Fischer projection, as in D -glyceraldehyde (Figure 1).

**Note that the designation D or L merely relates the configuration of a given molecule to that of glyceraldehyde and does not specify the sign of rotation of plane-polarized light. If the sign of optical rotation is to be specified in the name, the Fischer convention of D or L*

designations may be used along with a (plus) or (minus) sign.

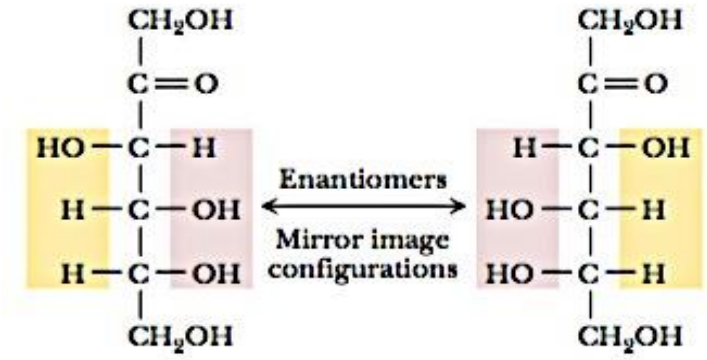
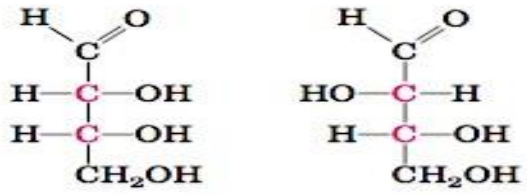


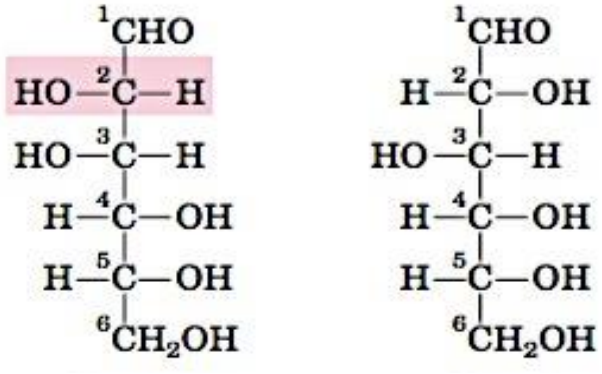
Figure 3 D-Fructose and,L-fructose, an enantiomeric pair.

D-FructoseL-fructose

The four and five-carbon ketoses are designated by inserting "ul" into the name of a corresponding aldose; for example, D-ribulose is the ketopentose corresponding to the aldopentose D-ribose. The keto- hexoses are named otherwise: for example, fructose and sorbose. According to convention, the D- and L-forms of a monosaccharide are *mirror images* of each other, as shown in Figure 3 for fructose. Stereoisomers that are mirror images of each other are called **enantiomers,** or sometimes *enantiomeric pairs.* For molecules that possess two or more chiral centers, more than two stereoisomers can exist. Pairs of isomers that have opposite configurations at one or more of the chiral centers but that are not mirror images of each other are called **diastereomers** or diastereomeric pairs (Fig.4 A). Two sugars that differ in configuration at only one chiral center are described as **epimers.** For exampleD-glucose and D-mannose are epimers (Fig. 4 B)



(A) D-Erythrose D-Threose



(B)D-Mannose **D-Glucose**

Figure 4 (A) Diastereomeric pair (B) Epimeric pair (epimer at C-2)

4.3.3.2Cyclic Structures and Anomeric Forms

Fischer projections are useful for presenting the structures of particular monosaccharides and their stereoisomers, but they ignore one of the most interesting facets of sugar structure—*the ability to form cyclic structures with formation of an additional asymmetric center*.In aqueous solution, aldotetroses and all monosaccharides with five or more carbon atoms in the backbone occur predominantly as cyclic (ring) structures in which the carbonyl group has formed a covalent bond with the oxygen of a hydroxyl group along the chain. The formation of these ring structures is the result of a general reaction between alcohols and aldehydes or ketones to form derivatives called hemiacetals or hemiketals (Fig. 5), which contain an additional asymmetric carbon atom and thus can exist in two stereoisomeric forms. The British carbohydrate chemist Sir Norman Haworth showed that the linear form of glucose (and other aldohexoses) could undergo a*intramolecular* reaction to form a *cyclic hemiacetal.* The resulting six-membered, oxygen-containing ring is similar to *pyran* and is designated a pyranose. The reaction is catalyzed by acid (H^+) or base (OH^-) and is readily reversible. The systematic names for the two ring forms of D-glucose are α-D-glucopyranose and β-D-glucopyranose.

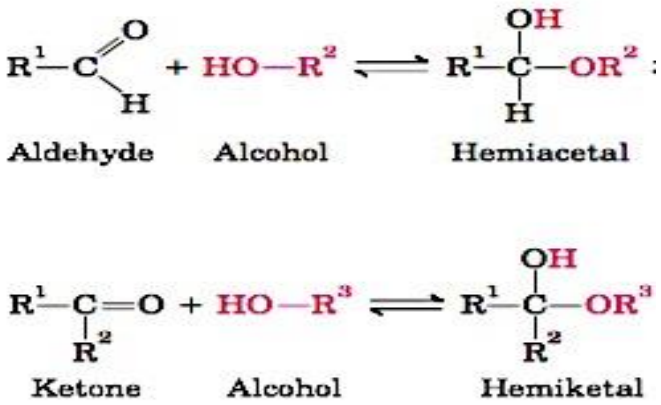


Figure 5 Formations of Hemiacetals and Hemiketals

Aldohexoses also exist in cyclic forms having five- membered rings, which, because they resemble the five- membered ring compound furan, are called furanoses. However, the six-membered aldopyranose ring is much more stable than the aldofuranose ring and predominates in aldohexose solutions. Only aldoses having five or more carbon atoms can form pyranose rings.

Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called anomers. The hemiacetal (or carbonyl) carbon atom is called the anomeric carbon. The αandβ anomers ofD-glucose interconvert in aqueous solution by a process called mutarotation. Thus, a solution of α-D-glucose and a solution of β-D-glucose eventually form identical equilibrium mixtures having identical optical properties. Mutarotation involves interconversion of αand βforms of the monosaccharide with intermediate formation of the linear aldehyde or ketone (Fig 6 & 7).

Ketohexoses also occur in and anomeric forms. In these compounds the hydroxyl group at C-5 (or C-6) reacts with the keto group at C-2, forming a furanose (or pyranose) ring containing a hemiketal linkage. D-Fructose readily forms the furanose ring (Fig. 7); the more common anomer of this sugar in combined forms or in derivatives is β-D-fructofuranose.

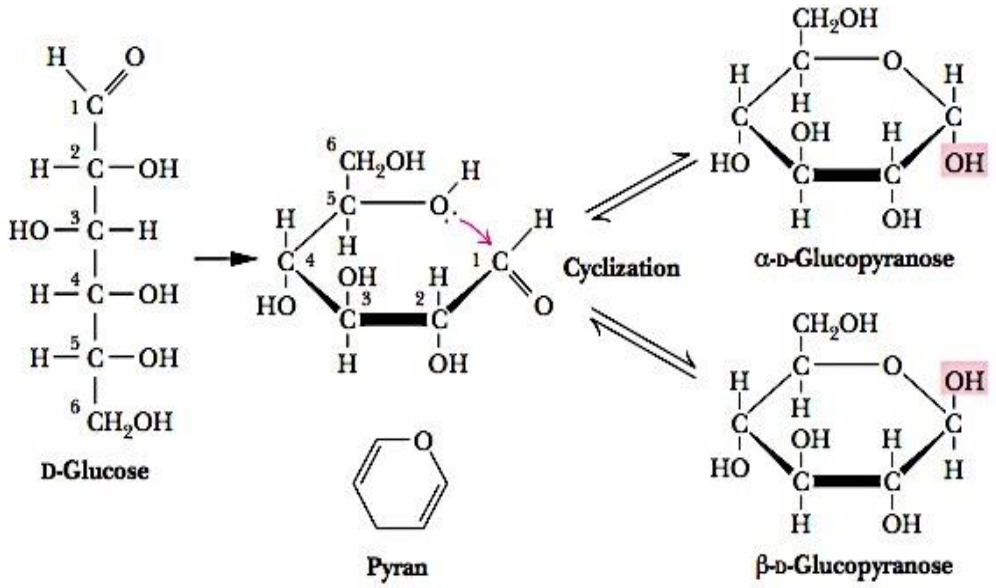


Figure 6 Formation of the two cyclic forms of D-glucose.

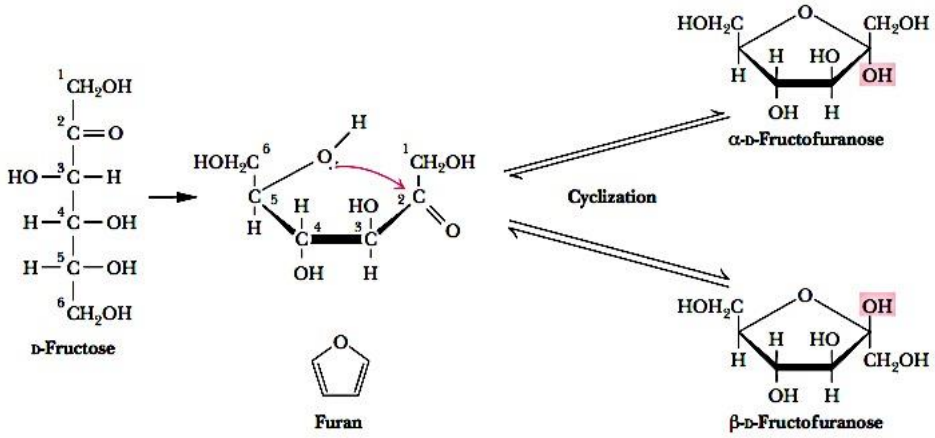


Figure 7 Formation of the two cyclic forms of D-Fructose

4.3.3.3 Haworth Projections

Haworth projections, represent pyranose and furanose structures as hexagonal and pentagonal rings lying perpendicular to the plane of the paper, with thickened lines indicating the side of the ring closest to the reader. Such **projections, that** are now widely used to represent saccharide structures (Figures 6 and 7), show substituent groups extending either above or below the ring.

The rules previously mentioned for assignment of α- and β-configurations can be applied to Haworth projection formulas. For the D-sugars, the anomeric hydroxyl group is below the

ring in the α-anomer and above the ring in the β-anomer. For L-sugars, the opposite relationship holds.

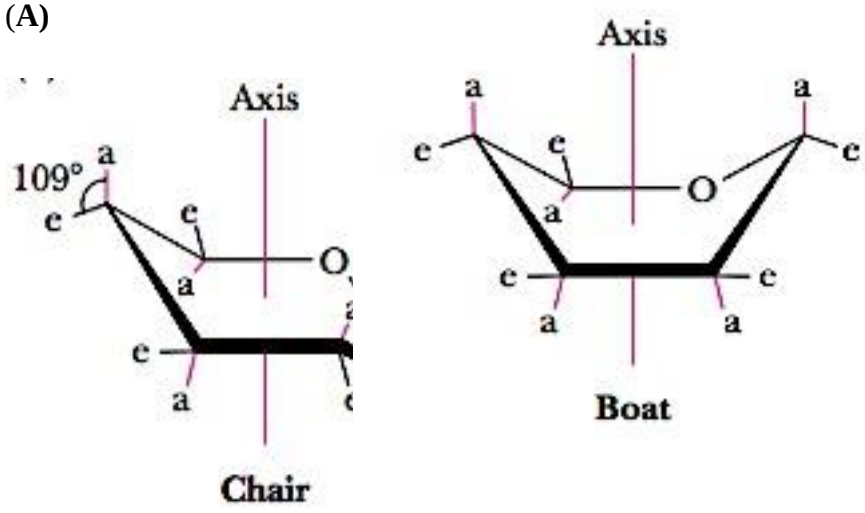
Although Haworth projections are convenient for display of monosaccharide structures, they do not accurately portray the conformations of pyranose and furanose rings. Given C-C-C tetrahedral bond angles of 109° and C-O-C angles of 111°, neither pyranose nor furanose rings can adopt true planar structures. Instead, they take on puckered conformations, and in the case of pyranose rings, the two favored structures are the **chair conformation** and the **boat conformation (**Fig 8A). The ring substituents in these structures can be **equatorial,** which means approximately coplanar with the ring, or **axial,** that is, parallel to an axis drawn through the ring as shown. Two general rules dictate the conformation to be adopted by a given saccharide unit.

(1) Bulky substituent groups on such rings are more stable when they occupy equatorial positions rather than axial positions.

(2) Chair conformations are slightly more stable than boat conformations.

For a typical pyranose, such as β-D-glucose, there are two possible chair conformations (Figure 9B). Interestingly, of all the D-aldohexoses, β-D-glucose is the only one that can adopt a conformation with all its bulky groups in an equatorial position. With this advantage of stability, β-D-glucose is the most widely occurring organic group in nature and the central hexose in carbohydrate metabolism.

(**A)**



a = axial bond e = equatorial bond

(B)

Figure 8(A) Chair and boat conformations of a pyranose sugar. (B) Two possible chair conformations of β-D-glucose.

4.3.3.4Derivatives of Monosaccharides

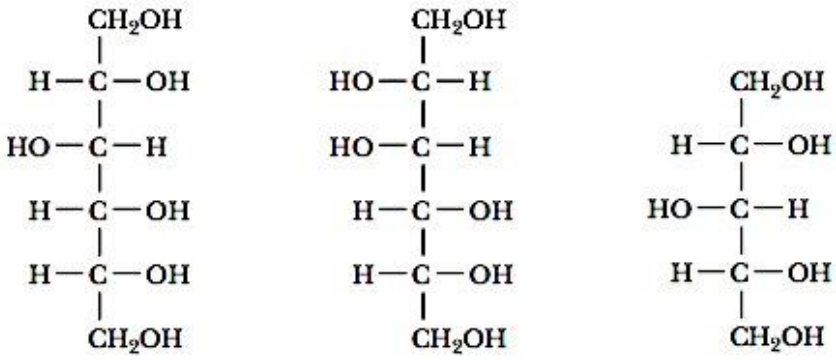
Sugar Acids

Sugars with free anomeric carbon atoms are rationally good reducing agents and reduce hydrogen peroxide, ferricyanide, certain metals and other oxidizing agents, resulting in the conversion of the sugar to a **sugar acid.**

Monosaccharides can be oxidized enzymatically at C-6, yielding **uronic acids,** such as **D-glucuronic** and **L-iduronic** acids (Figure9). L-Iduronic acid is similar to D-glucuronic acid, except for having an opposite configuration at C-5. Oxidation at both C-1 and C-6 produces **aldaric acids,** such as **D-glucaric acid.**

Sugar Alcohols

Sugar alcohols can be prepared by the mild reduction of the carbonyl groups of aldoses and ketoses. Sugar alcohols, or **alditols,** are designated by the addition of *-itol* to the name of the parent sugar (Figure 10). The alditols are linear molecules that cannot cyclize in the manner of aldoses. Nonetheless, alditols are characteristically sweet tasting, and **sorbitol, mannitol,** and **xylitol** are widely used to sweeten sugarless gum and mints.**Figure 9. Oxidation of Monosaccharides**

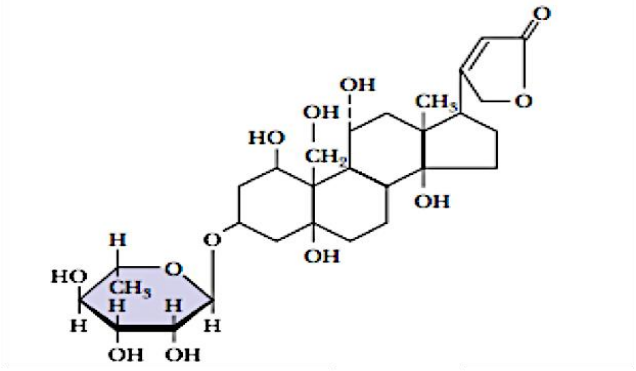


D-Glucitol (sorbitol) **D-MannitolD-Xylitol**

Figure 10Structures of some sugar alcohols

Deoxy Sugars

The **deoxy sugars** are monosaccharides with one or more hydroxyl groups replaced by hydrogens. 2-Deoxy-**D-**ribose (Figure 11), whose systematic name is 2-deoxy-**D-**erythropentose, is a constituent of DNA in all livingbeings. Deoxy sugars also occur frequently in glycoproteins and polysaccharides.**L-**Fucose and **L-**rhamnose, both 6-deoxy sugars, are components of some cell walls, and rhamnose is a component of **ouabain,** a highly toxic *cardiac glycoside* found in the bark and root of the ouabaio tree.



Ouabain

Figure 11*Deoxy sugars and ouabain. Hydrogen atoms highlighted in red show "deoxy" positions.*

Sugar Esters

Phosphate esters of glucose, fructose, and other monosaccharides are important metabolic intermediates, and the ribose moiety of nucleotides such as ATP and GTP is phosphorylated at the 5' position (Fig 12)

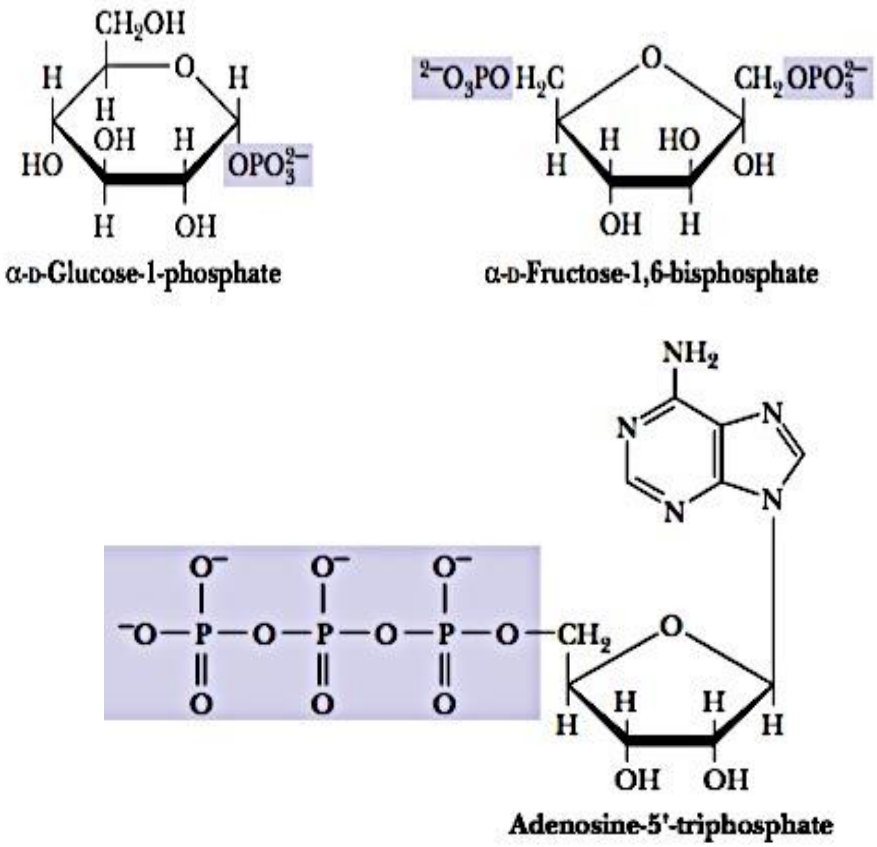


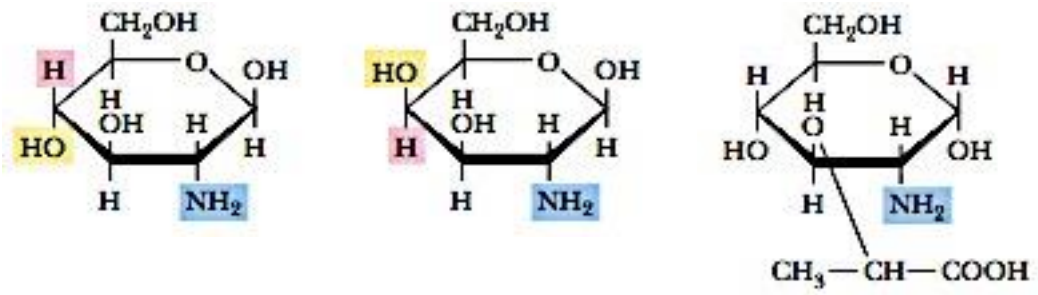
Figure 12 Sugar esters important in metabolism

Amino Sugars

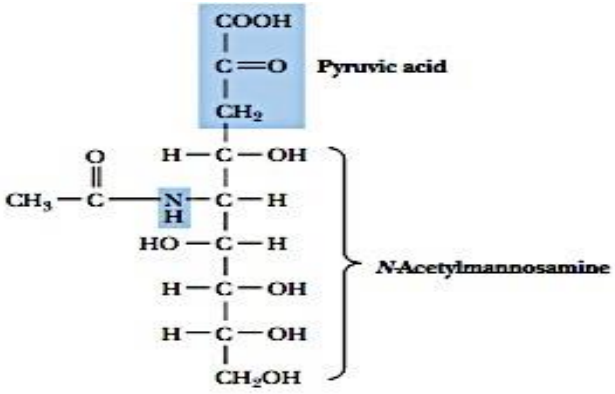
D-Glucosamineand **D-Galactosamine**-contain an amino group (instead of a hydroxyl group) at the C-2 position (Figure 13). They are found in many oligo- and polysaccharides, including *chitin,* a polysaccharide in the exoskeletons of crustaceans and insects.

Muramic acid and **Neuraminic acid-** components of the polysaccharides of cell membranes of higher organisms and also bacterial cell walls, are having glucosamines linked to three-carbon acids at the C-1 or C-3 positions. In muramic acid the hydroxyl group of a lactic acid moiety makes an ether linkage to the C-3 of glucosamine. Neuraminic acid (an *ami*ne isolated from *neur*al tissue) forms a C-C bond between the C-1 of *N*-acetylmannosamine and the C-3 of pyruvic acid (Figure13).

Sialic acids- The *N*-acetyl and *N*-glycolyl derivatives of neuraminic acid are collectively known as **Sialic acids** found widely in bacteria and animal systems.



β-D-Glucosamine β-D-Galactosamine Muramic acid



N-Acetyl-D-neuraminic acid (NeuNAc)

Figure 13 Structure of amino sugars

Acetals, Ketals, and Glycosides

Hemiacetals and hemiketals can react with alcohols in the presence of acid to form **acetals** and **ketals** (Figure 14). This reaction is a type of a *dehydration synthesis.*The pyranose and furanose forms of monosaccharides react with alcohols in the same way to form **glycosides** with retention of the α- and β-configuration at the C-1 carbon. The new bond between the anomeric carbon atom and the oxygen atom of the alcohol is called a **glycosidic bond.** Glycosides are named according to the parent monosaccharide.

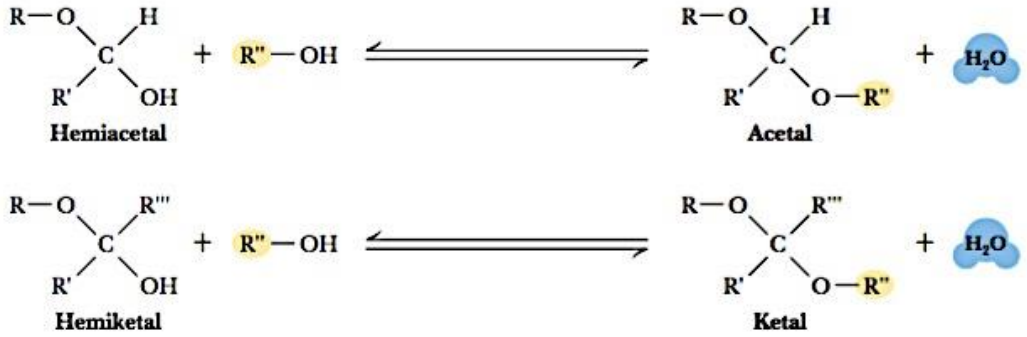


Figure 14 *Formation of Acetals and ketals from hemiacetals and hemiketals*

4.3.4OLIGOSACCHARIDE: STRUCTURE AND NOMENCLATURE

An **oligosaccharide** (from the Greekolígos, "a few", andsácchar, "sugar") is a saccharidepolymer containing a small number (typically two to ten) of simple sugars (monosaccharides).Monosaccharide units include the hexoses (glucose, fructose, mannose, and galactose) and the pentoses (ribose and xylose)

Disaccharides

Disaccharides are the simplest oligosaccharides consist of two monosaccharide units linked by a glycosidic bond.The disaccharides commonly found in nature are sucrose, maltose, and lactose. Each is a mixed acetal, when a hydroxyl group of one sugar reacts with the anomeric carbon of the other. Except for sucrose, each of these structures possesses one free unsubstituted anomeric carbon atom, and thus each of these disaccharides is a reducing sugar. The end of the molecule containing the free anomeric carbon is called the **reducing end,** and the other end is called the **nonreducing end.** In the case of sucrose, both of the anomeric carbon atoms are substituted, that is, neither has a free -OH group. The substituted anomeric carbons cannot be converted to the aldehyde configuration and thus cannot participate in the oxidation–reduction reactions characteristic of reducing sugars. Thus, sucrose is *not* a reducing sugar.

Maltose, isomaltose, and cellobiose (Figure 15) are all **homodisaccharides** because they each contain only one kind of monosaccharide, namely, glucose. **Maltose** is produced from starch (a polymer of -**D**-glucose produced by plants) by the action of amylase enzymes and is a component of malt (a substance obtained by allowing grain to soften in water and germinate). The enzyme **diastase,** produced during the germination process, catalyzes the hydrolysis of starch to maltose. Maltose is used in beverages and because it is fermented

readily by yeast, it is important in the brewing of beer. In both maltose and cellobiose, the glucose units are **1□4 linked,** meaning that the C-1 of one glucose is linked by a glycosidic bond to the C-4 oxygen of the other glucose. The only difference between them is in the configuration at the glycosidic bond. Maltose exists in the α- configuration, whereas cellobiose is β. **Isomaltose** is obtained in the hydrolysis of some poly- saccharides (such as dextran), and **cellobiose** is obtained from the acid hydrolysis of cellulose. Isomaltose also consists of two glucose units in a glycosidic bond, but in this case, C-1 of one glucose is linked to C-6 of the other, and the configuration isα.

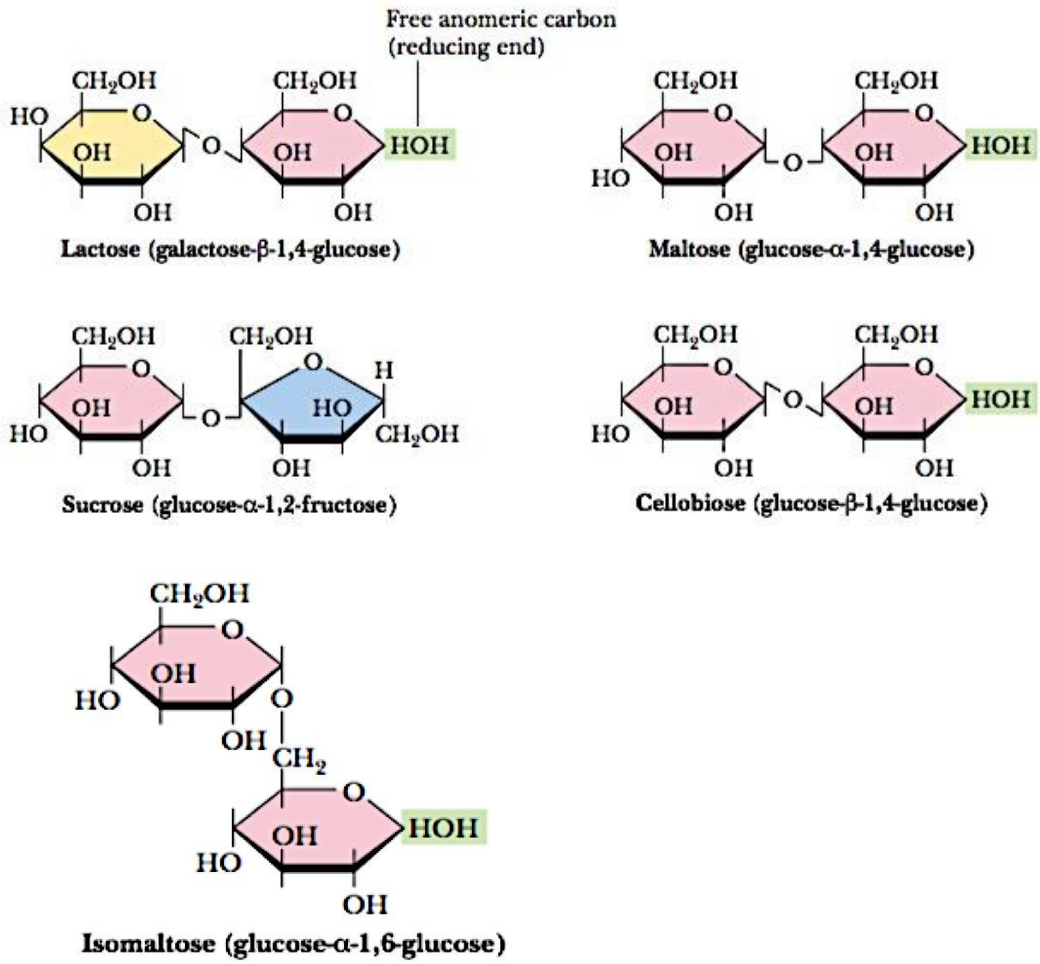


Figure 15*The structures Disaccharides (*Note: Pink colored ring= Glucose, yellow colored ring= galactose, blue colored ring = fructose)*

Rules of Nomenclature of Disaccharides:

To name reducing disaccharides several rules are followed. By convention, the name describes the compound with its nonreducing end to the left, and we can "build up" the name in the following order. (1) Give the configuration (α or β) at the anomeric carbon

joining the first monosaccharide unit (on the left) to the second. (2) Name the nonreducing residue; to distinguish five- and six-membered ring structures, insert “furano” or “pyrano” into the name. (3) Indicate in parentheses the two carbon atoms joined by the glycosidic bond, with an arrow connecting the two numbers; for example, (1□**4**) shows that C-1 of the first-named sugar residue is joined to C-4 of the second. (4) Name the second residue. If there is a third residue, describe the second glycosidic bond by the same conventions.

β-**D-lactose** (O-β-**D**-Galactopyranosyl-(1□**4**)**D**-glucopyranose) (Figure 15) is the prime carbohydrate in milk and is of prime nutritional importance to mammals in the early stages of their lives. It is formed from D-galactose and D-glucose via a (1□**4**) link, and because it has a free anomeric carbon available for oxidation, it is capable of mutarotation and is a reducing sugar. Its abbreviated name is Gal(β1□**4**)Glc. It is an interesting peculiarity of nature that lactose cannot be absorbed directly into the bloodstream. It must first be broken down into galactose and glucose by **lactase,** an intestinal enzyme that exists in young, nursing mammals but is not produced in significant quantities in the mature mammal. Most humans, with the exception of certain groups in Africa and northern Europe, produce only low levels of lactase. For most individuals, this is not a problem, but some cannot tolerate lactose and experience intestinal pain and diarrhea upon consumption of milk.

Sucrose, in contrast, is a disaccharide of almost universal appeal and tolerance. Produced by many higher plants and commonly known as *table sugar,* it is one of the products of photosynthesis and is composed of fructose and glucose. Sucrose is hydrolyzed by the enzyme **invertase,** and also easily hydrolyzed by dilute acid, apparently because the fructose in sucrose is in the relatively unstable furanose form. Contradictory to maltose and lactose, sucrose contains no free anomeric carbon atom; the anomeric carbons of both monosaccharide units are involved in the glycosidic bond (Fig. 15). Sucrose is therefore a nonreducing sugar. In the abbreviated nomenclature, a double-headed arrow connects the symbols specifying the anomeric carbons and their configurations. For example, the abbreviated name of sucrose is either Glc(α1↔2β)Fru or Fru(β2↔1α)Glc. Although sucrose and maltose are important to the human diet, they are not taken up directly in the body. In a manner similar to lactose, they are first hydrolyzed by **sucrase** and **maltase,** respectively, in the human intestine.

4.3.5 POLYSACCHARIDE: STRUCTURE AND NOMENCLATURE

Polysaccharides, also called glycans, differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the degree of branching. Basically they are of two types:

Homopolysaccharides-contains only a single type of monomer. Some serve as storage forms of monosaccharides that are used as fuels (starch and glycogen), Other (cellulose and chitin) serve as structural elements in plant cell walls and animal exoskeletons.Homopolysaccharides are often named for the sugar unit they contain, so that glucose homopolysaccharides are called **glucans,** while mannose homopolysaccharides are **mannans.** Other homopolysaccharide names are just as obvious: *galacturonans*, *arabinans,* and so on. Homopolysaccharides of uniform linkage type are often named by including notation to denote ring size and linkage type.

Heteropolysaccharides-contains two or more different kinds of monomer.They provide extracellular support for organisms of all kingdoms (peptidoglycan in the bacterial cell envelope, the extracellular matrix in animal tissues).

Interestingly, polysaccharides include not only those substances composed only of glycosidically linked sugar residues but also molecules that contain polymeric saccharide structures linked via covalent bonds to amino acids, peptides, proteins, lipids, and other structures. The most common constituent of polysaccharides is **D**-glucose, but **D**-fructose, **D**-galactose, L-galactose, **D**-mannose, L-arabinose, and **D**-xylose are also common. Common monosaccharide derivatives in polysaccharides include the amino sugars (**D**-glucosamine and **D**-galactosamine), their derivatives (*N*-acetylneuraminic acid and *N*-acetylmuramic acid), and simple sugar acids (glucuronic and iduronic acids). Polysaccharides differ not only in the nature of their component monosaccharides but also in the length of their chains and in the amount of chain branching that occurs. Although a given sugar residue has only one anomeric carbon and thus can form only one glycosidic linkage with hydroxyl groups on other molecules, each sugar residue carries several hydroxyls, one or more of which may be an acceptor of glycosyl substituents. This ability to form branched structures distinguishes polysaccharides from proteins and nucleic acids, which occur only as linear polymers.

4.3.6.1 POLYSACCHARIDE FUNCTIONS

- Typically asstorage materials, structural components, or protective substances.
- Variety of cellular recognition and intercellular communication events.

4.3.6.2 STORAGE POLYSACCHARIDES

The most important storage polysaccharides are starch in plant cells and glycogen in animal cells. Both polysaccharides occur intracellularly as large clusters or granules. Starch and glycogen molecules are heavily hydrated, because they have many exposed hydroxyl groups available to hydrogen bond with water. Most plant cells have the ability to form starch, but it is especially abundant in tubers, such as potatoes, and in seeds.

It is noteworthy to tell you that*organisms store carbohydrates in the form of polysaccharides rather than as monosaccharides to lower the osmotic pressure of the sugar reserves. Because osmotic pressures depend only on numbers of molecules, the osmotic pressure is greatly reduced by formation of a few polysaccharide molecules out of thousands (or even millions) of monosaccharide units.*

STARCH

By far the most common storage polysaccharide in plants is **starch,** which exists in two forms: α**-amylose** and **amylopectin (**Figure 16). Most forms of starch in nature are 10 to 30% α-amylose and 70 to 90% amylopectin. -Amylose is composed of linear chains of **D-**glucose in (1→4) linkages. The chains are of varying length, having molecular weights from several thousand to half a million. The chain has a reducing end and a nonreducing end. Although poorly soluble in water, α-amylose forms micelles in which the polysaccharide chain adopts a helical conformation. Iodine reacts with α-amylose to give a characteristic blue color, resulted from the insertion of iodine into the middle of the hydrophobic amylose helix.

In contrast to α-amylose, amylopectin is a highly branched chain of glucose units (Figure 16). Branches occur in these chains every 12 to 30 residues. The average branch length is between 24 and 30 residues, and molecular weights of amylopectin molecules can range up to 100 million. The linear linkages in amylopectin are (1→4), whereas the branch linkages are (1→6). As is the case for α-amylose, amylopectin forms micellar suspensions in water; iodine reacts with such suspensions to produce a red-violet color.

Glycogen

The major form of storage polysaccharide in animals is **glycogen.** Glycogen is found mainly in the liver and skeletal muscle. Like amylopectin, glycogen is a polymer of (1→4)-linked subunits of glucose, with (1→6)- linked branches, but glycogen is more extensively branched (on average, every 8 to 12 residues) and more compact than starch. Like amylopectin, glycogen yields a red-violet color with iodine. Glycogen can be hydrolyzed by both α- and β-amylases, yielding glucose and maltose, respectively, as products and can also be hydrolyzed by **glycogen phosphorylase,** an enzyme present in liver and muscle tissue, to release glucose-1-phosphate.

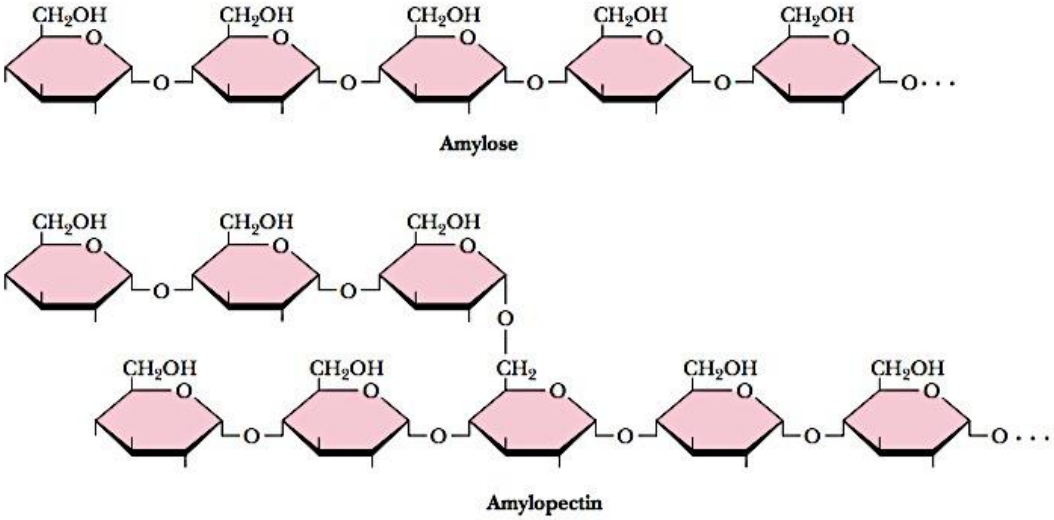


Figure 16 Amylose and amylopectin, the polysaccharides of starch [linear linkages are (1→4), but the branches in amylopectin are (1→6)]

Note*:Glycogen has a similar structure but is more highly branched and more compact.

Dextran

Dextrans are (1→6)-linked polysaccharides of **D**-glucose with branched chains found in yeast and bacteria (Figure 17). Because the main polymer chain is (1→6) linked, the repeating unit is *isomaltose,* Glc (1→6)-Glc. The branch points may be 1→2, 1→3, or 1→4 in various species. The degree of branching and the average chain length between branches depend on the species and strain of the organism. Bacteria growing on the surfaces of teeth produce extracellular accumulations of dextrans, an important component of *dental plaque.*

Synthetic dextrans are frequently used in research laboratories as the support medium for column chromatography of macromolecules.

Structural polysaccharides

Cellulose

Cellulose, the most abundant natural polymer is a fibrous, tough, water-insoluble substance, found in the cell walls of plants (stalks, stemsand trunks). Cellulose constitutes much of the mass of wood, and cotton is almost pure cellulose. Cellulose is one of the principal components providing physical structure and strength.Cellulose also has its delicate side, as*Cotton* woven fibers make some of our most comfortable clothing fabrics, is almost pure celluloseLike amylose and the main chains of amylopectin and glycogen, the cellulose molecule is a linear, unbranched homopolysaccharide, of D-glucose units. But there is a very important difference: in cellulose the glucose residues have the βconfiguration (Fig. 18), whereas in amylose, amylopectin, and glycogen the glucose is in the αconfiguration. The glucose residues in cellulose are linked byβ (1→4) glycosidic bonds, in contrast to the α(1→4) bonds of amylose, starch, and glycogen. This difference gives cellulose and amylose very different structures and physical properties.

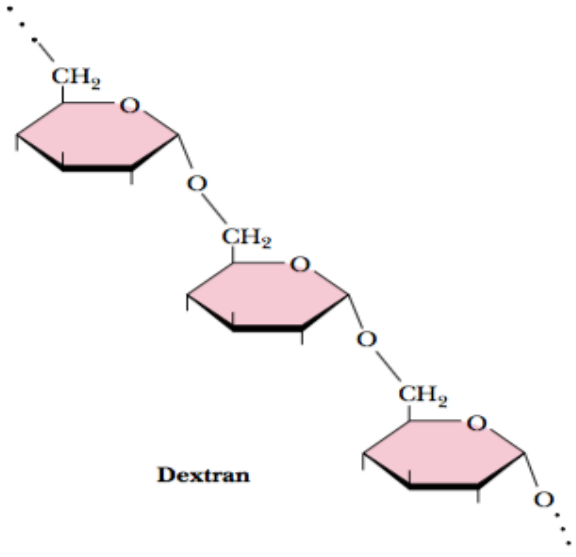


Figure 17 The structure of Dextran

The α(1→4)-linkage sites of amylose are naturally bent, conferring a gradual turn to the polymer chain, which results in the helical conformation whereas the most stable conformation about the β (1→4) linkage involves alternating 180° flips of the glucose units along the chain so that the chain adopts a fully extended conformation, referred to as an **extended ribbon.** Juxtaposition of several such chains permits efficient interchain hydrogen bonding, the basis of much of the strength of cellulose. The flattened sheets of the chains lie side by side and are joined by hydrogen bonds and are laid on top of one another in a way to give strength and stability to a wall. Cellulose is extremely resistant to hydrolysis, whether by acid or by the digestive tract amylases. Consequently, most animals (including humans) cannot digest cellulose to any significant degree. Ruminant animals (cattle, deer, giraffes, and camels) are an exception because bacteria that live in the rumen secrete the enzyme **cellulase,** a β-glucosidase effective in the hydrolysis of cellulose. The resulting glucose is then metabolized in a fermentation process to the benefit of the host animal. Termites similarly digest cellulose because their digestive tracts also contain bacteria that secrete cellulase.

Chitin

Chitin, earth's second most abundant carbohydrate polymer (after cellulose) is ahomopolysaccharide that is similar to cellulose, both in its biological function and its primary, secondary, and tertiary structure, is **chitin.** Chitin is present in the cell walls of fungi and is the fundamental material in the exoskeletons of crustaceans, insects, and spiders. The structure of chitin, an extended ribbon, is identical to cellulose, except that the -OH group on each C-2 is replaced by N-acetylglucosamine (-NHCOCH3), so that the repeating units are *N-acetyl-D-glucosamines* in β(1→4)linkage. Like cellulose (Figure 18), the chains of chitin form extended ribbons (Figure 19) and pack side by side in a crystalline, strongly hydrogen-bonded form. One

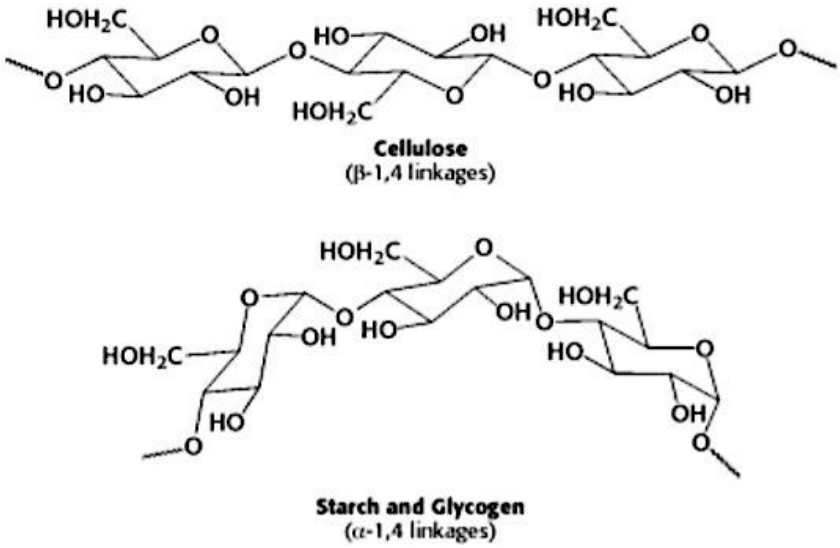


***Figure 18Cellulose, with** β(1→4)**glycosidic linkages, can adopt a fully extended conformation**, which are optimal for structural purposes. The α(1→4) linkages (starch & Glycogen) favor bent structures, which are more suitable for storage*

significant difference between cellulose and chitin is whether the chains are arranged in parellel(all the reducing ends together at one end of a packed bundle and all the nonreducing ends together at the other end) or **antiparallel** (each sheet of chains having the chains arranged oppositely from the sheets above and below). Natural cellulose seems to occur only in parallelarrangements. Chitin, however, can occur in three forms, sometimes all in the same organism. α-*Chitin* is an all-parallel arrangement of the chains, whereas β-*chitin* is an antiparallel arrangement. In δ-*chitin,* the structure is thought to involve pairs of parallel sheets separated by single antiparallel sheets.

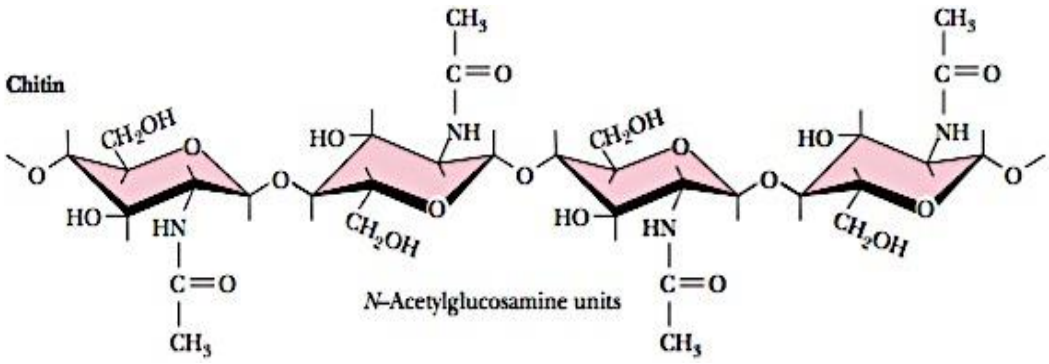


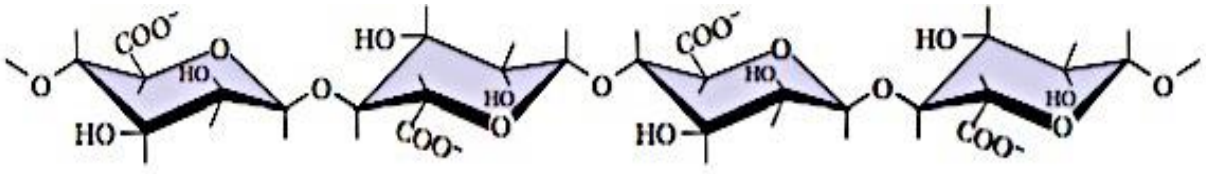
Figure 19A short segment of Chitin

Chitin's availability and abundance offer opportunities for industrial and commercial applications. Chitin-based coatings can extend the shelf life of fruits, and a chitin derivative

that binds to iron atoms in meat has been found to slow the reactions that cause rancidity and flavor loss. Without such a coating, the iron in meats activates oxygen from the air, forming reactive free radicals that attack and oxidize polyunsaturated lipids, causing most of the flavor loss associated with rancidity. Chitin-based coatings coordinate the iron atoms, preventing theirinteraction with oxygen.

Alginates

Alginate, is an anionicpolysaccharide distributed widely in the cell walls of brown algae*(Phaeophyceae)*, where through binding with water it forms a viscous gum.These polysaccharides have extended ribbon structures that bind metal ions, particularly calcium, in their structure. These include **poly (β-D-mannuronate)** and **poly (α-L-guluronate) (figure 20)** which are(1→4) linked chains formed from **β**-*mannuronic acid* and **α-L-***guluronic acid,* respectively. Both of these homopolymers are found together in most marine alginates, although mixed chains containing both monomer units are also found. The conformation of poly**(β-D**-mannuronate) is similar to that of cellulose. Alginate absorbs water quickly, which makes it useful as an additive in dehydrated products such as slimming aids, and in the manufacture of paper and textiles. It is also used for waterproofing and fireproofing fabrics, in the food industry as a thickening agent for drinks, ice cream and cosmetics, and as a gelling agent for jellies.



poly (β-D-mannuronate)

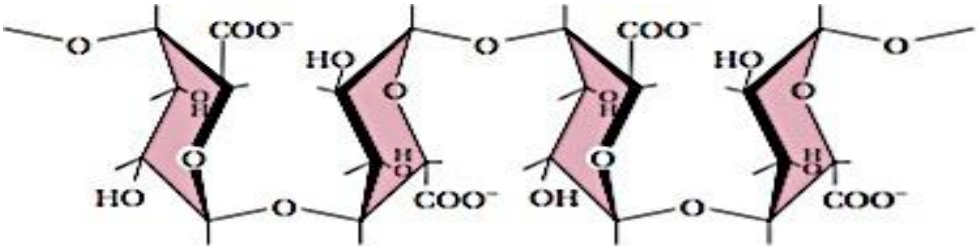


Figure 20 Structures of Alginates poly (α-L-guluronate)

Agarose

An important polysaccharide mixture isolated from marine red algae (*Rhodophyceae*) is agar, a mixture of sulfated heteropolysaccharides made up of D-galactose and an L-galactose derivative ether-linked between C-3 and C-6 (Fig. 21). The two major components of agar are the unbranched polymer agarose (Mr ~120,000) and a branched component, agaropectin. The three-dimensional structure of agarose is a double helix with a threefold screw axis. The central cavity is large enough to accommodate water molecules. Agarose and agaropectin readily form gels containing large amounts (up to 99.5%) of water. Agarose can be processed to remove most of the charged groups, yielding a material (trade name Sepharose) useful for purification of macromolecules in gel exclusion chromatography.

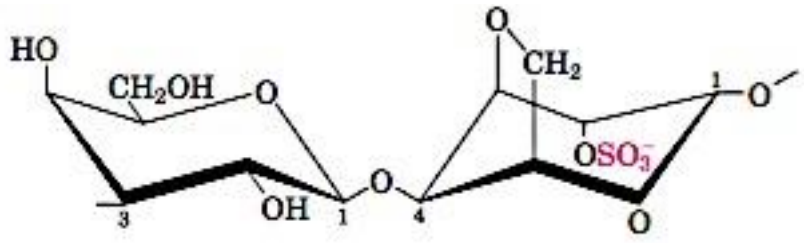


Figure 21 The structure of Agarose

Interesting is to note that the rigid component of bacterial cell walls is a heteropolymer of alternating β(1→4)-linked N-acetylglucosamine and N-acetylmuramic acid residues. The linear polymers lie side by side in the cell wall, cross-linked by short peptides, the exact structure of which depends on the bacterial species. The peptide cross-links weld the polysaccharide chains into a strong sheath that envelops the entire cell and prevents cellular swelling and lysis due to the osmotic entry of water.

Glycosaminoglycans

The extracellular matrix is composed of an interlocking meshwork of heteropolysaccharides and fibrous proteins such as collagen, elastin, fibronectin, and laminin. theseheteropolysaccharides known as **glycosaminoglycans** is involved in a variety of extracellular (and sometimes intracellular) functions, consist of linear chains of repeating disaccharides (figure 22) in which one of the monosaccharide units is an amino sugar (either N-acetylglucosamine or N-acetyl- galactosamine) the other is in most cases a uronic acid (usually D-glucuronic or L-iduronic acid) and one (or both) of the monosaccharide units

contains at least one negatively charged sulfate or carboxylate group. The combination of sulfate groups and the carboxylate groups of the uronic acid residues gives glycosaminoglycans a very high density of negative charge. To minimize the repulsive forces among neighboring charged groups, these molecules assume an extended conformation in solution. The specific patterns of sulfated and nonsulfated sugar residues in glycosaminoglycans provide for specific recognition by a variety of protein ligands that bind electrostatically to these molecules. **Heparin,** with the highest net negative charge of the disaccharides, is a natural anticoagulant substance. It binds strongly to *antithrombin III* (a protein involved in terminating the clotting process) and inhibits blood clotting. **Hyaluronate** molecules may consist of as many as 25,000 disaccharide units, with molecular weights of up to 107. Hyaluronates are important components of the vitreous humor in the eye and of *synovial fluid*, the lubricant fluid of joints in the body. The **chondroitins** and **keratan sulfate** are found in tendons, cartilage, and other connective tissue, whereas **dermatan sulfate,** as its name implies, is a component of the extracellular matrix of skin. Glycosaminoglycans are fundamental constituents of *proteoglycans* (discussed below).

4.3.7. Glycoconjugates: Proteoglycans, Glycoproteins, and Glycolipids

In addition to their imperative roles as stored fuels and as structural materials polysaccharides and oligosaccharides are information carriers:

They serve as target labels for some proteins and as facilitators of specific cell-cell interactions and interactions between cells and the extracellular matrix. Specific carbohydrate containing molecules act in cell-cell recognition and adhesion, cell migration during development, blood clotting, the immune response, and wound healing. In most of these cases, the informational carbohydrate is covalently joined to a protein or a lipid to form a glycoconjugate, which is the biologically active molecule.

Proteoglycans

These are macromolecules of the cell surface or extracellular matrix in which one or more glycosaminoglycan chains are joined covalently to a membrane protein or a secreted protein. The glycosaminoglycan moiety commonly forms the greater fraction (by mass) of the proteoglycan molecule, dominates the structure, and is often the main site of biological activity. In many cases the biological activity is the provision of multiple binding sites, rich

in opportunities for hydrogen bonding and electrostatic interactions with other proteins of the cell surface or the extracellular matrix. Proteoglycans are major components of connective tissue such as cartilage, in which their many noncovalent interactions with other proteoglycans, proteins, and glycosaminoglycans provide strength and toughness. These molecules act as tissue organizers, influence the development of specialized tissues, mediate the activities of various growth factors, and regulate the extra- cellular assembly of collagen fibrils.

Glycoproteins

These have one or several oligosaccharides of variable complexity joined covalently to a protein. They are found on the outer face of the plasma membrane, in the extracellular matrix, and in the blood. Inside cells they are found in specific organelles such as Golgi complexes, secretory granules, and lysosomes. The oligosaccharide portions of glycoproteins are less monotonous than the glycosaminoglycan chains of proteoglycans; they are rich in information, forming highly specific sites for recognition and high-affinity binding by other proteins.

Glycolipids

These are membrane lipids in which the hydrophilic head groups are oligosaccharides, which, as in glycoproteins, act as specific sites for recognition by carbohydrate-binding proteins. Gangliosides are membrane lipids of eukaryotic cells in which the polar head group, the part of the lipid that forms the outer surface of the membrane, is a complex oligosaccharide containing sialic acid and other monosaccharide residues. Some of the oligosaccharide moieties of gangliosides, such as those that determine human blood groups, are identical with those found in certain glycoproteins, which therefore also contribute to blood group type determination.

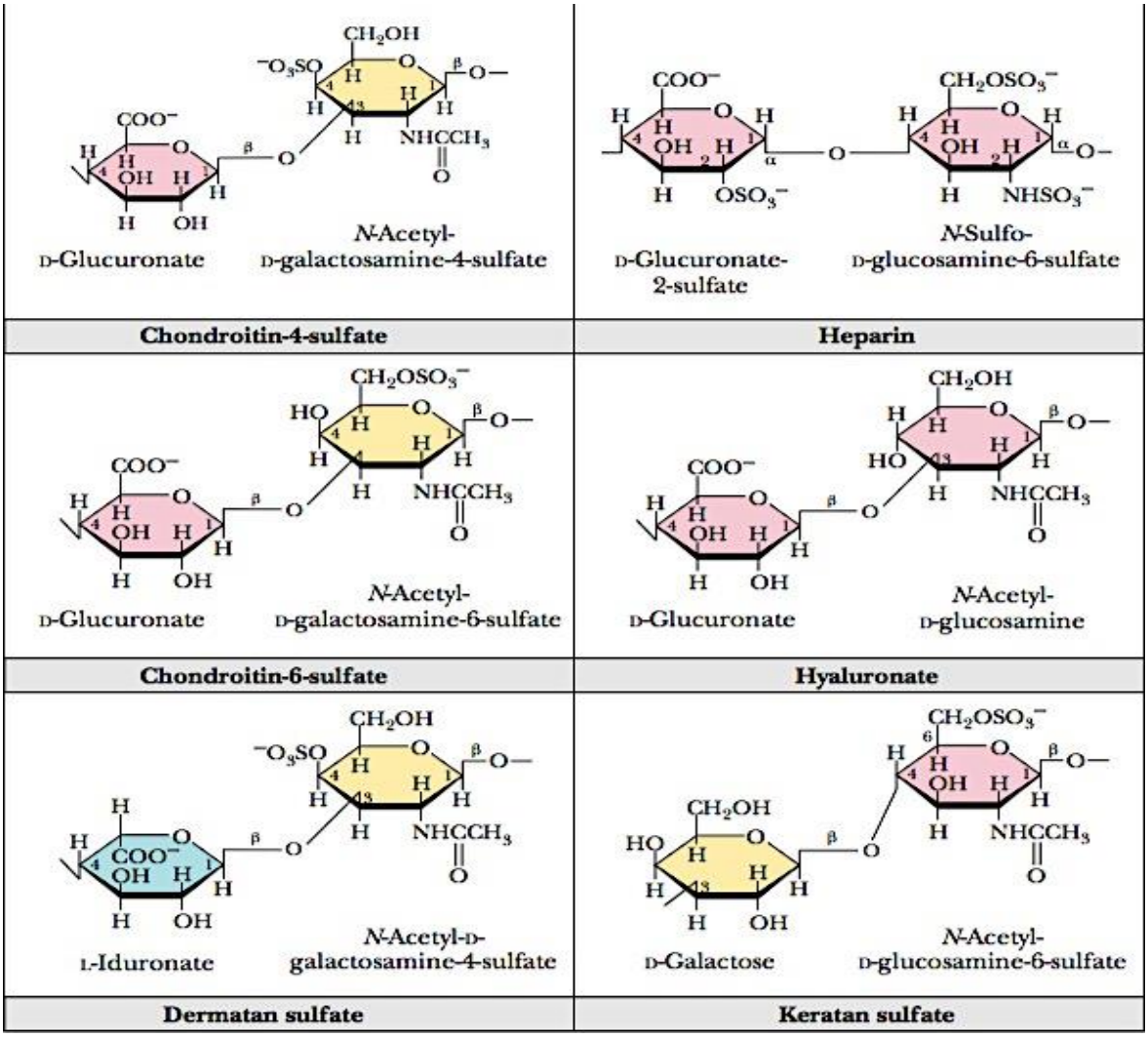


Figure 22 Glycosaminoglycans

4.4 METABOLISM OF CARBOHYDRATES

Glucose occupies a crucial position in the metabolism of plants, animals, and many microorganisms being rich in potential energy, and thus a good fuel. By storing glucose as a high molecular weight polymer such as starch or glycogen, a cell can stockpile large quantities of hexose units while maintaining a relatively low ***cytosolic osmolarity***. When there is need of egergy it can be released from these intracellular storage polymers and used to produce ATP (aerobically or anaerobically).

Glucose is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions (Escherichia coli can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other

metabolic intermediate needed for growth). In animals and vascular plants, glucose has three major fates:

- It may be stored (as a polysaccharide or as sucrose)
- Oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates
- Oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.

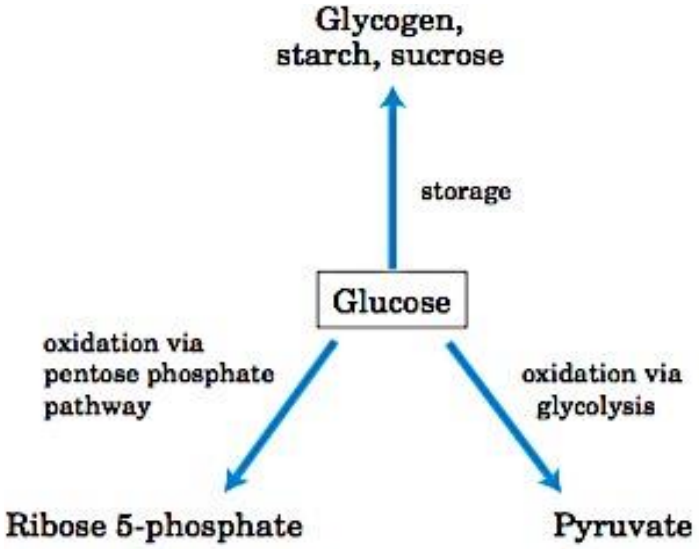


Figure 23 Pathways of glucose utilization

Organisms that do not have access to glucose from other sources must make it

Photosynthetic organisms- makes glucose by first reducing atmospheric CO2 to trioses, then converting the trioses to glucose).

Non-photosynthetic cells- makes glucose from simpler three and four-carbon precursors by the process of gluconeogenesis.

In this section we will study the individual reactions of glycolysis, gluconeogenesis, and the pentose phosphate pathway and the functional significance of each pathway.

4.4.1- GLYCOLYSIS: OVERVIEW

Glycolysis (from the Greek glykys, meaning "sweet," and lysis, meaning "splitting"), involves the degradation of a molecule of glucose in a series of enzyme-catalyzed reactions

to yield two molecules of the three-carbon compound pyruvate. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH. The complete glycolytic pathway was elucidated by 1940, largely through the pioneering contributions of Gustav Embden, Otto Meyerhof, Carl Neuberg, Jacob Parnas, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis is also known as the ***Embden-Meyerhof pathway.***

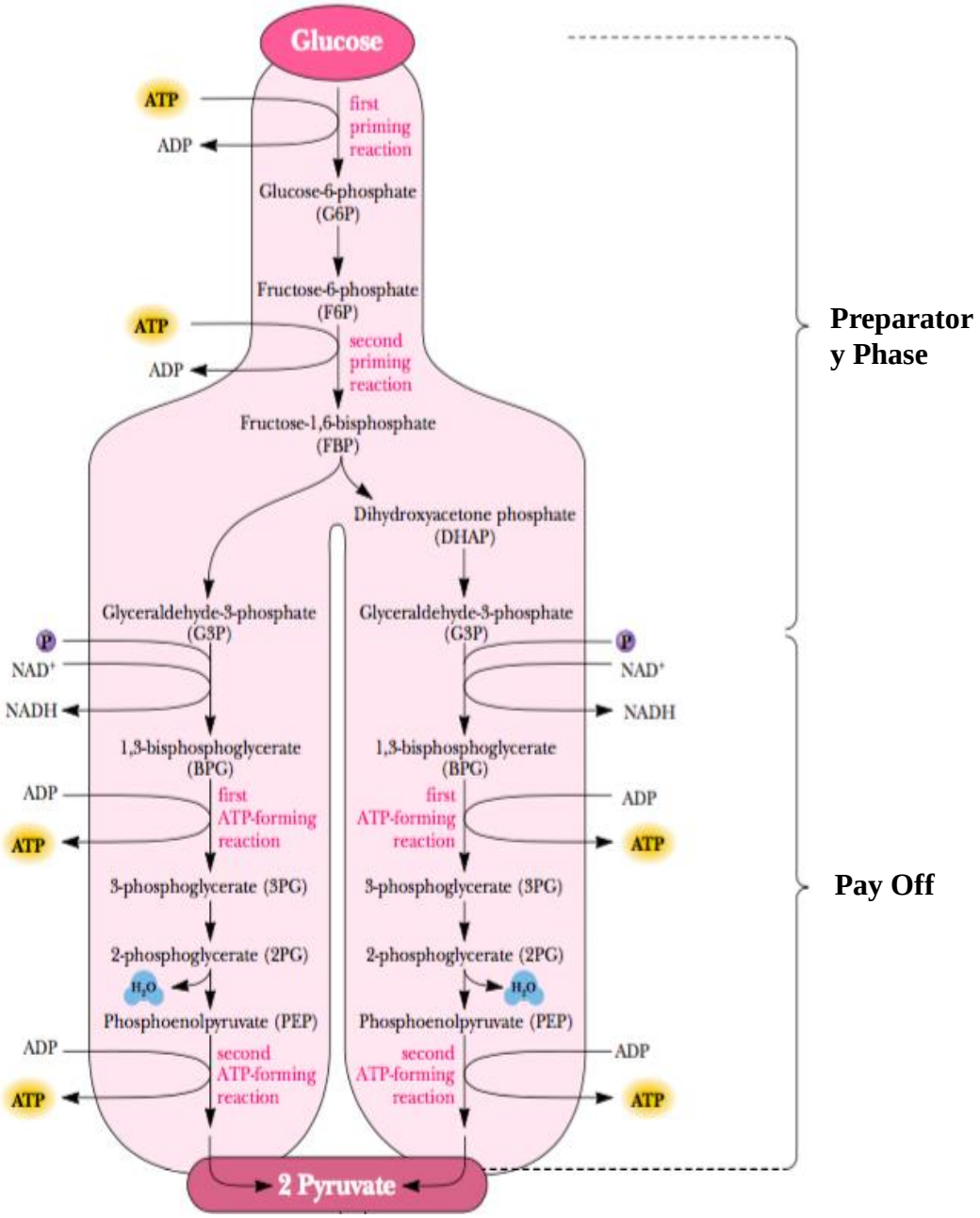


Figure 24 The glycolytic pathway

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps (fig. 24), the first five of which constitute the **preparatory phase** in which he energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate.

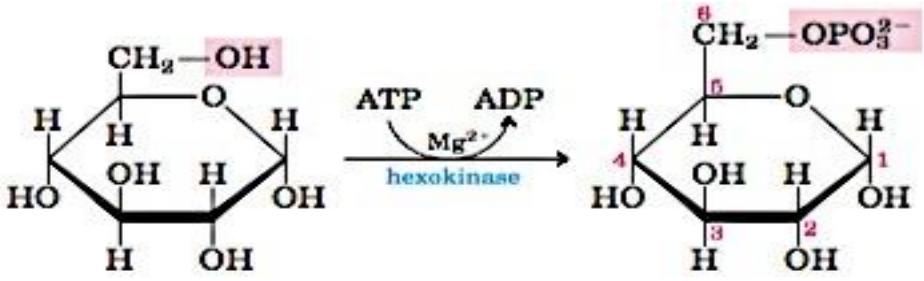
In the payoff phase, each of the two molecules of glyceraldehyde 3-phosphate derived from glucose undergoes oxidation; the energy of this oxidation reaction is conserved in the formation of one NADH and two ATP per triose phosphate oxidized. The net equation for the overall process is

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2\text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

THE FIRST PHASE OF GLYCOLYSIS

Reaction 1: Phosphorylation of Glucose by Hexokinase or Glucokinase—The First Priming Reaction

The initial reaction of the glycolysis pathway involves phosphorylation of glucose at carbon atom 6 by either hexokinase or glucokinase. The formation of such a phosphoester is thermodynamically unfavorable and requires energy in form of ATP to operate in the forward direction. The hexokinase or glucokinase reaction is one of two **priming reactions** in the cycle that requires two priming ATP molecules to start the sequence of reactions and delivers four molecules of ATP in the end.



Glucose Glucose 6-phosphate ΔG'°=-16.7 kJ/mol

This *reaction, which is irreversible under intracellular conditions, is catalyzed by hexokinase.*

*NOTE *In most animal, plant, and microbial cells, the enzyme that phosphorylates glucose is **hexokinase.** Magnesium ion (Mg^{2+}) is required for this reaction, as for the other kinase*

enzymes in the glycolytic pathway. The true substrate for the hexokinase reaction is $MgATP^{2-}$ Liver contains an enzyme called ***glucokinase,*** *which also carries out this reaction in but is highly specific for D-glucose, has a much higher Km for glucose (approximately 10.0 mM), and is not product-inhibited. With such a high Km for glucose, glucokinase becomes important metabolically only when liver glucose levels are high (for example, when the individual has consumed large amounts of sugar). When glucose levels are low, hexokinase is primarily responsible for phosphorylating glucose. However, when glucose levels are high, glucose is converted by glucokinase to glucose-6-phosphate and is eventually stored in the liver as glycogen.*

Importance of Phosphorylated Intermediates *Each of the nine glycolytic intermediates between glucose and pyruvate is phosphorylated. The phosphoryl groups appear to have three functions.*

Phosphorylated glycolytic intermediates cannot leave the cell (plasma membrane generally lacks transporters for phosphorylated sugars). After the initial phosphorylation, no further energy is necessary to retain phosphorylated intermediates in the cell, despite the large difference in their intracellular and extracellular concentrations.

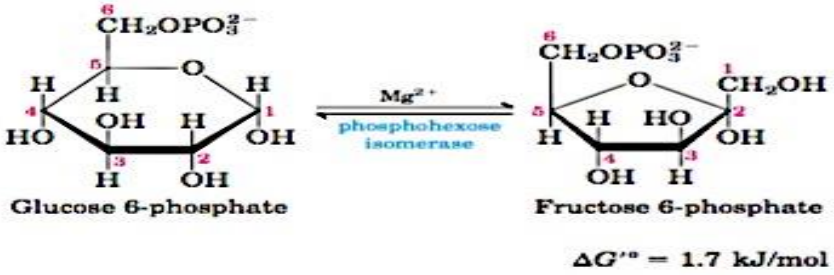
Energy released in the breakage of phosphoanhydride bonds (such as those in ATP) is partially conserved in the formation of phosphate esters such as glucose 6-phosphate. High-energy phosphate compounds formed in glycolysis (1,3-bisphos- phoglycerate and phosphoenolpyruvate) donate phosphoryl groups to ADP to form ATP.

Binding energy resulting from the binding of phosphate groups to the active sites of enzymes lowers the activation energy and increases the specificity of the enzymatic reactions.

Reaction 2: Phosphoglucoisomerase Catalyzes the Isomerization of Glucose-6-Phosphate

The second step in glycolysis is a common type of metabolic reaction: the isomerization of a sugar. In this particular case, the carbonyl oxygen of glucose- 6-phosphate is shifted from C-1 to C-2. This amounts to isomerization of an aldose (glucose-6-phosphate) to a ketose—fructose-6-phosphate. The reaction is necessary for two reasons. First, the next step in glycolysis is phosphorylation at C-1, and the hemiacetal -OH of glucose would be more difficult to phosphorylate than a simple primary hydroxyl. Second, the isomerization to

fructose (with a carbonyl group at position 2 in the linear form) activates carbon C-3 for cleavage in the fourth step of glycolysis. The enzyme responsible for this isomerization is **phosphoglucoisomerase,** also known as **glucose phosphate isomerase. The reaction proceeds readily in either direction, as might be expected from the relatively small change in standard free energy.**



Reaction 3: Phosphofructokinase—The Second Priming Reaction

The action of phosphoglucoisomerase, "moving" the carbonyl group from C-1 to C-2, creates a new primary alcohol function at C-1. The next step in the glycolytic pathway is the phosphorylation of this group by **phosphofructokinase (PFK-1).**

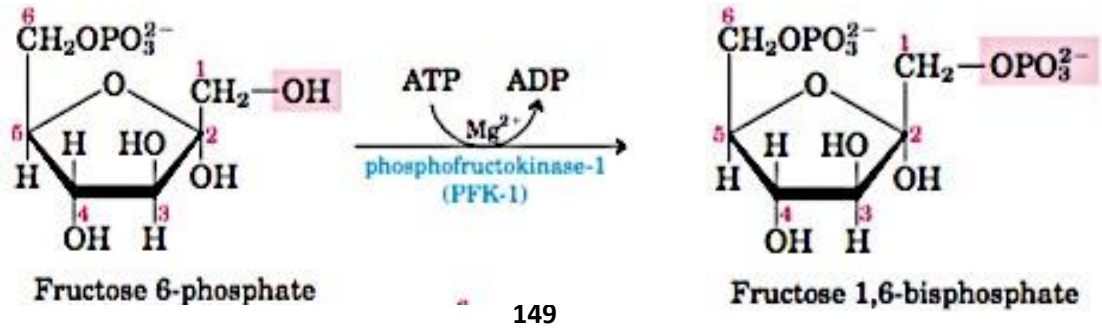
Note*- This enzyme is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway.

Once again, the substrate that provides the phosphoryl group is ATP. Like the hexokinase, glucokinase reaction, the phosphorylation of fructose-6-phosphate is a priming reaction and is endergonic:

Fructose-6-P + P_i →fructose-1,6-bisphosphate $\Delta G'°$= 16.3 kJ/mol

When coupled (by phosphofructokinase) with the hydrolysis of ATP, the overall reaction is strongly exergonic:

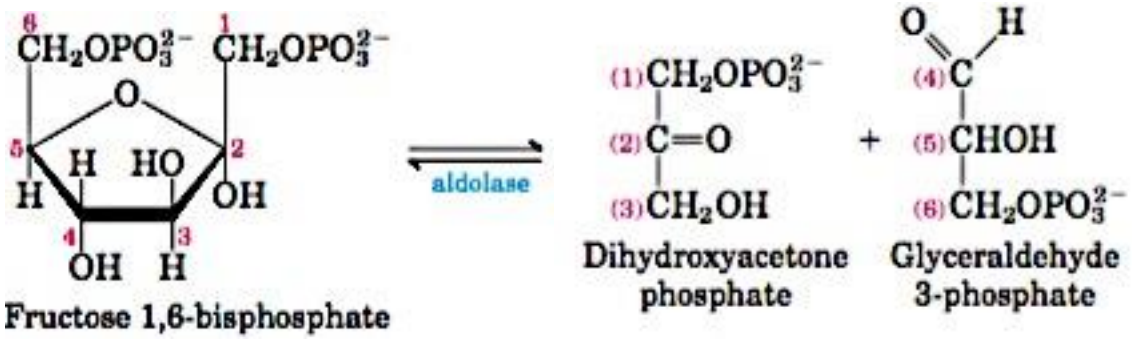
Fructose-6-P + ATP →fructose-1,6-bisphosphate ADP $\Delta G'°$=-14.2 kJ/mol



This reaction is essentially irreversible under cellular conditions and it is the first "committed" step in the glycolytic pathway. Glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis.Just as the hexokinase reaction commits the cell to taking up glucose, *the phosphofructokinase reaction commits the cell to metabolizing glucose* rather than converting it to another sugar or storing it. Similarly, just as the large free energy change of the hexokinase reaction makes it a likely candidate for regulation, so the phosphofructokinase reaction is an important site of regulation— indeed, the most important site in the glycolytic pathway. The activity of PFK-1 is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. The enzyme is inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids. In some organisms, fructose 2,6-bisphosphate (not to be confused with the PFK-1 reaction product, fructose 1,6- bisphosphate) is a potent allosteric activator of PFK-1.

Reaction 4: Cleavage of Fructose-1,6-bisphosphate

Fructose bisphosphate aldolase catalyzes a reversible aldol condensation that involves thecleavage of fructose-1,6-bisphosphate between the C-3 and C-4 carbons to yield two triose phosphates. The products are dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate.



Fructose 1,6-bisphosphate

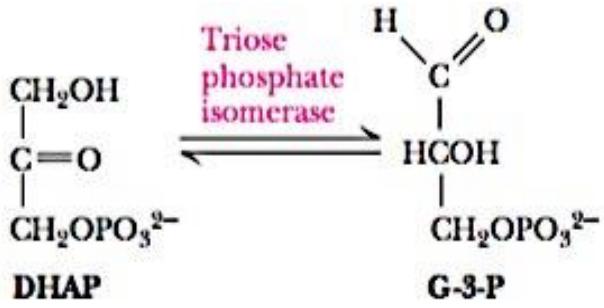
$\Delta G'^{\circ}$=23.8 kJ/mol

Reaction 5: Triose Phosphate Isomerase

Of the two products of the aldolase reaction, only glyceraldehyde-3-phosphate goes directly into the second phase of glycolysis. The other triose phosphate, dihydroxyacetone

phosphate, must be converted to glyceraldehyde-3-phosphate by the enzyme **triose phosphate isomerase.** This reaction thus permits both products of the aldolase reaction to continue in the glycolytic pathway, and in essence makes the C-1, C-2, and C-3 carbons of the starting glucose molecule equivalent to the C-6, C-5, and C-4 carbons, respectively.

The triose phosphate isomerase reaction completes the first phase of glycolysis, each glucose that passes through being converted to two molecules of glyceraldehyde-3-phosphate. Although the last two steps of the pathway are energetically unfavorable, the overall five-step reaction sequence has a net $\Delta G'°$ of +2.2 kJ/mol. It is the free energy of hydrolysis from the two priming molecules of ATP that brings the overall equilibrium constant close to 1 under standard-state conditions. The net ΔG under cellular conditions is quite negative.



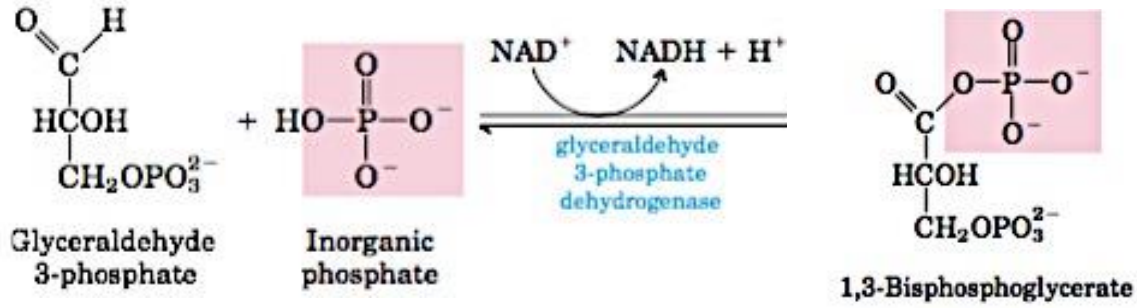
THE PAYOFF PHASE

The payoff phase of glycolysis includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP.one molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; both halves of the glucose molecule follow the same pathway in the second phase of glycolysis. The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is accompanied by the formation of four molecules of ATP from ADP. However, the net yield of ATP per molecule of glucose degraded is only two, because two ATP were devoted in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

Reaction 6: Glyceraldehyde-3-Phosphate Dehydrogenase

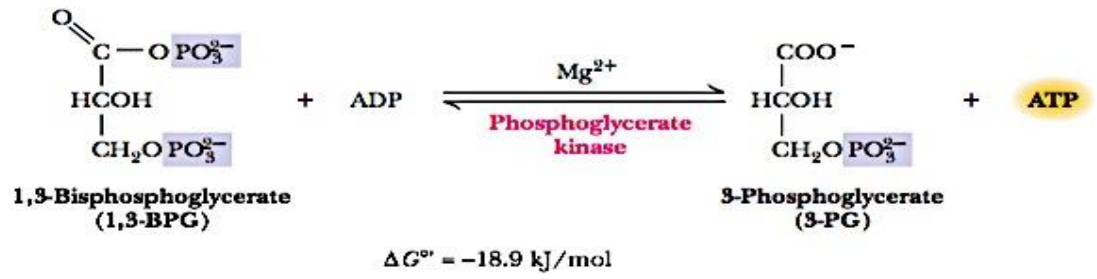
In the first glycolytic reaction to involve oxidation–reduction, glyceraldehyde- 3-phosphate is oxidized to 1,3-bisphosphoglycerate by **glyceraldehyde-3-phosphate dehydrogenase.** Although the oxidation of an aldehyde to a carboxylic acid is a highly exergonic reaction,

the overall reaction involves both formation of a carboxylic-phosphoric anhydride and the reduction of NAD^+ to NADH and is therefore slightly endergonic at standard state, with a $\Delta G'°$of 6.30 kJ/mol.



Reaction 7: Phosphoglycerate Kinase

The glycolytic pathway breaks even in terms of ATPs consumed and produced with this reaction. The enzyme **phosphoglycerate kinase** transfers a phosphoryl group from 1,3-bisphosphoglycerate to ADP to form an ATP. Because each glucose molecule sends two molecules of glyceraldehyde-3-phosphate into the second phase of glycolysis and because two ATPs were consumed per glucose in the first-phase reactions, the phosphoglycerate kinase reaction "pays off" the ATP debt created by the priming reactions. As might be expected for a phosphoryl transfer enzyme, Mg^{2+} ion is required for activity, and the true nucleotide substrate for the reaction is $MgADP^-$. It is appropriate to view the sixth and seventh reactions of glycolysis as a coupled pair, with 1,3-bis-phosphoglycerate as an intermediate. The phosphoglycerate kinase reaction is sufficiently exergonic at standard state to pull the G-3-P dehydrogenase reaction along. (In fact, the aldolase and triose phosphate isomerase are also pulled forward by phosphoglycerate kinase.) The net result of these coupled reactions is



Glyceraldehyde 3-phosphate +ADP +P_i +NAD^+⟺3-phosphoglycerate + ATP +NADH +H^+

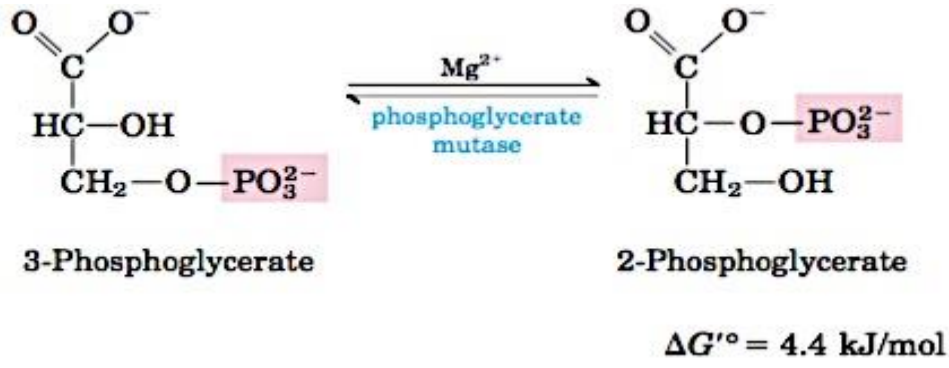
$\Delta G'°$=-12.5kJ/mol

Thus the overall reaction is exergonic.The outcome of these coupled reactions, both reversible under cellular conditions, is that the energy released on oxidation of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and Pi. The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a ***substrate-level phosphorylation.***

Reaction 8: Phosphoglycerate Mutase

The remaining steps in the glycolytic pathway prepare for synthesis of the second ATP equivalent. This begins with the **phosphoglycerate mutase** reaction, in which the phosphoryl group of 3-phosphoglycerate is moved from C-3 to C-2. (The term *mutase* is applied to enzymes that catalyze migration of a functional group within a substrate molecule.) The free energy change for this reaction is very small under cellular conditions ($\Delta G'^{\circ}$= 0.83 kJ/mol in erythrocytes).

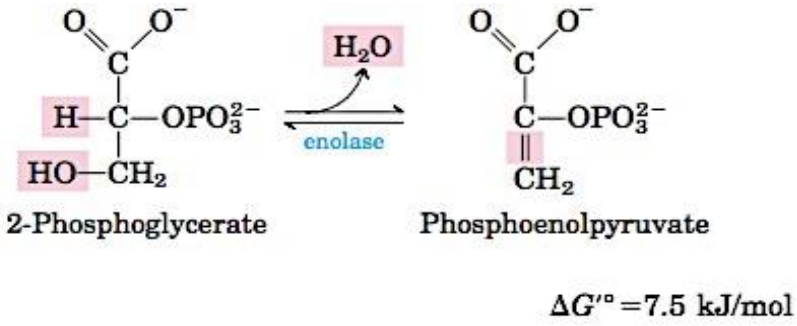
The reaction occurs in two steps: A phosphoryl group initially attached to a His residue of the mutase is transferred to the hydroxyl group at C-2 of 3-phosphoglycerate, forming 2, 3-bisphosphoglycerate (2, 3-BPG). The phosphoryl group at C-3 of 2,3-BPG is then transferred to the same His residue, producing 2-phosphoglycerate and regenerating the phosphorylated enzyme. Phosphoglycerate mutase is initially phosphorylated by phosphoryl transfer from 2,3-BPG, which is required in small quantities to initiate the catalytic cycle and is continuously regenerated by that cycle. Although in most cells 2,3-BPG is present in only trace amounts, it is a major component of erythrocytes, where it regulates the affinity of hemoglobin for oxygen.



9. Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

Enolase catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate. The reaction in essence involves dehydration—the removal of a water molecule—to form the

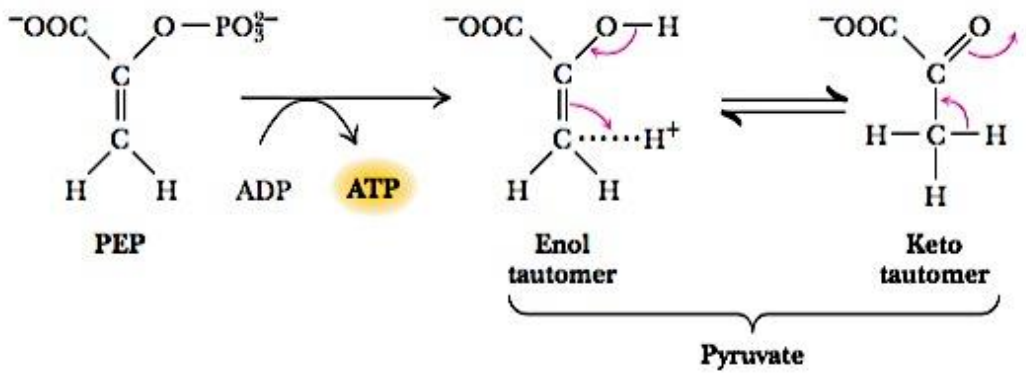
enol structure of PEP.



The enzyme is strongly inhibited by fluoride ion in the presence of phosphate. Inhibition arises from the formation of *fluorophosphate (FPO_3^{2-}),* which forms a complex with Mg^{2+} at the active site of the enzyme.

Reaction 10: Pyruvate Kinase

The second ATP-synthesizing reaction of glycolysis is catalyzed by **pyruvate kinase,** which brings the pathway at last to its pyruvate branch point. Pyruvate kinase mediates the transfer of a phosphoryl group from phosphoenolpyruvate to ADP to make ATP and pyruvate. The reaction requires Mg^{2+} ion and is stimulated by K^+ and certain other monovalent cations. The high free energy change for the conversion of PEP to pyruvate is due largely to the highly favorable and spontaneous conversion of the enol tautomer of pyruvate to the more stable keto form following the phosphoryl group transfer step. The overall reaction has a large, negative standard free- energy change, due in large part to the spontaneous con- version of the enol form of pyruvate to the keto form. The $\Delta G'°$ of phosphoenolpyruvate hydrolysis is -61.9 kJ/mol; about half of this energy is conserved in the formation of the phosphoanhydride bond of ATP ($\Delta G'°$= -30.5 kJ/mol), and the rest ($\Delta G'°$= -31.4 kJ/mol) constitutes a large driving force pushing the reaction toward ATP synthesis. The pyruvate kinase reaction is essentially irreversible under intra- cellular conditions and is an important site of regulation.



THE OVERALL BALANCE SHEET OF GLYCOLYSIS

The left-hand side of the following equation shows all the inputs of ATP, NAD^+, ADP, and Pi and the right-hand side shows all the outputs (Note*- each molecule of glucose yields two molecules of pyruvate):

Glucose +2ATP+ $2NAD^+$+4ADP +2Pi $\rightarrow$2 pyruvate+ 2ADP+ 2NADH +$2H^+$ +4ATP+ $2H_2O$

Canceling out common terms on both sides of the equation gives the overall equation for glycolysis under aerobic conditions:

Glucose + $2NAD^+$ + 2ADP +2Pi $\rightarrow$2 pyruvate+ 2NADH +$2H^+$ + 2ATP+ $2H_2O$

The two molecules of NADH formed by glycolysis in the cytosol are, under aerobic conditions, reoxidized to NAD^+ by transfer of their electrons to the electron- transfer chain, which in eukaryotic cells is located in the mitochondria. The electron-transfer chain passes these electrons to their ultimate destination, O_2:

2NADH +$2H^+$+ $O_2\rightarrow$ $2NAD^+$+ $2H_2O$

Electron transfer from NADH to O_2in mitochondria provides the energy for synthesis of ATP by respiration- linked phosphorylation.

In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (the pathway of carbon). Two molecules of ADP and two of Pi are converted to two molecules of ATP. Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehyde 3-phosphate to two of NAD^+ (the pathway of electrons).

4.4.1.1 THE METABOLIC FATES OF THE PRODUCTS OF GLYCOLYSIS: NADH AND PYRUVATE

In addition to ATP, the products of glycolysis are NADH and pyruvate. Their processing depends upon other cellular pathways. NADH must be recycled to NAD^+, lest NAD^+become limiting in glycolysis.What a given cell does with the pyruvate produced in glycolysis depends in part on the availability of oxygen. The pyruvate formed by glycolysis is further metabolized via one of two catabolic routes.

AEROBIC PATHWAY

Under aerobic conditions, pyruvate can be sent into the citric acid cycle (also known as the tricarboxylic acid cycle), where it is oxidized to CO_2 with the production of additional NADH (and $FADH_2$). Under aerobic conditions, the NADH produced in glycolysis and the citric acid cycle is reoxidized to NAD^+in the mitochondrial electron transport chain.

ANAEROBIC PATHWAY

Under anaerobic conditions, the pyruvate produced in glycolysis is processed differently. In yeast, it is reduced to ethanol; in other microorganisms and in animals, it is reduced to lactate. These processes are examples of**fermentation**—the production of ATP energy by reaction pathways in which organic molecules function as donors and acceptors of electrons. In either case, reduction of pyruvate provides a means of reoxidizing the NADH produced in the glyc- eraldehyde-3-phosphate dehydrogenase reaction of glycolysis.

LACTIC ACID FERMENTATION

In animal tissues experiencing anaerobic conditions, pyruvate is reduced to lactate. Pyruvate reduction occurs in tissues that normally experience minimal access to blood flow (e.g., the cornea of the eye) and also in rapidly contracting skeletal muscle. When skeletal muscles are exercised strenuously, the available tissue oxygen is consumed, and the pyruvate generated by glycolysis can no longer be oxidized in the TCA cycle and NADH cannot be reoxidized to NAD^+, but $NAD^+$is required as an electron acceptor for the further oxidation of pyruvate. Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NADnecessary for glycolysis to continue. Instead, excess pyruvate is reduced to lactate by lactate dehydrogenase (Figure 25). In anaerobic muscle tissue,

lactate represents the end of glycolysis. Most of this lactate must be carried out of the muscle by the blood and transported to the liver, where it can be resynthesized into glucose in gluconeogenesis.

Lactate produced by anaerobic microorganisms during **lactic acid fermentation** is responsible for the taste of sour milk and for the characteristic taste and fragrance of sauerkraut, which in reality is fermented cabbage.

ETHANOL (ALCOHOL) FERMENTATION

In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO_2, a process called ethanol (alcohol) fermentation (Fig. 26). In yeast, **alcoholic fermentation** is a two-step process. Pyruvate is decarboxylated to acetaldehyde by **pyruvate decarboxylase** in an essentially irreversible reaction. Thiamine pyrophosphate is a required cofactor for this enzyme. The second step, the reduction of acetaldehyde to ethanol by NADH, is catalyzed by **alcohol dehydrogenase**. At pH 7, the reaction equilibrium strongly favors ethanol. The end products of alcoholic fermentation are thus ethanol and carbon dioxide. Alcoholic fermentations are the basis for the brewing of beers and the fermentation of grape sugar in wine making

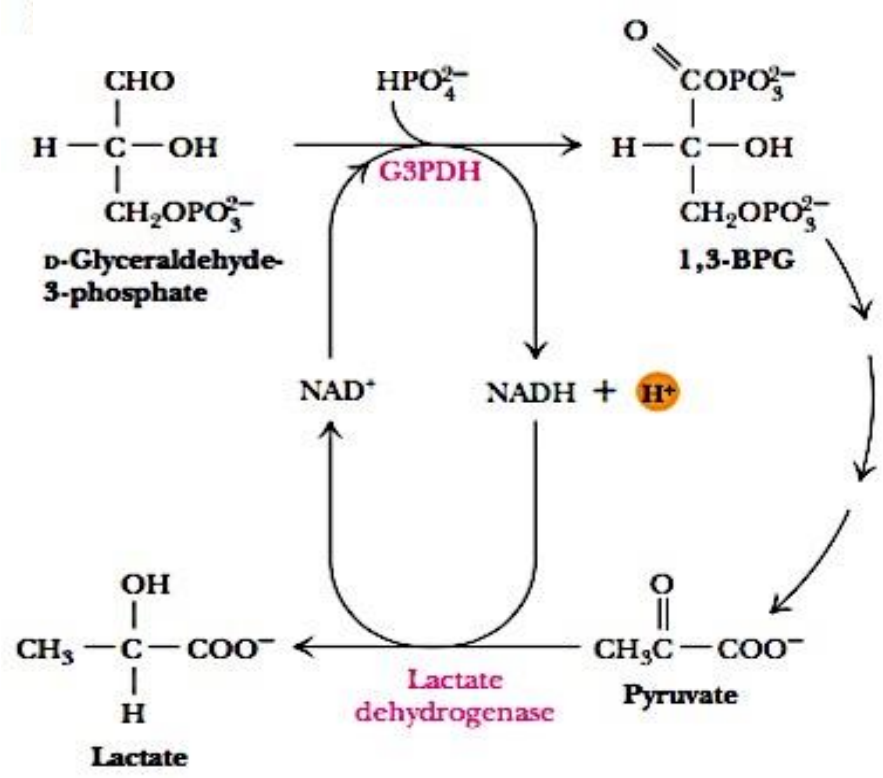


Figure 25Lactic acid fermentation

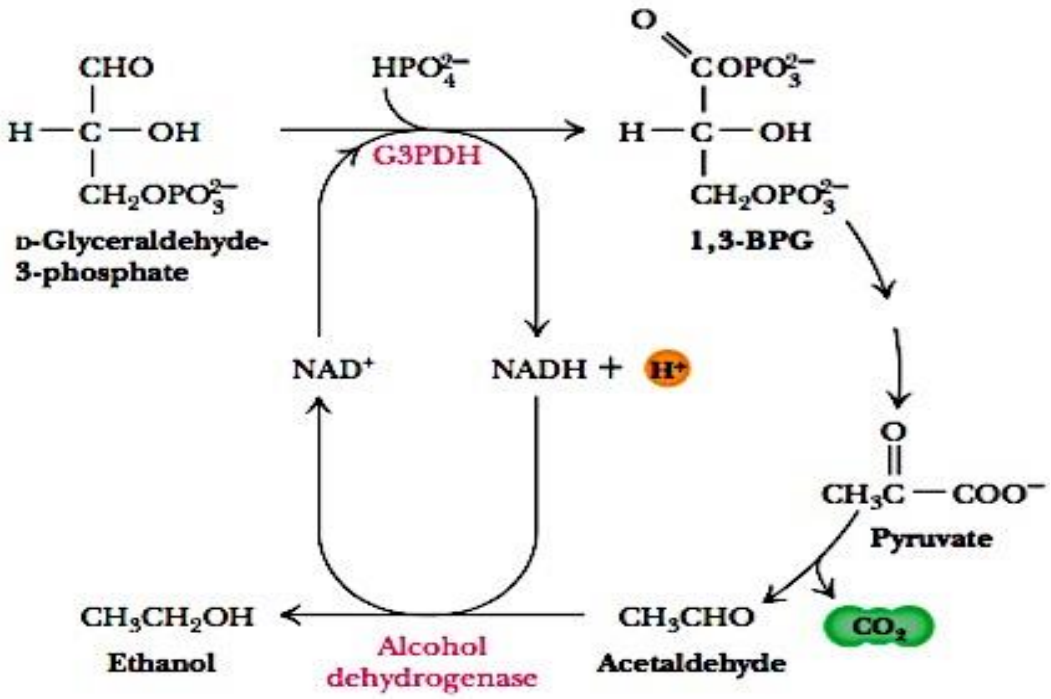


Figure 26 Alcoholic fermentation

4.4.1.2 UTILIZATION OF OTHER SUBSTRATES IN GLYCOLYSIS

Sugars other than glucose(both simple &complex), can enter the cycle if they can be converted by appropriate enzymes, to one of the intermediates of glycolysis. The most significant are the storage polysaccharides glycogen and starch; the disaccharides maltose, lactose, trehalose, and sucrose; and the monosaccharides fructose, mannose, and galactose (figure 27)

Entry of Glycogen and Starch

Glycogen in animal tissues and in microorganisms (and starch in plants) can be mobilized for use within the same cell by a phosphorolytic reaction catalyzed by glycogen phosphorylase (starch phosphorylase in plants). These enzymes catalyze an attack by Pi on the ($\alpha 1 \rightarrow 4$) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter. Glycogen

phosphorylase (or starch phosphorylase) acts repetitively until it approaches a($\alpha 1 \rightarrow 6$) branch point where its action stops. A debranching enzyme removes the branches

Glucose 1-phosphate produced by glycogen phosphorylase is converted to glucose 6-phosphate by phosphoglucomutase, which catalyzes the reversible reaction

Glucose 1-phosphate $\Leftrightarrow$glucose 6-phosphate

The glucose 6-phosphate thus formed can enter glycolysis or another pathway such as the pentose phosphate pathway.

Entry of fructose

Fructose produced by breakdown of sucrose, may participate in glycolysis by at least two different routes. In the liver, fructose is phosphorylated at C-1 by the enzyme fructokinase:

D-Fructose + $ATP^{4-} \rightarrow$D-fructose-1-phosphate^{2-}+ ADP^{3-}+ H^{+}

Subsequent action by fructose-1-phosphate aldolase cleaves fructose-1-P in a manner like the fructose bisphosphate aldolase reaction to produce dihydroxyacetone phosphate and D-glyceraldehyde:

D-Fructose-1-$P^{2-} \rightarrow$D-glyceraldehyde + dihydroxyacetone phosphate^{2-}

Dihydroxyacetone phosphate is an intermediate in glycolysis. D-Glyceraldehyde can be phosphorylated by triose kinase in the presence of ATP to form D-glyceraldehyde-3-phosphate, another glycolytic intermediate.

In the kidney and in muscle tissues, fructose is readily phosphorylated by hexokinase, which, as pointed out above, can utilize several different hexose substrates. The free energy of hydrolysis of ATP drives the reaction forward:

D-Fructose + $ATP^{4-} \rightarrow$D-fructose-1-phosphate^{2-}+ ADP^{3-}+ H^{+}

Fructose-6-phosphate generated in this way enters the glycolytic pathway directly in step 3, the second priming reaction. This is the principal means for channeling fructose into glycolysis in adipose tissue, which contains high levels of fructose.

Entry of Galactose

A somewhat more complicated route into glycolysis is followed by galactose, another simple hexose sugar. The process, called the Leloir pathway after Luis Leloir, its discoverer, begins with phosphorylation from ATP at the C-1 position by galactokinase:

D-Galactose + $ATP^{4-} \rightarrow$ D-galactose-1-phosphate^{2-} + ADP^{3-} + H^{+}

Galactose-1-phosphate is then converted into *UDP-galactose* (a sugar nucleotide) by galactose-1-phosphate uridylyltransferase, with concurrent production of glucose-1-phosphate and consumption of a molecule of UDP-glucose (figure 27). The glucose-1-phosphate produced by the transferase reaction is a substrate for the phosphoglucomutase reaction, which produces glucose-6-phos-phate, a glycolytic substrate. The other transferase product, UDP-galactose, is converted to UDP-glucose by UDP-glucose-4-epimerase. The combined action of the uridylyltransferase and epimerase thus produces glucose-1-P from galactose-1-P, with regeneration of UDP-glucose.

A rare hereditary condition known as galactosemia involves defects in galactose-1-P uridylyltransferase that render the enzyme inactive. Toxic levels of galactose accumulate in afflicted individuals, causing cataracts and permanent neurological disorders.

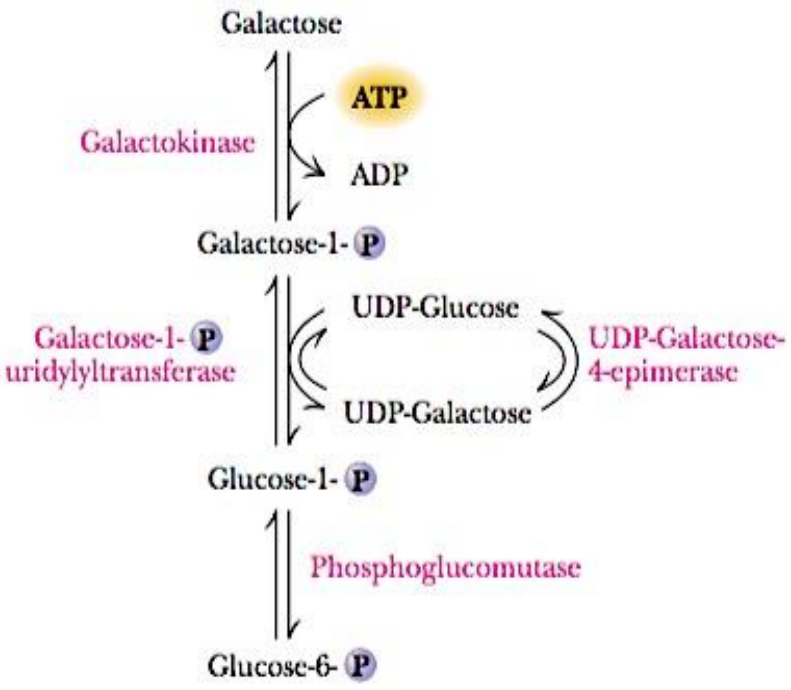


Figure 27 Galactose metabolisms via the Leloir pathway

Lactose Intolerance

A much more common metabolic disorder, lactose intolerance, occurs commonly in most parts of the world. Lactose intolerance is an inability to digest lactose because of the absence of the enzyme lactase in the intestines of adults. Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea. The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine. In most parts of the world where lactose intolerance is prevalent, adults do not use milk as a food, although milk products predigested with lactase are commercially available in some countries.

Entry of Disaccharides

Disaccharides must be hydrolyzed to monosaccharides before entering cells. Intestinal disaccharides and dextrins are hydrolyzed by enzymes attached to the outer surface of the intestinal epithelial cells:

$$\text{Dextrin} + n\text{H}_2\text{O} \xrightarrow[\text{dextrinase}]{} n \text{ D-glucose}$$

$$\text{Maltose} + \text{H}_2\text{O} \xrightarrow[\text{maltase}]{} 2 \text{ D-glucose}$$

$$\text{Lactose} + \text{H}_2\text{O} \xrightarrow[\text{lactase}]{} \text{D-galactose} + \text{D-glucose}$$

$$\text{Sucrose} + \text{H}_2\text{O} \xrightarrow[\text{sucrase}]{} \text{D-fructose} + \text{D-glucose}$$

$$\text{Trehalose} + \text{H}_2\text{O} \xrightarrow[\text{trehalase}]{} 2 \text{ D-glucose}$$

The monosaccharides so formed are actively transported into the epithelial cells, then passed into the blood to be carried to various tissues, where they are phosphorylated and funneled into the glycolytic sequence.

Entry of glycerol

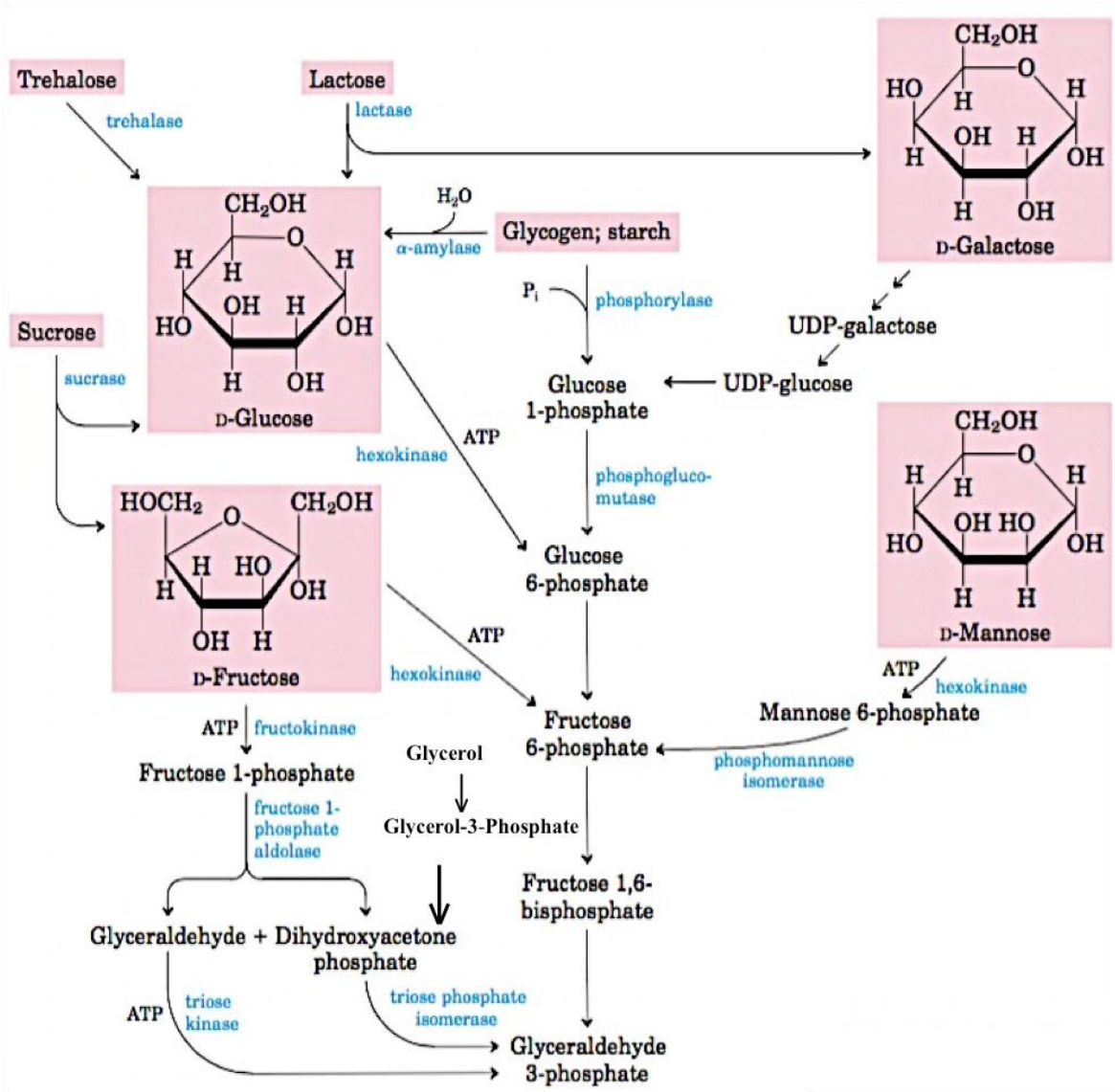
Glycerol is the last important simple metabolite, which is produced in substantial amounts by the decomposition of *triacylglycerols.* Itcan be converted to glycerol-3-phosphate by the action of glycerol kinase and then oxidized to dihydroxyacetone phosphate by the action of glycerol phosphate dehydrogenase, with NAD^+ as the required coenzyme. The

dihydroxyacetone phosphate thereby produced enters the glycolytic pathway as a substrate for triose phosphate isomerase.

The glycerol kinase reaction -

Glycerol +ATP → Glycerol-3-Phosphate + ADP

The glycerol phosphate dehydrogenase reaction -



Glycerol-3-phosphate + NAD^+→Dihydroxyacetone phosphate + NADH + H^+

Figure 28 Entry of glycogen, starch, disaccharides, hexoses and glycerol into the preparatory stage of glycolysis.

4.4.2- GLUCONEOGENESIS

The human body carries only a little more than a one-day supply of glucose. If glucose is not obtained in the diet, the body must produce new glucose from noncarbohydrate precursors,the term for this activity is **gluconeogenesis,** which means the generation (*genesis*) of new (*neo*) glucose. Additionally, muscles consume large amounts of glucose via glycolysis, producing large amounts of pyruvate. In vigorous exercise, muscle cells become anaerobic and pyruvate is converted to lactate. Gluconeogenesis salvages this pyruvate and lactate and reconverts it to glucose.

The Substrates of Gluconeogenesis

In addition to pyruvate and lactate, other noncarbohydrate precursors can be used as substrates for gluconeogenesis in animals including amino acids, glycerol and all the TCA cycle intermediates. On the other hand, fatty acids are not substrates for gluconeogenesis in animals, because most fatty acids yield only acetyl-CoA upon degradation, and animals cannot carry out net synthesis of sugars from acetyl-CoA. Lysine and leucine are the only amino acids that are not substrates for gluconeogenesis. These amino acids produce only acetyl-CoA upon degradation.

Note: Acetyl-CoA is a substrate for gluconeogenesis when the glyoxylate cycle is operating.

Site of Gluconeogenesis

The major sites of gluconeogenesis are the liver and kidneys, which account for about 90% and 10% of the body's gluconeogenic activity, respectively. Glucose produced by gluconeogenesis in the liver and kidney is released into the blood and is subsequently absorbed by brain, heart, muscle, and red blood cells to meet their metabolic needs. In turn, pyruvate and lactate produced in these tissues are returned to the liver and kidney to be used as gluconeogenic substrates.

The Pathway

Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share several steps, seven of the ten enzymatic reactions of gluconeogenesis are the reverse of glycolytic reactions. ***However, three reactions of***

glycolysis are essentially irreversible in vivo and cannot be used in gluconeogenesis: the conversion of glucose to glucose 6-phosphate by hexokinase, the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate by phosphofructokinase-1, and the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase.In cells, these three reactions are characterized by a large negative free-energy change, ΔG, whereas other glycolytic reactions have a ΔG near 0. In gluconeogenesis, the three irreversible steps are bypassed by a separate set of enzymes, catalyzing reactions that are sufficiently exergonic to be effectively irreversible in the direction of glucose synthesis. Thus, both glycolysis and gluconeogenesis are irreversible processes in cells. In animals, both pathways occur largely in the cytosol, necessitating their reciprocal and coordinated regulation.The processes of glycolysis and gluconeogenesis must be regulated in a reciprocal fashion so that when glycolysis is active, gluconeogenesis is inhibited, and vice- versa. Separate regulation of the two pathways is brought about through controls exerted on the enzymatic steps unique to each.

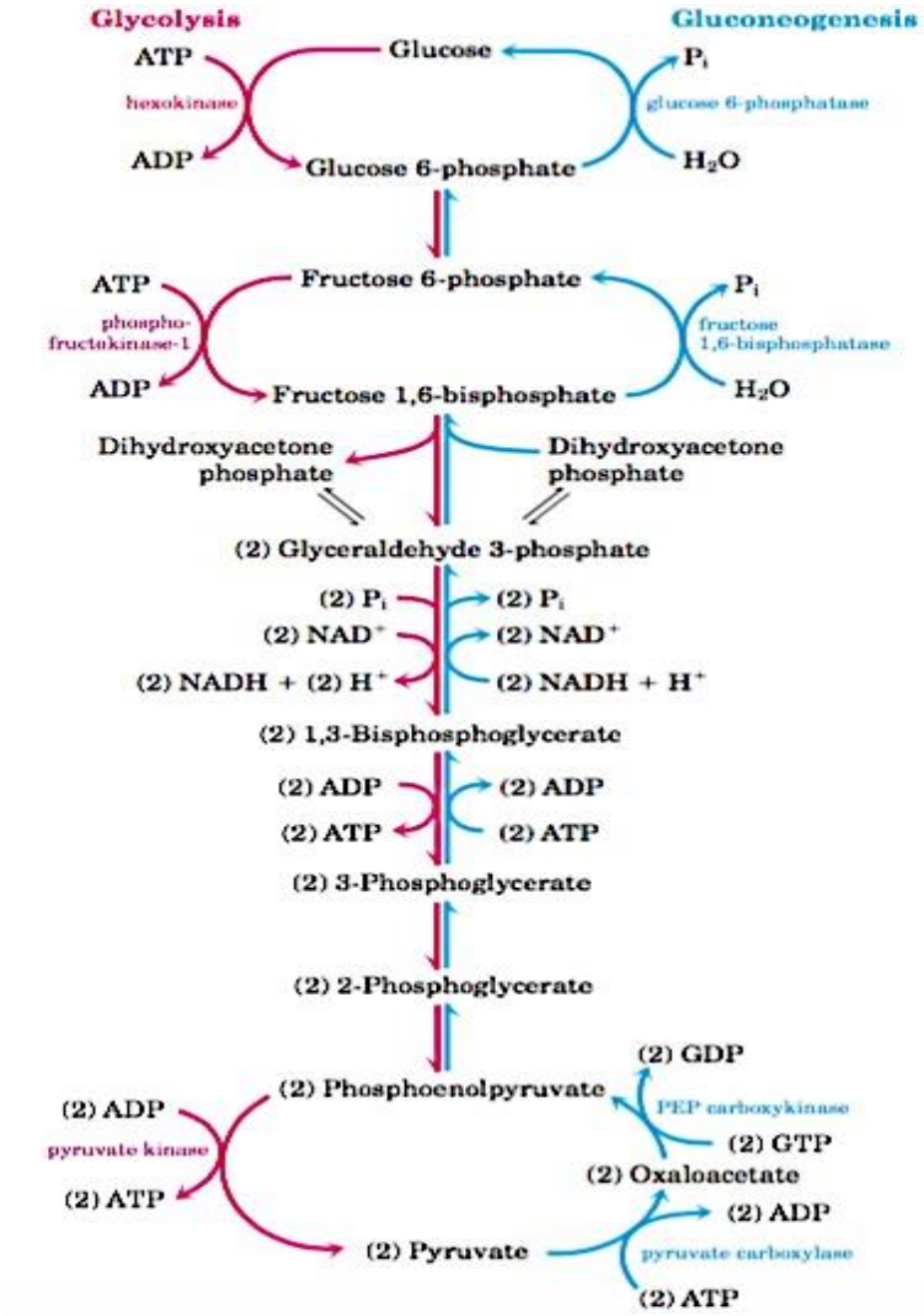


Figure 29 Opposing pathways of glycolysis and gluconeogenesis

The Unique Reactions of Gluconeogenesis

(1) Pyruvate Carboxylase—A Biotin-Dependent Enzyme & PEP Carboxykinase

Initiation of gluconeogenesis occurs by the pyruvate carboxylase reaction —**the conversion of pyruvate to oxaloacetate.**Route from pyruvate to PEP is the predominant path when pyruvate or alanine is the glucogenic precursor. A second pathway, described later, predominates when lactate is the glucogenic precursor.

The reaction takes place in two discrete steps, involves ATP and bicarbonate as substrates. Pyruvate is first transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, in which the α-amino group is removed from alanine (leaving pyruvate) and added to anα-keto carboxylic acid. Then pyruvate carboxylase, a mitochondrial enzyme converts the pyruvate to oxaloacetate:

$$\text{Pyruvate} + HCO^-_3 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

Pyruvate carboxylase a tetrameric enzyme (500 kD)is the first regulatory enzyme in the gluconeogenic pathway, utilizes biotin as a coenzyme and acetyl-coenzyme A as an allosteric activator. The pyruvate carboxylase reaction can replenish intermediates in another central metabolic pathway, the citric acid cycle.

Because the mitochondrial membrane has no transporter for oxaloacetate, before export to the cytosol the oxaloacetate formed from pyruvate must be reduced to malate by mitochondrial malate dehydrogenase, at the expense of NADH:

$$\text{Oxaloacetate} + \text{NADH} + H^+ \Leftrightarrow \text{L-malate} + NAD^+$$

Malate leaves the mitochondrion through a specific transporter in the inner mitochondrial membrane, and in the cytosol it is reoxidized to oxaloacetate, with the production of cytosolic NADH:

$$\text{Malate} + NAD^+ \rightarrow \text{oxaloacetate} + \text{NADH} + H^+$$

The oxaloacetate is then converted to PEP by phosphoenolpyruvate carboxykinase. This Mg^{2+}dependent reaction requires GTP as the phosphoryl group donor:

$$\text{Oxaloacetate} + \text{GTP} \Leftrightarrow \text{PEP} + CO_2 + \text{GDP}$$

The reaction is reversible under intracellular conditions; the formation of one high-energy phosphate compound (PEP) is balanced by the hydrolysis of another (GTP).

A second pyruvate→PEP pathway predominates when lactate is the glucogenic precursor. This pathway makes use of lactate produced by glycolysis in erythrocytes or anaerobic muscle, (after vigorous exercise). The lactate thus produced can be transported from muscle to the liver, where it is reoxidized by liver lactate dehydrogenase to yield pyruvate, which is

converted eventually to glucose. In this way, the liver shares in the metabolic stress created by vigorous exercise. It exports glucose to muscle, which produces lactate, which can be processed by the liver into new glucose. This is referred to as the **Cori cycle.** The conversion of lactate to pyruvate in the cytosol of hepatocytes yields NADH, and the export of reducing equivalents (as malate) from mitochondria is therefore unnecessary. After the pyruvate produced by the lactate dehydrogenase reaction is transported into the mitochondrion, it is converted to oxaloacetate by pyruvate carboxylase, as described above. This oxaloacetate, however, is converted directly to PEP by a mitochondrial isozyme of PEP carboxykinase, and the PEP is transported out of the mitochondrion to continue on the gluconeogenic path.

(2) Fructose-1,6-Bisphosphatase

The second glycolytic reaction that cannot participate in gluconeogenesis is the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate, like all phosphate ester hydrolyses, is a thermodynamically favorable (exergonic) reaction under standard-state conditions. Under physiological conditions in the liver, the reaction is also exergonic. **Fructose-1,6-bisphosphatase** is an allosterically regulated enzyme. Citrate stimulates bisphosphatase activity, but *fructose-2,6-bisphosphate* is a potent allosteric inhibitor. AMP also inhibits the bisphosphatase; the inhibition by AMP is enhanced by fructose-2, 6-bisphosphate.

Fructose 1,6-bisphosphate + $H_2O \rightarrow$ fructose 6-phosphate + P_i

(3) Glucose-6-Phosphatase

The final step in the gluconeogenesis pathway is the dephosphorylation of glucose-6-phosphate to glucose by the action of **glucose-6-phosphatase.** The reaction catalyzed by glucose 6-phosphatase does not require synthesis of ATP; it is a simple hydrolysis of a phosphate ester:

Glucose 6-phosphate + $H_2O \rightarrow$ glucose + Pi

This enzyme is present in the lumenal side of the membranes of the endoplasmic reticulum of liver and kidney cells, but is absent in muscle and brain. For this reason, **gluconeogenesis is not carried out in muscle and brain.** Its membrane association is important to its function because the substrate is hydrolyzed as it passes into the endoplasmic reticulum

itself. Vesicles form from the endoplasmic reticulum membrane and diffuse to the plasma membrane and fuse with it, releasing their glucose contents into the bloodstream.

4.4.2.1 COORDINATED REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS

Hexokinase, which catalyzes the entry of free glucose into the glycolytic pathway, is a regulatory enzyme. There are four isozymes (designated I to IV), encoded by four different genes. Muscle hexokinases I and II are allosterically inhibited by their product, glucose 6-phosphate, so whenever the cellular concentration of glucose 6-phosphate rises above its normal level, these isozymes are temporarily and reversibly inhibited, bringing the rate of glucose 6-phosphate formation into balance with the rate of its utilization and reestablishing the steady state.

The different hexokinase isozymes of liver and muscle reflect the different roles of these organs in carbohydrate metabolism: muscle consumes glucose, using it for energy production, whereas liver maintains blood glucose homeostasis by removing or producing glucose, depending on the prevailing glucose concentration.

Phosphofructokinase-1 Is under Complex Allosteric Regulation

The metabolically irreversible reaction catalyzed by PFK-1 is the step that commits glucose to glycolysis. In addition to its substrate-binding sites, this complex enzyme has several regulatory sites at which allosteric activators or inhibitors bind. ATP is not only a substrate for PFK-1 but also an end product of the glycolytic pathway. When high cellular [ATP] signals that ATP is being produced faster than it is being consumed, ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for fructose 6-phosphate. ADP and AMP, which increase in concentration as consumption of ATP outpaces production, act allosterically to relieve this inhibition by ATP. These effects combine to produce higher enzyme activity when ADP or AMP accumulates and lower activity when ATP accumulates.

Citrate (the ionized form of citric acid), a key intermediate in the aerobic oxidation of pyruvate, fatty acids, and amino acids, also serves as an allosteric regulator of PFK-1; high citrate concentration increases the inhibitory effect of ATP, further reducing the flow of glucose through glycolysis. In this case, as in several others encountered later, citrate serves

as an intracellular signal that the cell is meeting its current needs for energy-yielding metabolism by the oxidation of fats and proteins.

The most significant allosteric regulator of PFK-1 is fructose 2,6-bisphosphate, which strongly activates the enzyme.

Pyruvate Kinase Is Allosterically Inhibited by ATP

High concentrations of ATP, acetyl-CoA, and long-chain fatty acids (signs of abundant energy supply) allosterically inhibit all isozymes of pyruvate kinase. The liver isozyme (L form), but not the muscle isozyme (M form), is subject to further regulation by phosphorylation. When low blood glucose causes glucagon release, cAMP-dependent protein kinase phosphorylates the L isozyme of pyruvate kinase, inactivating it. This slows the use of glucose as a fuel in liver, sparing it for export to the brain and other organs. In muscle, the effect of increased [cAMP] is quite different. In response to epinephrine, cAMP activates glycogen breakdown and glycolysis and provides the fuel needed for the fight-or-flight response.

Gluconeogenesis Is Regulated at Several Steps

In the pathway leading from pyruvate to glucose, the first control point determines the fate of pyruvate in the mitochondrion. Pyruvate can be converted either to acetyl-CoA to fuel the citric acid cycle, or to oxaloacetate (by pyruvate carboxylase) to start the process of gluconeogenesis. When fatty acids are readily available as fuels, their breakdown in liver mitochondria yields acetyl-CoA, a signal that further oxidation of glucose for fuel is not necessary. Acetyl-CoA is a positive allosteric modulator of pyruvate carboxylase and a negative modulator of pyruvate dehydrogenase, through stimulation of a protein kinase that inactivates the dehydrogenase. When the cell's energetic needs are being met, oxidative phosphorylation slows, NADH rises relative to NAD and inhibits the citric acid cycle, and acetyl-CoA accumulates. The increased concentration of acetyl-CoA inhibits the pyruvate dehydrogenase complex, slowing the formation of acetyl-CoA from pyruvate, and stimulates gluconeogenesis by activating pyruvate carboxylase, allowing excess pyruvate to be converted to glucose.

The second control point in gluconeogenesis is the reaction catalyzed by FBPase-1, which is strongly inhibited by AMP. The corresponding glycolytic enzyme, PFK-1, is stimulated by

AMP and ADP but inhibited by citrate and ATP. Thus these opposing steps in the two pathways are regulated in a coordinated and reciprocal manner. In general, when sufficient concentrations of acetyl-CoA or citrate (the product of acetyl- CoA condensation with oxaloacetate) are present, or when a high proportion of the cell's adenylate is in the form of ATP, gluconeogenesis is favored. AMP promotes glycogen degradation and glycolysis by activating glycogen phosphorylase (via activation of phosphorylase kinase) and stimulating the activity of PFK-1. Another set of regulatory processes is triggered from outside the cell by the hormones insulin and glucagon, which signal too much or too little glucose in the blood, respectively, or by epinephrine, which signals the impending need for fuel for a fight-or-flight response. These hormonal signals bring about covalent modification (phosphorylation or dephosphorylation) of target proteins inside the cell; this takes place on a somewhat longer time scale than the internally driven allosteric mechanisms—seconds or minutes, rather than milliseconds.

Fructose 2,6-Bisphosphate Is a Potent Regulator of Glycolysis and Gluconeogenesis

The hormonal regulation of glycolysis and gluconeogenesis is mediated by fructose 2,6-bisphosphate, an allosteric effector for the enzymes PFK-1 and FBPase-1.When fructose 2,6-bisphosphate binds to its allosteric site on PFK-1, it increases that enzyme's affinity for its substrate, fructose 6-phosphate, and reduces its affinity for the allosteric inhibitors ATP and citrate. At the physiological concentrations of its substrates ATP and fructose 6-phosphate and of its other positive and negative effectors (ATP, AMP, citrate), PFK-1 is virtually inactive in the absence of fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate activates PFK-1 and stimulates glycolysis in liver and, at the same time, inhibits FBPase-1, thereby slowing gluconeogenesis.

The cellular concentration of fructose 2,6-bisphosphate is set by the relative rates of its formation and breakdown. It is formed by phosphorylation of fructose 6-phosphate, catalyzed by phosphofructokinase-2 (PFK-2), and is broken down by fructose 2,6-bisphosphatase (FBPase-2). (PFK-2 and FBPase-2 are two distinct enzymatic activities of a single, bifunctional protein. The balance of these two activities in the liver, which determines the cellular level of fructose 2,6- bisphosphate, is regulated by glucagon and insulin.Glucagon stimulates the adenylyl cyclase of liver to synthesize 3,5 -cyclic AMP (cAMP) from ATP. Then cAMP activates cAMP-dependent protein kinase, which transfers a phosphoryl group from ATP to the bifunctional protein PFK-2/FBPase-2. Phosphorylation

of this protein enhances its FBPase-2 activity and inhibits its PFK-2 activity. Glucagon thereby lowers the cellular level of fructose 2,6-bisphosphate, inhibiting glycolysis and stimulating gluconeogenesis. The resulting production of more glucose enables the liver to replenish blood glucose in response to glucagon. Insulin has the opposite effect, stimulating the activity of a phosphoprotein phosphatase that catalyzes removal of the phosphoryl group from the bifunctional protein PFK-2/FBPase-2, activating its PFK-2 activity, increasing the level of fructose 2,6-bisphosphate, stimulating glycolysis, and inhibiting gluconeogenesis.

Xylulose 5-Phosphate, a Key Regulator of Carbohydrate

Another recently discovered regulatory mechanism also acts by controlling the level of fructose 2,6-bisphosphate. In the mammalian liver, xylulose 5-phosphate, a product of the hexose monophosphate pathway, mediates the increase in glycolysis that follows ingestion of a high-carbohydrate meal. The xylulose 5-phosphate concentration rises as glucose entering the liver is converted to glucose 6-phosphate and enters both the glycolytic and hexose monophosphate pathways. Xylulose 5-phosphate activates a phosphoprotein phosphatase, PP2A, that dephosphorylates the bifunctional PFK-2/FBPase-2 enzyme. Dephosphorylation activates PFK-2 and inhibits FBPase-2, and the resulting rise in [fructose 2,6-bisphosphate] stimulates glycolysis and inhibits gluconeogenesis. The increased glycolysis boosts the production of acetyl-CoA, while the increased flow of hexose through the hexose monophosphate pathway generates NADPH. Acetyl-CoA and NADPH are the starting materials for fatty acid synthesis, which has long been known to increase dramatically in response to intake of a high-carbohydrate meal.

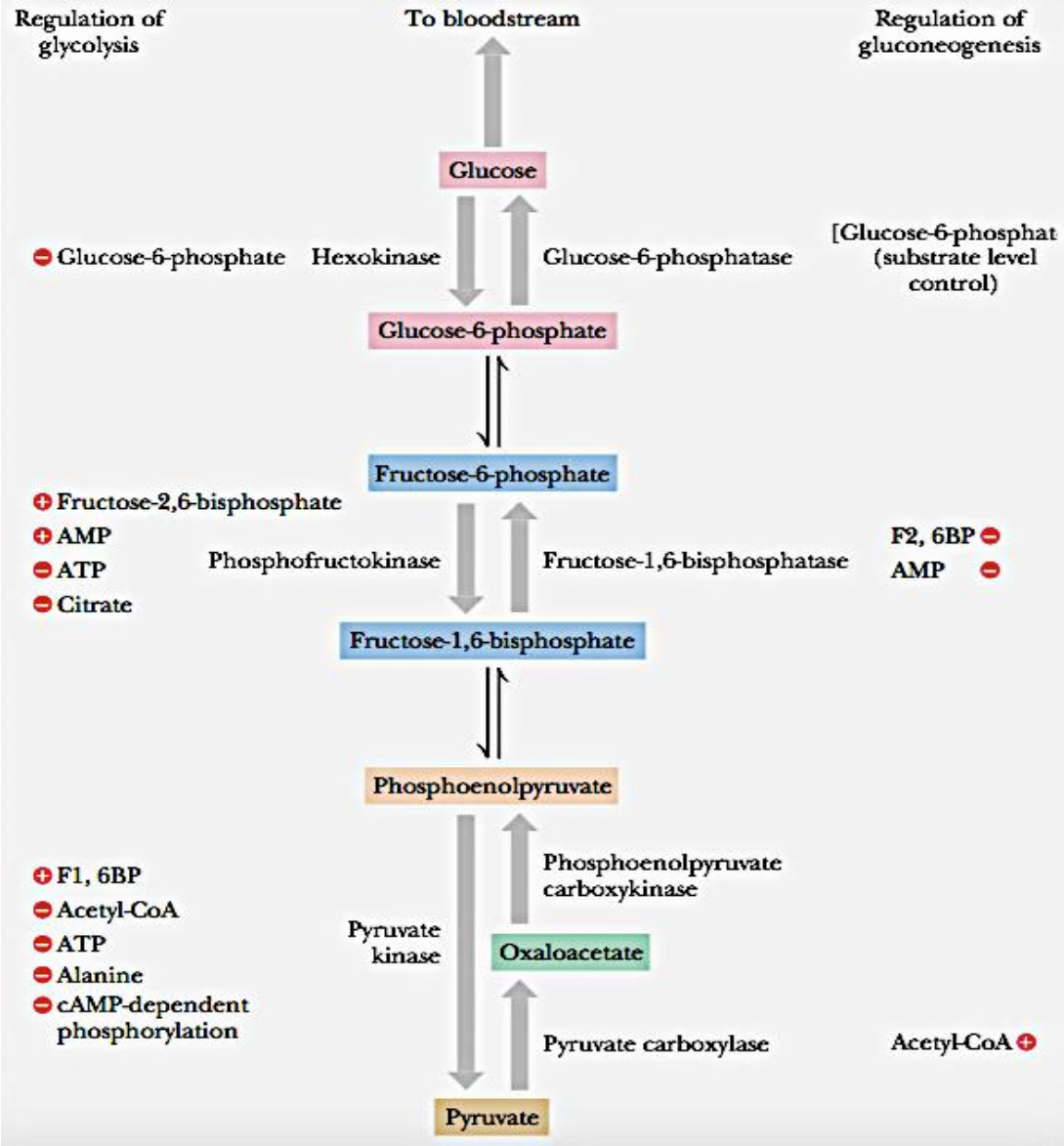


Figure 30 Regulation mechanisms in glycolysis and gluconeogenesis. Acitvators are depicted by plus sign and inhibitors by minus sign

4.4.3 PENTOSE PHOSPHATE PATHWAY

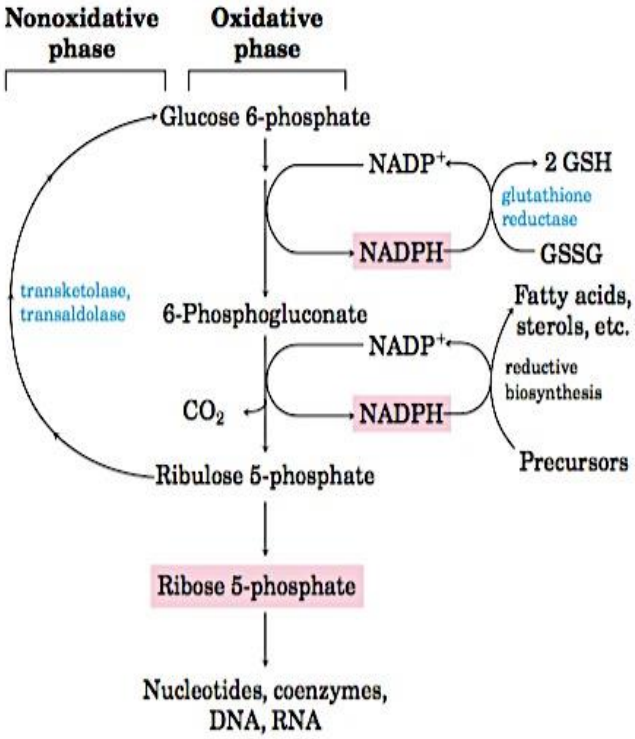
Cells require a constant supply of NADPH for reductive reactions vital to biosynthetic purposes. Much of this requirement is met by a glucose-based metabolic sequence variously called **the pentose phosphate pathway, the hexose mono-phosphate shunt, or the phosphogluconate pathway**. In addition to providing NADPH for biosynthetic processes, this pathway produces *ribose-5-phosphate*, which is essential for nucleic acid synthesis. Several metabolites of the pentose phosphate pathway can also be shuttled into glycolysis. Rapidly dividing cells, such as those of bone marrow, skin and intestinal mucosa, use the pentoses to make RNA, DNA, and such coenzymes as ATP, NADH, $FADH_2$, and coenzyme

A.

In other tissues, the essential product of the pentose phosphate pathway is not the pentoses but the electron donor NADPH, needed for reductive biosynthesis or to counter the damaging effects of oxygen radicals. By maintaining a reducing atmosphere (a high ratio of NADPH to $NADP^+$ and a high ratio of reduced to oxidized glutathione), they can prevent or undo oxidative damage to proteins, lipids, and other sensitive molecules.

An Overview

The pentose phosphate pathway begins with glucose-6-phosphate, a six-carbon sugar, and produces three-, four-, five-, six-, and seven-carbon sugars. Two successive oxidations lead to the reduction of $NADP^+$to NADPH and the release of CO_2. Five subsequent non-oxidative steps produce a variety of carbohydrates, some of which may enter the glycolytic pathway. The enzymes of the pentose phosphate pathway are particularly abundant in the cytoplasm of liver and adipose cells. **These enzymes are largely absent in muscle**, where glucose-6-phosphate is utilized primarily for energy production via glycolysis and the TCA cycle. These pentose phosphate pathway enzymes are located in the cytosol, which is the site of fatty acid synthesis, a pathway heavily dependent on NADPH for reductive reactions.

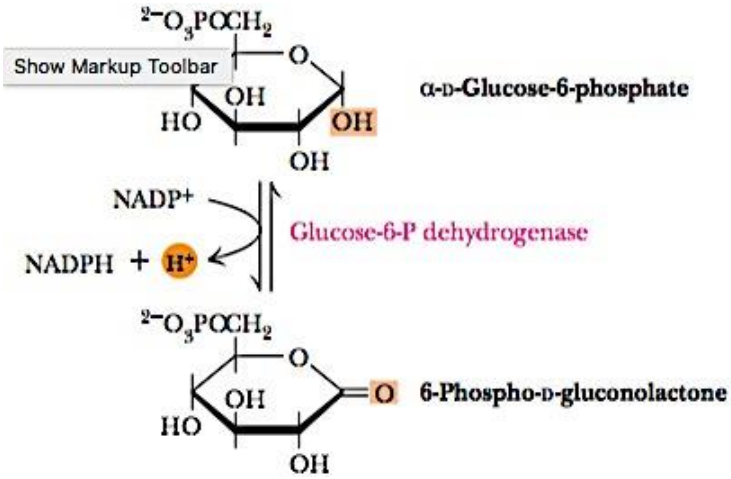


The Oxidative Steps of the Pentose Phosphate Pathway

(1) Glucose-6-Phosphate Dehydrogenase

The pentose phosphate pathway begins with the oxidation of glucose-6-phosphate. The products of the reaction are**6-Phospho-D-gluconolactone (**cyclic ester) and NADPH (Figure 31). Glucose-6-phosphate dehydrogenase, which catalyzes this reaction, is highly specific for $NADP^+$. As the first step of a major pathway, the reaction is irreversible and highly regulated. Glucose-6-phosphate dehydrogenase is strongly inhibited by the product coenzyme, NADPH, and also by fatty acid esters of coenzyme A (which are intermediates of fatty acid biosynthesis).

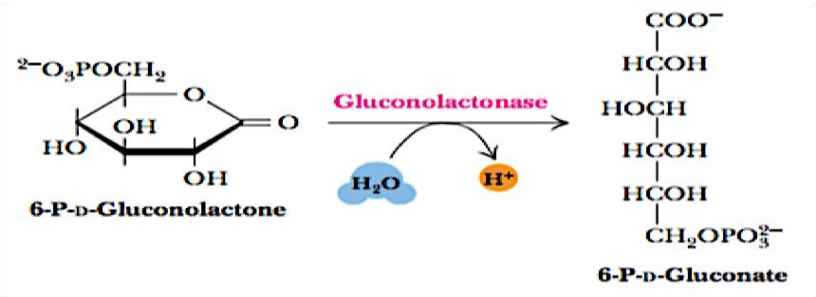
(Step 1)



(2) Gluconolactonase

The **6-Phospho-D-gluconolactone** produced in step 1 is hydrolytically unstable and readily undergoes a spontaneous ring-opening hydrolysis, although an enzyme, gluconolactonase, accelerates this reaction. The linear product, the sugar acid 6-phospho-D-gluconate, is further oxidized in step 3.

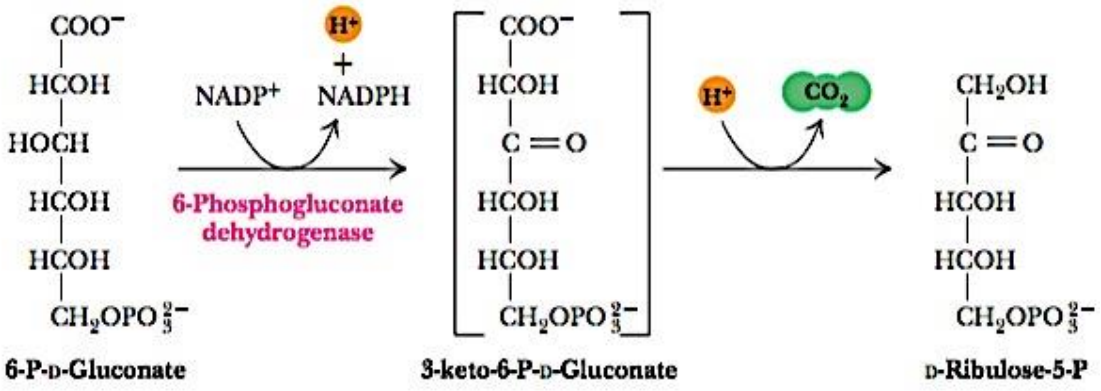
(Step 2)



(3) 6-Phosphogluconate Dehydrogenase

The oxidative decarboxylation of 6-phosphogluconate by 6-phosphogluconate dehydrogenase yields D-ribulose-5-phosphate and another equivalent of NADPH. There are two distinct steps in this reaction: the initial NADP -dependent dehydrogenation yields a -keto acid, 3-keto-6-phosphogluconate, which is very susceptible to decarboxylation (the second step). The resulting product, D-ribulose-5-P, is the substrate for the nonoxidative reactions composing the rest of this pathway.

(Step 3)



The Nonoxidative Steps of the Pentose Phosphate Pathway

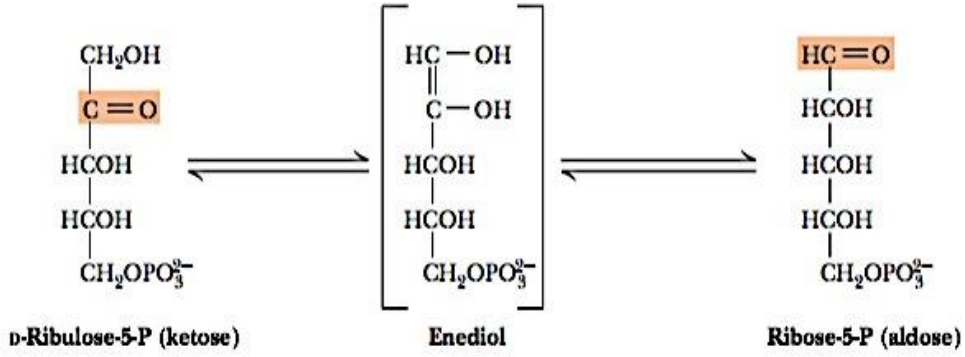
This portion of the pathway begins with an isomerization and an epimerization, and it leads to the formation of either D-ribose-5-phosphate or D-xylulose- 5-phosphate. These intermediates can then be converted into glycolytic intermediates or directed to biosynthetic processes.

(4) Phosphopentose Isomerase

This enzyme interconverts ribulose-5-P and ribose-5-P via an enediol intermediate. The reaction (and mechanism) is quite similar to the phosphoglucoisomerase reaction of glycolysis, which interconverts glucose-6-P and fructose-6-P. The ribose-5-P produced in this reaction is utilized in the biosynthesis of coenzymes (including NADH, NADPH, FAD, and B_{12}), nucleotides, and nucleic acids (DNA and RNA). The net reaction for the first four steps of the pentose phosphate pathway is

Glucose-6-P + 2 $NADP^+ \rightarrow$ ribose-5-P +2 NADPH + $2H^+$+ CO_2

(Step 4)



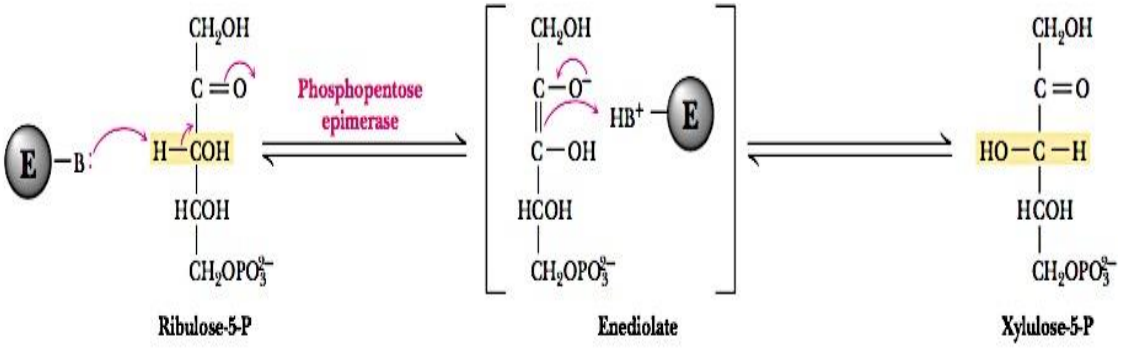
(5) Phosphopentose Epimerase

This reaction converts ribulose-5-P to another ketose, namely, xylulose-5-P. This reaction also proceeds by an enediol intermediate, but involves an inversion at C-3. In the reaction, an acidic proton located α- to a carbonyl carbon is removed to generate the enediolate, but the proton is added back to the same carbon from the opposite side.

Note* -Interchange of groups on a single carbon is an epimerization, and interchange of groups between carbons is referred to as an isomerization.

To this point, the pathway has generated a pool of pentose phosphates. The $\Delta G'°$ for each of the last two reactions is small, and the three pentose-5-phosphates coexist at equilibrium. The pathway has also produced two molecules of NADPH for each glucose-6-P converted to pentose-5-phosphate. The next three steps rearrange the five-carbon skeletons of the pentoses to produce three-, four-, six-, and seven-carbon units, which can be used for various metabolic purposes.

(Step 5)

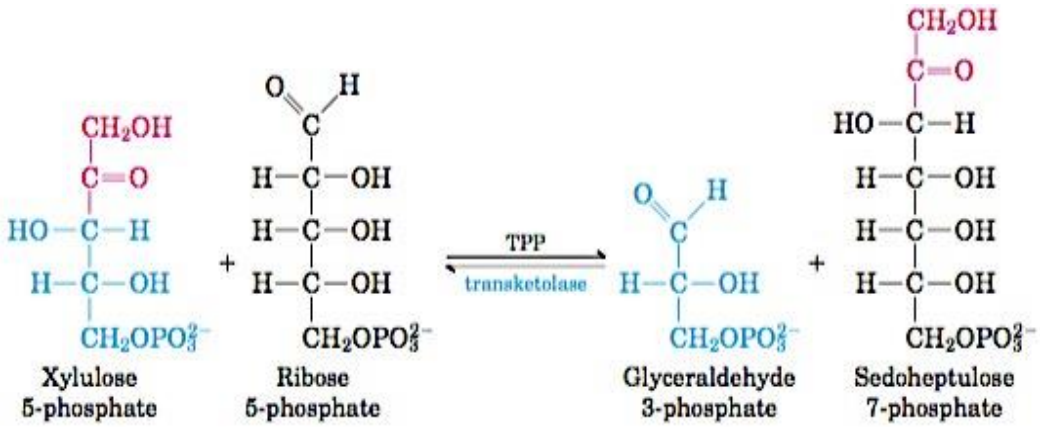


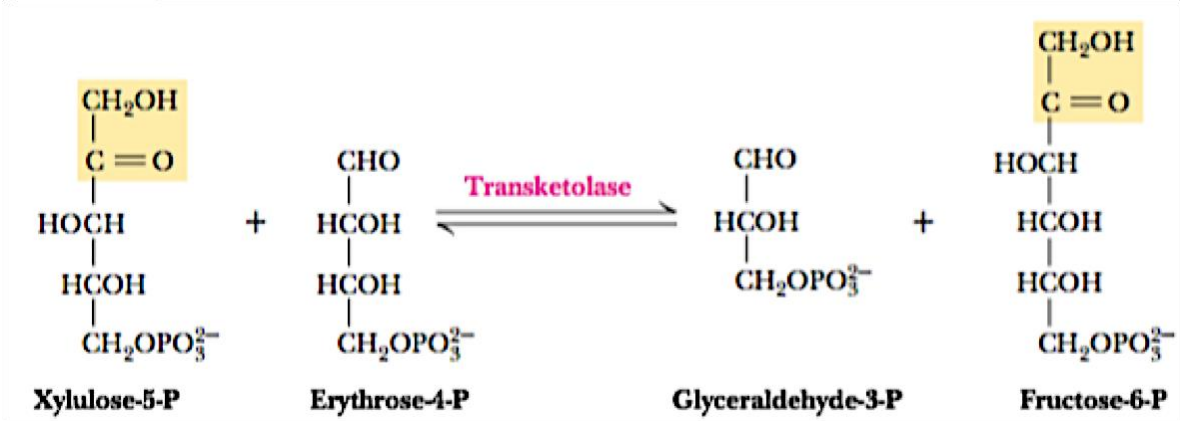
(6) and (8) Transketolase

The transketolase enzyme acts at both steps 6 and 8 of the pentose phosphate pathway. In

both cases, the enzyme catalyzes the transfer of two-carbon units. In these reactions (and also in step 7, the transaldolase reaction, which transfers three-carbon units), the donor molecule is a ketose and the recipient is an aldose. In step 6, xylulose-5-phosphate transfers a two-carbon unit to ribose-5-phosphate to form glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate. Step 8 involves a two-carbon transfer from xylulose-5-phosphate to erythrose 4-phosphate to produce another glyceraldehyde- 3-phosphate and a fructose-6-phosphate. Three of these products enter directly into the glycolytic pathway. Transketolase is a thiamine pyrophosphate–dependent enzyme, and the mechanism involves abstraction of the acidic thiazole proton of TPP, attack by the resulting carbanion at the carbonyl carbon of the ketose phosphate substrate, expulsion of the glyceraldehyde-3-phosphate product, and transfer of the two-carbon unit. Transketolase can process a variety of 2-keto sugar phosphates in a similar manner. It is specific for ketose substrates with the configuration shown, but can accept a variety of aldose phosphate substrates.

(Step 6)

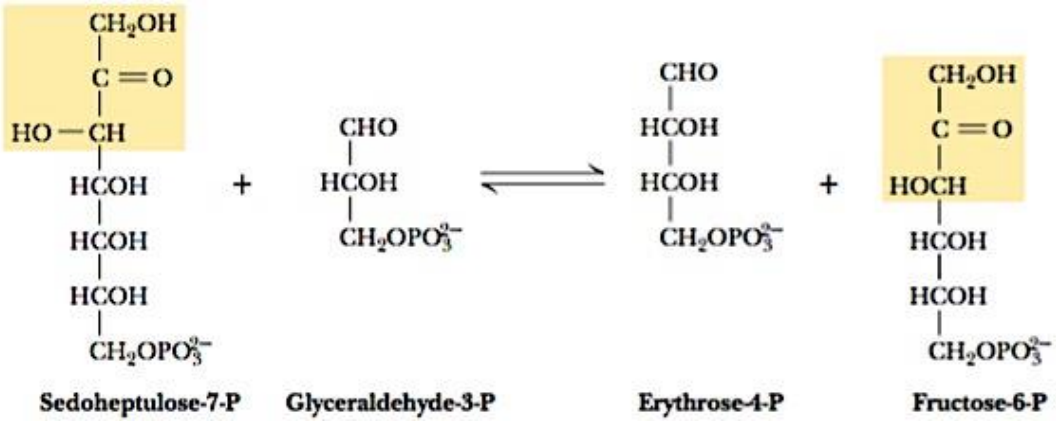




(7) Transaldolase

The transaldolase functions primarily to make a useful glycolytic substrate from the sedoheptulose-7-phosphate produced by the first transketolase reaction. This reaction is quite similar to the aldolase reaction of glycolysis, involving formation of a Schiff base intermediate between the sedoheptulose-7-phosphate and an active-site lysine residue. Elimination of the erythrose-4-phosphate product leaves an enamine of dihydroxyacetone, which remains stable at the active site (without imine hydrolysis) until the other substrate comes into position. Attack of the enamine carbanion at the carbonyl carbon of glyceraldehyde-3-phosphate is followed by hydrolysis of the Schiff base (imine) to yield the product fructose-6-phosphate.

(Step 7)



4.4.3.1 REGULATION OF PENTOSE PHOSPHATE PATHWAY

It is clear that glucose-6-phosphate can be used as a substrate either for glycolysis or for the pentose phosphate pathway. The cell makes this choice on the basis of its relative needs for biosynthesis and for energy from metabolism. ATP can be produced in abundance if glucose-6-phosphate is channeled into glycolysis. On the other hand, if NADPH or ribose-5-phosphate is needed, glucose-6-phosphate can be directed to the pentose phosphate pathway. The molecular basis for this regulatory decision depends on the enzymes that metabolize glucose-6-phosphate in glycolysis and the pentose phosphate pathway. In glycolysis, phosphoglucoisomerase converts glucose-6-phosphate to fructose-6-phosphate, which is utilized by phosphofructokinase (a highly regulated enzyme) to produce fructose-1, 6-bisphosphate. In the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (also highly regulated) produces gluconolactone from glucose-6-phosphate. Thus, the fate of glucose-6-phosphate is determined to a large extent by the relative activities of

phosphofructokinase and glucose-6-P dehydrogenase. Recall that PFK is inhibited when the ATP/AMP ratio increases, and that it is inhibited by citrate but activated by fructose-2, 6-bisphosphate. Thus, when the energy charge is high, glycolytic flux decreases. Glucose-6-P dehydrogenase, on the other hand, is inhibited by high levels of NADPH and also by the intermediates of fatty acid biosynthesis. Both of these are indicators that biosynthetic demands have been satisfied. If that is the case, glucose-6-phosphate dehydrogenase and the pentose phosphate pathway are inhibited. If NADPH levels drop, the pentose phosphate pathway turns on, and NADPH and ribose-5-phosphate are made for biosynthetic purposes.

Even when the latter choice has been made, however, the cell must still be "aware" of the relative needs for ribose-5-phosphate and NADPH (as well as ATP). Depending on these relative needs, the reactions of glycolysis and the pentose phosphate pathway can be combined in novel ways to emphasize the synthesis of needed metabolites.

4.4.4 GLYCOGEN METABOLISM

In a wide range of organisms, excess glucose is converted to polymeric forms for storage—glycogen in vertebrates and many microorganisms, starch in plants. In vertebrates, glycogen is found primarily in the liver and skeletal muscle. The glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for the neurons of the brain, which cannot use fatty acids as fuel. In humans, the total amount of energy stored as glycogen is far less than the amount stored as fat (triacylglycerol), but fats cannot be converted to glucose in mammals and cannot be catabolized anaerobically.

Glycogen granules are complex aggregates of glycogen and the enzymes that synthesize it and degrade it, as well as the machinery for regulating these enzymes. The general mechanisms for storing and mobilizing glycogen are the same in muscle and liver, but the enzymes differ in subtle yet important ways that reflect the different roles of glycogen in the two tissues. Glycogen is also obtained in the diet and broken down in the gut, and this involves a separate set of hydrolytic enzymes that convert glycogen (and starch) to free glucose.

4.4.4.1 GLYCOGENOLYSIS(GLYCOGEN BREAKDOWN)

In skeletal muscle and liver, the glucose units of the outer branches of glycogen enter the glycolytic pathway through the action of three enzymes: glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase. Glycogen phosphorylase catalyzes the reaction in which an$\alpha(1\rightarrow4)$ glycosidic linkage between two glucose residues at a nonreducing end of glycogen undergoes attack by inorganic phosphate (Pi), removing the terminal glucose residue as α-D-glucose 1-phosphate (figure 32). The cleavage of a bond by the addition of orthophosphate is referred to as *phosphorolysis*. This phosphorolysis reaction is different from the hydrolysis of glycosidic bonds by amylase during intestinal degradation of dietary glycogen and starch. In phosphorolysis, some of the energy of the glycosidic bond is preserved in the formation of the phosphate ester, glucose 1-phosphate.

NOTE*- *The phosphorolytic cleavage of glycogen is energetically advantageous because the released sugar is already phosphorylated. In contrast, a hydrolytic cleavage would yield glucose, which would then have to be phosphorylated at the expense of the hydrolysis of a molecule of ATP to enter the glycolytic pathway. An additional advantage of phosphorolytic cleavage for muscle cells is that glucose 1-phosphate, negatively charged under physiological conditions, cannot diffuse out of the cell. Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction.*

Glycogen phosphorylase acts repetitively on the nonreducing ends of glycogen branches until it reaches a point four glucose residues away from an $\alpha(1\rightarrow6)$ branch point, where its action stops. How can the remainder of the glycogen molecule be mobilized for use as a fuel? Two additional enzymes, a *transferase* and α-*1,6-glucosidase*, remodel the glycogen for continued degradation by the phosphorylase (Figure 33). *The transferase shifts a block of three glycosyl residues from one outer branch to the other.* This transfer exposes a single glucose residue joined by an α-$\alpha(1\rightarrow6)$-glycosidic linkage. $\alpha(1\rightarrow6)$-Glucosidase, also known as the debranching enzyme, hydrolyzes the $\alpha(1\rightarrow6)$glycosidic bond, resulting in the release of a free glucose molecule.

This free glucose molecule is phosphorylated by the glycolytic enzyme hexokinase. Thus, the transferase and $\alpha(1\rightarrow6)$glucosidase convert the branched structure into a linear one, which paves the way for further cleavage by phosphorylase. It is noteworthy that, in eukaryotes, thetransferase and the $\alpha(1\rightarrow6)$glucosidase activities are present in a single 160-

kd polypeptidechain, providing yet another example of a bifunctional enzyme .

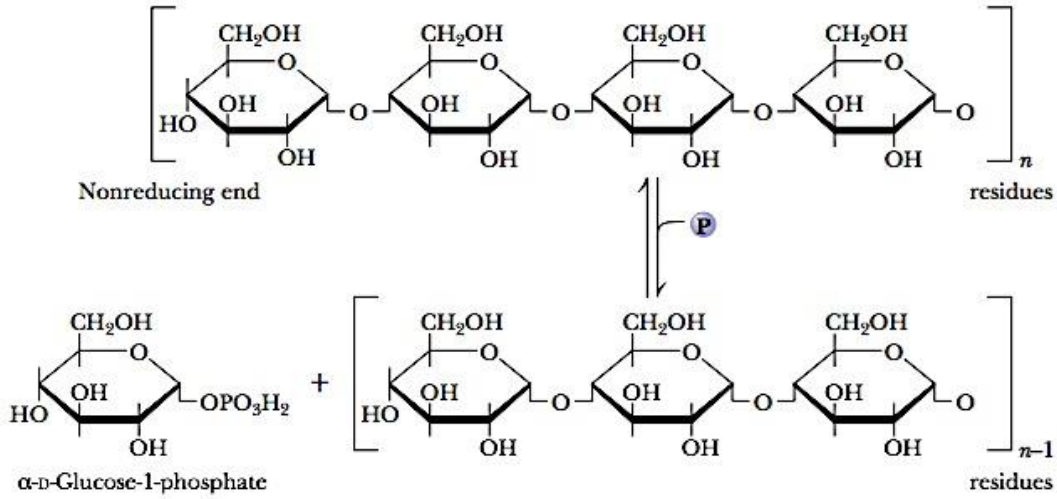


Figure 32 The glycogen phosphorylase reaction

Glucose 1-phosphate, the end product of the glycogen phosphorylase reaction, is converted to glucose 6-phosphate to enter the metabolic mainstream by phosphoglucomutase, which catalyzes the reversible reaction

Glucose 1-phosphate ⇔glucose 6-phosphate

The catalytic site of an active mutase molecule contains a phosphorylated serine residue. The phosphoryl group is transferred from the serine residue to the C-6 hydroxyl group of glucose 1-phosphate to form glucose 1,6-bisphosphate. The C-1 phosphoryl group of this intermediate is then shuttled to the same serine residue, resulting in the formation of glucose 6-phosphate and the regeneration of the phosphoenzyme.

The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction. In liver, glycogen breakdown serves a different purpose: to release glucose into the blood when the blood glucose level drops, as it does between meals. **This requires an enzyme, glucose 6-phosphatase, that is present in liver and kidney but not in other tissues.** The enzyme is an integral membrane protein of the endoplasmic reticulum, with its active site on the lumenal side of the ER. Glucose 6-phosphate formed in the cytosol is transported into the ER lumen by a specific transporter (T1) and hydrolyzed at the lumenal surface by the glucose 6-phosphatase. The

resulting Pi and glucose are thought to be carried back into the cytosol by two different transporters (T2 and T3), and the glucose leaves the hepatocyte via yet another transporter in the plasma membrane (GLUT2). **Note* that by having the active site of glucose 6-phosphatase inside the ERlumen, the cell separatesthis reaction from the process of glycolysis, which takes place in the cytosoland would be aborted by the action of glucose 6-phosphatase**.

Genetic defects in either glucose 6-phosphatase or T1 lead to serious derangement of glycogen metabolism, resulting in type Ia glycogen storage disease.

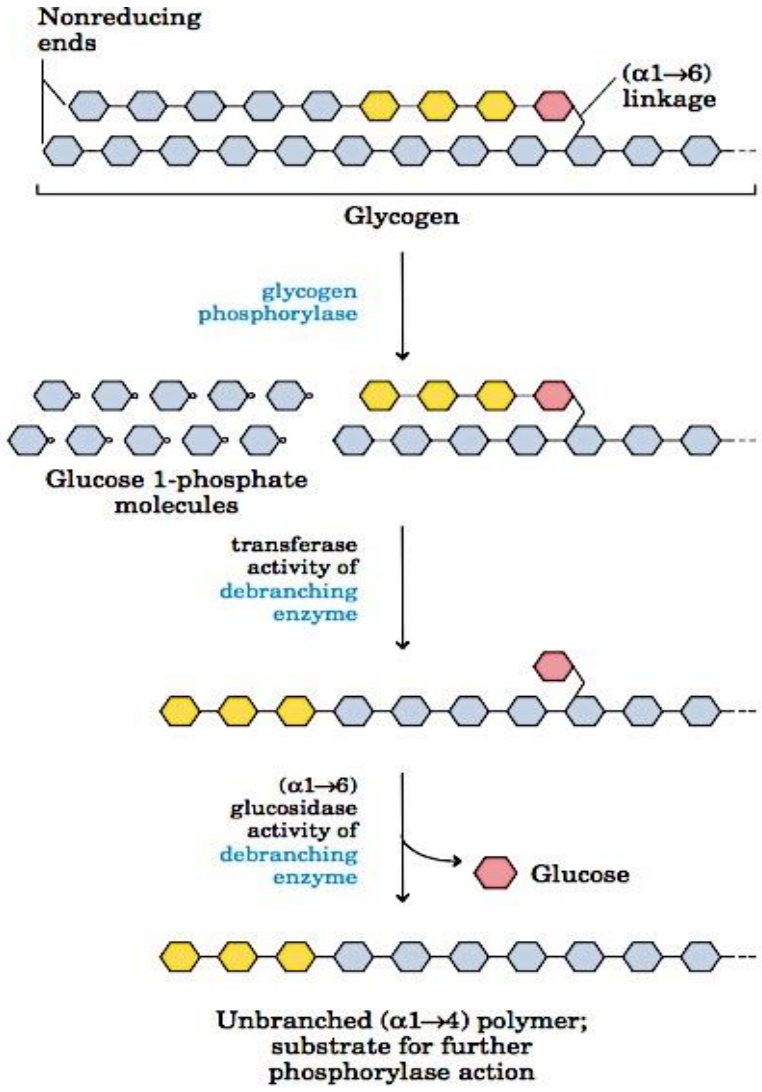


Figure 33 The reaction of the glycogen debranching enzyme

4.4.4.2- GLYCOGEN SYNTHESIS (GLYCOGENESIS)

Animals synthesize and store glycogen when glucose levels are high, but the synthetic pathway is not merely a reversal of the glycogen phosphorylase reaction. High levels of

phosphate in the cell favor glycogen breakdown and prevent the phosphorylase reaction from synthesizing glycogen *in vivo*, in spite of the fact that $\Delta G'°$ for the phosphorylase reaction actually favors glycogen synthesis. Hence, another reaction pathway must be employed in the cell for the net synthesis of glycogen. In essence, this pathway must activate glucose units for transfer to glycogen chains.

Glucose Units Are Activated for Transfer by Formation of Sugar Nucleotides

Glycogen synthesis depended upon **sugar nucleotides,** which may be thought of as activated forms of sugar units. For example, formation of an ester linkage between the C-1 hydroxyl group and the β-phosphate of UDP activates the glucose moiety of **UDP-glucose.**

UDP-Glucose Synthesis Is Driven by Pyrophosphate Hydrolysis

Sugar nucleotides are formed from sugar-1-phosphates and nucleoside triphosphates by specific **pyrophosphorylase** enzymes. For example, **UDP-glucose pyrophosphorylase** catalyzes the formation of UDP-glucose from glucose-1-phosphate and uridine 5 – triphosphate (Figure 34):

Glucose-1-P + UTP $\rightarrow$ UDP-glucose pyrophosphate

The reaction proceeds via attack by a phosphate oxygen of glucose-1-phosphate on the α-phosphorus of UTP, with departure of the pyrophosphate anion. The reaction is a reversible one, butit is driven forward by subsequent hydrolysis of pyrophosphate:

Pyrophosphate + $H_2O \rightarrow$ 2 Pi

The net reaction for sugar nucleotide formation (combining the preceding two equations) is thus Glucose-1-P + UTP + $H_2O \rightarrow$ UDP-glucose +2 Pi

Sugar nucleotides of this type act as donors of sugar units in the biosynthesis of oligo- and polysaccharides. In animals, UDP-glucose is the donor of glucose units for glycogen synthesis, but ADP-glucose is the glucose source for starch synthesis in plants.

Glycogen Synthase Catalyzes Formation of α-(1→4) Glycosidic Bonds in Glycogen

The very large glycogen polymer is built around a tiny protein core. The first glucose residue is covalently joined to the protein **glycogenin** via an acetal linkage to a tyrosine–OH group on the protein. Sugar units are added to the glycogen polymer by the action of

glycogen synthase. The reaction involves transfer of a glucosyl unit from UDP-glucose to the C-4 hydroxyl group at a nonreducing end of a glycogen strand. The mechanism proceeds by cleavage of the C-O bond between the glucose moiety and the β-phosphate of UDP-glucose, leaving an oxonium ion intermediate, which is rapidly attacked by the C-4 hydroxyl oxygen of a terminal glucose unit on glycogen (Figure 35).

Glycogen Branching Occurs by Transfer of Terminal Chain Segments

Glycogen is a branched polymer of glucose units. The branches arise from α-(1→6) linkages which occur every 8 to 12 residues. The branches provide multiple sites for rapid degradation or elongation of the polymer and also increase its solubility. Glycogen branches are formed by amylo-(1→4) to (1→6) transglycosylase or glycosyl- (4→6) transferasealso known as *branching enzyme.* The reaction involves the transfer of a six- or seven-residue segment from the nonreducing end of a linear chain at least 11 residues in length to the C-6 hydroxyl of a glucose residue of the same chain or another chain (Figure 36). For each branching reaction, the resulting polymer has gained a new terminus at which growth can occur. Further glucose residues may be added to the new branch by glycogen synthase.

Note-.The biological effect of branching is to make the glycogen molecule more soluble and to increase the number of nonreducing ends. This increases the number of sites accessible to glycogen phosphorylase and glycogen synthase, both of which act only at nonreducing ends.*

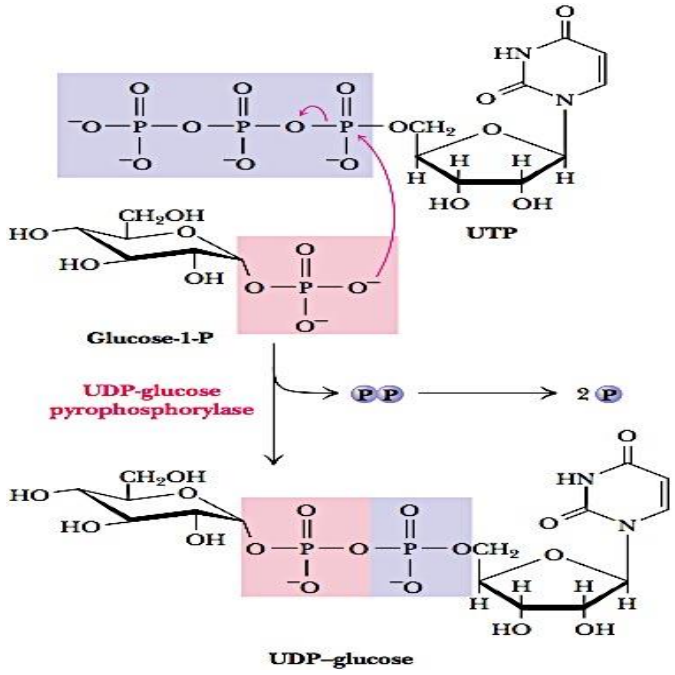


Figure 34 The UDP-glucose pyrophosphorylase reaction

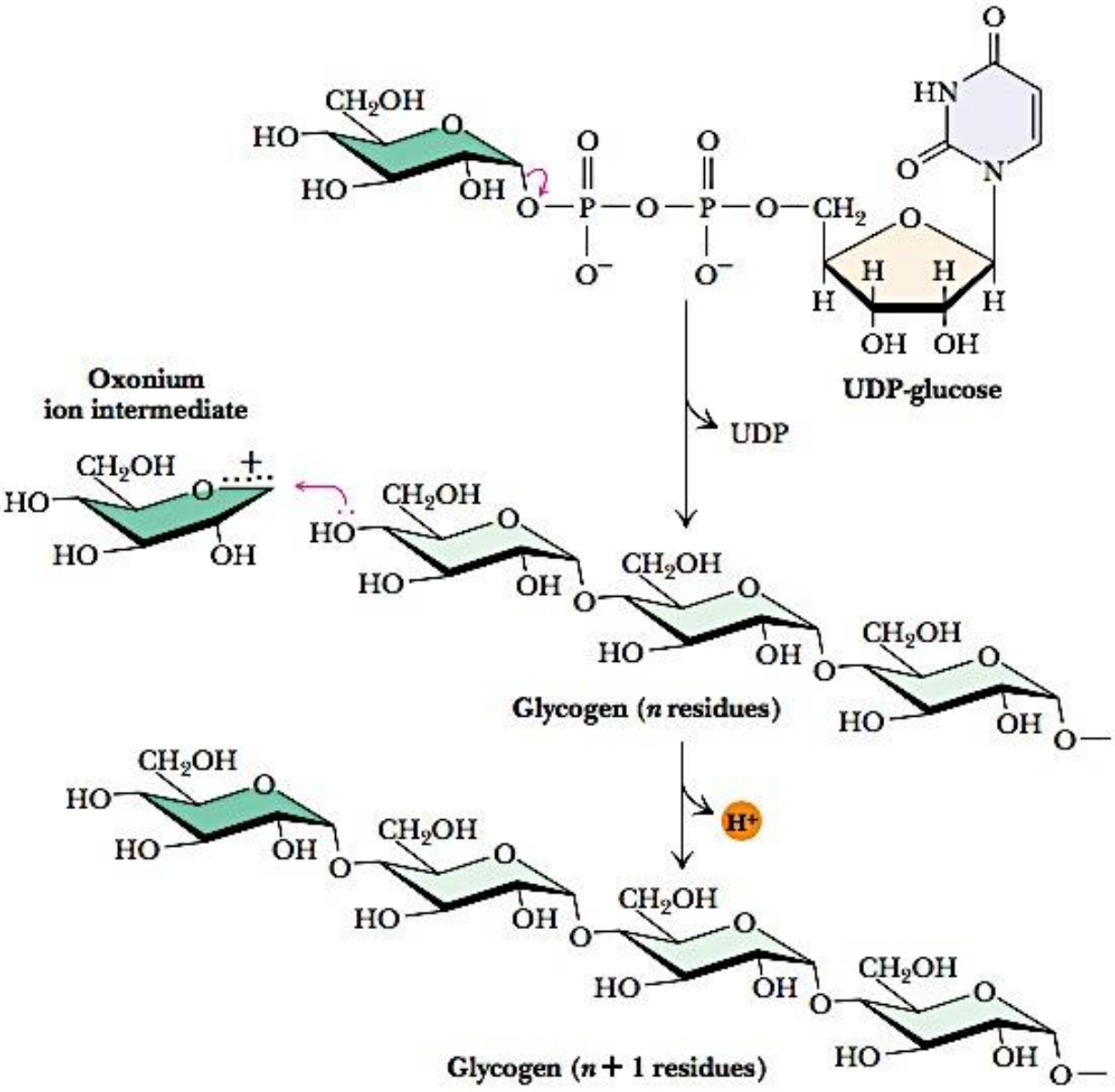


Figure 35 The glycogen synthase reaction

4.4.4.3 REGULATION OF GLYCOGEN METABOLISM

Control of glycogen metabolism is effected via reciprocal regulation of glycogen phosphorylase and glycogen synthase. Thus, activation of glycogen phosphorylase is tightly

linked to inhibition of glycogen synthase, and vice versa. Regulation involves both allosteric control and covalent modification, with the latter being under hormonal control.

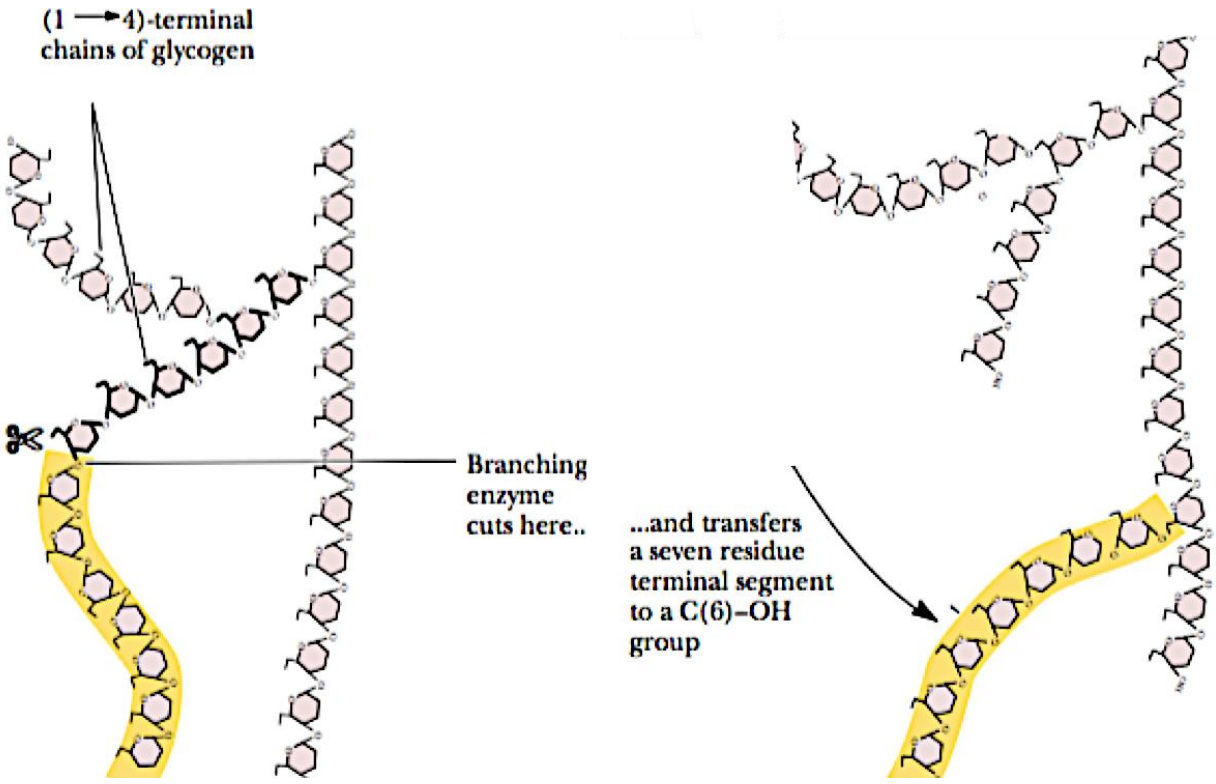


Figure 36 Formation of glycogen branches

Glycogen Phosphorylase

Glycogen metabolism is precisely controlled by multiple interlocking mechanisms, and the focus of this control is glycogen phosphorylase. *Phosphorylase is regulated by several allosteric effectors that signal the energy state of the cell as well as by reversible phosphorylation, which is responsive to hormones such as insulin, epinephrine, and glucagon.* There are differences in the control of glycogen metabolism in two tissues: skeletal muscle and liver. These differences are due to the fact that *the muscle uses glucose to produce energy for itself, whereas the liver maintains glucose homeostasis of the organism as a whole.*

The binding of the substrate *inorganic phosphate* (Pi) to muscle glycogen phosphorylase is highly cooperative, which allows the enzyme activity to increase markedly over a rather narrow range of substrate concentration. Pi is a *positive homotropic effector* with regard to its interaction with glycogen phosphorylase.

Feedback inhibition of glycogen phosphorylase by ATP and glucose-6-P provides a very effective way to regulate glycogen breakdown. Both ATP and glucose-6-P act by decreasing the affinity of glycogen phosphorylase for its substrate Pi. Because the binding of ATP or glucose-6-P has a negative effect on substrate binding, these substances act as *negative heterotropic effectors. When concentrations of ATP or glucose-6-P accumulate to high levels, glycogen phosphorylase is inhibited; when [ATP] and [glucose- 6-P] are low, the activity of glycogen phosphorylase is regulated by availability of its substrate, Pi.*

AMP also provides a regulatory signal to glycogen phosphorylase. It binds to the same site as ATP, but it stimulates glycogen phosphorylase rather than inhibiting it. AMP acts as a *positive heterotropic effector*, meaning that it enhances the binding of substrate to glycogen phosphorylase. Significant levels of AMP indicate that the energy status of the cell is low and that more energy (ATP) should be produced. Reciprocal changes in the cellular concentrations of ATP and AMP and their competition for binding to the same site (the *allosteric site*) on glycogen phosphorylase, with opposite effects; allow these two nucleotides to exert *rapid and reversible control* over glycogen phosphorylase activity. Such reciprocal regulation ensures that the production of energy (ATP) is commensurate with cellular needs.

To summarize, muscle glycogen phosphorylase is allosterically activated by AMP and inhibited by ATP and glucose-6-P; caffeine can also act as an allosteric inhibitor. When ATP and glucose-6-P are abundant, glycogen breakdown is inhibited. When cellular energy reserves are low (i.e., high [AMP] and low [ATP] and [G-6-P]), glycogen catabolism is stimulated.

The dimeric skeletal muscle phosphorylase exists in two interconvertible forms: the active form of the enzyme designated the **R state** and the inactive form denoted as the **T state**. Thus, AMP promotes the conversion to the active R state, whereas ATP, glucose-6-P, and caffeine favor conversion to the inactive T state.

Regulation of Glycogen Phosphorylase by Covalent Modification

As early as 1938, it was known that glycogen phosphorylase existed in two forms: the less active **phosphorylase *b*** and the more active **phosphorylase *a.*** In 1956, Edwin Krebs and Edmond Fischer reported that a "converting enzyme" could convert phosphorylase *b* to phosphorylase *a*. Three years later, Krebs and Fischer demonstrated that the conversion of phosphorylase *b* to phosphorylase *a* involved covalent phosphorylation.

Enzyme Cascades Regulate Glycogen Phosphorylase

The phosphorylation reaction that activates glycogen phosphorylase is mediated by an **enzyme cascade**. The first part of the cascade leads to hormonal stimulation ofadenylyl **cyclase,** a membrane-bound enzyme that converts ATP to *adenosine-3,5-cyclic monophos-phate,* denoted as *cyclic AMP* or simply *cAMP*. This regulatory molecule is found in all eukaryotic cells and acts as an intracellular messenger molecule, controlling a wide variety of processes. Cyclic AMP is known as a **second messenger** because it is the intracellular agent of a hormone (the "first messenger").

Cyclic AMP is an essential activator of *cAMP-dependent protein kinase (PKA).* This enzyme is normally inactive because its two catalytic subunits (C) are strongly associated with a pair of regulatory subunits (R), which serve to block activity. Binding of cyclic AMP to the regulatory subunits induces a conformation change that causes the dissociation of the C monomers from the R dimer. The free C subunits are active and can phosphorylate other proteins. One of the many proteins phosphorylated by PKA is *phosphorylase kinase*. Phosphorylase kinase is inactive in the unphosphorylated state and active in the phosphorylated form. As its name implies, phosphorylase kinase functions to phosphorylate (and activate) glycogen phosphorylase. Thus, stimulation of adenylyl cyclase leads to activation of glycogen breakdown.

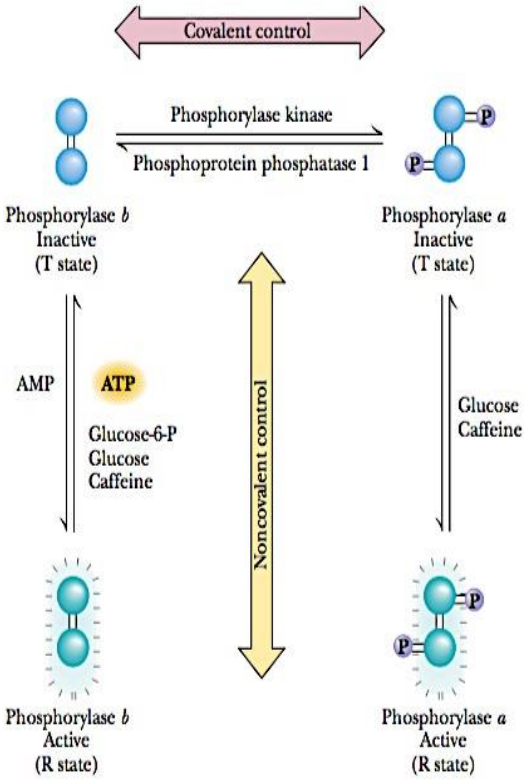


Figure 37 The mechanism of covalent modification and allosteric regulation of glycogen phosphorylase. The T states are blue and the R states blue-green.

Regulation of Glycogen Synthase by Covalent Modification

Glycogen synthase also exists in two distinct forms which can be interconverted by the action of specific enzymes: active, dephosphorylated **glycogen synthase I** (glucose-6-P-independent) and less active phosphorylated **glycogen synthase D**(glucose-6-P-dependent). The nature of phosphorylation is more complex with glycogen synthase. As many as nine serine residues on the enzyme appear to be subject to phosphorylation, each site's phosphorylation having some effect on enzyme activity.

Dephosphorylation of both glycogen phosphorylase and glycogen synthase is carried out by **phosphoprotein phosphatase 1.** The action of phosphoprotein phosphatase 1 inactivates glycogen phosphorylase and activates glycogen synthase.

Hormones Regulate Glycogen Synthesis and Degradation

Storage and utilization of tissue glycogen, maintenance of blood glucose concentration, and other aspects of carbohydrate metabolism are meticulously regulated by hormones, including *insulin*, *glucagon*, *epinephrine*, and the *glucocorticoids.*

Insulin Is a Response to Increased Blood Glucose

The primary hormone responsible for conversion of glucose to glycogen is **insulin**. Insulin is secreted by special cells in the pancreas called the **islets of Langerhans.** Secretion of insulin is a response to increased glucose in the blood. Insulin acts to rapidly lower blood glucose concentration in several ways. Insulin stimulates glycogen synthesis and inhibits glycogen breakdown in liver and muscle.

Several other physiological effects of insulin also serve to lower blood and tissue glucose levels. Insulin stimulates the active transport of glucose (and amino acids) across the plasma membranes of muscle and adipose tissue. Insulin also increases cellular utilization of glucose by inducing the synthesis of several important glycolytic enzymes, namely, glucokinase, phosphofructokinase, and pyruvate kinase. In addition, insulin acts to inhibit several enzymes of gluconeogenesis. These various actions enable the organism to respond quickly to increases in blood glucose levels.

Glucagon and Epinephrine Stimulate Glycogen Breakdown

Catabolism of tissue glycogen is triggered by the actions of the hormones **epinephrine** and **glucagon.** In response to decreased blood glucose, glucagon is released from the cells in pancreatic islets of Langerhans. This peptide hormone travels through the blood to specific receptors on liver cell membranes. (Glucagon is active in liver and adipose tissue, but not in other tissues.) Similarly, signals from the central nervous system cause release of epinephrine —also known as adrenaline—from the adrenal glands into the bloodstream. Epinephrine acts on liver and muscles. When either hormone binds to its receptor on the outside surface of the cell membrane, a cascade is initiated that activates glycogen phosphorylase and inhibits glycogen synthase. The result of these actions is tightly coordinated stimulation of glycogen breakdown and inhibition of glycogen synthesis.

Cortisol and Glucocorticoid Effects on Glycogen Metabolism

Glucocorticoids are a class of steroid hormones that exert distinct effects on liver, skeletal

muscle, and adipose tissue. The effects of cortisol, a typical glucocorticoid, are best described as *catabolic* because cortisol promotes protein breakdown and decreases protein synthesis in skeletal muscle. In the liver, however, it stimulates gluconeogenesis and increases glycogen synthesis. Cortisol- induced gluconeogenesis results primarily from increased conversion of amino acids into glucose. Specific effects of cortisol in the liver include increased gene expression of several of the enzymes of the gluconeogenic pathway, activation of enzymes involved in amino acid metabolism, and stimulation of the urea cycle, which disposes of nitrogen liberated during amino acid catabolism.

4.4.5 CITRIC ACID CYCLE

Glucose can be metabolized to pyruvate anaerobically to synthesize ATP through the glycolytic pathway. Glycolysis, however, harvests a fraction of the ATP available from glucose. Whereas, aerobic processing of glucose, is the source of most of the ATP generated in metabolism. This aerobic phase of catabolism is called respiration or more precisely cellular respiration. The aerobic processing of glucose starts with the complete oxidation of glucose derivatives to carbon dioxide. This oxidation takes place in the *citric acid cycle,* a series of reactions also known as the *tricarboxylic acid (TCA) cycle* or the *Krebs cycle.* **The citric acid cycle is the *final common pathway for the oxidation of fuel molecules* — amino acids, fatty acids, and carbohydrates. Most fuel molecules enter the cycle as acetyl coenzyme A.**

Cellular respiration occurs in three major stages.

In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl group of acetyl-coenzyme A (acetyl-CoA).

In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.

In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons. The electrons are transferred to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain. In the

course of electron transfer, the large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation

Production of Acetyl-CoA

The formation of acetyl CoA from carbohydrates is less direct than from fat. Recall that carbohydrates, most notably glucose, are processed by glycolysis into pyruvate. Under anaerobic conditions, the pyruvate is converted into lactic acid or ethanol, depending on the organism. Note that, in the preparation of the glucose derivative pyruvate for the citric acid cycle, an oxidative decarboxylation takes place and high transfer-potential electrons in the form of NADH are captured. Thus, the pyruvate dehydrogenase reaction has many of the key features of the reactions of the citric acid cycle itself.

In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain. Before entering the citric acid cycle, the carbon skeletons of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input. Many amino acid carbons also enter the cycle this way, although several amino acids are degraded to other cycle intermediates. Under aerobic conditions, the pyruvate is transported into mitochondria in exchange for OH^- by the pyruvate carrier, an antiporter. In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the *pyruvate dehydrogenase complex* to form acetyl CoA. *This irreversible reaction is the link between glycolysis and the citric acid cycle.*the pyruvate dehydrogenase (PDH) complex, is a cluster of enzymes—multiple copies of each of three enzymes—located in the mitochondria of eukaryotic cells and in the cytosol of prokaryotes.

Pyruvate Is Oxidized to Acetyl-CoA and CO_2

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA . The NADH formed in this reaction gives up a hydride ion (:H) to the respiratory chain, which carries the two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate. The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

The Pyruvate Dehydrogenase Complex Requires Five Coenzymes

The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA requires the sequential action of three different enzymes and five different coenzymes or prosthetic groups—thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A, nicotinamide adenine dinucleotide (NAD), and lipoate. *thiamine pyrophosphate (TPP), lipoic acid,* and *FAD* serve as catalytic cofactors, and CoA and NAD+ are stoichiometric cofactors.

The PDH complex contains three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3)—each present in multiple copies. E2 is the point of connection for the prosthetic group lipoate, attached through an amide bond to the ε-amino group of a Lys residue. E2 has three functionally distinct domains: the amino-terminal lipoyl domain, containing the lipoyl-Lys residue(s); the central E1- and E3-binding domain; and the inner-core acyltransferase domain, which contains the acyltransferase active site. The active site of E1 has bound TPP, and that of E3 has bound FAD. Also part of the complex are two regulatory proteins, a protein kinase and a phosphoprotein phosphatase. This basic E1-E2-E3 structure has been conserved during evolution.

pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate.

Step 1 -C-1 of pyruvate is released as CO_2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group. This first step is the slowest and therefore limits the rate of the overall reaction. It is also the point at which the PDH complex exercises its substrate specificity.

Step 2 the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate). The two electrons removed in this reaction reduce the –S-S- of a lipoyl group on E2 to two thiol (-SH) groups. The acetyl moiety produced in this oxidation-reduction reaction is first esterified to one of the lipoyl -SH groups, then transesterified to CoA to form acetyl-CoA.

Step 3 Thus the energy of oxidation drives the formation of a high-energy thioester of acetate. The remaining reactions catalyzed by the PDH complex (by E3, in steps 4 and 5) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of

E2 to prepare the enzyme complex for another round of oxidation. The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD^+.

Central to the mechanism of the PDH complex are the swinging lipoyllysyl arms of E2, which accept from E1 the two electrons and the acetyl group derived from pyruvate, passing them to E3. All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex. The five-reaction sequence is thus an example of substrate channeling. The intermediates of the multistep sequence never leave the complex, and the local concentration of the substrate of E2 is kept very high. Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate.

Reactions of the Citric Acid Cycle

The citric acid cycle is central to energy-yielding metabolism its role is not limited to energy conservation. Four- and five-carbon intermediates of the cycle serve as precursors for a wide variety of products. To replace intermediates removed for this purpose, cells employ anaplerotic (replenishing) reactions

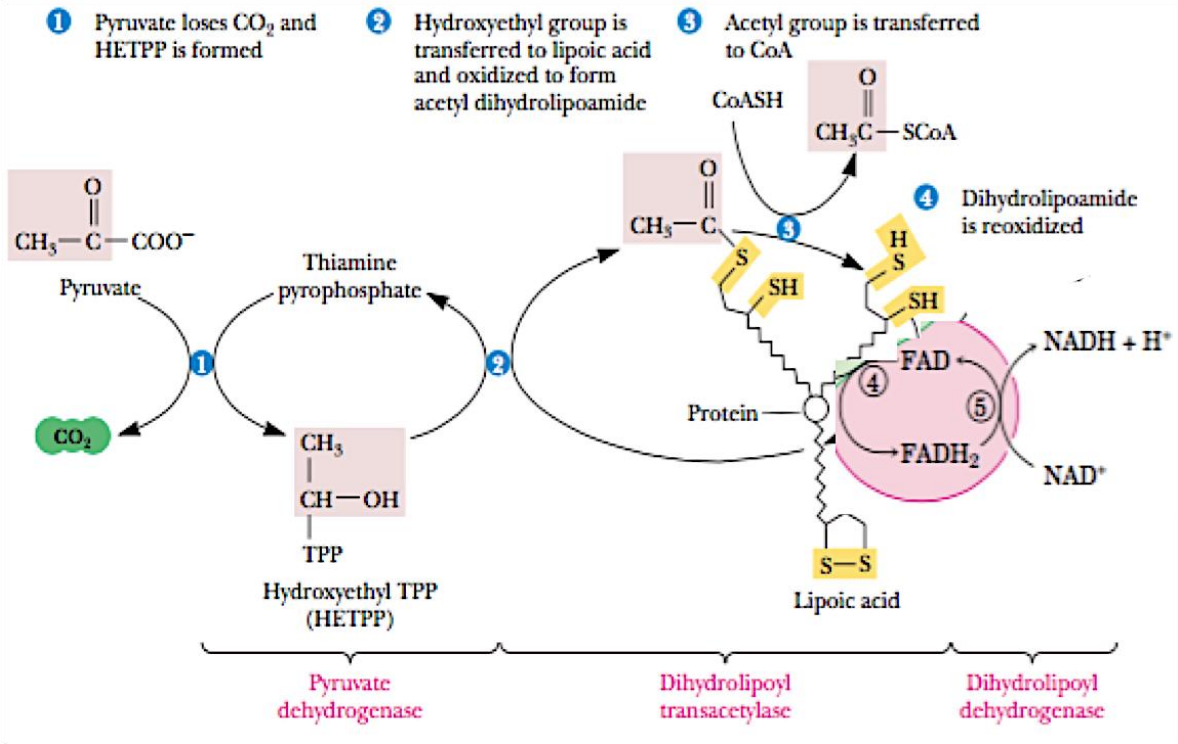


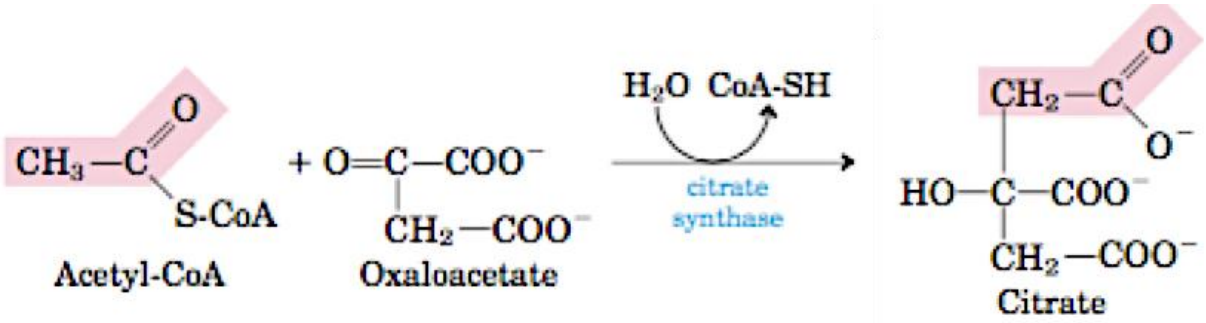
Figure 38 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex

The Citrate Synthase Reaction

The first reaction within the TCA cycle, the one by which carbon atoms are introduced, is the **citrate synthase reaction**. Here acetyl-CoA reacts with oxaloacetate in a **Perkin condensation** (a carbon–carbon condensation between a ketone or aldehyde and an ester). In this reaction the methyl carbon of the acetyl group is joined to the carbonyl group (C-2) of oxaloacetate. Citroyl-CoA is a transient intermediate formed on the active site of the enzyme. It rapidly undergoes hydrolysis to free CoA and citrate, which are released from the active site. The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic. The large, negative standard free-energy change of the citrate synthase reaction is essential to the operation of the cycle because, as noted earlier, the concentration of oxaloacetate is normally very low. The CoA liberated in this reaction is recycled to participate in the oxidative decarboxylation of an- other molecule of pyruvate by the PDH complex.

Citrate synthase in mammals is a dimer of 49-kD subunits. On each subunit, oxaloacetate and acetyl-CoA bind to the active site, which lies in a cleft between two domains and is surrounded mainly by α-helical segments. Binding of oxaloacetate induces a conformational change that facilitates the binding of acetyl-CoA and closes the active site, so that the reactive carbanion of acetyl-CoA is protected from protonation by water.

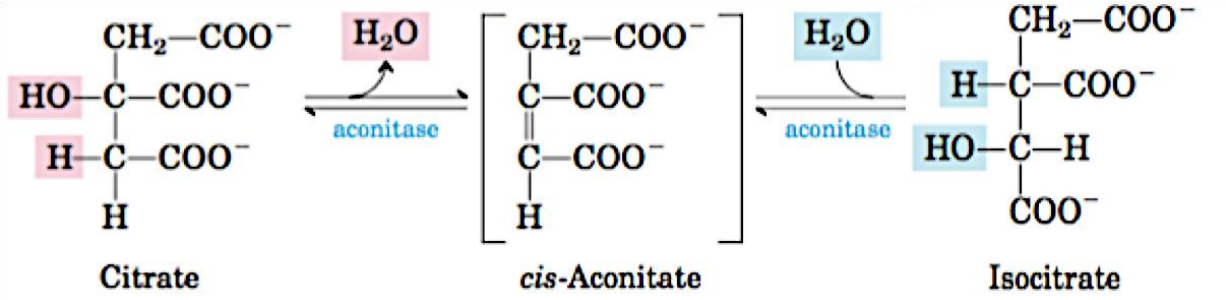
Citrate synthase is the first step in this metabolic pathway, and as stated the reaction has a large negative $\Delta G'^{\circ}$. As might be expected, it is a highly regulated enzyme. NADH, a product of the TCA cycle, is an allosteric inhibitor of citrate synthase, as is succinyl-CoA, the product of the fifth step in the cycle (and an acetyl-CoA analog).



Formation of Isocitrate via cis-Aconitate

Citrate itself poses a problem: it is a poor candidate for further oxidation because it contains a tertiary alcohol, which could be oxidized only by breaking a carbon–carbon bond. An obvious solution to this problem is to isomerize the tertiary alcohol to a secondary alcohol, which the cycle proceeds to do in the next step

The enzyme aconitase catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of the tricarboxylic acid cis-aconitate, which normally does not dissociate from the active site. Aconitase can promote the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate in two different ways, one leading to citrate and the other to isocitrate:



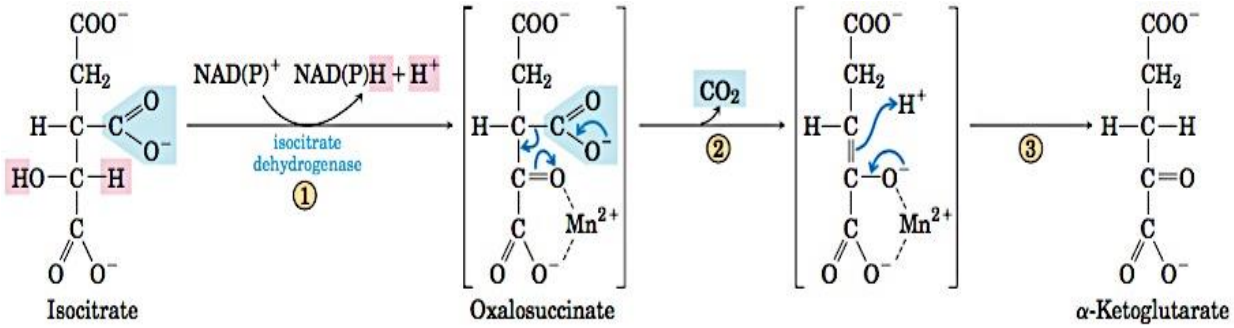
Isocitrate Dehydrogenase—The First Oxidation in the Cycle

In the next step of the TCA cycle, isocitrate is oxidatively decarboxylated to yield α-ketoglutarate, with concomitant reduction of NAD to NADH in the isocitrate dehydrogenase reaction. This two-step reaction involves (1) oxidation of the C-2 alcohol of isocitrate to form oxalosuccinate, followed by (2) a β-decarboxylation reaction that expels the central carboxyl group as **CO_2**, leaving the product α-ketoglutarate. Oxalosuccinate, the β-keto acid produced by the initial dehydrogenation reaction, is unstable and thus is readily decarboxylated.

Isocitrate Dehydrogenase Links the TCA Cycle and Electron Transport

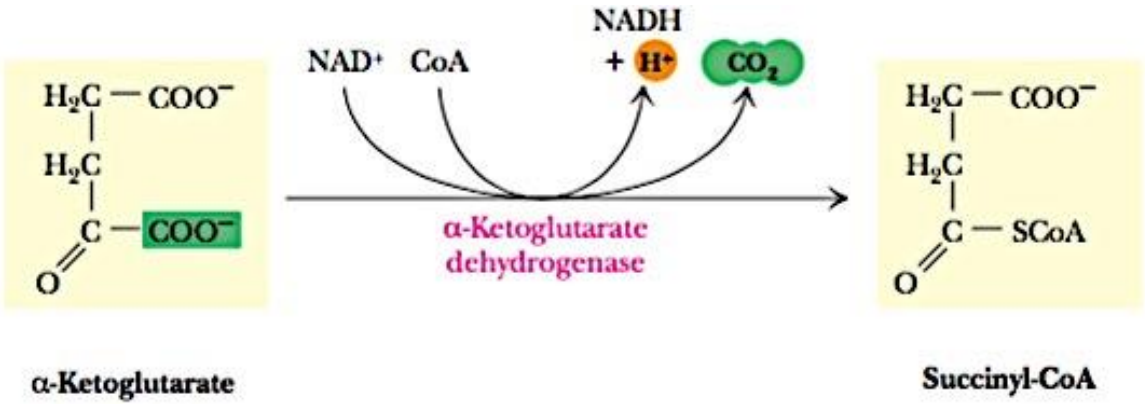
Isocitrate dehydrogenase provides the first connection between the TCA cycle and the electron transport pathway and oxidative phosphorylation, via its production of NADH. As a connecting point between two metabolic pathways, isocitrate dehydrogenase is a regulated reaction.NADH and ATP are allosteric inhibitors, whereas ADP acts as an allosteric activator. The enzyme is virtually inactive in the absence of ADP.Also, the product, α-

ketoglutarate, is a crucial α-keto acid for aminotransferase reactions, connecting the TCA cycle (that is, carbon metabolism) with nitrogen metabolism.



α-Ketoglutarate Dehydrogenase— A Second Decarboxylation

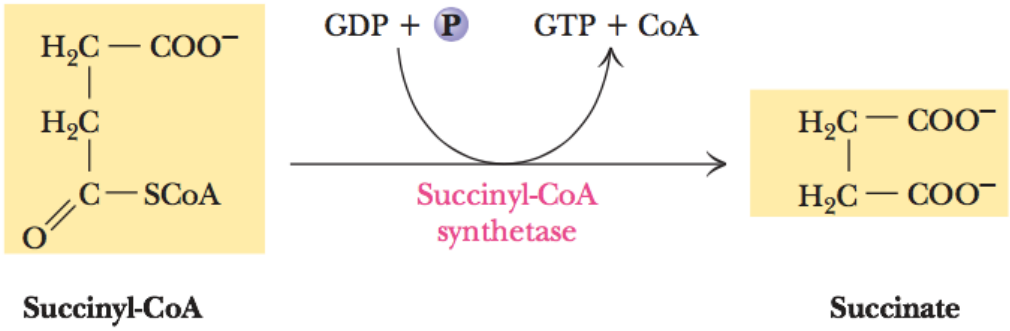
A second oxidative decarboxylation occurs in the **α-ketoglutarate dehydrogenase reaction**. Like the pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase is a multienzyme complex—consisting of *α-ketoglutarate dehydrogenase, dihydrolipoyl transsuccinylase,* and *dihydrolipoyl dehydrogenase*—that employs five different coenzyme. The dihydrolipoyl dehydrogenase in this reaction is identical to that in the pyruvate dehydrogenase reaction. The mechanism is analogous to that of pyruvate dehydrogenase. As with the pyruvate dehydrogenase reaction, this reaction produces NADH and a thioester product—in this case, succinyl-CoA. Succinyl-CoA and NADH products are energy-rich species that are important sources of metabolic energy in subsequent cellular processes.



Succinyl-CoA Synthetase—A Substrate- Level Phosphorylation

The NADH produced in the foregoing steps can be routed through the electron transport pathway to make high-energy phosphates via oxidative phosphorylation. However, succinyl-

CoA is itself a high-energy intermediate and is utilized in the next step of the TCA cycle to drive the phosphorylation of GDP to GTP (in mammals) or ADP to ATP (in plants and bacteria). The reaction is catalyzed by **succinyl-CoA synthetase,** sometimes called **succinate thiokinase.** The free energies of hydrolysis of succinyl-CoA and GTP or ATP are similar.



Succinyl-CoA **Succinate**

Succinyl-CoA synthetase provides another example of a **substrate-level phosphorylation**, in which a substrate, rather than an electron transport chain or proton gradient, provides the energy for phosphorylation. It is the only such reaction in the TCA cycle. The GTP produced by mammals in this reaction can exchange its terminal phosphoryl group with ADP via the **nucleoside diphosphate kinase reaction**

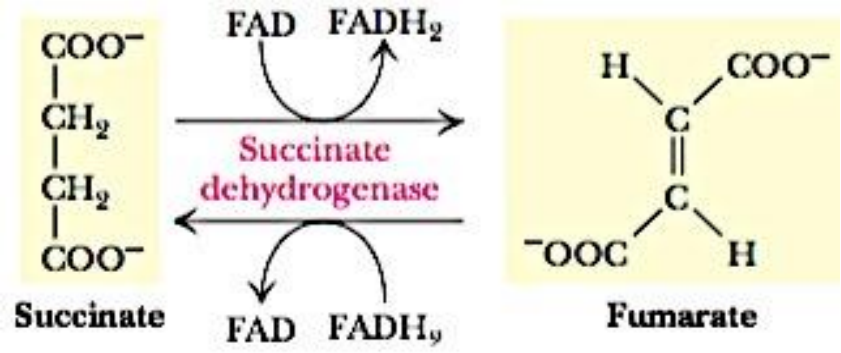
$$GTP + ADP \rightleftharpoons GDP + ATP$$

Succinate Dehydrogenase—An Oxidation Involving FAD

The oxidation of succinate to fumarate is carried out by **succinate dehydrogenase,** a membrane-bound enzyme that is actually part of the succinate–coenzyme Q reductase of the electron transport chain. In contrast with all of the other enzymes of the TCA cycle, which are soluble proteins found in the mitochondrial matrix, succinate dehydrogenase is an integral membrane protein tightly associated with the inner mitochondrial membrane. Succinate oxidation involves removal of H atoms across a C-C bond, rather than a C-O or C-N bond, and produces the *trans*- unsaturated fumarate. This reaction (the oxidation of an alkane to an alkene) is not sufficiently exergonic to reduce NAD^+, but it does yield enough energy to reduce [FAD].

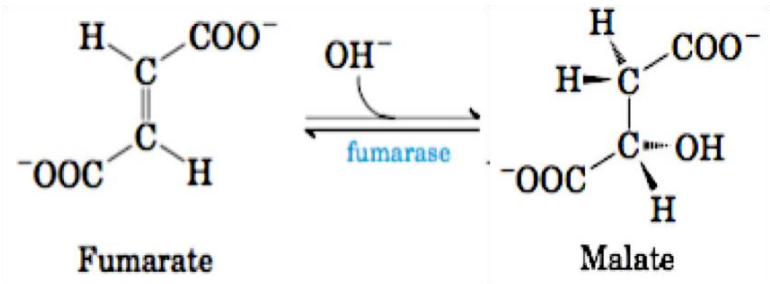
Succinate dehydrogenase is a dimeric protein, with subunits of molecular masses 70 kD and 27 kD.The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD. Electrons pass from succinate through the FAD and iron-sulfur centers before entering the chain of electron carriers in the mitochondrial inner membrane

(or the plasma membrane in bacteria). Electron flow from succinate through these carriers to the final electron acceptor, $\mathbf{O}_2$, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).



Fumarase Catalyzes *Trans*-Hydration of Fumarate

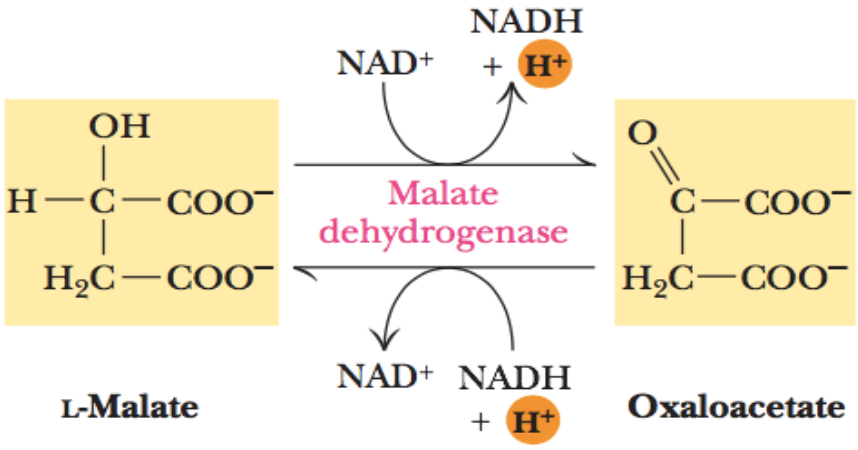
Fumarate is hydrated in a stereospecific reaction by fumarase to give L-malate. The reaction involves *trans*-addition of the elements of water across the double bond. Though the exact mechanism is uncertain, it may involve protonation of the double bond to form an intermediate carbonium ion or possibly attack by water or OH^- anion to produce a carbanion, followed by protonation. This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate). In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.



Malate Dehydrogenase—Completing the Cycle

In the last step of the TCA cycle, L-malate is oxidized to oxaloacetate by **malate dehydrogenase**. This reaction is very endergonic. Consequently, the concentration of oxaloacetate in the mitochondrial matrix is usually quite low. The reaction, however, is pulled forward by the favorable citrate synthase reaction. Oxidation of malate is coupled to reduction of yet another molecule of NAD^+, the third one of the cycle. Counting the [FAD]

reduced by succinate dehydrogenase, this makes the fourth coenzyme reduced through oxidation of a single acetate unit.



The Energy of Oxidations in the Cycle Is Efficiently Conserved

A two-carbon acetyl group entered the TCA cycle by combining with oxaloacetate. Two carbon atoms emerged from the cycle as CO_2 from the oxidation of isocitrate and α-ketoglutarate. The energy released by these oxidations was conserved in the reduction of three NAD^+ and one FAD and the production of one ATP or GTP. At the end of the cycle a molecule of oxaloacetate was regenerated.

Although the citric acid cycle directly generates only one ATP per turn (in the conversion of succinyl-CoA to succinate), the four oxidation steps in the cycle provide a large flow of electrons into the respiratory chain via NADH and $FADH_2$ and thus lead to formation of a large number of ATP molecules during oxidative phosphorylation.

The energy yield from the production of two molecules of pyruvate from one molecule of glucose in glycolysis is 2 ATP and 2 NADH. In oxidative phosphorylation, passage of two electrons from NADH to O_2 drives the formation of about 2.5 ATP, and passage of two electrons from $FADH_2$ to O_2 yields about 1.5 ATP. This stoichiometry allows us to calculate the overall yield of ATP from the complete oxidation of glucose. When both pyruvate molecules are oxidized to $6CO_2$ via the pyruvate dehydrogenase complex and the citric acid cycle, and the electrons are transferred to O_2 via oxidative phosphorylation, as many as 32 ATP are obtained per glucose.

The TCA Cycle Provides Intermediates for Biosynthetic Pathways

The citric acid cycle is an amphibolic pathway, that serves in both catabolic and anabolic processes. Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways.Four-, five-, and six-carbon species produced in the TCA cycle also fuel a variety of **biosynthetic processes.** α-Ketoglutarate, succinyl-CoA, fumarate and oxaloacetate are all precursors of important cellular species. A transamination reaction converts α-ketoglutarate directly to glutamate, which can then serve as a versatile precursor for proline, arginine, and glutamine. Succinyl-CoA provides most of the carbon atoms of the porphyrins. Oxaloacetate can be transaminated to produce aspartate. Aspartic acid itself is a precursor of the pyrimidine nucleotides and, in addition, is a key precursor for the synthesis of asparagine, methionine, lysine, threonine, and isoleucine. Oxaloacetate can also be decarboxylated to yield PEP, which is a key element of several pathways, namely (1) synthesis (in plants and microorganisms) of the aromatic amino acids phenylalanine, tyrosine, and tryptophan; (2) formation of 3-phospho- glycerate and conversion to the amino acids serine, glycine, and cysteine; and (3) *gluconeogenesis*

Finally, citrate can be exported from the mitochondria and then broken down by **ATP-citrate lyase** to yield oxaloacetate and acetyl-CoA, a precursor of fatty acids. Oxaloacetate produced in this reaction is rapidly reduced to malate, which can then be processed in either of two ways: it may be transported into mitochondria, where it is reoxidized to oxaloacetate, or it may be oxidatively decarboxylated to pyruvate by **malic enzyme,** with subsequent mitochondrial uptake of pyruvate. This cycle permits citrate to provide acetyl-CoA for biosynthetic processes, with return of the malate and pyruvate by-products to the mitochondria.

4.4.5.1The Anaplerotic, or "Filling Up," Reactions

In a sort of reciprocal arrangement, the cell also feeds many intermediates back into the TCA cycle from other reactions. Since such reactions replenish the TCA cycle intermediates, Hans Kornberg proposed that they be called **anaplerotic reactions** (literally, the "filling up" reactions). Thus, **PEP carboxylase** and **pyruvate carboxylase** synthesize oxaloacetate from pyruvate.

Pyruvate carboxylase is the most important of the anaplerotic reactions. It exists in the mitochondria of animal cells but not in plants, and it provides a direct link between glycolysis and the TCA cycle. The enzyme is tetrameric and contains covalently bound biotin and an Mg^{2+}site on each subunit. Pyruvate carboxylase has an absolute allosteric requirement for acetyl-CoA. Thus, when acetyl-CoA levels exceed the oxaloacetate supply, allosteric activation of pyruvate carboxylase by acetyl-CoA raises oxaloacetate levels, so that the excess acetyl-CoA can enter the TCA cycle.

PEP carboxylase occurs in yeast, bacteria, and higher plants, but not in animals. The enzyme is specifically inhibited by aspartate, which is produced by transamination of oxaloacetate. Thus, organisms utilizing this enzyme control aspartate production by regulation of PEP carboxylase. Malic enzyme is found in the cytosol or mitochondria of many animal and plant cells and is an NADPH- dependent enzyme.

It is worth noting that the reaction catalyzed by **PEP carboxykinase** could also function as an anaplerotic reaction, were it not for the particular properties of the enzyme. CO_2 binds weakly to PEP carboxykinase, whereas oxaloacetate binds very tightly, and, as a result, the enzyme favors formation of PEP from oxaloacetate.

The catabolism of amino acids provides pyruvate, acetyl-CoA, oxaloacetate, fumarate, α-ketoglutarate, and succinate, all of which may be oxidized by the TCA cycle. In this way, proteins may serve as excellent sources of nutrient energy.

4.4.5.2 Regulation of the TCA Cycle

Situated as it is between glycolysis and the electron transport chain, the TCA cycle must be carefully controlled by the cell. If the cycle were permitted to run unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzymes and ATP; conversely, if it ran too slowly, ATP would not be produced rapidly enough to satisfy the needs of the cell. Also, as just seen, the TCA cycle is an important source of precursors for biosynthetic processes and must be able to provide them as needed.

What are the sites of regulation in the TCA cycle? we might anticipate that some of the reactions of the TCA cycle would operate near equilibrium under cellular conditions (with $\Delta G = 0$), whereas others—the sites of regulation—would be characterized by large, negative ΔG values. Three reactions of the cycle—citrate synthase, isocitrate dehydrogenase, and α-

ketoglutarate dehydrogenase—operate with large, negative *G* values under mitochondrial conditions and are thus the primary sites of regulation in the cycle.

The principal regulatory "signals" are the concentrations of acetyl-CoA, ATP, NAD^+, and NADH, with additional effects provided by several other metabolites. The main sites of regulation are pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. NADH inhibits all of these enzymes, so that when the cell has produced all the NADH that can conveniently be turned into ATP, the cycle shuts down. For similar reasons, ATP is an inhibitor of pyruvate dehydrogenase and isocitrate dehydrogenase. The TCA cycle is turned on, however, when either the ADP/ATP or NAD^+ /NADH ratio is high, an indication that the cell has run low on ATP or NADH. Regulation of the TCA cycle by NADH, NAD^+, ATP, and ADP thus reflects the energy status of the cell. On the other hand, succinyl-CoA is an *intracycle regulator,* inhibiting citrate synthase and α-ketoglutarate dehydrogenase. Acetyl-CoA acts as a signal to the TCA cycle that glycolysis or fatty acid breakdown is producing two-carbon units. Acetyl-CoA activates pyruvate carboxylase, the anaplerotic reaction that provides oxaloacetate, the acceptor for increased flux of acetyl-CoA into the TCA cycle.

Regulation of Pyruvate Dehydrogenase

High levels of either product, acetyl-CoA or NADH, allosterically inhibit the pyruvate dehydrogenase complex. Acetyl-CoA specifically blocks dihydrolipoyl transacetylase, and NADH acts on dihydrolipoyl dehydrogenase. The mammalian pyruvate dehydrogenase is also regulated by covalent modifications. A Mg^{2+}-dependent **pyruvate dehydrogenase kinase** is allosterically activated by NADH and acetyl-CoA, and when levels of these metabolites rise in the mitochondrion, they stimulate phosphorylation of a serine residue on the pyruvate dehydrogenase subunit, blocking the first step of the pyruvate dehydrogenase reaction, the decarboxylation of pyruvate. Inhibition of the dehydrogenase in this manner eventually lowers the levels of NADH and acetyl-CoA in the matrix of the mitochondrion. Reactivation of the enzyme is carried out by **pyruvate dehydrogenase phosphatase,** a Ca^{2+}-activated enzyme that binds to the dehydrogenase complex and hydrolyzes the phosphoserine moiety on the dehydrogenase subunit. At low ratios of NADH to $NAD^+$and low acetyl-CoA levels, the phosphatase maintains the dehydrogenase in an activated state, but a high level of acetyl-CoA or NADH once again activates the kinase and leads to the

inhibition of the dehydrogenase. Insulin and Ca^{2+}ions activate dephosphorylation, and pyruvate inhibits the phosphorylation reaction.

Pyruvate dehydrogenase is also sensitive to the energy status of the cell. AMP activates pyruvate dehydrogenase, whereas GTP inhibits it. High levels of AMP are a sign that the cell may become energy-poor. Activation of pyruvate dehydrogenase under such conditions commits pyruvate to energy production.

Regulation of Isocitrate Dehydrogenase

The mechanism of regulation of isocitrate dehydrogenase is in some respects the reverse of pyruvate dehydrogenase. The mammalian isocitrate dehydrogenase is subject only to allosteric activation by ADP and NAD^+ and to inhibition by ATP and NADH. Thus, high NAD^+ /NADH and ADP/ATP ratios stimulate isocitrate dehydrogenase and TCA cycle activity. The *Escherichia coli* enzyme, on the other hand, is regulated by covalent modification. Serine residues on each subunit of the dimeric enzyme are phosphorylated by a protein kinase, causing inhibition of the isocitrate dehydrogenase activity. Activity is restored by the action of a specific phosphatase. When TCA cycle and glycolytic intermediates—such as isocitrate, 3-phosphoglycerate, pyruvate, PEP, and oxaloacetate—are high, the kinase is inhibited, the phosphatase is activated, and the TCA cycle operates normally.When levels of these intermediates fall, the kinase is activated, isocitrate dehydrogenase is inhibited, and isocitrate is diverted to the *glyoxylate pathway*.

4.4.6- THE GLYOXYLATE CYCLE OF PLANTS AND BACTERIA

Plants (particularly seedlings, which cannot yet accomplish efficient photosynthesis), as well as some bacteria and algae, acetate can serve both as an energy-rich fuel and as a source of phosphoenolpyruvate for carbohydrate synthesis.

Although we saw that the TCA cycle can supply intermediates for some biosynthetic processes, the cycle gives off $2CO_2$ for every two-carbon acetate group that enters and cannot effect the *net synthesis* of TCA cycle intermediates. Thus, it would not be possible for the cycle to produce the massive amounts of biosynthetic intermediates needed for acetate-based growth unless alternative reactions were available. In essence, the TCA cycle is geared primarily to energy production, and it "wastes" carbon units by giving off CO_2. Modification of the cycle to support acetate-based growth would require eliminating the CO_2-producing reactions and enhancing the net production of four-carbon units (i.e.,

oxaloacetate). Plants and bacteria employ a modification of the TCA cycle called the **glyoxylate cycle** to produce four-carbon dicarboxylic acids (and eventually even sugars) from two-carbon acetate units. The glyoxylate cycle catalyze the net conversion of acetate to succinate or other four- carbon intermediates of the citric acid cycle:

$2\ \text{Acetyl-CoA} + \text{NAD}^+ + 2\text{H}_2\text{O} \rightarrow \text{succinate} + 2\text{CoA} + \text{NADH} + \text{H}^+$

The site of the Glyoxylate Cycle

The enzymes of the glyoxylate cycle in plants are contained in **glyoxysomes,** organelles devoted to this cycle. Yeast and algae carry out the glyoxylate cycle in the cytoplasm.

The Cycle

In the glyoxylate cycle, acetyl-CoA condenses with oxaloacetate to form citrate, and citrate is converted to isocitrate, exactly as in the citric acid cycle. The next step, however, is not the breakdown of isocitrate by isocitrate dehydrogenase but the cleavage of isocitrate by isocitrate lyase, forming succinate and glyoxylate. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by malate synthase. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle. Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes. The succinate may be converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis.

Note*-Vertebrates do not have the enzymes specific to the glyoxylate cycle (isocitrate lyase and malate synthase) and therefore cannot bring about the net synthesis of glucose from lipids.

The Glyoxylate Cycle Helps Plants Grow in the Dark

The existence of the glyoxylate cycle explains how certain seeds grow underground (or in the dark), where photosynthesis is impossible. Many seeds (peanuts, soybeans, and castor beans, for example) are rich in lipids, most organisms degrade the fatty acids of lipids to acetyl- CoA. Glyoxysomes form in seeds as germination begins, and the glyoxylate cycle uses the acetyl-CoA produced in fatty acid oxidation to provide large amounts of oxaloacetate and other intermediates for carbohydrate synthesis. Once the growing plant

begins photosynthesis and can fix CO_2 to produce carbohydrates, the glyoxysomes disappear.

Glyoxysomes Must Borrow Three Reactions from Mitochondria

Glyoxysomes do not contain all the enzymes needed to run the glyoxylate cycle; succinate dehydrogenase, fumarase, and malate dehydrogenase are absent. Consequently, glyoxysomes must cooperate with mitochondria to run their cycle. Succinate travels from the glyoxysomes to the mitochondria, where it is converted to oxaloacetate. Transamination to aspartate follows because oxaloacetate cannot be transported out of the mitochondria. Aspartate formed in this way then moves from the mitochondria back to the glyoxysomes, where a reverse transamination with α-ketoglutarate forms oxaloacetate, completing the shuttle. Finally, to balance the transaminations, glutamate shuttles from glyoxysomes to mitochondria.

4.4.6.1The Citric Acid and Glyoxylate Cycles Are Coordinately Regulated

The sharing of common intermediates between **Citric Acid and Glyoxylate Cycles** requires that these pathways be coordinately regulated. Isocitrate is a crucial intermediate, at the branch point between the glyoxylate and citric acid cycles. Isocitrate dehydrogenase is regulated by covalent modification: a specific protein kinase phosphorylates and thereby inactivates the dehydrogenase. This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose. A phosphoprotein phosphatase removes the phosphoryl group from isocitrate dehydrogenase, reactivating the enzyme and sending more isocitrate through the energy-yielding citric acid cycle. The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.

The phosphoprotein phosphatase that activates isocitrate dehydrogenase is stimulated by intermediates of the citric acid cycle and glycolysis and by indicators of reduced cellular energy supply. The same metabolites inhibit the protein kinase activity of the bifunctional polypeptide. Thus, the accumulation of intermediates of the central energy-yielding pathways—indicating energy depletion—results in the activation of isocitrate dehydrogenase. When the concentration of these regulators falls, signaling a sufficient flux through the energy-yielding citric acid cycle, isocitrate dehydrogenase is inactivated by the protein kinase. The same intermediates of glycolysis and the citric acid cycle that activate isocitrate dehydrogenase are allosteric inhibitors of isocitrate lyase. When energy-yielding

metabolism is sufficiently fast to keep the concentrations of glycolytic and citric acid cycle intermediates low, isocitrate dehydrogenase is inactivated, the inhibition of isocitrate lyase is relieved, and isocitrate flows into the glyoxylate pathway, to be used in the biosynthesis of carbohydrates, amino acids, and other cellular components.

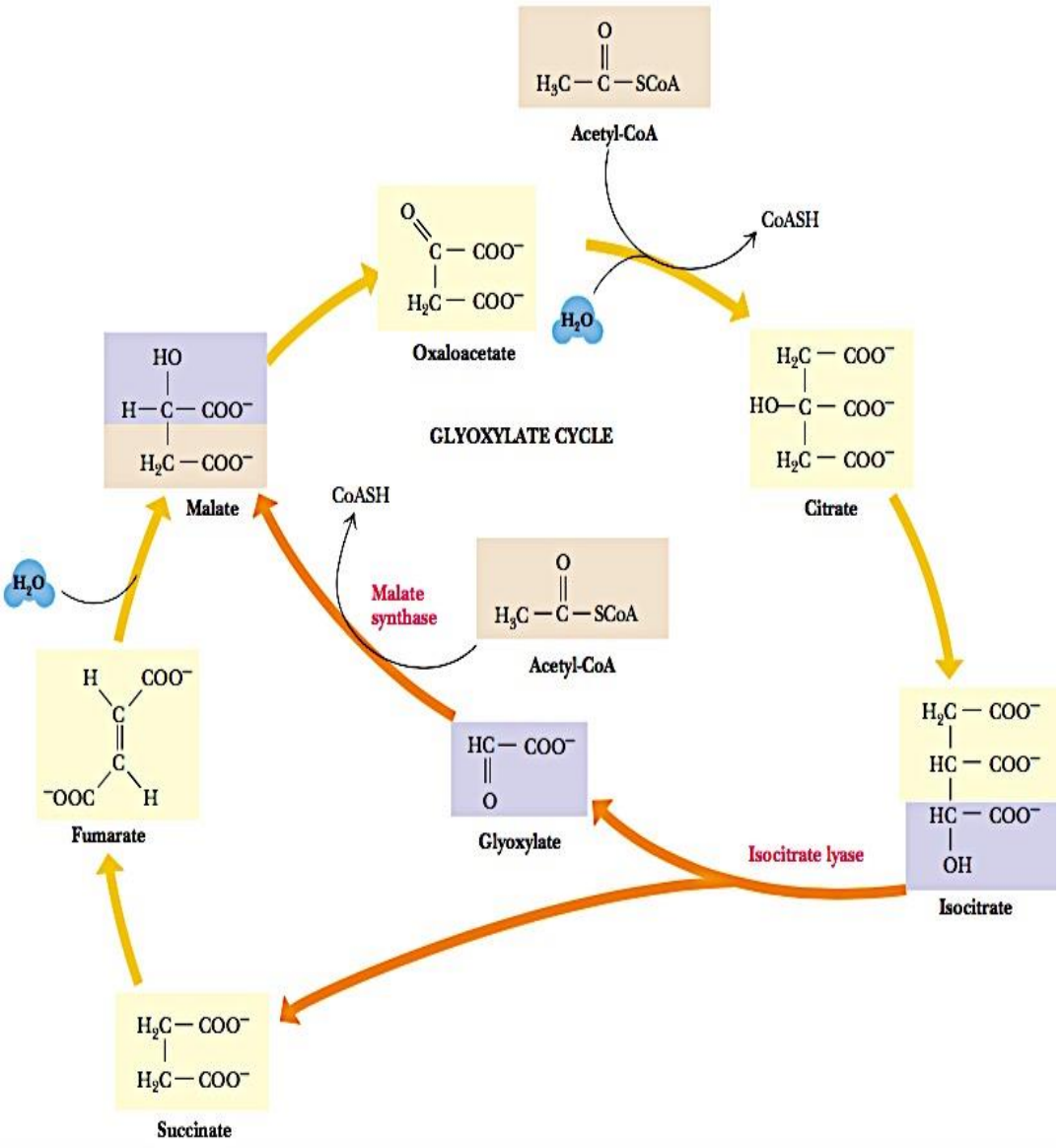


Figure 39The glyoxylate cycle

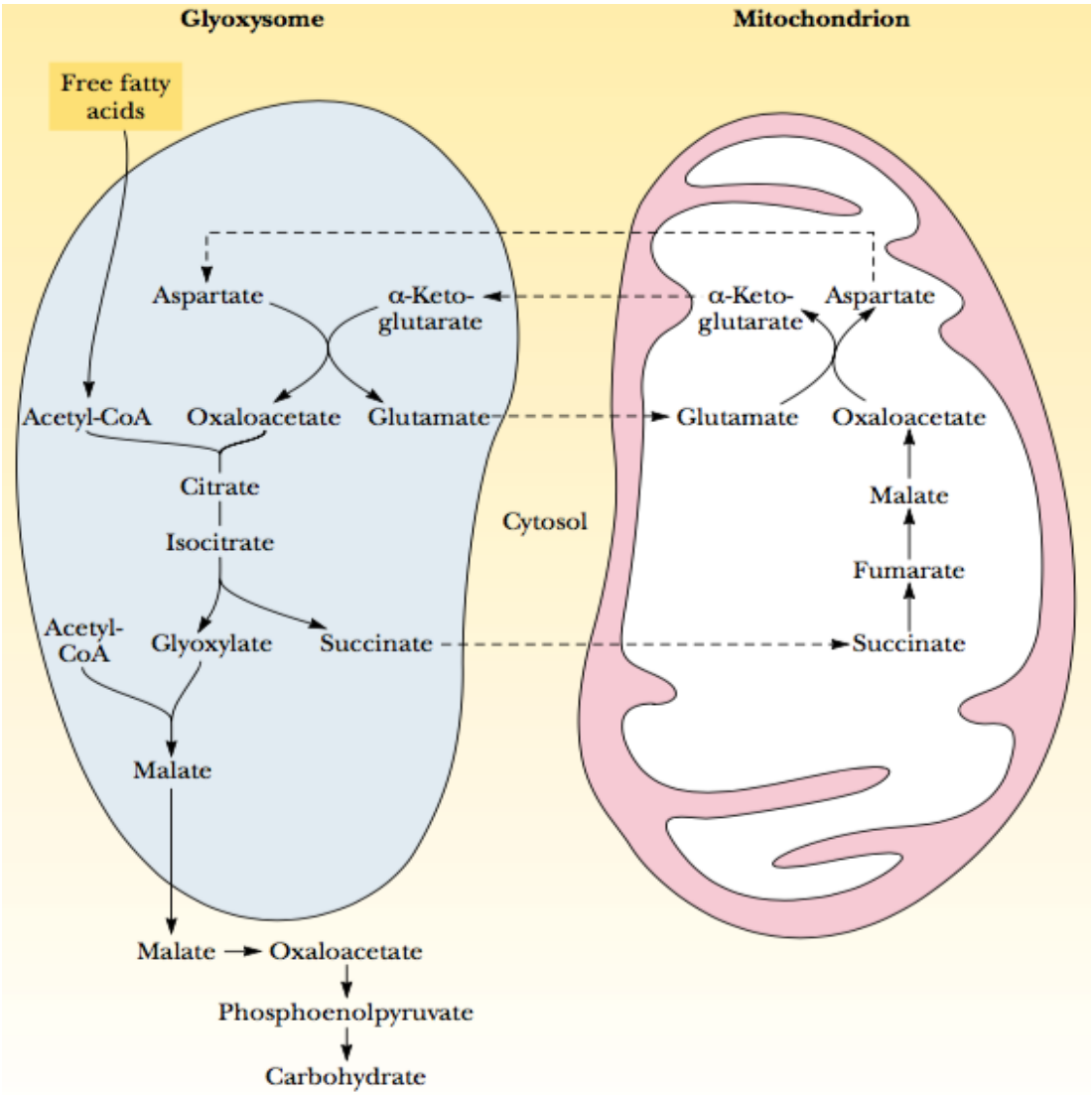


Figure 40 Relationship between the glyoxylate and citric acid cycles

4.4.7 - ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

Most of the metabolic energy that is obtainable from substrates entering glycolysis and the TCA cycle is funneled via oxidation–reduction reactions into NADH and reduced flavoproteins [$FADH_2$]. Whereas ATP made in glycolysis and the TCA cycle is the result of substrate-level phosphorylation, NADH-dependent ATP synthesis is the result of oxidative phosphorylation. Electrons stored in the form of the reduced coenzymes, NADH or [$FADH_2$], are passed through an elaborate and highly organized chain of proteins and coenzymes, the so-called **electron transport chain,** finally reaching O_2 (molecular oxygen), the terminal electron acceptor. Each component of the chain can exist in (at least) two oxidation states, and each component is successively reduced and reoxidized as electrons move through the chain from NADH (or [$FADH_2$]) to O_2. In the course of electron transport,

a proton gradient is established across theinner mitochondrial membrane. It is the energy of this proton gradient that drives ATP synthesis.

Electron Transport and Oxidative Phosphorylation Are Membrane-Associated Processes

The processes of electron transport and oxidative phosphorylation are **membrane-associated.** Bacteria are the simplest life form, and bacterial cells typically consist of a single cellular compartment surrounded by a plasma membrane and a more rigid cell wall. In such a system, the conversion of energy from NADH and [$FADH_2$] to the energy of ATP via electron transport and oxidative phosphorylation is carried out at (and across) the plasma membrane. In eukaryotic cells, electron transport and oxidative phosphorylation are localized in mitochondria, which are also the sites of TCA cycle activity and fatty acid oxidation.

Mitochondrion is surrounded by a simple outer membrane and more complex inner membrane. The space between the inner and outer membranes is referred to as the intermembrane space. The outer membrane functions mainly to maintain the shape of the mitochondrion. The inner membrane lacks cholesterol and is quite impermeable to molecules and ions. Species that must cross the mitochondrial inner membrane—ions, substrates, fatty acids for oxidation, and so on— are carried by specific transport proteins in the membrane. Notably, the inner membrane has extensive folds, known as cristae, providing the inner membrane with a large surface area in a small volume.

The Electron Transport Chain—An Overview

The electron transport chain involves several different molecular species, including:

(a) Flavoproteins, which contain tightly bound FMN or FAD as prosthetic groups, and which may participate in one- or two- electron transfer events.

(b) Coenzyme Q,also called **ubiquinone** (and abbreviated **CoQ** or **UQ**), which can function in either one- or two-electron transfer reactions.

(c) Several **cytochromes** (proteins containing heme prosthetic groups, which function by carrying or transferring electrons), including cytochromes *b*, *c*, c_1, *a*, and a_3. Cytochromes are one-electron transfer agents, in which the heme iron is converted from Fe^{2+} to Fe^{3+} and back.

(d) A number of **iron–sulfur proteins,** which participate in one-electron transfers involving the Fe^{2+} and Fe^{3+}states.

(e) Protein-bound **copper,** a one-electron transfer site, which converts between Cu^{+} and Cu^{2+}.

All these intermediates except for cytochrome *c* are membrane-associated (either in the mitochondrial inner membrane of eukaryotes or in the plasma membrane of prokaryotes). All three types of proteins involved in this chain— flavoproteins, cytochromes, and iron–sulfur proteins—possess electron-transferring **prosthetic groups.**

Electron Carriers Function in Multienzyme Complexes

The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes that can be physically separated. Gentle treatment of the inner mitochondrial membrane with detergents allows the resolution of four unique electron-carrier complexes, each capable of catalyzing electron transfer through a portion of the chain. (I) **NADH–coenzyme Q reductase,** (II) **succinate–coenzyme Q reductase,** (III) **coenzyme Q–cytochrome c reductase,** and (IV) **cytochrome c oxidase**

Complex I: NADH–Coenzyme Q Reductase

As name implies, this complex transfers a pair of electrons from NADH to coenzyme Q, a small, hydrophobic, yellow compound. Another common name for this enzyme complex is NADH:ubiquinone oxidoreductase or *NADH dehydrogenase*. The complex involves more than 42 polypeptide chains, one molecule of flavin mononucleotide (FMN), and as many as six Fe-S clusters. By virtue of its dependence on FMN, NADH–UQ reductase is a *flavoprotein*. Although the precise mechanism of the NADH–UQ reductase is not known, the first step involves binding of NADH to the enzyme on the *matrix* side of the inner mitochondrial membrane, and transfer of electrons from NADH to tightly bound FMN:

$$NADH + [FMN] + H^{+} \rightarrow [FMNH_2] + NAD^{+}$$

The second step involves the transfer of electrons from the reduced $[FMNH_2]$ to a series of Fe-S proteins, including both 2Fe-2S and 4Fe-4S clusters. NADH is a two-electron donor, whereas the Fe-S proteins are one-electron transfer agents. The flavin of FMN has three redox states—the oxidized, semiquinone, and reduced states. It can act as *either* a one-

electron *or* a two-electron transfer agent and may serve as a critical link between NADH and the Fe-S proteins.

The final step of the reaction involves the transfer of two electrons from iron–a sulfur cluster to coenzyme Q. Coenzyme Q is a **mobile electron carrier.** Its isoprenoid tail makes it highly hydrophobic, and it diffuses freely in the hydrophobic core of the inner mitochondrial membrane. As a result, it shuttles electrons from Complexes I and II to Complex III. The oxidation of one NADH and the reduction of one UQ by NADH–UQ reductase results in the net transport of protons from the matrix side to the cytosolic side of the inner membrane. The cytosolic side, where H accumulates, is referred to as the **P** (for *positive*) face; similarly, the matrix side is the **N** (for *negative*) face. Some of the energy liberated by the flow of electrons through this complex is used in a *coupled process* to drive the transport of protons across the membrane.

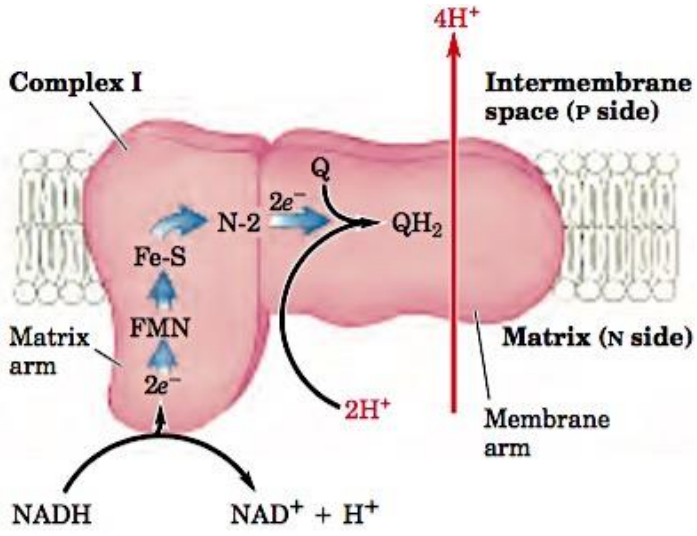


Figure 41 NADH: ubiquinoneoxidoreductase (ComplexI)

Complex II: Succinate–Coenzyme Q Reductase

Complex II is perhaps better known by its other name—**succinate dehydrogenase,** the only TCA cycle enzyme that is an integral membrane protein in the inner mitochondrial membrane. This enzyme is composed of four subunits: two Fe-S proteins, and two other peptides. Also known as *flavoprotein 2 (FP_2),* it contains an FAD covalently bound to a histidine residue, and three Fe-S centers: a 4Fe-4S cluster, a 3Fe-4S cluster, and a 2Fe-2S cluster. When succinate is converted to fumarate in the TCA cycle, concomitant reduction of

bound FAD to $FADH_2$occurs in succinate dehydrogenase. This $FADH_2$ transfers its electrons immediately to Fe-S centers, which pass them on to UQ. Electron flow from succinate to UQ,

Succinate $\rightarrow$ fumarate + $2H^+ + 2\ e^-$

$UQ + 2H^+ + 2\ e^- \rightarrow UQH_2$

Net reaction: Succinate +UQ$\rightarrow$fumarate + $UQH_2 \Delta G'° = -5.6$kJ/mol

The small free energy change of this reaction is not sufficient to drive the transport of protons across the inner mitochondrial membrane.

This is a crucial point because proton transport is coupled with ATP synthesis. Oxidation of one $FADH_2$ in the electron transport chain results in synthesis of approximately two molecules of ATP, compared with the approximately three ATPs produced by the oxidation of one NADH. Other enzymes can also supply electrons to UQ, including mitochondrial glycerophosphate dehydrogenase, an inner membrane–bound shuttle enzyme, and the fatty acyl–CoA dehydrogenases, three soluble matrix enzymes involved in fatty acid oxidation. The effect of each of these electron-transferring enzymes is to con- tribute to the pool of reduced ubiquinone. QH_2 from all these reactions is reoxidized by Complex III.

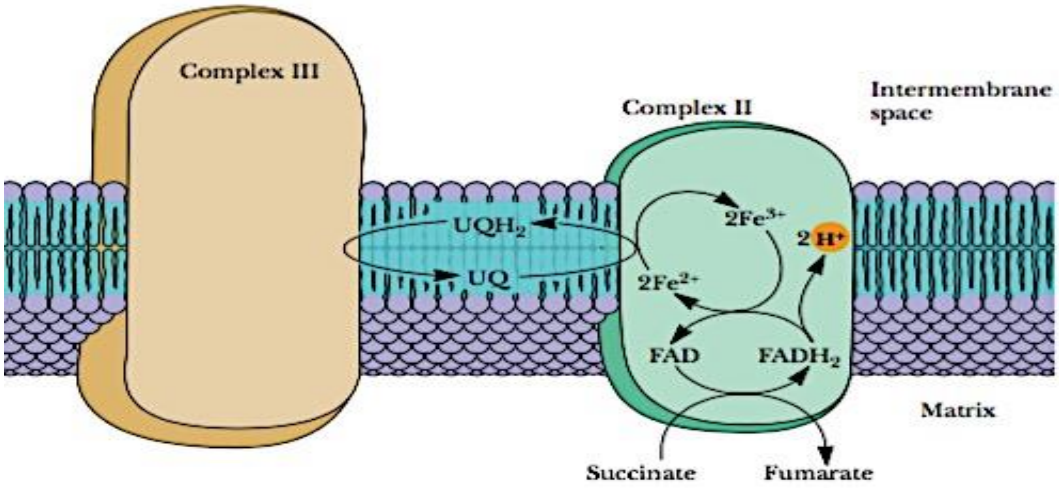


Figure 42 Scheme for electron flow in complex II

Complex III: Coenzyme Q–Cytochrome *c* Reductase

In the third complex of the electron transport chain, reduced coenzyme Q (UQH_2) passes its electrons to cytochrome *c* via a unique redox pathway known as the **Q cycle.** *UQ–cytochrome c reductase* (UQ–cyt *c* reductase) involves three different cytochromes and an

Fe-S protein. In the cytochromes of these and similar complexes, the iron atom at the center of the porphyrin ring cycles between the reduced Fe^{2+}ferrous) and oxidized Fe^{3+} (ferric) states.

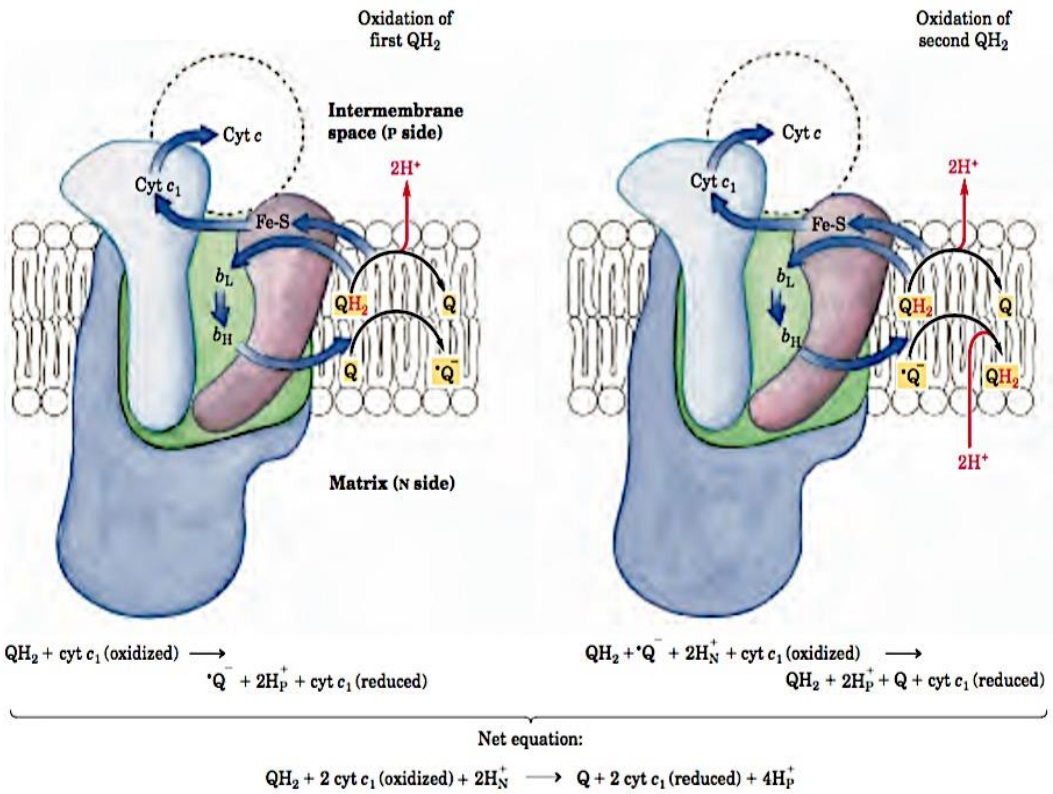
Cytochromes were first named and classified on the basis of their absorption spectra, which depend upon the structure and environment of their heme groups. The ***b* cytochromes** contain *iron–protoporphyrin IX*, the same heme found in hemoglobin and myoglobin. The ***c* cytochromes** contain *heme c,* derived from iron–protoporphyrin IX by the covalent attachment of cysteine residues from the associated protein. UQ–cyt *c reductase contains a b-type cytochrome, of 30 to 40 kD, with two different heme sites and one c-type cytochrome.*

The net equation for the redox reactions of this Q cycle is

QH_2+ 2 cyt c_1 (oxidized) + $2H^+_N \rightarrow$Q + 2 cyt c_1 (reduced) + $4H^+_P$

The Q Cycle

The Q cycle accommodates the switch between the two-electron carrier ubiquinone and the one-electron carriers—cytochromes b_{562}, b_{566}, c_1, and c—and explains the measured stoichiometry of four protons translocated per pair of electrons passing through the Complex III to



cytochrome c. Although the path of electrons through this segment of the respiratory chain is

complicated, the net effect of the transfer is simple: QH_2 is oxidized to Q and two molecules of cytochrome c are reduced.

Cytochrome c is a soluble protein of the intermembrane space. After its single heme accepts an electron from Complex III, cytochrome c moves to Complex IV to donate the electron to a binuclear copper center.

Complex IV: Cytochrome c to O_2In the final step of the respiratory chain, Complex IV, also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O. Complex IV is a large enzyme of the inner mitochondrial membrane. Bacteria contain a form that is much simpler, with only three or four subunits, but still capable of catalyzing both electron transfer and proton pumping. Comparison of the mitochondrial and bacterial complexes suggests that three subunits are critical to the function. Mitochondrial subunit II contains two Cu ions complexed with the -SH groups of two Cys residues in a binuclear center (Cu_A) that resembles the 2Fe-2S centers of iron-sulfur proteins. Subunit I contains two heme groups, designated a and a_3, and another copper ion (Cu_B). Heme a_3 and Cu_B form a second binuclear center that accepts electrons from heme a and transfers them to O_2 bound to heme a_3.

Electron transfer through Complex IV is from cytochrome c to the Cu_A center, to heme a, to the heme a_3–Cu_B center, and finally to O_2. For every four electrons passing through this complex, the enzyme consumes four "substrate" H^+ from the matrix (N side) in converting O_2 to $2H_2O$. It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space (P side) for each electron that passes through, adding to the electrochemical potential produced by redox-driven proton transport through Complexes I and III. The overall reaction catalyzed by Complex IV is

$$4 \text{ Cyt } c \text{ (reduced)} + 8\ H^+_N + O_2 \rightarrow 4 \text{ cyt } c \text{ (oxidized)} + 4H^+_P + 2H_2O$$

This four-electron reduction of O_2involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals—very reactive species that would damage cellular components. The intermediates remain tightly bound to the complex until completely converted to water.

For each pair of electrons transferred to O_2, four protons are pumped out by Complex I, four by Complex III, and two by Complex IV (Fig. 45). The vectorial equation for the process is therefore

$NADH + 11\ H^+_N + ½\ O_2 \rightarrow NAD^+ + 10H^+_P + H_2O$

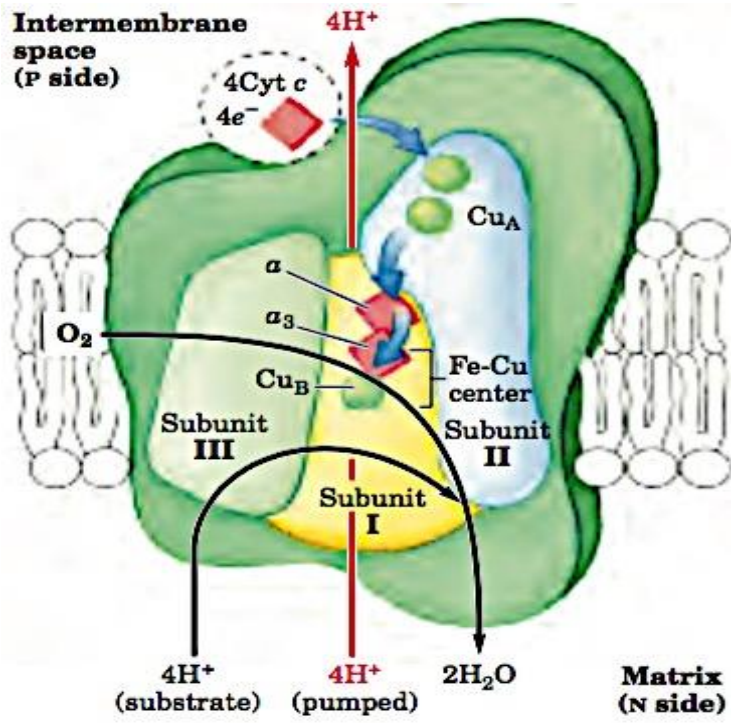


Figure 44 Path of electrons through Complex IV

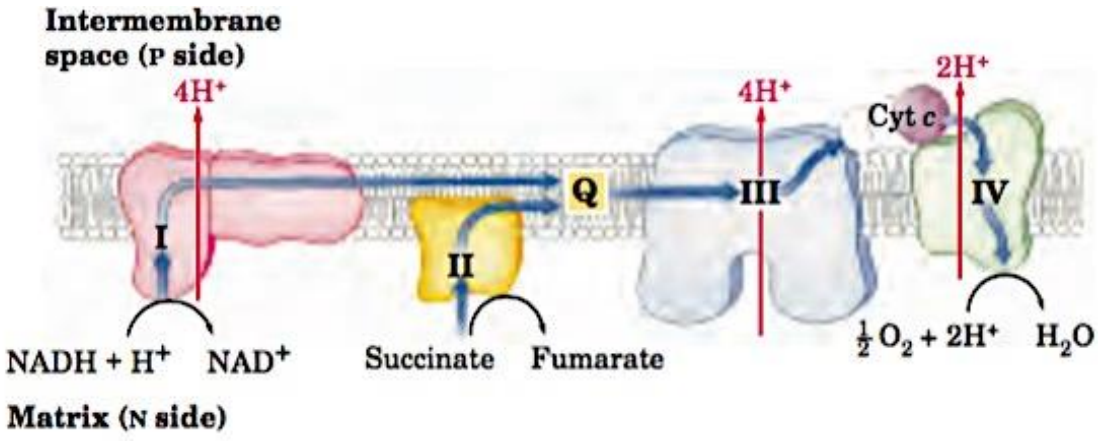


Figure 45 Summary of the flow of electrons and protons through the four complexes of the respiratory chain

The electrochemical energy inherent in this difference in proton concentration and separation of charge represents a temporary conservation of much of the energy of electron transfer. The energy stored in such a gradient, termed the proton-motive force, has two components: (1) the chemical potential energy due to the difference in concentration of a chemical species (H^+) in the two regions separated by the membrane, and (2) the electrical potential energy that results from the separation of charge when a proton moves across the membrane without a counterion

When protons flow spontaneously down their electrochemical gradient, energy is made available to do work. In mitochondria, chloroplasts, and aerobic bacteria, the electrochemical energy in the proton gradient drives the synthesis of ATP from ADP and Pi.

4.4.7.1 ATP Synthesis

How is a concentration gradient of protons transformed into ATP? Electron transfer releases, and the proton-motive force conserves, more than enough free energy per "mole" of electron pairs to drive the formation of a mole of ATP. Mitochondrial oxidative phosphorylation therefore poses no thermodynamic problem. But what is the chemical mechanism that couples proton flux with phosphorylation?

In 1961, Peter Mitchell proposed a novel coupling mechanism involving a proton gradient across the inner mitochondrial membrane. In Mitchell's **chemiosmotic hypothesis,** protons are driven across the membrane from the matrix to the intermembrane space and cytosol by the events of electron transport. This mechanism stores the energy of electron transport in an **electrochemical potential.** As protons are driven out of the matrix, the pH rises and the matrix becomes negatively charged with respect to the cytosol. Proton pumping thus creates a pH gradient and an electrical gradient across the inner membrane, both of which tend to attract protons back into the matrix from the cytoplasm. Flow of protons down this electrochemical gradient, an energetically favorable process, then drives the synthesis of ATP. To emphasize this crucial role of the proton-motive force, the equation for ATP synthesis is sometimes written

$$ADP + Pi + nH^+_P \rightarrow ATP + H_2O + nH^+_N$$

ATP Synthase

The mitochondrial complex that carries out ATP synthesis is called **ATP synthase** or sometimes **F_1F_O–ATPase** (for the reverse reaction it catalyzes). **It Consists of Two Complexes—F_1 and F_O.** The spheres observed in electron micrographs make up the **F_1 unit,** which catalyzes ATP synthesis. These F1 spheres are attached to an integral membrane protein aggregate called the **F_O unit.** F1 consists of five polypeptide chains named α, β, γ, δ and ε with a subunit stoichiometry $\alpha_3\beta_3 3\ \gamma\delta\varepsilon$. F_O consists of three hydrophobic subunits denoted by *a*, *b*, and *c*, with an apparent stoichiometry of $a_1b_2\ c_{9\text{-}12}$. F_O forms the transmembrane pore or channel through which protons move to drive ATP synthesis. The α andβ subunits are homologous, and each of these subunits bind a single ATP. The catalytic sites are in the βsubunits; the function of the ATP sites in the αsubunits is unknown.

Proton Flow Through ATP Synthase Leads to the Release of Tightly Bound ATP: The Binding-Change Mechanism

ATP synthase catalyzes the formation of ATP from ADP and orthophosphate.

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightleftharpoons ATP^{4-} + H_2O$$

The actual substrates are Mg^{2+} complexes of ADP and ATP, as in all known phosphoryl transfer reactions with these nucleotides. A terminal oxygen atom of ADP attacks the phosphorus atom of Pi to form a pentacovalent intermediate, which then dissociates into ATP and H_2O. The attacking oxygen atom of ADP and the departing oxygen atom of Pi occupy the apices of a trigonal bipyramid.

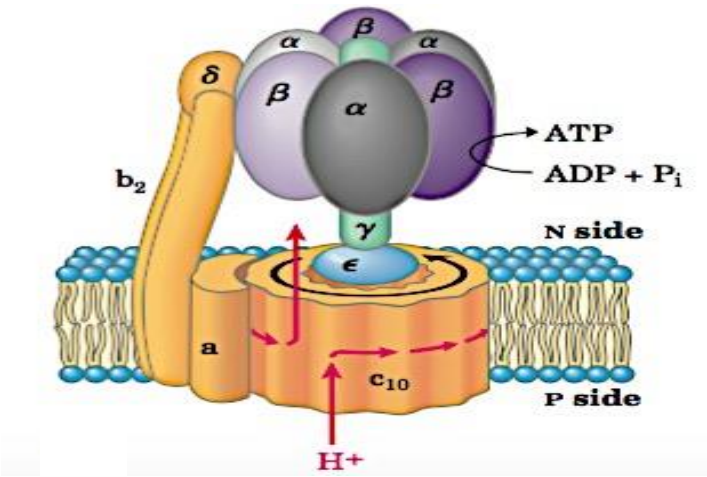


Figure 46 Diagram of the F_1F_O complex

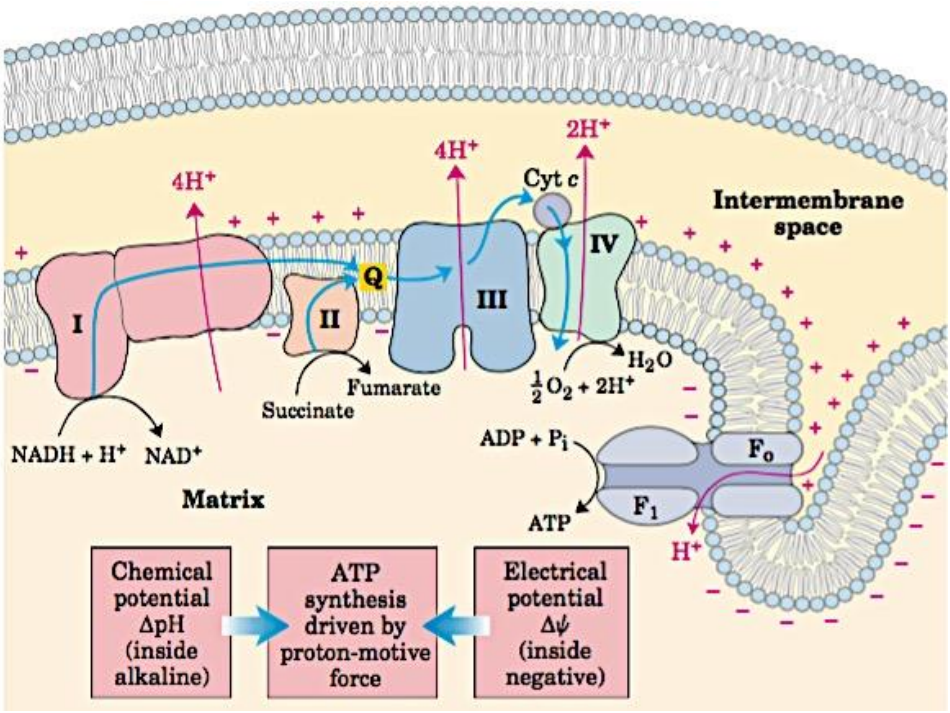


Figure 47 Chemiosmotic model

How does the flow of protons drive the synthesis of ATP?

Paul Boyer proposed a *binding-change mechanism* (Figure 48)for proton-driven ATP synthesis. This proposal states that changes in the properties of the three βsubunits allow sequential ADP and Pi binding, ATP synthesis, and ATP release. As already noted, interactions with the γsubunit make the three βsubunits inequivalent. One βsubunit can be in the T, or tight, conformation. This conformation binds ATP with great avidity. Indeed, its affinity for ATP is so high that it will convert bound ADP and Pi into ATP with equilibrium constant near 1. However, the conformation of this subunit is sufficiently constrained that it cannot release ATP. A second subunit will then be in the L, or loose, conformation. This conformation binds ADP and Pi. It, too, is sufficiently constrained that it cannot release bound nucleotides. The final subunit will be in the O, or open, form. This form can exist with a bound nucleotide in a structure that is similar to those of the T and L forms, but it can also convert to form a more open conformation and release a bound nucleotide.

The interconversion of these three forms can be driven by rotation of the γsubunit. Suppose the γsubunit is rotated 120 degrees in a counterclockwise direction (as viewed from the top). This rotation will change the subunit in the T conformation into the O conformation, allowing the subunit to release the ATP that has been formed within it. The subunit in the L conformation will be converted into the T conformation, allowing the transition of bound

ADP + Pi into ATP. Finally, the subunit in the O conformation will be converted into the L conformation, trapping the bound ADP and Pi so that they cannot escape. The binding of ADP and Pi to the subunit now in the O conformation completes the cycle.

This mechanism suggests that ATP can be synthesized by driving the rotation of the γsubunit in the appropriate direction. Likewise, this mechanism suggests that the hydrolysis of ATP by the enzyme should drive the rotation of the γsubunit in the opposite direction.

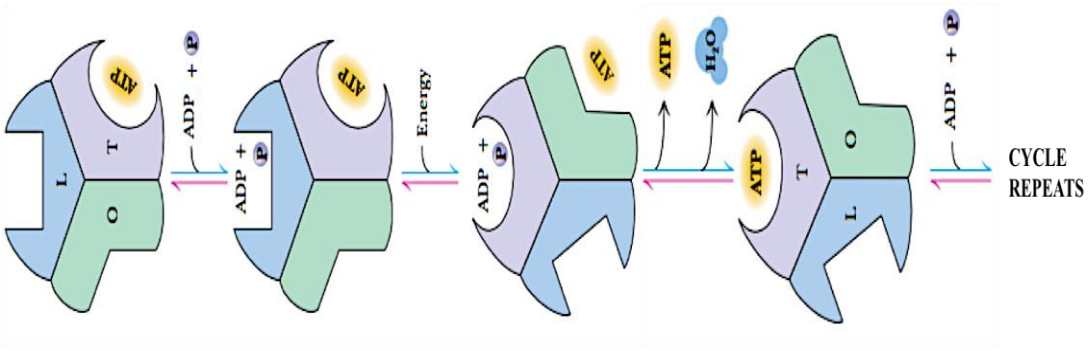


Figure 48 Binding-Change Mechanisms for ATP Synthase

4.4.7.2 Inhibitors of Oxidative Phosphorylation

Rotenone is a common insecticide that strongly inhibits the NADH–UQ reductase. Ptericidin, Amytal, and other barbiturates, mercurial agents, and the widely prescribed painkiller Demerol also exert inhibitory actions on this enzyme complex. All these substances appear to inhibit reduction of coenzyme Q and the oxidation of the Fe-S clusters of NADH–UQ reductase.

Complex IV, the cytochrome *c* oxidase, is specifically inhibited by cyanide (CN^-), azide (N_3^-), and carbon monoxide (CO). Cyanide and azide bind tightly to the ferric form of cytochrome a_3, whereas carbon monoxide binds only to the ferrous form.Inhibitors of ATP synthase include dicyclohexylcarbodiimide (DCCD) and oligomycin

4.4.7.3 Uncouplers

Another important class of reagents affects ATP synthesis, but in a manner that does not involve direct binding to any of the proteins of the electron transport chain or the $\mathbf{F_1F_O}$–ATPase. These agents are known as uncouplers because they disrupt the tight coupling between electron transport and the ATP synthase. Uncouplers act by dissipating the proton gradient across the inner mitochondrial membrane created by the electron transport system.

Typical examples include 2,4-dinitrophenol, dicumarol, and carbonyl cyanide-*p*-trifluoro-methoxyphenyl hydrazone (perhaps better known as fluorocarbonyl-cyanide phenylhydrazone or FCCP)

4.4.7.4 ATP Exits the Mitochondria via an ATP–ADP Translocase

ATP, the cellular energy currency, must exit the mitochondria to carry energy throughout the cell, and ADP must be brought into the mitochondria for reprocessing. Neither of these processes occurs spontaneously because the highly charged ATP and ADP molecules do not readily cross biological membranes. Instead, these processes are mediated by a single transport system, the **ATP– ADP translocase.** This protein tightly couples the exit of ATP with the entry of ADP so that the mitochondrial nucleotide levels remain approximately constant. For each ATP transported out, one ADP is transported into the matrix. Transport occurs via a single nucleotide-binding site, which alternately faces the matrix and the cytosol. It binds ATP on the matrix side, reorients to face the cytosol, and exchanges ATP for ADP, with subsequent movement back to the matrix face of the inner membrane.

4.4.7.5 Regulation of Oxidative Phosphorylation

Oxidative phosphorylation is regulated by cellular energy demands. The intracellular [ADP] and the mass-action ratio [ATP]/([ADP][Pi]) are measures of a cell's energy status. Normally this ratio is very high, so the ATP-ADP system is almost fully phosphorylated. When the rate of some energy-requiring process (protein synthesis, for example) increases, the rate of breakdown of ATP to ADP and Pi increases, lowering the mass-action ratio. With more ADP available for oxidative phosphorylation, the rate of respiration increases, causing regeneration of ATP. This continues until the mass-action ratio returns to its normal high level, at which point respiration slows again. The rate of oxidation of cellular fuels is regulated with such sensitivity and precision that the [ATP]/([ADP][Pi]) ratio fluctuates only slightly in most tissues, even during extreme variations in energy demand. In short, ATP is formed only as fast as it is used in energy-requiring cellular activities.

Note- In brown fat, which is specialized for the production of metabolic heat, electron transfer is uncoupled from ATP synthesis due to presence of a unique protein in their inner membrane. Thermogenin (uncoupling protein) provides a path for protons to return to the matrix without passing through the Fo F1 complex and the energy of fatty acid oxidation is dissipated as heat.*

ATP-Producing Pathways Are Coordinately Regulated

The major catabolic pathways arecoordinated regulated to function together in an economical manner to produce ATP and biosynthetic precursors. The relative concentrations of ATP and ADP control not only the rates of electron transfer and oxidative phosphorylation but also the rates of the citric acid cycle, pyruvate oxidation, and glycolysis. Whenever ATP consumption increases, the rate of electron transfer and oxidative phosphorylation increases. Simultaneously, the rate of pyruvate oxidation via the citric acid cycle increases, increasing the flow of electrons into the respiratory chain. These events can in turn evoke an increase in the rate of glycolysis, increasing the rate of pyruvate formation. When conversion of ADP to ATP lowers the ADP concentration, acceptor control slows electron transfer and thus oxidative phosphorylation. Glycolysis and the citric acid cycle are also slowed, because ATP is an allosteric inhibitor of the glycolytic enzyme phosphofructokinase-1 and of pyruvate dehydrogenase.

Phosphofructokinase-1 is also inhibited by citrate, the first intermediate of the citric acid cycle. When the cycle is "idling," citrate accumulates within mitochondriaand then spills into the cytosol. When the concentrations of both ATP and citrate rise, they produce a concerted allosteric inhibition of phosphofructokinase-1 that is greater than the sum of their individual effects, slowing glycolysis.

4.5 SUMMARY

- Sugars (also called saccharides) are compounds containing an aldehyde or ketone group and two or more hydroxyl groups.
- Monosaccharides generally contain several chiral carbons and therefore exist in a variety of stereochemical forms, represented on paper as Fischer projections. Epimers are sugars that differ in configuration at only one carbon atom.
- Monosaccharides commonly form internal hemiacetals or hemiketals, in which the aldehyde or ketone group joins with a hydroxyl group of the same molecule, creating a cyclic structure; this can be represented as aHaworth perspective formula.
- Oligosaccharides are short polymers of several monosaccharides joined by glycosidic bonds. At one end of the chain, the reducing end, is a monosaccharide unit whose anomeric carbon is not involved in a glycosidic bond.
- Polysaccharides (glycans) serve as stored fuel and as structural components of cell

walls and extracellular matrix. The homopolysaccharides starch and glycogen are stored fuels in plant, animal, and bacterial cells. They consist of D-glucose with linkages, and all three contain some branches.

- Glycosaminoglycans are extracellular heteropolysaccharides in which one of the two monosaccharide units is a uronic acid and the other an N-acetylated amino sugar.
- Proteoglycans are glycoconjugates in which a core protein is attached covalently to one or more large glycans. Glycoproteins contain covalently linked oligosaccharides that are smaller but more structurally complex, and therefore more information-rich, than glycosaminoglycans.
- Glycolysis is a near-universal pathway by which a glucose molecule is oxidized to two molecules of pyruvate, with energy conserved as ATP and NADH.It is tightly regulated in coordination with other energy-yielding pathways to assure a steady supply of ATP. Hexokinase, PFK-1, and pyruvate kinase are all subject to allosteric regulation.
- Gluconeogenesis is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose.Glycolysis and gluconeogenesis are reciprocally regulated to prevent wasteful operation of both pathways at the same time.
- The oxidative pentose phosphate pathway (phosphogluconate pathway, or hexose monophosphate pathway) brings about oxidation and decarboxylation at C-1 of glucose 6-phosphate, reducing $NADP^+$ to NADPH and producing pentose phosphates.
- Glycogen phosphorylase catalyzes phosphorolytic cleavage at the nonreducing ends of glycogen chains, producing glucose 1-phosphate. The debranching enzyme transfers branches onto main chains and releases the residue at the ($\alpha 1 \rightarrow 6$) branch as free glucose.Glycogen synthesis depended upon sugar nucleotides.Glycolysis and Gluconeogenesis are coordinatly Regulated.
- The citric acid cycle (Krebs cycle, TCA cycle) is a nearly universal central catabolic pathway in which compounds derived from the breakdown of carbohydrates, fats, and proteins are oxidized to CO_2, with most of the energy of oxidation temporarily held in the electron carriers $FADH_2$ and NADH. During aerobic metabolism, these electrons are transferred to O_2 and the energy of electron flow is trapped as ATP.

- In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the net formation of succinate, oxaloacetate, and other cycle intermediates from acetyl-CoA.
- Oxidative phosphorylation is the culmination of energy yielding metabolism in aerobic organisms.

4.6 GLOSSARY

Aldose-a sugar containing the aldehyde group or its hemiacetal equivalent.

Allosteric regulation-is the **regulation** of an enzyme by binding an effector molecule at a site other than the enzyme's active site. The site to which the effector binds is termed the **allosteric** site.

Anomers-are diastereoisomers of cyclic forms of sugars or similar molecules differing in the configuration at the anomeric carbon (C-1 atom of an aldose or the C-2 atom of a 2-ketose).

ATP Synthase-an enzyme complex of the inner mitochondrial membrane catalyzes the formation of ATP from ADP and Pi, accompanied by the flow of protons.

Carbohydrates-are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis having the empirical formula $(CH_2O)n$.

Chemiosmotic theory -the electrochemical energy inherent in the difference in proton concentration and separation of charge across the inner mitochondrial membrane—the proton-motive force—drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with ATP synthase.

Citric acid cycle- a central catabolic pathway in which compounds derived from the break-down of carbohydrates, fats, and proteins are oxidized to CO_2, with most of the energy of oxidation temporarily held in the electron carriers $FADH_2$ and NADH. During aerobic metabolism, these electrons are transferred to O_2 and the energy of electron flow is trapped as ATP.

Cytochromes-are iron containing hemeproteins central to which are heme groups that are primarily responsible for the generation of ATP via electron transport.

Epimers- sugars that differ only in the configuration around one carbon atom are called epimers.

Fermentation- Fermentation is a general term for the anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP.

Gluconoegenesis- a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates.

Glycan- compounds consisting of a large number of monosaccharides linked glycosidically

Glycolysis- is the metabolic pathway that converts glucose into pyruvate.

Glycogenolysis-is the biochemical breakdown of glycogen to glucose.

Glycogenesis- is the formation of glycogen from glucose.

Glycoprotein- any class of proteins that have carbohydrate groups attached to the polypeptide chain.

Glycosidic Bond- or glycosidic linkage is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.

Ketose- is a monosaccharide containing one ketone group per molecule

Mutarotaion- is the change in the optical rotation because of the change in the equilibrium between two anomers, when the corresponding stereocenters interconvert.

Oxidative Phosphorylation- is the culmination of energy-yielding metabolism in aerobic organisms. Oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP.

Proteoglycan- Proteoglycans are proteins that are heavily glycosylated. The basic proteoglycan unit consists of a "core protein" with one or more covalently attached glycosaminoglycan chain.

Proton-motive force-The proton motive force is the energy generated from transferring protons or electrons across an energy-transducing cell membrane.

Reducing sugar- Any carbohydrate, which is capable of being oxidized and causes the reduction of other substances is known as reducing sugar.

Substrate-level phosphorylation- is a metabolic reaction that results in the formation of ATP or GTP by the direct transfer of a phosphoryl group to ADP or GDP from another phosphorylated compound.

Vice versa-in reverse order from the way something has been stated.

4.7 SELF ASSESSMENT QUESTIONS

4.7.1 Long answer type questions:-

Q 1. What are carbohydrates? Describe their classification in detail with suitable examples.

Q 2. Give a detail account of the glycolysis and its regulation.

Q 3. Give comparative account of the process of glycogenolysis and glycogenesis.

Q 4. Describe pentose Phosphate pathway and its significance.

Q 5. Describe in detail the process of gluconeogenesis and its role in metabolism.

Q 6. Give a detail account of the Citric acid cycle and its regulation.

4.7.2 Short answer type questions:-

Q 1.Write short note on the types of fermentation.

Q 2. Write notes on electron transport chain.

Q 3. Write notes on glyoxylate cycle.

Q 4. Write short note on- Monosaccharide, Oligosaccharide and polysaccharide

Q 5. Write notes on starch and glycogen.

4.7.3Fill in the blanks

Q 1. Carbohydrates have the empirical formula _____.

Q 2. Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called _____.

Q 3. _____and_____ are reciprocally regulated to prevent wasteful operation of both pathways at the same time.

Q 4. _____is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose.

Q 5. Pyruvate dehydrogenase complex requires five coenzymes or prosthetic groups namely_____.

Q 6. _____replenish Citric Acid Cycle intermediates.

Q 7. The proton-motive force drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with_____.

Q 8. _____is also inhibited by citrate, the first intermediate of the citric acid cycle.

REFERENCES AND SUGGESTED READINGS

- Lehninger Principles of Biochemistry (5th Edition, W. H. Freeman) by David L. Nelson (Author), Michael M. Cox (Author)
- Biochemistry (5th edition, Wadsworth Publishing Co Inc) by Reginald H Garrett (Author), Charles M Grisham (Author)
- Harper's Biochemistry – Murray, Granner, Mayes, Rodwell – Prentice Hall International Inc.

ANSWERS

Fill in the blanks

1. $(CH_2O)_n$, 2. Anomers , 3. Glycolysis, Gluconeogenesis, 4.Gluconeogenesis, 5.Thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A, nicotinamide adenine dinucleotide (NAD), and lipoate.6. Anaplerotic Reactions, 7. ATP synthase, 8.Phosphofructokinase-1

UNIT 5- PROTEINS

Contents

5.1 INTRODUCTION

- Proteins are the most significant and abundant biological macromolecule, occurring in all cells and all parts of the cell. These form the fundamental structural components of the body, both protoplasmic and extracellular.
- The term Protein is derived from the Greek word *proteios*, meaning "of the first order". It was discovered by Jöns Jakob Berzelius in 1838.
- The functions of proteins include structural support, protection, transport, catalysis, defence, regulation, and movement in an organism.
- Proteins are assembled from combinations of biological α-amino acids, which contain Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N) and sometimes Phosphorus (P) and Sulphur (S).

5.2 GENERAL PROPERTIES

- Proteins are usually large molecules like the muscle protein titin or connectin has a single amino acid chain that is 27,000 subunits long. Such long chains of amino acids are almost totally referred to as proteins, but shorter sequences of amino acids are referred to as polypeptides, peptides, and oligopeptides.
- Variation in amino acids chains of proteins contributes to their functional diversity for instance, a shorter amino acid chain is more likely to act as a hormone (like insulin), rather than an enzyme (which depends on its defined three-dimensional structure for functionality).
- Proteins are colourless, usually tasteless, homogenous and crystalline. They are soluble in water, salt solution, dilute acids and alkaline due to their large size and they form colloids and exhibit colloidal properties.
- Proteins possess free ionic or electrically charged groups, so they can migrate in an electrical field.
- Proteins range in size from small ones such as the RNA digesting enzyme ribonuclease A, which has a molecular weight of 5,733 with 51 amino acid residues, to large molecules such as the cholesterol transport protein apolipoprotein B, which has a molecular weight of 513,000 and 4,636 amino acids.
-

5.3 AMINO ACIDS

- Amino acids are as monomers of proteins. These molecules are composed of a central or alpha carbon with three attachments: an amino group (-NH2), a carboxylic acid group (-COOH), and a unique R group, or side chain.
- In proteins, amino acids (specifically, α-amino acids) are linked together by peptide bonds, which is formed when the amino group of one amino acid reacts with the carboxyl group of a second amino acid to form a covalent bond after releasing a water molecule. All the amino acids except proline have both free α-amino and free α-carboxyl groups.
- In neutral solution (pH 7), the carboxyl group exists as $OC\bar{O}O$ and the amino group as ONH_3^+. The resulting amino acid contains one positive and one negative charge, it is a neutral molecule called a zwitterion.
- Scientists classify amino acids into different categories based on the nature of the side chain. A tetrahedral carbon atom with four distinct groups is called chiral. all of the amino acids have chiral carbon atom except for glycine amino acid.
- The ability of a molecule to rotate plane polarized light to the left, L (levorotary) or right, D (dextrorotary) gives it its optical and stereochemical fingerprint.
- All amino acids within polypeptides are configured in the L form. The L form corresponds to the absolute configuration of S, which is a system used to designate stereochemistry in the field of organic chemistry.
- Although D-amino acids (designated as R stereoisomers in the field of organic chemistry) exist naturally, they are not found in proteins

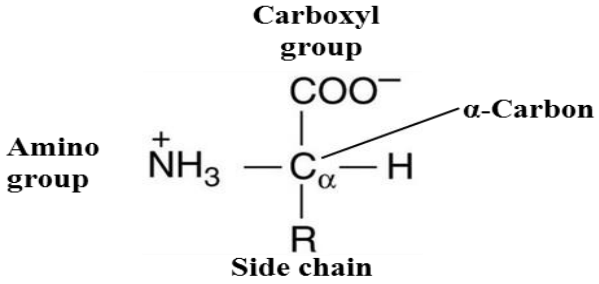


Figure 1 - Anatomy of an amino acid

- Yet, over 50 amino acids have discovered by scientists, only 20 amino acids are used to make proteins in your body. Out of twenty, ten are defined as essential. The other

ten amino acids can be synthesized by our body. All proteins are synthesized using different combinations of those twenty. Amino acids bond together to form long chains.

- **Essential Amino Acids**: Arginine (essential in children, not in adults), Leucine, Histidine, Isoleucine, Lysine, Threonine, Tryptophan, Methionine, Phenylalanine and Valine.
- **Nonessential Amino Acids**: Alanine, Asparagine, Aspartic Acid, Glutamic Acid, Cysteine, Glutamine, Glycine, Proline, Serine, and Tyrosine.
- Amino acids have several side groups which make different from each other, there are two main groups: polar and non-polar.

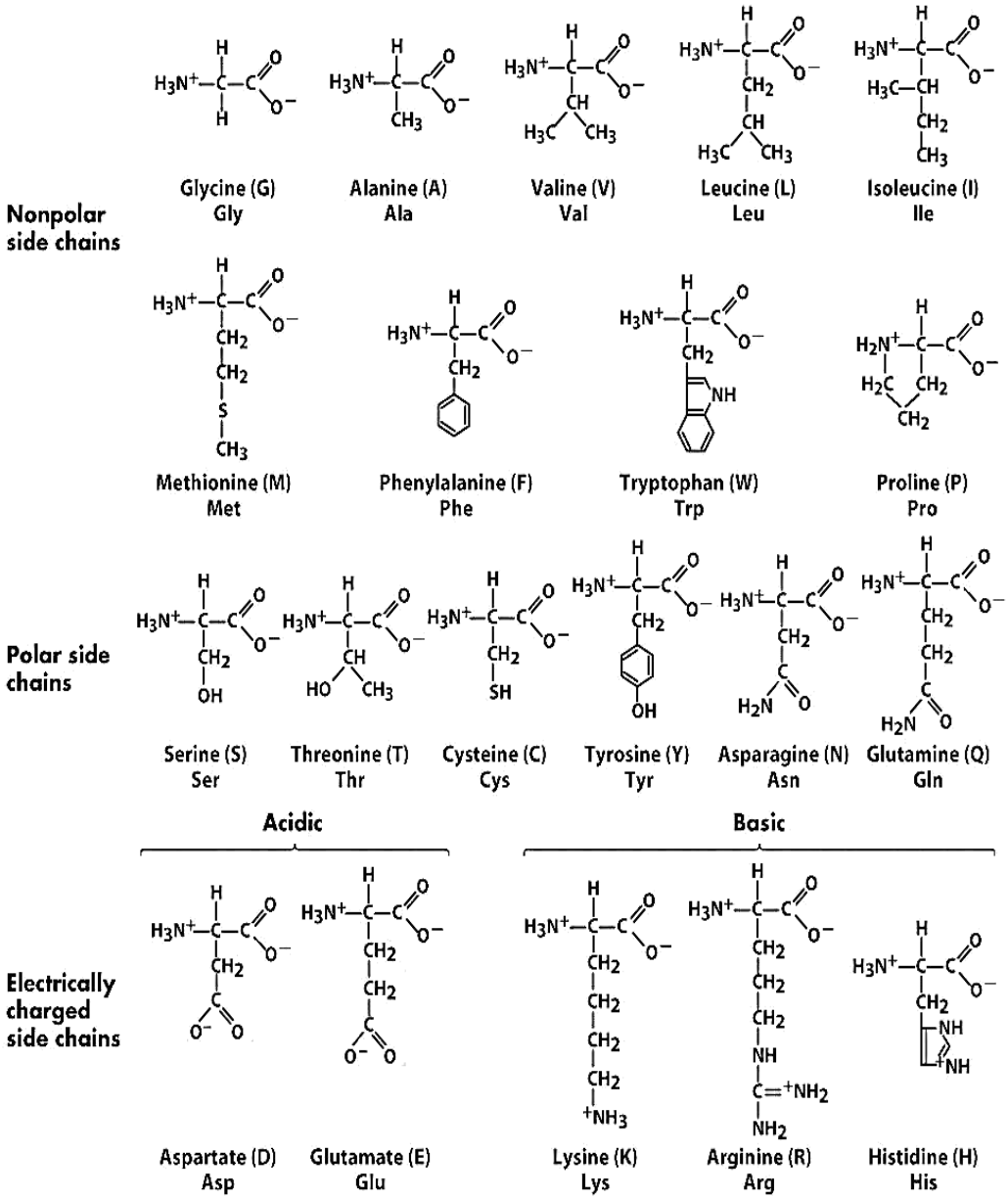
5.3.1 Uncommon Amino Acids

- Several amino acids occur only rarely in proteins. These include hydroxylysine and hydroxyproline, which are found mainly in the collagen and gelatin proteins, and thyroxine and 3,3, 5-triiodothyronine, iodinated amino acids that are found only in thyroglobulin, a protein produced by the thyroid gland. Thyroxine and 3,3, 5-triiodothyronine are produced by iodination of tyrosine residues in thyroglobulin in the thyroid gland.
- Degradation of thyroglobulin releases these two iodinated amino acids, which act as hormones to regulate growth and development. Certain muscle proteins contain methylated amino acids, including methylhistidine, ε-N-methyllysine, and ε-N, N, N-trimethyllysine. γ-Carboxyglutamic acid is found in several proteins involved in blood clotting, and pyroglutamic acid is found in a unique light-driven proton-pumping protein called bacteriorhodopsin.
- Certain proteins involved in cell growth and regulation are reversibly phosphorylated on the OH groups of serine, threonine, and tyrosine residues. Aminoadipic acid is found in proteins isolated from corn. Amino acids such as, N-methylarginine and N-acetyl lysine are found in histone proteins associated with chromosomes.

1.3.2 Amino Acid Classification

Amino acids can be classified into four general groups based on the properties of the "R" group in each amino acid. Amino acids can be polar, nonpolar, positively charged, or negatively charged. Polar amino acids have "R" groups that are

hydrophilic and nonpolar amino acids are hydrophobic. Figure-2 shows list of the 20 amino acids grouped on the basis of “R" group properties.



Source - www.pinterest.com

Figure 2 - Amino acid structure

5.3.2.1 Non-polar Amino Acids

- Aliphatic: glycine, alanine, valine, isoleucine, leucine
- Aromatic: phenylalanine, tryptophan.

- Cyclic: Proline
- Sulfur-Containing: methionine

5.3.2.2 Polar Amino Acids

- Sulfur-Containing: cysteine
- Hydroxyl-Containing: serine, threonine
- Aromatic: tyrosine
- Acidic Amide: asparagine, glutamine

5.3.2.3 Charged Amino Acids (at physiological pH)

- Acidic : aspartic acid, glutamic acid
- Basic : histidine, lysine, arginine

5.4 STRUCTURE OF PROTEINS

The four levels of protein structure are distinguished from one another by the degree of complexity in the polypeptide chain. A single protein molecule may contain one or more of the protein structures types.

5.4.1 Primary structure

- The primary structure of a protein is the level of protein structure that refers to the specific linear sequence of amino acids. Amino acids in a polypeptide (protein) are linked by peptide bonds. The amino acid sequence begins with the N-terminal amino acid (a free amino group) and ends at C-terminal (a free carboxyl group). The peptide bond is planar and cannot rotate freely due to a partial double bond character.

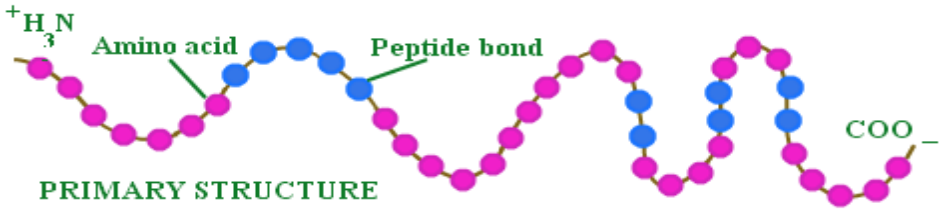


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Figure 3 - Primary (1°) structure of a protein

5.4.1.1 Ramachandran Plot

- A Ramachandran plot, also known as a Ramachandran diagram or a [φ,ψ] plot, was originally developed by Gopalasamudram Ramachandran, an Indian physicist, in 1963.
- Ramachandran plot is a way to visualize dihedral angles ψ against φ of amino acid residues in the protein structure.
- Ramachandran recognized that many combinations of angles in a polypeptide chain are forbidden because of steric collisions between atoms. His two-dimensional plot shows the allowed and disfavored values of ψ and φ: three-quarters of the possible combinations are excluded simply by local steric clashes.
- Steric exclusion is the fact that two atoms cannot be in the same place at the same time is the powerful organizing principle that propels the use of the Ramachandron plot forward.
- The two torsion angles of the polypeptide chain, also called Ramachandran angles, describe the rotations of the polypeptide backbone around the bonds between N-Cα (called Phi, φ) and Cα-C (called Psi, ψ).
- The Ramachandran plot provides an easy way to view the distribution of torsion angles of a protein structure. It also provides an overview of allowed and disallowed regions of torsion angle values, serving as an important factor in the assessment of the quality of protein three-dimensional structures.

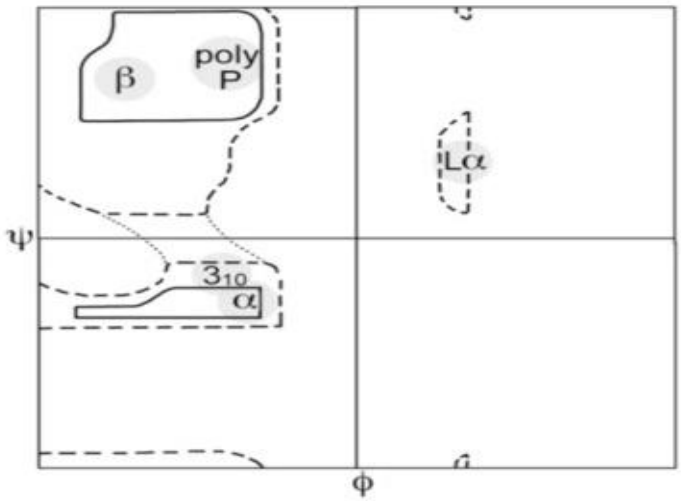


Figure 4- Ramachandran Plot

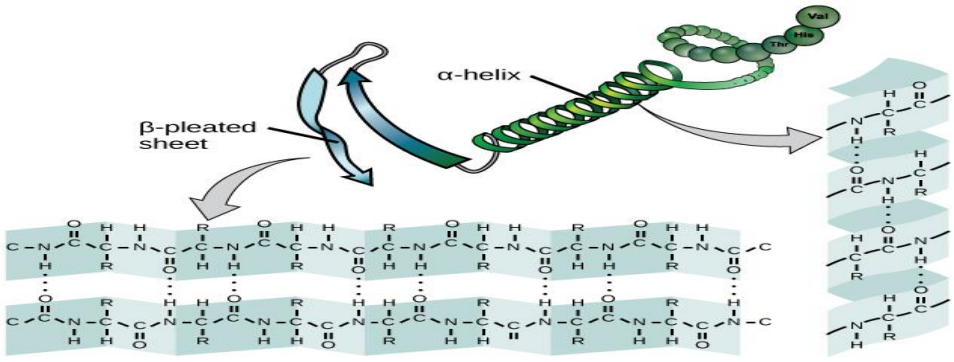
5.4.1.2 Regions in Ramachandran Plot

The Ramachandran Plot helps with the determination of secondary structures of proteins.

- Quadrant II shows the biggest region in the graph. This region has the most favorable conformations of atoms. It shows the sterically allowed conformations for beta strands.
- Quadrant III shows the next biggest region in the graph. This is where right-handed alpha helices lie.
- Quadrant IV has almost no outlined region. This conformation (ψ around -180 to 0 degrees, φ around 0-180 degrees) is disfavored due to steric clash.

5.4.2 Secondary structure

- The α-helix is a right-handed and tightly packed coiled structure with amino acid side chains extending outward from the central axis. Free rotation is possible about only two of the three covalent bonds of the polypeptide backbone: the α-carbon (Cα) to the carbonyl carbon (Co) bond and the Cα to nitrogen bond.
- The α-helix is stabilized by extensive hydrogen binding. It forms between hydrogen (H) atom attached to peptide nitrogen (N) and oxygen (O) atom attached to peptide carbon (C).
- ß-sheet is the second type of the structure of protein. They are composed of two or more segments of fully extended peptide chains. The polypeptide chains in the ß-sheet may be arranged parallel or anti-parallel.



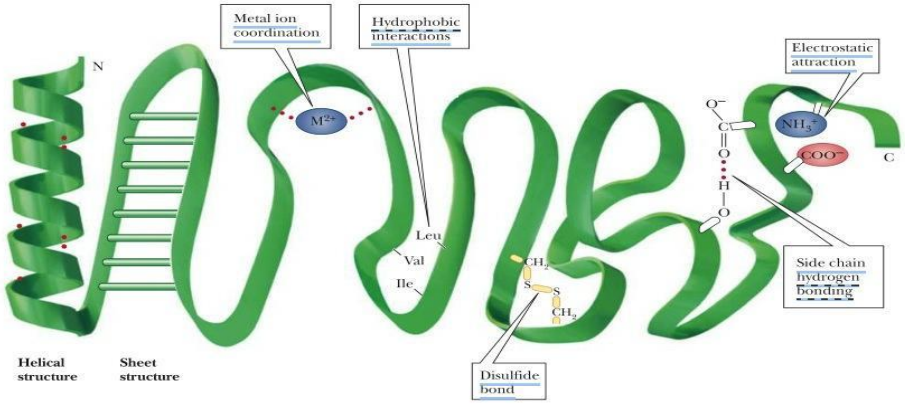
Source -https://www.boundless.com

Figure 6- Two structural motifs that arrange the primary structure of proteins into a higher level of organization predominate in proteins: the α -helix and the ß -pleated strand.

5.4.2. Tertiary Structure

The three-dimensional shape of a protein molecule is the tertiary structure... Although the three-dimensional shape of a protein may seem irregular and random, by many stabilizing forces due to bonding interactions between the side-chain groups of the amino acids.

It is a compact structure with hydrophobic side-chains held interior while the surface of the protein molecule.



Source - www.studyblue.com

Figure 7 - Tertiary Structure of protein

5.4.2 Quaternary Structure

- A great majority of the proteins are composed of single polypeptide chains. Some proteins, consist of two or more polypeptide which may be identical or dissimilar. The quaternary structure refers to how these protein subunits interact with each other and organize themselves to form a larger combined protein complex. The final shape of the protein complex is once again stabilized by various interactions, such as hydrogen-bonding, disulfide-bridges and salt bridges.
- The arrangement of these protein subunits in three-dimensional complexes constitutes the quaternary structure. Hemoglobin, which serves as an oxygen carrier in blood, has a quaternary structure of four subunits.

- While considering these higher levels of structure, it is useful to classify proteins into two major groups. Fibrous proteins, having polypeptide chains arranged in long strands or sheets, and globular proteins, having polypeptide chains folded into a spherical or globular shape.
- Fibrous proteins usually consist largely of a single type of secondary structure, globular proteins often contain several types of secondary structure. The two groups differ functionally in that the structures that provide support, shape, and external protection to vertebrates are made of fibrous proteins, whereas most enzymes and regulatory proteins are globular proteins.

- Certain fibrous proteins played a key role in the development of our modern understanding of protein structure and provide particularly clear examples of the relationship between structure and function.

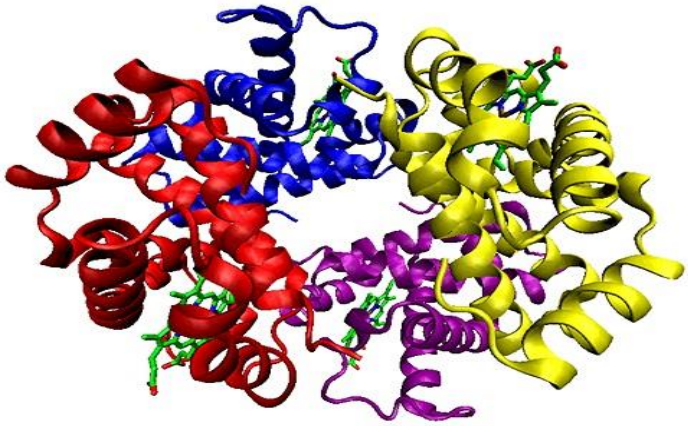


Figure 8 - Quaternary structure: A ribbon representation

5.5 CLASSIFICATION OF PROTEINS

5.5.1 Simple proteins

Albumins:

- Soluble in water, coagulated by heat and precipitated at high salt concentrations. Examples – Serum albumin, egg albumin, lactalbumin (Milk), leucosin (wheat), legumelin (soyabeans).

Globulins:

- Insoluble in water, soluble in dilute salt solutions and precipitated by half saturated salt solutions.

  Examples – Serum globulin, vitellin (egg yolk), tuberin (potato), myosinogen (muscle), legumin (peas).

Glutelins:

- Insoluble in water but soluble in dilute acids and alkalis. Mostly found in plants.

  Examples – Glutenin (wheat), oryzenin (rice).

Prolamines:

- Insoluble in water and absolute alcohol but soluble in 70 to 80 per cent alcohol.

  Examples – Gliadin (wheat), zein (maize).

Protamines:

- Basic proteins of low molecular weight. Soluble in water, dilute acids and alkalis, j Not coagulable by heat.

  Examples – Salmine (salmon sperm).

Histones:

- Soluble in water and insoluble in very dilute ammonium hydroxide.

  Examples – Globin of hemoglobin and thymus histones.

Scleroproteins:

- Insoluble in water, dilute acids and alkalis.

  Examples – Keratin (hair, horn, nail, hoof and feathers), collagen (bone, skin), elastin (ligament).

5.5.2 Conjugated Proteins

Define Conjugated Proteins

Nucleoproteins:

- Composed of simple basic proteins (pro-tamines or histones) with nucleic acids, I found in nuclei. Soluble in water.

  Examples – Nucleoprotamines and nucleohistones.

Lipoproteins:

- Combination of proteins with lipids, such ‘as fatty acids, cholesterol and 1 phospholipid etc.

  Examples – Lipoproteins of egg-yolk, milk and cell membranes, lipoproteins of blood.

Glycoproteins:

- Combination of proteins with carbohydrate (mucopolysaccharides).
  Examples – Mucin (saliva), ovomucoid (egg white), osseomucoid (bone), tendomucoid (tendon).

Phosphoproteins:

- Contain phosphorus radical as a prosthetic group.
  Examples – Caseinogen (milk), ovovitellin (egg yolk).

Metalloproteins:

- Contain metal ions as their prosthetic groups. The metal ions generally are Fe, I Co. Mg, Mn, Zn, Cu etc.
  Examples – Siderophilin (Fe), ceruloplasmin (Cu).

Chromoproteins:

- Contain porphyrin (with a metal ion) as their prosthetic groups.
  Examples – Haemoglobin, myoglobin, catalase, peroxidase, cytochromes.

Flavoproteins:

- Contain riboflavin as their prosthetic groups.
  Examples – Flavoproteins of liver and kidney.

5.5.3 Derived Protein Definition

5.5.3.1 Primary derivatives

Proteans:

- Derived in the early stage of protein hydrolysis by dilute acids, enzymes or alkalis.
  Examples – Fibrin from fibrinogen.

Metaproteins:

- Derived in the later stage of protein hydrolysis by slightly stronger acids and alkalis.
  Examples – Acid and alkali metaproteins.

Coagulated:

- They are denatured proteins formed by the action of heat. X-rays, ultraviolet rays etc.
  Examples – Cooked proteins, coagulated albumins.

Secondary derivatives

Proteoses:

Formed by the action of pepsin or trypsin. Precipitated by a saturated solution of ammonium sulphate, incoagulable by heat.

Examples – Albumose from albumin, globulose from globulin.

Peptones:

A further stage of cleavage than the proteoses. Soluble in water, incoagu-lable by heat and not precipitated by saturated ammonium sulphate solutions.

Peptides:

Compounds containing two or more amino acids. They may be di-, tri-, and polypeptides.

Examples – Glycyl-alanine, leucyl-glutamic acid.

5.6 BIOLOGICAL FUNCTIONS OF PROTEINS AND SOME REPRESENTATIVE

EXAMPLES: All the molecules encountered in living organisms, proteins have the most diverse functions, as given below:

Catalysis - Catalytic proteins enzymes accelerate thousands of biochemical reactions in several metabolic processes such as digestion, energy capture, and biosynthesis. These molecules have remarkable properties. For example, enzymes can increase reaction rates in order of 10^6 and 10^{12}. They can perform this feat under mild conditions of pH and temperature because they can induce or stabilize strained reaction intermediates. For example, ribulose bisphosphate carboxylase is an important enzyme in photosynthesis, and the protein complex nitrogenase is responsible for nitrogen fixation.

Structure- Structural proteins often have very specialized properties. For example, collagen (the major components of connective tissues) and fibroin (silkworm protein) have significant mechanical strength. Elastin, the rubber like protein found in elastic fibers, is found in blood vessels and skin.

Movement - Proteins are involved in all cell movements. Actin, tubulin, and other proteins comprise the cytoskeleton. Cytoskeletal proteins are active in cell division, endocytosis, exocytosis, and the ameboid movement of white blood cells.

Defense - A wide variety of proteins are protective. In vertebrates, keratin, a protein found in skin cells, aids in protecting the organism against mechanical and chemical injury. The blood-clotting proteins fibrinogen and thrombin prevent blood loss when blood vessels are damaged. The immunoglobulins (or antibodies) are produced by lymphocytes when foreign organisms such as bacteria invade an organism. Binding of antibodies to an invading organism is the first step in its destruction.

Regulation - Binding of a hormone molecule or a growth factor to cognate receptors on its target cell changes cellular function. For example, insulin and glucagon are peptide hormones that regulate blood glucose levels. Growth hormone stimulates cell growth and division. Growth factors are polypeptides that control animal cell division and differentiation. Examples include platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

Transport- Many proteins act as carriers of molecules or ions across membranes or between cells. Examples of membrane transport proteins include the enzyme Na-K ATPase and the glucose transporter. Other transport proteins include O_2 hemoglobin, which carries O2 to the tissues from the lungs, and the lipoproteins LDL and HDL, which transport water-insoluble lipids in the blood from the liver. Transferrin and ceruloplasmin are serum proteins that transport iron and copper, respectively.

Storage - Certain proteins serve as a reservoir of essential nutrients. For example, ovalbumin in bird eggs and casein in mammalian milk are rich sources of organic nitrogen during development. Plant proteins such as zein perform a similar role in the germinating seeds.

Stress response - The capacity of living organisms to survive a variety of abiotic stresses is mediated by certain proteins. Examples include cytochrome P450, a diverse group of enzymes found in animals and plants that usually convert a variety of toxic organic contaminants into less toxic derivatives, and metallothionein, a cysteine-rich intracellular protein found in virtually all mammalian cells that bind to and sequesters toxic metals such as cadmium, mercury, and silver. Excessively high temperatures and other stresses result in the synthesis of a class of proteins called the heat shock proteins (hsps) that promote the correct refolding of damaged proteins. If such proteins are severely damaged, hsps promote their degradation. Cells are protected from radiation by DNA repair enzymes.

5.7 SUMMARY

- Proteins are the workhorses of biochemistry, participating in essentially all cellular processes. Protein structure can be described at four levels.
- The primary structure refers to the amino acid sequence.
- The secondary structure refers to the conformation adopted by local regions of the polypeptide chain.
- Tertiary structure describes the overall folding of the polypeptide chain.
- Finally, quaternary structure refers to the specific association of multiple polypeptide chains to form multisubunit complexes.

5.8 GLOSSARY

Alpha helix: a coiled conformation, resembling a righthanded spiral staircase, for a stretch of consecutive amino acids in which the backbone –N–H group of every residue n donates a hydrogen bond to the C=O group of every residue n+4

Amide bond: a chemical bond formed when a carboxylic acid condenses with an amino group with the expulsion of a water molecule.

Antiparallel beta sheet: a beta sheet, often formed from contiguous regions of the polypeptide chain, in which each strand runs in the opposite direction from its immediate neighbors.

Beta sheet: a secondary structure element formed by backbone hydrogen bonding between segments of the extended polypeptide chain.

Beta-turn: a tight turn that reverses the direction of the polypeptide chain, stabilized by one or more backbone hydrogen bonds. Changes in chain direction can also occur by loops, which are peptide chain segments with no regular conformations.

Helix: A spiral structure with a repeating pattern.

Parallel beta sheet: a beta sheet, formed from noncontiguous regions of the polypeptide chain, in which every strand runs in the same direction.

Peptide bond: another name for amide bond, a chemical bond formed when a carboxylic acid condenses with an amino group with the expulsion of a water molecule. The term peptide bond is used only when both groups come from amino acids.

Polypeptide: a polymer of amino acids joined together by peptide bonds.

primary structure: the amino-acid sequence of a polypeptide chain.

Ramachandran plot: a two-dimensional plot of the values of the backbone torsion angles phi and psi, with allowed regions indicated for conformations where there is no steric interference. Ramachandran plots are used as a diagnosis for accurate structures: when the phi and psi torsion angles of an experimentally determined protein, structure are plotted on such a diagram, the observed values should fall predominantly in the allowed regions.

Secondary structure: folded segments of a polypeptide chain with repeating, characteristic phi, psi backbone torsion angles, that are stabilized by a regular pattern of hydrogen bonds between the peptide –N–H and C=O groups of different residues.

Side chain: a chemical group in a polymer that protrudes from the repeating backbone. In proteins, the side chain, which is bonded to the alpha carbon of the backbone, gives each of the 20 amino acids its particular chemical identity. Glycine has no side chain, and the end of the side chain of proline is fused to the nitrogen of the backbone, creating a closed ring.

Tertiary structure: the folded conformation of a protein, formed by the condensation of the various secondary elements, stabilized by a large number of weak interactions.

Van der Waals interaction: a weak attractive force between two atoms or groups of atoms, arising from the fluctuations in electron distribution around the nuclei. Van der Waals forces are stronger between less electronegative atoms such as those found in hydrophobic group

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5.10 TERMINAL QUESTIONS AND ANSWER

Ques. 1 - What is the importance of proteins for living organisms?

Ans. - Proteins play a fundamental role in nearly all biological processes. Due to their diversity, they can take on many different configurations and can play varied roles in cells and tissues. Some protein functions are worth noting: they have a structural function (cell membrane proteins, cytoskeleton proteins, connective tissue proteins), an enzymatic function (enzymes are proteins), an energy storage function (proteins can be broken down into acetyl-CoA to "feed" the Krebs cycle), an osmotic regulation function (albumin), a transportation function (membrane channels, respiratory pigments), an immune protection function (antibodies), a movement function (contractile proteins), an endocrine integration function (hormones) and a informative function (membrane receptors, intracellular signalers). There are also many proteins whose biological functions are not yet known.

Ques. 2 -What unit are proteins composed of?

Ans. - The units that makeup proteins are amino acids.

Ques. 3-What is an oligopeptide? How is it different from a polypeptide?

Ans. - The peptide molecule is the molecule formed by the bonding of amino acids through the peptide bond. An oligopeptide is a peptide composed of few amino acids (oligo = few). Polypeptides are peptides that contain many amino acids (poly = many), in general, more than 50.

Ques. 4 - How can the binding of two amino acids into a peptide formation be described?

Ans. - A peptide is formed when a carbon atom from the carboxyl group of one amino acid is connected to the nitrogen atom of the amine group of another amino acid. By the formation of this bond, the hydroxyl group of the carboxyl group and one hydrogen atom of the amine are lost resulting in the release of one water molecule.

Ques. 5- What is the bond between two amino acids called?

Ans. - The chemical bond between two amino acids is called a peptide bond.

Ques. 6-What is the difference between alpha-helix and beta-sheet protein conformations?

Ans. - Alpha-helix and beta-sheet conformations are the two main types of secondary structures of a protein molecule. Depending on the primary protein structure, its secondary structure can be of one type or the other. In the alpha-helix structure, the polypeptide curls longitudinally through the action of hydrogen bonds, forming a spiral or helix. In the beta-

sheet conformation, the protein is more extended and the hydrogen bonds form a zig-zag-shaped protein structure called a beta-strand. Many beta-strands put together to make a beta-sheet.

Ques. 7-What is the quaternary structure of a protein? Do all proteins have a quaternary structure?

Ans. - The quaternary structure of a protein is the spatial conformation caused by interactions between the polypeptide chains that form the protein. Only proteins made up of two or more polypeptide chains have a quaternary structure. Insulin (two chains), hemoglobin (four chains) and immunoglobulins (antibodies, four chains) are some of the examples of protein with a quaternary structure.

5.10.1 Multiple Choice Questions1. The repeating units of proteins are

A. glucose units
B. amino acids
C. fatty acids
D. peptides

2. Amino acids are joined by

peptide bond
hydrogen bond
ionic bond
glycosidic bond

3. The primary structure of protein represents

A. Linear sequence of amino acids joined by peptide bond
B. the 3-dimensional structure of the protein
C. the helical structure of the protein
D. subunit structure of the protein

4. Peptide bond is

A. rigid with partial double bond character
B. planar, covalent
C. covalent
D. all of the above

5. Enzymes are

A. proteins
B. carbohydrates
C. nucleic acids
D. DNA molecule

6. A dipeptide has

A. 2 amino acids and 1 peptide bond
B. 2 amino acids and 2 peptide bonds
C. 2 amino acids and 3 peptide bonds
D. 2 amino acids and 4 peptide bonds

7. The most common secondary structure is

A. α-helix
B. β-pleated sheet
C. β-pleated sheet parallel
D. β-pleated sheet nonparallel

8. Myoglobin is a

A. protein with a primary structure
B. protein with a secondary structure
C. protein with a tertiary structure
D. protein with quaternary structure

9. Fibrous protein such as silk fibroin consists of polypeptide chains arranged in

α-helix
β-pleated sheet
β-helix
none of these

10. Tertiary structure is maintained by

A. peptide bond
B. hydrogen bond

C. di-sulphide bond
D. all of the above

11. Haemoglobin has

A. primary structure
B. secondary structure
C. tertiary structure
D. quaternary structure

12. Disulphide bonds are formed between

A. cysteine residues that are close together
B. cystine residues that are close together
C. proline residues that are close together
D. histidine residues that are close together

Answers -: 1-B 2-A 3-A 4-D 5-A 6-A 7-A 8-C 9-B 10-D 11-D 12-A

UNIT 6 LIPIDS

Contents

6.1 INTRODUCTION

- Lipids (fats and oils) are esters formed by condensation of trihydroxy alcohol glycerol and fatty acids.
- Certain lipids include functional groups derived from phosphoric acid, carbohydrates, amino alcohols, and steroid molecules such as cholesterol.
- Lipids are a class of biological molecules defined by low solubility in water and high solubility in nonpolar solvents such as ether or benzene. As molecules that are largely hydrocarbon in nature, lipids represent highly reduced forms of carbon and, upon oxidation in metabolism, yield large amounts of energy. Lipids are thus the molecules of choice for metabolic energy storage.
- Lipids found in biological systems are either hydrophobic (containing only nonpolar groups) or amphipathic, which means they possess both polar and nonpolar groups. The hydrophobic nature of lipid molecules allows membranes to act as effective barriers to more polar molecules.
- Some lipids although present in relatively small quantities play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchor for proteins chaperones. They also help membrane protein fold, act as emulsifying agents in the digestive tract, also act as hormones and intracellular messengers.
- Lack of solubility of lipids in water is an important property because our body chemistry is firmly based on water. The body also needs compounds that are insoluble for many purposes like separation of compartments containing aqueous solutions form each other.
- Lipids are insoluble in water because the polar groups they contain are much smaller than their alkane like (nonpolar) portion. These nonpolar portions provide water hydrophobic property to lipids.
- Although the term lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways both to break down and to synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

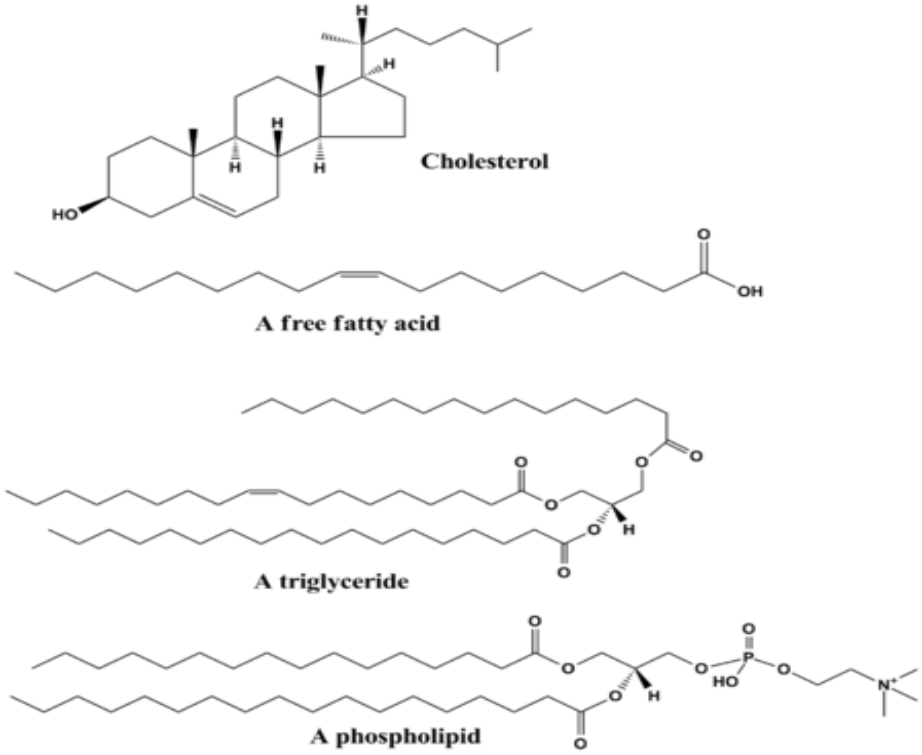


Figure 1 - Structures of some common lipids

6.2 PROPERTIES OF LIPIDS

A natural fat consists of a mixture of several types of triacylglycerols (TAG). Natural fats have colour, odour and taste.

6.2.1 Melting point

- Triacylglycerols of higher saturated fatty acids have high melting points. Fats rich in such saturated triacylglycerols are solid at room temperature ex- lard, butter, and margarine. Triacylglycerols of unsaturated fatty acids have lower melting points because the bend at each cis double bond of their fatty acid chain prevents the close packing of their fatty acid tails and consequently lowers the interchain hydrophobic interaction.

6.2.2 Solubility

- All fats are soluble in nonpolar organic fat solvents such as chloroform, carbon tetrachloride, benzene and ether, because of their nonpolar hydrocarbon fatty acid tails. They are also soluble in hot ethanol.

6.2.3 Saponification

- Hydrolysis of triacylglycerols with KOH or NaOH is called saponification or soap formation. These soaps are the household soaps. Sodium soaps are hard and potassium soaps are soft. Detergents have anacidic group like sulphuric acid attached to the fatty acids.

6.2.4 Saponification Number

- It is the number of milligrams of KOH required to saponify the free and combined fat in 1 gram of a given fat. A high saponification number indicates that the fat is made up of low molecular weight fatty acids and vice versa.

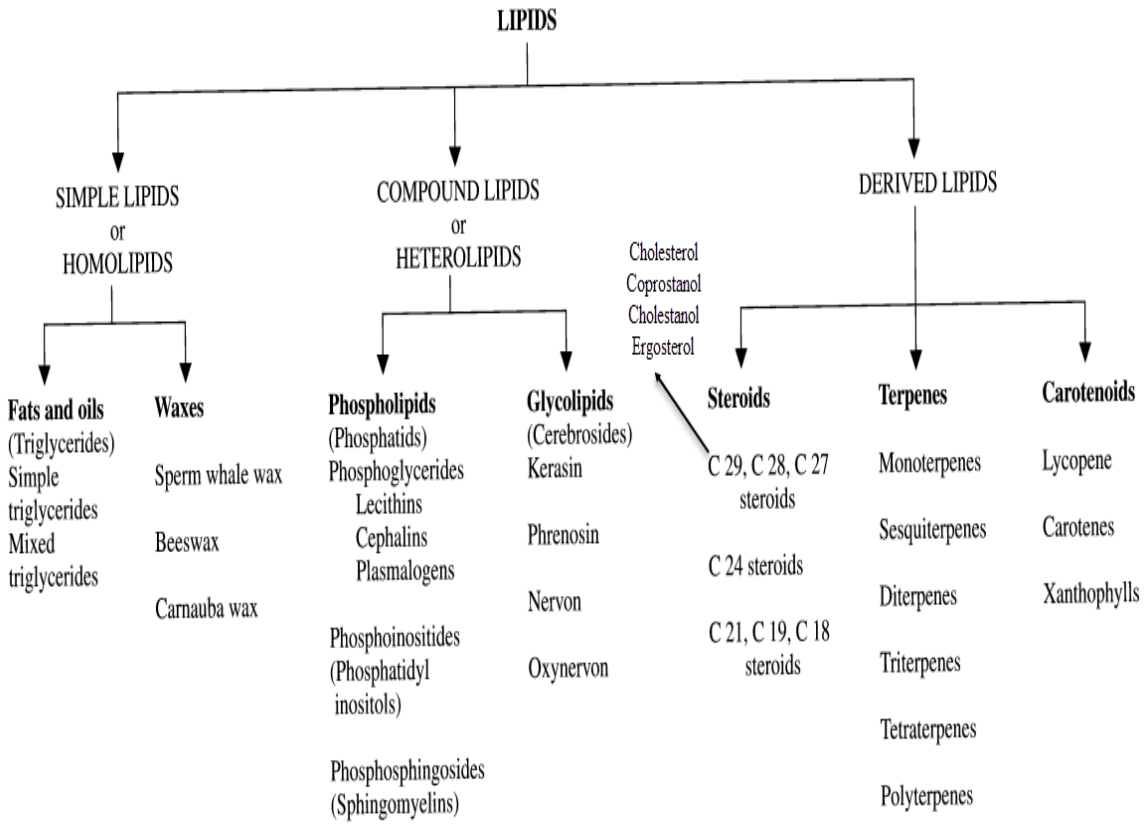
6.2.5 Iodine Number

- It is the grams of iodine required to saturate 100 grams of fat. It is an indication of unsaturation.

6.2.6 Rancidity

- Fats with enzymes like lipase undergo partial hydrolysis and oxidation of unsaturated fatty acids at the double bonds. This is even brought about by the atmospheric moisture and temperature. Release of hydrogen peroxide giving a bad odour and taste to the fat. This fat is said to be rancid and the process is known as rancidity. Rancidity can be prevented by antioxidants like vitamin E, vitamin C, phenols, hydroquinone’s, etc.

6.3 CLASSIFICATION OF LIPIDS

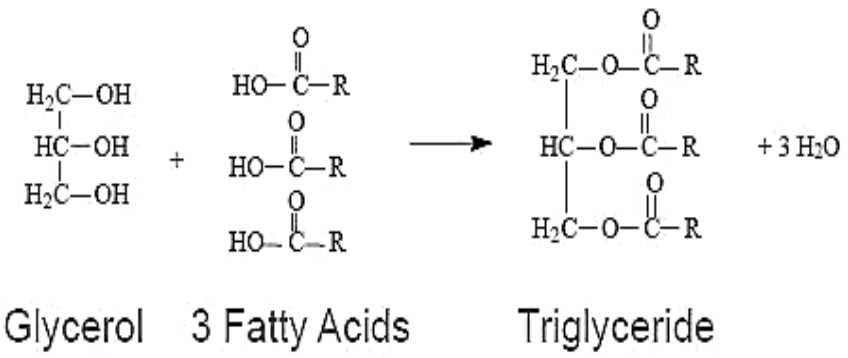


6.3.1 Simple Lipids or Homolipids [mention table in running text]

Simple lipids are esters of a fatty acid linked with various alcohols.

6.3.1.1 Fats and oils (triglycerides, triacylglycerols)

- These esters of fatty acid have glycerol, a trihydroxy alcohol. Fat is solid at room temperature, while oil is in liquid form.
- Triglycerides are abundant and constitute about 98 percent of all dietary lipids. The rest consists of cholesterol, its esters and phospholipids. Unlike carbohydrates, which can be stored only for a short time in the body, triglycerides are stored in the body in large amounts as body fat, which can last for years.
- An average man weighing about 70 kg, has at least 10 to 20 percent of his body weight in lipid, most of which is triacylglycerol. This is found in adipose (fat) tissue, as well as all other organs of the body. Body fat is a reservoir of chemical energy.



Source - http://www.cmecorner.com

Figure 2 - Triglyceride

6.3.1.2 Waxes

. Waxes are long-chain saturated and unsaturated fatty acid esters with monohydroxy alcohols, which have high molecular weight. The fatty acids range in between C_{14} and C_{36} and the alcohols range from C_{16} to C_{36}.

. Waxes are produced naturally by skin glands as a protection, to keep it lubricated, pliable, and water-proof. Wax also covers hair, feathers, and wool.

- The term `wax' originates in the Old English word weax, meaning “the material of the honeycomb”, reminding of beeswax, the honeycomb is made of.
- Bees wax is secreted by honey bee to form honey comb. The chief ingredient is myricyl palmitate.

$$CH_3(CH_2)_{14}COO\text{-}CH_2(CH_2)_{28}CH_3$$

Myricyl palmitate

- Waxes also serve as the chief storage form of fuel in planktons. Since marine organisms (whale, herring, salmon) consume planktons in large quantities, waxes act as major food and storage lipids in them. The sperm whale wax is rich in cetyl palmitate.

$$CH_3(CH_2)_{14}COO\text{-}CH_2(CH_2)_{14}CH_3$$

Cetyl palmitate

- Chinese wax is the secretion of an insect.

- Carnauba wax is found on the leaves of carnauba palm of Brazil. This is the hardest known wax which consists mainly of fatty acids esterified with tetracosanol.

$$CH_3(CH_2)_{32}CH_2OH$$

Tetracosanol

- Lanoline is also known as ‘wool wax’. It is obtained from wool and is used in making ointment.

6.3.2 Compound Lipids or Heterolipids

Heterolipids are fatty acid esters with alcohol and additional groups.

6.3.2.1 Phospholipids (phosphatids)

- Phospholipids contain fatty acids, glycerol, nitrogen bases, phosphoric acid, and other substituents.
- They are most abundant in cell membranes and serve as structural components. They are not stored in large quantities. As their name implies, phospholipids contain phosphorus in the form of phosphoric acid groups.
- Their molecular structure is polar, consisting of one hydrophilic head group and two hydrophobic tails.
- Thus, phospholipids are amphipathic, whereas the storage lipids (triglycerides and waxes) are not. In phospholipids, two of the OH groups in glycerol are linked to fatty acids while the third OH group is linked to phosphoric acid. The phosphate is further linked to one of a variety of small polar head groups (alcohols).
- Phospholipids are classified into phosphoglycerides, phosphoinositides and phosphosphingosides.

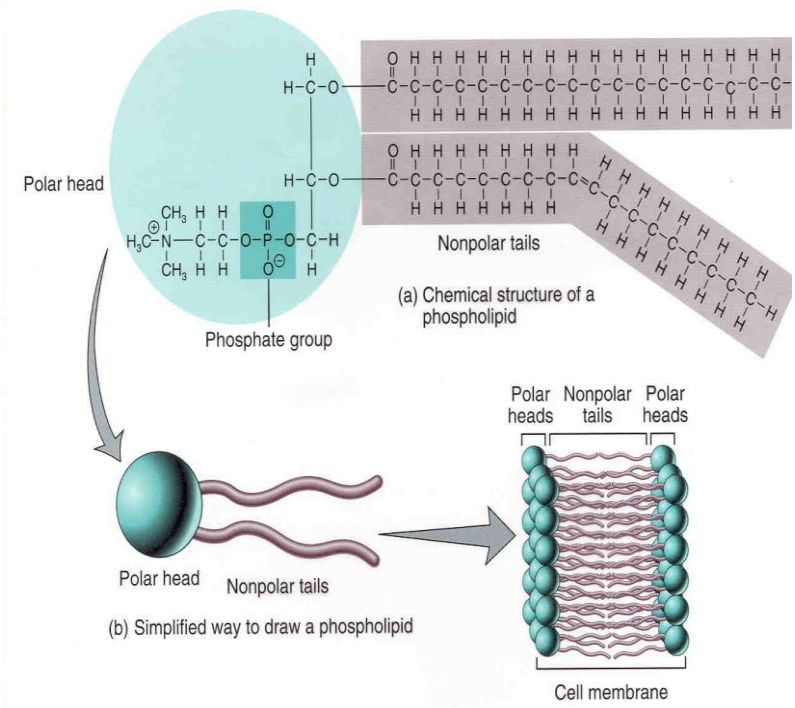
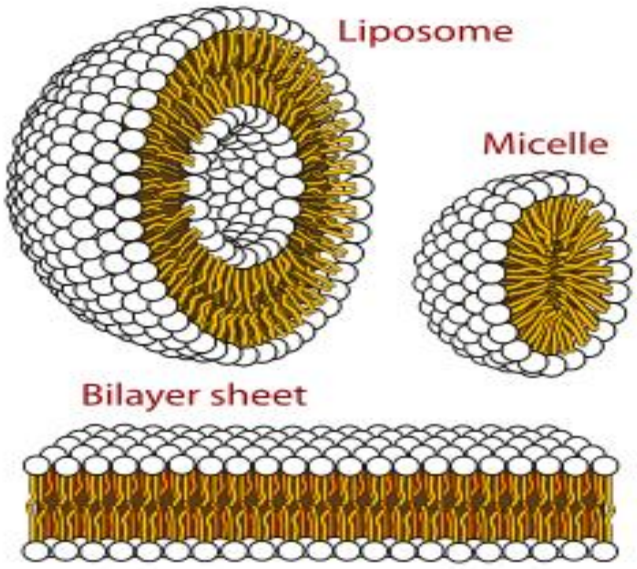


Figure 3 - Molecular structure of Phospholipids

6.3.2.1.1 Phospholipids are the main lipid constituents of membranes

- Phospholipids may be regarded as derivatives of phosphatidic acid, in which the phosphate is esterified with the OH of alcohol. Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols as well as phosphoglycerols but is not found in large quantity in tissues. Amphipathic lipids self-orient at oil:water interfaces.
- They form membranes, micelles, liposomes, and emulsions. In general, lipids are insoluble in water since they contain a predominance of nonpolar (hydrocarbon) groups. However, fatty acids, phospholipids, sphingolipids, bile salts, and, to a lesser extent, cholesterol contain polar groups. Therefore, part of the molecule is hydrophobic, or water-insoluble; and part is hydrophilic, or water-soluble. Such molecules are described as amphipathic. They become oriented at oil:water interfaces with the polar group in the water phase and the nonpolar group in the oil phase.



Source- www.en.wikipedia.org/wiki/Lipid

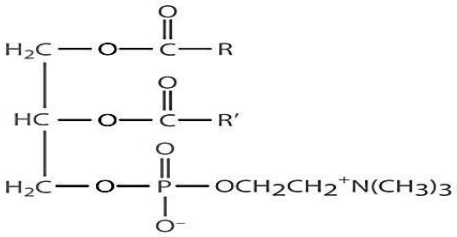
Figure 4 - A spherical Liposome, A Micelle, and A Lipid bilayer

- A bilayer of such amphipathic lipids has been regarded as a basic structure in biologic membranes. When a critical concentration of these lipids is present in an aqueous medium, they form micelles. Aggregations of bile salt into micelles and liposomes and the formation of mixed micelles with the products of fat digestion are important in facilitating absorption of lipids from the intestine.
- Liposomes are formed by sonicating an amphipathic lipid in an aqueous medium. They consist of spheres of lipid bilayers that enclose part of the aqueous medium. They are of potential clinical use – particularly when combined with tissue-specific antibodies – as carriers of drugs in the circulation, targeted to specific organs, eg, in cancer therapy. In addition, they are being used for gene transfer into vascular cells and as carriers for topical and transdermal.

6.3.2.2 Phosphoglycerides

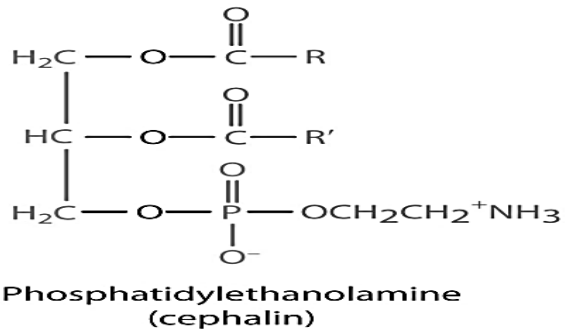
- Lecithins (phosphatidylcholines) (likithos = yolk) are widely distributed in nature. Various oil seeds like soybean and the yeasts are important sources from the plant world. In animals, the glandular and nervous tissues are rich in these lipids. Lecithins are required for the normal transport and utilization of as other lipids especially, in the liver of animals. In their absence, accumulation of lipids occurs in the liver to as much as 30% against a normal value of 3-4%, giving rise to a condition called “fatty

liver". This fatty infiltration may lead to fibrotic changes, characteristic of the liver disease cirrhosis.



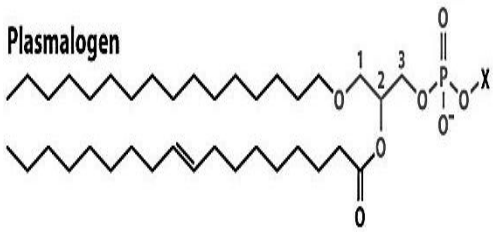
Source - MikeBlaber.org

- Cephalins (kephalus = head) are closely associated with lecithins in animal tissues. These have also been identified from soybean oil. These are similar in structure to the lecithins except that the choline is replaced by either ethanolamine or serine. Serine is the biochemical precursor of ethanolamine.



Source - MikeBlaber.org

- Plasmalogens (Phosphoglyceracetals) constitute about 10% of the phospholipids of the brain and muscle. These are apparently not found in significant quantities in plant tissues. Structurally, these resemble lecithins and cephalins but have one of the fatty acids replaced by unsaturated ether. Since the nitrogen base can be choline, ethanolamine or serine, three types of plasmalogens are accordingly distinguished: phosphatidal choline, phosphatidalethanolamine and phosphatidal serine.



Source - MikeBlaber.org

6.3.2.3 Phosphoinositides (Phosphatidyl inositols)

- Phosphoinositides have been found to occur in phospholipids of brain tissue and of soybeans and are of considerable importance because of their role in transport processes in cells. These are phospholipids where a cyclic hexahydroxy alcohol called inositol replaces the base.
- The inositol is present as the stereoisomer, myo-inositol. On hydrolysis, the phosphoinositides yield 1 mole of glycerol, two moles of fatty acid, 1 mole of inositol and 1, 2, or 3 moles of phosphoric acid. Accordingly, mono-, di- or triphosphoinositides are found. Phosphoinositides are also known as glycolipids, as they contain carbohydrate residues.

6.3.2.4 Phosphosphingosides (Sphingomyelins)

- These compounds are commonly found in nerve tissue specially, in the myelin sheath of the nerve (hence their name, sphingomyelins). They consist of phosphocholine & ceramide. In a syndrome called Niemann−Pick disease, the sphingomyelins are stored in the brain in large quantities. These differ from other phospholipids in lacking glycerol as well as the presence of another nitrogenous base sphingosine or a closely related dihydrosphingosine, besides choline, in place of glycerol. Sphingomyelins are electrically charged molecules and contain phosphocholine as their polar head groups.

6.3.2.5 Glycolipids (Cerebrosides or Glycosphingosides)

Glycolipids are fatty acids with carbohydrates and nitrogen but without phosphoric acid.

These cerebrosides are important constituents of the brain and other tissues. They consist of at least one sugar unit, so they are also called glycosphingosides.

They are like phospholipids because they have a hydrophobic region, with a polar region and two long hydrocarbon tails. Like phospholipids, glycolipids form lipid bilayers and form the structure of cellular membranes.

Glycolipids also include compounds such as sulfolipids, gangliosides, and sulfatids.

Sulfolipids are glycolipids that contain sulfur and are widely distributed in plants. It is localized in the chloroplasts but is also found in the chromatophores of photosynthetic bacteria. As the sulfur in this compound is present as a sulfonic group in a hexose, this may be included under a class of compounds called sulfolipids.

In 1955, Klenk isolated a new type of glycolipid from brain tissue and named it as ganglioside. These are found in significant concentrations in ganglion cells of nervous tissue (hence so named) and also in most parenchymatous tissues like spleen and erythrocytes. They make up about 6% of the membrane lipids in the gray matter of the brain. They are also found in lesser amounts in the membranes of nonneural tissues. Gangliosides are thought to act as receptors for toxic agents like the pathogens such as vibrio cholerae influenza virus and tetanus toxin. They are also implicated to play a role in the cell-cell interaction.

A sulfate ester analogue of phrenosin is abundant in the white matter of brain. It is another sulfur-containing glycolipid. In this molecule sulfate is present in ester linkage at C 3 of the galactose portion of the molecule. Members of this group of cerebroside sulfuric esters have been designated as sulfatides.

6.3.3 Derived Lipids

Derived lipid is a “catch-all” group in Bloor’s classification. It includes thehydrolytic products of simple and compound lipids and also various other compounds such as steroids, terpenes, fatty acids, alcohols, fatty aldehydes, ketones etc.

6.3.3.1 Steroids

The steroids (stereos = solid) are one of the most studied classes of biological compounds and are often found in association with fat. Since they contain no fatty acids, they are no saponifiable, i.e., cannot be hydrolyzed by heating with alkali to yield soaps of their fatty acid components. Fats, on the other hand, are saponifiable and form soaps when hydrolyzed with alkali. The steroids may be separated from the fat after the latter is saponified since they occur in ‘no saponifiable residue’. All steroids may be considered as derivatives of a fused and fully saturated ring system

called cyclopentanoperhydrophenanthrene or sterane. This system consists of 3 cyclohexane rings (A, B and C) fused in nonlinear or phenanthrene manner and a terminal cyclopentane ring (D). Certain common steroids are described below.

6.3.3.1.1 C 29, C 28 and C 27 Steroids

6.3.3.1.1.2 Cholesterol (chole = bile)

Cholesterol is a well-studied lipid, because of its strong correlation with the incidence cardiovascular disease.

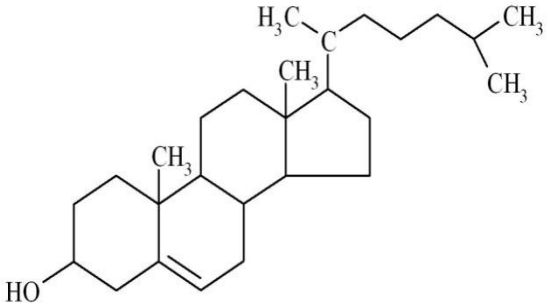


Figure 6 - cholesterol

It is an important component of cell membranes and plasma lipoproteins and is an important precursor of many biologically important substances like bile acids and steroid hormones. It is abundant in nerve tissues and is associated with gallstones.

Dietary cholesterol is found in saturated fats of animals (as butter and lard), but vegetable oils do not contain cholesterol. Only a small portion of your body cholesterol comes from the diet. Most of it is produced in the body.

Eating unsaturated fatty acids from vegetable oil helps lower blood cholesterol levels by reducing cholesterol synthesis in the body. However, eating saturated fats from animal fat elevates blood cholesterol and triglycerides and reduce our good to bad cholesterol ratio.

There are 2 types of cholesterol, the low-density lipoprotein cholesterol. (LDL-C) and the high-density lipoprotein cholesterol (HDL-C).

6.3.3.1.2 C 24 Steroids or Bile Acids

The bile acids are the important end products of cholesterol metabolism in higher plants. About 20 natural bile acids have been characterized. All these are derived from a C 24 parent steroid, cholanic acid and resemble coprostanol in having rings A

and B in cis form. The most abundant bile acids in human bile are cholic acid (25-60% of the total bile acids), chenodeoxycholic acid (30-50%) and deoxycholic acid (5-25%). Various bile acids differ from each other in the number and position of OH groups which are all in a configuration. In the bile acids, the number of OH group(s) may be 1, 2, or 3 and the position of OH group(s) may be any of the following: 3, 6, 7, 11, 12 and 23. The side chain is usually made up of 5 carbon atoms and bears the carboxyl group.

6.3.3.1.3 C 21, C 19 and C 18 Steroids

Several important poisons are based on the steroid structure.

Digitoxin or digitalin is present in the foxglove plant (*Digitalis purpurea*) and has a powerful heart stimulating action.

Another cardiac glycoside, Quabain, is isolated from the East African Ouabio tree and has the sugar, rhamnose, attached to a modified sterol nucleus. It is of interest to note that it is a powerful inhibitor of the 'sodium pump', a device which normally ensures that the cell content of potassium is higher and that of sodium is lower than in the circumambient fluid.

6.3.3.1.3.1 Steroid hormone

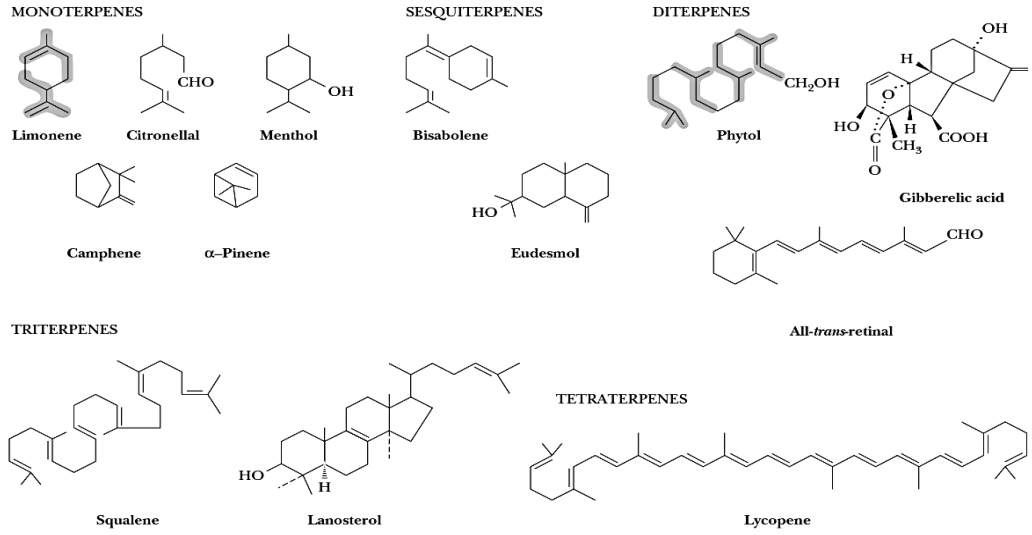
- A steroid hormone is a steroid that acts as a hormone. Steroid hormones can be grouped into two classes: corticosteroids (typically made in the adrenal cortex, hence cortico-) and sex steroids (typically made in the gonads or placenta).
- Those two classes are five types according to the receptors to which they bind: glucocorticoids, mineralocorticoids (corticosteroids), androgens, estrogens, and progestogens (sex steroids).
- Vitamin D derivatives are a sixth closely related hormone system with homologous receptors. They have some of the characteristics of true steroids as receptor ligands.

6.3.4 Terpenes

- Among the nonsaponifiable lipids found in plants are many hydrocarbons known as terpenes (from turpentine). In general, these hydrocarbons and their oxygenated derivatives have lesser than 40 carbon atoms. The simplest terpenes are called

monoterpenes and conform to the formula $C_{10}H_{16}$ (equivalent to 2 isoprene units), those with the formula $C_{15}H_{24}$ are called as sesquiterpenes, with $C_{20}H_{32}$ as diterpenes and with $C_{30}H_{48}$ as triterpenes. Terpenes with 40 carbon atoms (tetraterpenes) include compounds called carotenoids.

- O. Wallach (1910 Nobel Laureate in Chemistry) was the first to point out, in 1887, that nearly all the terpenoids are made of varying number of repetitive units (C_5H_8), called isoprene units. His finding later came to be known isoprene rule. Structurally, isoprene is a 5-carbon diene.



Source – garret and grasha

Figure 7 - Many monoterpenes are readily recognized by their characteristic flavors or odors. The diterpenes, which are C20 terpenes, include retinal (the essential light-absorbing pigment in rhodopsin, the photoreceptor protein of the eye), phytol (a constituent of chlorophyll), and the gibberellins (potent plant hormones). The triterpene lanosterol is a constituent of wool fat. Lycopene is a carotenoid found in ripe fruit, especially tomatoes.

Hemiterpenes are single isoprene unit. Isoprene is considered the only hemiterpene, but oxygen-containing derivatives such as prenol and isovaleric acid are hemiterpenoids.

Monoterpenes consist of two isoprene units and have the molecular formula $C_{10}H_{16}$. Monoterpenes and monoterpenoids include geraniol, terpineol (present in lilacs),

Limonene (present in citrus fruits), myrcene (present in hops), Linalool (present in lavender) or pinene (present in pine trees). Iridoids derive from monoterpenes.

Sesquiterpenes consist of three isoprene units and have the molecular formula $C_{15}H_{24}$. Examples of sesquiterpenes and sesquiterpenoids include humulene, farnesenes, farnesol. (The sesqui- prefix means one and a half.)

Diterpenes are made of four isoprene units and have the molecular formula $C_{20}H_{32}$. They obtain from geranylgeranyl pyrophosphate. Diterpenes and diterpenoids are cafestol, kahweol, cembrene and taxadiene (precursor of taxol). Diterpenes also form the basis for biologically important compounds such as retinol, retinal, and phytol.

Sesterterpenes, terpenes having 25 carbons and 5 isoprene units, are unusual relative to the other sizes. (The sester- prefix means half to three, i.e. two and a half.) Geranylfarnesol is an example of a sesterterpenoid.

Triterpenes consist of six isoprene units and have the molecular formula $C_{30}H_{48}$. The linear triterpene squalene, the mainelement of shark liver oil, is derived from the reductive pairing of two molecules of farnesyl pyrophosphate. Squalene is then processed biosynthetically to produce any lanosterol or cycloartenol, the structural precursors to all the steroids.**Sesquarterpenes** are collected of 7 isoprene units and have the molecular formula $C_{35}H_{56}$. Sesquarterpenes are naturally microbial in their origin. Sesquarterpenoids are ferrugicadiol and tetraprenylcurcumene.

Tetraterpenes contain eight isoprene units and have the molecular formula $C_{40}H_{64}$. Biologically important tetraterpenoids include the acyclic lycopene, the monocyclic gamma-carotene, and the bicyclic alpha- and beta-carotene.

6.4FUNCTIONS OF LIPIDS

Lipids are stored in adipose tissue in form of triglycerides and are one of the main sources of energy. They are the best energy source for humans and provide lots of calories. If carbohydrates, on average, give 4 kcal/g, as proteins, lipids, gives on average, 9 kcal/g.

Lipids are utilized as “bricks” for building of biological membranes (phospholipids, cholesterol and glycolipids together with proteins), and contributes to structure of cell wall/ cell membrane that separates intracellular environment from extracellular one. Inside the cell, lipids bilayer forms component of organelles like mitochondria,

Golgi apparatus or nucleus. Moreover, they are also vital for the maintenance, physiochemical properties, and repair of cell membranes themselves.

Many hormones are lipids. Steroid hormones, like estrogens, androgens, and cortisol, are produced from cholesterol, prostaglandins, prostacyclin, leukotrienes, thromboxanes, and other compounds from omega-3 and omega-6 polyunsaturated fatty acids with 20 carbon atoms.

Many lipids (diacylglycerol, ceramides, sphingosine and platelet-activating factor) act as regulators of intracellular processes.

Fat deposits are classified as structural fat, that functions to protecto organs against traumatic injuries and shock, fat pads on the palms and buttocks protect the bones from mechanical pressure.

In several animals, some lipids are secreted into external environment and act as pheromones that attract or repel other organisms.

They affect the texture and flavor of food and so its palatability.

Food manufacturers use fat for its textural properties, e.g., in baked goods fat increase the tenderness of the product. Chefs know that fat addiction adds to the palatability of meal and increase satiety after a meal.

6.5 SUMMARY

- Almost all fatty acids, the hydrocarbon components of many lipids, have an even number of carbon atoms (usually 12 to 24); they are either saturated or unsaturated, with double bonds almost always in the cis configuration.
- Triacylglycerols contain three fatty acid molecules esterified to the three hydroxyl groups of glycerol. Simple triacylglycerols contain only one type of fatty acid; mixed triacylglycerols, two or three types. Triacylglycerols are primarily storage fats; they are present in many foods.
- Lipids are water-insoluble cellular components.

6.6 GLOSSARY

Bilayer: A double layer of lipid molecules with the hydrophilic ends oriented outward, in contact with water, and the hydrophobic parts oriented inward.

cephalins (kephalins): Compounds derived from glycerol in which a primary and the secondary hydroxyl groups are esterified with long-chain fatty acids, and the remaining

primary one with the mono(2-aminoethyl) ester of phosphoric acid, or with the monoserine ester of phosphoric acid.

fatty acids: Aliphatic monocarboxylic acids derived from or contained in esterified form in an animal or vegetable fat, oil or wax.

glycerides: Esters of glycerol (propane-1,2,3-triol) with fatty acids, widely distributed in nature. They are by long-established custom subdivided into triglycerides, 1,2- or 1,3-diglycerides, and 1- or 2-monoglycerides, according to the number and position of acyl groups (not, as one might suppose, the number of glycerol residues).

glycolipids: Naturally occurring 1,2-di-O-acylglycerols joined at oxygen 3 by a glycosidic linkage to a carbohydrate part (usually a mono-, di-, or tri-saccharide). Some substances classified as bacterial glycolipids have the sugar part acylated by one or more fatty acids and the glycerol part may be absent.

lecithin's: Choline esters of phosphatidic acids. These compounds should be named systematically.

leukotrienes: Linear C20 endogenous metabolites of arachidonic acid (icosa-5,8,11,14-tetraenoic acid) containing a terminal carboxy function and four or more double bonds (three or more of which are conjugated) as well as other functional groups. A subclass of icosanoids.

lipids: A loosely defined term for substances of biological origin that are soluble in nonpolar solvents. They consist of saponifiable lipids, such as glycerides (fats and oils) and phospholipids, as well as no saponifiable lipids, principally steroids.

Lipid bilayer: Model for the structure of the cell membrane based on the interaction between the hydrophobic regions of phospholipids.

Micelle: An aggregate of lipids in which the polar head groups face outward and the hydrophobic tails face inward; no solvent is trapped in the center.

Monolayer: A single layer of oriented lipid molecules.

phosphoglycerides: Phosphoric diesters, esters of phosphatidic acids, generally having a polar head group (OH or NH2) on the esterified alcohol which typically is 2-aminoethanol, choline, glycerol, inositol, serine.

Steroids: Compounds that are derivatives of a tetracyclic structure composed of a cyclopentane ring fused to a substituted phenanthrene nucleus.

Sulfatides: Hydrogen sulfate esters of glycosphingolipids. The related compounds are called as glycerosphingolipid derivatives.

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6.8 TERMINAL QUESTIONS AND ANSWER

Ques. 1- What are the main types of lipids?

Ans. - The main types of lipids are triglycerides (fats and oils), phospholipids, waxes and steroids.

Ques. 2- What is the composition of triglycerides?

Ans. - Triglycerides, which are fats or oils, are made up of three molecules of fatty acids bound to one molecule of glycerol.

Ques. 3-What are phospholipids?

Ans. - Phospholipids are molecules made up of one molecule of glycerol bound to two long molecules of fatty acids and to one phosphate group. Therefore, phospholipids are amphipathic molecules, meaning that they have a non-polar portion, due to the long fatty acid chains, and a polar portion, due to the phosphate group. Phospholipids are the main component of cell membranes. Sphingomyelin, that forms the myelin sheath of axons in the nervous system, is a phospholipid.

Ques. 4-What are steroids? What are some examples of steroids with a biological function?

Ans. - Steroids are lipids that consist of an angular combination of four carbon rings, three of which are made of six carbon atoms and one of which, located at the end, made of five carbon atoms in the extremity. The bond between each ring and the adjacent ring is made through by the sharing of two adjacent carbon atoms which belong to both rings.

Bile salts, cholesterol, the sex hormones estrogen, progesterone and testosterone, corticosteroids and pro-vitamin D are examples of steroids.

Ques. 5-What are hydrophobic molecules and hydrophilic molecules? Describe in relation to their polarity?

Ans. - Hydrophobic molecules are molecules with little or no propensity to dissolve in water (hydro = water, phobia = fear). Hydrophilic molecules are those that have a large propensity to dissolve in water (philia = friendship). Water is a polar substance. Remembering the rule that "equal dissolves equal", it is easy to conclude that hydrophobic substances are non-polar molecules whereas hydrophilic molecules are polar molecules.

Ques.6- How are lipids classified according to solubility?

Ans. - Fats and oils are hydrophobic molecules, meaning that they are non-polar and insoluble in water. Lipids, in general, are molecules with a large non-polar extension, making them soluble in non-polar solvents, such as benzene, ether and chloroform. Amphipathic lipids, are lipids with both a hydrophilic portion, which gives them the property of water-solubility, as well as a hydrophobic portion, which is non-polar.

Ques.7- Why do fats have thermal insulation properties?

Ans. - Triglycerides are poor heat conductors and, in addition, they form thick layers of fatty tissue when accumulated in an organism. That is why they are good thermal insulators.

In animals that live in cold climates, such as polar bears, seals and whales, Adipose tissue helps the maintenance of internal body temperature.

Ques.8- How are lipids used as an energy source by the body?

Ans. - Carbohydrates are the main energy source for aerobic cell respiration. When such substances are absent or deficient, the body can use lipid stores since fats (like proteins) can be broken down into acetyl-CoA to feed the Krebs cycle (a stage of aerobic cellular respiration).

6.8.1 Multiple Choice Questions

1. Triacylglycerols are
   A. soluble in water
   B. insoluble in water
   C. soluble in water at elevated temperature
   D. partially soluble in water
2. In mammals, the majorfat in adipose tissues is
   Phospholipid
   Cholesterol

Sphingolipids

Triacylglycerol

3. The cholesterol molecule is

Benzene derivative

Quinoline derivative

Steroid

Straight chain acid

4. A large and important group of compounds which can be made by repetition of simple isoprenoid units is called

A. Terpenoids
B. steroids
C. carotenoids
D. terpenes

5. Derivatives of phosphatidic acid, which are composed of glycerol, fatty acids and phosphoric acids are known as

A. phospholipids
B. acylglycerol
C. triglycerides
D. esters

6. HDLs are synthesized in

A. blood
B. liver
C. intestine
D. pancreas

7. If thefatty acidis esterified with an alcohol of high molecular weight instead of glycerol, the resulting compound is

A. Lipositol
B. Plasmalogen
C. Wax
D. Cephalin

8. Natural lipids are readily soluble in

A. Oil
B. Mercury
C. Water

D. None of these

9. High content of Triglycerides are seen in

A. LDL
B. HDL
C. VLDL
D. Chylomicrons

10. In humans, a dietary essential fatty acid is

A. Palmitic acid
B. Stearic acid
C. Oleic acid
D. Linoleic acid

Answers -: 1-B 2-D 3-A 4-B 5-A 6-B 7-C 8-D 9-D 10-D

Note: It will be better if some introduction on biological oxidation and its significance be written. Starting with protein metabolism is rather abrubt.

UNIT: 7 BIOLOGICAL OXIDATION

Contents

7.1 PROTEIN METABOLISM

7.1.1 Decarboxylation

- Some amino acids undergo decarboxylation (removal of carboxyl group). The result is the formation of biogenic amines (monoamines) that exhibit a broad spectrum of functions in the human body.
- Decarboxylation is a chemical reaction that releases carbon dioxide (CO_2). Usually, decarboxylation refers to a reaction of carboxylic acids, removing a carbon atom from the chain. The reverse process, which is the first chemical step in photosynthesis, is called carbonation, the addition of CO_2 to a compound. Enzymes that catalyze decarboxylation called decarboxylases or, the more formal term, carboxy-lyases

7.1.2 Transamination

- Transamination is freely reversible reactions catalyzed by transaminases (aminotransferases). The amino group of α-amino acid is exchanged with ketonyl α-carbon of keto-acid whereby the original amino acid is changed to a new α-keto-acid while the original α-keto-acid forms a new amino acid.

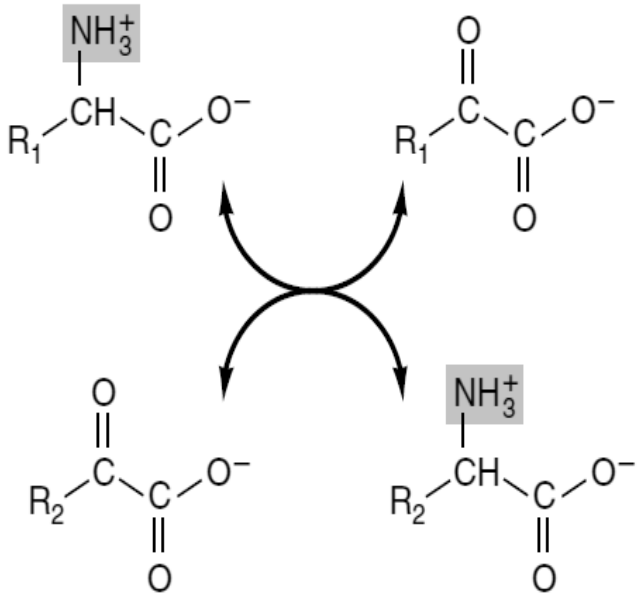

Figure 1 - Transamination

7.1.3 Deamination of Amino Acids

- Deamination is also an oxidative reaction that occurs under aerobic conditions in all tissues but especially the liver. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia and the amine functional group is replaced by the ketone group. The ammonia eventually goes into the urea cycle.
- Oxidative deamination occurs primarily on glutamic acid because glutamic acid was the end product of many transamination reactions.

7.2 LIPID METABOLISM

7.2.1 Fatty acid metabolism

- Fatty acid metabolism consists of catabolic processes that generate energy, and anabolic processes that create biologically important molecules (triglycerides, phospholipids, second messengers, local hormones and ketone bodies).
- . One role of fatty acids in animal metabolism is energy production, captured in the form of adenosine triphosphate (ATP).
- In comparison to other macronutrient classes (carbohydrates and protein), fatty acids yield the most ATP on an energy per gram basis, when they are completely oxidized to CO_2 and water by β-oxidation and the citric acid cycle.

7.2.1.1 β-oxidation

- β-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA, which enters the citric acid cycle, and NADH and $FADH_2$, which are co-enzymes used in the electron transport chain. It is named as such because the beta carbon of the fatty acid undergoes oxidation to a carbonyl group.

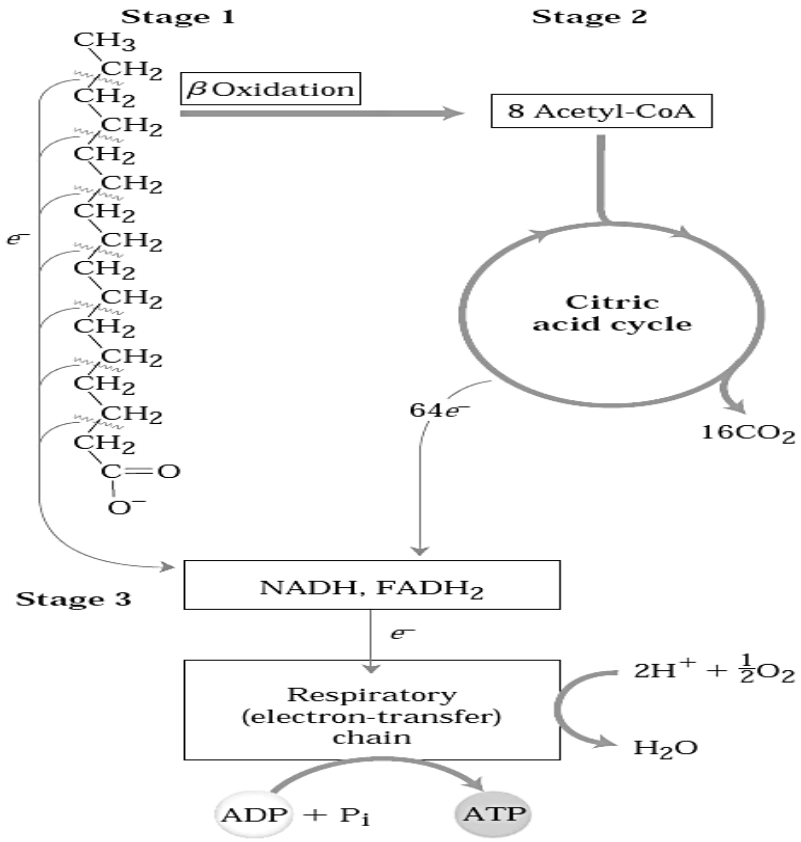

Figure 2 - Stages of fatty acid oxidation. Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA. This process is called β-oxidation. Stage 2: The acetyl groups are oxidized to CO_2 via the citric acid cycle. Stage 3: Electrons derived from the oxidations of stages 1 and 2 pass to O2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

- Beta-oxidation is primarily facilitated by the mitochondrial trifunctional protein, an enzyme complex associated with the inner mitochondrial membrane, although some fatty acids are oxidized in peroxisomes.
- Fatty acids can also be cleaved, or partially cleaved, from their chemical attachments in the cell membrane to form second messengers within the cell, and local hormones in the immediate vicinity of the cell. The prostaglandins made from arachidonic acid stored in the cell membrane, are probably the most well-known group of these local hormones.

7.2.2 Ketone bodies

- During fasting or carbohydrate starvation, oxaloacetate gets depleted in the liver because it is used for gluconeogenesis. This impedes entry of acetyl-CoA into Krebs cycle. Acetyl-CoA then is converted in liver mitochondria to ketone bodies, acetoacetate and β-hydroxybutyrate. Three enzymes are involved in thesynthesis of ketone bodies:
- β –Ketothiolase perform the final step of the β-oxidation pathway runs backward, condensing 2 acetyl-CoA to produce acetoacetyl-CoA, with the release of one CoA.
- HMG-CoA synthase catalyzes the condensation of a third acetate moiety (from acetyl-CoA) with acetoacetyl-CoA to form hydroxymethylglutaryl-CoA (HMG-CoA). HMG-CoA lyase cleaves HMG-CoA to yield acetoacetate plus acetyl-CoA.
- β -Hydroxybutyrate dehydrogenase catalyzes inter-conversion of the ketone bodies acetoacetate and β -hydroxybutyrate.
- Ketone bodies are transported in the blood to other cells, where they are converted back to acetyl-CoA for catabolism in Krebs cycle, to generate ATP. While ketone bodies thus function as an alternative fuel, amino acids must be degraded to supply input to gluconeogenesis when hypoglycemia occurs, since acetate cannot be converted to glucose.

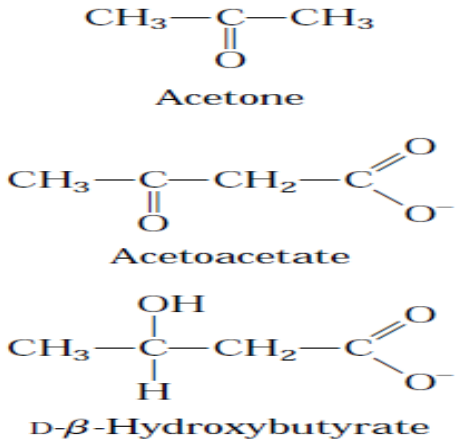

Figure 3 - ketone bodies

Note: Pl. explain oxidative phosphorylation following Respiratory chain. The sections need to be modulated.

7.3 ELECTRON TRANSPORT CHAIN AND PRODUCTION OF ATP

- The electron transport chain is shown in abbreviated form. In each turn of the citric acid cycle, four pairsof hydrogen atoms are, one each from isocitrate, ketoglutarate, succinate and malate, by the action of specific dehydrogenases. These hydrogen atoms donate their electrons to the electron transport chain and become H^+ ions, which escape into the aqueous medium.
- These electrons are transported along a chain of electron - carrying molecules to ultimately reach cytochrome aa_3 or cytochrome oxidase, which promotes the transfer of electrons to oxygen, the final electron acceptor in aerobic organisms. As each atom of oxygen accepts two electrons from the chain, two H^+ are taken up from the aqueous medium to form water.

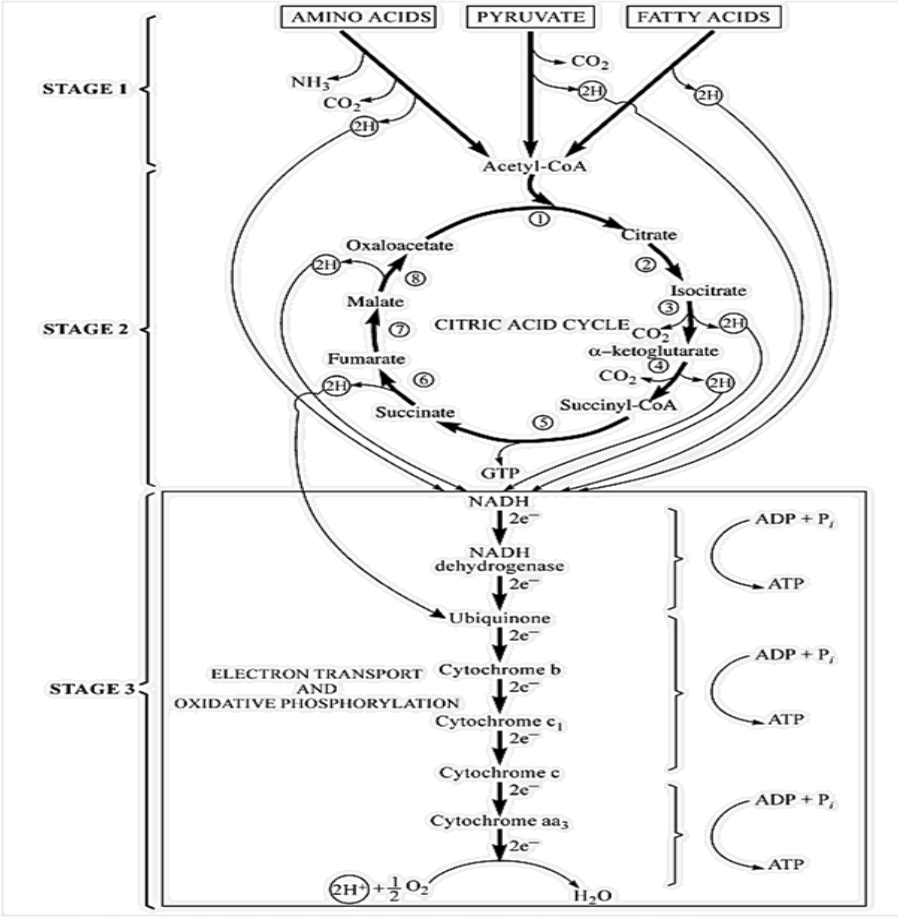

Figure 4 -The flow sheet of respiration with special reference to the electron transport and oxidative phosphorylation.

Please explain the role of cytochromes on next page, they are discussed briefly

Oxidative phosphorylation occurs in the inner mitochondrial membrane where NADH and $FADH_2$ are oxidized back to NAD^+ and FAD. They transfer their e- in a series of steps and ultimately to O_2.

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

The energy released in these e^- transfers is used to pump H^+ (protons) out of the matrix into the intermembrane space. This produces a proton gradient (different $[H^+]$ on each side) a state of high potential energy.

- At enzyme complex I, NADH is oxidized to NAD^+ and e^- are transferred between different
- $FADH_2$ is oxidized (transfers its e^-) to the CoQ at enzyme complexII. The reduced CoQ joins the rest of the "chain".
- The reduced CoQ travels to enzyme complex III where the e^- are transferred between proteins and then to cytochrome c..
- Cytochrome c travels to the enzyme complex IV where the e- are transferred between proteins and then to O^2 to form water.

The H+ ions that have been pumped into the intermembrane space can only get back into the matrix through ATP Synthase. The energy released as H+ flow back to the matrix is coupled with the formation of ATP:

$ADP + Pi \rightarrow ATP + H_2O$

(Oxidative phosphorylation)

Each NADH that enters the electron transport chain produces three ATP molecules (i.e H^+ ions are pumped at complexes I, III and IV) whereas each $FADH_2$ (that joins the "chain" at complex two) produces 2 ATP molecules (i.e H^+ ions are pumped only at complexes III and IV)

7.3.1 Site of oxidative phosphorylation

- Electron microscopic studies by George Palade and Fritjof Sjöstrand have revealed that each mitochondrion has two membrane systems: an outer membrane and an extensive inner membrane, which is highly-folded into a series of internal ridges called cristae.

- There are two compartments in mitochondria: the intermembrane space between the outer and inner membranes and the matrix, which is bounded by the inner membrane.
- The outer membrane is freely permeable to most small molecules and ions and contains some enzymes. In contrast, the inner membrane is impermeable to nearly all ions and most uncharged molecules and contains the electron-transport chains, succinate dehydrogenase and ATP-synthesizing enzymes.
- The inner membrane of a single liver mitochondrion may have over 10,000 sets of electron-transport chains and ATP synthetase molecules. The heart mitochondria have profuse cristae and therefore contain about three times more sets of electron-transport chains than that of liver mitochondria.
- The intermembrane space contains adenylate kinase and some other enzymes whereas the matrix compartment contains most of the citric acid cycle enzymes, the pyruvate dehydrogenase system and the fatty acid oxidation system. It also contains ATP, ADP, AMP, phosphate, NAD, NADP, coenzyme A and various ions such as K+, Mg2+, and Ca2+.

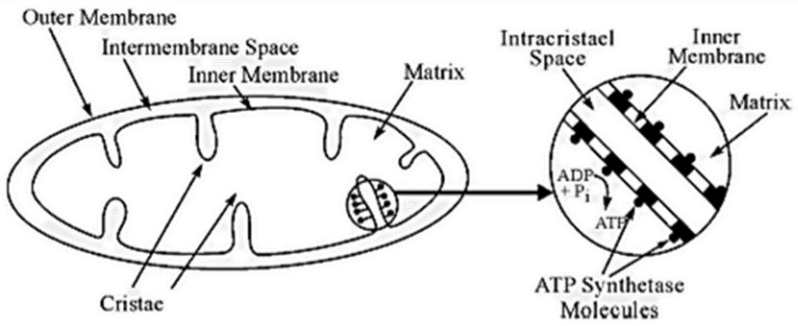

Figure 5 - Biochemical anatomy of a mitochondrion: The base pieces of ATP synthetase molecules are located with the inner membrane.

7.4 SUMMARY

- During digestion, proteins are hydrolyzed into amino acids, which are then absorbed by the capillaries of villi and enter the liver via the hepatic portal vein.
- Amino acids, under the influence of human growth hormone and insulin, enter the body cells by active transport.

- Inside cells, amino acids are synthesized into protein that function as enzymes, transport molecules, antibodies, clotting chemicals, hormones, contractile elements in muscle fibers and structural elements such as hair. REWRITE summary (above 3 points have not been discussed at all in the unit)
- Before amino acids can be catabolized, they must be converted to substances that can enter the TCA cycle. These conversions involve deamination, decarboxylation, and hydrogenation. Amino acids can be converted into glucose, fatty acids and ketone bodies.
- The ketone bodies—acetone, acetoacetate, and D--hydroxybutyrate—are formed in the liver. The latter two compounds serve as fuel molecules in extrahepatic tissues, through oxidation to acetyl-CoA and entry into the citric acid cycle.
- Overproduction of ketone bodies in uncontrolled diabetes or severely reduced calorie intake can lead to acidosis or ketosis.
- The flow of electrons through Complexes I, III, and IV results in pumping of protons across the inner mitochondrial membrane, making the matrix alkaline relative to the intermembrane space. This proton gradient provides the energy (in the form of the proton-motive force) for ATP synthesis from ADP and Pi by ATP synthase in the inner membrane.

7.4 GLOSSARY

ATP: Adenosine triphosphate, a nucleotide that is the main energy source in cells.

Beta-oxidation (β-oxidation): Oxidative degradation of fatty acids that occurs by the successive oxidation of the β-carbon atom.

Bile salts: Derivatives of cholesterol with detergent properties that aid in the solubilization of lipid molecules in the digestive tract.

Tricarboxylic acid (TCA) cycle: The cyclical process whereby acetate is completely oxidized to CO2 and water, and electrons are transferred to NAD^+ and flavine. The TCA cycle is localized to the mitochondria in eukaryotic cells and to the plasma membrane in prokaryotic cells. Also called the Krebs or citric acid cycle.

Fatty acid: A long-chain hydrocarbon containing a carboxyl group at one end. Saturated fatty acids have completely saturated hydrocarbon chains. Unsaturated fatty acids have one or more carbon-carbon double bonds in their hydrocarbon chains.

Ketone bodies: Refers to acetoacetate, acetone, and b-hydroxybutyrate made from acetyl-CoA in the liver and used for energy in nonhepatic tissue.

Ketosis: A condition in which the concentration of ketone bodies in the blood or urine is unusually high.

Metabolism: The sum total of the enzyme-catalyzed reactions that occur in a living organism.

Oxidation: The loss of electrons from a compound.

Oxidative phosphorylation: The formation of ATP as the result of the transfer of electrons to oxygen.

Proton motive force (Dp): The thermodynamic driving force for proton translocation.

7.5 REFERENCES

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7.6 TERMINAL QUESTIONS AND ANSWER

Ques. 1 - What is the metabolism?

Ans. - The metabolism is the set of physical and chemical processes upon which the life of the cells of a living organism depends.

Ques. 2 - What is the difference between anabolism and catabolism?

Ans. - The metabolism is comprised of two different processes, anabolism, and catabolism. Anabolism is a metabolic process that transform simpler compounds into organic molecules, normally consuming energy. Catabolism is a metabolic process

that break down organic molecules into simpler and less complex substances, normally releasing energy. The energy released in catabolism may be used in vital processes of the body, including anabolism.

7.6.1 Multiple Choice Questions

1. In deamination, amino acid is converted in to
   A. aldol acid
   B. keto acid
   C. hydrochloric acid
   D. carboxylic acid

2. Ketone bodies include
   A. acetone
   B. acetoacetic acid
   C. beta-hydroxybutyric acid
   D. all of above

3. Ketone bodies are produced by
   A. stomach
   B. liver
   C. kidneys
   D. gal bladder

4. Ketone bodies are made from
   A. acetone
   B. hydrochloric acid
   C. acetyl-CoA
   D. acetoacetic acid

5. β-oxidation takes place in
   A. mitochondrial
   B. cytoplasm
   C. chloroplast
   D. nucleus

Answers -: 1-B 2-D 3-B 4-C 5-A