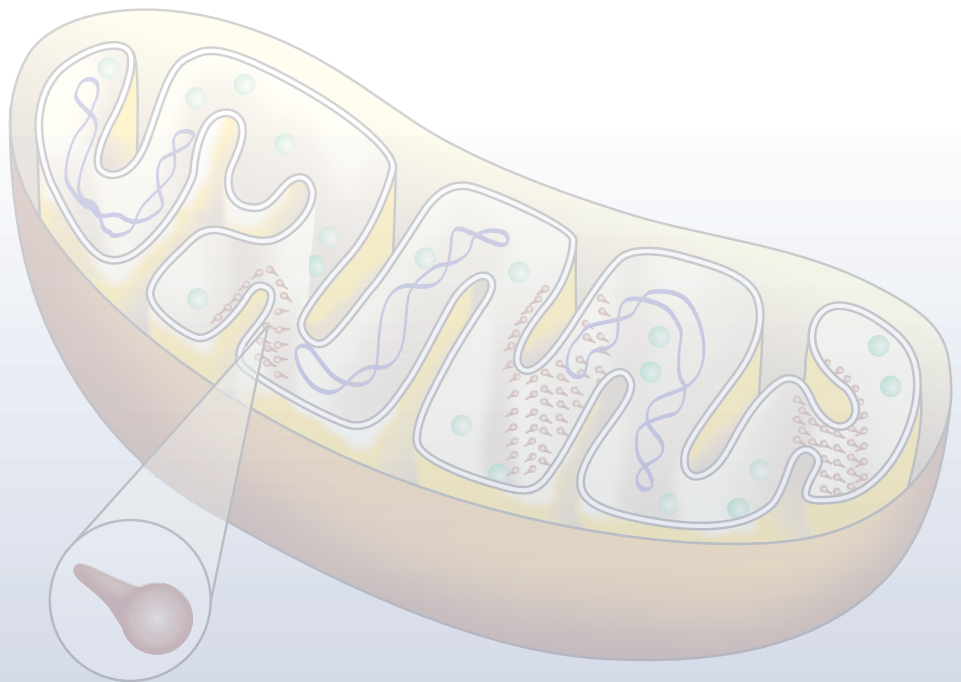


Section

1

Cell and its Biomolecules

- Cell and its Organelles 3
- Enzymes 8
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Cell and its Organelles

COMPETENCY BI 1.1

At the end of this chapter learner should be able to describe the molecular and functional organization of a cell and its sub-cellular components.

Specific Learning Objectives

BI 1.1.1 Enumerate subcellular components of a cell.

BI 1.1.2 Describe functions of various components of the cell.

All living cell may be classified into two broad categories: Prokaryote and Eukaryote.

Prokaryotes

Prokaryotic cells are characterized by lack of well defined nucleus and internal membranous structures like mitochondria, peroxisomes, etc. They are mostly unicellular. They have dense area in the cell known as nucleoid, where single strand DNA is segregated in discrete mass (Fig. 1.1).

Eukaryotes

Eukaryotic cell may be single cell (yeast, fungi) or may be multicellular (plants, animals). Eukaryotic cell is characterized by well defined nucleus, and other well defined organelles like mitochondria, lysosomes, peroxisomes surrounded by membranes. Membrane system is well defined in eukaryotic cells. This membrane meshwork is organized in important systems like endoplasmic reticulum and Golgi apparatus. Major advantage of presence of organelles in eukaryotes is that the concentration of chemical intermediates can be maximized locally and relatively lower amount of reactants will be desired to get the same outcome (Fig. 1.2).

Basic composition and fundamental chemical reaction is same in both prokaryotic and eukaryotic cells. They

do have remarkable differences, e.g. histone protein is found in eukaryotic cells but prokaryotic cells do not contain histone proteins.

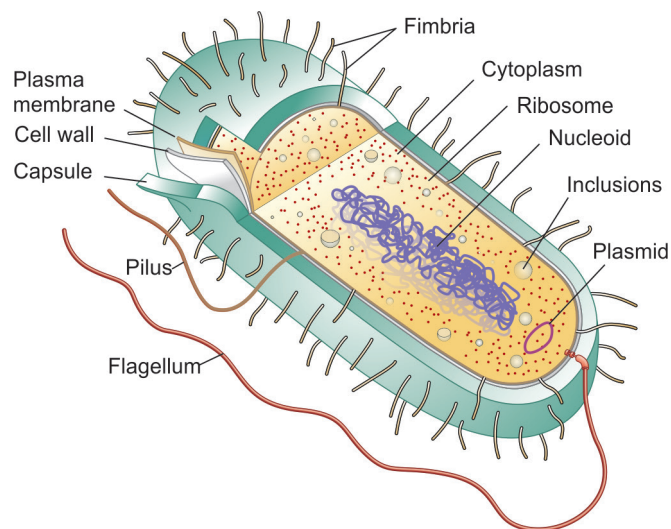


Fig. 1.1: A prokaryotic cell

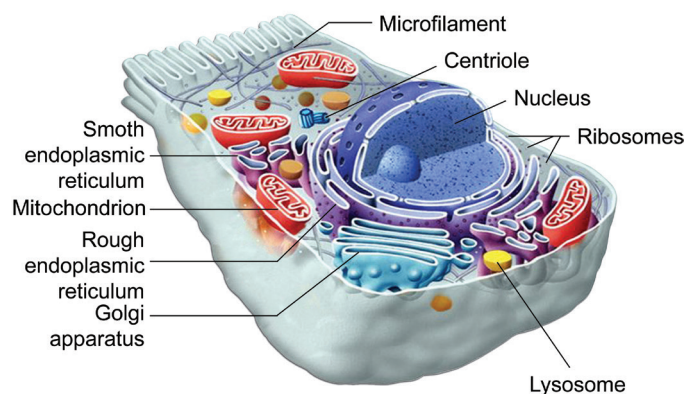


Fig. 1.2: Basic structure of an eukaryotic cell

PLASMA MEMBRANE

Outermost covering of the cell is plasma membrane which consists of mainly lipid and protein. It has numerous functions like

- Cell morphology and movement
- Transportation of small molecule and ions
- Cell-to-cell interaction
- Recognition
- Receptor for small molecule.

Detail of structure and function of plasma membrane will be dealt separately in Chapter 2.

DESCRIPTION OF INDIVIDUAL ORGANELLE IN THE EUKARYOTIC CELL

Cell contains many intracellular organelles dispersed in the cytosol. Each organelle has a specific function which is very important to make the cell a functional unit. Such organelles are:

- Nucleus
- Mitochondria
- Endoplasmic reticulum (ER)
- Golgi apparatus
- Peroxisome
- Lysosome

Nucleus

- It is the largest organelle of the cell (diameter 10 μm).
- Its main function is the storage, replication and expression of the genetic material.
- Nucleus is surrounded by an envelop which contains outer and inner nuclear membranes. Inner membrane contains a number of pores of approximately 90 Å diameter, and outer membrane is continuous with the rough endoplasmic reticulum and studded with ribosomes (Fig. 1.3).

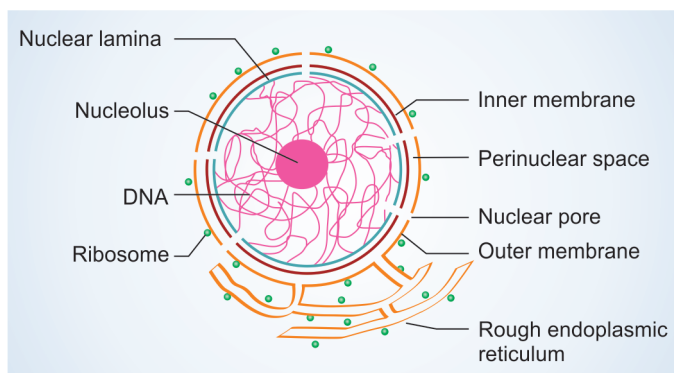


Fig. 1.3: Nucleus

- Perinuclear space (the space between outer and inner membrane of the nucleus) is continuous with the lumen of rough endoplasmic reticulum.
- In eukaryotic cell, nucleus contains DNA which together with the histone and other structural proteins form chromatin. During cell division chromatin further condenses to form chromosome.
- Nucleus sometimes contains one or more electron dense region known as nucleolus. DNA in nucleolar area contains gene for rRNA.
- Nucleus is responsible for DNA replication and transcription of various RNA.
- Nucleus also carry a special metabolic task where NAD^+ is synthesized in the nucleolus from its precursor NMN^+ (nicotinamide mononucleotide). NMN^+ is transported to nucleolus from the cytosol where it is converted to NAD^+ . Finally, NAD^+ from nucleus is transported to the cytosol. Protein synthesis does not take place in the nucleus. Histone and nonhistone proteins, which are needed in the nucleus are, synthesized in cytosol and are transported to the nucleus.

Mitochondria

- Size of the mitochondria is about 0.5–1.0 μm in diameter and 7 μm in length. Each cell contains a large number of mitochondria (approx. 2000), which constitute 25% of total cell volume.
- In an electron micrograph, mitochondria appear as a rod, sphere or filamentous body which is surrounded by an outer and an inner membrane (OMM and IMM).
- Outer membrane is smooth, but inner membrane contains a number of folds or cristae.
- Between outer and inner membranes, there is intermembranous space (Fig. 1.4). Inner membrane is rich in cardiolipin, but has no cholesterol.

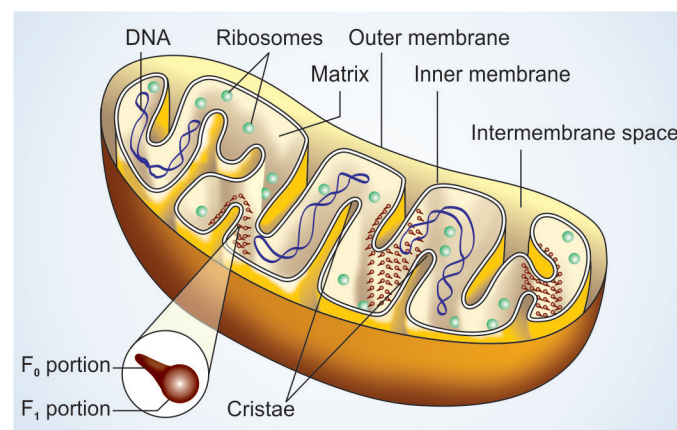


Fig. 1.4: Mitochondrion

- Human mitochondria contain small circular DNA which has code for two rRNA, 22 tRNA and 13 proteins. Outer membrane allows particle less than 10 kDa to pass through it, but inner membrane is completely impermeable, even to small molecule. Inner membrane of mitochondria contains numerous transporters, which allow transportation of many metabolites.
- IMM is the host for many enzymes including enzymes of ETC, which are involved in the process of oxidative phosphorylation.
- Mitochondria are involved in various biochemical processes which are summarized in Table 1.1.

TABLE 1.1 Biochemical processes in which mitochondria are involved

Inner membrane	Mitosol (matrix)
Oxidative phosphorylation	Fatty acid oxidation
	Heme biosynthesis
	Gluconeogenesis
	Urea synthesis
	TCA cycle
	Amino acid oxidases

- In addition to the roles described above, mitochondria also play a very important role in the process of apoptosis. Cytochrome *c* (a component of ETC) is an initiator of apoptosis.
- Mitochondria also possess 2–10 copies of double stranded circular DNA, which are maternally transmitted.
- Replication of mitochondrial DNA occurs without proofreading, hence it is very much prone for mutation. There are many diseases associated with mitochondrial DNA mutation (Table 1.2).

Endoplasmic Reticulum (ER)

- It is a membrane-bound tubular organelle, which is continuous with the outer membrane of the nucleus. It is a structure which looks like interconnected mesh of membrane-bound tubules.
- There are two types of ER in a cell.
 - Rough endoplasmic reticulum (RER):** These are studded with multiple ribosomes on its outer surface, giving it a rough appearance.

- Smooth endoplasmic reticulum (SER):** These are devoid of ribosome on the outer surface and hence it is a smooth structure.

Rough Endoplasmic Reticulum (RER)

This is the site of protein synthesis. Those proteins which are destined for lysosome, membrane and for export from the cell (secretory proteins) are synthesized in the ribosome of the rough endoplasmic reticulum. Other proteins are synthesized on the ribosome which are lying free in the cytosol. ER is also involved in protein folding (Fig. 1.5).

Smooth Endoplasmic Reticulum (SER)

These are not studded with the ribosome and are not involved in the biosynthesis of the protein, rather they are involved in lipid synthesis and detoxification reactions (Fig. 1.5).

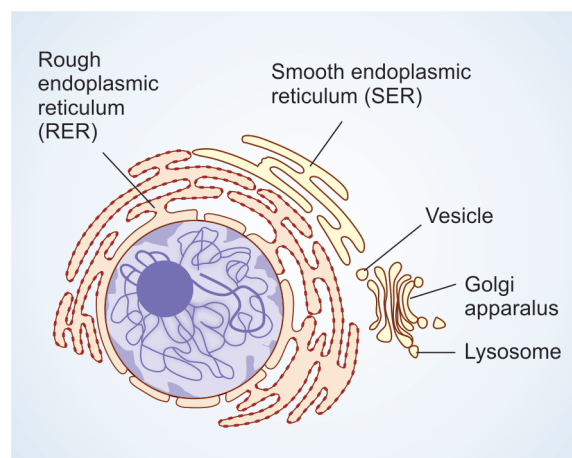


Fig. 1.5: Rough and smooth endoplasmic reticulum

Generally, number of SER is small in a cell, but in cells like hepatocyte and Leydig cell it is abundant. Membrane-bound enzyme of the SER is responsible for phospholipid, cholesterol and steroid hormone synthesis. This also contains enzyme cytochrome P450 which is involved in hydroxylation reactions during biotransformation.

ER and Golgi apparatus are concurrently involved in formation of lysosome and peroxisome and Ca^{++} signaling.

TABLE 1.2 Diseases associated with mitochondrial DNA gene mutation

Leber hereditary optic neuropathy (LHON)	Single base change in mitochondrial gene encoding three subunits (ND1, ND4, ND6) of complex I (ubiquinone oxidoreductase), which lowers the activity of NADH.
MERRF	Myoclonic epilepsy and ragged red fiber
MELAS	Mitochondrial encephalopathy, lactic acidosis and stroke like activity.

ER meshwork is fragmented during cell fractionation and small vesicles called *microsomes* are produced. These microsomes are not present in intact cell.

Golgi Apparatus

- Golgi apparatus is also known as Golgi complex. They are network of flattened smooth membrane stacks-cistern-vesicles.
- They are involved in modification and sorting of various proteins which are to be incorporated into various membranes and organelles or have to be secreted out.
- They also have enzymes which are involved in the process of transfer of carbohydrate residues on newly synthesized protein (glycoconjugation as a post-translational modification). This process of conjugation of carbohydrate on the protein is important in deciding the ultimate destination of the protein.
- Golgi apparatus is the major site of new membrane synthesis which helps in formation of lysosomes and peroxisomes (Fig. 1.6).

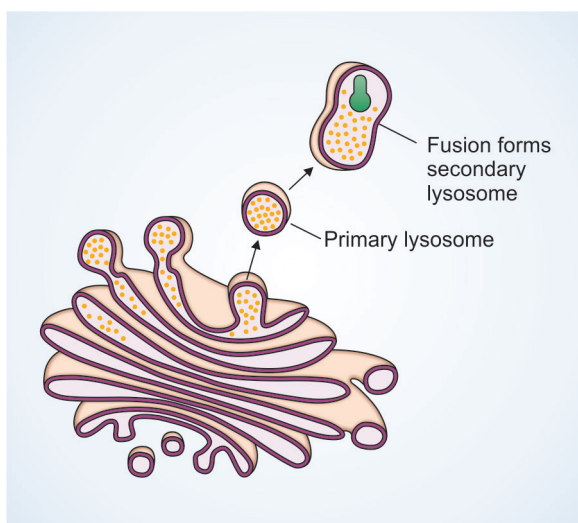


Fig. 1.6: Golgi apparatus

Peroxisome

It is also called microbodies (not to be confused with microsome, which is produced due to fragmentation of ER during cell fractionation) (Fig. 1.7).

As the name implies, these organelles are involved in the production or utilization of hydrogen peroxide. Peroxisomes are spherical as well as oval in shape and surrounded by a single layer of membrane. Their size is small (0.3–1.5 μm).

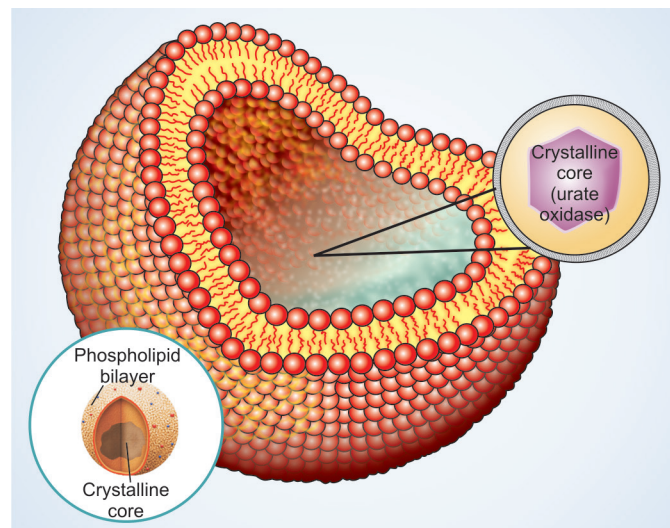


Fig. 1.7: Peroxisome

They play a very important role in

1. Very long chain fatty acid (VLCFA) oxidation
2. Synthesis of glycerolipid
3. Synthesis of glycerol ether lipid (plasmalogen)
4. Synthesis of isoprenoid

Catalase enzyme is found in peroxisome which is involved in conversion of H_2O_2 to H_2O and O_2 molecules.

Zellweger syndrome: This is a severe neurological disorder which is due to absence of functional peroxisome in various cells of the body. Death occurs by age of 6 months. Underlying problem in this disorder is the *defect of mechanism of protein import in the lumen of the peroxisome*. It is an autosomal recessive disorder.

Lysosome

- These organelles are rich in hydrolase class of enzymes (class III) which cleave the carbon-oxygen, carbon-nitrogen, carbon-sulphur, oxygen-phosphorous bonds in lipids, protein, carbohydrate and nucleic acid. The enzymes of lysosome act best at acidic pH, hence intralysosomal pH is 5.
- Primary lysosome fuses with vesicle containing external material which may have been ingested in the cell by phagocytosis, pinocytosis or endocytosis. This creates secondary lysosome in the cell which has both the material as well as the hydrolase enzyme to digest them.
- Lysosomes are involved in the process called autophagy whereby they hydrolyse cellular components like proteins, nucleic acids, lipids and organelles like mitochondria (Fig. 1.6).

I CELL DISEASE (INCLUSION CELL DISEASE)

Here, the defect lies in the targeting of newly synthesised lysosomal enzymes to the lysosome which is due to lack of enzyme 'N-acetyl D-glucosamine phosphotransferase'. This enzyme is responsible for transfer of N-acetylo glucosamine phosphate to high mannose type oligosaccharide of the proteins destined for lysosome.

Fibroblast of affected individual shows dense inclusion bodies (I cell) and lack phosphotransferase activity.

This disease is characterized by severe psychomotor retardation, skeletal abnormality, coarse facial features, and restricted joint movement.

Symptoms are present at birth and death occurs by 8 years of age (Fig. 1.8).

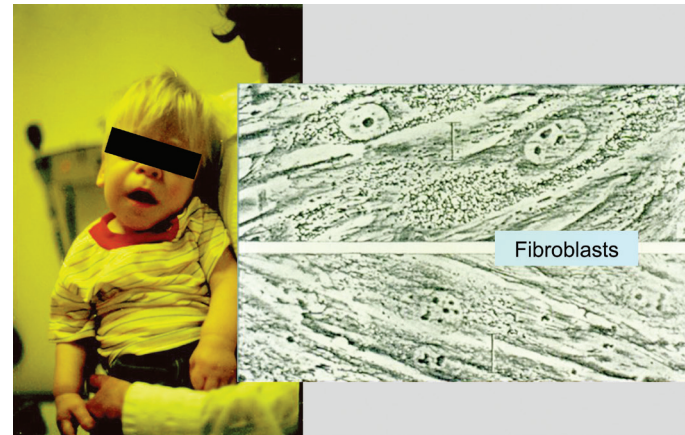


Fig. 1.8: I cell disease (Inclusion cell disease)

Enzymes

COMPETENCY BI 2.1

At the end of this chapter learner should be able to explain fundamental concepts of enzyme, isoenzyme, alloenzyme, coenzyme and cofactors. Enumerate the main classes of IUBMB nomenclature.

COMPETENCY BI 2.3

At the end of this chapter learner should be able to describe and explain the basic principles of enzyme activity.

COMPETENCY BI 2.4

At the end of this chapter learner should be able to describe and discuss enzyme inhibitors as poisons and drugs and as therapeutic enzymes.

COMPETENCY BI 2.5

At the end of this chapter learner should be able to describe and discuss the clinical utility of various serum enzymes as markers of pathological conditions.

COMPETENCY BI 2.6

At the end of this chapter learner should be able to discuss use of enzymes in laboratory investigations (enzyme-based assays).

Specific Learning Objectives

- BI 2.1.1** Define enzymes, isoenzymes, coenzyme, alloenzymes, cofactors.
- BI 2.1.2** Describe functions of isoenzymes, alloenzymes, and coenzymes.
- BI 2.3.1** Describe mechanism of action of different enzymes.
- BI 2.3.2** Describe 'lock and key' hypothesis.
- BI 2.3.3** Describe Koshland's induced fit theory.
- BI 2.3.4** Describe Michelis Menten theory.

Contd.

Specific Learning Objectives (Contd.)

- BI 2.4.1** Describe competitive inhibition.
- BI 2.4.2** Describe noncompetitive inhibition.
- BI 2.4.3** Describe uncompetitive inhibition.
- BI 2.4.4** Describe therapeutic action of enzyme inhibition.
- BI 2.5.1** Describe various enzymes used as diagnostic markers of pathological condition.
- BI 2.6.1** Enumerate important enzyme based assays used in lab investigation.

Frederich W. Kühne coined the term 'enzyme'.

What is an Enzyme?

- Enzymes are biocatalysts which enhance the rate of a biochemical reaction which otherwise progress very slowly in absence of enzyme.
- Enzymes are neither changed nor lost during or after the reaction and are recovered intact at the end of reaction.

What is the Biochemical Nature of the Enzyme?

Enzymes are mostly proteins. There are certain RNAs which are known to possess enzymatic activity. Such type of RNAs which have catalytic activity, are known as ribozymes.

GENERAL CHARACTERISTIC OF ENZYMES

Enzymes are mostly proteins which are generally heat labile and are water soluble.

CLASSIFICATION OF ENZYMES

According to International Union of Biochemistry and Molecular Biology, 1964 (IUBMB) enzymes are divided into six major classes:

- | | |
|-------------------|--------------|
| i. Oxidoreductase | iv. Lyase |
| ii. Transferase | v. Isomerase |
| iii. Hydrolase | vi. Ligase |

Oxidoreductase

Enzymes in this class are involved in transfer of hydrogen ion from one substrate to other. The substrate which is donating the hydrogen is oxidized and the one which is accepting the hydrogen is reduced.

Transferase

Enzymes in this class are involved in transfer of groups other than hydrogen. Various examples of such enzymes are:

- Transaminase/aminotransferase
- Methyltransferase
- Transaldolase
- Transketolase
- Kinase
- Pyruvate dehydrogenase complex
- Branching enzyme

Hydrolase

Enzymes of this class use water and cleave the bonds so that the substrate is cleaved into simpler products. These enzymes act in irreversible manner.

All digestive enzymes belong to this class.

Following are the examples of such enzymes:

1. Lipase (cleaves the ester bond)
2. Amylase (cleaves the glycosidic bond)
3. Pepsin (cleaves the peptide bond)
4. Urease (cleaves C–N bond other than peptide bond)

Lyase

Enzymes of this class are involved in cleavage of C–C, C–O and C–N bonds. These bonds are cleaved due to atom elimination. At times the bond is not even cleaved after the atoms are eliminated, rather double bond is left at that place. Nature of enzyme may be reversible or irreversible. Important examples of such enzymes are:

- Aldolase
- Fumarase
- Arginosuccinate lyase
- HMG-CoA lyase
- ATP citrate lyase

Isomerase

This class of enzymes rearranges the atoms within the same molecule. This results in synthesis of isomeric form of the original molecule. Important examples of such enzymes are:

- Methyl malonyl CoA mutase
- Triose phosphate isomerase
- Retinene isomerase

- Epimerase
- Phosphohexose isomerase

Ligase

This class of enzymes catalyzes the joining together of two molecules coupled to the hydrolysis of ATP. Important examples of such enzymes are:

- Acetyl CoA carboxylase (all carboxylases)
- PRPP synthetase
- Glutamine synthetase
- Aminoacyl-tRNA synthetase
- Arginosuccinate synthetase
- Carbamoyl phosphate synthetase I and II

MODE OF ACTION OF ENZYME

Enzymes act via lowering the 'activation energy'.

What is 'Activation Energy' and how Enzyme Facilitates its Lowering?

Whenever a substrate is converted to a product, a transient intermediate is produced which is known as transition state.

Difference of energy of the substrate and the transition state is known as activation energy (Fig. 2.1).

Enzyme Lowers Activation Energy.

It is said that whenever the substrate is binding the active site of the enzyme, certain amount of energy known as binding energy is released, which lowers the activation energy.

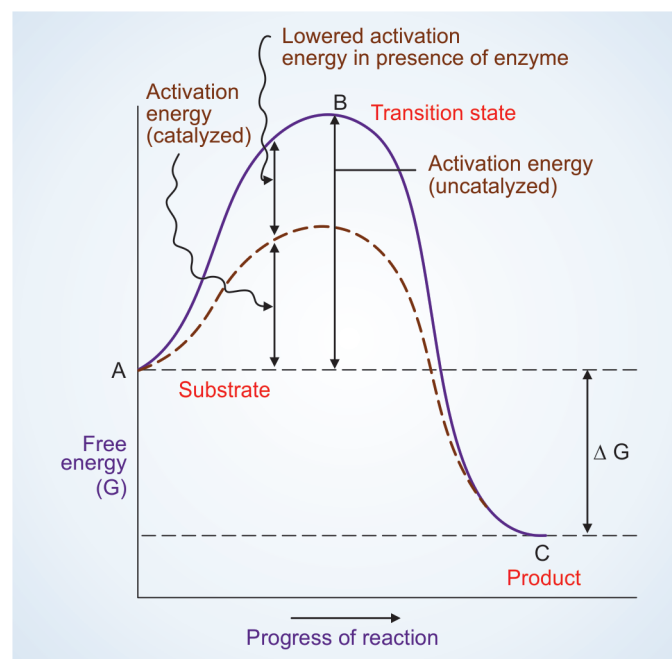


Fig. 2.1: Lowering of activation energy by enzyme

What is the Advantage of Lowering of Activation Energy?

In the presence of enzyme when the activation energy is lowered, the reaction proceeds faster and substrate is quickly converted to product.

Models to Explain the Binding of Substrate to the Active Site of the Enzyme

Substrate binds the enzyme at its active site. To explain the binding of substrate to the active site of the enzyme, there are two theories.

1. **Lock and key model (rigid template model):** By Emil Fisher (1890) (Fig. 2.2)
2. **Induced fit model (hand in glove model):** By Daniel E. Koshland (1958) (Fig. 2.3)

In 'lock and key model', it was proposed that the active site of the enzyme has predetermined shape which correctly fits the substrate into it, facilitating the reaction. This was compared to lock and key where key correctly fits into rigid groove in the lock.

This model could explain the specificity with which the enzyme functions, but could not explain the action of allosteric modifiers on to the enzyme.

Emil Fisher's 'lock and key model' was undebated till 1958, when Daniel E. Koshland proposed 'induced fit model' to explain the binding of the substrate to the enzyme.

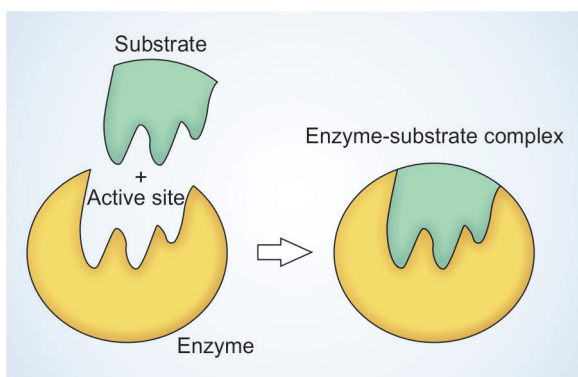


Fig. 2.2: Lock and key model

In this model Koshland proposed that active site may not be having a fixed structure, rather will show the flexibility and can be modulated according to the shape of the substrate to accommodate it perfectly.

Steady state of reaction: The state at which rate of synthesis of enzyme-substrate complex (ES complex) is equal to the rate of its degradation, is known as steady state of the reaction.

Turnover number or catalytic constant (k_{cat}): Number of substrate molecules converted to product by an enzyme

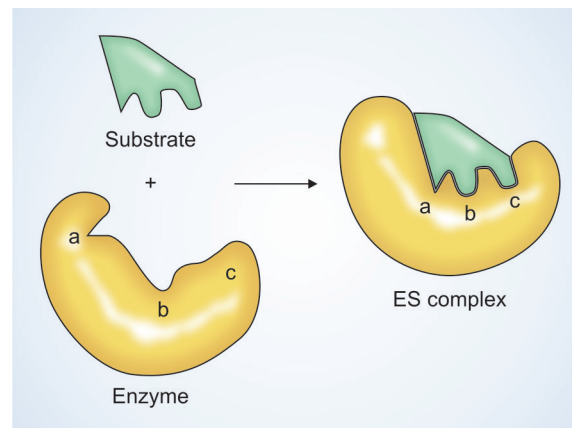


Fig. 2.3: Induced fit model

in unit time is called as turnover number or catalytic constant (k_{cat}).

ENZYME KINETICS

Enzyme kinetics is the study of reaction rate and their response to the changes of experimental parameters.

There are many factors which affect the enzyme kinetics. They are:

1. Substrate concentration
2. Temperature
3. pH
4. Enzyme concentration
5. Product concentration
6. Inhibitors

Effect of Substrate Concentration on Enzyme Kinetics

The effect of substrate concentration on velocity of reaction in a fixed concentration of enzyme is shown in Fig. 2.4.

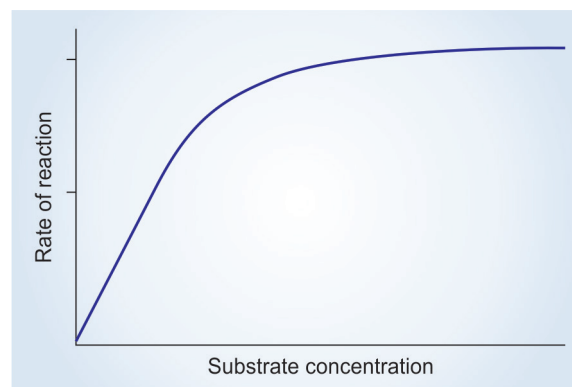


Fig. 2.4: Effect of substrate concentration on reaction velocity

Fig. 2.4 shows, when the substrate concentration is increased, initially the velocity is increased in direct proportion (linear part of the graph/first order kinetics) till all the active site of the enzyme is saturated with the substrate.

Further increase of substrate no more increases the velocity (hyperbolic part of the graph/zero order kinetics), as all the active site of the enzyme is already saturated with the substrate.

Michaelis Constant (K_m)

- It is the substrate concentration at which the velocity of reaction is half of the maximum velocity (Fig. 2.5).
- K_m is the measure of substrate concentration which is required for significant catalysis to occur.
- K_m signifies that half of the active sites of the enzyme is saturated with the substrate.
- K_m also signifies the affinity of substrate to the enzyme. Numerically K_m value is inversely proportional to the affinity of the substrate.

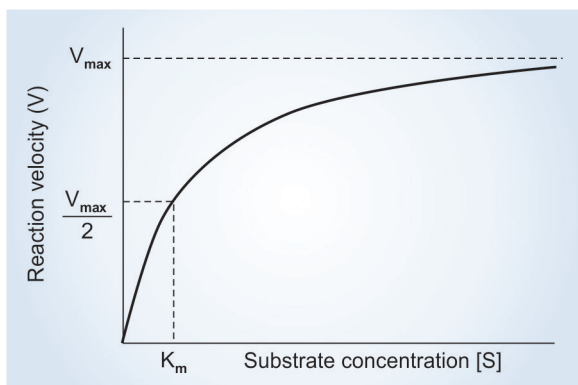


Fig. 2.5: Plot to show K_m value (Michaelis Menten constant)

- K_m is the numerical value which has a unit. Unit of K_m is same as that of substrate concentration.
- K_m is said to be the 'signature of enzyme' as it is used to identify the unknown enzyme which is separated from a protein mixture.
- K_m is sensitive to pH, temperature and ionic strength of the solution.
- Isoenzymes of an enzyme may have different substrate affinity and hence different K_m values.

Lineweaver-Burk Plot (Double Reciprocal Plot)

When $1/S$ concentration and $1/V$ is plotted on X- and Y-axes, respectively, we get Lineweaver-Burk plot (double reciprocal plot). The point at which line intersects the X-axis represents $-1/K_m$ numerically and the point at which line intersects the Y-axis represents $1/V_{max}$ numerically (Fig. 2.6).

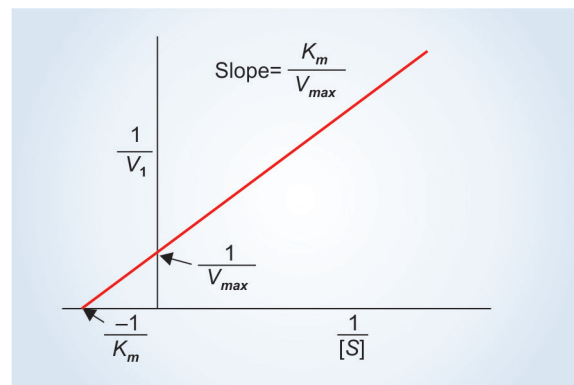


Fig. 2.6: Lineweaver-Burk plot (double reciprocal plot)

Major use of this plot resides in the fact that kinetic mechanism of enzyme inhibitor can be determined with greater ease using this plot compared to Michaelis Menten graph.

Effect of Temperature on Enzyme Kinetics (Bell Shaped)

The effect of temperature on velocity of reaction in a fixed concentration of enzyme is shown in Fig. 2.7.

Fig. 2.7 shows that when the temperature is increased, initially the velocity is increased in direct proportion till the maximum velocity is achieved. Further increase of temperature decreases the velocity of the reaction resulting in a bell-shaped curve.

The temperature at which the velocity of the reaction is maximum is known as optimum temperature. Rise of temperature initially increases the velocity of reaction due to the fact that this temperature overcomes the energy barrier, but further increase of temperature denatures the active site of the enzyme which leads to lowering of the velocity of enzyme catalyzed reaction.

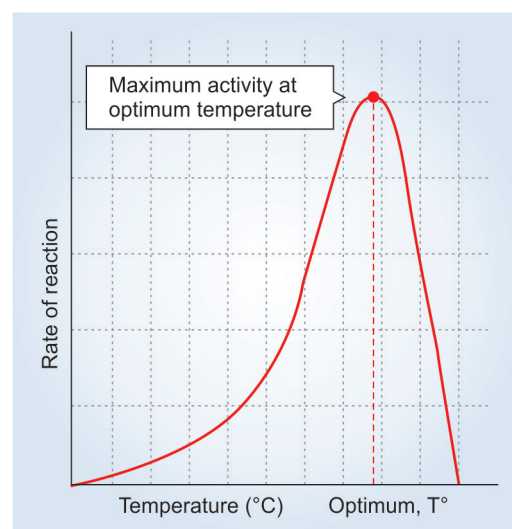


Fig. 2.7: Bell-shaped curve to show optimum T°

Q10 (Temperature Coefficient)

For each 10 degree rise of temperature the reaction velocity is doubled.

Effect of H^+ Concentration or pH on Enzyme Kinetics

The effect of pH on velocity of reaction in a fixed concentration of enzyme is shown in Fig. 2.8.

Fig. 2.8 shows that when the pH is increased, initially the velocity is increased in direct proportion till the maximum velocity is achieved. Further increase of pH decreases the velocity of the reaction resulting in a bell-shaped curve. The pH at which the velocity of the reaction is maximum is known as optimum pH.

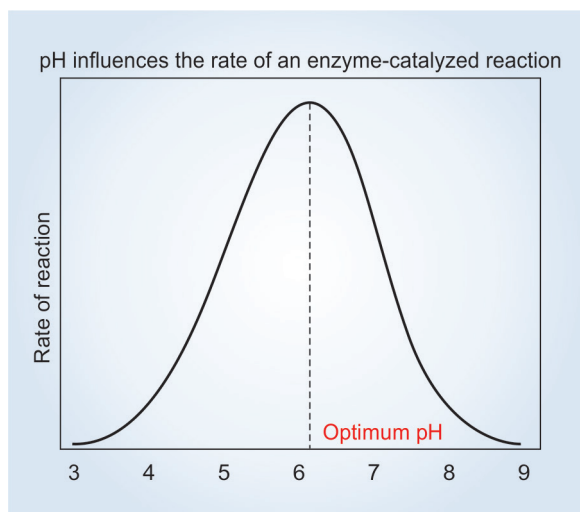


Fig. 2.8: Bell-shaped curve to show optimum pH

Changing H^+ concentration and so the pH, affects the enzyme activity in many ways:

- pH affects the ionization of the amino acids at the active site.
- pH affects the ionization of the substrate which binds at the active site.
- Extreme of pH may lead to denaturation of enzyme.

Effect of Enzyme Concentration on Enzyme Kinetics

Velocity of reaction is dependent on quantity of the enzyme provided that all the active sites of enzyme get sufficient substrate to bind, that means the substrate is present in sufficient quantity and is not the limiting factor.

The graph is linear when the velocity of reaction is plotted against concentration of enzyme (Fig. 2.9).

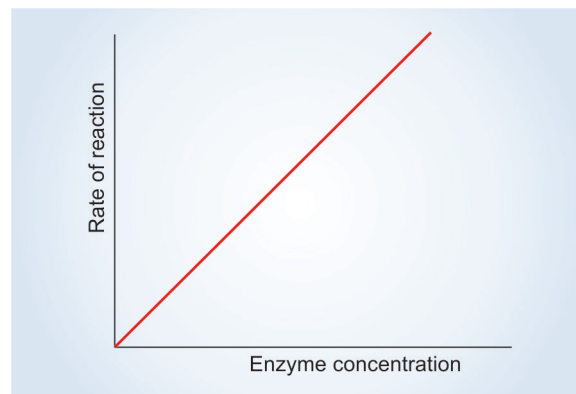


Fig. 2.9: Effect of enzyme concentration on reaction velocity

inhibition is not generally observed as the product of one enzymatic reaction acts as a substrate for another enzymatic reaction.

Effect of Inhibitors on Enzyme Kinetics

An inhibitor is any substance which decreases the velocity of an enzyme catalyzed reaction. In other words in presence of an inhibitor velocity of enzyme catalyzed reaction is decreased.

Study of types of inhibitors and their effect on enzyme kinetics is important as there are many enzymes in biological system which are under control of these various types of inhibitors. Moreover, many of the drugs which are used therapeutically are designed based on these kinetics of inhibitor.

Inhibitors may act in a reversible or irreversible manner.

Reversible Inhibitors

Such type of inhibitors bind the enzyme in a reversible fashion mostly in noncovalent bond.

Full function of enzyme is restored once the inhibitor is dissociated from the enzyme.

There are various types of inhibitors which act in a reversible fashion:

- Competitive inhibitor
- Noncompetitive inhibitors
- Uncompetitive inhibitors

Competitive inhibitors

- Such type of inhibitors are structurally similar to the substrate and hence they compete with the substrate to bind at the active site.
- The binding of such inhibitors at the active site is through noncovalent bond.
- Inhibitors may get dissociated at the active site as the noncovalent bond is weak and hence it is a reversible type of inhibition.

Effect of Product Concentration on Enzyme Kinetics

Product imparts inhibitory affect on the activity of the enzyme. This is called product inhibition. This type of

- In this type of inhibition when the inhibitor binds the active site, no product is formed.
- At very high concentration of substrate the effect of inhibitor will be negligible as practically all the substrate will get the opportunity to bind the active site resulting in the achievement of maximum velocity which is possible for that enzyme.

New maximum velocity and new K_m value in the presence of inhibitor is known as apparent V_{max} and apparent K_m value respectively (V'_{max} and K'_m).

Following is the effect of competitive inhibitors on the kinetics of enzyme:

1. **Effect on V_{max} :** The effect of a competitive inhibitor is reversed by increasing $[S]$. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor.
2. **Effect on K_m :** A competitive inhibitor increases the apparent K_m for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve $\frac{1}{2} V_{max}$.
3. **Effect on Lineweaver-Burk plot:** Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited and uninhibited reactions intersect on the Y-axis at $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different X-axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor (Fig. 2.10).

Examples of competitive inhibitors:

1. Sulphonamide as para-aminobenzoic acid analogue
2. Methotrexate as dihydrofolate reductase inhibitor
3. Dicumarol as vitamin K analogue
4. Statins as HMG-CoA reductase analogue
5. Ethanol in methanol poisoning
6. 5-Fluorouracil as an inhibitor of thiamidylate synthase.
7. Isoniazid (INH) as vitamin B₆ analogue

■ Noncompetitive inhibition (Mixed type of inhibition)

This type of inhibition is recognized by its characteristic effect on V_{max} and occurs when the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme or the ES complex, thereby preventing the reaction from occurring.

1. **Effect on V_{max} :** Noncompetitive inhibition cannot be overcome by increasing the concentration of

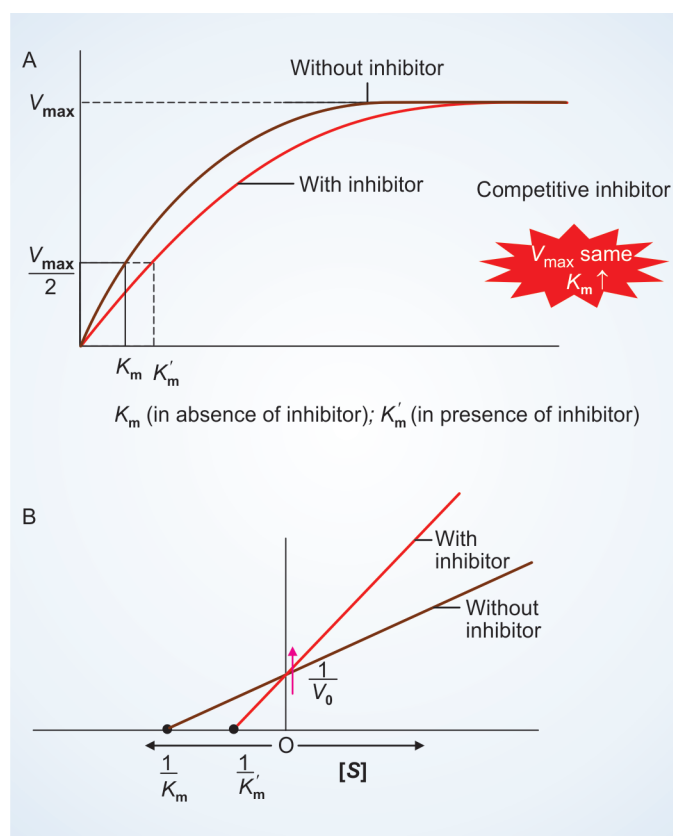


Fig. 2.10: Effect of competitive inhibitors (A = effect shown on Michaelis Mentem plot, B = effect shown on Lineweaver-Burk plot)

substrate. Thus, noncompetitive inhibitors decrease the V_{max} of the reaction.

2. **Effect on K_m :** Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.
3. **Effect on Lineweaver-Burk plot:** Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting $1/V_0$ vs $1/[S]$ and noting that V_{max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged (Fig. 2.11).

Examples of noncompetitive inhibitors:

1. Cyanide as cytochrome oxidase inhibitor
2. Fluoride as enolase inhibitor in glycolysis
3. Iodoacetate as inhibitor of glyceraldehyde-3-phosphate dehydrogenase
4. British anti-Lewisite (BAL) as antidote of heavy metal poisoning
5. Organophosphorus poisoning as an inhibitor of acetylcholinesterase.

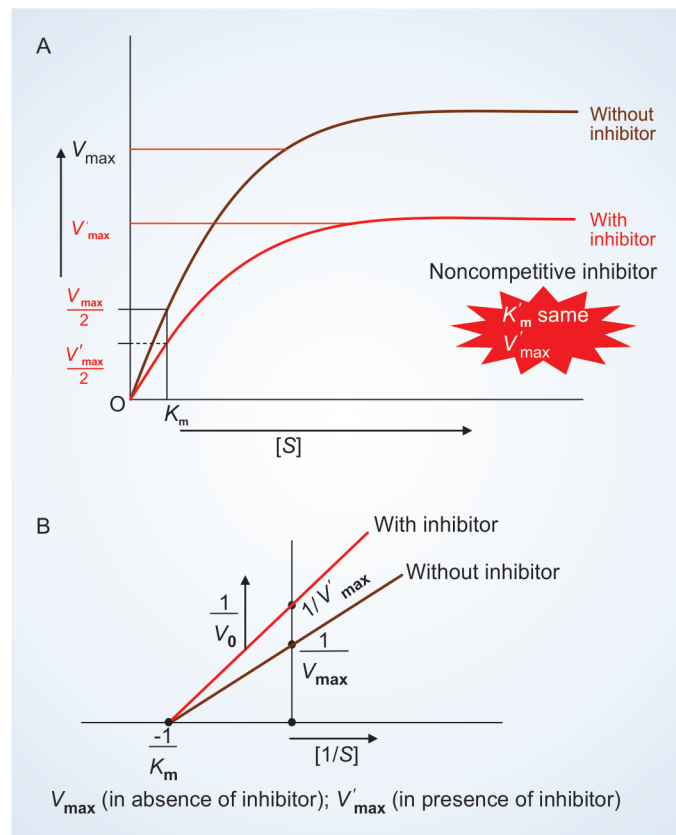


Fig. 2.11: Effect of noncompetitive inhibitors (A = effect shown on Michaelis Menten plot; B = effect shown on Lineweaver-Burk plot)

Uncompetitive inhibition

This type of inhibition is recognized by decrease of both V_{max} and K_m value. It is a very rare type of inhibition.

Example: Inhibition of placental ALP by phenyl alanine (Fig. 2.12).

Irreversible Inhibitors

This type of inhibitors bind the enzyme covalently and tightly and are not dissociated from them leading to irreversible type of inhibition.

Irreversible inhibitors may be of following types:

1. Group specific inhibitors
2. Substrate analogue inhibitors (affinity labels)
3. Suicidal inhibitors (mechanism based inactivation)

SUICIDAL INHIBITION

Suicidal inhibition is also called as 'mechanism based inactivation' as in this type of inhibition of enzymes, the enzyme's own activity is utilized first, to convert a less potent inhibitor to more potent inhibitor. This more

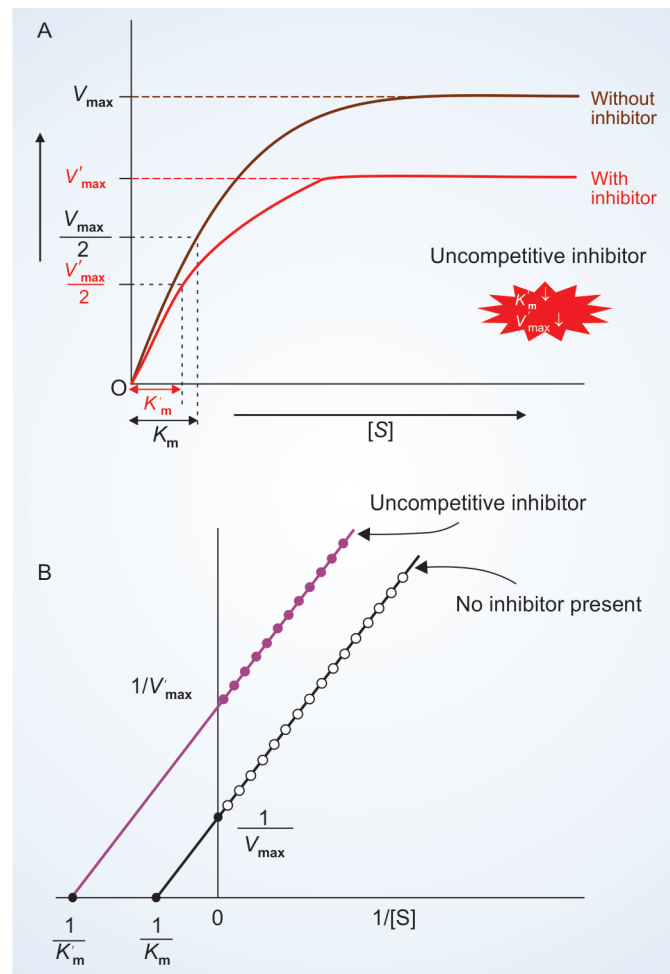


Fig. 2.12: Effect of uncompetitive inhibitors (A = effect shown on Michaelis Menten plot; B = effect shown on Lineweaver-Burk plot)

potent inhibitor in turn inactivates the enzyme which actually had synthesized it.

In other words, enzyme synthesizes its own poison.

Allopurinol given to reduce hyperuricemia shows suicidal inhibition of xanthine oxidase.

Xanthine oxidase is the enzyme which converts hypoxanthine to xanthine and xanthine to uric acid (Fig. 2.13).

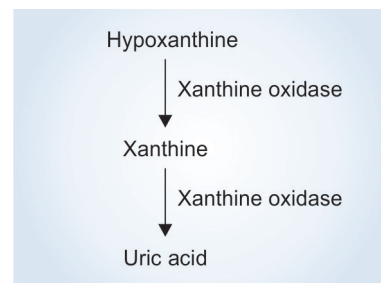


Fig. 2.13: Role of xanthine oxidase in uric acid synthesis

Allopurinol as such does not inhibit the action of xanthine oxidase. It is first converted to alloxanthine by action of xanthine oxidase (Fig. 2.14).

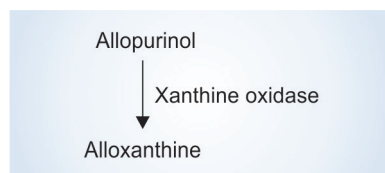


Fig. 2.14: Activation of allopurinol by xanthine oxidase

This alloxanthine now competes with xanthine for xanthine oxidase and inhibits it. It is a classic example of suicidal inhibition.

COFACTOR AND COENZYME

Some enzymes require an additional chemical component for their activity, this additional component is known as cofactor.

Cofactor may be inorganic ions, such as Fe^{++} , Mg^{++} , Mn^{++} or Zn^{++} ; or it may be a complex organic or metallo-organic molecule called a coenzyme (Tables 2.1 and 2.2).

Coenzyme may be covalently or noncovalently linked.

Prosthetic group denotes covalently bound cofactor.

Example:

1. Biotin as prosthetic group for carboxylase enzymes.
2. Heme as prosthetic group for cytochromes.

Serine proteases: These are proteolytic enzymes with serine residue at its active center. Trypsin, chymotrypsin, thrombin and elastin are examples of such enzymes.

TABLE 2.1	Inorganic elements as a cofactor for certain enzymes
Cu^{++}	Superoxide dismutase (SOD) Monoamino oxidase (MAO) Lysyl oxidase Cytochrome oxidase ALA synthase Tyrosinase
$\text{Fe}^{++}/\text{Fe}^{+++}$	Cytochrome oxidase Catalase Peroxidase Proline hydroxylase
K^{+}	Pyruvate kinase
Mg^{++}	Hexokinase Glucose-6-phosphatase Pyruvate kinase
Mn^{++}	Arginase Superoxide dismutase (SOD) Ribonucleotide reductase
Se	Glutathione peroxidase (GPO) Deiodinase
Zn^{++}	Carbonic anhydrase Alcohol dehydrogenase Carboxypeptidase A and B ALA synthase Superoxide dismutase (SOD) RNA polymerase ALP LDH
Mo (molybdenum)	Xanthine oxidase Sulphite oxidase Aldehyde oxidase

TABLE 2.2	Coenzymes and the group they transfer	
Coenzyme	Group they transfer	Dietary precursor
Biocytin	CO_2	Biotin
Coenzyme A	Acyl group	Pantothenic acid and other compound
FAD	Electron	Riboflavin (vit. B_2)
Lipoate	Electron and acyl group	Not required in diet
NAD	Hydride ion (H^-)	Nicotinic acid (niacin)
Pyridoxal phosphate (PLP)	Amino group	Pyridoxine (B_6)
THF	1 carbon group	Folate
TPP	Aldehyde	Thiamine
Coenzyme B_{12} (5'-deoxyadenosylcobalamin)	H atom and alkyl group	Vit. B_{12}

Regulation of Enzyme Activity

Overall activity of an enzyme in a rate-limiting step of a pathway depends upon two important factors:

- Concentration of the enzyme
- Intrinsic catalytic efficiency of the enzyme

Concentration of the Enzyme and Regulation of its Level

Enzymes in a biochemical system are constantly undergoing turnover. It means there is constant synthesis and degradation of the enzymes at a particular rate.

The net concentration of enzyme may be altered by changing the rate constant of synthesis (K_s) or degradation (K_{deg}) or both.

Induction of enzyme synthesis: Enzyme transcription from its gene may be enhanced by an inducer which may be its own substrate, structurally related compound or totally irrelevant molecule.

There are many enzymes which are inducible in human. They are:

- HMG CoA reductase
- Tryptophan pyrrolase
- ALA (aminolevulinic acid) synthase
- Cytochrome P450
- Threonine dehydratase
- Urea cycle enzymes

Repression of enzyme synthesis: Many enzymes are under repression by many factors. Gene of ALA synthase (the rate limiting enzyme of heme biosynthesis) is under repression by heme.

When the gene is derepressed in lack of heme then only the enzyme ALA synthase is synthesized and the heme synthesis can take place (Fig. 2.15).

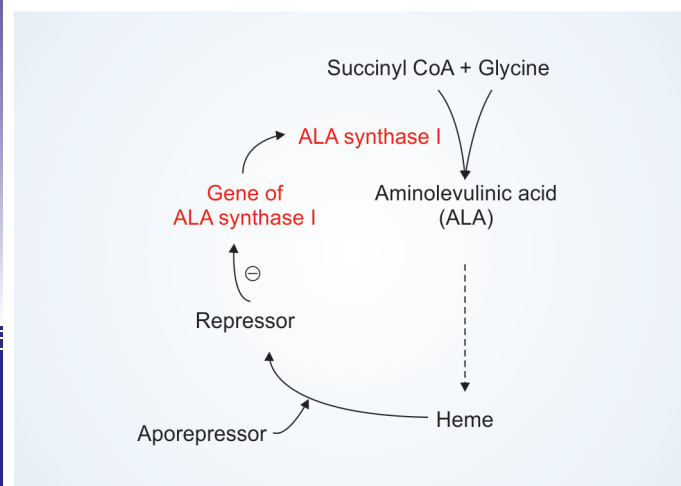


Fig. 2.15: Repression and derepression of enzyme

Degradation of enzyme by endoplasmic reticulum associated degradation (ERAD) (ubiquitin-mediated degradation): Regulatory enzymes having short half-life are important substrate for ubiquitin-mediated proteasomal pathway degradation. ERAD is an energy dependent process.

Intrinsic Catalytic Efficiency of the Enzyme and its Regulation

Two important ways by which intrinsic catalytic efficiency of an enzyme may be altered are:

- Allosteric regulation
- Covalent modification.

In contrast to hours/days needed for changing the concentration of enzyme by regulating its synthesis and degradation, the time duration needed for changing the intrinsic catalytic activity by allosteric or covalent regulation of the enzyme is quite less (seconds/minutes).

Regulation of intrinsic catalytic efficiency is a short term rapid response while regulation of enzyme concentration is a long-term adaptive response.

Allosteric Regulation of Enzyme Activity

- All enzymes have active site where substrate binds.
- Some enzymes have other sites in addition to active site where allosteric modifier bind and change the activity of active site.
- Allosteric modifier do not have any structural resemblance to substrate.
- The activity of enzymes that catalyzes key regulatory reactions (committed steps) of metabolic pathways are often subject to allosteric regulation. Their activity can be modulated by the binding of allosteric effectors to a site on the enzyme that is distinct from the active site (i.e. allosteric site). Effectors are positive, if they enhance the rate of a reaction (i.e. activators) and negative, if they decrease the rate of reaction (i.e. inhibitors).
- Allosteric modifier may be positive or negative depending upon whether they are increasing the activity of active site or decreasing the activity of active site.
- Allosteric modifier may be homotropic or heterotropic. when substrate itself acts as an allosteric modifier, it is called *homotropic effect*, and when allosteric effectors are different from substrate, it is called *heterotropic effect*.
- Allosteric enzymes do not obey Michaelis Menten behavior. They do not produce hyperbolic substrate saturation curve rather they produce sigmoidal saturation kinetic curve.

- Allosteric enzymes may be classified into *k series* enzyme and *v series* enzyme.

k series: Here substrate saturation kinetics is like competitive inhibition (V_{max} same but K_m increased).

v series: Here substrate saturation kinetics is like noncompetitive inhibition (V_{max} decreased but K_m same).

What is feed-forward reaction?

ATP synthesized in purine nucleotide biosynthesis stimulates pyrimidine nucleotide biosynthesis by allosteric activation of aspartate transcarbamoylase (ATC) enzyme. It is an example of feed-forward reaction.

Regulation of Enzyme Activity by Covalent Modification

- May be reversible or irreversible
- To regulate the catalytic activity of enzyme the types of covalent modification which are observed, are:
 - Partial proteolysis
 - Phosphorylation
- Histone and other DNA binding protein undergo various covalent modifications like methylation, acetylation, phosphorylation, ADP-ribosylation. Such modification of histone protein changes their interaction with DNA and hence chromatin structure (euchromatin *vs* heterochromatin). This certainly has effect on gene transcription and DNA replication.
- Phosphorylation of protein occurs at specific amino acid like serinyl, threonyl or tyrosyl by protein kinase. Such phosphate group may be removed by protein phosphatase enzyme. In certain enzymes, the addition of a phosphate group to a specific amino acid residue dramatically enhances or depresses the enzymatic activity.
- Other residues which may be target for phosphorylation may be histidyl, lysyl, arginyl and aspartyl residue.

List of enzymes where catalytic activity is altered by phosphorylation/dephosphorylation is given in Table 2.3.

TABLE 2.3 List of enzymes active in phosphorylated and dephosphorylated states	
Active in phosphorylated state	Active in dephosphorylated state
Glycogen phosphorylase	Acetyl CoA carboxylase
Citrate lyase	Glycogen synthase
Phosphorylase β kinase	PDH
HMG-CoA reductase kinase	HMG CoA reductase
Fructose-2,6-bisphosphatase	PFK-2
Hormone sensitive lipase	Pyruvate kinase

- Most common types of covalent modification are phosphorylation, dephosphorylation and acetylation and deacetylation. Other types are glycosylation, hydroxylation and prenylation.
- Protein phosphorylation may have following effect on effect of protein:
 - Catalytic efficiency of the enzyme may get effected.
 - Alteration of protein location in the cell
 - Susceptibility of protein for degradation
 - Response to allosteric regulator may vary.

Compartmentalization

Sometimes the various enzymes of a pathway are distributed in different compartments of the cell. For example, enzymes of urea biosynthesis, heme biosynthesis and gluconeogenesis are distributed both in mitochondria and cytosol. Such kind of physical barrier which separates the enzyme of a pathway in different compartments of the cell helps in better regulation of enzymes.

ISOENZYMES

Isoenzymes are different molecular forms of enzymes that may be isolated from the same or different tissues.

Isoenzymes are physically distinct and separable forms of given enzymes.

Types of Isoenzymes

- True isoenzymes**: Here the genes of isoenzymes are different which may be located on same or different chromosomes.
 - Malate dehydrogenase isoenzymes (cytosolic and mitochondrial), are derived from different genes located on the same chromosome.
 - Salivary and pancreatic amylase are derived from different genes located on different chromosomes.

TABLE 2.4 Hybrid isoenzymes	
LDH1	HHHH
LDH2	HHHM
LDH3	HHMM
LDH4	HMMM
LDH5	MMMM
CPK-1	BB
CPK-2	MB
CPK-3	MM

- b. **Hybrid isoenzymes:** Here isoenzymes are made up of more than two subunits which are different. It is a varied subunit combination which gives rise to different isoenzymes, e.g. LDH isoenzymes, CPK isoenzymes (LDH is made up of four subunits either of all H, or all M or HM in varied combination) (Table 2.4).
- c. **Allozymes/Allelozymes:** Here isoenzymes are derived from different alleles of the same gene, e.g. G6PD. There are more than 300 alleles known for G6PD of human species.
- d. **Isoforms:** These isoforms are derived after different post-translational modifications, e.g. sialic acid content of ALP in various isoenzymes is different.

Characteristics of Isoenzymes

1. Same function/biochemical role
2. Different structure
3. Different electrophoretic mobility
4. Different immunological characteristics
5. Different affinity to substrate
6. Different K_m value.

Certain Definitions

1. **Specific activity of enzyme:** On separation of enzyme from a protein mixture it is important to assess the purity of preparation. This is assessed by 'specific activity of enzyme'. 'Specific activity of enzyme' is defined as 'number of enzyme units in each mg of separated protein'. Its unit is IU/mg. Higher the specific activity of the enzyme, purer the preparation is.
2. **Katal:** One katal is defined as 'amount of enzyme which is required to convert one mole of substrate to its product in one second time'.

3. **International unit:** One IU is defined as amount of enzyme required to convert one mole of substrate to its product in one minute duration.

Relation between IU and Katal

1 IU = 60 millikatal (m kat)

We need to have lesser amount of enzyme in one IU compared to amount of enzyme required to make one Katal unit.

ENZYMES IN DIAGNOSTICS AND THERAPEUTICS

In addition to their role in enhancing the rate of reaction, enzymes also are used in diagnosis and therapeutics (Table 2.5).

TABLE 2.5 Enzymes in diagnostics and therapeutics	
Enzymes in diagnostics	Used for
Alanine aminotransferase (ALT)	• Hepatocyte damage
Aspartate aminotransferase (AST)	• Hepatocyte damage • Myocardial infarction
Amylase	• Pancreatic disease
Alkaline phosphatase (ALP)	• Hepatobiliary disorder • Bone disease
Acid phosphatase	• Prostate cancer
Prostate specific antigen (PSA)	• Prostate cancer
Creative kinase	• MI muscle dystrophy
Enzymes in therapeutics	Used for
Streptokinase • Bacterial asparaginase	To lyse blood clot In treatment of leukemia
Enzymes in analysis	Used for
GDD-POD	Glucose estimation
Uricase	Uric acid estimation
Urease	Urea estimation
CHOD and POD	Cholesterol estimation

EXERCISE

LONG QUESTIONS (10 MARKS EACH)

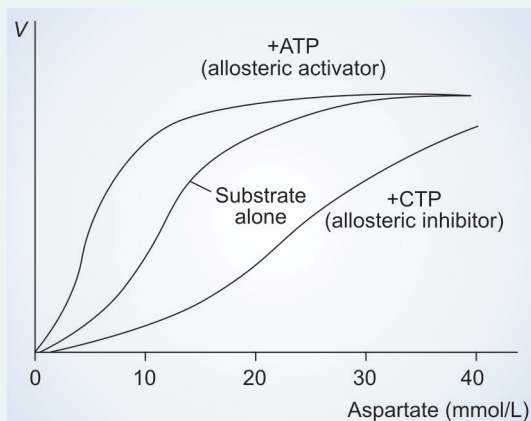
- Q 1. Describe different types of inhibition of enzymes. Show their effect on enzyme kinetics with the help of double reciprocal graph.
- Q 2. What are isoenzymes? How many varieties of isoenzymes you know? Describe the diagnostic significance of the isoenzymes of lactate dehydrogenase.

SHORT NOTES (5 MARKS EACH)

- Q 1. Profile of serum enzymes in diagnosis of myocardial infarction
- Q 2. Classification of enzymes (IUPAC system) with two examples in each class
- Q 3. Diagrams of double reciprocal plot (Lineweaver-Burk plot) of enzyme activity *vs* substrate concentration in presence and absence of a noncompetitive inhibitor of an enzyme and mark $1/V_{\max}$ and $-1/K_m$ on the diagrams
- Q 4. Michaelis Menten equation and its importance
- Q 5. Isoenzymes and their importance in the diagnosis of myocardial infarction
- Q 6. Differences between cofactors and coenzymes
- Q 7. Covalent modification of enzymes and its metabolic significance, using glycogen turnover as an example
- Q 8. Factors affecting rate of enzyme catalysed reaction
- Q 9. Role of metal ions in enzyme catalysis
- Q 10. Enzyme inhibition, two examples of competitive inhibition and any two drugs which are based on competitive inhibition
- Q 11. General properties of an allosteric enzyme and one example of the reaction catalysed by an allosteric enzyme

MULTIPLE CHOICE QUESTIONS

2.1 The substrate saturation curve given below is the characteristic of allosteric enzyme. True statement is:



- a. Allosteric modifier binds in a concentration dependent manner
- b. Modifier can affect the catalytic site by binding to the allosteric site
- c. Adding more substrate to the enzyme can displace the allosteric modifier
- d. Allosteric modifiers change the binding constant of the enzyme but not the velocity of reaction

2.2 Activator of sulphite oxidase is:

- a. Molybdenum
- b. Copper
- c. Selenium
- d. Zinc

2.3 Treatment of multiple carboxylase deficiency is:

- a. Biotin
- b. Pyridoxine
- c. Thiamine
- d. Folic acid

2.4 All are true about oxygenase enzymes, except:

- a. Incorporate one oxygen atom in the substrate
- b. Incorporate two oxygen atoms in the substrate

- c. Involved in hydroxylation reaction
- d. Involved in carboxylation of drugs

2.5 All of the following enzymes are regulated by calcium or calmodulin, except:

- a. Adenylate cyclase
- b. Glycogen synthase
- c. Guanylyl cyclase
- d. Hexokinase

2.6 The predominant isoenzyme of LDH in the cardiac muscle is:

- a. LDH-1
- b. LDH-2
- c. LDH-3
- d. LDH-5

2.7 All are nonfunctional enzymes, except:

- a. Alkaline phosphatase
- b. Acid phosphatase
- c. Lipoprotein lipase
- d. Gamma glutamyl transpeptidase

2.8 Refsum's disease is due to deficiency of which of the following enzymes?

- a. Malonate dehydrogenase
- b. Thiophorase
- c. Succinate thiokinase
- d. Phytanic acid alpha oxidase

2.9 Which of the following enzymes is active in dephosphorylated state?

- a. HMG-CoA reductase
- b. Glycogen phosphorylase
- c. Glycogen phosphorylase kinase
- d. Citrate lyase
- e. Glycogen synthase

2.10 Zinc is a cofactor for:

- a. Pyruvate dehydrogenase
- b. Pyruvate decarboxylase
- c. α -ketoglutarate dehydrogenase
- d. Alcohol dehydrogenase

ANSWERS

2.1 (b) Modifier can affect the catalytic site by binding to the allosteric site

- Allosteric enzyme does not bind the modifier in concentration dependent manner as exemplified by sigmoidal shape of such curve.
- Allosteric modifier binds the allosteric site and addition of more substrate as such does not displaces the allosteric modifier from allosteric sites.
- Allosteric modifier changes both the binding constant of the enzyme and velocity of reaction.

2.2 (a) Molybdenum**2.3 (a) Biotin**

Biotin is a water-soluble vitamin and acts as a coenzyme for carboxylase group of enzymes.

2.4 (d) Involved in carboxylation of drugs

- Oxygenases are oxidoreductase class of enzymes where, oxygen is incorporated into the substrate.
- Mono-oxygenase incorporates one atom of the oxygen into the substrate.
- Addition of hydroxyl group is catalysed by mono-oxygenase enzymes.
- Dioxygenase incorporates two atoms of the oxygen into the substrate.
- Carboxylation is catalysed by carboxylase group of enzyme which incorporates CO_2 into the substrate.

2.5 (d) Hexokinase

Following is the list of enzymes which are regulated by calcium or calmodulin:

1. Adenylyl cyclase
2. Guanylyl cyclase
3. Glycogen synthase
4. Phospholipase A2
5. Pyruvate carboxylase
6. Pyruvate dehydrogenase
7. Pyruvate kinase
8. Phosphodiesterase
9. Glycerol-3-phosphate dehydrogenase

2.6 (a) LDH-1

In normal plasma, LDH-2 is more in concentration than LDH-1.

In myocardial infarction level of LDH-1 increases and this leads to altered ratio of LDH isoenzymes. It means LDH-1 becomes more than LDH-2 ($\text{LDH-1} > \text{LDH-2}$).

This altered ratio of the LDH is known as **flipped pattern**.

2.7 (c) Lipoprotein lipase

Nonfunctional plasma enzymes are those which normally do not function/reside in the plasma, rather they come to plasma only due to damage of respective cell where they are normally reside.

Example:

- Lipoprotein lipase
- Clotting factor
- 5'-nucleotidase

2.8 (d) Phytanic acid alpha oxidase**2.9 (a) HMG-CoA reductase and (e) Glycogen synthase****Enzymes active in dephosphorylated state**

- Glycogen synthase
- Glucokinase
- Phosphofructokinase
- Pyruvate kinase
- HMG-CoA reductase

Enzymes active in phosphorylated state

- Glycogen phosphorylase
- Phosphorylase kinase
- HMG-CoA reductase kinase
- Hormone sensitive lipase
- Citrate lyase

2.10 (d) Alcohol dehydrogenase

Enzymes requiring Zn are:

- a. Carbonic anhydrase
- b. Alcohol dehydrogenase
- c. Carboxypeptidase A and B
- d. ALA synthase
- e. Superoxide dismutase (SOD)
- f. RNA polymerase
- g. ALP
- h. LDH

Carbohydrates and their Chemistry

COMPETENCY BI 3.1

At the end of this chapter learner should be able to discuss and differentiate monosaccharides, disaccharides and polysaccharides giving examples of main carbohydrates as energy fuel, structural element and storage in the human body.

Specific Learning Objectives

- BI 3.1.1** Describe the classification of carbohydrates.
- BI 3.1.2** Discuss and differentiate monosaccharides, disaccharides and polysaccharides.
- BI 3.1.3** Enumerate various carbohydrates which play structural role.
- BI 3.1.4** Enumerate various carbohydrates which play role in energy production.
- BI 3.1.5** Enumerate various carbohydrates which play storage role.

CARBOHYDRATE

Carbohydrates are aldehyde or keto derivatives of polyhydroxy alcohols or substances which yield such compounds on hydrolysis.

Glycerol having three hydroxyl groups is the parent alcohol from which carbohydrates are derived.

Classification

Carbohydrates are classified based on number of individual monosaccharide units derived from complete hydrolysis of the carbohydrate compound.

Accordingly they are classified into following four major groups:

- 1. Monosaccharides $[C_n(H_2O)_n]$:** They are carbohydrates which cannot be further hydrolyzed into simple carbohydrates. Monosaccharides are subdivided further depending on (i) number of carbon atoms and (ii) presence of aldehyde/ketone group (Table 3.1).

TABLE 3.1 Monosaccharides

General formula	Aldosugar	Ketosugar
Trioses	Glyceraldehyde	Dihydroxyacetone
Tetroses	Erythrose	Erythrulose
Pentoses	Ribose	Ribulose
Hexoses	Glucose	Fructose
Heptoses	Glucoheptose	Sedoheptulose
Nanoses	Sialic acid (NANA)	—

- 2. Disaccharides $[C_n(H_2O)_{n-1}]$:** Yield two molecules of same/different monosaccharides on hydrolysis (Fig. 3.1 and Table 3.2).

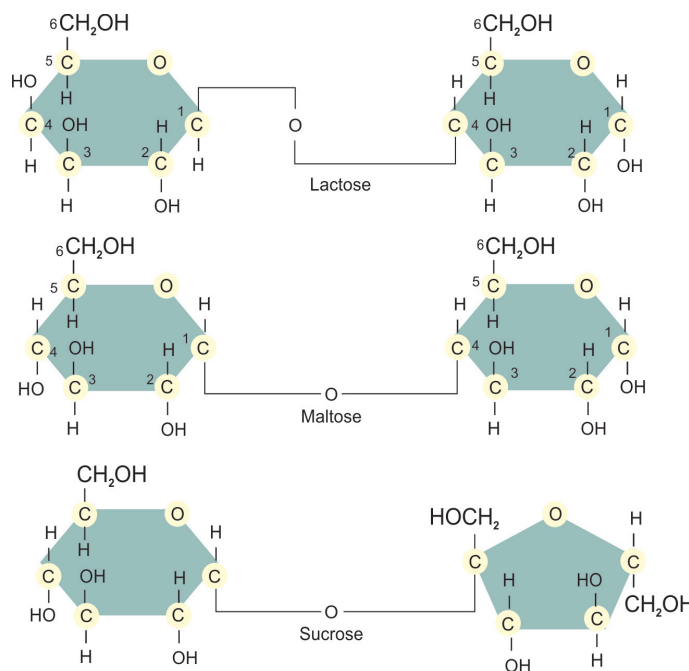


Fig. 3.1: Disaccharides

TABLE 3.2 List of disaccharides

Disaccharides	Reducing (R) or nonreducing (NR)	Individual monosaccharide units	Bonds
Sucrose	NR	Glucose + Fructose	α -D-glucopyranosyl β -D-fructofuranoside
Trehalose	NR	Glucose + Glucose	α -1,1-glycosidic
Maltose	R	Glucose + Glucose	α -1,4-glycosidic
Isomaltose	R	Glucose + Glucose	α -1,6-glycosidic
Lactose	R	Glucose + Galactose	β -1,4-glycosidic
Lactulose	R	Fructose + Galactose	β -1,4-glycosidic

3. **Oligosaccharides:** Yield 3–10 molecules of monosaccharide units on hydrolysis.

4. **Polysaccharides:** Yield more than 10 molecules of monosaccharides on hydrolysis.

Polysaccharides may be classified as homopolysaccharides or heteropolysaccharides depending on whether the same or different individual monosaccharides are produced on complete hydrolysis of the compound.

- Homopolysaccharides (homoglycans):** Polymers of same monosaccharide units, e.g. starch, glycogen, inulin, cellulose, etc. (Table 3.3).
- Heteropolysaccharides:** Mucopolysaccharides are important example of heteropolysaccharides (HPS). They are also known as glycosaminoglycans (GAG).

Other examples of heteropolysaccharides are:

- Heparin
- Heparan sulphate
- Chondroitin sulphate
- Dermatan sulphate
- Keratan sulphate I and II
- Hyaluronic acid

Important characteristics of various heteropolysaccharides are given in Table 3.4.

ISOMERS

Compounds which have same chemical formula but differ in their spatial configuration are known as stereoisomers. Number of possible isomers depends on number of asymmetric carbon atoms (n). If a compound has ' n ' number of asymmetric C-atom, it will have total n^2 stereoisomers.

Types of Isomers

- D- and L-isomers:** In this type of isomers the difference in the structure is in the orientation of $-H$ and $-OH$ groups around carbon atom adjacent to terminal primary alcohol group (penultimate carbon/reference carbon atoms). If $-OH$ group on this carbon atom is towards right, carbohydrate is called D-isomer, when $-OH$ group is on left, it is a member of L-series (Fig. 3.2).

Optical activity: Presence of asymmetric carbon atom confers optical activity on the compound. When a

TABLE 3.3 List of homopolysaccharides

Homopolysaccharides	Units of monosaccharides	Bonds
Starch	Glucose	α -1,4 and α -1,6 glycosidic bond
Glycogen	Glucose	α -1,4 and α -1,6 glycosidic bond
Cellulose	Glucose	β -1,4 glycosidic bond
Inulin	Fructose	β -1,2 glycosidic bond
Dextran	Glucose	α -1,6 glycosidic bond α -1,4 glycosidic bond α -1,3 glycosidic bond
Chitin	N-acetyl D-glucosamine	β -1,4 glycosidic bond

beam of plane polarized light is passed through a solution exhibiting optical activity, it will be rotated to right/left:

- If rotated to right, the compound is called dextrorotatory (*d* or + sign),
- When rotated to left, compound is called levorotatory (*l* or –sign).

Racemic mixture: When equal amount of dextrorotatory and levorotatory isomers are present in a mixture, the

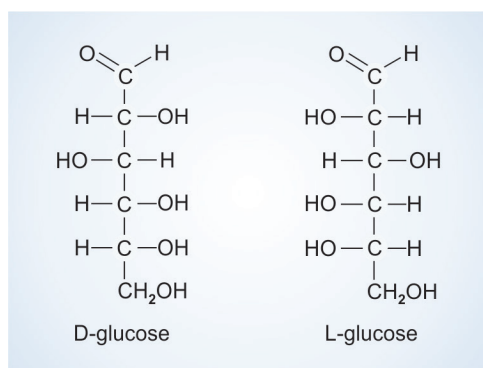


Fig. 3.2: D- and L-isomers of glucose

resulting mixture has no net optical activity, such a mixture is called racemic mixture.

2. **Aldo-keto isomers:** If the reactive group of the carbohydrate is aldehyde, it is an aldose and if the reactive group of the carbohydrate is ketone, it is a ketose (Fig. 3.3).
3. **Pyranose and furanose ring structures:** Pyranose is heterocyclic, hexacyclic ring structure and furanose ring is heterocyclic pentacyclic ring structure. In physiological state 99% of the glucose is in pyranose form, and 99% of the fructose is in furanose form (Fig. 3.4).

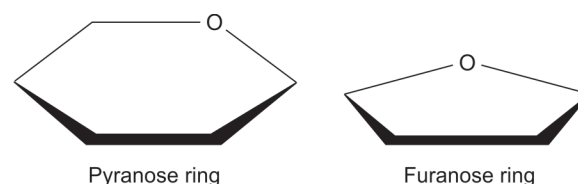


Fig. 3.4: Pyranose and furanose rings

4. **Alpha and beta anomers:** In the ring structure form, if the –OH group on anomeric carbon atom is above the plane of ring, the anomeric form is β and if –OH

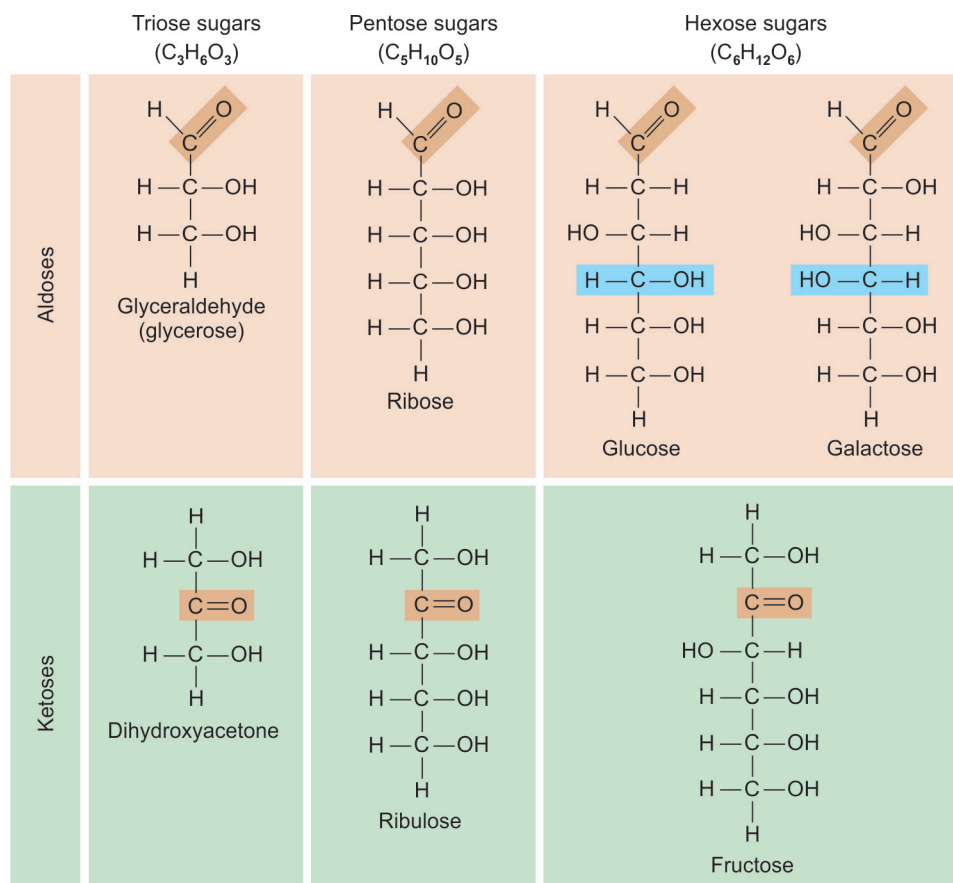


Fig. 3.3: Aldo-keto isomers

TABLE 3.4 Important characteristics of various glycosaminoglycans

Name	Location	Role they play	Remarks
Hyaluronic acid	Skin, synovial fluid, vitreous humor, bone, cartilage, embryonic tissue	Cell migration during morphogenesis and wound healing	No sulphation
Heparin	Mast cell, liver, lung, skin	Anticoagulant (bind factor IX and XI)	Releases lipoprotein lipase from capillary endothelial wall
Heparan sulphate	Skin, kidney, basement membrane	Cell growth, cell-to-cell communication receptor role	IdUA* is also found
Chondroitin sulphate	Cartilage, bone, CNS	Structure of ECM, cartilage	—
Dermatan sulphate	Skin, wide distribution	Blood coagulation, wound repair, resistance to infection	IdUA* is also found
Keratan sulphate I and II	Cornea, cartilage, loose connective tissue	Corneal transparency	No uronic acid

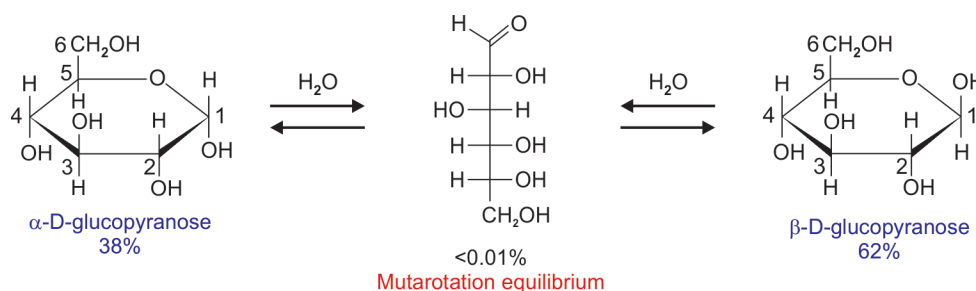
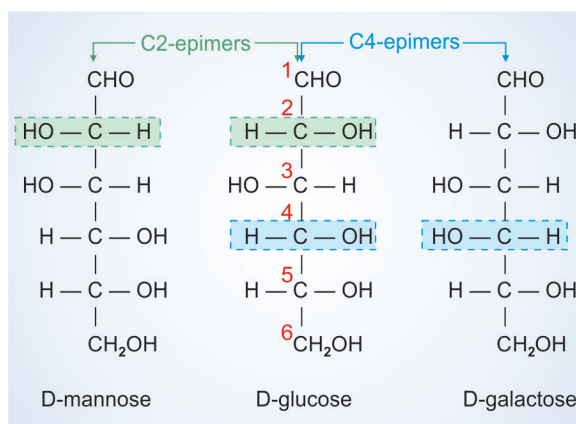
* IdUA: Iduronic acid (it is 5' epimer of 'D' glucuronic acid)

group on anomeric carbon atom is below the plane of ring, the anomeric form is α . Anomeric carbon atom is the carbon at which hemiacetal or hemiketal group is present.

Mutarotation: Interconversion of α and β forms of D-glucose in aqueous medium is known as mutarotation (Fig. 3.5).

5. **Epimers:** Isomers differing as a result of variation in configuration of the hydroxyl group on only one of the asymmetric carbon atoms (carbon 2, 3, 4 or 5) of glucose are known as epimers.

Glucose and mannose are C2-epimers and glucose and galactose are C4-epimers (Figs 3.6a to c).

**Fig. 3.5:** Mutarotation (in glucose)**Fig. 3.6a:** C2 and C4-epimers of glucose

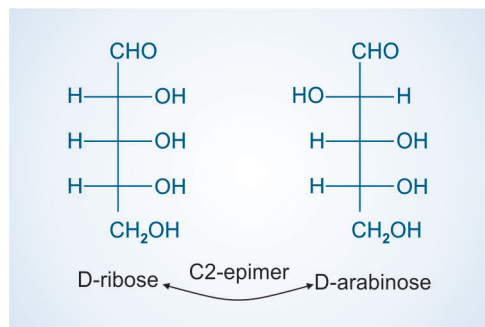


Fig. 3.6b: C2-epimers of ribose

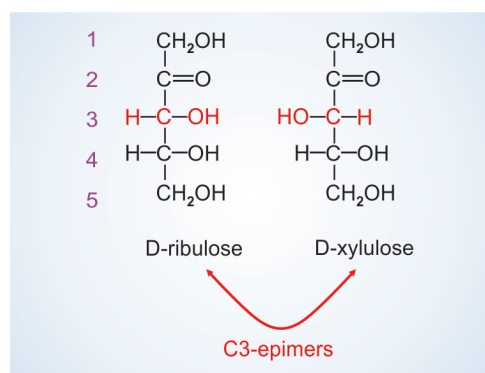


Fig. 3.6c: C3-epimers of ribulose

Aldonic acid: Oxidation of carbonyl (aldehyde) carbon of the glucose produces carboxyl group at that position. This new structure is gluconic acid (Fig. 3.7).

Uronic acid: Oxidation of the last carbon atom of the chain (C6 in case of glucose and other hexoses) produces uronic acid, e.g. glucuronic acid, etc. (Fig. 3.8).

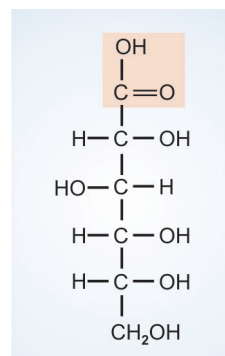


Fig. 3.7: D-gluconic acid (aldonic acid)

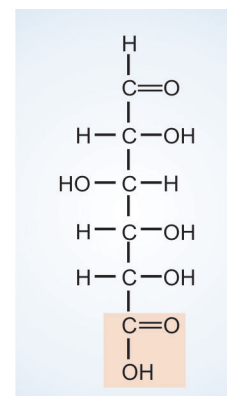


Fig. 3.8: D-glucuronic acid (uronic acid)

Invert sugar: Sucrose is known as invert sugar. Sucrose has specific optical rotation of +66.5°. On hydrolysis sucrose solution yields equimolar mixture of D-glucose (specific rotation of +52.5°) and D-fructose (specific rotation of -92°), this results in net levorotation. Hence, sucrose is known as invert sugar.

In other words, fructose is strongly levorotatory and changes (inverts) the weaker dextrorotatory action of sucrose.

Amino Acids and their Chemistry

Amino acids are the building blocks of the protein. There are 20 standard amino acids.

The general structure of a standard amino acid contains a central alpha (α) carbon atom to which following groups are attached (Fig. 4.1):

- Carboxylic group
- Amino group
- Hydrogen atom
- Side chain (R)

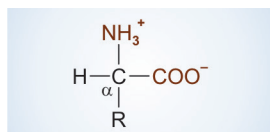


Fig. 4.1: Structure of an amino acid

Proline is the only exception of the above rule where imino group (not the amino group) is attached to the alpha carbon atom.

Stereoisomer of Amino Acid

All amino acids except glycine have two stereoisomers, D and L forms.

In proteins, it is mainly L form of amino acid. D-amino acids are very rare, they are found in bacterial cell wall and antibiotics (Fig. 4.2).

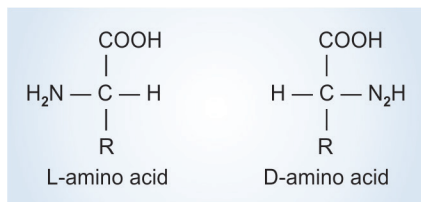


Fig. 4.2: Amino acids

Physical and chemical characteristics of stereoisomers are mostly same, but they differ in their optical activity,

where D form rotates the optical light in right direction and L form rotates the optical light in left direction.

Peptide bond: Amino acids are linked together through peptide bonds to make a long stretch of protein. It is the α -amino group of one amino acid which is linked with the α -carboxyl group of another amino acid to form a peptide bond (Fig. 4.3).

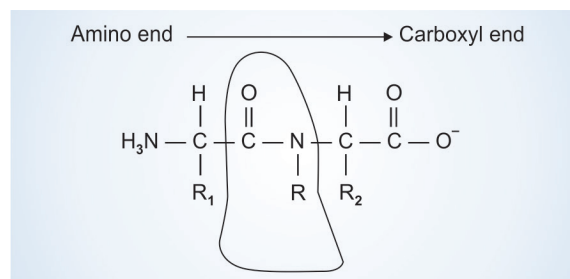


Fig. 4.3: Peptide bond formation

Characteristics of peptide bond

- Partial double bond (distance is 1.32Å)
- Rigid and planar
- The hydrogen of amino group and oxygen of the carbonyl group are *trans* (opposite) in nature, rather than in *cis* (adjacent).
- Uncharged but polar

The free NH_2 group of the terminal amino acids is called N-terminal end and free COOH end is called as C-terminal end.

Ionization of Amino Acids

All standard amino acids have two ionizable groups:

- α -amino group
- α -carboxyl group

In addition to above two ionizable groups, most of the amino acids also have ionizable acid or base group in their side chain.

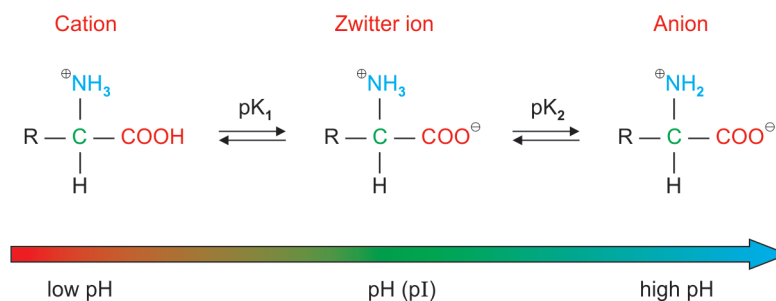


Fig. 4.4: Isoelectric pH (pI) and zwitter ion form

Zwitter ions (ampholyte): Amino acids in solution are predominantly present as dipolar ions (zwitter ions/ampholytes) rather than as unionized molecules. Net charge on the amino acid depends upon the pH of the medium.

pH at which amino acids are electrically neutral (equal positive and negative charges present) is known as isoelectric pH (pI).

At isoelectric pH, amino acids show following characteristics (Fig. 4.4):

1. No net charge.
2. No movement on electrophoresis
3. Minimum solubility
4. Maximum precipitability
5. Least buffering capacity

Each amino acid and protein are having their specific isoelectric pH at which they are in zwitter ion form.

For albumin, isoelectric pH is 4.7. This is the reason of negative charge on albumin at plasma pH (7.4) and at urine pH (6.5).

Structure of a Typical Amino Acid (Fig. 4.5)

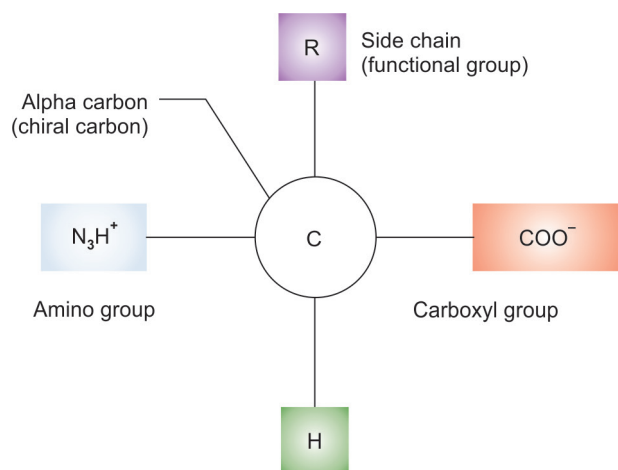


Fig. 4.5: Structure of a typical amino acid

CLASSIFICATION OF AMINO ACIDS (Fig. 4.6)

They are classified based on following four characters:

1. Based on structure
2. Based on side chain character
3. Based on metabolic fate
4. Based on nutritional requirement

1. Classification of Amino Acids Based on Structure

a. Aliphatic amino acids

1. Monoamino monocarboxylic acid

- *Simple:* Glycine, alanine
- *Branched:* Valine, leucine, isoleucine
- *Hydroxyl:* Serine, threonine
- *Sulphur containing:* Cysteine, methionine
- *Amide group containing:* Glutamine, asparagine

2. Monoamino dicarboxylic acid: Aspartic acid, glutamic acid

3. Dibasic monocarboxylic acid: Arginine, lysine

b. Aromatic amino acids: Phenylalanine, tyrosine

c. Heterocyclic amino acids: Histidine, tryptophan

d. Imino amino acid: Proline

e. Derived amino acids: Hydroxyproline, hydroxylysine, ornithine, citrulline, homocysteine.

Special Groups Present in Some Amino Acids (Table 4.1)

TABLE 4.1 Special groups of some amino acids		
Amino acid	Group	Present at following carbon atom
Arginine	Guanidium	δ carbon
Tryptophan	Indole	β
Histidine	Imidazole	β
Proline	Pyrrolidine	α
Tyrosine	Phenol	β

2. Classification of Amino Acids Based on Side Chain Character (Fig. 4.6)

a. Nonpolar

- *Aliphatic*: Glycine, alanine, valine, leucine, isoleucine, methionine, proline.
- *Aromatic*: Phenylalanine, tryptophan.

b. Polar

- *Uncharged*: Serine, threonine, cysteine, asparagine, glutamine, tyrosine.
- *Acidic*: Aspartic acid, glutamic acid
- *Basic*: Arginine, lysine, histidine

3. Classification of Amino Acids Based on Metabolic Fate

- **Purely ketogenic**: Leucine
- **Ketogenic and glucogenic**: PITTL (phenylalanine, isoleucine, tyrosine, tryptophan, lysine)
- **Pure glucogenic**: Rest 14 amino acids are glucogenic.

4. Classification of Amino Acids Based on Nutritional Requirement

Essential

- Threonine
 - Valine
 - Tryptophan
 - Isoleucine
 - Leucine
 - Lysine
 - Phenyl alanine
 - Methionine
- (Pneumo: TV TILL 8 PM)

Semi-essential

- Two amino acids are said to be semi-essential as depending upon physiological status, they are either essential or nonessential.
 - Arginine and histidine are two amino acids which fall in this category.
- Histidine is stored in carnosine in the muscle.

Nonessential

- Rest 10 amino acids are nonessential.

Lipids and their Chemistry

COMPETENCY BI 4.1

At the end of this chapter learner should be able to describe and discuss main classes of lipids (essential/nonessential fatty acids, cholesterol and hormonal steroids, triglycerides, major phospholipids and sphingolipids) relevant to human system and their major functions.

Specific Learning Objectives

BI 4.1.1 Define lipids.

BI 4.1.2 Discuss the classification of lipids.

BI 4.1.3 Describe biochemical importance of various lipids in human body.

WHAT IS LIPID?

- Lipids are heterogeneous groups of compounds which are soluble in nonpolar solvents such as ether, chloroform and benzene and relatively insoluble in water and other polar solvents.
- Lipids are either the esters of fatty acids with the alcohol or are compounds which are capable of forming such esters.

CLASSIFICATION OF LIPIDS

As lipids are heterogeneous groups of compounds, classifying them is little challenging. There is no

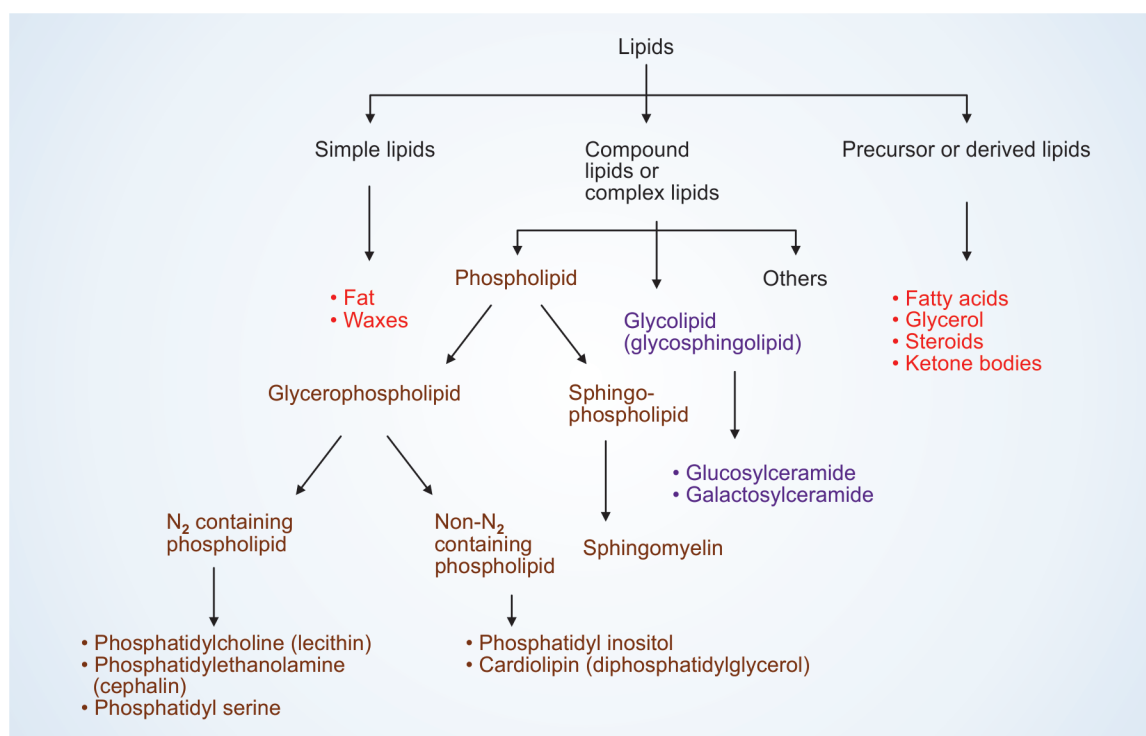


Fig. 5.1: Modified Bloor's classification

internationally accepted classification for the lipid. The most widely accepted classification is Bloor's Classification which is described below:

Modified Bloor's Classification (Fig. 5.1)

Simple Lipids

These are fatty acids esterified with various alcohols. Depending on alcoholic moiety, they are known as either fat or wax.

- Fats or triacylglycerol:** Esters of fatty acids with glycerol. A fat in the liquid state is known as oil.
- Waxes:** Esters of fatty acids with higher molecular weight monohydric alcohols like sphingosine.

Compound or Complex Lipids

Esters of fatty acids containing 'groups' in addition to an alcohol and fatty acids.

Depending upon additional group, complex lipids are further classified into:

- Phospholipids:** Lipids containing a fatty acid, an alcohol and a phosphoric acid residue, e.g. glycerophospholipids and sphingophospholipids.
- Glycolipids:** Lipids containing a fatty acid, alcohol and carbohydrates.
- Other complex lipids:** Sulpholipids, aminolipids and lipoproteins.

Precursor and Derived Lipids

These include fatty acids, glycerol, steroids and ketone bodies.

DESCRIPTION OF AN INDIVIDUAL LIPID COMPOUND

Fat or Triacylglycerol (TAG)

Fatty acids get esterified with the hydroxyl group of glycerol resulting in formation of triglycerides. Triglycerides may be:

- Simple triglycerides:** When all the three fatty acids which are attached to glycerol are same, the resulting triglyceride is simple triglyceride.
- Mixed triglyceride:** When more than one variety of fatty acids is esterified with the glycerol, the resulting triglyceride becomes mixed triglyceride.

TAG is also called neutral fat because esterification of the fatty acid with the glycerol mask the polar group of both the fatty acid and glycerol (carboxyl and hydroxyl group respectively).

Stored TAG provides energy for longer duration compared to stored glycogen, during period of fasting or starvation (Fig. 5.2).

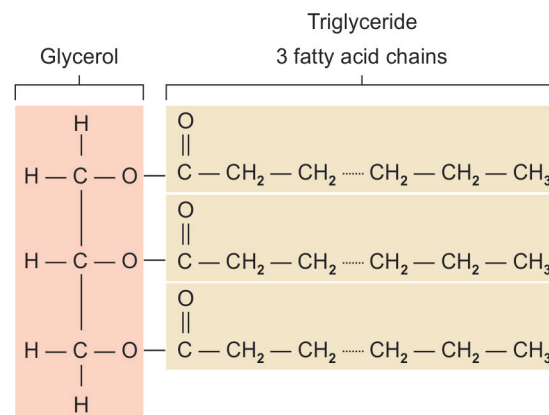


Fig. 5.2: Structure of triacylglycerol

Waxes

Waxes are esters of fatty acids with high molecular weight alcohol like sphingosine. They are also known as ceramide.

Phospholipids

Phospholipids are major constituents of plasma membrane. Phospholipids may be glycerophospholipid or sphingophospholipid depending upon whether they have glycerol or sphingosine as their component alcohol.

Plasmalogens are special types of phospholipids with special structural characteristics which are described later.

A. Glycerophospholipids

Glycerophospholipids are classified into nitrogen containing and non-nitrogen containing phospholipids based on presence or absence of nitrogen in the base moiety of the phospholipid (Fig. 5.3).

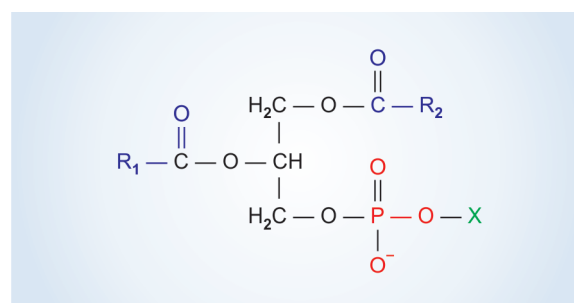


Fig. 5.3: Structure of glycerophospholipid

Nitrogen Containing Glycerophospholipid

1. Phosphatidylcholine (lecithin):

- Most abundant phospholipid of membrane, store of choline (choline is important for nerve transmission and acts as a source of methyl group).

- It is important for lipoprotein synthesis and is also important for esterification of cholesterol.
 - Dipalmitoyl lecithin (DPL) acts as lung surfactant.
2. *Phosphatidylethanolamine (cephalin)*
 - Clotting factor III (thromboplastin) is composed mainly of cephalin
 3. *Phosphatidylserine*

Non-nitrogen Containing Glycerophospholipid

1. *Phosphatidylinositol*: Acts as a precursor of second messenger. Phospholipase C acts on phosphatidyl inositol to convert it to inositol triphosphate and diacylglycerol.
2. *Cardiolipin (diphosphatidyl glycerol)*:
 - Cardiolipin is abundantly found in the inner membrane of mitochondria.
 - This phospholipid has a key role in mitochondrial structure and function.
 - Cardiolipin is antigenic in nature.

- It is thought to be involved in programmed cell death (apoptosis) (Fig. 5.4).

B. Sphingophospholipids

These phospholipids have got sphingosine alcohol, e.g. sphingomyelin. It is further described under the heading sphingolipids.

C. Plasmalogens

Plasmalogens are rare form of phospholipids which are found in brain myelin and cardiac muscle. They have important structural difference compared to a classical glycerophospholipid. Plasmalogens have an unsaturated alcohol linked through ether linkage at S_N1 carbon of glycerol instead of a saturated fatty acid esterified there.

Important examples of plasmalogens are:

- Choline plasmalogen (cardiac muscle)
- Ethanolamine plasmalogens (myelin)
- Platelet activating factor (PAF)

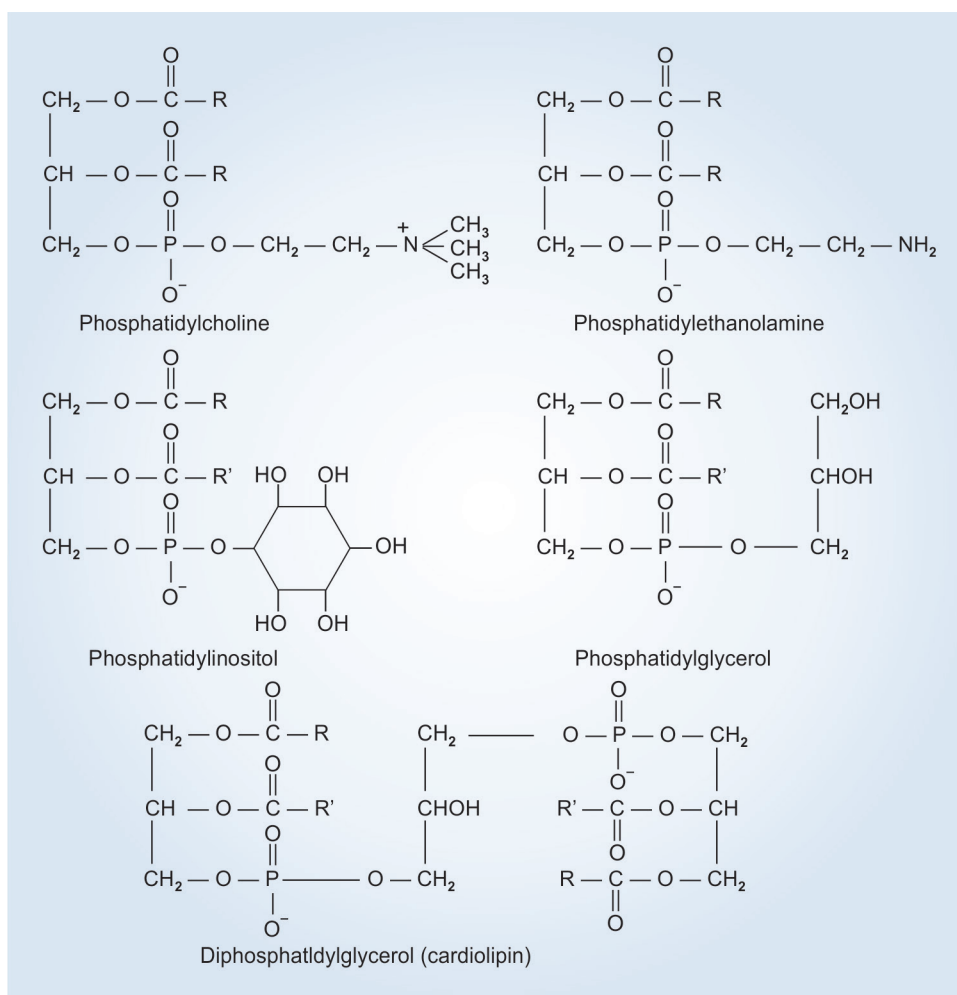


Fig. 5.4: Various phospholipids

Glycolipid (Glycosphingolipid)

Lipids with carbohydrate component are called glycolipids (Fig. 5.5).

They are:

- Cerebrosides** are ceramide monohexosides (e.g. galactocerebroside and glucocerebroside).
 - *Galactosylceramide* abundant in brain and nervous system.
 - *Glucosylceramide* abundant in extraneural tissue.
 - *Sulphatidated cerebroside* that contains sulphated sugars, e.g. sulphogalactocerebroside.
- Globosides** are ceramide oligosaccharides that contain two or more sugar molecules, most often galactose, glucose or N-acetylgalactosamine, attached to ceramide.
- Gangliosides** are glycosphingolipids that contain one or more sialic acid residue mainly N-acetyl derivative of neuraminic acid (Fig. 5.5).

GM1 is the receptor of cholera toxin.

GM3 is the simplest ganglioside.

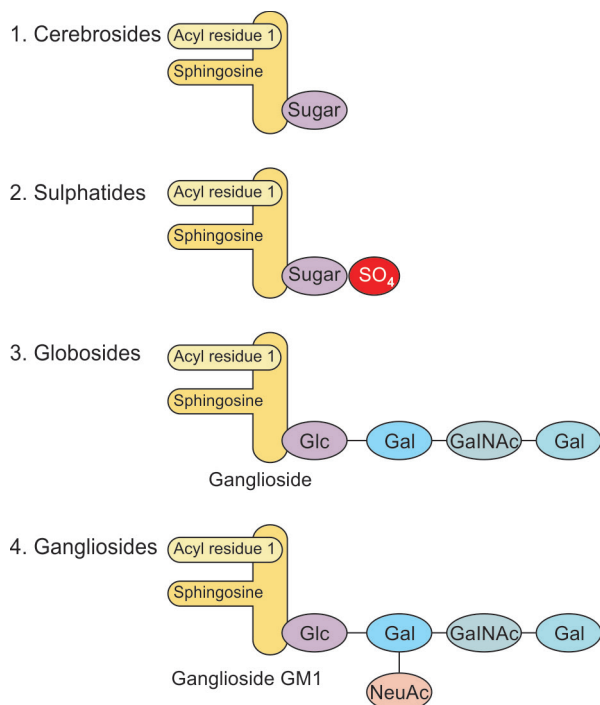


Fig. 5.5: Various gangliosides

Functions of Glycolipids

1. Found at outer leaflet of cell membrane.
2. It is the receptor for various drugs/viruses.
3. It is antigenic (source of blood group antigens).
4. Immunological reaction determinant.

SPHINGOLIPIDS

Sphingolipids are found in central nervous system and specially in white matter. They are those compound lipids which have sphingosine alcohol in them. They are:

- Sphingophospholipids:** Important example is sphingomyelin which on hydrolysis yields a fatty acid, phosphoric acid, choline and a complex amino alcohol, sphingosine (Fig. 5.6).
 - The combination of sphingosine plus fatty acids is known as ceramide.
 - Fatty acid component of sphingomyelin may be:
 - Lignoceric acid
 - Nervonic acid
 - Stearic acid

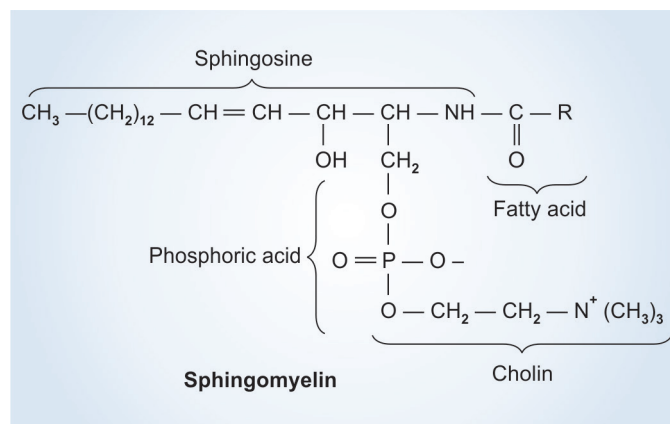


Fig. 5.6: A sphingophospholipid

- Sphingoglycolipid (glycolipids):** Sphingolipids that contain carbohydrates moieties. All glycolipids are infact sphingolipids only. So sphingoglycolipid and glycolipids terminologies are used interchangeably (Fig. 5.7).

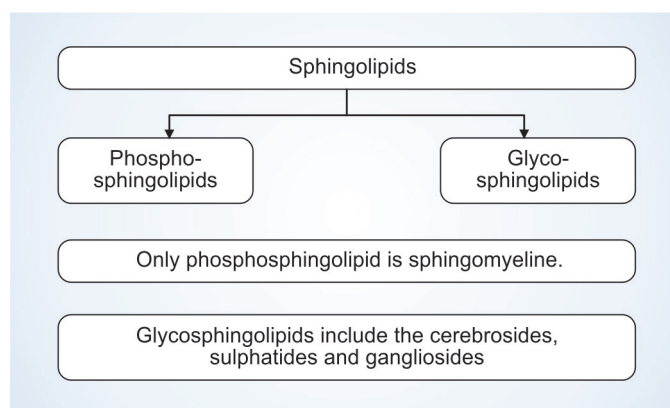


Fig. 5.7: Classification of sphingolipids

Sterols

- Sterols are structural lipids of the membrane.
- Cholesterol is an important sterol in the animal tissue.
- Cholesterol has cyclo-pentano-perhydro-phenantherene ring (CPPP) (Fig. 5.8).
- Cholesterol is found in animal lipid but not in plant lipid.

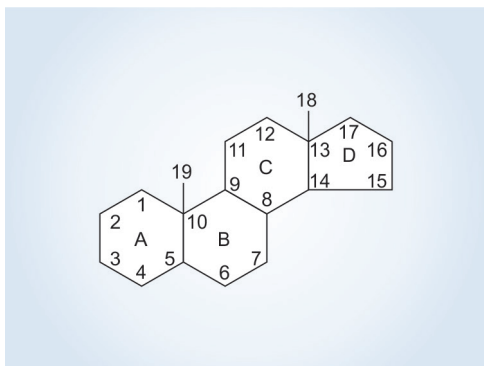


Fig. 5.8: Cyclo-pentano-perhydro-phenantherene ring

Functions of the Cholesterol

1. Formation of cell membrane
2. Cholesterol is the precursor of steroids (progesterone, androgens, estrogens, mineralocorticoids, glucocorticoids)
3. Cholesterol is the precursor of bile acid.
4. Cholesterol is the precursor of vitamin D.

LIPOPROTEINS

Lipoprotein is an important macromolecular complex which acts as vehicle for transport of various lipids in the plasma from one place to other.

It is a water soluble structure having amphipathic shell and nonpolar core (Fig. 5.9).

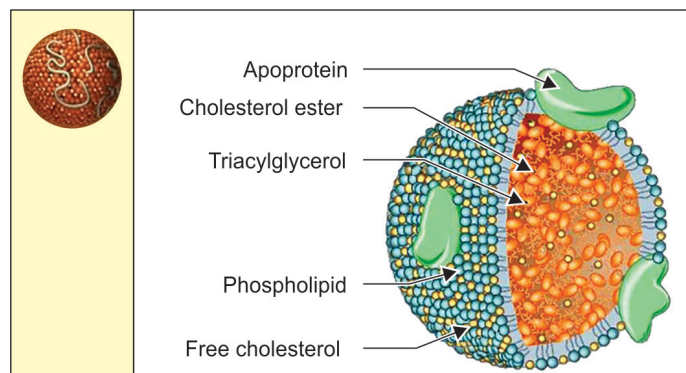


Fig. 5.9: Structure of a typical lipoprotein

Phospholipids and free cholesterol are lipid components of shell which also contain certain protein known as apoprotein or apolipoprotein. Apoproteins may be integrated or peripheral depending upon whether they are embedded in the shell or simply sitting on the surface of the lipoprotein just like a cap.

Core has nonpolar lipids like triacylglycerol and cholesterol ester in varied proportion among various lipoproteins. Some lipoproteins like chylomicron and VLDL have excess quantity of triacylglycerol and others like LDL have excess amount of cholesterol ester in the core section.

Role of Phospholipids in Formation of Micelles, Lipid Bilayers and Liposomes

Micelle

Phospholipids are amphipathic molecules having a polar head and a nonpolar tail. These phospholipids tend to aggregate in the aqueous medium as to make a structure which has a polar head exposed to the surface and a nonpolar tail sandwiched in the center of the structure (Fig. 5.10).

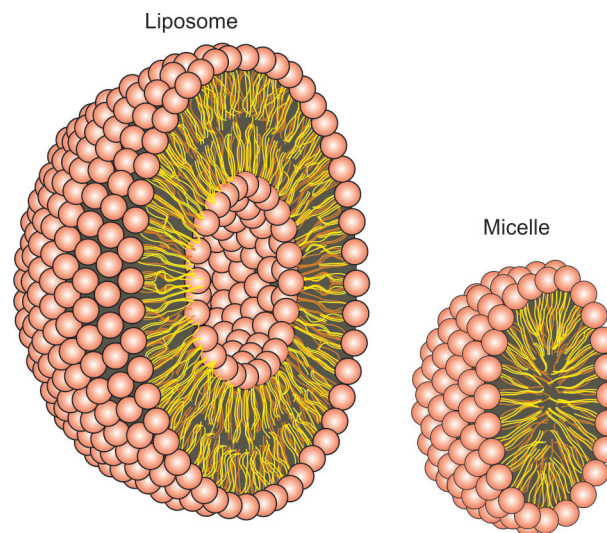


Fig. 5.10: Micelle structure

Lipid Bilayer

Phospholipids are capable of forming lipid bilayers. In this bilayer hydrophobic tail is approximated and polar head is exposed outwards as to face the aqueous media (Fig. 5.11).

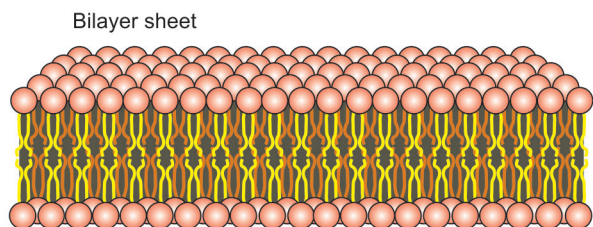


Fig. 5.11: Lipid bilayer

Liposomes

Liposomes are artificial structures which are produced in the laboratories when phospholipids are suspended in the aqueous media and are sonicated (high frequency sound waves are applied) (Fig. 5.12).

These liposomes act as vehicle for transportation of drugs for therapeutic use and DNA for gene therapy. They are injected in patients where lipid bilayer is metabolized by reticuloendothelial cell and the material in the vesicles are delivered locally.

Liposomes are used for following:

- To study the membrane permeability
- To deliver the drugs
- To deliver the DNA for gene therapy

Liposomes are coated sometimes with the specific material as to deliver its content at the precise location.

Fatty Acids

- Fatty acids are aliphatic carboxylic acid which is represented as RCOOH .
- They are amphipathic lipids which means they have both polar (hydrophilic) and nonpolar (hydrophobic) moiety in them.
- Fatty acids are good source of energy as they generate good number of ATP on oxidation.

Classification of Fatty Acids

Fatty acids are classified based on following criteria:

- Length of hydrocarbon chain
 - Odd or even number of carbon atoms
 - Degree of unsaturation
 - Linear/branched chain fatty acid
1. Depending on the length, fatty acid chain may be:
- *Short chain fatty acid (SCFA)*: 4–6 number of carbon atoms.
 - *Medium chain fatty acid (MCFA)*: 8–14 number of carbon atoms.
 - *Long chain fatty acid (LCFA)*: 16–22 number of carbon atoms.
 - *Very long chain fatty acid (VLCFA)*: >24 number of carbon atoms.

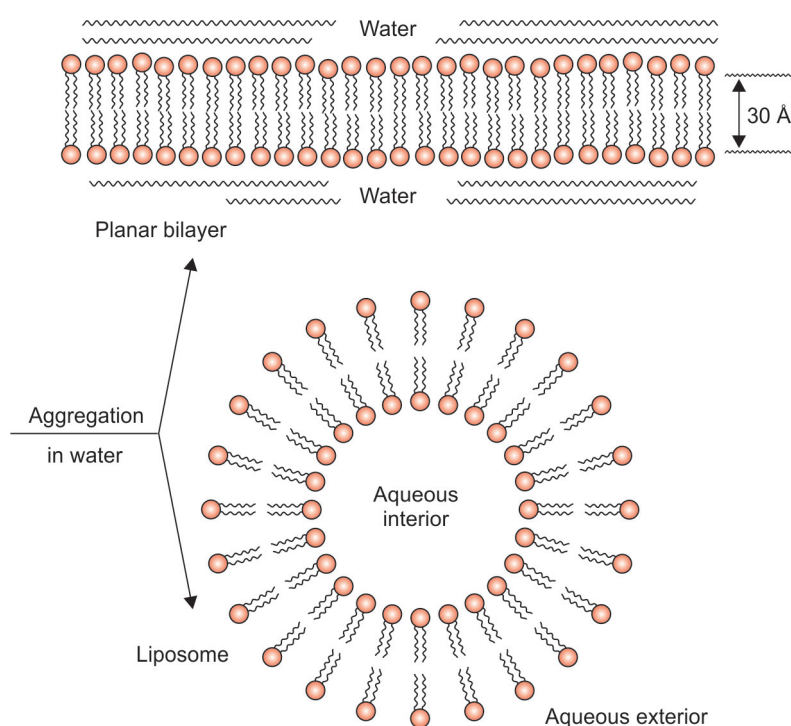


Fig. 5.12: Liposome

- Depending on odd or even number of carbon atoms, fatty acid chain may be:
 - Odd chain fatty acid
 - Even chain fatty acid
- Depending on the degree of unsaturation the fatty acid chain may be:
 - **Saturated fatty acids:** They do not contain double bond in the chain. Suffix is —anoic acid.
 - **Unsaturated fatty acids:** They have one or more double bonds. Suffix is —enoic acid.
- Depending upon presence or absence of branching, the fatty acid chain may be:
 - Linear
 - Branched
 - Cyclic

Isomers of Fatty Acids

Unsaturated fatty acids may be *cis* or *trans* depending on the configuration of hydrocarbon chain beyond the double bond (Fig. 5.13).

- **Cis:** When the fatty acid tail beyond the double bond is organized in the same direction. Naturally occurring fatty acids in human, bacteria and plants are mainly *cis* type.
- **Trans:** When the fatty acid tail beyond the double bond is oriented in the extended fashion.

Trans fatty acids are not naturally found, rather they are produced due to artificial hydrogenation of vegetable oil to make margarine.

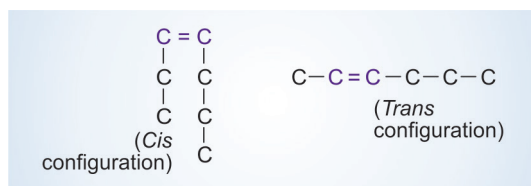


Fig. 5.13: *Cis* and *trans* fatty acids

Numbering System of Carbon in Fatty Acids

Numbering of carbon atom of fatty acid chain is important to delineate the number and the position of double bond in an unsaturated fatty acid. There are two ways described as follows:

- C numbering system:** The carbon atoms are numbered from the carboxyl carbon. Carboxyl carbon is given number 1, further upward the carbon atoms are given number in sequential order 2, 3, 4, 5, 6, etc.
- Omega (Ω) or n numbering system:** The carbon adjacent to carboxyl carbon is known as α-carbon. Carbon atom numbers 3 and 4 are the β and γ, respectively.

- The terminal methyl carbon is known as ω-carbon (Fig. 5.14).

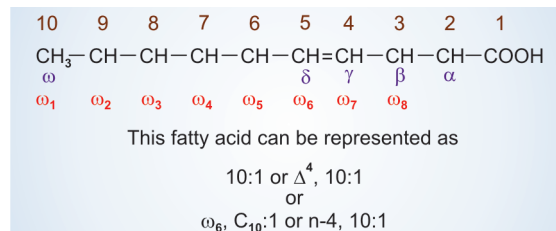


Fig. 5.14: Δ and ω systems of nomenclature

To nomenclate the fatty acid both the systems are utilized.

Delta system: This system of numbering not only tells the position of double bond but also indicates the number of double bonds in a fatty acid, e.g. Δ⁷ fatty acid means, there is a single double bond present between carbon atoms 7 and 8.

Omega system: This system of numbering shows position of first double bond starting from ω end. ω₃ family means that the first double bond is between ω₃ and ω₄ carbon atoms, ω₇ family means that the first double bond is between ω₇ and ω₈ carbon atoms.

ω system of numbering does not give idea about total number of double bonds present in a fatty acid chain.

Some important fatty acids and the ω family they belong to are as follows:

ω ₉	ω ₆	ω ₃	ω ₇
Oleic acid	Linoleic acid	α-linolenic acid	Palmitoleic acid
Elaidic acid	γ-linolenic acid	Timnodonic acid	
	Arachidonic acid	Cervonic acid	

List of predominant fatty acids of the mammalian system, with their number of carbon atoms and double bonds is shown in Table 5.1.

Important dietary sources of linoleic acid are (decreasing order of predominance):

- Safflower oil
- Corn oil
- Sunflower oil
- Soya bean oil

Functions of the Fatty Acids

- From building blocks of phospholipids and glycolipids
- Fatty acid produces prostaglandin.
- Fatty acid is an important source of energy.

TABLE 5.1 List of important fatty acids (with their number of carbon atoms and double bonds)		
Fatty acids with double bond(s)	Numbers of carbon atoms	Number of double bonds
Lauric	12	0
Myristic	14	0
Palmitic	16	0
Stearic	18	0
Palmitoleic	16	1
Oleic	18	1
Linoleic	18	2
Linolenic	18	3
Arachidonic	20	4
Timnodonic	20	5
Cervonic	22	6

Essential Fatty Acids

They are essential fatty acids as they can not be synthesized in the human cell. They are:

- Linoleic acid
- Alpha linolenic acid

Semi-essential Fatty Acid

- Arachidonic acid is a semi-essential fatty acid. It is important to know why arachidonic acid is called semi-essential fatty acid. Dietary linoleic acid is capable of synthesizing arachidonic acid by following a pathway in human cell (Fig. 5.15). When a person is taking an adequate quantity of linoleic acid in the diet, arachidonic acid becomes nonessential, but when a person is deprived of dietary linoleic acid, arachidonic acid becomes essential. Hence, it is called semi-essential fatty acid.

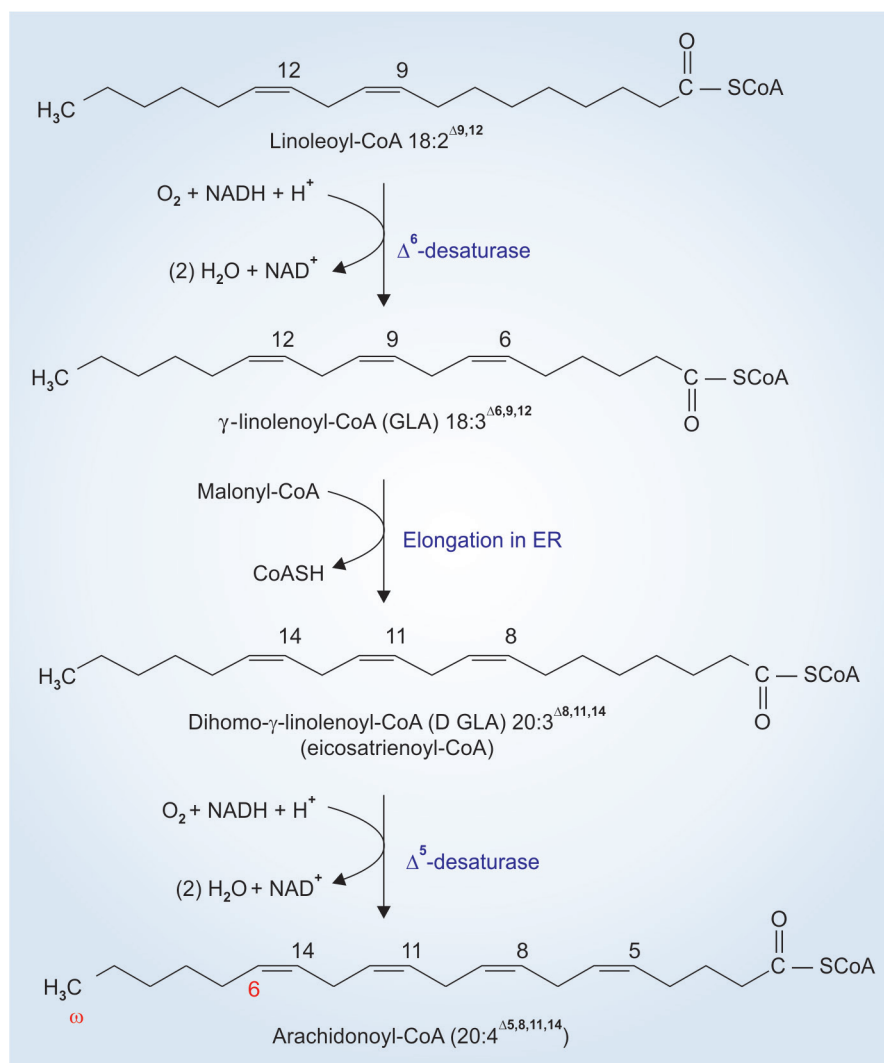


Fig. 5.15: Pathway in human cell to synthesize arachidonic acid from linoleic acid

Functions of Essential and Semi-essential Fatty Acids

- **Important for synthesis of phospholipid of the membrane:** Arachidonic acid constitutes 15% of total fatty acid which may be there in phospholipids.
- **Important for retina and brain development:** Docosahexaenoic acid is derived from fish oil or is synthesized from linoleic acid and is very important for retina and brain development.
- **Important for cholesterol esterification:** Polyunsaturated fatty acid is involved in esterification of cholesterol which helps in its excretion from the body.
- **Precursors for prostaglandins:** Arachidonic acid is an important fatty acid which help in prostaglandin synthesis.

Deficiency of Essential Fatty Acid

Deficiency of essential fatty acid is associated with

- Poor wound healing
- Dermatitis
- $\omega 3$ fatty acid is good for health.

Eicosanoids

Eicosanoids are group of 20 carbon compounds. Various compounds which are collectively named as eicosanoids are (Fig. 5.16):

1. Prostaglandins (PG)
2. Prostacyclins (PGI)
3. Thromboxanes (TX)
4. Leukotrienes (LT)
5. Lipoxins (LX)

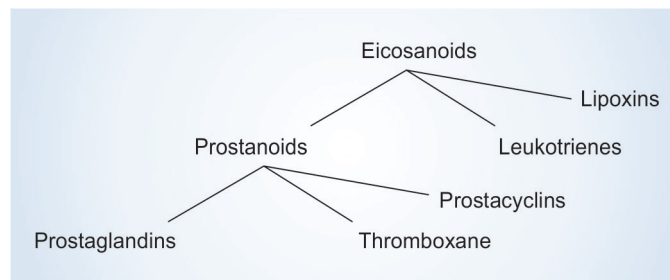


Fig. 5.16: Classification of eicosanoids

There are three groups of eicosanoids which are derived from different fatty acids. For example, group 1 eicosanoids are derived from linoleic acid. Group 2 eicosanoids are derived from arachidonic acid and group 3 eicosanoids are derived from α -linolenic acid.

Collectively eicosanoids play a very important role in human health and disease. For example, in blood clotting, maintenance of blood pressure, muscle contraction, inflammation, pain, asthma, fever, etc. (Table 5.2).

Synthesis of Group 2 Eicosanoids

Arachidonic acid is released from membrane phospholipid by action of phospholipase A₂. Arachidonic acid may enter in cyclo-oxygenase pathway where it undergoes oxidative-cyclization to produce various prostanoids or it may enter in lipoxygenase pathway to produce various leukotrienes and lipoxins (Fig. 5.17).

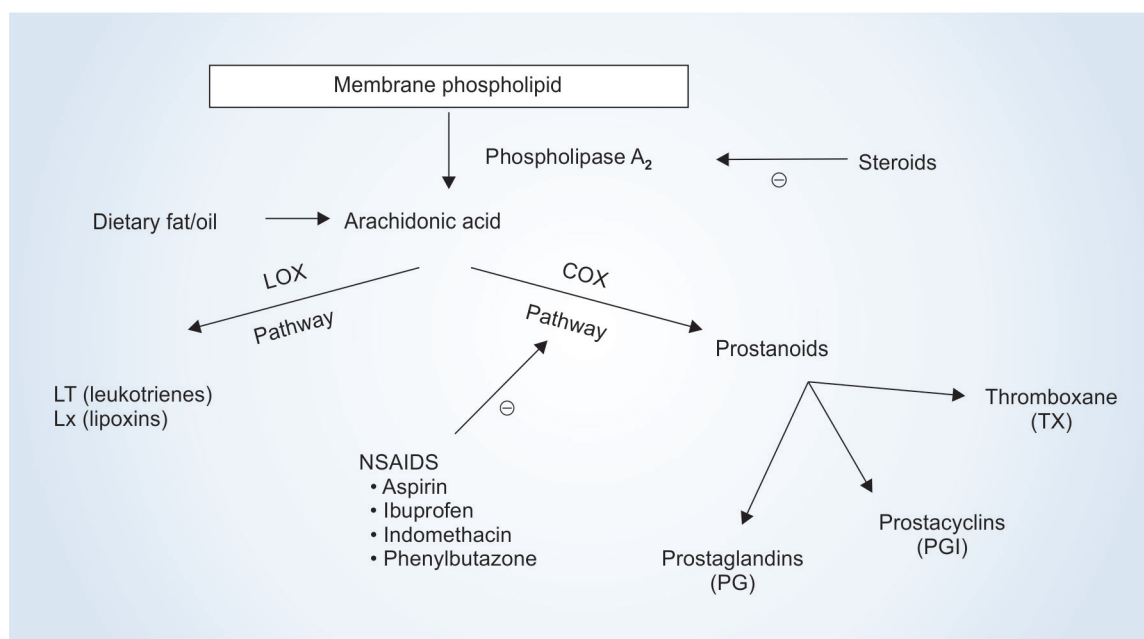


Fig. 5.17: Phospholipase A₂ releasing arachidonic acid from membrane phospholipid

TABLE 5.2 Different groups of eicosanoids and their members				
Group of eicosanoids	Fatty acid which give rise to them	Members		
		Prostanoids	Leukotrienes	Lipoxin**
1.	Linoleic acid	PGE ₁ PGF ₁ TXA ₁	LTA ₃ LTC ₃ LTD ₃	Nil
2.*	Arachidonic acid	PGD ₂ PGE ₂ PGF ₂ PGI ₂ TXA ₂	LTA ₄ LTB ₄ LTC ₄ LTD ₄ LTE ₄	LXA ₄ LXB ₄ LXC ₄ LXD ₄ LXE ₄
3.	α -linolenic acid	PGD ₃ PGE ₃ PGF ₃ PGI ₃ TXA ₃	LTA ₅ LTB ₅ LTL ₅	Nil

* Group 2 eicosanoids are biologically most important group of eicosanoids in human.
 ** Lipoxins are produced only by arachidonic acid.

COX and LOX Pathways of Arachidonic Acid

These pathways are shown in Fig. 5.18.

IMPORTANT CHARACTERISTICS AND BIOLOGICAL ROLE OF VARIOUS EICOSANOIDS

A. Prostaglandin

Though originally isolated from human seminal fluid, prostaglandin now is isolated from all the mammalian tissues. Various prostaglandins (PGs) though differ in their structures, they have certain common structural features like:

1. Hydroxy group at C-15
2. All PGs are having cyclopentane ring
3. 'Trans' double bond at position 13.

Biological Role

1. Prostaglandins induce uterine contraction, hence used for:
 - Induction of labor
 - Termination of pregnancy
 - Prevention of conception
2. Causes vasodilation and hence lowers BP
3. PG inhibits gastric secretion
4. PG decreases intraocular pressure and is used in treatment of glaucoma
5. PG used in treatment of erectile dysfunction
6. PG induces pain, inflammation and fever.
7. Daily excretion of prostaglandin and their metabolites is 1 mg.

COX inhibitors inhibit prostaglandin synthesis and help relieving pain, inflammation and fever.

COX inhibitors are NSAIDS (nonsteroidal anti-inflammatory drugs) like:

- Aspirin (acetylsalicylic acid)
- Ibuprofen
- Indomethacin
- Phenylbutazone
- Naproxen

Aspirin is irreversible inhibitor while other NSAIDS are reversible inhibitors.

Steroids are helpful in relieving pain and inflammation via their inhibitory effect on phospholipase A₂.

B. Thromboxane

Thromboxanes are synthesised in platelets (thrombocytes) and hence the name. They are synthesized from PGH₂ by action of thromboxane synthase.

Thromboxanes have following role:

1. Vasoconstriction
2. Platelet aggregation
3. Mobilization of intracellular Ca⁺⁺.

Aspirin when given in low dose (75–150 mg/day) reduces the risk of thrombus (blood clot) formation as it inhibits thromboxane synthesis and hence prevents platelet aggregation.

Low dose aspirin is therapeutically given in patients of cardiovascular disease to reduce the risk of myocardial infarction.

C. Prostacyclins

Enzyme prostacyclin synthase produces prostacyclins from PGH_2 in vascular endothelial cells.

Prostacyclins inhibit platelet aggregation and leads to vasodilatation.

D. Leukotrienes and Lipoxins (LT and LX)

LT and LX play role in vasoconstriction, bronchoconstriction, vascular permeability, smooth muscle contraction, WBC chemotaxis, etc. and are important in hypersensitivity and inflammation.

LIPIDS AND THEIR DIVERSE ROLE

Lipids play diverse role in biological system ranging from storage form of energy to being a component of plasma membrane.

Following Table 5.3 represents important functions of various members of lipids.

TABLE 5.3 Important lipids and their functions in human body	
Lipid(s)	Function(s)
Triacylglycerol	Storage of energy
Phospholipids, glycolipids, sterols	Structural role
Cholesterol	Precursor of steroid hormone, precursor of vitamin D
Myelin	Electroinsulator
Phospholipids	Activator of enzymes
Phospholipids, bile salts	Absorption of fat soluble vitamins
Bile acid	Digestion of lipid
Bile acid	Absorption of lipid
Phospholipids	Precursor of second messenger

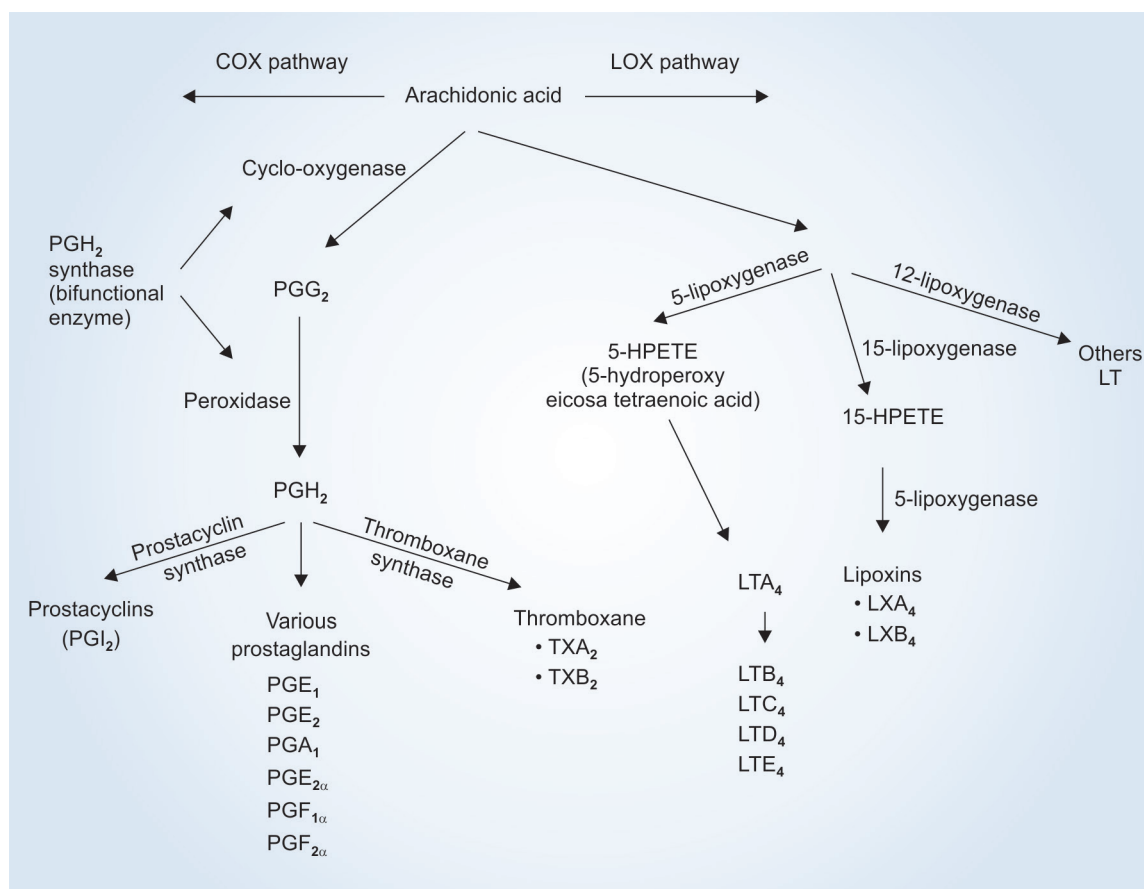


Fig. 5.18: COX and LOX pathways of arachidonic acid