Section 1

Cell, its Organelles and Enzymes

- Biochemical and Clinical Significance of Cell and its Various Organelles
- Enzymes: Various Aspects
- Clinical Enzymology

Chapter 1

Biochemical and Clinical Significance of Cell and its Various Organelles

Competency achievement: After reading this chapter, learner should be able to:

BI1.1 Explain molecular and functional organization of a cell and its subcellular components.

Specific Learning Objectives

BI1.1.1 Enumerate subcellular components of a cell.

BI1.1.2 Describe functions of various components of the cell.

All living cells may be classified into two broad categories: Prokaryotes and eukaryotes.

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 of 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 reactions are same in both prokaryotic and eukaryotic cells. At the same time, they have remarkable differences, e.g. histone protein is found in eukaryotic cells but prokaryotic cells do not contain histone proteins.

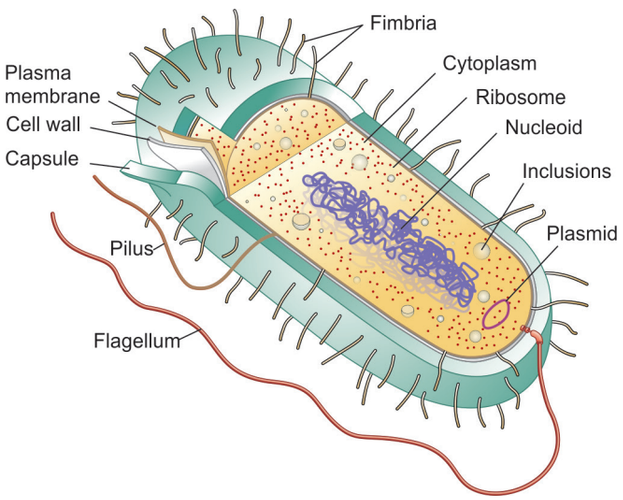


Fig. 1.1: A prokaryotic cell

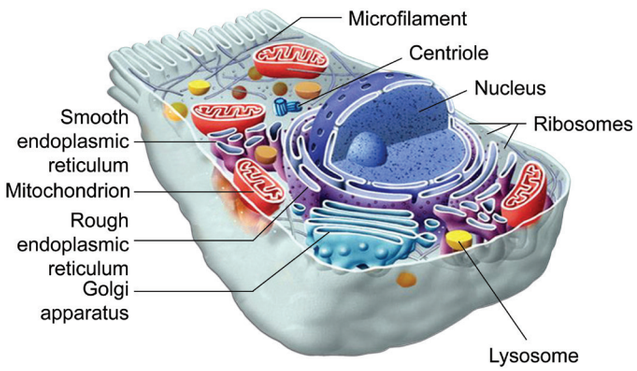


Fig. 1.2: Basic structure of an eukaryotic cell

The differences between prokaryotic cell and eukaryotic cell are mentioned in Table 1.1.

TABLE 1.1 Difference between prokaryotic and eukaryotic cell

Eukaryotic cell	*Prokaryotic cell*
Size is bigger (10–100 μm)	Size is smaller (1–10 μm)
Cell is surrounded by flexible plasma membrane	Cell is surrounded by rigid cell wall
Well-defined nucleus	Nucleus absent, nucleoid present
Cell divison by mitosis	Cell division by fission
Well-defined subcellular organelles	Lack of organelle
Well-defined cytoskeleton (tubules and filament) found in cytosol	Lack of cytoskeleton in cytosol

DESCRIPTION OF INDIVIDUAL ORGANELLES 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 (Fig. 1.1):

1. Nucleus
2. Mitochondria
3. Endoplamic reticulum (ER)
4. Golgi apparatus
5. Peroxisome
6. Lysosome

Nucleus

- It is the largest organelle of the cell (diameter 10 μm).
- Its main functions are 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).
- Perinuclear space (the space between outer and inner membranes 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 forms chromatin. During cell divison chromatin further condenses to form chromosome.
- Nucleus sometimes contains one or more electron dense region(s) known as nucleolus. DNA in nucleolar area contains gene for rRNA.

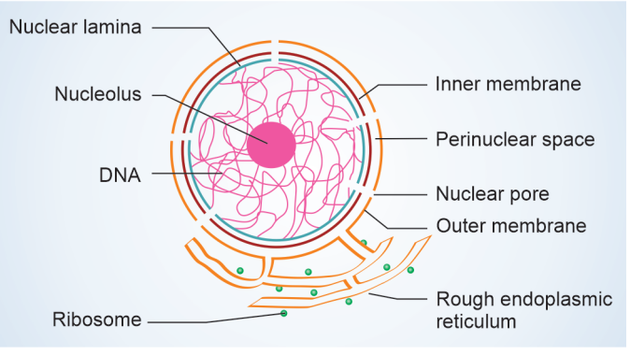


Fig. 1.3: Nucleus

- Nucleus is responsible for DNA replication and transcription of various RNA.
- Nucleus also carries a special metabolic task where NAD^+ is synthesized in the nucleolus from its precursor nicotinamide mononucleatide (NMN^+). 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 transported to the nucleus.

Mitochondria

- Size of a mitochondrion 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, mitochondrion appears 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.
- Human mitochondria contain small circular DNA which has code for 2 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 molecules. Inner membrane of mitochondria contains numerous transporters, which allow transportation of many metabolites.

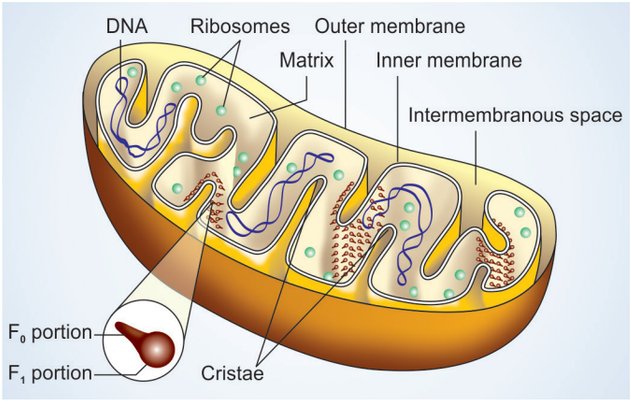


Fig. 1.4: Mitochondrion

- Inner mitochondrial membrane 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 1.2.

TABLE 1.2 Biochemical processes in which different portions of mitochondria are involved

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

Various enzymes and their distribution in mitochondria are summarized in Table 1.3.

- 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.

Inherited Disorders Related to Mitochondrial DNA Mutation

Thirteen out of total 67 proteins required for oxidative phosphorylation are synthesized *de novo* by gene of mitochondrial DNA. Rest other proteins are synthesized in the cytosol coded by nuclear DNA gene; and are then being imported to mitochondria.

As the mitochondrial DNA does not undergo proofreading while replication, rate of mutation is considerably high here.

Following are disorders associated with mutation of mtDNA:

Clinical Correlation

Diseases associated with mutation of mitochondrial DNA

1. *Leber Hereditary Optic Neuropathy (LHON)*

LHON affects central nervous system including optic nerve and it causes sudden onset blindness in early adulthood. This is due to single base change in mitochondrial gene encoding three subunits (ND1, ND4 and ND6) of complex I (ubiquinone oxidoreductase), which lowers the activity of NADH.

Severity of the disease varies according to the amount of mutant mitochondrial DNA. If this amount of mutated mitochondrial DNA is less, patient develops sudden onset blindness and if the amount of mutant mitochondrial DNA is high, then patient develops severe disease characterized by early onset of generalized movement disorder, impaired speech and mental retardation.

2. *Myoclonic Epilepsy with Ragged Red Fibers (MERRF)*

MERRF disease is due to point mutation of gene which encodes **tRNA of lysine**.

This disease is characterized by myopathy, generalized seizures and ataxia. Abnormal-shaped mitochondrion is found in skeletal muscle which has got paracrystalline structures giving the appearance of **ragged red fibers** and hence the name. Cytochrome *c* oxidase activity is also decreased.

3. *Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like Activity (MELAS)*

MELAS is due to point mutation of the gene which encodes **tRNA of leucine**. Here, skeletal muscle also shows ragged red fibers but retains cytochrome *c* oxidase activity.

- This is due to mutation of gene for NADH-CoQ oxidoreductase (complex I) or both complex I and complex IV.
- Characterized by blindness, tremor and ataxia.

Severity of the disease varies according to percentage of mutated mt DNA. Typical central nervous symptoms appear when percentage of mutated mtDNA is >85%. In less severe form, patient may

TABLE 1.3 Various enzymes and thier distribution in mitochondria

Enzymes in outer mitochondrial membrane	*Enzymes in intermembranous space*	*Enzymes on inner mitochondrial membrane*	*Enzymes in the matrix of mitochondria*
Monoamino oxidase	Adenylate kinase	• β-hydroxybutyrate dehydrogenase • Succinate dehydrogenase	• ALA synthase • Protoporphyrinogen IX oxidase • Ferrochelatase • All TCA cycle enzymes except succinate dehydrogenase
Fatty acyl-CoA synthetase		F_0–F_1 ATP synthase (complex V of ETC)	
Carbamoyl phosphate synthetase I	Nucleoside diphosphate kinase	NADH-CoQ oxidoreductase	• PDH complex
Kynurenine hydroxylase		Glycerol-3-phosphate dehydrogenase	• All enzymes of fatty acid β-oxidation ▪ Acyl-CoA dehydrogenase ▪ Enoyl-CoA hydratase ▪ β-hydroxy acyl-CoA dehydrogenase ▪ Thiolase
Nucleoside diphosphate kinase	Creatine phosphokinase (CPK)	Carnitine palmitoyltransferase II Cytochrome *b*, c_1, *c*, *a*, a_3	
Phospholipase A		Adenine nucleotide translocase	• Glutamate dehydrogenase
		Monocarboxylate transporter	• Carbamoylphosphate synthetase I (urea cycle)
		Dicarboxylate transporter	• Ornithine transcarbamoylase (urea cycle)
		Tricarboxylate transporter	

present with deafness, diabetes mellitus, dementia, lactic acidosis and stroke.

4. Mutation of Cytochrome b and Exercise Intolerance

Mutation of cytochrome *b* is not maternally inherited rather it is somatic mutation. In this type of mutation, patient develops exercise intolerance.

Case 1.1: Mitochondrial Encephalopathy, Lactic Acidosis and Stroke (MELAS)

A 14-year-old girl presents with history of severe headache with vomiting for past two days. She complains of generalized weakness and fatigue for past one week. She has weakness in arms and finds it difficult to do her routine work. Mother complains that she has hard of hearing as well. She is born to nonconsanguineous marriage and mother says that she has delayed milestones.

Blood investigation revealed the following results:

Serum lactate: 22.8 mg/dl (normal = 5–18 mg/dl)

Lactate: Pyruvate ratio: 29: 1 (normal = 10:1 to 20:1)

mtDNA mutation was found in the DNA material extracted from peripheral blood from her and her mother showed mutation of nucleotide at position 3243.

Q1. What may be the diagnosis in this case?

Ans. Classical triad of encephalopathy, lactic acidosis and stroke like features are strongly suggestive of mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS). In addition to these features, bilateral neurosensory hearing loss and diabetes mellitus are associated features in this syndrome. In 80% cases of MELAS, there occur A to G transition mutation at 3243 nucleotide in the dihydrouridine arm (DHU) of tRNA for leucine.

Q2. Which all complexes of ETC are affected in various mutations of mitochondrial DNA?

Ans. In event of mtDNA mutation following complexes of ETC will be affected: Complex I, III, IV, V

Total thirteen proteins of ETC are encoded by mitochondrial DNA:

Complex I: 7 proteins

Complex III: 1 protein

Complex IV: 3 proteins

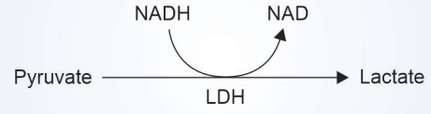
Complex V: 2 proteins

Q3. Which all complexes are involved in mitochondrial disease mentioned in this case?

Ans. In MELAS, the respiratory complex predominantly involved is complex I with secondary involvement of complex IV.

Q4. What is the reason of lactic acidosis in this patient?

Ans. In event of defect in ETC , NADH is accumulated which is not oxidized to the extent as expected. This results in reduction of pyruvate to lactate in following reaction:



Q5. Name the diseases which are associated with mitochondrial DNA mutation.

Ans. Following are the diseases which are linked to mitochondrial DNA mutation.

a. MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke)
b. LHON (Leber hereditary optic neuropathy)
c. MERRF (myoclonic epilepsy with ragged red fibers)
d. Kearns-Sayre syndrome

Various genes of mitochondrial DNA are represented in Fig. 1.5.

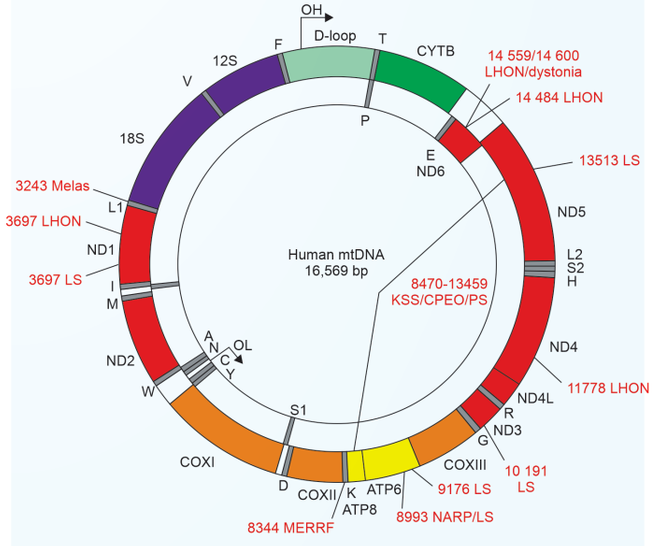


Fig. 1.5: Mitochondrial DNA with its various genes

Q6. What is ragged red fiber?

Ans. Ragged red muscle fiber: It is common to MELAS, Leber hereditary optic neuropathy, myoclonic epilepsy with ragged red fiber, Kearns-Sayre syndrome. It is due to abnormal proliferation of mitochondria in muscle cell under the sarcolemma.

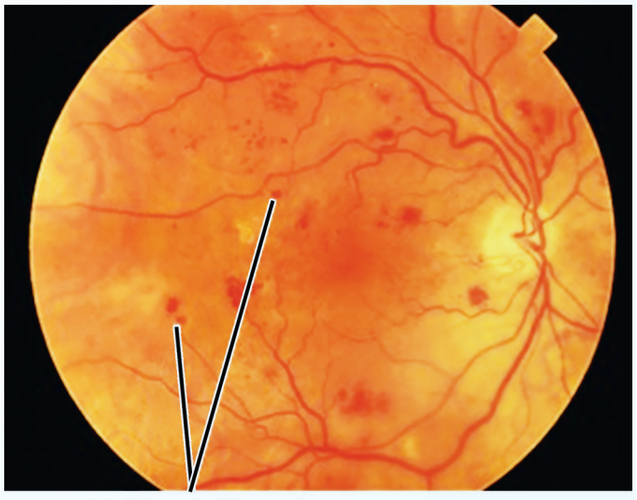
Some patients suffering with MELAS show negative finding on muscle biopsy, specially during early course of the disease.

Case 1.2: Leber Hereditary Optic Neuropathy (LHON)

A 23-year-old male is presenting with sudden onset blindness in both eyes for past one week. Blindness first started as loss of central vision in left eye followed by involvement of right eye within 5 days. He does not have any pain in either of the eyes.

He is nondiabetic, normotensive and nonsmoker on mixed diet. No history of trauma to either eyes.

Diagnosis of LHON (Leber hereditary optic neuropathy) was made based on clinical history and ophthalmoscopic examination which revealed dilated, tortuous, and telangiectatic retinal vessels on and around the disc along with hemorrhagic patches in nerve fiber layer (Fig. 1.6).



Dot/blot intraretinal hemorrhages

Fig. 1.6: Ophthalmoscopic examination

Q1. What is the etiopathogenesis of Leber hereditary optic neuropathy (LHON)?

Ans. Leber hereditary optic neuropathy (LHON) is a mitochondrial genetic disease that preferentially causes blindness in young adult males. LHON affects central nervous system including optic nerve and it causes sudden onset blindness in early adulthood/ young adults. It was first reported by Theodor Leber in 1871.

It is characterized by bilateral subacute loss of central vision owing to focal degeneration of the retinal ganglion cell layer and optic nerve.

Over 95% of LHON cases are primarily the result of one of the three mitochondrial DNA (mtDNA) point mutations, G3460A, G11778A, and T14484C, which all involve genes encoding complex I subunits of the respiratory chain.

Severity of the disease varies according to the amount of mutant mitochondrial DNA. If this amount of mutated mitochondrial DNA is less, patient develops sudden onset blindness and if the amount of mutant mtDNA is high then patient develops severe disease characterized by early onset of generalized movement disorder, impaired speech and mental retardation.

Q2. What are the other diseases associated with mitochondrial DNA mutation?

Ans. Other diseases associated with mitochondrial DNA mutation are:

a. Myoclonic epilepsy and ragged red fiber (MERRF):

MERRF disease is due to point mutation of gene which encodes tRNA of lysine.

This disease is characterized by myopathy, generalized seizures and ataxia. Abnormal-shaped mitochondria is found in skeletal muscle which has got para-crystalline structures giving the appearance of ragged red fiber and hence the name. Cytochrome *c* oxidase activity is also decreased.

b. Mitochondrial encephalopathy lactic acidosis and stroke-like activity (MELAS):

MELAS is due to point mutation of the gene which encodes tRNA of leucine. In this disease also skeletal muscle shows ragged red fibers but retains cytochrome *c* oxidase activity.

Severity of the disease varies according to percentage of mutated mtDNA. Typical central nervous symptoms appear when percentage of mutated mtDNA is >85%. In less severe form, patient may present with deafness and diabetes mellitus.

c. Mutation of cytochrome *b* and exercise intolerance:

Mutation of cytochrome *b* is not maternally inherited rather it is somatic mutation. In this type of mutation patient develops exercise intolerance

Q3. What is the inheritance of mitochondrial diseases?

Ans. Inheritance of mitochondrial diseases is maternal. Affected mother transmits disease to all her children as mitochondria in zygote is coming from ovum.

Pattern of inheritence of mitochondrial disease is represented in Fig. 1.7.

Fig. 1.7: Maternal inheritance of mitochondrial diseases

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.

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. 1.8).

Smooth Endoplasmic Reticulum (SER)

It is not studded with the ribosome and is not involved in the biosynthesis of the protein, rather it is involved in lipid synthesis and detoxification reactions (Fig. 1.8).

Generally, number of SER is small in a cell, but in cells like hepatocyte and Leydig cells it is found abundantly. 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^{2+} signaling.

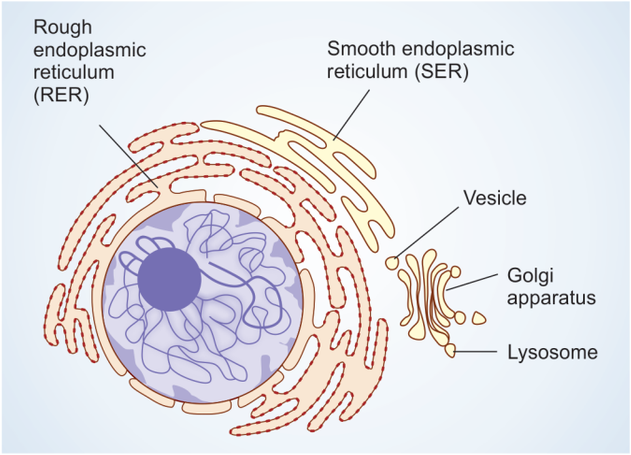


Fig. 1.8: Rough and smooth endoplasmic reticulum

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. There are networks of flattened smooth membrane-bound structures known as cisternae.
- 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. 1.9).

Peroxisomes

They are also called microbodies (not to be confused with microsome, which is produced due to fragmentation of ER during cell fractionation) (Fig. 1.10).

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).

They play a very important role in

1. Very long chain fatty acid (VLCFA) oxidation

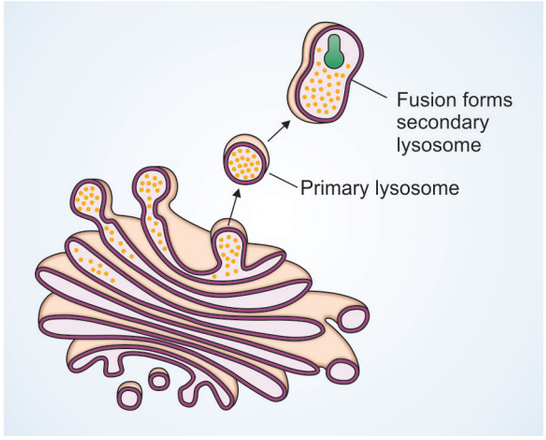


Fig. 1.9: Golgi apparatus

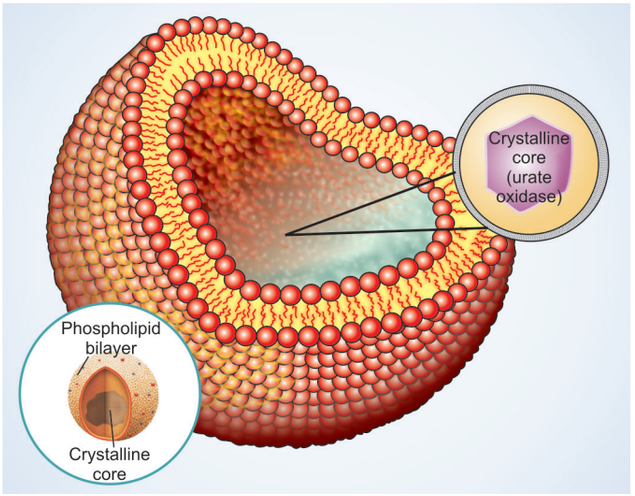


Fig. 1.10: Peroxisome

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.

Clinical Correlation

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 six months of age. 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.

Case 1.3: Zellweger Syndrome

A young child was found to have increased level of very long chain fatty acid, low level of glycerol ether lipid (plasmalogen) and increased concentration of cholestanoic acid (precursor of bile acid) in the blood.

Liver, brain, kidney and skeletal muscle were involved. Baby had hypotonia of lower limb muscles.

Diagnosis of Zellweger syndrome was made.

Q. What is the biochemical defect in this disorder?

Ans. Zellweger syndrome is the most severe presentation of a group of disorder known as peroxisomal biogenesis disorder (PBD). PBD comprises more than 25 genetically-related disorders where there is malfunction of peroxisome either partially or fully.

In **Zellweger syndrome** which is autosomal recessive disorder, lack of peroxisome is seen which results in lack of all the functions for which peroxisome is required, e.g. VLCFA oxidation, bile acid synthesis, branched chain fatty acid oxidation, synthesis of ether lipid (plasmalogen) are all affected.

Intermediate form of PBD is XALD (X-linked adrenoleukodystrophy) where defect lies in VLCFA transporter protein (ALD protein) in the peroxisomal membrane. This affects young boys. In this disease only problem occurs is accumulation of VLCFA due to absence of its transport protein in the membrane. Remaining other functions of peroxisome remain intact.

Refsum disease is the mildest form of PBD where the enzyme 'phytanoyl-CoA hydroxylase' is defective resulting in impaired oxidation of branched chain fatty acid (phytanic acid). This disease presents with severe neurological dysfunction along with blindness and deafness.

Lysosomes

- These organelles are rich in hydrolase class of enzymes (class III) which cleave the carbon–oxygen, carbon–nitrogen, carbon–sulphur, oxygen–phosphorus 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. 1.4).

Clinical Correlation

I Cell Disease (Inclusion Cell Disease or Mucolipidosis II)

I cell disease is an important lysosomal storage disorder. In this disease, lysosomal function is defective due to lack of acid hydrolase enzyme in the lysosome. Acid hydrolase is a glycoprotein which is glycosylated in the Golgi apparatus with the help of enzyme N-acetyl-D-glucosamine phosphotransferase (GlcNAc phosphotransferase).

GlcNAc phosphotransferase helps in generation of mannose-6-phosphate residue on acid hydrolase enzyme which is important for transport of this enzyme within the lysosome.

In inclusion cell disease, this tagging of lysosomal enzyme with mannose-6-phosphate marker is not occurring, resulting in secretion of this enzyme in extracellular compartment rather than targeting this enzyme to lysosomal lumen. Abnormal high level of lysosomal enzyme is thus found in plasma.

This disease is named as 'I cell disease' due to presence of inclusion bodies (Fig. 1.11) in the cells which are cultured from patients. Disease is characterized by psychomotor retardation, bone dysplasia, coarse facies, muscle hypotonia.

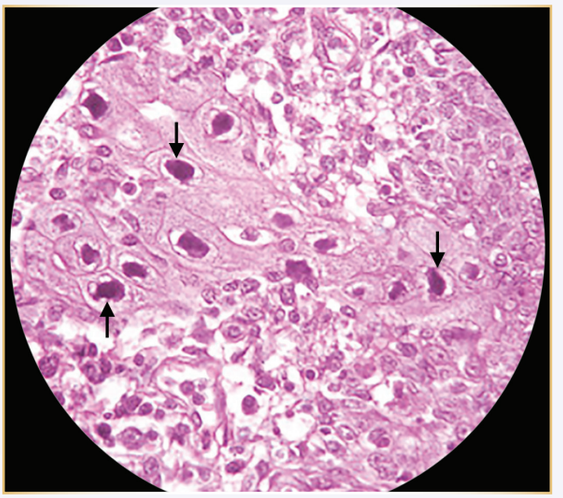


Fig. 1.11: Inclusion bodies

Symptoms are present at birth and death occurs by 8 years of age (Fig. 1.12).

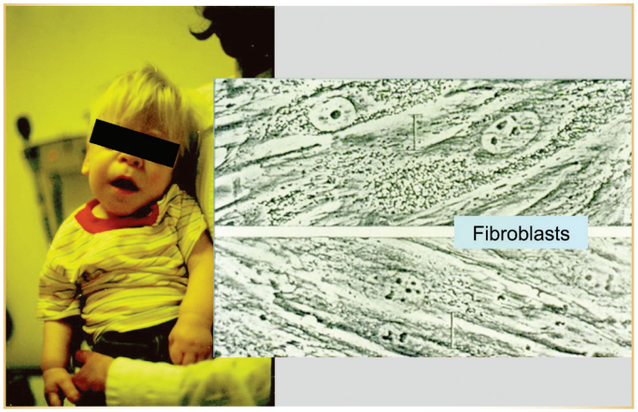


Fig. 1.12: A child with I cell (inclusion cell) disease, fibroblasts having inclusion bodies are shown in inset

Case 1.4: I Cell Disease (Inclusion Cell Disease) or Mucolipidosis II

A nine-month-old child born to consanguineous marriage, presented with severe psychomotor retardation and skeletal anomaly and coarse facial features (Fig. 1.13).

Lysosomal enzyme tests (including alpha-mannosidase, alpha-fucosidase, beta-glucuronidase and beta- galactosidase) were performed on the leukocytes, skin fibroblasts and in the plasma. Activities of all the four enzymes were low in both leukocytes and fibroblasts. Plasma was found to have unusual high level of certain lysosomal enzymes.

The diagnosis of I cell disease (inclusion cell disease) was made.

Fig. 1.13: Coarse facial features of a child with I cell disease

Q1. Comment on the diagnosis.

Ans. I cell disease is a lysosomal storage disorder. It is also known as mucolipidosis II. Enzyme deficient in this disease is 'N-acetyl-D-glucosamine phosphotransferase (GlcNAc phosphotransferse)' which is a Golgi apparatus enzyme responsible for mannose-6-phosphate tagging of lysosomal enzyme which helps in correct localization of these enzymes in the lysosmes.

This disease is characterized by coarse facila features, puffed eyelids, macroglossia, flat nasal bridge, psychomotor retardation, bone dysplasia, muscle hypotonia, and death by 4 to 6 years of age. This is inherited as an autosomal recessive disorder.

Q2. Why lysosomal enzymes are not in lysosome rather they are secreted in plasma? Explain.

Ans. Enzymes found in Golgi apparatus, which are responsible for mannose-6-phosphate tagging of lysosomal enzymes are two, i.e.

a. N-acetyl-D-glucosamine phosphotransferase (GlcNAc phosphotransferse)
b. N-acetyl-D-glucosaminidase

In deficiency of 'N-acetyl-D-glucosamine phosphotransferase (GlcNAc phosphotransferse)', the lysosomal enzymes though are being synthesized normally in rough endoplasmic reticulum are not getting localised in the lysosome, rather are secreted outside the cell due to lack of recognition marker 'mannose-6-phosphate' tagging.

Hence, abnormal high level of lysosomal enzyme is found in plasma.

Q3. Why this disease is nomenclated as 'I cell disease'?

Ans. This disease is named as 'I cell disease' due to presence of inclusion bodies in the cells which is cultured from patients (Fig. 1.14). These inclusion bodies are spherical, dense, cytoplasmic vesicles which are seen around the nucleus and juxtaglomerular Golgi apparatus.

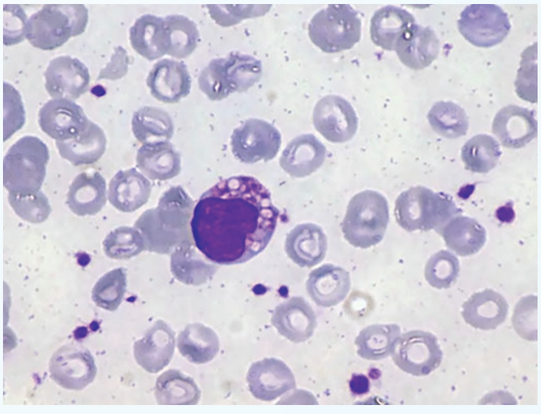


Fig. 1.14: Inclusion bodies

Q4. What is the line of treatment in I cell therapy?

Ans. Therapy is symptomatic. ERT has not proven promising result in this disease. Gene replacement therapy with cDNA carrying lysosmal enzyme encoding genes using retroviral vectors have shown promising results.

Clinical Correlation

Clinical conditions associated with dysfunction of lysosome:

a. **Lung fibrosis in silicosis:** The inhalation of silica particles in lung results in lysosomal membrane damage. The released lysosomal enzyme then tend to stimulate fibroblast which proliferates and deposits collagen fibers in the lung resulting in lung fibrosis and reduced compliance.

b. **Arthritis in gout:** The sodium monourate crystals which are deposited in gout when phagocytosed by phagocyte tend to damage the lysosome which releases its enzyme which causes inflammation and arthritis seen in gout.

c. **Lysosomal cathepsin and tumor metastasis:** Cathepsins are lysosomal proteases which are normally located within the lysosome. These cathepsin are released from their intralysosomal location by certain cancer cells. Released cathepsins tend to damage the basal lamina of cell by hydrolysing elastin and collagen. This helps in tumor metastasis.

d. **Postmortem autolysis:** Rupture of lysosomes after cell death releases all its enzymes and causes postmortem autolysis.

e. **Various lysosomal storage disorders:** There are many diseases which are due to deposition of abnormal quantity of certain biomolecules in lysosome which tend to lack the enzymes responsible for their degradation. These diseases fall under broad category of disorders known as lysosomal storage disorders (LSDs). Some of important LSDs are:

i. All mucopolysaccharoidosis
ii. Pompe disease
iii. All sphingolipidosis
iv. I cell disease (mucolipidosis II)

VARIOUS COMPARTMENTS IN THE CELL AND PATHWAYS WHICH OCCUR IN CELL

There are many pathways which take place in specific compartment/s of the cell. Most of the pathways involve single compartment, but there are pathways which involve multiple compartments.

As exemplified in description above, the summary of functions of various cell organelles is summarized in Table 1.4.

TABLE 1.4 Functions of various cell organelles

Organelle	*Function*
Nucleus	• Contains genetic material
Ribosomes	• Protein synthesis (translation)
Rough endoplasmic reticulum	• Synthesis/modification and transport of proteins and lipids • Folding of protein molecules in sacs called cisternae • Transport of synthesized proteins in vesicles to the Golgi apparatus
Smooth endoplasmic reticulum	• Synthesizes lipids, phospholipids, and steroids • Plays a major role in excitation–contraction coupling in skeletal muscles • Metabolism of carbohydrates, drug detoxification, attachment of receptors on cell membrane proteins, and steroid metabolism
Golgi apparatus	• Processing, distribution of proteins and lipids • Major collection and dispatch station of protein products received from the endoplasmic reticulum • Post-translational modification of proteins
Lysosomes	• Digestion of substances in cell • Contain acid hydrolase enzymes • Digest excess or worn-out organelles, food particles, and engulf viruses or bacteria
Peroxisomes	• Lipid metabolism and detoxification • Breakdown of very long chain fatty acids through beta-oxidation
Centriole	• Cytoskeletal organization

Details of distribution of various pathways in cell compartment are enlisted in Table 1.5.

TABLE 1.5 Various components and pathways which occur in the cell

Cytosol	Mitochondrial matrix	Partly mitochondrial/partly cytosolic	Peroxisome	Smooth endoplasmic reticulum (SER)	Rough endoplasmic reticulum (RER)	Golgi apparatus	Nucleus	Lysosome
Glycolysis	TCA cycle	Gluconeogenesis	Fatty acid α-oxidase	Fatty acid ω-oxidation	Protein synthesis	Post-translational modification of protein	DNA replication Transcription	Protein degradation
HMP shunt	Ketogenesis	Urea synthesis	VLCFA beta-oxidation	Steroid hormone synthesis				Glycogen degradation
Glycogenesis	Ketone body utilization	Heme synthesis	Branched chain fatty acid oxidation	Cholesterol synthesis				
Glycogenolysis	Electron transport chain (ETC)							
Fructose metabolism	Fatty acid β-oxidation							
Galactose metabolism								
Uronic acid pathway								

PLASMA MEMBRANE

Outermost covering of the cell is known as plasma membrane which consists of lipid, protein and carbohydrate. It has numerous functions which are enumerated below:

1. It acts as a barrier around the cell which separates intercellular and extracellular environments.
2. It selectively allows the movement of molecules and ions from both within and outside of the cell.
3. It determines cell morphology and movement.
4. It has receptor role to play.
5. It helps in cell-to-cell recognition.
6. Cell-to-cell interaction and cell communication.
7. It helps in secretion and absorption of various substances.

Detail of structure and function of plasma membrane will be dealt separately in Chapter 9, page 122.

KEYPOINTS

1. Cell may be classified into two broad categories: Prokaryote and eukaryote.
2. Prokaryotic cells are mostly unicellular, characterized by lack of well-defined nucleus and internal membranous structures.
3. Eukaryotic cell is characterized by well-defined nucleus, and other well-defined organelles like mitochondria, lysosomes, peroxisomes, etc. surrounded by membranes.
4. Eukaryotic cell may be single cell (yeast, fungi) or may be multicellular (plants, animals).
5. Nucleus is the largest organelle of the cell (diameter 10 μm). Its main function is the storage, replication and expression of the genetic material.
6. Nucleus sometimes contains one or more electron dense region known as nucleolus. DNA in nucleolar area contains gene for rRNA.
7. In an electron micrograph of an eukaryotic cell, mitochondria appear as a rod, sphere or filamentous body which is surrounded by an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM).
   - Outer membrane is smooth, but inner membrane contains a number of folds or cristae.
   - Between outer and inner membranes, there is intermembranous space.
8. Human mitochondria contain small circular DNA which has code for 2 rRNA, 22 tRNA and 13 proteins. Replication of mitochondrial DNA occurs without proofreading, hence it is very much prone for mutation. There are many diseases associated with mitochondrial DNA mutation.
9. Endoplasmic reticulum is a membrane-bound tubular organelle. There are two types of ER in a cell, rough endoplasmic reticulum and smooth endoplasmic reticulum. This is the site of protein synthesis, lipid synthesis and various detoxification reactions.
10. Golgi apparatus is also known as Golgi complex. They are networks of flattened smooth membrane-bound structures known as cisternae. It is involved in modification and sorting of various proteins and also glycoconjugation.
11. Peroxisome is also called microbodies. These organelles are involved in the production or utilization of hydrogen peroxide. They play a very important role in
    - Very long chain fatty acid (VLCFA) oxidation
    - Synthesis of glycerolipid
    - Synthesis of glycerol-ether lipid (plasmalogen)
    - Synthesis of isoprenoid
12. Lysosomes are rich in enzymes belonging to hydrolase class of enzymes (class III of enzyme classification) which cleave the carbon–oxygen, carbon–nitrogen, carbon–sulphur, oxygen–phosphorous bonds in lipids, protein, carbohydrate and nucleic acid.
13. I cell disease results due to defect in the targeting of newly synthesised lysosomal enzymes to the lysosome. This is due to lack of enzyme 'N-acetyl D-glucosamine phosphotransferase'.

Chapter 2

Enzymes: Various Aspects

Competency achievement: After reading this chapter, learner should be able to:

BI2.1	Explain fundamental concepts of enzyme, isoenzyme, alloenzyme, coenzyme and cofactors. Enumerate the main classes of IUBMB nomenclature.
BI2.3	Describe and explain the basic principles of enzyme activity.
BI2.4	Explain enzyme inhibitors as poisons and drugs and as therapeutic enzymes.
BI2.5	Explain clinical utility of various serum enzymes as markers of pathological conditions.
BI2.6	Use of enzymes in laboratory investigations (enzyme-based assays).

Specific Learning Objectives

BI2.1.1	Define enzymes, isoenzymes, coenzyme, alloenzymes, cofactors.
BI2.1.2	Describe functions of isoenzymes, alloenzymes, and coenzymes.
BI2.3.1	Describe mechanism of action of different enzymes.
BI2.3.2	Describe 'lock and key' hypothesis.
BI2.3.3	Describe Koshland's induced-fit theory.
BI2.3.4	Describe Michaelis-Menten theory.
BI2.4.1	Describe competitive inhibition.
BI2.4.2	Describe noncompetitive inhibition.
BI2.4.3	Describe uncompetitive inhibition.
BI2.4.4	Describe therapeutic action of enzyme inhibition.
BI2.5.1	Describe various enzymes used as diagnostic markers of pathological condition.
BI2.6.1	Enumerate important enzyme-based assays used in lab investigation.

What is an Enzyme?

- Friedrich W. Kühne coined the term 'enzyme'.
- Enzymes are biocatalysts which enhance the rate of a biochemical reaction which otherwise progresses 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, hence they are generally heat labile and are soluble in water and other polar solvents.
- Like proteins, enzymes also possess nitrogen (16%) of its weight.
- Enzymes do not decide the direction of reaction, rather they just enhance the rate of reaction.

CLASSIFICATION OF ENZYMES

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

I. Oxidoreductase
II. Transferase
III. Hydrolase
IV. Lyase
V. Isomerase
VI. Ligase

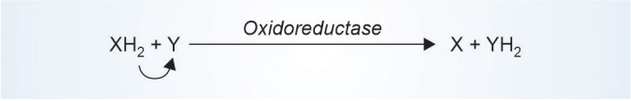
In August 2018, the International Union of Biochemistry and Molecular Biology classified enzymes which are involved in catalysing the movement of ions

or molecules across membranes or their separation within membranes under a new enzyme class (EC) of translocases (EC 7).

Many of such enzymes use ATP hydrolysis for their action and previously were described as ATPase.

Class I: 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.



Class II: 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

Class III: 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)
5. Acetylcholinesterase
6. Fumaryl-acetoacetate hydrolase

Class IV: 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

Class V: 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:

- Methylmalonyl-CoA mutase
- Triose-phosphate isomerase
- Retinene isomerase
- Epimerase
- Phosphohexose isomerase
- Racemase

Class VI: Ligase

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

- All carboxylases, acetyl-CoA carboxylase, pyruvate carboxylase, etc.
- Phosphoribosyl pyrophosphate synthetase (PRPP)
- Glutamine synthetase
- Aminoacyl-tRNA synthetase
- Argininosuccinate synthetase
- Carbamoyl phosphate synthetase I
- Carbamoyl phosphate synthetase II

Class VII: Translocase

Certain enzymes involved in translocation of biomolecules are categorized in a new class of enzyme classification (class VII), named as 'translocase'.

Important examples of such enzymes are:

1. ATP–ADP translocase
2. Phosphate translocase
3. Carnitine–acylcarnitine translocase (CACT)

Coding of Enzyme

As per IUBMB, enzyme can be coded as EC.n1.n2.n3.n4, where

- n1 is class
- n2 is subclass

- n3 is sub-subclass (subgroup)
- n4 is number of particular enzyme in the list

Difference between Synthase and Synthetase

Synthase belongs to class IV and synthetase belongs to class VI.

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).

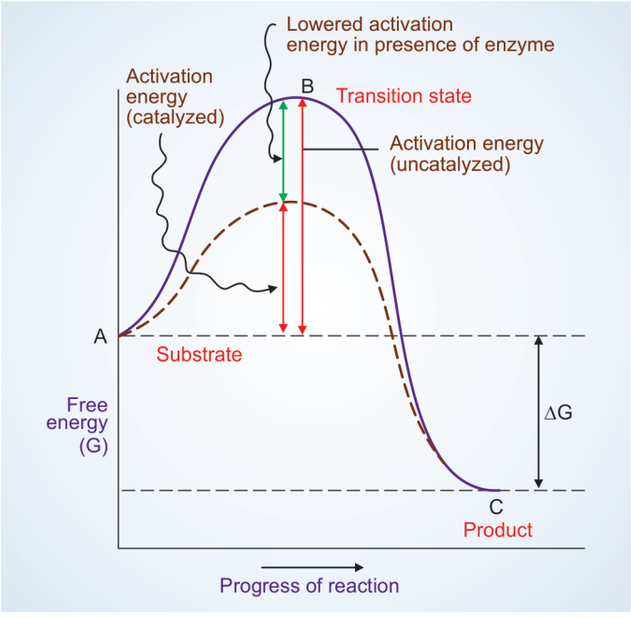


Fig. 2.1: Lowering of activation energy by enzyme

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.

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 Fischer (1894) (Fig. 2.2)
2. **Induced fit model (hand in glove model):** By Daniel E. Koshland (1958) (Fig. 2.3)

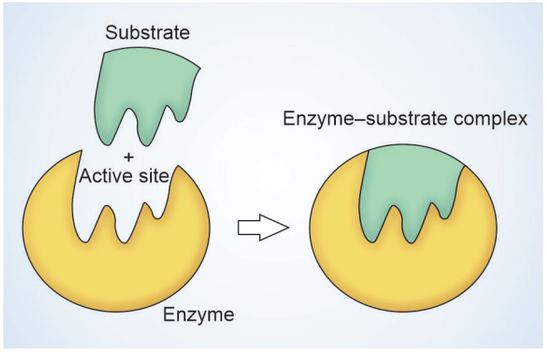


Fig. 2.2: Lock and key model (Emil Fischer, 1894)

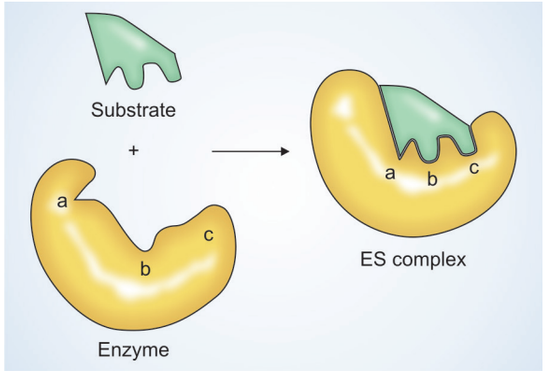


Fig. 2.3: Induced fit model (Daniel E. Koshland, 1958)

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 onto the enzyme.

Emil Fischer'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.

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 formation of enzyme–substrate complex (ES complex) is equal to the rate of its degradation, is known as steady state of the reaction.

Mechanism of Enzyme Action

Following mechanisms are described to explain enzyme action.

a. Acid–base catalysis
b. Covalent catalysis
c. Metal ion catalysis
d. Substrate strain
e. Effect of entropy
f. Product–substrate orientation

Out of the above mechanisms, acid–base catalysis, covalent catalysis and metal ion catalysis are explained in detail below:

a. **Acid–base catalysis:** Here, during the reaction, proton transfer occurs resulting in formation of unstable charged intermediate which breaks down easily to produce product.
   Water may or may not be available as proton donor or acceptor in acid–base catalysis. If water is available it is 'specific acid–base catalysis' and if water is not available rather weak acid or weak base tends to get involved in proton transfer, it is known as 'general acid–base catalysis'.
b. **Covalent catalysis:** In this, a transient covalent bond is formed between substrate and enzyme. Either the functional group of cofactor or the side chain of amino acid acts as nucleophiles and plays role in bond formation between enzyme and the substrate.
c. **Metal ion catalysis:** Metal either as a part of enzyme or when taken in free form froms the reaction solution participate in catalysis in several ways:
   i. Ionic interaction between metal and substrate, orients the substrate at active site of enzyme such that the reaction is facilitated.
   ii. Metal may undergo oxidation–reduction by changing the metal ion oxidation state.

Certain enzyme (i.e. chymotrypsin) may use more than one mechanism to catalyse the reaction.

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

1. 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.

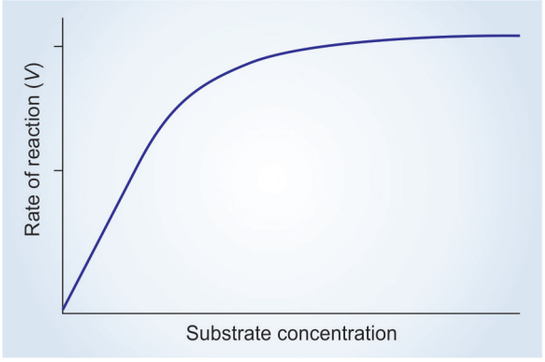


Fig. 2.4: Effect of substrate concentration on reaction velocity

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 concentration does not increase the velocity of the reaction (hyperbolic part of the graph/zero order kinetics), as the active site of the enzyme is already saturated completely with the substrate.

Michaelis-Menten 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.

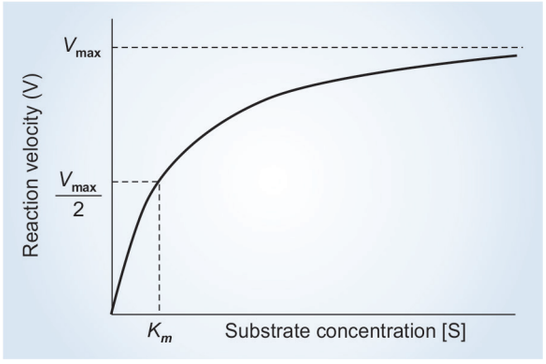


Fig. 2.5: Plot to show K_m value (Michaelis-Menten constant)

- K_m also signifies the affinity of substrate to the enzyme. Numerically, K_m value is inversely proportional to the affinity of the substrate to the enzymes.
- 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 nature of unknown enzyme which is isolated 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, e.g. glucokinase and hexokinase.

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).

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. Description of inhibitors effect on enzyme kinetics is discussed ahead.

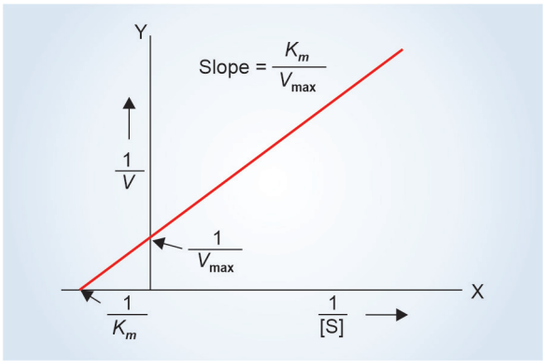


Fig. 2.6: Lineweaver-Burk plot (double reciprocal plot)

2. Effect of Temperature on Enzyme Kinetics (Bell-shaped Curve)

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.

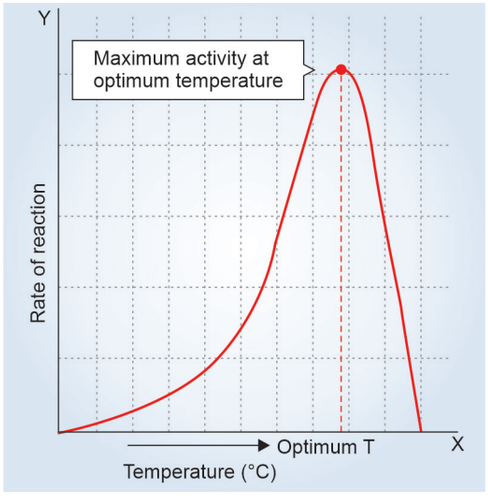


Fig. 2.7: Bell-shaped curve to show optimum T

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.

Temperature Coefficient (Q10)

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

3. 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

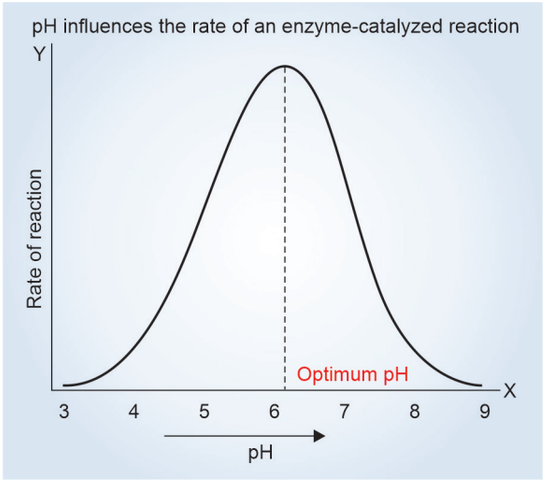


Fig. 2.8: Bell-shaped curve to show optimum pH

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.

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

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

4. 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).

5. Effect of Product Concentration on Enzyme Kinetics

Product imparts inhibitory effect on the activity of the enzyme. This is called product inhibition. This type of inhibition is not generally observed as the product of one enzymatic reaction acts as a substrate for another enzymatic reaction.

6. 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.

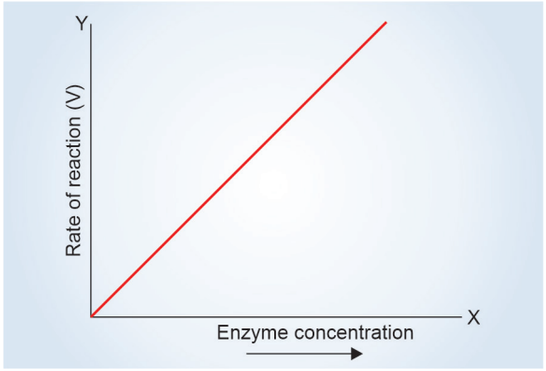


Fig. 2.9: Effect of enzyme concentration on reaction velocity

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 inhibitors.

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:

1. Competitive inhibitor
2. Noncompetitive inhibitors
3. 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.
- 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 values, respectively (V'_{max} and K'_m).

Following are the effects of competitive inhibitors on the kinetics of enzyme:

1. *Effect on V_{max}*: The effect of a competitive inhibitor is reversed by increasing concentration [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 ½ V_{max}.
3. *Effect on Lineweaver-Burk plot:* Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the graphs 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).

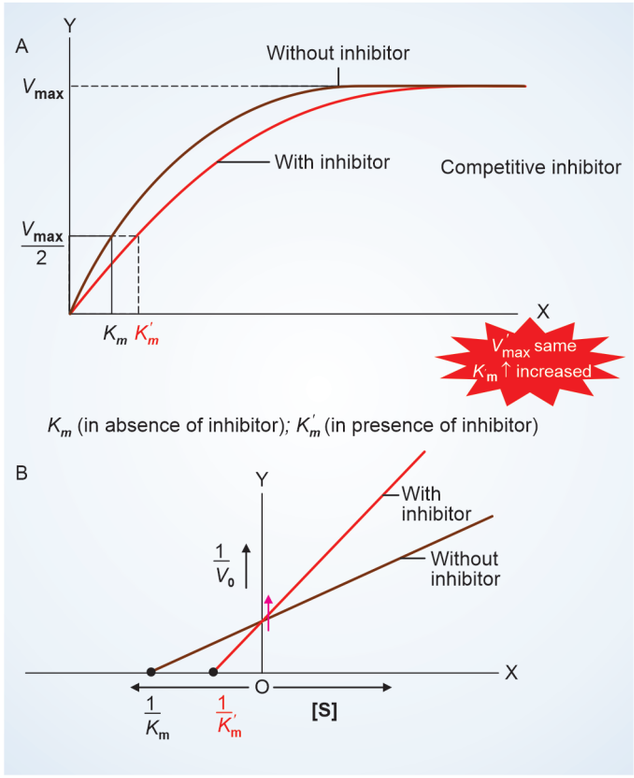


Fig. 2.10: Effect of competitive inhibitors (A = effect shown on Michaelis-Mentem plot, B = effect shown on Lineweaver-Burk plot)

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 thymidylate synthase
7. Isoniazid (INH) as vitamin B_6 analogue

Case 2.1: Ethanol Used as Therapeutic Agent in Methanol Poisoning

A 29-year-old male came to emergency unit with difficulty in vision and gasping respiration. His friend gave the history of alcohol intake from a local shop. Blood investigation revealed high anion gap metabolic acidosis.

HCO_3^-: 4.3 mmol/L (normal = 12 mmol/L)
pH: 6.89 (normal = 7.35–9.45)
Serum osmolality was 379 mOsm/kg.
Na^+ : 146 mmol/L (normal = 135–145 mmol/L)
K^+ : 7.7 mmol/L (normal = 3.5–5 mmol/L)
Urea: 32 mg/dl (normal = 15–45 mg/dl)
Glucose: 108 mg/dl (normal random = 80–140 mg/dl)
Cl^-: 116 mmol/L (normal = 90–110 mmol/L)
Lactate: 14.2 mmol/L (normal = 0.5–1.0 mmol/L)

Patient was given IV ethanol for treatment in addition to dialysis and other supportive care.

Q1. What is the rationale behind giving ethanol in methanol poisoning?

Ans. Methanol is a toxic alcohol. Toxicity of methanol is due to formation of toxic compounds formaldehyde during its metabolism. Formaldehyde is a toxic gas which damages the retina which may result in blindness.

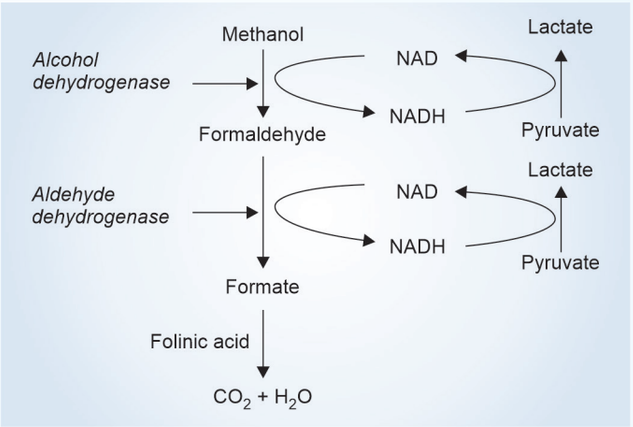
Ethanol acts as a competitive inhibitor of alcohol dehydrogenase enzyme. In presence of ethanol, enzyme alcohol dehydrogenase preferentially binds ethanol which is converted to acetaldehyde. Acetaldehyde thus produced is not of immediate toxicity, hence infusion of ethanol provides protection against toxicity of formaldehyde. Goal of therapy is to maintain serum level of ethanl as 80 to 120 mg/dl.

Hemodialysis is also useful in treatment of methanol poisoning to get rid of toxic compounds already formed.

Q2. Explain the occurrence of lactic acidosis in methanol poisoning.

Ans. Methanol metabolism produces excess amount of NADH. This is due to the fact that alcohol dehydrogenase requires NAD as coenzyme which produces NADH.

Further metabolism of formaldehyde needs aldehyde dehydrogenase which also requires NAD as a cofactor producing NADH. Figure below explains this process:



This high level of NADH thus produced tends to reduce pyruvate to lactate which results in lactic acidosis.

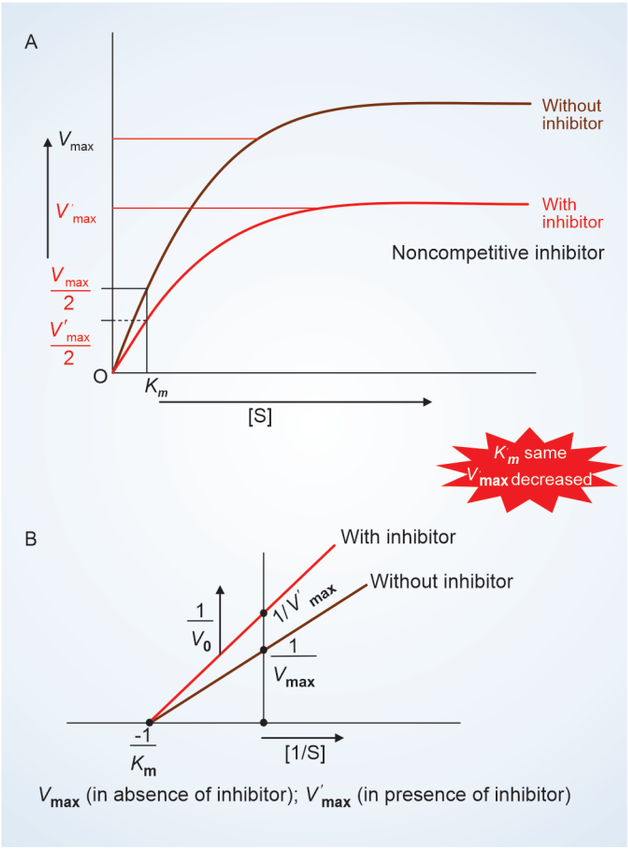


Fig. 2.11: Effect of noncompetitive inhibitors (A = effect shown on Michaelis-Menten plot; B = effect shown on Lineweaver-Burk plot)

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 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.

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)

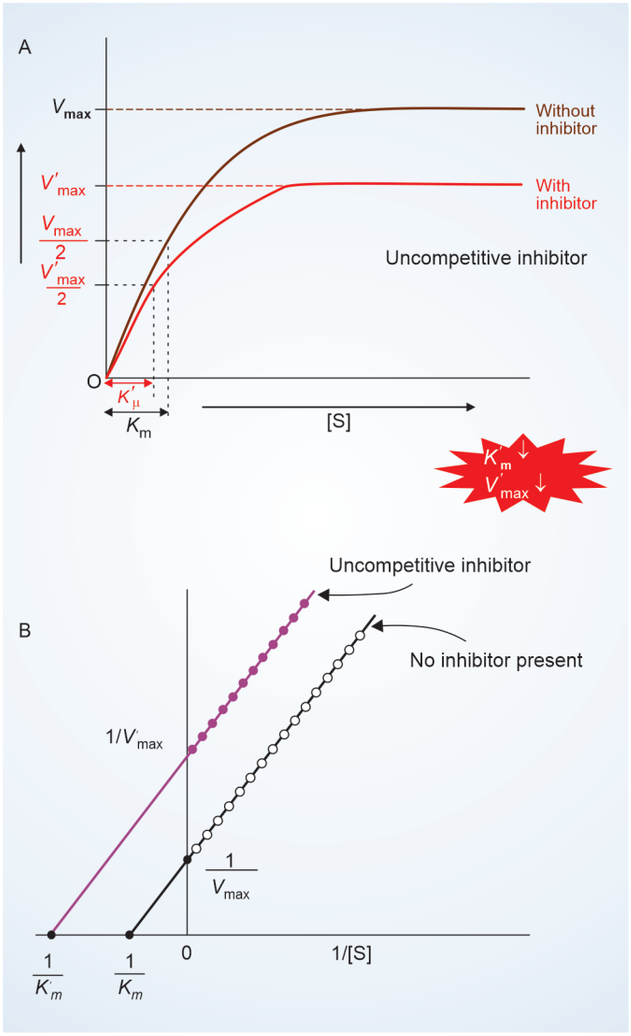


Fig. 2.12: Effect of uncompetitive inhibitors (A = effect shown on Michaelis-Menten plot; B = effect shown on Lineweaver-Burk plot

Suicidal Inhibition

Suicidal inhibition is also called '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 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).

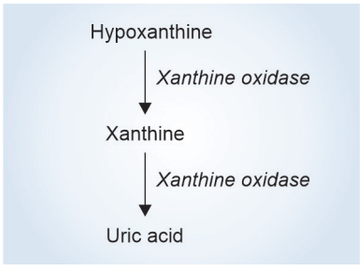


Fig. 2.13: Role of xanthine oxidase in uric acid synthesis

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).

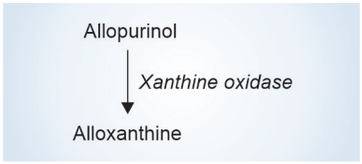


Fig. 2.14: Activation of allopurinol by xanthine oxidase

This alloxanthine now compete with substrate xanthine for xanthine oxidase active site and inhibits it in a competitive manner. It is an example of suicidal inhibition as the enzyme is preparing its own poison.

COFACTOR AND COENZYME

Some enzymes require an additional chemical component for their activity, this additional component is known as cofactor. Cofactor is the nonprotein component of the holoenzyme. This nonprotein cofactor may be organic compound or may be inorganic metal cofactor.

Organic cofactor is also called coenzyme. Inorganic cofactor may be metal or ion. Metals like Cu, Zn, Mg, Mn, Fe, Ca, Mo and ions like chloride may be required for enzyme action.

METALLOENZYMES

These are enzymes where metals are always associated with enzyme. Following are examples (Tables 2.1 and 2.2):

Metalloenzymes	*Metals tightly bound as prosthetic group*
Alcohol dehydrogenase	Zinc
Carbonic anhydrase	Zinc
Alkaline phosphatase	Zinc
Carboxypeptidase	Zinc
Aldolase	Zinc
Phenol oxidase	Copper
Cytochrome oxidase	Iron and copper (within the heme)
Pyruvate oxidase	Manganese
Xanthine oxidase	Molybdenum

TABLE 2.1 Inorganic elements as a cofactor for certain enzymes

Copper (Cu^{2+})	• Superoxide dismutase (SOD) • Monoamino oxidase (MAO) • Lysyl oxidase • Cytochrome oxidase • ALA synthase • Tyrosinase
Iron (Fe^{2+}/Fe^{3+})	• Cytochrome oxidase • Catalase • Peroxidase • Proline hydroxylase
Potassium (K^{+})	• Pyruvate kinase
Magnesium (Mg^{2+})	• Hexokinase • Glucose-6-phosphatase • Pyruvate kinase
Manganese (Mn^{2+})	• Arginase • Superoxide dismutase (SOD) • Ribonucleotide reductase
Selenium (Se)	• Glutathione peroxidase (GPO) • Deiodinase
Zinc (Zn^{2+})	• Carbonic anhydrase • Alcohol dehydrogenase • Carboxypeptidase A and B • ALA synthase • Superoxide dismutase (SOD) • RNA polymerase • ALP • LDH
Molybdenum (Mo)	• Xanthine oxidase • Sulphite oxidase • Aldehyde oxidase • Dinitrogenase
Ni	• Urease

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 compounds
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)
Tetrahydrofolic acid (THF)	1 carbon group	Folate
Thiamine pyrophosphate (TPP)	Aldehyde	Thiamine
Coenzyme B_{12} (5′-deoxyadenosyl-cobalamin)	H atom and alkyl group	Vit. B_{12}

Coenzyme may be covalently or noncovalently linked.

Prosthetic group denotes covalently bound cofactor.

Prosthetic Groups

Organic cofactor (coenzyme) or inorganic metallic cofactor may act as prosthetic group.

Unlike nonprosthetic cofactors, prosthetic cofactors do not dissociate with enzyme even when enzyme is not acting.

Examples of prosthetic group are:

- Heme as prosthetic group of cytochrome oxidase
- Biotin as prosthetic group for carboxylase enzyme
- Copper, in tyrosinase enzyme, is the prosthetic group.

Coenzymes

Coenzymes are classified in two groups based on whether they transfer hydrogen or any other group other than hydrogen. Those coenzymes which transfer hydrogen are classified as group 1, and those coenzymes which transfer any other group other than hydrogen are classified as group 2.

They are enlisted in Table 2.3.

Those coenzymes which are involved in transfer of hydrogen, play very important role in oxidoreaductase reactions.

TABLE 2.3 Coenzymes belonging to group 1 and group 2

Group 1 (transfers H^+/electron)	*Group 2 (transfers group other than H^+)*
NAD^+	ATP (transfers phosphate)
$NADP^+$	Biotin (transfers carbon dioxide)
FAD	Folic acid (transfers one carbon moiety)
FMN	TPP (transfers hydroxyethyl group)
Coenzyme Q	Coenzyme A (transfers acyl group) PLP (transfers amino group)

REGULATION OF ENZYME ACTIVITY

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

a. Concentration of the enzyme and its regulation
b. Intrinsic catalytic efficiency of the enzyme and its regulation

a. Concentration of the Enzyme and its Regulation

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
- Aminolevulinic acid (ALA) 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).

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. Details regarding ERAD process for protein degradation are described below.

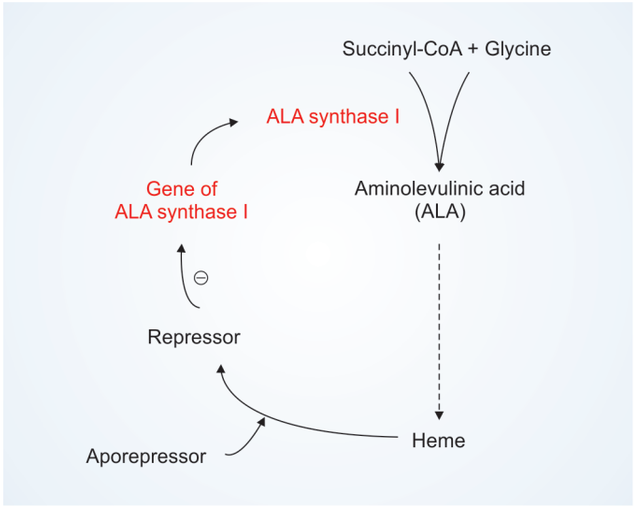


Fig. 2.15: Repression enzyme. ALA synthase I in heme biosynthesis

Endoplasmic Reticulum (ER)-associated Degradation (ERAD)

Endoplasmic reticulum (ER) is an extensive network of flattened, membrane-enclosed tubes or sacs that extends throughout the cytosol. It plays important roles in many biochemical processes required for cell survival and normal cellular functions.

In addition to protein synthesis, various other significant cellular activities such as protein transport and folding, protein quality control processes, lipid and steroid biosynthesis, carbohydrate metabolism and calcium storage occur in the ER.

The ER is equipped with a stringent quality control system that monitors the proteins that are synthesized and folded in the ER.

This ER quality control system is able to discriminate between the correctly folded proteins that exit the ER to their final destinations and the misfolded or unfolded proteins that are retained and refolded in the ER.

The accumulation of misfolded or unfolded polypeptides in the ER activates the unfolded protein response (UPR), which induces the expression of molecular chaperones and ERAD components that increase the folding capacity of the ER and activate the clearance of accumulating misfolded proteins.

Proteins that are terminally misfolded are selectively transported from the ER into the cytosol,

and subsequently ubiquitinated and degraded by the proteasome, a process called ER-associated degradation (ERAD). In other words, ERAD is a secretory protein quality control process that results in the removal of aberrant proteins from the ER and involves following steps (Fig. 2.16):

a. Recognition and targeting of substrates
b. Ubiquitination
c. Retrotranslocation
d. Shuttling to the proteasome
e. Proteasomal degradation

Nearly, all ERAD substrates are modified with ubiquitin, a 76-amino acid peptide that helps target proteins to the proteasome. Specific E3 ubiquitin ligases are required for ERAD and reside in or near the ER membrane. ERAD substrates are degraded by the proteasome, a large multi-catalytic protease that resides in the cytoplasm. Although integral membrane proteins in the ER can readily access the proteasome, soluble ERAD substrates (that reside within the lumen) must be retrotranslocated or dislocated from the ER to the cytoplasm before they are degraded.

ERAD plays important role in cell homeostasis as evidenced by erratic ERAD function, is associated in the pathology of many diseases such as cystic fibrosis, α1-antitrypsin (AAT) insufficiency, diabetes, neurodegenerative diseases (Parkinson, Alzheimer's and Huntington's diseases), viral infection and albinism.

b. Intrinsic Catalytic Efficiency of the Enzyme and its Regulation

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

i. Allosteric regulation
ii. 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.

i. Allosteric Regulation of Enzyme Activity

- All enzymes have an active site where substrate binds.
- Some enzymes have other sites in addition to active site where allosteric modifier binds and changes the activity of active site.
- Allosteric modifiers do not have any structural resemblence 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) (Fig. 2.17).

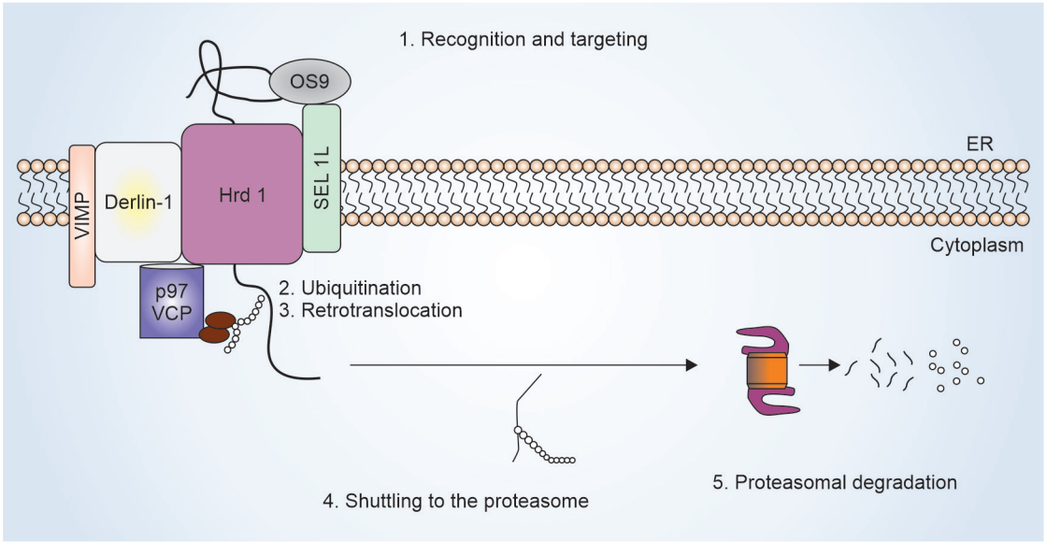


Fig. 2.16: Key steps involved in ERAD: Recognition, targeting, ubiquitination, retrotranslocation, shuttling to the proteosome and proteasomal degradation

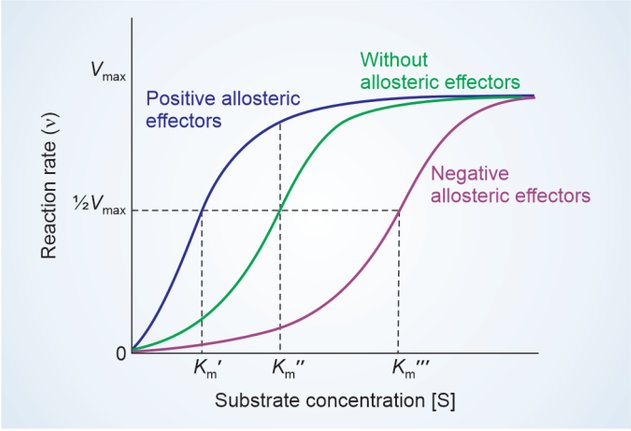


Fig. 2.17: : Effect of positive and negative allosteric effectors on allosteric enzyme saturation kinetics

- Allosteric modifiers may be positive or negative depending upon whether they are increasing the activity of active site or decreasing the activity of active site.
- Allosteric modifiers 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 (Fig. 2.18).
- Allosteric enzymes may be classified into *k series* enzyme and *v series enzyme*.

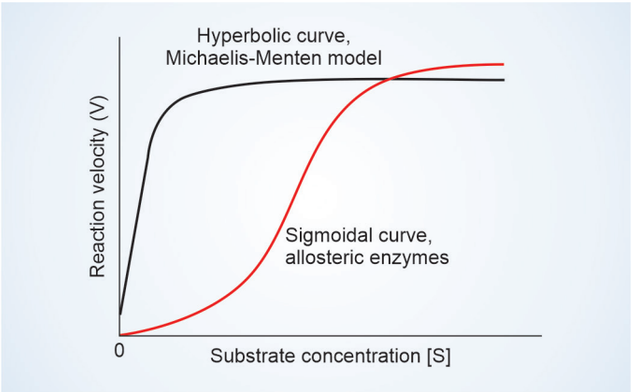


Fig. 2.18: Sigmoidal saturation kinetic curve of allosteric enzyme

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

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

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.

ii. Regulation of Enzyme Activity by Covalent Modification

- May be reversible or irreversible
- To regulate the catalytic activity of enzyme, the types of covalent modifications which are observed, are:
  a. Partial proteolysis
  b. Phosphorylation
- Histone and other DNA-binding proteins undergo various covalent modifications like methylation, acetylation, phosphorylation, ADP-ribosylation. Such modification of histone protein changes its interaction with DNA and hence chromatin structure (euchromatin *vs* heterochromatin). This certainly has an effect on gene transcription and DNA replication.
- Phosphorylation of protein occurs at specific amino acids 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 mentioned in Table 2.4.

TABLE 2.4 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 modifications are phosphorylation, dephosphorylation and acetylation and deacetylation. Other types are glycosylation, hydroxylation and prenylation.
- Protein phosphorylation may have following effect on protein:
  - Catalytic efficiency of the enzyme may get affected.
  - Alteration of protein location in the cell.
  - Susceptibility of protein for degradation.
  - Response to allosteric regulator may vary.

Compartmentalization

Sometimes, 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.

Following Table 2.5 summarizes the specific compartmental distribution of various enzymes.

TABLE 2.5 Compartmental distribution of various enzymes

Cell compartment	*Enzymes*
Plasma membrane	
Cytosol	Glycolytic enzymes
Outer mitochondrial membrane	
Inner mitochondria membrane	
Intermembranous space	
Mitochondrial matrix	TCA cycle enzymes
Peroxisome	
Lysosome	

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

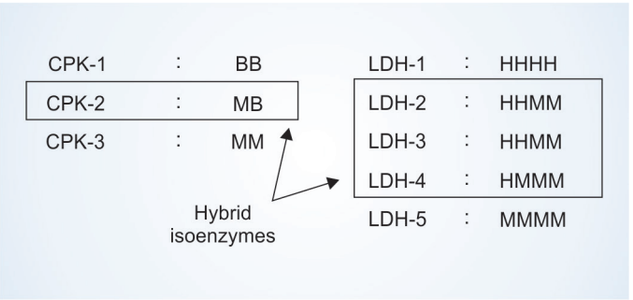
a. **True isoenzymes:** Here, the genes of isoenzymes are different which may be located on same or different chromosomes.
   - Malate dehydrogenase isoenzymes (cytosolic and mitochondrial) are derived from different genes located on the same chromosome.
   - Salivary and pancreatic amylase are derived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

b. **Hybrid isoenzymes:** For the enzymes which are oligomeric (means which possess more than one subunit), and for which there are more than one type of subunits, the differential combination of subunits gives rise to wide range of isoenzymes. Such isoenzymes are called hybrid isoenzymes. Examples of hybrid isoenzymes are CPK-2 and LDH-2, LDH-3 and LDH-4 of LDH. LDH is made-up of four subunits either of all H, or all M or H and M in varied combinations) (Table 2.6).

TABLE 2.6 Hybrid isoenzymes

LDH-1	HHHH
LDH-2	HHHM
LDH-3	HHMM
LDH-4	HMMM
LDH-5	MMMM
CPK-1	BB
CPK-2	MB
CPK-3	MM

CPK is a dimer made-up of two subunits 'B' and 'M' type. Both subunits may be same (either B type or both may be M type) or one may be B type and other may be of M type. Accordingly, CPK has three isoenzymes:
- CPK-1[BB]
- CPK-2[MB]
- CPK-3[MM]



c. **Allozymes/allelozymes:** Different allelic forms of the same gene when produce isoenzymes, are termed as allozymes. For glucose-6-phosphate dehydrogenase (G6PD) enzyme there are more than 400 mutations of its gene, has been identified on X-chromosome. Here, isoenzymes are derived from different alleles of the same gene, e.g. G6PD.

d. **Isoforms:** These are special type of isoenzymes which are originating due to nongenetic causes. Such

post-translational modifications when give rise to isoenzymes, are called isoforms. For example, following post-translational modifications may occur:

1. Content of sialic acid residue on liver and bone ALP may differ.
2. Oxidation of sulfhydryl groups in adenosine deaminase, acid phosphatase may give rise to isoforms.
3. Partial cleavage of enzyme may give rise to different isoenzymes.
4. Deamidation may give rise to different isoenzymes.
5. Phosphorylation of an enzyme may give rise to various isoforms for the same enzyme.

These isoforms are derived after different post-translational modifications, e.g. sialic acid content of ALP in various isoenzymes is different.

Characteristics of Isoenzymes

Physical characteristics which may differ in isoenzymes are:

1. **Isoenzymes may differ in structure:** The primary structure itself may differ in isoenzyme, which may be either due to difference in gene loci or due to different alleles of the same gene.
2. **Isoenzymes may differ in electrophoretic mobility:** Electrophoretic mobility depends upon ratio of charge to the mass. Isoenzymes tend to differ in their ionic charges and hence their charge to mass ratio differ, resulting in differential mobility of component isoenzymes on electrophoretic plate.
3. **Isoenzymes may differ in their stability:** True isoenzymes (whether originating from multiple gene loci or from different alleles of same gene), specifically have difference in resistance to denaturation (e.g. by detergents, urea and heat).
4. **Isoenzymes derived from multiple gene loci** usually differ considerably in their catalytic properties. For example, in substrate affinity, K_m value, antigenicity, charge, etc. Difference in net molecular charge of isoenzyme makes a ground for their separation by techniques like electrophoresis, ion-exchange chromatography.

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 milligram of separated protein". Its unit is IU/mg. Higher the specific activity of the enzyme, purer the preparation is.
2. **Turnover number or catalytic constant (K_{cat}):** Number of substrate molecules converted to product by an enzyme in a unit time, is called turnover number or catalytic constant (K_{cat}).

Difference between Enzyme Activity and Specific Activity

Enzyme activity and specific activity are different terminologies altogether. As described earlier *specific activity of enzyme* denotes the purity of enzyme (refer above), and on the other hand *enzyme activity* denotes the 'potency' of enzyme as specified by units of enzyme in one international unit and one Katal unit as discussed below.

One international unit (U): It is defined as "quantity of enzyme needed to convert 1 µmol of substrate to product in one minute time (1 µmol/min)."

One katal unit: Defined as "quantity of enzyme needed to convert 1 mol of substrate to product in one second time (mol/sec)".

As per current recommendation by International Union of Pure and Applied Chemistry (IUPAC) and International Union of Biochemistry (IUB), enzyme activity should be represented in mol per second and the enzyme concentration to be expressed in terms of katal per liter (kat/L).

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

SERINE PROTEASE

Serine protease is an important category of proteolytic enzymes which has got serine amino acid in addition to some other amino acid at their active site. Large number of proteolytic enzymes are seen to possess serine at their active site.

Serine protease is a group of enzymes which cleaves peptide bonds in proteins where serine acts as nucleophilic amino acid at the active site of enzyme. Three amino acids which make catalytic triads are:

- Serine
- Histidine
- Aspartate

Serine acts as nucleophilic amino acid and donates electron to carbonyl carbon of peptide bond which is to be hydrolyzed.

Serine proteases are abundant class of proteases. Approximaely, one-third of all known proteolytic enzymes are serine proteases.

Examples of serine proteases are:

- Trypsin
- Chymotrypsin
- Elastase
- Plasmin
- Thrombin
- Acrosomal protease

Proteolytic enzyme	*Amino acid at the active site*
Alkaline phosphatase	Serine
Acetylcholinesterase	Serine
Phosphoglucomutase	Serine
Trypsin	Serine, histidine
Thrombin	Serine, histidine
Chymotrypsin	Serine (195), aspartic acid (102), histidine (57) (catalytic triads)

In addition to these amino acids enlisted above, some other amino acids like lysine (in aldolase), cysteine (in carbonic anhydrase) and histidine (in hexokinase), are also found at active site of various enzymes.

Mostly, serine proteases are endopeptidases that catalyze bond hydrolysis in the middle of polypeptide chain. However, some are exopeptidases also.

RIBOZYME

Initially, it was thought that catalytic activity resided only in polypeptides, until 'ribozymes' were discovered. In ribozymes, catalytic activity resided in RNA. In other words, enzymes whose RNA subunits carry out catalytic reactions are known as ribozymes. There are five classes of ribozymes:

1. **23S rRNA and 28S rRNA:** These are ribosomal RNAs which posses 'peptidyltransferase' activity in prokaryotes and eukaryotes, respectively.
2. **Ribonuclease P:** It acts on tRNA precursor and produces mature 5'-end of tRNA.
3. **Group I intron:** Seen in fungal mitochondria, bacteria and bacteriophage T4.
4. **Group II intron (self-splicing intron):** Seen in mitochondria, RNA of yeast and other fungi.
5. **Group III intron (self-cleaving RNA):** Examples are genomic RNA of viruses.

Enzymes which are intracellular, have been proved as an important marker in disease diagnosis. Damage of cells where these enzymes normally reside, results in release of such enzymes in the plasma. Assessment of these enzymes in plasma thus acts as an important marker of the disease involved.

FUNCTIONAL *vs* NONFUNCTIONAL ENZYMES

Functional Plasma Enzymes

Enzymes which predominantly found in the plasma rather than their intracellular location, are known as functional plasma enzymes. These enzymes are synthesized elsewhere (mostly in liver) and are secreted in the plasma.

Examples are lipoprotein lipase, cholinesterase, ceruloplasmin, plasmin and thrombin.

Nonfunctional Plasma Enzymes

These enzymes are present mainly intracellularly. Their plasma level is very low or negligible compared to the concentration which is present in intracellular location. Site of action of these enzymes is intracellular and these enzymes are not functional in plasma.

Examples are:

AST, ALT, ALP, LDH, CPK, amylase, lipase.

Excessive plasma level of these enzymes serves as a marker of those disease which tend to damage/destroy respective cell containing these enzymes. Thus, nonfunctional enzymes prove very important for diagnosing cell/organ damage, extent of damage and also prove useful in follow-up to assess the response to therapy.

Active Site or Active Centre of Enzyme

The whole area on an enzyme is catalytically not active. The small region on the enzyme where substrate binds and actual catalysis occurs, is known as active site or acive center of the enzyme. Active site constitutes a small portion of the enzyme. Rest of the enzymes which are not actively partipating in the reaction are in fact important for making three-dimensional structure of the enzyme.

Most of the time, the active site is located in cleft/crevices of enzyme. The substrate bind in the crevices at these active sites. For substrate binding at these active sites, Koshland proposed induced-fit model and Fischer proposed lock and key model.

The lock-and-key model is the earliest and also the simplest model to explain molecular recognition in enzyme actions. It was first proposed by Fischer in 1894.

The lock-and-key model assumes that the enzyme (lock) recognizes the substrate (key) through the shape complementarity between the enzyme's active site and the substrate.

The comparison between the structures of a protein in complexes (i.e. holo structures) and unbound protein structures (i.e. apo structures) demonstrates that the protein often undergoes certain conformational changes when forming the complex structure with its binding partner.

The induced-fit model was first proposed by Koshland in 1958 to explain the protein conformational changes in the binding process. This model suggests that an enzyme, when binding with its substrate, optimizes the interface through physical interactions to form the final complex structure. The induced-fit model is supported by the fact that many ligands are buried in the protein-binding sites in the protein–ligand complex structures in the protein data bank (PDB).

Koshland's Induced-fit Model

As per this model, there occurs conformational change at the active site after initial binding of substrate there. This conformation change then accommodates the substrate rather more easily and this results in catalysis.

Fischer's Lock and Key Model/Template Theory

As per this model, the active site is a rigid structure and its shape is complimentary to the shape of substrate which then fits the active site as key fits into a lock. This model could not explain flexibility shown by enzyme.

Free Energy Change of Exergonic, Endergonic and Isothermic Reactions

Free energy change is calculated as difference of free energy of product and free energy of substrate.

In *exergonic reactions*, there occurs loss of energy when substrate is converted to product. This results in free energy change which has some value in negative. So, if the reaction has got free energy change value in negative it means that it is exergonic reaction and will go till completion spontaneously (Fig. 2.19).

Example:

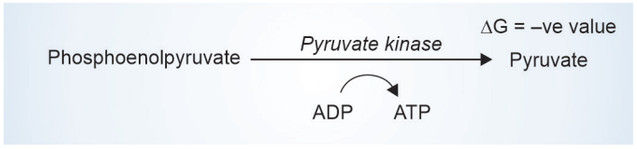


Fig. 2.19: Exergonic reaction

On the other hand, in *endergonic reactions*, energy is used when substrate is converted to product. This results in free energy change which has some value in positive. So, if the reaction has got free energy change value in positive, it means that it is an endergonic reaction and will go till completion, only when energy is provided from some outside source (Fig. 2.20).

Example:

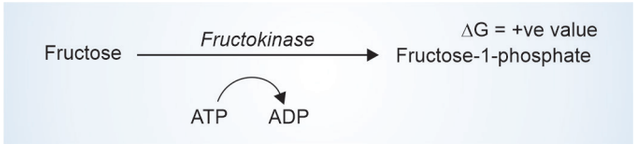


Fig. 2.20: Endergonic reactions

In *isothermic reactions*, there is no net change in energy when the reaction is occurring and free energy change of such reaction is zero (Fig. 2.21).

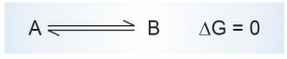


Fig. 2.21: Isothermic reaction

Best examples of isothermic reactions are reversible reactions in state of equilibrium, when rate of forward reaction is exactly same as rate of backward reaction. In such a case, whatever energy is consumed/produced to proceed the reaction in forward direction, same amount of energy is produced/consumed for reaction to occur in backward direction.

Chapter 3

Clinical Enzymology

ENZYME AS A MARKER OF THE DISEASE

ENZYME AS A MARKER IN MYOCARDIAL INFARCTION

Assessment of plasma enzyme is an indispensable tool to diagnose myocardial infarction. Enzymes are easy, quick and reliable method for not only diagnosing the disease but also give important information on the severity of the disease.

Number of enzymes are useful in diagnosing myocardial infarction. Their time of appearance in plasma, peak level presentation, duration of their existence in plasma are specific and knowledge of this helps in assessment of disease at any given time after the episode of myocardial infarction.

Following are the enzymes which are used in assessment of myocardial infarction:

a. Creatine phosphokinase (CPK): Total and MB fraction
b. Lactate dehydrogenase (LDH)
c. Aspartate transaminase (AST)

Table 3.1 illustrates the onset of appearance of these enzymes in plasma, time of their peak appearance, and the time by which they decline to basal level.

TABLE 3.1 Enzymes in assessment of myocardial infarction (time of appearance, peak level and decline)

Enzymes	*Appearance in plasma (post-MI)*	*Time of peak level*	*Decline to low level*
CPK (MB)	4–8 hrs	1 day (24 hrs)	2–3 days
AST	24–36 hrs	2 days (48 hrs)	4–6 days
LDH	8–10 hrs	2–3 days	10–15 days

In addition of enzyme markers described above certain cardiac proteins also act as a marker of myocardial infarction. They are:

a. Cardiac troponin I
b. Cardiac troponin T
c. Myoglobin

Troponin T and troponin I are cardiac proteins which are involved in muscle contractility. Troponin I is released earlier than troponin T.

Normal range of troponin T = 0–0.01 ng/ml

Normal range of troponin I = 0–0.04 ng/ml

Myoglobin is though an earlier marker than troponin T and I, it is a nonspecific marker.

Case 3.1: Myocardial Infarction and Enzyme Marker

A 56-year-old chronic smoker for past 30 years who is a lawyer by profession presents with heaviness in chest with pain on left upper limb for past 12 hours (Fig. 3.1).

Fig. 3.1: Male presenting with chest pain

He is known hypertensive on regular antihypertensive medication. He is not a known diabetic.

ECG was done immediately which showed significant ST-segment elevation in I, II and V1 to V6 leads (Fig. 3.2).

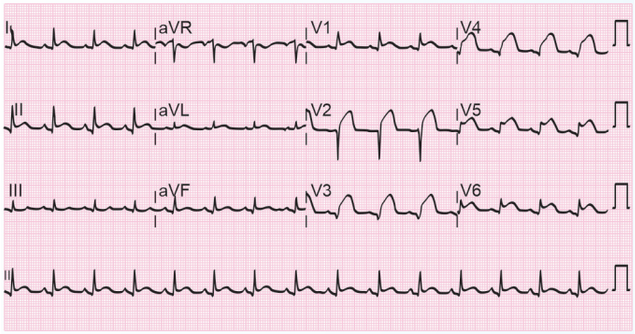


Fig. 3.2: ECG finding

Blood biochemistry revealed following results:
Troponin T = 134 ng/ml
Troponin I = 240 ng/ml
CPK total = 2000 IU/L
CPK MB = 340 IU/L
AST = 765 IU/L
LDH = 323 IU/L

Angiography revealed 95% block in proximal portion of left anterior descending (LAD) artery (Fig. 3.3).

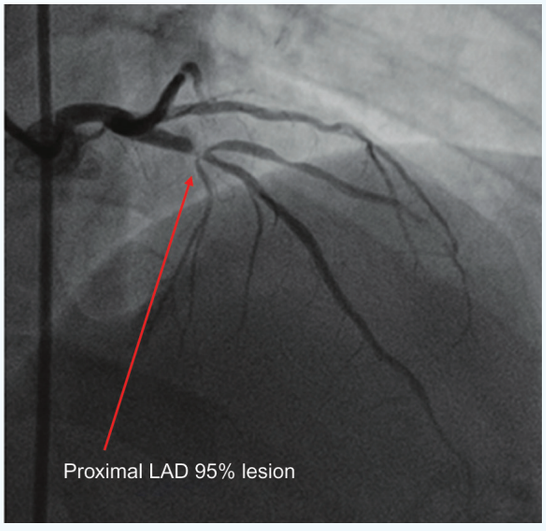


Fig. 3.3: LAD artery showing block

Q1. What may be the diagnosis in above case?

Ans. The clinical presentation and biochemical finding in this case suggest that this patient is suffering with myocardial infarction. A condition when cardiac myocyte undergoes necrosis due to lack of blood supply to those cells.

Q2. What is angina?

Ans. Angina is often described as a burning or compression sensation or difficulty in breathing, located in the precordial region or any other region of the chest, radiating to the neck, shoulder and left arm. It usually increases in intensity within minutes and may be accompanied by symptoms such as nausea and sweating. It can be triggered by physical or emotional stress and relieved by rest or use of nitrates.

Q3. What are the various enzymes and nonenzymatic markers for assessment of MI which can be assessed to diagnose and monitor MI? What is time of their appearance, peak and persistence in the plasma?

Ans. There are various enzymes which are used in assessment of MI. They are:

CPK total, CPK–MB, AST, LDH.

Out of these, AST and LDH are nonspecific markers of MI as these enzymes are found in other cells also in addition to heart. They have a wide tissue distribution that significantly limits the specificity for myocardial necrosis.

CPK is the enzyme which serves as carrier of high energy phosphate and has got three isoenzymes, namely CPK-BB, CPK-MB, CCPK-MM. Creatine kinase MB (CK-MB) is an isoenzyme of creatine kinase that is the most abundant in the heart and should be assessed to rule in or rule out the myocardial infarction.

Nonenzymatic markers of MI are troponin T and troponin I and myoglobin.

Below mentioned graph is well representing the time of onset, peak and duration of persistence of various enzymes in a typical case of MI (Fig. 3.4).

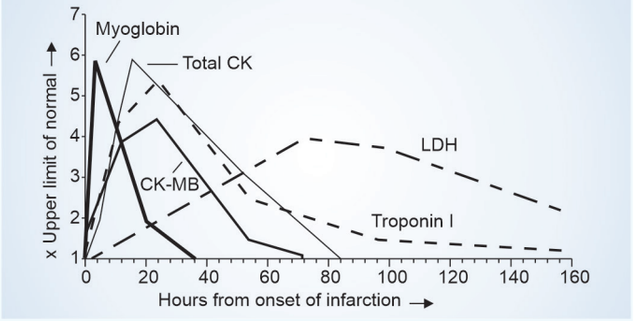


Fig. 3.4: Graph representing the onset, peak and decline of various enzyme markers in a typical case of myocardial infarction

Q4. Why troponin is more specific for MI? Which troponin is better for detection of MI—troponin T or troponin I?

Ans. Troponin I is better than troponin T.

Q5. Name the enzyme/s which may be used for treatment in such cases.

Ans. There are few enzymes which are being used as thrombolytic agents in such cases of MI. They are streptokinase, urokinase, etc. (read clinical correlation given below).

Q6. What are the risk factors for myocardial infarction?

Ans. Important risk factors for myocardial infarction are: Dyslipidemia, male gender, diabetes mellitus, hypertension, smoking, sedentary lifestyle and advanced age.

Clinical Correlation

Streptokinase and Myocardial Infarction

Fibrinolytic agents are useful adjunct in treatment of myocardial infarction and should ideally be initiated within 30 minutes of initiation of symptoms. Following are the fibrinolytic agents used:

a. Streptokinase
b. Tissue plasminogen activator (tPA)
c. Tenecteplase (TNK)
d. Reteplase (rPA)

All above drugs promote conversion of plasminogen to plasmin which then causes fibrinolysis.

Tissue plasminogen activator (tPA), and other fibrin specific plasminogen activator TNK and rPA are more effective than streptokinase in restoring full perfusion.

Research has shown that streptokinase could lyse over 50% of a thrombus in 5 to 10 minutes.

Origin and Use of Streptokinase

Streptokinase is a protein which is derived from beta-hemolytic streptococci of Lancefield group C bacteria. It is approved by Food and Drug Administration (FDA) for the treatment of acute ST-segment elevation myocardial infarction (STEMI), arterial thrombosis or embolism, deep vein thrombosis, pulmonary embolism, and arteriovenous cannula occlusion.

Mechanism of Action of Streptokinase

Streptokinase forms a complex with plasminogen, which then converts to the proteolytic enzyme plasmin. This process results in a cascade that ultimately leads to the lysis of fibrin clots. Once administered, streptokinase causes a systemic thrombolytic state that usually resolves within 48 hours of administration.

Thrombolytic therapy should be initiated as soon as the symptoms are present (within 30 minutes) and no later than 6 hours.

Table 3.2 illustrates the onset of appearance of these proteins in the plasma, time of their peak appearance, and the time by which they decline to basal level.

TABLE 3.2 Proteins used in assessment of MI with their duration of appearance, peak level and decline

Cardiac proteins	*Appearance in plasma (post-MI)*	*Time of peak level*	*Decline to low level*
Troponin I	4–10 hrs	12–24 hrs	5–9 days
Troponin T	18–36 hrs	36–48 hrs	5–14 days
Myoglobin	1–4 hrs	4–6 hrs	2 to 3 days

Vertical Integration: ECG Finding in Myocardial Infarction

Notice normal ECG waves in Fig. 3.5 and Fig. 3.6A. Also note the ST-segment elevation and T wave inversion in myocardial infarction in Figs 3.6B and 3.6C.

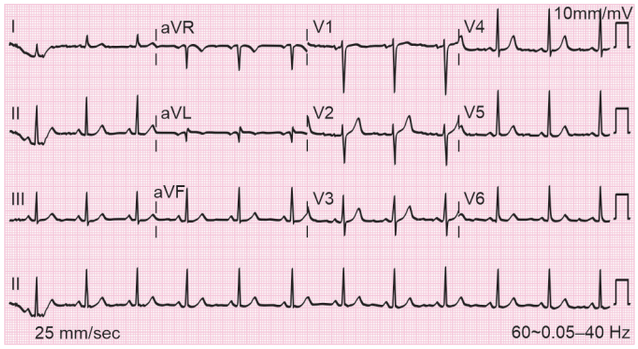


Fig. 3.5: Normal ECG pattern

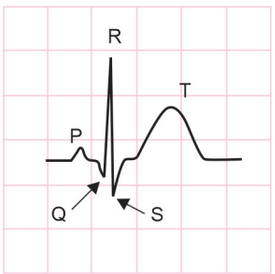


Fig. 3.6A: Normal

Fig. 3.6B: ST-segment elevation

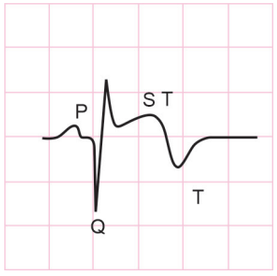


Fig. 3.6C: ST-elevation and T wave inversion

An ECG strip showing changes in inferior wall MI in various ECG leads are shown in Fig. 3.7.

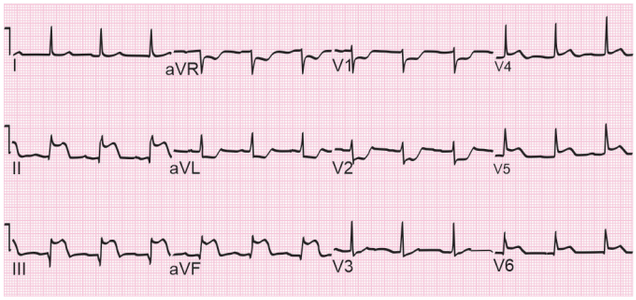


Fig. 3.7: ST-elevation in inferior leads, i.e. II, III, aVF in a case of inferior wall MI

ENZYME AS A MARKER IN LIVER DISEASE

Following are the enzymes which are being used as a marker of liver disease:

a. **AST (SGOT):** Aspartate transaminase
b. **ALT (SGPT):** Alanine transaminase
c. **ALP:** Alkaline phosphatase
d. **γ-GT:** Gamma-glutamyltransferase

ALT is more specific than AST for liver disease. ALP and γ-GT are important to diagnose obstructive hepatobiliary disease. Gamma-glutamyltransferase (γ-GT) is a marker of alcoholic liver disease.

For detail of the role of these enzymes as a marker in liver disease, reader is suggested to refer Chapter 34 'Liver Function Tests (LFTs)' at page number 433, of this book.

ENZYME AS A MARKER IN PANCREATIC DISEASE

Following enzymes are being assessed for acute pancreatitis.

a. **Serum amylase:** Amylase is the enzyme which is secreted by exocrine pancreas, and it reaches peak level in the plasma at 5 to 12 hours after the onset of acute episode of pancreatitis and return to basal level by 2 to 4 days once the acute episode is over.
b. **Serum lipase:** This enzyme also tends to elevate in plasma in acute pancreatitis and pancreatic cancer.

Case 3.2: Acute Pancreatitis

A 45-year-old male presents with intense pain in epigastric and periumbilical region. Pain is radiating to back and is associated with vomiting. He had two episodes of vomiting while coming to hospital. He has low grade fever (99° fare height). He is chronic alcoholic indulged in alcoholism for past 15 years (Fig. 3.8).

Fig. 3.8: Epigastric pain radiating to back in acute pancreatitis

On palpation abdomen was found to be distended and tender.

On auscultation bowel sounds were absent.

Blood biochemistry revealed

- Serum amylase: 220 U/L (normal is 30 to 100 U/L)
- Serum lipase: 180 U/L (normal is 15 to 80 U/L)

Q1. What is the diagnosis based on clinical and biochemical findings?

Ans. This patient is having an acute pancreatitis as diagnosed based on clinical and biochemical findings.

Q2. What is the criteria of diagnosis of acute pancreatitis?

Ans. Two of the following three criteria to be fulfilled for diagnosis of acute pancreatitis are:

a. Epigastric pain radiating to back
b. Three fold or greater increase of serum lipase and/or amylase
c. Abdominal imaging studies giving the conclusive findings

Q3. What is the single best enzyme to diagnose pancreatitis?

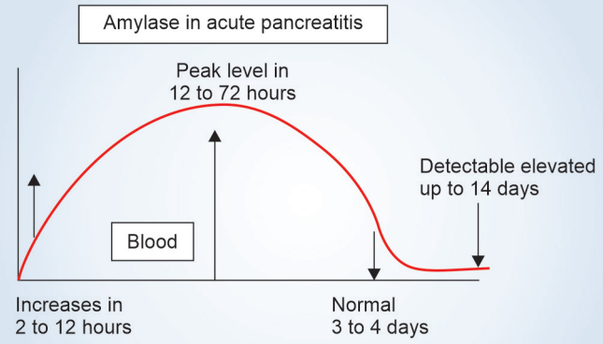
Ans. Single best enzyme to measure in acute pancreatitis is lipase which rises parallel to amylase but tend to stay longer in plasma than amylase.

Acute pancreatitis is a rapid onset of inflammation of the pancreas causing mild-to-severe life-threatening conditions. It is diagnosed when two of the following three criterias are met with.

1. Level of amylase or lipase is equal or more than three times of upper limit
2. Upper abdominal pain
3. Cross-sectional imaging finding on CT or MRI

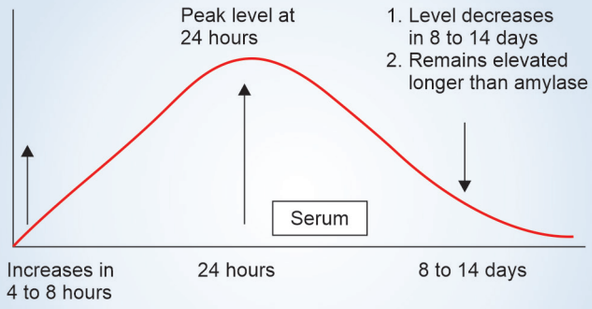
Both amylase and lipase, belong to the class III of enzyme classification, i.e. hydrolases. Amylase is an enzyme that catalyzes the hydrolysis of starch into monosaccharide and disaccharide by acting on glycosidic linkage while lipase is an esterase which catalyzes the hydrolysis of triacylglycerol and releases fatty acid by cutting ester bond.

For diagnosing acute pancreatitis serum lipase offers a higher sensitivity and specificity than serum amylase. Lipase also offers a larger diagnostic window than amylase since it is elevated for a longer time, thus allowing it to be a useful diagnostic biomarker in early and late stages of acute pancreatitis.



Serum amylase: Timeline of onset of increase, peak and decline in a case of acute pancreatitis

Several recent evidence-based guidelines recommend the use of lipase over amylase. Simultaneous assessment of both the enzymes has shown little to no increase in the diagnostic sensitivity and specificity. Thus, unnecessary testing and laboratory expenditures can be reduced by testing lipase alone.



Serum lipase: Timeline of onset of increase, peak and decline in a case of acute pancreatitis

Following table summarises the rise, peak and decline of serum amylase and lipase in a typical case of acute pancreatitis.

Timeline	*Amylase*	*Lipase*
Onset	2–12 hrs	4–8 hrs
Peak	12–72 hrs	24 hrs
Decline	3–4 days	8–14 days

Lipase is specific and amylase is not specific for pancreas.

Raised amylase may be seen in following conditions:

- Acute pancreatitis
- Chronic pancreatitis (ductal obstruction)
- Pancreatic pseudocyst
- Pancreatic trauma
- Pancreatic cancer
- Renal insufficiency
- Cancer lung, ovary, breast, oesophagus (tumor hyperamylasaemia)
- Macro amylasemia
- Burns
- Diabetic ketoacidosis
- Pregnancy
- Opiates

Q4. What is the most sensitive test to detect pancreatitis in early stages?

Ans. Direct invasive test to detect pancreatic secretory function (secretin test) is the most sensitive and specific test to detect early chronic pancreatic disease when imaging is inconclusive or normal.

Following imaging tests are done in a case of pancreatitis:

- **Non-invasive test:** USG, CT, magnetic resonance cholangiopancreatography (MRCP)
- **Invasive test:** Endoscopic retrograde cholangio-pancreatography (ERCP), endoscopic ultrasonography (EUS)

Q5. What are the common causes which are associated with acute pancreatitis?

Ans. Causes of acute pancreatitis (Fig. 3.9):

- Gallstone
- Alcohol: Alcoholism and cigarette smoking compound the effect on incidence of pancreatitis.
- Hypertriacylglyceredemia (>1000 mg/dl)
- Abdominal trauma
- ERCP induced

Fig. 3.9: Factors precipitating the acute pancreatitis

Q6. Which type of hyperlipidaemia is associated with chances of getting acute pancreatitis?

Ans. Hypertriacylglyceredemia either because of deficiency of lipoprotein lipase enzyme or because of apo-CII results in raised chylomicron in blood. This causes hypertriacylglyceredemia in lipid profile. This is associated with pancreatitis. Reason of association of hypertriglyceridemia and incidences of pancreatitis are obscure.

ENZYME AS A MARKER IN PROSTATIC CANCER

Enzymes which are being assessed for diagnosing prostatic cancer are:

a. Prostate specific antigen b. Acid phosphatase.

Table 3.3 summarizes all the enzymes involved in various diseases with their reference range.

TABLE 3.3 Enzymes involved in various diseases with their reference range

Diseases	*Serum enzyme as marker*	*Normal range*
Myocardial infarction	CPK (MB) AST LDH	<5 g/L 8–20 IU/L 125–220 U/L
Liver disease	AST (SGOT) ALT (SGPT) ALP γ-GT	8–20 IU/L 15–40 IU/L 100–280 IU/L 10–30 IU/L
Acute pancreatitis	Amylase Lipase	80–180 Somogyi units/dl <40 IU/L
Prostate cancer	PSA (prostate specific antigen) Acid phosphatase	1–5µg/L 3.5–12 IU/L

ENZYMES USED IN VARIOUS ANALYTICAL TECHNIQUES

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzymes are useful in designing analytical tools. ELISA (enzyme-linked immunosorbent assay) is a technique which is a good example where enzyme labeled antibodies are utilized in designing the assay.

This is the techniques which is based on immunological principles where antigen interacts with antibody to produce immune complex. This is a useful technique which is commonly used in clinical and research laboratories for quantitative detection of even minute quantity of antigen and antibodies in the biological fluids.

Day to day, this technique is being used in quantifying various hormones like T_3, T_4, TSH, fertility hormones like LH, FSH, prolactin, tumor markers , ferritin, LDH, etc.

Enzyme is used to tag the antibody which is then identified using substrate.

Commonly used enzymes for tagging are:

- Horse radish peroxidase (HRP)
- Alkaline phosphatase (ALP)
- Glucose-6-phosphate dehydrogenase (G6PD)
- Beta-D-galactosidase

In this technique, antibody is bound to solid phase and sample (patient serum) is allowed to interact with solid phase antibody, as to make antigen–antibody complex. After washing, when excess of unbound antigen of the sample is washed off, a second antibody

which is labeled with enzyme is added in the reaction well and is allowed to interact with antigen. After incubation unbound labeled antibody is washed off and substrate of enzyme is added which interacts with enzyme to generate colored product. The intensity of color is directly proportional to the amount of enzyme which in turn is directly proportional to the amount of antigen present in the serum. Color is then read to quantify the amount of antigen present in the patient's serum.

Activity of enzyme is assessed either by measuring the product formed or by measuring the effect of product formed on a coupled reaction. Product generated can be measured photometrically, fluorometrically or using chemiluminiscence assay.

ENZYMES USED IN BIOASSAY

There are many assessment methods where specific enzymes are used to quantitatively analyse certain biochemical parameters like glucose, urea, uric acid and cholesterol. The enzymes used in these methods, specifically act on desired substrate. This kind of substrate has great advantage of specifity for the substance which is being measured. Important examples are given below.

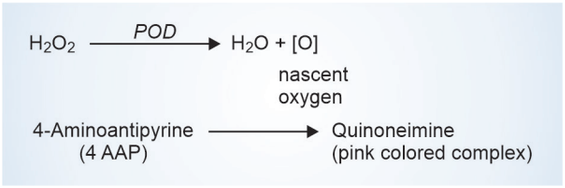
A. **Hexokinase:** It is used as a reference method for quantitatively estimating the glucose. Here,
- Glucose is converted to glucose-6-phosphate by hexokinase enzyme, and glucose-6-phosphate is next acted upon by enzyme glucose-6-phosphate dehydrogenase with simultaneous generation of NADPH.
- This NADPH is read at 340 nm. Amount of NADPH produced is directly proportional to glucose in serum or plasma.

B. **Glucose oxidase-peroxidse (GOD-POD) method:** It is the most commonly used method for estimation of glucose in any biological fluid.
- Here, glucose is converted to gluconic acid by enzyme glucose oxidase. H_2O_2 is produced as by-product in this reaction.

Reaction 1

- H_2O_2 is then acted upon by peroxidase to produce nascent oxygen which interacts with dye 4-aminoantipyrine (4AAP) which gives pink colored complex of quinoneimine.

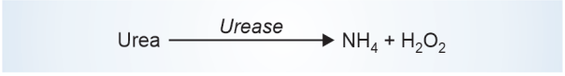
Reaction 2



- Pink color is read colorimetrically at 540 nm and color absorbance (OD) is directly proportional to the amount of nascent oxygen which in turn is directly proportional to the amount of glucose in biological fluid.

C. **Urease method:** This method is used for assessment of urea.
- In this method, the enzyme urease first hydrolyzes urea into ammonium ion (NH_4^+) and carbonate (CO_3^{2-}).
- Ammonium ion then interacts with 2-oxoglutarate in presence of glutamate dehydrogenase (GLDH) and rate of disappearance of NADH is measured at 340 nm.

Reaction 3



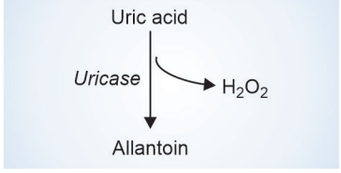
The released ammonia can also be quantified by other methods also like

1. Color change associated with pH indicator
3. Glutamine synthetase
3. Pyruvate kinase
4. Pyruvate oxidase

D. **Uricase method:** Enzyme uricase estimates uric acid in biological fluid.

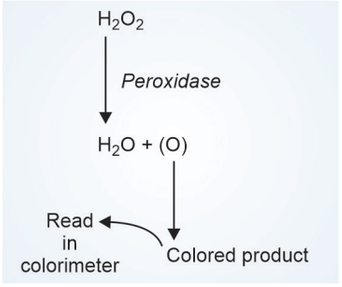
In this method, uricase acts on uric acid to convert it to allantoin, H_2O_2 is the by-product in this reaction.

Reaction 4



H_2O_2 is next acted upon by peroxidase enzyme which converts it to H_2O and nascent oxygen [O].
Nascent oxygen [O] acts on indicator dye which is converted to colored complex. Color thus produced is directly proportional to the amount of uric acid and OD is measured calorimetrically, then calculated for uric acid concentration.

Reaction 5



E. **Cholesterol oxidase-peroxidase (CHOD-POD) method:** Cholesterol esterase first of all converts cholesterol ester to free cholesterol.
- Free cholesterol is then acted upon by cholesterol oxidase. H_2O_2 is the by-product in this reaction.

Reaction 6

Reactions for enzymatic method of cholesterol estimation:

Cholesterol ester + H_2O $\xrightarrow[\text{Esterase}]{\text{Cholesterol}}$ Cholesterol + Fatty acid

Cholesterol + O_2 $\xrightarrow[\text{oxidase}]{\text{Cholesterol}}$ Cholestenone + H_2O_2

H_2O_2 $\xrightarrow{\text{Peroxidase}}$ H_2O + [O]

H_2O_2 + 4-Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine (pink color) → Read at 540 nm

This H_2O_2 is then acted upon by peroxidase which produces nascent oxygen as a reactive species.

Nascent oxygen then acts on 4-aminoantipyrine to produce pink-colored complex 'quinoneimine' the concentration of which is read colorimetrically at 540 nm.

We thus see that basic principle of glucose estimation by GOD–POD method and cholesterol estimation by CHOD–POD method is same, where colour of quinoneimine dye is measured at 540 nm.

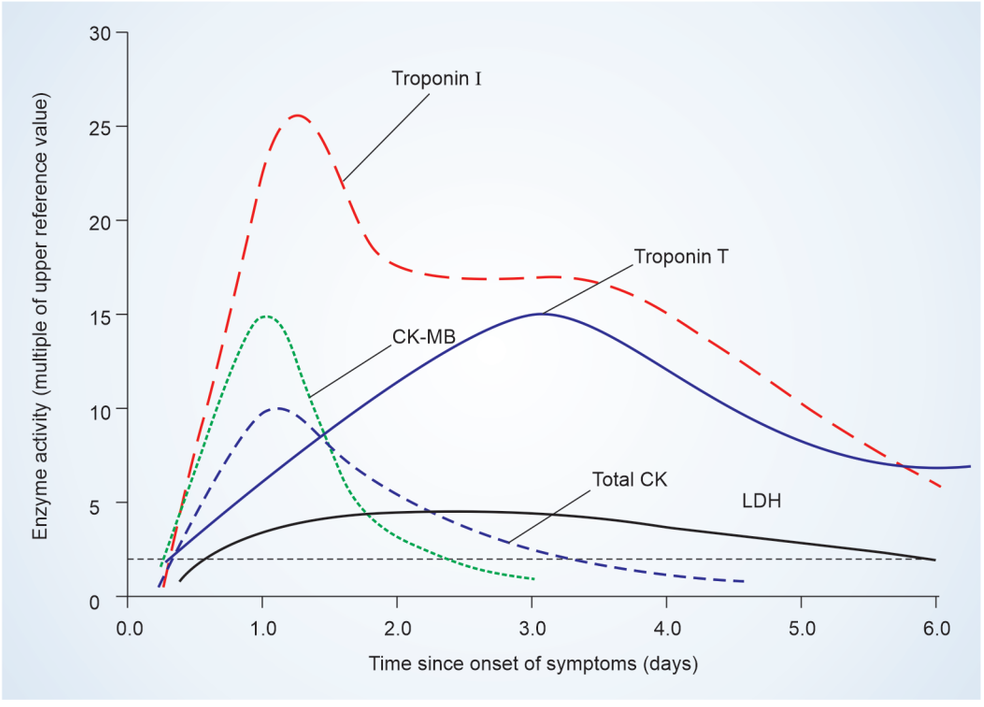


Fig. 3.10: Graphical representation of various enzyme patterns in a typical case of myocardial infarction

ENZYMES USED IN THERAPEUTICS

Enzymes are effective as therapeutic agents as well. Following are certain examples where enzymes are being used as a treatment modality.

A. **For clot lysis in myocardial infarction:** Streptokinase and urokinase (refer Clinical Corelation given on page 34)
B. **As an anticancer drug:** Asparaginase used in treatment of leukemia.
C. **For treatment of indigestion:** Trypsin and pepsin are being used as digestive syrups and capsules.
D. **During treatment of emphysema:** Alpha-1 antitrypsin is used in the treatment of emphysema, underlying etiology of which is deficiency of alpha-1 antitrypsin.
E. **For the treatment of odema:** Hyaluronidase is being used.
F. **Anti-inflammatory role:** Papain is being used.
G. **To degrade collagen in severe burn:** Collagenase is being used.

Summary of diverse role of enzyme in assessment, diagnosis and therapeutics is shown in Table 3.4.

TABLE 3.4 Enzymes used in diagnostics and therapeutics

Enzymes in assessment	*Used for*
Glucose oxidase (GOD) and peroxidase (POD)	• Glucose estimation
Cholesterol oxidase (CHOD) and peroxidase (POD)	• Cholesterol estimation
Uricase	• Uric acid estimation
Urease	• Urea estimation
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
Creatine phosphokinase (CPK)	• Myocardial infarct • Muscular dystrophy
Enzymes in therapeutics	*Used for*
Streptokinase • Bacterial asparginase	• To lyse blood clot • In treatment of leukemia

KEYPOINTS

1. Enzymes are biocatalysts which enhance the rate of a biochemical reaction and they are neither changed nor lost during or after the reaction.
2. Enzymes are mostly proteins. RNAs which have catalytic activity, are known as ribozymes.
3. Enzymes are divided into six major classes according to International Union of Biochemistry and Molecular Biology (IUBMB):
   i. Oxidoreductase
   ii. Transferase
   iii. Hydrolase
   iv. Lyase
   v. Isomerase
   vi. Ligase
4. Enzymes belonging to oxidoreductase class are involved in transfer of hydrogen ion from one substrate to other.
5. Enzymes belonging to transferase class are involved in transfer of groups other than hydrogen.
6. Enzymes belonging to hydrolase class use water and cleave the bonds so that the substrate is cleaved into simpler products.
7. Enzymes belonging to lyase class are involved in cleavage of C–C, C–O and C–N bonds.
8. Enzymes belonging to isomerase class of enzymes rearrange the atoms within the same molecule. This results in synthesis of isomeric form of the original molecule.
9. Enzymes belonging to ligase class, catalyze the joining together of two molecules coupled to the hydrolysis of ATP.
10. Enzymes act via lowering the 'activation energy'. Activation energy is the amount of energy which is required to convert the substrate to high energy intermediate.
11. To explain the binding of substrate to the active site of the enzyme, there are two theories:
    a. Lock and key model (rigid template model): By Emil Fischer.
    b. Induced-fit model (hand in glove model): By Daniel E Koshland.
12. 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.
13. In 'induced-fit 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.
14. **Turnover number or catalytic constant (K_{cat}):** Number of substrate molecules converted to product by an enzyme in unit time is called turnover number or catalytic constant (K_{cat}).
15. Many factors affect the enzyme kinetics. They are: Substrate concentration, temperature, pH, enzyme concentration, product concentration and inhibitors.
16. **Michaelis constant (K_m):** It is the substrate concentration at which the velocity of reaction is half of the maximum velocity.
17. K_m signifies that half of the active sites of the enzyme are saturated with the substrate. Numerically, K_m value is inversely proportional to the affinity of the substrate to the enzymes.
18. Lineweaver-Burk plot (double reciprocal plot) is derived when 1/S concentration and 1/*V* is plotted on *x*- and *y*-axes, respectively.
19. The temperature at which the velocity of the reaction is maximum is known as *optimum temperature*.
20. The pH at which the velocity of the reaction is maximum is known as optimum pH.
21. An inhibitor is any substance which decreases the velocity of an enzyme-catalyzed reaction. Inhibitors may act in a reversible or irreversible *manner*.
22. Inhibitors which act in a reversible fashion are:
    - Competitive inhibitors
    - Noncompetitive inhibitors
    - Uncompetitive inhibitors
23. Competitive inhibition: In presence of competitive inhibitors, V_{max} remains same and K_m increases.
    Examples of competitive inhibitors:
    - Sulphonamide as para-aminobenzoic acid analogue
    - Methotrexate as dihydrofolate reductase inhibitor
    - Dicumarol as vitamin K analogue
    - Statins as HMG-CoA reductase analogue
    - Ethanol in methanol poisoning
    - 5-Fluorouracil as an inhibitor of thymidylate synthase
    - Isoniazid (INH) as vitamin B_6 analogue
24. Noncompetitive inhibition (mixed type of inhibition): In presence of noncompetitive inhibitors V_{max} lowers and K_m remains same.

Examples of noncompetitive inhibitors:

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

25. **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.

26. Irreversible inhibitors may be of following types:
    - Group-specific inhibitors
    - Substrate analogue inhibitors (affinity labels)

27. **Suicidal inhibitors:** Suicidal inhibition is also called '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.

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

28. Some enzymes require an additional chemical component for their activity, this additional component is known as cofactor which may be inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+} or Zn^{2+}; or it may be a complex organic or metallo-organic molecule called a *coenzyme*.

29. **Prosthetic group** denotes covalently bound cofactor.

30. Enzyme activity is determined by two primary factors:
    a. Concentration of the enzyme
    b. Intrinsic catalytic efficiency of the enzyme

31. Concentration of enzyme vary via induction and repression of gene:
    i. 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

    ii. There are many enzymes which are under repression by their corresponding factors. For example, gene of ALA synthase is under repression by repressor made-up of heme.

32. Two important ways by which intrinsic catalytic efficiency of an enzyme may be altered are: Allosteric regulation and covalent modification.

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

EXERCISE

Chapter 1
Biochemical and Clinical Significance of Cell and its Various Organelles

SHORT ANSWER QUESTIONS (5 MARKS EACH)

Write short notes on the following.

1. Difference between prokaryotic cell and eukaryotic cell
2. Mitochondrial mutation
3. Endoplasmic reticulum
4. Lysosome
5. Inclusion cell disease
6. Ribosome

Chapter 3
Clinical Enzymology

LONG ANSWER QUESTIONS (10 MARKS EACH)

1. Describe different types of inhibition of enzymes. Show their effect on enzyme kinetics with the help of double reciprocal graph.
2. What are isoenzymes? How many varieties of isoenzymes you know? Describe the diagnostic significance of the isoenzymes of lactate dehydrogenase.
3. Discuss various mechanisms by which enzyme activity can be regulated.
4. Classify enzymes with suitable examples in each class. Describe various modalities by which enzyme activity is regulated. Write a note on isoenzyme with their use in diagnostics.

SHORT ANSWER QUESTIONS (5 MARKS EACH)

Write short notes on the following.

1. Profile of serum enzymes in diagnosis of myocardial infarction
2. Classification of enzymes (IUPAC system) with two examples in each class
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
4. Michaelis-Menten equation and its importance
5. Isoenzymes and their importance in the diagnosis of myocardial infarction
6. Differences between cofactors and coenzymes
7. Covalent modification of enzymes and its metabolic significance, using glycogen turnover as an example
8. Factors affecting rate of enzyme catalyzed reaction
9. Role of metal ions in enzyme catalysis
10. Enzyme inhibition, two examples of competitive inhibition and any two drugs which are based on competitive inhibition
11. General properties of an allosteric enzyme and one example of the reaction catalyzed by an allosteric enzyme
12. Metallic cofactor
13. Activation energy
14. Suicidal inhibition
15. Competitive *vs* noncompetitive inhibition
16. Allosteric enzymes
17. Covalent enzymes
18. Role of enzyme in disease diagnosis
19. Enzyme markers in liver disease

MULTIPLE CHOICE QUESTIONS

Chapter 1
Biochemical and Clinical Significance of Cell and its Various Organelles

1.1 Eukaryotic cell membrane contains all of the following, except:

a. Cholesterol
b. Riglycerides
c. Carbohydrates
d. Lecithin

1.2 Size of nucleus is:

a. 10 mm
b. 10 µm
c. 10 nm
d. 100 mm

1.3 Inclusion cell disease is associated with:

a. Lysosome
b. Nucleus
c. Mitochondria
d. Peroxisome

1.4 Which of the following is not the disease associated with mitochondrial DNA mutation?

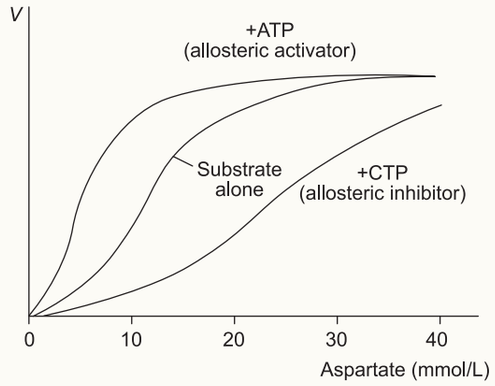
a. MELAS
b. MERRF
c. Zellweger syndrome
d. Mutation of cytochrome *b*

1.5 For which of the following processes mitochondrial matrix (either partially or fully) is not involved?

a. Fatty acid beta-oxidation
b. Gluconeogenesis
c. Urea cycle
d. Fatty acid synthesis

Chapter 2
Enzymes: Various Aspects

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-glutamyltranspeptidase

2.8 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

2.9 The active site of an enzyme:

a. Is formed only after addition of a specific substrate
b. Is directly involved in binding of allosteric inhibitors
c. Resides in a few adjacent amino acid residues in the primary sequence of the polypeptide chain
d. Binds competitive inhibitors

2.10 Zinc is a cofactor for:

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

2.11 By which of the following actions Gs receptor can be stimulated to induce watery diarrhea?

a. Phosphorylation
b. Dephosphorylation
c. ATP–ADP exchange
d. ADP ribosylation

ANSWERS AND EXPLANATIONS OF MCQS

Chapter 1
Biochemical and Clinical Significance of Cell and its Various Organelles

1.1 (b) Triglycerides

A note on eukaryotic cell membrane:

- The cytoplasm is enclosed within a plasma membrane composed of protein and phospholipid, similar to the prokaryotic cell membrane.
- Most animal cells have no other surface layers; however, plant cells have an outer cell wall composed of cellulose.

1.2 (b) 10 µm

Nucleus is the largest organelle having diameter of 10 µm.

1.3 (a) Lysosome

Inclusion cell disease is associated with lysosome.

1.4 (c) Zellweger syndrome

Zellweger syndrome is associated with peroxisomal defect in very long chain fatty acid (VLCFA) oxidation.

1.5 (d) Fatty acid synthesis

Synthesis of fatty acid is a cytosolic process.

Chapter 2
Enzymes: Various Aspects

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 displace 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 catalyzed by mono-oxygenase enzymes.
- Dioxygenase incorporates two atoms of the oxygen into the substrate.
- Carboxylation is catalyzed 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 (LDH-1 > 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 (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

2.9 (d) Binds competitive inhibitors

- The active site is formed when the enzyme folds into its three-dimensional configuration and may involve amino acid residues that are far apart in the primary sequence.
- Substrate molecules bind at the active site.
- Competitive inhibitors compete with the substrate. (Both bind at the active site.)
- Allosteric inhibitors bind at a site other than the active site.

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

2.11 (d) ADP ribosylation

- Cholera is an important infectious disease, endemic in certain Asian countries and other parts of the world. Fecal contamination of water and food is the principal method of transmission.
- It is due to *Vibrio cholerae*, a bacterium that secretes a protein enterotoxin.
- The enterotoxin is made-up of one A-subunit (composed of one A1- and one A2-peptide joined by a disulfide link) and five B-subunits and has a molecular mass of approximately 84 kDa. In the small intestine, the toxin attaches by means of the B-subunits binding to the ganglioside GM1 present in the plasma membrane of mucosal cells.
- The A-subunit then dissociates, and the A1 peptide passes across to the inner aspect of the plasma membrane.
- It catalyzes the ADP-ribosylation (using NAD^+ as donor) of the GTP-binding regulatory component (Gs) of adenylate cyclase, upregulating the activity of this enzyme.
- Thus, adenylyl cyclase becomes chronically activated. This results in an elevation of cAMP, which activates protein kinase A (PKA). This in turn via phosphorylation of CFTR and of a Na^+–H^+ exchanger leads to inhibition of absorption of Na^+ and enhancement of secretion of Cl^-. Thus, massive amounts of NaCl accumulate inside the lumen of the intestine, attracting water by osmosis and contributing to the liquid stools.