

# ENZYMES

6

Define :- catalyst

Enzymes are biocatalysts—the catalysts of life. A catalyst is defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any change in the overall process.

Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature, colloidal and thermolabile in character, and specific in their action.

Define :- Enzymes

In recent years, certain non-protein enzymes (chemically RNA) have also been identified.

## NOMENCLATURE AND CLASSIFICATION

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act.

Define : Inter & Extra cellular enzymes

Enzymes are sometimes considered under two broad categories : (a) Intracellular enzymes—They are functional within cells where they are synthesized. (b) Extracellular enzymes—These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since

Based on International union of Bio-chemistry

1964, [The IUB system of enzyme classification has been in force. Enzymes are divided into six major classes (in that order).] Each class on its own represents the general type of reaction brought about by the enzymes of that class.

OTHLIL

1. Oxidoreductases : Enzymes involved in oxidation-reduction reactions.

2. Transferases : Enzymes that catalyse the transfer of functional groups.

3. Hydrolases : Enzymes that bring about hydrolysis of various compounds.

4. Lyases : Enzymes specialised in the addition or removal of water, ammonia, CO<sub>2</sub> etc.

5. Isomerases : Enzymes involved in all the isomerization reactions.

6. Ligases : Enzymes catalysing the synthetic reactions (Greek : ligate—to bind) where two molecules are joined together and ATP is used.

[The word OTHLIL (first letter in each class) may be memorised to remember the six classes of enzymes in the correct order].

Each class in turn is subdivided into many subclasses which are further divided. A four digit Enzyme Commission (E.C.) number is assigned to each enzyme representing the class (first digit), sub-class (second digit), sub-sub class (third digit) and the individual enzyme (fourth digit).

In the Table 6.1, selected examples for the six classes of enzymes are given.

Although the IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the **trivial names, along with the E.C. numbers** as and when needed, are **commonly used and widely accepted**.

### CHEMICAL NATURE OF ENZYMES

All the enzymes are invariably proteins. In recent years, however, a few RNA molecules have been shown to function as enzymes. Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as **holoenzyme** which is often made up of

**apoenzyme** (the protein part) and a **coenzyme** (non-protein organic part).

### FACTORS AFFECTING ENZYME ACTIVITY

The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity. The important factors that influence the velocity of the enzyme reaction are discussed hereunder.

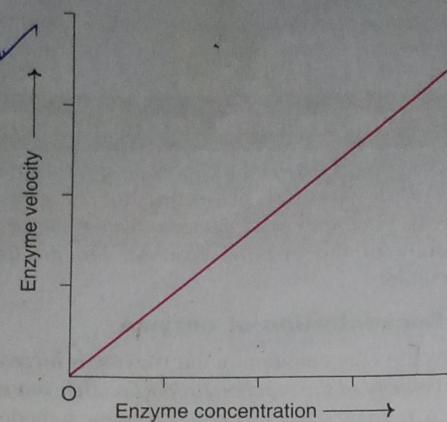
#### 1. Concentration of enzyme

As the concentration of the **enzyme is increased, the velocity of the reaction proportionately increases** (Fig.6.1). In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases.

TABLE 6.1 Classification of enzymes

Enzyme class with examples*	Reaction catalysed
1. Oxidoreductases Alcohol dehydrogenase (alcohol : NAD <sup>+</sup> oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation → Reduction $AH_2 + B \rightarrow A + BH_2$
2. Transferases Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \rightarrow A + B - X$
3. Hydrolases Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \rightarrow AH + BOH$
4. Lyases Aldolase (fructose-bisphosphate aldolase, E.C. 4.1.2.13), fumarase, histidase	Addition → Elimination $A - B + X - Y \rightarrow AX - BY$
5. Isomerases Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \rightarrow A'$
6. Ligases Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP]{\quad} A - B + ADP + Pi$

\* For one enzyme in each class, systematic name along with E.C. number is given in the brackets.

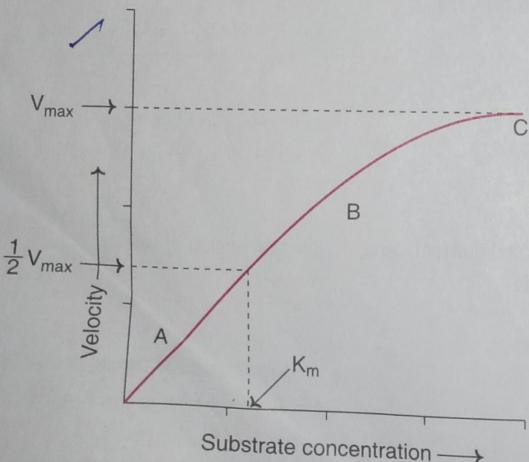


**Fig.6.1 : Effect of enzyme concentration on enzyme velocity.**

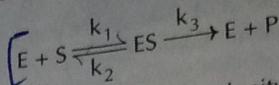
## 2. Concentration of substrate

[Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels.] A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration [Fig.6.2]. Three distinct phases of the reaction are observed in the graph.

**Enzyme kinetics and  $K_m$  value :** The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).



**Fig.6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).**



Here  $k_1$ ,  $k_2$  and  $k_3$  represent the velocity constants for the respective reactions, as indicated by arrows.

( $K_m$ , the **Michaelis-Menten constant** (or **Briggs and Haldane constant**), is given by the formula

$$K_m = \frac{k_2 + k_3}{k_1}$$

The following equation is obtained after suitable algebraic manipulation.

$$v = \frac{V_{max} [S]}{K_m + [S]} \quad \text{equation (1)}$$

where  $v$  = Measured velocity,

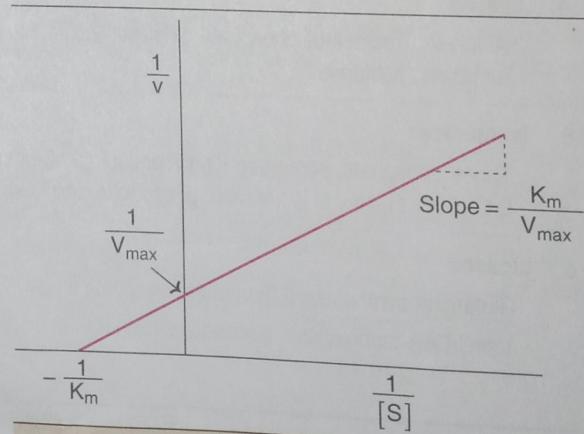
$V_{max}$  = Maximum velocity,

$S$  = Substrate concentration,

$K_m$  = Michaelis - Menten constant.]

$K_m$  or the **Michaelis-Menten constant** is defined as the **substrate concentration** (expressed in moles/lit) to produce half-maximum velocity in an enzyme catalysed reaction. It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the  $K_m$  value.

$K_m$  value is a constant and a characteristic feature of a given enzyme (comparable to a thumb impression or signature). It is a representative for measuring the strength of ES complex. A **low  $K_m$  value indicates a strong affinity between enzyme and substrate**, whereas a high  $K_m$  value reflects a weak affinity between them. For majority of enzymes, the  $K_m$  values are in the range of  $10^{-5}$  to  $10^{-2}$  moles. It may, however,



**Fig.6.3 : Lineweaver-Burk double reciprocal plot.**

be noted that  $K_m$  is not dependent on the concentration of enzyme.

**Lineweaver-Burk double reciprocal plot :** For the determination of  $K_m$  value, the substrate saturation curve (Fig.6.2) is not very accurate since  $V_{max}$  is approached asymptotically. By taking the reciprocals of the equation (1), a straight line graphic representation is obtained.

The Lineweaver-Burk plot is shown in Fig.6.3. It is much easier to calculate the  $K_m$  from the intercept on x-axis which is  $-(1/K_m)$ . Further, the double reciprocal plot is useful in understanding the effect of various inhibitions (discussed later).

### 3. Effect of temperature

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed (Fig.6.4).

The optimum temperature for most of the enzymes is between  $40^\circ\text{C}$ – $45^\circ\text{C}$ . However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at  $100^\circ\text{C}$ .

In general, when the enzymes are exposed to a temperature above  $50^\circ\text{C}$ , denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above  $70^\circ\text{C}$ ).

### 4. Effect of pH

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a

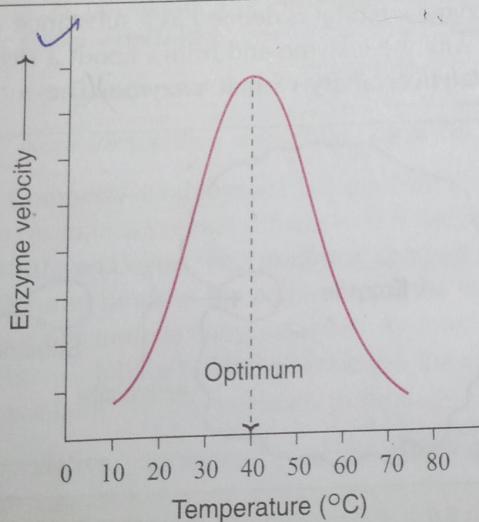


Fig.6.4 : Effect of temperature on enzyme velocity.

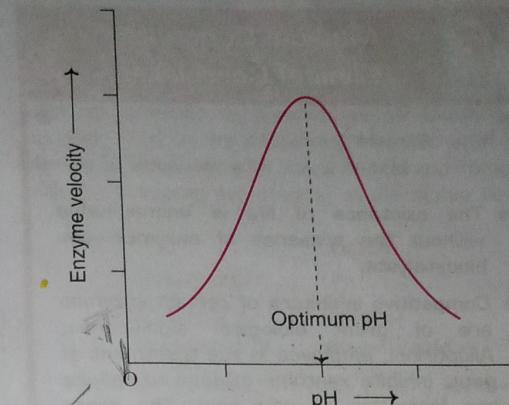


Fig.6.5 : Effect of pH on enzyme velocity.

bell-shaped curve is normally obtained (Fig.6.5). Each enzyme has an optimum pH at which the velocity is maximum.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11) for optimum pH.

### 5. Effect of product concentration

The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

### 6. Effect of activators

Some of the enzymes require certain inorganic metallic cations like  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion ( $\text{Cl}^-$ ) for amylase.

Two categories of enzymes requiring metals for their activity are distinguished

(a) **Metal-activated enzymes :** The metal is not tightly held by the enzyme and can be exchanged easily with other ions e.g. ATPase ( $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ) Enolase ( $\text{Mg}^{2+}$ ).



## Medical Concepts/ Clinical Correlates

- The existence of life is unimaginable without the presence of enzymes—the biocatalysts.
- Competitive inhibitors of certain enzymes are of great biological significance. Allopurinol, employed in the treatment of gout, inhibits xanthine oxidase to reduce the formation of uric acid. The other competitive inhibitors include aminopterin used in the treatment of cancers, sulfanilamide as antibacterial agent and dicumarol as an anticoagulant.
- Penicillin antibiotics irreversibly inhibit serine containing enzymes of bacterial cell wall synthesis.

(b) **Metalloenzymes :** These enzymes hold the metals rather tightly which are not readily exchanged. e.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase and aldolase contain zinc.

Phenol oxidase (copper);

Pyruvate oxidase (manganese);

Xanthine oxidase (molybdenum);

Cytochrome oxidase (iron and copper).

### ACTIVE SITE

Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis (Fig.6.6).

*(The active site (or active centre) of an enzyme is defined as the small region at which the substrate(s) binds and participates in the catalysis.)*

*Define: Active site*

### Salient features of active site

1. The existence of active site is due to the tertiary structure of protein resulting in three dimensional native conformation.

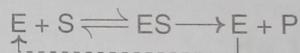
2. The active site is made up of amino acids (known as **catalytic residues**) which are far from each other in the linear sequence of amino acids (primary structure of protein). For instance, the enzyme **lysozyme** has 129 amino acids. The **active site** is formed by the contribution of amino acid residues numbered 35, 52, 62, 63 and 101.

3. The active site is not rigid in structure and shape. It is rather **flexible** to promote the specific substrate binding.

4. Generally, the active site possesses a **substrate binding site** and a **catalytic site**. The latter is for the catalysis of the specific reaction.

5. Of the 20 amino acids that could be present in enzyme structure, only some of them are **repeatedly found at the active sites** of various enzymes. These amino acids are **serine**, **aspartate**, **histidine**, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, **serine** is the most frequently found at the active sites.

6. The substrate [S] binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available for reuse.



### ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a **decrease in catalytic activity** of that **enzyme** (The inhibitor

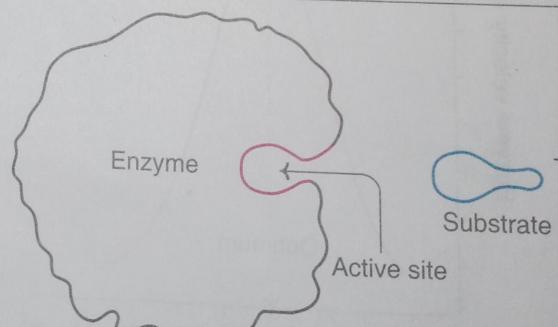
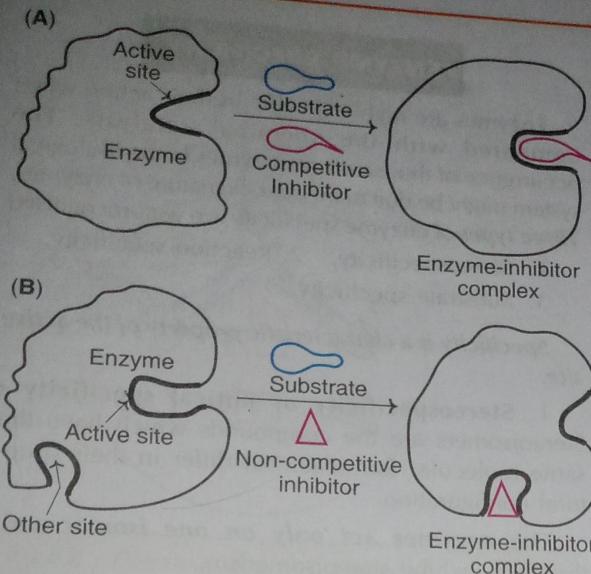


Fig.6.6 : A diagrammatic representation of an enzyme with active site.



**Fig.6.7 : A diagrammatic representation of an (A) Competitive and (B) Non-competitive inhibition.**

may be organic or inorganic in nature. There are three broad categories of enzyme inhibition

1. Reversible inhibition.
2. Irreversible inhibition.
3. Allosteric inhibition.

### 1. Reversible inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

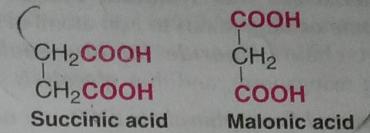
- I. Competitive inhibition (Fig.6.7A)
- II. Non-competitive inhibition (Fig.6.7B)

**I. Competitive inhibition :** The inhibitor ( $I$ ) which closely resembles the real substrate ( $S$ ) is regarded as a **substrate analogue**. The **inhibitor competes with substrate** and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind.

The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate

concentration. In competitive inhibition, the  $K_m$  value **increases** whereas  $V_{max}$  remains **unchanged**.

The enzyme **succinate dehydrogenase (SDH)** is a classical example of competitive inhibition with succinic acid as its substrate. **Malonic acid has structural similarity with succinic acid** and compete with the substrate for binding at the active site of SDH.



**Methanol** is **toxic** to the body when it is converted to formaldehyde by the enzyme alcohol dehydrogenase (ADH). Ethanol can compete with methanol for ADH. Thus, **ethanol can be used in the treatment of methanol poisoning**,

Some more examples of the enzymes with substrates and competitive inhibitors of biological significance are given in **Table 6.2**.

**Antimetabolites** : These are the chemical **compounds that block the metabolic reactions** by their inhibitory action on enzymes. Antimetabolites are usually structural analogues of substrates, and thus are competitive inhibitors (**Table 6.2**). They are in use for the treatment of cancer, gout etc. The term **antivitamins** is used for the antimetabolites which block the biochemical actions of vitamins causing deficiencies. e.g., sulphanilamide, dicumarol.

**II. Non-competitive inhibition** : The inhibitor binds at a site other than the active site on the enzyme surface (Fig.6.7B). This binding impairs the enzyme function. The **inhibitor has no structural resemblance with the substrate**. However, there usually exists a strong affinity for the inhibitor to bind at the second site. In fact, the inhibitor does not interfere with the enzyme-substrate binding. But the catalysis is prevented, possibly due to a distortion in the enzyme conformation.

The non-competitive inhibitor generally binds with the enzyme as well as the ES complex.

For non-competitive inhibition, the  $K_m$  value is **unchanged** while  $V_{max}$  is **lowered**.

Heavy metal ions ( $\text{Ag}^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$  etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulphhydryl groups.

## 2. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible. These inhibitors are usually toxic substances which may be present naturally or man-made.

(The penicillin antibiotics act as irreversible inhibitors of serine-containing enzymes, and block the bacterial cell wall synthesis) Cyanide inhibits cytochrome oxidase (binds to iron atom) of electron transport chain. Fluoride inhibits enolase (by removing manganese), and thus glycolysis.)

*Diisopropyl fluorophosphate* (DFP) is a nerve gas developed by the Germans during Second World War. DFP irreversibly binds with enzymes containing serine at the active site, e.g. *serine proteases*, *acetylcholine esterase*.

**Suicide inhibition** : In this type of irreversible inhibition, the original inhibitor is converted to a more potent form by the same enzyme that ought to be inhibited e.g., *allopurinol*, an inhibitor of xanthine oxidase, gets converted to *alloxanthine*, a more effective inhibitor of the enzyme (Refer Chapter 17).

## 3. Allosteric inhibition

The details of this type of inhibition are given under allosteric regulation as a part of the regulation of enzyme activity in the living system.

## ENZYME SPECIFICITY

Enzymes are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes. Three types of enzyme specificity are well-recognised

1. Stereospecificity,
2. Reaction specificity,
3. Substrate specificity

*Specificity is a characteristic property of the active site.*

**1. Stereospecificity or optical specificity :** Stereoisomers are the compounds which have the same molecular formulae, but differ in their structural configuration.

The enzymes act only on one isomer and, therefore, exhibit stereoisomerism.

e.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids respectively.

Hexokinase acts on D-hexoses;

Glucokinase on D-glucose;

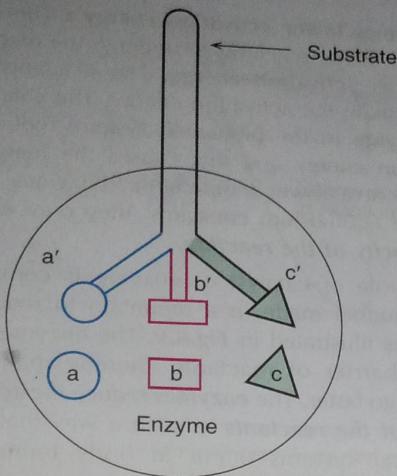
Amylase acts on  $\alpha$ -glycosidic linkages;

Cellulase cleaves  $\beta$ -glycosidic bonds.

Stereospecificity is explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the

**TABLE 6.2 Selected examples of enzymes with their respective substrates and competitive inhibitors**

Enzyme	Substrate(s)	Inhibitor(s)	Significance of inhibitor(s)
Xanthine oxidase	Hypoxanthine xanthine	Allopurinol	Used in the control of gout to reduce excess production of uric acid from hypoxanthine.
Monoamine oxidase	Catecholamines (epinephrine, norepinephrine)	Ephedrine, amphetamine	Useful for elevating catecholamine levels.
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, amethopterin, methotrexate	Employed in the treatment of leukemia and other cancers.
Acetylcholine esterase	Acetylcholine	Succinyl choline	Used in surgery for muscle relaxation, in anaesthetised patients.
Dihydropteroate synthase	Para aminobenzoic acid (PABA)	Sulfonilamide	Prevents bacterial synthesis of folic acid.
Vitamin K epoxide reductase	Vitamin K	Dicumarol	Acts as an anticoagulant.
HMG CoA reductase	HMG CoA	Lovastatin, compactin	Inhibit cholesterol biosynthesis



**Fig.6.8 :** Diagrammatic representation of stereospecificity ( $a'$ ,  $b'$ ,  $c'$ )—three point attachment of substrate to the enzyme ( $a$ ,  $b$ ,  $c$ ).

surface of the enzyme (Fig.6.8). The class of enzymes belonging to **isomerases** do not exhibit stereospecificity since they are specialized in the interconversion of isomers.

2. **Reaction specificity :** The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc. The enzymes however, are different for each of these reactions.

3. **Substrate specificity** The substrate specificity varies from enzyme to enzyme. It may be either absolute, relative or broad.

(a) **Absolute substrate specificity** : Certain enzymes act only on one substrate e.g. **glucokinase** acts on glucose to give glucose 6-phosphate, urease cleaves urea to ammonia and carbon dioxide.

(b) **Relative substrate specificity** : Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin and chymotrypsin is a good example for **group specificity** (Refer Fig.8.4). Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino

acids (phenylalanine, tyrosine and tryptophan). Examples of **bond specificity**—glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester bonds of lipids etc.

(c) **Broad specificity** : Some enzymes act on closely related substrates which is commonly known as broad substrate specificity, e.g. **hexokinase** acts on glucose, fructose, mannose and glucosamine and not on galactose.

## COENZYMES

Many enzymes require certain **non-protein** small additional factors, collectively referred to as cofactors for catalysis. The cofactors may be **organic or inorganic** in nature. Define ; Coenzyme

The non-protein, organic, low molecular weight and dialysable substance associated with enzyme function is known as coenzyme.

The functional enzyme is referred to as **holoenzyme** which is made up of a protein part (**apoenzyme**) and a non-protein part (**coenzyme**). The term **activator** is referred to the inorganic cofactor (like  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$  etc.) necessary to enhance enzyme activity.

**Coenzymes are second substrates** : Coenzymes are often regarded as the second substrates or **co-substrates**, since they have affinity with the enzyme comparable with that of the substrates. Coenzymes undergo alterations during the enzymatic reactions, which are later regenerated. This is in contrast to the substrate which is converted to the product.

Coenzymes participate in various reactions involving transfer of atoms or groups like hydrogen, aldehyde, keto, amino, acyl, methyl, carbon dioxide etc. Coenzymes play a decisive role in enzyme function.

**Coenzymes from B-complex vitamins** : Most of the coenzymes are the derivatives of water soluble B-complex vitamins. In fact, the biochemical functions of B-complex vitamins are exerted through their respective coenzymes. The chapter on vitamins gives the details of structure and function of the coenzymes (Chapter 7). In Table 6.3, a summary of the vitamin related coenzymes with their functions is given.

**Non-vitamin coenzymes :** Not all coenzymes are vitamin derivatives. There are some other organic substances, which have no relation with vitamins but function as coenzymes. They may be considered as non-vitamin coenzymes e.g. **ATP, CDP, UDP** etc.

**Nucleotide coenzymes :** Some of the coenzymes possess nitrogenous base, sugar and phosphate. Such coenzymes are, therefore, regarded as nucleotides e.g. **NAD<sup>+</sup>, NADP<sup>+</sup>, FMN, FAD, coenzyme A, UDP** etc.

**Coenzymes do not decide enzyme specificity :** A particular coenzyme may participate in catalytic reactions along with different enzymes. For instance, **NAD<sup>+</sup>** acts as a coenzyme for lactate dehydrogenase and alcohol dehydrogenase. In both the enzymatic reactions, **NAD<sup>+</sup>** is involved in hydrogen transfer. The **specificity of the enzyme is mostly dependent on the apoenzyme and not on the coenzyme.**

### MECHANISM OF ENZYME ACTION

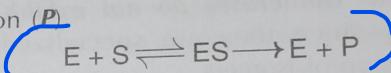
Catalysis is the prime function of enzymes. The nature of catalysis taking place in the biological system is similar to that of non-biological catalysis. For any chemical reaction to occur, the reactants have to be in an activated state or transition state.

**Enzymes lower activation energy :** The energy required by the reactants to undergo the reaction is known as **activation energy**. The reactants when heated attain the activation energy. The catalyst (or the enzyme in the biological system) reduces the activation energy and this causes the reaction to proceed at a lower temperature. Enzymes do not alter the equilibrium constants, they only **enhance the velocity of the reaction.**

The role of catalyst or enzyme is comparable with a tunnel made in a mountain to reduce the barrier as illustrated in Fig.6.9. The enzyme lowers energy barrier of reactants, thereby making the reaction go faster. The **enzymes reduce the activation energy of the reactants** in such a way that all the biological systems occur at body temperature (below 40°C).

### Enzyme-substrate complex formation

The prime requisite for enzyme catalysis is that the substrate (**S**) must combine with the enzyme (**E**) at the active site to form enzyme-substrate complex (**ES**) which ultimately results in the product formation (**P**)

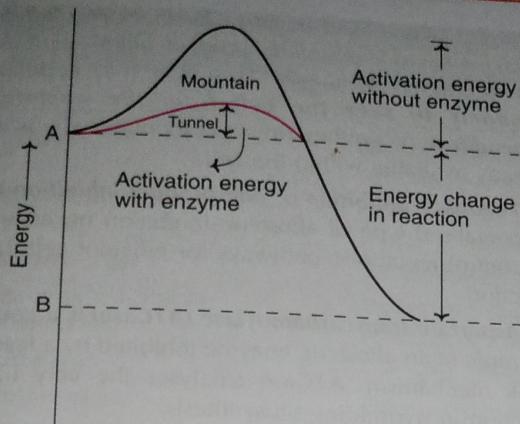


A few theories have been put forth to explain mechanism of enzyme-substrate complex formation.

TABLE 6.3 Coenzymes of B-complex vitamins\*

Coenzyme (abbreviation)	Derived from vitamin	Atom or group transferred	Dependent enzyme (example)
Thiamine pyrophosphate (TPP)	Thiamine	Aldehyde or keto	Transketolase
Flavin mononucleotide (FMN)	Riboflavin	Hydrogen and electron	L - Amino acid oxidase
Flavin adenine dinucleotide (FAD)	Riboflavin	"	D - Amino acid oxidase
Nicotinamide adenine dinucleotide(NAD <sup>+</sup> )	Niacin	"	Lactate dehydrogenase
Nicotinamide adenine dinucleotide phosphate (NADP <sup>+</sup> )	"	"	
Lipoic acid	Lipoic acid	"	Glucose 6-phosphate dehydrogenase
Pyridoxal phosphate (PLP)	Pyridoxine	Amino or keto	Pyruvate dehydrogenase complex
Coenzyme A (CoA)	Pantothenic acid	Acyl	Alanine transaminase
Tetrahydrofolate (FH <sub>4</sub> )	Folic acid	One carbon (formyl, methenyl etc.)	Thiokinase Formyl transferase
Biotin coenzyme	Biotin	CO <sub>2</sub>	
Methylcobalamin; Deoxyadenosyl cobalamin	Cobalamin	Methyl/isomerisation	Pyruvate carboxylase Methylmalonyl CoA mutase

\* Details for each coenzyme are given in the chapter 7 on vitamins



**Fig.6.9 : Effect of enzyme on activation energy of a reaction (A is the substrate and B is the product. Enzyme decreases activation energy).**

### Lock and key model or Fischer's template theory

According to this model, the structure or conformation of the **enzyme is rigid**. The substrate fits to the binding site (now active site) just as a key fits into the proper lock. Thus the **active site** of an enzyme is **a rigid** and pre-shaped template where only a specific substrate can bind (**Fig.6.10A**). This model does not give any scope for the flexible nature of enzymes, hence the model totally **fails to explain** many facts of **enzymatic reactions**, the most important being the effect of allosteric modulators.

### Induced fit theory or Koshland's model

Koshland, in 1958, proposed a more acceptable and realistic model for enzyme-substrate complex formation. As per this model, the **active site is not rigid and pre-shaped**. The essential features of the substrate binding site are present at the nascent active site. The interaction of the **substrate** with the enzyme **induces** a fit or a **conformation change in the enzyme**, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis (**Fig.6.10B**).

Induced fit model has **sufficient experimental evidence** from the X-ray diffraction studies. Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

### Substrate strain theory

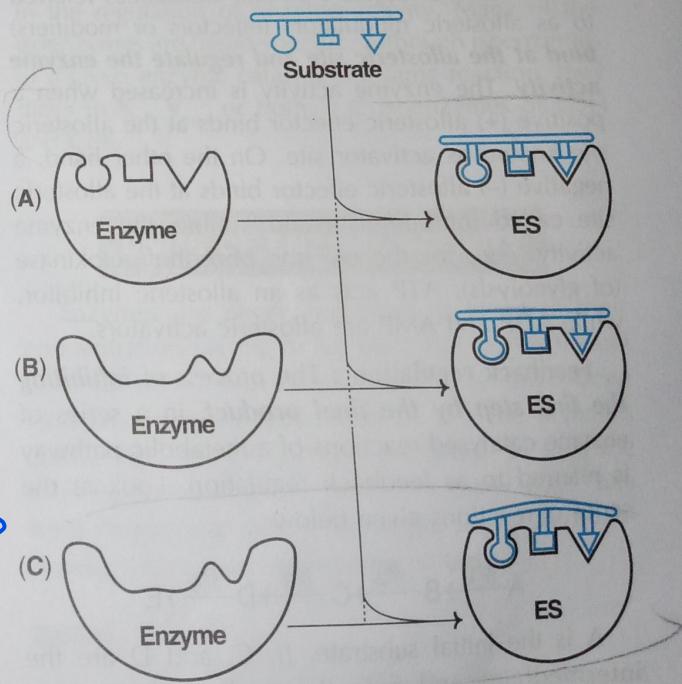
In this model, the substrate is strained due to the induced conformation change in the enzyme (**Fig.6.10C**). It is also possible that when a substrate binds to the preformed active site, the **enzyme induces a strain to the substrate**. The strained substrate leads to the formation of product. The concept of substrate strain explains the role of enzyme in increasing the rate of reaction.

In fact, a **combination of the induced fit model with the substrate strain** is considered to be **operative** in the enzymatic action.

### MECHANISM OF ENZYME CATALYSIS

The formation of an enzyme-substrate complex (ES) is very crucial for the catalysis to occur. It is estimated that an enzyme catalysed reaction proceeds  $10^6$  to  $10^{12}$  times faster than a non-catalysed reaction. It is worthwhile to briefly understand the ways and means through which the catalytic process takes place leading to the product formation. The enhancement in the rate of the reaction is mainly due to four processes :

1. Acid-base catalysis;
2. Substrate strain;
3. Covalent catalysis;
4. Entropy effects.



**Fig.6.10 : Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model; (B) Induced fit theory (C) Substrate strain theory.**

## REGULATION OF ENZYME ACTIVITY IN THE LIVING SYSTEM

The various factors that influence enzyme activity in the laboratory (*in vitro*) have been discussed. Some of these factors like temperature and pH are quite constant in the living cell. In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways to achieve cellular economy.

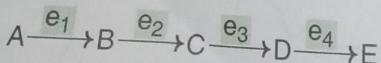
1. Allosteric inhibition
2. Activation of latent enzymes
3. Compartmentation of metabolic pathways
4. Control of enzyme synthesis
5. Enzyme degradation
6. Isoenzymes

### 1. Allosteric regulation or allosteric inhibition

Some of the enzymes possess additional sites, known as allosteric sites (Greek : *allo*—other), besides the active site. Such enzymes are known as allosteric enzymes. The **allosteric sites** are unique places on the enzyme molecule.

**Allosteric effectors :** Certain substances referred to as allosteric modulators (effectors or modifiers) **bind at the allosteric site and regulate the enzyme activity.** The enzyme activity is increased when a positive (+) allosteric effector binds at the allosteric site known as activator site. On the other hand, a negative (-) allosteric effector binds at the allosteric site called inhibitor site and inhibits the enzyme activity. e.g., for the enzyme phosphofructokinase (of glycolysis), ATP acts as an allosteric inhibitor, while ADP and AMP are allosteric activators.

**Feedback regulation :** The process of **inhibiting the first step by the final product**, in a series of enzyme catalysed reactions of a metabolic pathway is referred to as feedback regulation. Look at the series of reactions given below

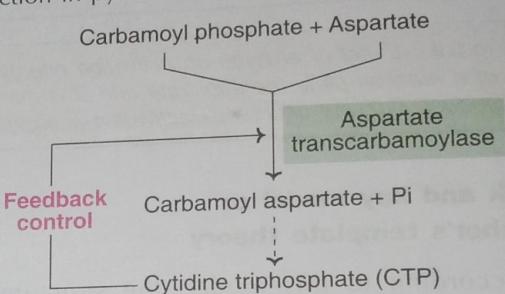


A is the initial substrate, B, C, and D are the intermediates and E is the end product, in a pathway catalysed by four different enzymes ( $e_1, e_2, e_3, e_4$ ). The very first step ( $A \rightarrow B$  by the enzyme  $e_1$ ) is the most effective for regulating the pathway, by the final end product E. This type of

control is often called **negative feedback regulation** since increased levels of end product will result in its ( $e_i$ ) decreased synthesis. This is a real **cellular economy to save the cell from the wasteful expenditure** of synthesizing a compound which is already available within the cell.

**Feedback inhibition or end product inhibition** is a specialised type of allosteric inhibition necessary to control metabolic pathways for efficient cellular function.

**Aspartate transcarbamoylase (ATCase)** is a good example of an allosteric enzyme inhibited by a feedback mechanism. ATCase catalyses the very first reaction in pyrimidine biosynthesis.



Carbamoyl phosphate undergoes a sequence of reactions for synthesis of the end product, CTP. When CTP accumulates, it allosterically inhibits the enzyme aspartate transcarbamoylase by a feedback mechanism.

### 2. Activation of latent enzymes

Latent enzymes, as such, are inactive. Some enzymes are synthesized as **proenzymes** or **zymogens** which undergo irreversible covalent activation by the breakdown of one or more peptide bonds. For instance, proenzymes—namely chymotrypsinogen, pepsinogen and plasminogen, are respectively—converted to the active enzymes chymotrypsin, pepsin and plasmin.

### 3. Compartmentation

There are certain substances in the body (e.g., fatty acids, glycogen) which are synthesized and also degraded. There is no point for simultaneous occurrence of both the pathways. Generally, the **synthetic** (anabolic) and **breakdown** (catabolic) pathways are **operative in different cellular organelles** to achieve maximum economy. For instance, enzymes for fatty acid synthesis are found in the cytosol whereas enzymes for fatty acid oxidation are present in the mitochondria.

**TABLE 6.4 Distribution of certain enzymes and metabolic pathways in cellular organelles**

<i>Organelle</i>	<i>Enzyme/metabolic pathway</i>
Cytoplasm	Aminotransferases; peptidases; glycolysis; hexose monophosphate shunt; fatty acid synthesis; purine and pyrimidine catabolism.
Mitochondria	Fatty acid oxidation; amino acid oxidation; Krebs cycle; urea synthesis; electron transport chain and oxidative phosphorylation.
Nucleus	Biosynthesis of DNA and RNA.
Endoplasmic reticulum (microsomes)	Protein biosynthesis; triacylglycerol and phospholipid synthesis; steroid synthesis and reduction; cytochrome P <sub>450</sub> ; esterase.
Lysosomes	Lysozyme; phosphatases; phospholipases; hydrolases; proteases; lipases; nucleases.
Golgi apparatus	Glucose 6-phosphatase; 5'-nucleotidase; glucosyl-and galactosyl-transferases.
Peroxisomes	Catalase; urate oxidase; D-amino acid oxidase; long chain fatty acid oxidation.

Mitochondrion is regarded as the power house of the cell where most of the energy producing pathways are located. The intracellular location of certain enzymes and metabolic pathways is given in **Table 6.4**.

#### 4. Control of enzyme synthesis

Most of the enzymes, particularly the rate limiting ones, are present in very low concentration. Nevertheless, the amount of the enzyme directly controls the velocity of the reaction, catalysed by that enzyme. **Many rate limiting enzymes have short half-lives.** This helps in the efficient regulation of the enzyme levels.

There are two types of enzymes—(a) **Constitutive enzymes** (house-keeping enzymes)—the levels of which are not controlled and remain fairly constant. (b) **Adaptive enzymes**—their concentrations increase or decrease as per body needs and are well-regulated. The synthesis of enzymes (proteins) is regulated by the genes (*Chapter 26*).

**Induction and repression :** The term induction is used to represent increased synthesis of enzyme while repression indicates its decreased synthesis. Induction or repression which ultimately determines the enzyme concentration at the gene level through the mediation of hormones or other substances.

#### 5. Enzyme degradation

Enzymes are not immortal, since it will create a series of problems. There is a lot of variability in the half-lives of individual enzymes. For some, it is in days while for others in hours or in minutes,

e.g. LDH<sub>4</sub>—5 to 6 days; LDH<sub>1</sub>—8 to 12 hours; amylase—3 to 5 hours.

In general, the key and **regulatory enzymes are most rapidly degraded**. Though not always true, an enzyme with long half-life is usually sluggish in its catalytic activity.

#### 6. Isoenzymes

**Multiple forms** of the same **enzyme** will also help in the regulation of enzyme activity. Many of the isoenzymes are tissue-specific. Although isoenzymes of a given enzyme catalyse the same reaction, they differ in K<sub>m</sub>, V<sub>max</sub> or both. e.g. isoenzymes of LDH and CPK.

#### UNITS OF ENZYME ACTIVITY

Enzymes are never expressed in terms of their concentration (as mg or µg etc.), but are expressed only as activities. Various methods have been introduced for the estimation of enzyme activities (particularly for the plasma enzymes). In fact, the activities have been expressed in many ways, like **King-Armstrong units**, **Somogyi units**, **Reitman-Frankel** units, spectrophotometric units etc.

#### Katal

In order to **maintain uniformity** in the expression of enzyme activities (as units) worldover, the **Enzyme Commission of IUB** has suggested radical changes. A new unit—namely katal (abbreviated as kat)—was introduced. **One kat denotes the conversion of one**

**mole substrate per second** (mol/sec). Activity may also be expressed as millikatals (mkat), microkatals ( $\mu$ kat) and so on.

### International Units (IU)

Some workers prefer to use standard units or SI units (System International). One SI unit or International Unit (IU) is defined as the amount of enzyme activity that catalyses the **conversion of one micromol of substrate per minute**. SI units and katal are interconvertible.

$$1 \text{ IU} = 16.67 \text{ nkat}$$

### Laboratory use of enzyme units

In the clinical laboratories, however, the units—namely katal or SI units—are yet to find a place. **Many investigators still use the old units** like King-Armstrong units, Somogyi units etc., while expressing the enzyme activities.

### PLASMA/SERUM ENZYMES

Enzymes in the circulation are divided into two groups – plasma functional and plasma non-functional.

#### 1. Plasma specific or plasma functional enzymes

Certain enzymes are normally present in the plasma and they have specific functions to perform. Generally, these enzyme activities are higher in plasma than in the tissues. They are mostly synthesized in the liver and enter the circulation e.g. lipoprotein lipase, plasmin, thrombin, choline esterase, ceruloplasmin etc.

#### 2. Non-plasma specific or plasma non-functional enzymes

These enzymes are either totally absent or present at a low concentration in plasma compared to their levels found in the tissues. The digestive enzymes of the gastrointestinal tract (e.g. amylase, pepsin, trypsin, lipase etc.) present in the plasma are known as secretory enzymes. All the other plasma enzymes associated with metabolism of the cell are collectively referred to as **constitutive enzymes** (e.g. lactate dehydrogenase, transaminases, acid and alkaline phosphatases, creatine phosphokinase).

### DIAGNOSTIC IMPORTANCE OF ENZYMES

Estimation of enzyme activities in biological fluids (particularly plasma/serum) is of great clinical

importance. Particularly non-plasma specific enzymes are very important for the diagnosis and prognosis of several diseases.

The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels could be due to cellular damage, increased rate of cell turnover, proliferation of cells, increased synthesis of enzymes etc. Serum enzymes are conveniently used as **markers** to detect the cellular damage which ultimately helps *in the diagnosis of diseases*.

(Note : The term **biomarker** refers to any **laboratory analyte** (enzyme, protein, antigen, antibody, metabolite etc.) **that is useful for the diagnosis/prognosis of any disease**. Biomarker is a vague term, and less frequently used by biochemists.)

A summary of the important enzymes useful for the diagnosis of specific diseases is given in **Table 6.5**.

Define, Isoenzymes ↗

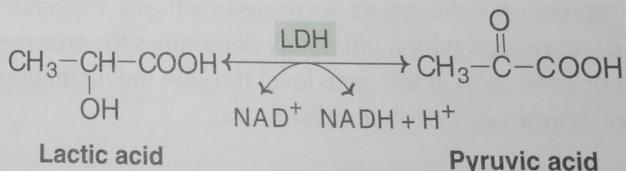
### ISOENZYMES

The **multiple forms of an enzyme** catalysing the same reaction are **isoenzymes** or **isozymes**. They, however, differ in (their physical and chemical properties) which include the structure, electrophoretic and immunological properties,  $K_m$  and  $V_{max}$  values, pH optimum, relative susceptibility to inhibitors and degree of denaturation.

#### Isoenzymes of lactate dehydrogenase (LDH)

Among the isoenzymes, LDH had been the most thoroughly investigated.

LDH, whose systematic name is L-lactate-NAD<sup>+</sup> oxidoreductase (E.C. 1.1.1.27), catalyses the interconversion of lactate and pyruvate as shown below



LDH has five distinct isoenzymes  $\text{LDH}_1$ ,  $\text{LDH}_2$ ,  $\text{LDH}_3$ ,  $\text{LDH}_4$  and  $\text{LDH}_5$ . They can be separated by electrophoresis (cellulose or starch gel or agarose gel).  $\text{LDH}_1$  has more positive charge and fastest in electrophoretic mobility while  $\text{LDH}_5$  is the slowest.



### Medical Concepts/ Clinical Correlates

- Feedback (or end product) inhibition is a specialized form of allosteric inhibition that controls several metabolic pathways e.g. CTP inhibits aspartate transcarbamoylase; Cholesterol inhibits HMG CoA reductase. The end product inhibition is utmost important to cellular economy since a compound is synthesized only when required.
- A few RNA molecules (ribozymes) function as non-protein enzymes. It is believed that ribozymes were functioning as biocatalysts before the occurrence of protein enzymes during evolution.
- Elevation in serum enzyme activities is of great importance for the diagnosis of several diseases. e.g. amylase—acute pancreatitis; ALT—hepatitis; alkaline phosphatase—obstructive jaundice; CPK-MI;

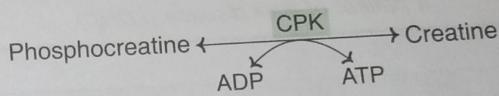
**Structure of LDH isoenzymes :** LDH is an oligomeric (tetrameric) enzyme made up of four

polypeptide subunits. Two types of subunits namely M (for muscle) and H (for heart) are produced by different genes. M—subunit is basic while H subunit is acidic. The isoenzymes contain either one or both the subunits giving  $LDH_1$  ( $H_4$ ) to  $LDH_5$  ( $M_4$ ).

**Diagnostic importance of LDH :** Isoenzymes of LDH have immense value in the diagnosis of heart and liver related disorders (Fig.6.11). In healthy individuals, the activity of  $LDH_2$  is higher than that of  $LDH_1$  in serum. In the case of **myocardial infarction**,  $LDH_1$  is much greater than  $LDH_2$  and this happens within 12 to 24 hours after infarction. Increased activity of  $LDH_5$  in serum is an indicator of **liver diseases**. LDH activity in the RBC is 80-100 times more than that in the serum. Hence for estimation of LDH or its isoenzymes, serum should be totally free from hemolysis or else false positive results will be obtained.

### Isoenzymes of creatine phosphokinase

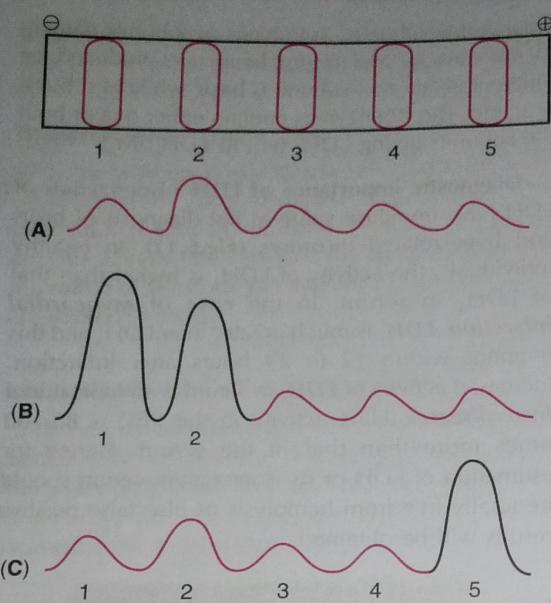
Creatine kinase (CK) or **creatine phosphokinase (CPK)** catalyses the interconversion of phosphocreatine (or creatine phosphate) to creatine.



CPK exists as **three isoenzymes**. Each isoenzyme is a dimer composed of two subunits—M (muscle) or B (brain) or both.

**Table 6.5 Important enzymes in the diagnosis of diseases**

Serum enzyme (elevated)	Disease (most important)
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5'-Nucleotidase	Hepatitis
$\gamma$ -Glutamyl transpeptidase (GGT)	Alcoholism



**Fig.6.11 :** Electrophoresis of lactate dehydrogenase with relative proportions of isoenzymes (A) Normal serum (B) Serum from a patient of myocardial infarction ( $LDH_1$  and  $LDH_2''$ ) (C) Serum from a patient of liver disease ( $LDH_5''$ ).

Isoenzyme	Subunit	Tissue of origin
CPK <sub>1</sub>	BB	Brain
CPK <sub>2</sub>	MB	Heart
CPK <sub>3</sub>	MM	Skeletal muscle

In healthy individuals, the isoenzyme CPK<sub>2</sub> (MB) is almost undetectable in serum with less than 2% of total CPK. After the myocardial infarction (MI), within the first 6-18 hours, CPK<sub>2</sub> increases in the serum to as high as 20% (against 2% normal). CPK<sub>2</sub> isoenzyme is not elevated in skeletal muscle disorders. Therefore, estimation of **CPK<sub>2</sub> (MB) is the earliest reliable indication of myocardial infarction.**

### Isoenzymes of alkaline phosphatase

As many as **six isoenzymes** of alkaline phosphatase have been identified. The enzyme is a monomer, the isoenzymes are due to the difference in the carbohydrate content (sialic acid residues). The most important ALP isoenzymes are  $\alpha_1$ -ALP,  $\alpha_2$ -heat labile ALP,  $\alpha_2$ -heat stable ALP, pre- $\beta$  ALP,  $\gamma$ -ALP etc.

Increase in  $\alpha_2$ -heat labile ALP suggests hepatitis whereas pre- $\beta$ -ALP indicates bone diseases.

### Isoenzymes of alcohol dehydrogenase

Alcohol dehydrogenase (ADH) has two heterodimer isoenzymes. Among the white Americans and Europeans, ' $\alpha\beta_1$ ' isoenzyme is predominant whereas in Japanese and Chinese (Orientals) ' $\alpha\beta_2$ ' is mostly present. The isomer  $\alpha\beta_2$  more rapidly converts alcohol to acetaldehyde.

Accumulation of acetaldehyde is associated with tachycardia (increase in heart rate) and facial flushing among Orientals which is not commonly seen in whites. It is believed that **Japanese and Chinese have increased sensitivity to alcohol due to the  $\alpha\beta_2$ -isoenzyme of ADH.**

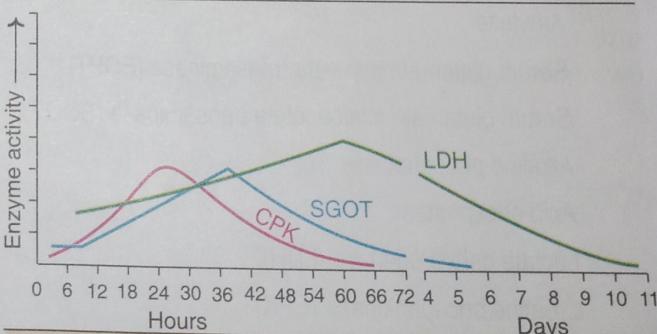
### ENZYME PATTERN IN DISEASES

For the right diagnosis of a particular disease, it is always better to estimate a few (three or more) serum enzymes, instead of a single enzyme. Examples of enzyme patterns in important diseases are given here.

### Enzymes in myocardial infarction

The enzymes—namely creatine phosphokinase (CPK), aspartate transaminase (AST) and lactate dehydrogenase (LDH)—are important in the diagnosis of myocardial infarction (MI). The elevation of these enzymes in serum in relation to hours/days of MI is given in the **Fig.6.12**.

**Creatine phosphokinase** (precisely isoenzyme **MB**) is the first enzyme to be released into circulation within **6-18 hours after the infarction**. Therefore, CPK estimation is highly useful for the early diagnosis of MI. This enzyme reaches a peak value within 24-30 hours and returns to normal level by the 2nd or 3rd day.



**Fig.6.12 :** Enzyme pattern in myocardial infarction (CPK-Creatine phosphokinase; SGOT-Serum glutamate oxaloacetate transaminase; LDH-Lactate dehydrogenase).

**Aspartate transaminase** (AST or SGOT) rises sharply after CPK, and reaches a peak within 48 hours of the myocardial infarction. AST takes 4-5 days to return to normal level.

**Lactate dehydrogenase** (LDH<sub>1</sub>) generally rises from the second day after infarction, attains a peak by the 3rd or 4th day and takes about 10-15 days to reach normal level. Thus, LDH is the last enzyme to rise and also the last enzyme to return to normal level in MI.

[Note : Certain non-enzymatic biomarkers (e.g. **troponin I**, **myoglobin**, **brain natriuretic peptide**) are in recent use for the diagnosis of MI. The concentration of troponin I shots up in serum within 4 hours of MI and remains elevated for about 2 weeks.]

### Enzymes in liver diseases

The following enzymes—when elevated in serum—are useful for the diagnosis of liver dysfunction due to viral hepatitis (jaundice), toxic hepatitis, cirrhosis and hepatic necrosis

1. **Alanine transaminase**;
2. Aspartate transaminase;
3. Lactate dehydrogenase.

The enzymes that markedly increase in intrahepatic and extrahepatic cholestasis are

1. Alkaline phosphatase,
  2. 5'-Nucleotidase
- Serum  **$\gamma$ -glutamyl transpeptidase** is useful for the diagnosis of **alcoholic liver diseases**.

### Enzymes in muscle diseases

In the muscular dystrophies, probably due to the increased leakage of enzymes from the damaged cells, serum levels of certain muscle enzymes are increased. These include creatine phosphokinase, aldolase and aspartate transaminase. Of these, CPK is the most reliable indicator of muscular diseases, followed by aldolase.

### Enzymes in cancers

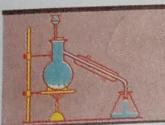
Increase in the serum acid phosphatase (tartarate labile) is specific for the detection of prostatic carcinoma.

[Note : **Prostate specific antigen** (PSA; Mol Wt. 32kD), though not an enzyme, is a more reliable marker for the detection of prostate cancer. Normal serum concentration of PSA is 1-4 ng/ml.]

A non-specific increase in certain enzymes like LDH, alkaline phosphatase and transaminase may be associated with malignancy in any part of the body.

$\beta$ -Glucuronidase estimation in urine is useful in detecting the cancers of urinary bladder, pancreas etc.

## SUMMARY

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1. Enzymes are the protein biocatalysts synthesized by the living cells. They are classified into six major classes—oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.
  2. Factors like concentration of enzyme, substrate, temperature, pH etc. influence enzyme activity. The substrate concentration to produce half-maximal velocity is known as Michaelis constant ( $K_m$  value).
  3. Enzyme activities are inhibited by reversible (competitive, non-competitive and uncompetitive), irreversible and allosteric manner.
  4. Many enzymes require the presence of non-protein substances called cofactors (coenzymes) for their action. Most of the coenzymes are derivatives of B-complex vitamins (e.g. NAD<sup>+</sup>, FAD, TPP etc.)
  5. Estimation of serum enzymes is of great help in the diagnosis of several diseases. Serum amylase and lipase are increased in acute pancreatitis; alanine transaminase in hepatitis; aspartate transaminase, lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in myocardial infarction; alkaline phosphatase in rickets and hyperparathyroidism; acid phosphatase in prostatic carcinoma;  $\gamma$ -glutamyl transpeptidase in alcoholism.
  6. Isoenzymes are the multiple forms of an enzyme catalysing the same reaction which however, differ in their physical and chemical properties. LDH has five isoenzymes while CPK has three. LDH<sub>1</sub> and CPK<sub>2</sub> are very important in the diagnosis of myocardial infarction.