Enzymes

INTRODUCTION

Enzymes are biological materials with catalytic properties, i.e. they increase the rate of chemical reactions in biological and *in vitro* (in the laboratory) systems, that otherwise proceed very slowly. The study of enzymes and of the changes in the enzyme activity that occur in body fluids has become a valuable diagnostic tool for the elucidation of various diseases and for testing organ function.

DEFINITION

Enzymes are biological catalyst produced by living tissues. They are proteins (except a small group of RNA acting as ribozyme) that have the property of accelerating specific chemical reactions without being consumed in the process.

ZYMOGEN OR PROENZYME

- A number of proteolytic enzymes found in the blood or in the digestive tract are present in an inactive (precursor) form, called ***zymogen*** or **proenzymes.**
- For example, chymotrypsin is secreted by the pancreas as **chymotrypsinogen.** It is activated in the digestive tract by the proteolytic enzyme trypsin.
- Precursor proteins or inactive enzyme names have the prefix *"pro"* like prothrombin, proelastase, etc. or suffix *"ogen"* like chymotrypsinogen, trypsinogen, pepsinogen, etc.

COFACTORS (COENZYME AND ACTIVATOR)

- Some enzymes require an additional nonprotein component for its optimum activity. This additional component is called ***cofactor*** which may be either loosely or tightly bound to the protein portion of the enzyme.
- These cofactors may be:
  - Organic compounds, called ***coenzymes***
  - Inorganic ions, called ***activators.***
- Enzymes without its cofactor is referred to as an **apoenzyme;** the complete catalytically active enzyme is called **holoenzyme.**

  Apoenzyme + cofactor = holoenzyme.
- Many vitamins function as coenzymes. Coenzymes derived from vitamins will be considered for more details ***in chapter 7.***
- The lists of coenzyme and activators are given in **Tables 6.1 and 6.2** respectively.

Table 6.1: Some common coenzymes and their functions

Vitamin	Coenzyme	Function as coenzyme
Thiamine (Vit B_1)	TPP (Thiamine pyrophosphate)	Oxidative decarboxylation and transketolase reaction
Riboflavin (Vit B_2)	FAD and FMN (Flavin Adenine Dinucleotide and Flavin Mononucleotide)	Oxidation and reduction reactions
Niacin (B_3)	NAD^+ (Nicotinamide Adenine Dinucleotide), $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate)	Oxidation and reduction reactions
Pyridoxine (Vit B_6)	PLP (Pyridoxal phosphate)	Transamination, deamination decarboxylation reactions of amino acids
Biotin (B_7)	Biocytin	Carboxylation reactions
Folic acid (Vit B_9)	THF (Tetrahydrofolate)	Carrier of one carbon group
Pantothenic acid (B_5)	Coenzyme A	Acyl carrier
Cynocobalamine (B_{12})	Methylcobalamine and Deoxyadenosylcobalamine	Transfer of CH_3 group and isomerizations

Table 6.2: Enzymes requiring or containing inorganic elements as cofactors (activators)

Enzyme	Cofactor (activator)
Ferroxidase (ceruloplasmin), Ascorbic acid oxidase	Copper
Carbonic anhydrase, DNA-polymerase, Porphobilinogen synthase, Carboxypeptidase	Zinc
Cytochrome oxidase, Catalase	Iron
Glucose-6-Phosphatase, Hexokinase	Magnesium
Glutathione peroxidase	Selenium
Arginase, Pyruvate carboxylase	Manganese
Xanthine oxidase	Molybdenum

HOW ENZYMES WORK

Energy Changes Occur During the Reaction

Virtually all chemical reactions have an energy barrier, separating the reactants and the products. This barrier, called the *free energy of activation*, is the energy difference between the energy of the reactant and high energy intermediates that occurs during the formation of a product. **Figure 6.1** shows the changes in energy during the conversion of a molecule of reactant 'S' to product 'P' through the transition state S*.

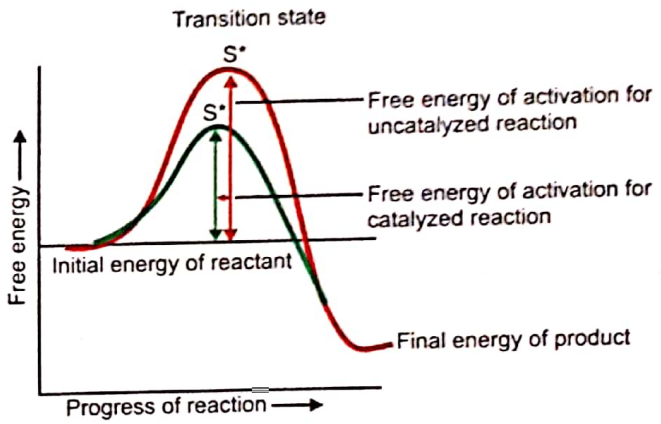


Figure 6.1: Comparison of the free energy of activation of a catalyzed and uncatalyzed reaction, S*: Transition state

$$S \rightleftharpoons S^* \rightleftharpoons P$$

(Reactant) (Product)

The peak of free energy activation, represents the transition state, in which the high energy intermediates (S*) are formed during the conversion of a reactant to a product **(Figure 6.1).**

- An enzyme lowers the energy required for activation to the transition state.
- Without a catalyst, the reaction will occur only if enough heat energy is added to the reaction system.
- ***With an enzyme as a catalyst, the reaction may easily proceed at the normal physiological temperature.***

MECHANISM OF ENZYME ACTION

- Formation of an enzyme-substrate (ES) complex is the first step in enzymatic catalysis.

- **Substrate is bound through multiple noncovalent interactions at the active site of the enzyme forming an enzyme-substrate (ES) complex which is subsequently converted to product and free enzyme.**

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

- The active site of an enzyme is the region that binds the substrate and which contains the specific amino acid residues.
- Two models for substrate binding to the active site of the enzyme, have been proposed to explain the specificity that an enzyme has for its substrate:
  1. Lock and key model or rigid template model of Emil Fisher.
  2. Induced fit model or hand-in-glove model of Daniel E Koshland.

Lock and Key Model or Rigid Template Model of Emil Fisher

- This model was proposed by Emil Fisher in 1890.
- In this model, enzyme is preshaped and the active site has a rigid structure that is complementary to that of the substrate **(Figure 6.2).**
- This model is called lock and key model, because in this model the substrate fits into the active site in much the same way that a key fits into a lock.
- This model has been useful in understanding how some enzymes can bind only a specific substrate but will not bind another compound with an almost identical structure. For example, most enzymes in carbohydrate metabolism can bind the D-isomer of hexoses but cannot bind the corresponding L-isomer, which differs only in the configuration around a single carbon atom.
- This model explains all mechanisms but do not explain the changes in the enzyme activity in the presence of allosteric modulators.

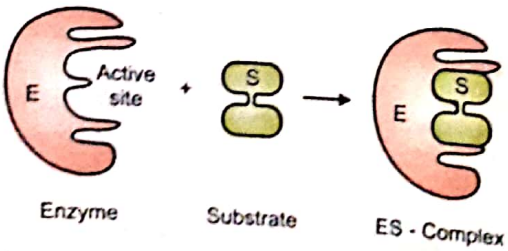


Figure 6.2: Representation of formation of an ES-complex according to the Fisher's lock and key model. The active site of the enzyme is complementary in shape to that of substrate

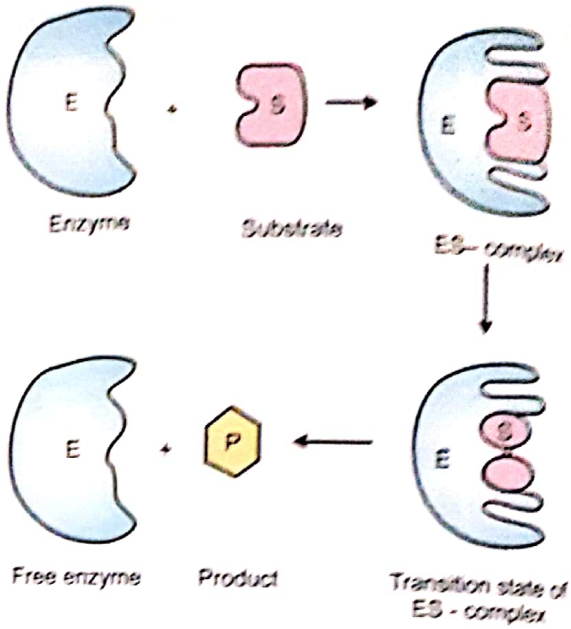


Figure 6.3: Schematic representation of induced fit model of Koshland

Induced Fit Model or Hand-in-glove Model of Daniel E Koshland (Figure 6.3)

Fisher's model explained the specificity of enzyme substrate interaction but the implied rigidity of the enzymes active site failed to explain the dynamic changes that must take place during catalysis. A model that accounts for both of these aspects of enzyme catalysis is the **induced fit model of Daniel E Koshland.**

- In the Fisher's model the catalytic site is presumed to be preshaped to fit the substrate. However, Daniel E Koshland in 1958 postulated that the **enzymes are flexible and shapes of the active site can be modified by the binding of the substrate.**
- In the induced fit model, the substrate induces a conformational change in the enzyme, in the same manner in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape. Therefore, this model is also known as **hand-in-glove model.**
- Conformational change in enzyme in turn induces reciprocal changes in its bound substrate that alters their orientation and configuration and strains the structure of the bound substrate. Due to such changes energy is librated, which is called **intrinsic binding energy (Figure 6.3).**
- This intrinsic binding energy due to the substrate-enzyme interaction is made available for the transformation of the substrate into product.

- This model is believed to describe more accurately the specificity of substrate binding than does lock and key model of E Fisher.

ENZYME CLASSIFICATION

The classification of enzyme was described in 1961 by enzyme commission of the International Union of Biochemistry (IUB). According to this classification, each enzyme is characterized by a code number called **enzyme code number** or **'EC' number,** consisting of four digits.

According to the IUB system, enzymes are classified into six major classes as follows:

1. EC-1 : Oxidoreductases
2. EC-2 : Transferases
3. EC-3 : Hydrolases
4. EC-4 : Lyases
5. EC-5 : Isomerases
6. EC-6 : Ligases.

Some common examples of different classes of enzymes are given in **Table 6.3.**

Table 6.3: Examples of enzymes with their main class

Class	Example
EC-1:Oxidoreductases	Lactate dehydrogenase (LDH) Glucose 6-phosphate dehydrogenase (G-6-PD) Cytochrome oxidase
EC-2: Transferases	Aspartate aminotransaminase(AST) Alanine aminotransaminase (ALT) Hexokinase
EC-3: Hydrolases	Lipase α-Amylase Trypsin Lactase Sucrase Pepsin
EC-4: Lyases	Aldolase Argininosuccinase Carbonic anhydrase
EC-5: Isomerases	Phosphoglucomutase Triphosphate isomerase Phosphohexose isomerase
EC-6: Ligases	Glutamine synthetase Pyruvate carboxylase DNA ligases

EC-1 Oxidoreductases

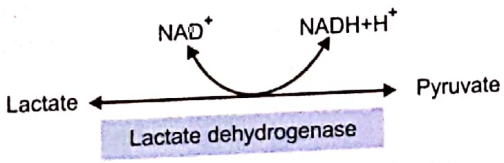
Those enzymes that catalyze oxidation-reduction reactions, are included in this class which can be illustrated schematically as follows:

$$\underset{\text{(red)}}{A} + \underset{\text{(ox)}}{B} \rightleftharpoons \underset{\text{(ox)}}{A} + \underset{\text{(red)}}{B}$$

Enzymes in this category include :

- Dehydrogenases
- Reductases
- Oxidases
- Peroxidases.

Specific Example



EC-2 Transferases

Those enzymes that catalyze the transfer of a group such as, *amino, carboxyl, methyl* or *phosphoryl,* etc. from one molecule to another are called transferases. These reactions can be illustrated as follows:

$$A\text{–}X + B \rightleftharpoons A + B\text{–}X$$

Some common enzymes in this category include :

- Amino transferase or transaminase
- Kinase
- Transcarboxylase.

Specific Example

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons[\text{PLP}]{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

where, ALT : Alanine aminotransferase
PLP : Pyridoxal phosphate (coenzyme)

EC-3 Hydrolases

Enzymes of this class catalyze the cleavage of C-O, C-N, C-C and some other bonds with the addition of water. These can be illustrated as follows:

$$A\text{-}B + H_2O \longrightarrow A\text{-}OH + B\text{-}H$$

Some common enzymes in this category are:

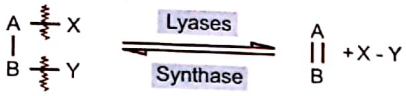
- Acid phosphatase
- All digestive enzymes like α-amylase, pepsin, trypsin, chymotrypsin, etc.

Specific Example

Glucose-6-phosphate + H_2O —(Glucose-6-phosphatase)→ Glucose + Pi

EC-4 Lyases

Lyases catalyze the cleavage of **C-O, C-C** and **C-N** bonds by means other than hydrolysis or oxidation, giving rise to compound with double bonds or catalyze the reverse reaction, by the addition of group to a double bond. In cases where reverse reaction is important, then synthase, (not synthetase of group EC-6) is used in the name. This type of reaction is illustrated as follows:



Specific Example

Fructose 1,6-bisphosphate —(Aldolase)→ Glyceraldehyde- 3-phosphate + Dihydroxy actone phosphate

EC-5 Isomerases

Isomerases catalyze intramolecular structural rearrange ment in a molecule. They are called **epimerases, isomerases** or **mutases,** depending on the type of isomerism involved. This reaction can be illustrated as follows:

A B C ⇌ C A B

Specific Example

Glucose-6-phosphate —(Glucose-6-phosphate isomerase)→ Fructose-6-phosphate

EC-6 Ligases (Synthetases)

Ligases catalyze the joining of two molecules coupled with the hydrolysis of ATP. The reaction is illustrated as follows:

A + B + ATP ⟶ A B + ADP + Pi

Specific Example

Glutamate + NH_3 + ATP —(Glutamine synthetase)→ Glutamine + ADP + Pi

SPECIFICITY OF ENZYME ACTION

Specificity refers to the ability of an enzyme to discriminate between two competing substrates. Enzymes are highly specific both in the reaction catalyzed and in their choice of substrates. Specificity makes it possible for a number of enzymes to co-exist in the cell without interfering in each other's actions.

Types of Specificity

The following types of specificity have been recognized:

1. Substrate specificity
2. Reaction specificity
3. Stereo specificity.

Substrate Specificity

There are following types of substrate specificity:

i. Absolute substrate specificity
ii. Relative substrate specificity
iii. Broad substrate specificity.

Absolute substrate specificity

Certain enzymes will act on only one substrate and catalyze one reaction, e.g. Glucokinase, lactase, urease, etc.

Glucose —(Glucokinase)→ Glucose-6-phosphate

Lactose —(Lactase)→ Glucose +Galactose

Urea —(Urease)→ Ammonia + CO_2

Relative substrate specificity

In this case, enzyme acts on more than one substrate. The relative substrate specificity is of two types:

a. Group specificity
b. Bond specificity.

- In **group specificity,** an enzyme acts on more than one substrate containing a particular group, e.g. chymotrypsin acts on several proteins by hydrolyzing peptide bonds attached to aromatic amino acids. Trypsin hydrolyzes peptide linkages involving arginine or lysine.
- In **bond specificity,** an enzyme acts on more than one substrate containing a particular kind of bond, e.g. salivary α-amylase cleaves α-(1→4) glycosidic bonds of carbohydrates, lipase hydrolyzes ester bonds of lipids.

Broad substrate specificity

In this case, an enzyme acts on more than one structurally related substrates, e.g. hexokinase catalyzes the phosphorylation of more than one kind of hexoses such as glucose, fructose and mannose.

Reaction Specificity

In this case, an enzyme is specific to a particular reaction but not to substrate (s) and catalyzes only one type of reaction. For example, pyruvate can undergo several reactions. Each reaction is catalyzed by a separate enzyme, which catalyzes only that reaction and none other as shown in **Figure 6.4.**

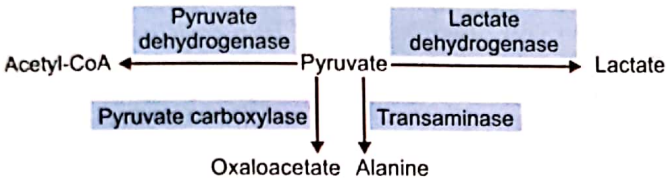


Figure 6.4: Example of reaction specificity

Stereo Specificity

Many enzymes show specificity towards stereoisomers, i.e. they act on only one type of isomer. For example,

- L-lactate dehydrogenase will act only on L-lactic acid and not D-lactic acid.
- Likewise, L-amino acid oxidase and D-amino acid oxidase are distinct enzymes which act only on L and D-amino acids respectively.
- D-glucose oxidase can similarly act only on D-glucose and not on L-glucose.
- Salivary α-amylase acts on the α-1,4 glycoside linkage and is inactive on β-1,4 glycoside linkage.
- Isomerase and epimerase do not show stereospecificity.

FACTORS AFFECTING THE VELOCITY OF ENZYME REACTION

Various factors that affect enzyme activity are:

- Substrate concentration
- Enzyme concentration
- pH i.e. H^+ ion concentration
- Temperature
- Product concentration
- Activators and coenzymes
- Time
- Physical agents
- Inhibitors

Effect of Substrate Concentration

For a given quantity of enzyme, the velocity of the reaction increases as the concentration of the substrate is increased. At first, this relationship is almost linear but later, the reaction curve becomes hyperbolic in shape **(Figure 6.5).**

- At relatively low concentrations of substrate, V_0 increases almost linearly with an increase in substrate concentration [S], a condition known as ***First order kinetics.***
- At higher substrate concentrations, V_0 increases by smaller amounts in response to increase in substrate concentration [S].
- Finally, a part is reached beyond which there are only vanishingly small increase in V_0 with increasing [S], a condition known as ***zero order kinetics*** and a plateau is called ***maximum velocity, V_{max}*** **(Figure 6.5).** Under these conditions, all the free enzymes will have been converted into ES form so that any further increase in substrate concentration has no effect on the rate and the reaction achieves a steady state.

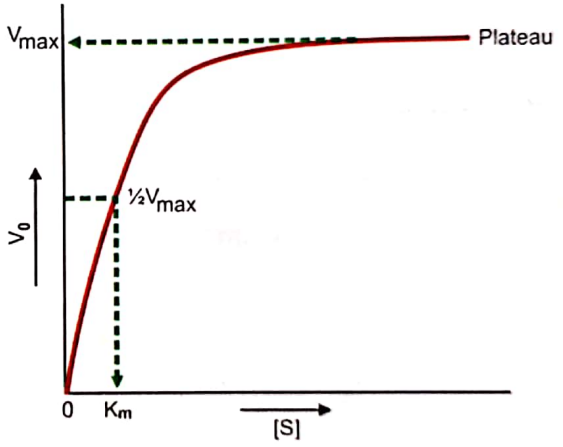


Figure 6.5: Effects of substrate concentration [S] on enzyme activity keeping enzyme concentration constant
where, V_0 : initial velocity
V_{max} : maximum velocity
K_m : 1/2 V_{max} = Michaelis Menten constant
[S] : substrate concentration

Effect of Enzyme Concentration

- The velocity of a reaction is directly proportional to the amount of enzyme present as long as the amount of substrate is not limiting.
- The substrate must be present at a concentration sufficient to ensure that all of the enzyme molecules have substrate bound to their active site **(Figure 6.6).**

Effect of Hydrogen Ion Concentration pH

- Each enzyme has an ***optimum pH,*** i.e. a pH at which the enzyme activity is maximum. Below or above this pH, enzyme activity is decreased. The optimum pH

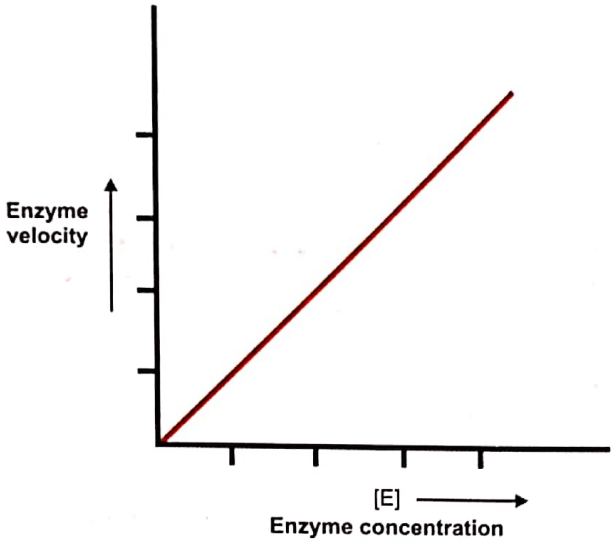


Figure 6.6: Effect of enzyme concentration on enzyme activity

differs from enzyme to enzyme, e.g. optimum pH for:
- Pepsin = 1.2
- Trypsin = 8.0

- A bell shaped curve is obtained when enzyme velocity is plotted against pH **(Figure 6.7)**.
- Changes in pH can alter the following:
  - Ionization state of the amino acid residues present in the active site of the enzyme. Enzyme activity is related to the ionic state of active site of the enzyme.
  - The ionization state of the substrate. The active site of an enzyme may require particular ionic state of the substrate for optimum activity.
  - Drastic change in pH denatures the enzyme protein.

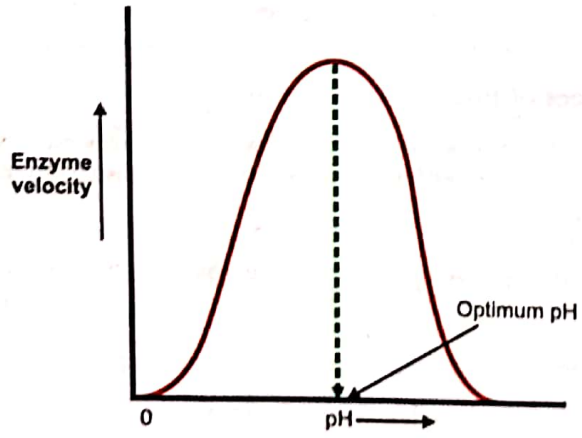


Figure 6.7: Effect of pH on enzyme activity

All these affect the enzyme substrate complex formation and decrease the rate of enzyme reaction. *As pH affects the enzyme activity, in enzyme studies, buffers are used to keep enzyme at an optimum or at least a favorable H^+ ion concentration.*

Effect of Temperature

- Enzyme catalyzed reactions show an increase in rate with increasing temperature only within a relatively small and low temperature range.
- Each enzyme shows the highest activity at a particular temperature called *optimum temperature.*
- The activity progressively declines both above and below this temperature.
- A bell shaped curve is obtained when we plot the enzyme velocity Vs. temperature **(Figure 6.8).**

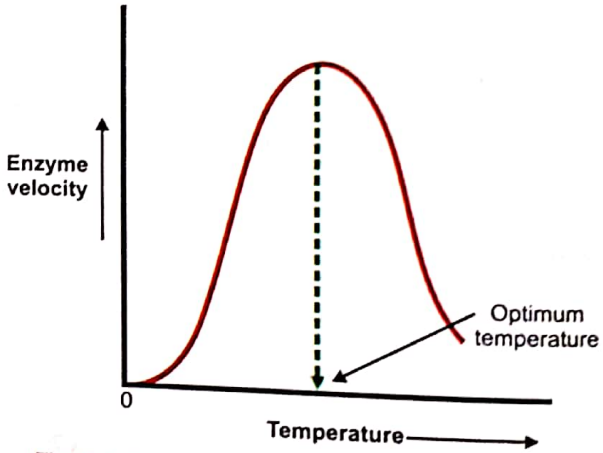


Figure 6.8: Effect of temperature on enzyme activity

- Increase in velocity is due to the increase in the kinetic energy. Further elevation of the temperature results in a decrease in reaction velocity due to denaturation of the enzyme protein.
- Low temperature also decreases enzyme activity and enzymes may be completely inactive at temperature of 0°C and below.
- The inactivity at low temperature is reversible. So, many enzymes in tissues or extracts may be preserved for months by storing at –20°C or –70°C.
- The reaction velocity of most chemical reactions increases with temperature approximately doubles for each 10°C rise called temperature coefficient Q_{10}.
- Most of the body enzymes have the optimum temperature close to 37°C to 38°C and have progressively less activity as the temperature rises.

Effect of Product

Accumulation of products of the reaction causes the inhibition of enzyme activity for some enzymatic reactions, this form of control will limit the rate of formation of the product when the product is under used. In biological systems, however, the product is usually removed as it becomes a substrate for a succeeding enzyme in a metabolic pathway.

Effect of Activators and Co-enzymes

The activity of many enzymes is dependent on the activators (metallic ions) like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, etc. and coenzymes for their optimum activity. In absence of these activators and coenzymes, enzymes become functionally inactive.

Effect of Time

Under optimum conditions of pH and temperature, time required for an enzyme reaction is less. The time required for the completion of an enzyme reaction increases with changes in temperature and pH from its optimum.

Effect of Physical Agents

Physical agent like light rays can inhibit or accelerate certain enzyme reactions. For example, the activity of salivary amylase is increased by red and blue light. On the other hand, it is inhibited by ultraviolet rays.

Effect of Inhibitors

The substances which stop the enzymatic reaction are called inhibitors. Presence of these substances in reaction medium decreases the rate of enzyme reaction. Different types of enzyme inhibitors are discussed separately later.

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ENZYME KINETICS

The study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as ***kinetics.***

One of the key factors affecting the rate of a reaction catalyzed by an enzyme *in vitro* (in laboratory) is the amount of substrate present, [S]. The effect on V_0 (initial velocity) of varying substrate [S] concentration, when enzyme concentration is held constant, is shown in **Figure 6.5.**

Michaelis-Menten Equation

Enzyme catalyzed reactions occur in two stages as shown in the following equations

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \quad \text{.....1)}$$

$$ES \xrightarrow{K_3} E + P \quad \text{.....2)}$$

K_1, K_2 and K_3 are rate constant. The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration. The relationship between reaction rate and substrate concentration shown in **Figure 6.5** is described mathematically by the 'Michaelis-Menten equation' as follows:

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

where,

V_0 = initial reaction velocity (is the rate of reaction as soon as enzyme and substrates are mixed).

V_{max} = maximum velocity (is observed when all active sites on the enzyme are filled with substrate).

K_m = Michaelis-Menten constant, [is the substrate concentration, at which the reaction rate is half of its maximum velocity (V_{max})].

[S] = Substrate concentration

Every enzyme has the characteristics V_{max} and K_m which are sensitive to change in pH, temperature and ionic strength.

Significance of K_m (Michaelis Constant)

- K_m, the Michaelis constant is equal to the substrate concentration at which the reaction rate is half of its maximal value. The K_m value of an enzyme depends on the substrate and environmental conditions such as pH, temperature and ionic strength.
- The Michaelis constant, K_m has two significances:
  1. K_m provides a measure of the substrate concentration required for significant catalysis to occur.
  2. It is a measure of the **affinity of the enzyme for its substrate,** a high K_m indicates weak binding and a low K_m indicates strong binding with its substrate.

Significance of V_{max} (Maximal Velocity)

- The V_{max} of a reaction is an index of the catalytic efficiency of an enzyme. The V_{max} is useful in comparing the activity of one enzyme with that of another.

Lineweaver-Burk Plot or Double-Reciprocal Plot

Lineweaver-Burk plots are used to obtain values for V_{max} and K_m. A more accurate method of determining

values for V_{max} and K_m uses Lineweaver-Burk equation shown below. This equation is obtained by taking the reciprocal of the Michaelis-Menten equation.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

- When $1/V_0$ is plotted against $1/[S]$, a straight line is obtained **(Figure 6.9)**, with a slope equal to K_m/V_{max}.
- The point, at which line intersects the y-axis, is numerically equal to $1/V_{max}$.
- The point at which the line intersects the x-axis is numerically equal to $-1/K_m$.
- This plot is useful to determine the mechanism of action of enzyme inhibitors.

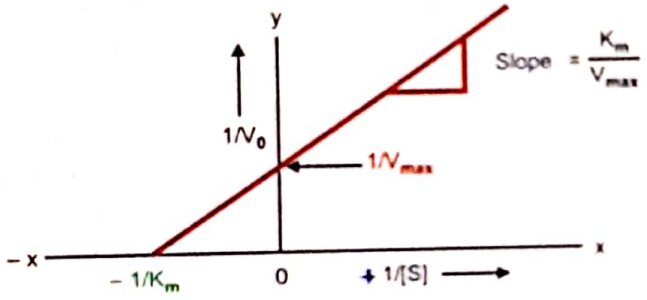


Figure 6.9: Lineweaver-Burk plot (Double reciprocal plot)

ENZYME INHIBITION

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called inhibitor. Two general classes of inhibitors are recognized according to whether the inhibitor action is **reversible** or **irreversible (Figure 6.10)**

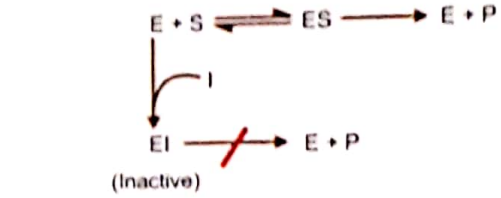
1. Reversible inhibitor
2. Irreversible inhibitor.

Reversible Inhibitor

- Reversible inhibitors bind to enzymes through **non-covalent** bonds and the activity of the enzyme is restored fully when the inhibitor is removed from the system.
- Different types of reversible inhibitors are:
  i. Competitive or substrate analogue inhibitor
  ii. Noncompetitive inhibitor
  iii. Uncompetitive inhibitor.

Competitive or Substrate Analogue Inhibitor

- A competitive inhibitor is usually a structural analogue of the substrate.
- The chemical structure of the inhibitor (I) closely resembles that of the substrate (S) and binds to the enzyme at the active site, forming an EI complex rather than ES-complex **(Figure 6.11).**



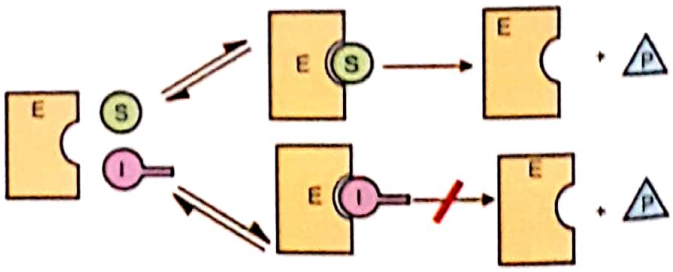


Figure 6.11: Diagrammatic representation of competitive inhibition where, E: Enzyme; S: Substrate; I: Competitive inhibitor; P: Product

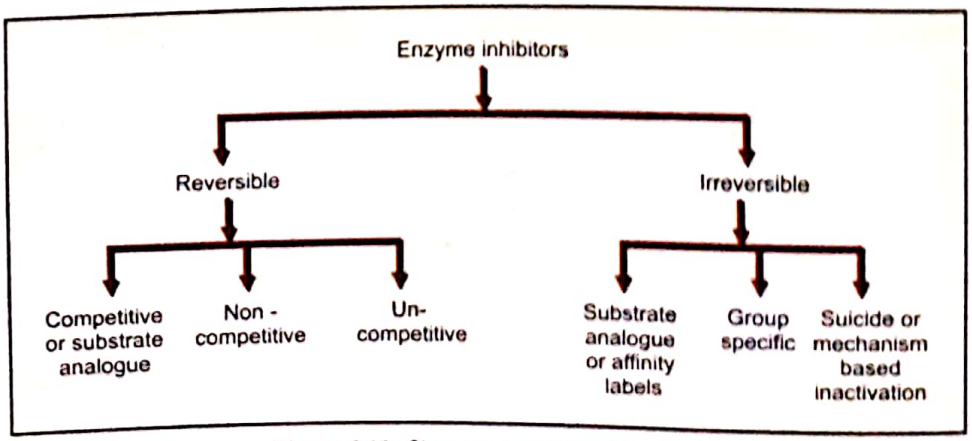


Figure 6.10: Classes of enzyme inhibitors

- But because it is not identical with the substrate, breakdown into products does not take place.
- When both the substrate and this type of inhibitor are present, they compete for the same binding site on the enzyme.
- The inhibition could be overcome by increasing substrate concentration. In competitive inhibition, the **K_m increases** whereas **V_{max} remains unchanged (Figure 6.12).**

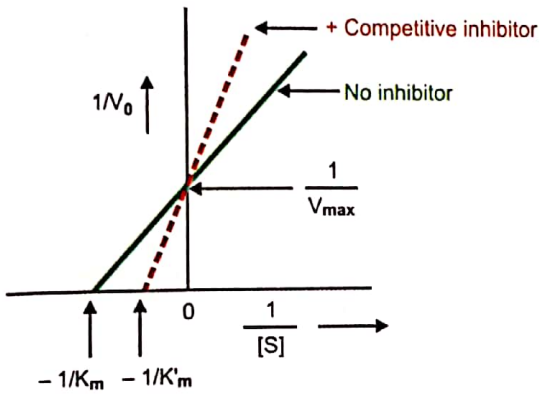


Figure 6.12: A double reciprocal plot of enzyme kinetics in presence and absence of competitive inhibitor, V_{max} is unaltered whereas K_m is increased

- The classical example is the competitive inhibition of **succinate dehydrogenase** by the **malonate.**
- Succinate dehydrogenase is one of the enzymes of the citric acid cycle. Succinate dehydrogenase is inhibited by malonate which resembles succinate.
- Many drugs which act as competitive inhibitors are given below and some more are given in **Table 6.4.**

Sulfonamide

- Sulfonamide is an analogue of P-aminobenzoic acid (PABA) and inhibits **pteroid synthetase** enzyme required for the synthesis of **folic acid** in microorganisms.
- Since folic acid is involved in the biosynthesis of purines and thymine, sulfonamides inhibit growth of the pathogenic organisms.

Isoniazide [Isonicotinic acid hydrazine (INH)]

It is an antituberculous drug, an analogue of nicotinamide.

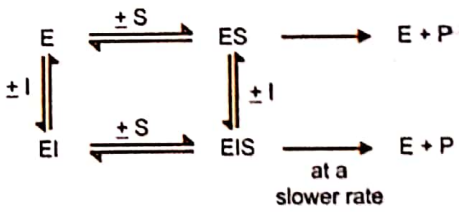
INH interferes with the biosynthesis of NAD and restrict the growth of the organisms that cause tuberculosis.

Dicumarol

It is an anticoagulant drug structurally similar to vitamin K. It inhibits the vitamin K activity and inhibits the formation of prothrombin.

Noncompetitive Inhibitors

- As the name implies, in this type of inhibition no competition occurs between substrate and inhibitor. Inhibitor is usually structurally different from the substrate.
- It binds at a site on the enzyme molecule other than the substrate-binding site and thus there is no competition between inhibitor and substrate.
- Since inhibitor and substrate may bind at different sites; formation of both EI and EIS-complexes is possible.
- Since EIS may breakdown to form product at a slower rate than does ES, the reaction is slowed but not halted. The following reactions may occur:



- For noncompetitive inhibition, the **K_m value is unchanged** while **V_{max} is lowered (Figure 6.13)**

Table 6.4: Commonly used drugs that are enzyme inhibitors

Drug	*Type of inhibition*	*Target enzyme*	*Therapeutic use*
Mevinolin Lovastatin	Competitive	HMG-CoA reductase (3-Hydroxy-3-Methyl-Glutaryl CoA-reductase)	Hypercholesterolemia
Allopurinol	Competitive	Xanthine oxidase	Gout
Methotrexate	Competitive	Dihydrofolate reductase	Cancer
5-Fluorouracil	Suicide	Thymidylate synthase	Cancer
Aspirin	Suicide	Cyclo-oxygenase	Anti-inflammatory
Penicillin	Suicide	Bacterial transpeptidase	Antibacterial

- Examples of noncompetitive inhibitors are:
  - **Ethanol** or certain **narcotic drugs** are noncompetitive inhibitor of **acid phosphatase**.
  - **Trypsin inhibitors** occur in **soybean** and **raw egg white**, inhibit activity of trypsin noncompetitively.
  - As well as ***Ascaris* parasites** (worm) contain **pepsin** and **trypsin inhibitors**, which inhibit non-competitively action of pepsin and trypsin, that is why ascaris worm is not digested in human intestine.

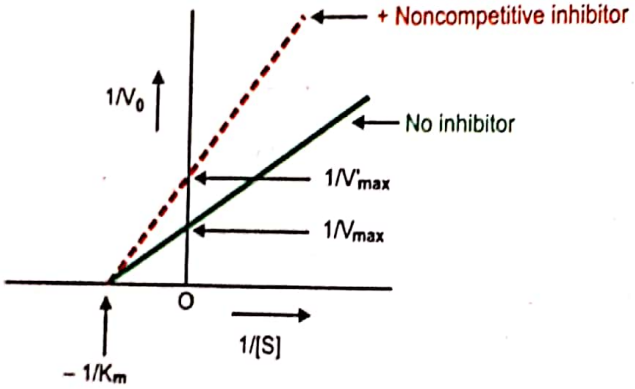


Figure 6.13: A double reciprocal plot of enzyme kinetics in presence and absence of noncompetitive inhibitor; K_m is unaltered by noncompetitive inhibitor, whereas V_{max} is decreased

Uncompetitive Inhibitor

- Uncompetitive inhibitor can bind only to the enzyme-substrate (ES) complex.
- It does not have affinity for free enzyme. Uncompetitive inhibitor **decreases both V_{max}** and **K_m (Figure 6.14).**
- This form of inhibitor is rare with single substrate but more common in multiple substrate reaction.

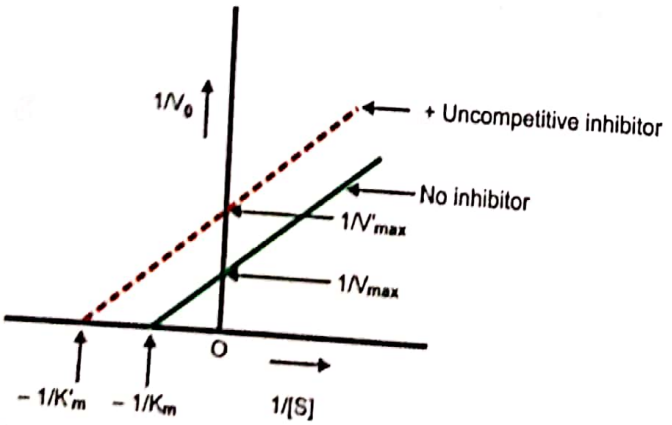


Figure 6.14: Effect of uncompetitive inhibitor on the double reciprocal plot; it shows parallel lines with decrease in both V_{max} and K_m

Irreversible Inhibitor

- An irreversible inhibitor binds with an enzyme tightly **covalently** and forms a stable complex.
- An irreversible inhibitor cannot be released by dilution or dialysis or simply by increasing the concentration of substrate.
- Irreversible inhibitors can be divided into three categories:
  i. Group specific inhibitors
  ii. Substrate analogue inhibitor or affinity labels
  iii. Suicide inhibitor or mechanism based inactivation.
- In terms of enzyme kinetics, the effect of an irreversible inhibitor is like that of the **reversible noncompetitive** inhibitors resulting in a **decreased** in V_{max} but having **no effect on the K_m (Table 6.5).**

Table 6.5: Effect of inhibitors on kinetic properties of enzymes

Type of inhibitor	*Km*	*V_{max}*
Irreversible	No effect	Decreased
Reversible competitive	Increased	No effect
Reversible noncompetitive	No effect	Decreased
Reversibe uncompetitive	Decreased	Decreased

Group Specific Irreversible Inhibitor

These inhibitors react with specific R-groups (side chain) of amino acid residues in the active site of enzyme. Examples of group specific irreversible inhibitors are:

Di-isopropylphosphofluoride (DIPF)

- DIPF can inhibit an enzyme **acetylcholine esterase** by covalently reacting with **hydroxyl group** of a **serine** residue present at the active site of the enzyme **(Figure 6.15)**

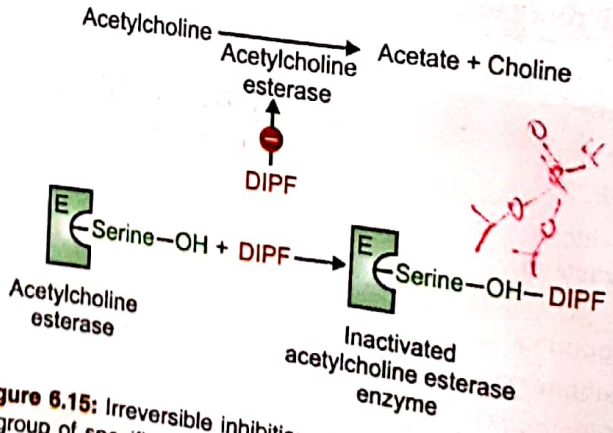


Figure 6.15: Irreversible inhibition of acetylcholine esterase by a group of specific inhibitor, diisopropylphosphofluoride (DIPF)

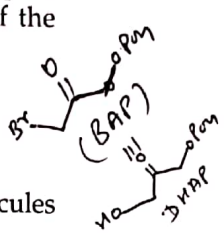
- DIPF has also been found to inhibit **trypsin, chymotrypsin, elastase** and **phosphoglucomutase**.

Iodoacetamide and heavy metals

- Iodoacetamide and heavy metals like, Pb^{2+}, Ag^{+}, Hg^{2+}, etc. which react with **sulfhydryl (-SH)** group of **cysteine** residues present at the active site of the enzyme and makes them inactive.

Substrate Analogue Irreversible Inhibitor or Affinity Labels

- Substrate analogues or affinity labels are molecules that are structurally similar to the substrate.
- These substrate analogues possess a highly reactive group which is not present in the natural substrate.
- The reactive group of substrate analogues covalently reacts with amino acid residues of the active site of the enzyme and permanently block the active site of the enzyme, e.g. 3-Bromoacetol phosphate (BAP).
- BAP is a substrate analogue of the normal substrate dihydroxyacetone phosphate (DHAP) for the enzyme phosphotriose isomerase of glycolysis.



Suicide Inhibitor or Mechanism Based Inactivation

- These compounds are relatively unreactive until they bind to the active site of a specific enzyme.
- On binding to the active site of the enzyme they carry out the first few catalytic activities of the normal enzyme reaction.
- Instead of being transformed into a normal product, however, the inhibitor is converted to a very reactive compound that combines irreversibly with the enzyme leading to its irreversible inhibition.
- The enzyme literally commits suicide. These are also called **mechanism based inactivation** because they utilize the normal enzyme reaction mechanism to inactivate the enzyme.
- These inhibitors act as drugs for example.

Penicillin

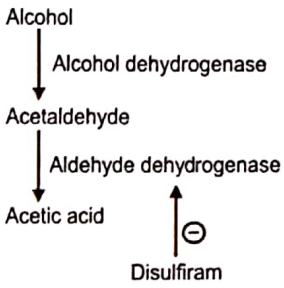
- Penicillin irreversibly inactivates an essential bacterial enzyme **glycopeptidyl transpeptidase** involved in the formation of bacterial cell wall.

Aspirin

- Aspirin inactivates an enzyme **cyclo-oxygenase** which catalyzes the first reaction in the biosynthesis of prostaglandins from arachidonic acid.

Disulfiram (antabuse)

- Disulfiram is a drug used in the treatment of alcoholism. It inhibits irreversibly **aldehyde dehydrogenase** enzyme resulting in accumulation of acetaldehyde in the body. Accumulation of acetaldehyde in the tissue leads to alcohol avoidance.



Clinical Application of Enzyme Inhibitor

Enzyme inhibitors have therapeutic applications. Most antibiotics and anticancer drugs that are used therapeutically are either competitive inhibitor or mechanism based suicide inhibitor. Some commonly used drugs that exert their effects by inhibiting enzymes are described in **Table 6.4.**

ALLOSTERIC ENZYME

Allosteric enzyme is a **regulatory enzyme.** The term allosteric derives from Greek word, **allo** means **other** and **steros** means **space** or **site.** ***Allosteric enzymes are those having other site.***

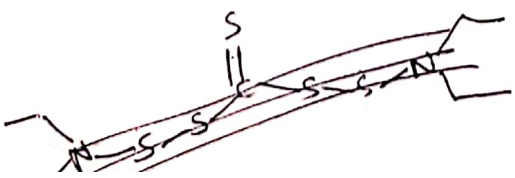
Like all enzymes, allosteric enzymes have active site for binding of the substrate but they also have one or more regulatory (or allosteric) sites for binding regulatory metabolites which is called ***modulator.***

- Allosteric enzymes may be inhibited or stimulated by their modulators **(Table 6.6)**.
- Modulators that inhibit enzyme activity are termed ***negative modulators.*** Whereas those that increase enzyme activity are called ***positive modulators.***
- Just as the active site of an enzyme is specific for its substrate, the allosteric site is specific for its modulators. Allosteric enzymes are generally larger and more complex than those of simple enzymes.

Feedback Allosteric Inhibition

In some multienzyme systems, the first enzyme of the sequence is the regulatory enzyme and has distinctive characteristics.

- It is inhibited by the end product of the multienzyme system whenever the end product of such metabolic reaction produced in excess of the cells needs. The end product of the pathway acts as a specific inhibitor of the first or regulatory enzyme in the pathway.



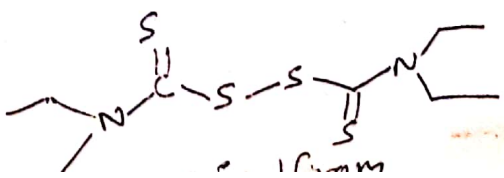


Table 6.6: Allosteric enzymes and its modulators

Pathway	Enzyme	Inhibitor	Activator
Glycolysis	Phosphofructokinase-I	ATP	AMP
Pyruvate to acetyl-CoA	Pyruvate dehydrogenase	ATP	-
TCA cycle	Isocitrate dehydrogenase	ATP	ADP
Gluconeogenesis	Pyruvate carboxylase	-	Acetyl-CoA
Fatty acid synthesis	Acetyl-CoA carboxylase	-	Citrate

- The whole enzyme system thus slows down to bring the rate of production of its end product back into balance with the cell's needs.

This type of regulation is called ***feedback inhibition.*** For example, first enzyme **δ-aminolevulenic acid synthase** is an allosteric enzyme in heme synthesis is inhibited by its end product heme. **(Figure 6.16).**

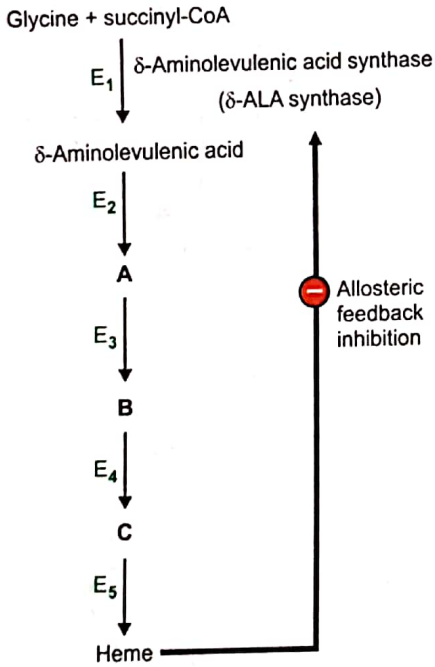


Figure 6.16: Feedback inhibition. First enzyme δ-ALA-synthase is an allosteric enzyme inhibited by its end product heme

ISOENZYME

- Isoenzymes or isozymes are multiple forms (isomers) of the same enzyme that catalyze the same biochemical reaction. Isoenzymes show different chemical and physical properties like electrophoretic mobility and kinetic properties.
- Not all enzymes have isoenzymes. In fact, it was found that only those enzymes, which are active in polymeric form demonstrate isoenzyme. For example:
  1. Lactate dehydrogenase (LDH)
  2. Creatine kinase (CK) (formerly called creatine phosphokinase (CPK).
- Some more examples of isoenzymes are given in **Table 6.7.**

Table 6.7: Examples of isoenzymes

Enzyme	Isoenzyme forms
Acid phosphatase	Prostate, erythrocytes, platelets, liver, spleen, kidney and bone marrow
Alkaline phosphatase	Bone, liver, placenta, intestine and kidney
Amylase	Salivary and pancreatic
Hexokinase	Liver (glucokinase) and muscle

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme that catalyzes the oxidation of L-lactate to pyruvate.

- LDH has five isoenzymes:
  - LDH_1 (H_4)
  - LDH_2 (H_3M)
  - LDH_3 (H_2M_2)
  - LDH_4 (HM_3)
  - LDH_5. (M_4)
- Since LDH is a **tetramer,** made up of two types of polypeptide **M *(muscle)*** type and ***H (heart)*** type, five combinations are possible with varying ratios of two kinds of polypeptides **(Table 6.8).**
- Five isoenzymes of LDH can be detected by electrophoresis as they have different electrophoretic mobilities.
- **LDH_1** is the **fastest moving** fraction towards the anode and **LDH_5** is the **slowest moving** isoenzyme of LDH **(Figure 6.17).**

- LDH_1 predominates in cells of cardiac muscle, and erythrocytes and LDH_5 is the most abundant form in the liver and in skeletal muscle **(Table 6.8).**

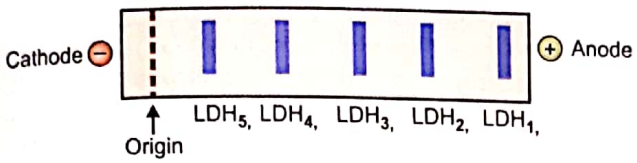


Figure 6.17: Electrophoretic separation of LDH isoenzyme

Clinical Applications of LDH

The LDH isoenzyme analysis may be useful in the following clinical situations **(Table 6.8)**:

- Significant elevation of LDH_1 and LDH_2 (LDH_1>LDH_2) occurs within 24 to 48 hours after myocardial infarction.
- Predominant elevation of LDH_2 and LDH_3 occur in leukemia. LDH_3 is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of LDH_5 occurs after damage to the liver or skeletal muscle.

Creatine Kinase (CK)

- Creatine kinase isoenzymes are **dimer** that are made up of two types of polypeptide chains, which may be either ***M (muscle)*** type or **B *(brain)*** type, generating three isoenzymes **(Table 6.8).**
  - CK_1 (BB) : It is present in the brain
  - CK_2 (MB) : Cardiac tissue is the only tissue which has the mixed MB (CK_2) isoenzyme
  - CK_3 (MM) : It is present in skeletal muscle.

Clinical Application

- CK_1 may be elevated in neonates particularly in damaged brain or very low birth weight newborn.
- Increased level of CK_2 in blood is characteristic of **damage of heart** tissue from myocardial infarction because cardiac tissue is the only tissue which has the mixed MB (CK_2) isoenzyme.
- Elevated levels of CK_3 in serum occur in all types of **dystrophies** and **myopathies.**

CLINICAL SIGNIFICANCE OF ENZYMES

Certain enzymes are used:

- For the diagnosis of the disease
- As therapeutic agents
- As analytical reagents.

Diagnostic Use of Enzymes

Enzymes are known as marker of **cellular damage** and their measurement in plasma is used in the investigation of diseases of **liver, heart, skeletal muscle,** the **biliary tract** and the **pancreas.** The enzymes that are found in plasma can be categorized into two major groups: The **plasma specific** enzyme and the **plasma nonspecific** enzyme.

- The **plasma specific** enzymes are present in higher concentration in plasma than in most tissues; e.g.
  - The enzymes involved in blood coagulation
  - Ferroxidase
  - Pseudocholinesterase
  - Lipoprotein lipase.
- These enzymes are clinically of interest when their concentration decreases in plasma.

Table 6.8: Type, composition, location and diagnostic importance of lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes

Type	*Composition*	*Location*	*Diagnostic importance (cause of elevated level)*
LDH_1	HHHH	Heart, RBC	Myocardial infarction
LDH_2	HHHM	Heart, RBC	Megaloblastic anemia
LDH_3	HHMM	Brain	Leukemia, malignancy
LDH_4	HMMM	Lung, spleen	Pulmonary infarction
LDH_5	MMMM	Liver, muscle	Liver diseases, Muscle damage/diseases
CK_1	BB	Brain	Neurological injury
CK_2	BM	Heart	Myocardial infarction
CK_3	MM	Skeletal muscle	Muscular dystrophies and myopathies

- The **plasma nonspecific** enzymes are present in very high concentration in tissues than in the plasma. Estimation of plasma nonspecific enzymes is very important for the diagnosis of several disease.
- The important enzymes useful for the diagnosis of specific diseases are listed in **Table 6.9**. A brief account of selected diagnostic enzymes is discussed.

Alanine transaminase (ALT)

- Alanine transaminase was known formerly as glutamate pyruvate transaminase (GPT).
- The plasma ALT normal value for adult is 10 to 40 U/L.
- ALT level is elevated in **liver diseases** (viral or toxic hepatitis), **jaundice** and **cirrhosis** of liver.

Aspartate transaminase (AST)

- It was known formerly as glutamate oxaloacetate transminase (GOT).
- The plasma AST normal value for adults is 10 to 30 U/L.
- Increased AST level occurs after **myocardial infarction.** The plasma AST level starts increasing after 6 to 8 hours after the onset of chest pain with peak values 18 to 24 hours and the values fall to normal level by the fourth or fifth day.
- It is moderately elevated in liver disease.

Alkaline phosphatase (ALP)

- ALP hydrolyzes organic phosphate at alkaline pH.
- Normal serum level for adults is 3-13 KA units/dl.
- It is elevated in certain **bone** and **liver disease.**
- Very high levels may be noticed in **obstructive jaundice, bone diseases** such as **Paget's disease,** rickets, osteomalacia, carcinoma of bone and hyperparathyroidism.

Acid phosphatase (ACP)

- It hydrolyzes phosphoric acid ester at pH 5 to 6.
- Normal serum value for ACP is 0.5 to 4 KA units/dL.
- Prostatic acid phosphatase enzyme is useful for the diagnosis and prognosis of **prostate cancer.** ACP is therefore an important **tumor marker.**

Amylase

- It catalyzes hydrolysis of starch and glycogen.
- Normal serum value is 50-120 U/L.
- The activity of serum amylase is increased in **acute pancreatitis.**
- Elevated activity of amylase is also found in urine of the patient of acute pancreatitis.
- Increase in serum levels are also seen in **chronic pancreatitis, mumps** and **obstruction of pancreatic duct.**

Creatine kinase (CK)

Refer isoenzyme.

Lactate dehydrogenase (LDH)

Refer isoenzyme.

Prostate specific antigen (PSA Seminogelase)

- It is a glycoprotein with mild protease activity and involved in the liquification of the seminal coagulum formed after ejaculation.

Table 6.9: Enzymes of diagnostic importance

Enzyme	*Clinical Application*
Acid phosphatase	Prostatic cancer
Alanine aminotransferase	Liver disease (viral or toxic hepatitis), jaundice and liver cirrhosis
Aldolase	Muscle diseases
Alkaline phosphatase	Obstructive jaundice, bone diseases such as Paget's disease, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism
Amylase	Acute pancreatitis, mumps, obstruction in pancreatic duct
Aspartate transaminase	Myocardial infarction, liver diseases
Cholinesterase	Organophosphorus insecticide poisoning, hepatic parenchymal diseases
Creatine kinase	Myocardial infarction, muscle diseases
γ-Glutamyl transferase	Hepatobiliary disease, alcoholism
Lactate dehydrogenase	Myocardial infarction, leukemia, muscular dystrophy, hepatic diseases
5'-Nucleotidase	Hepatitis, obstructive jaundice
Prostate specific antigen	Prostate cancer
Trypsin	Pancreatic disease, cystic fibrosis

- It is produced exclusively by prostate glands.
- Elevated blood levels of PSA occur in prostate cancer.

Enzyme Assay in Myocardial Infarction

The enzymes' assay, which are most helpful in the diagnosis of myocardial infarction are listed in **Table 6.10** and **Figure 6.18.**

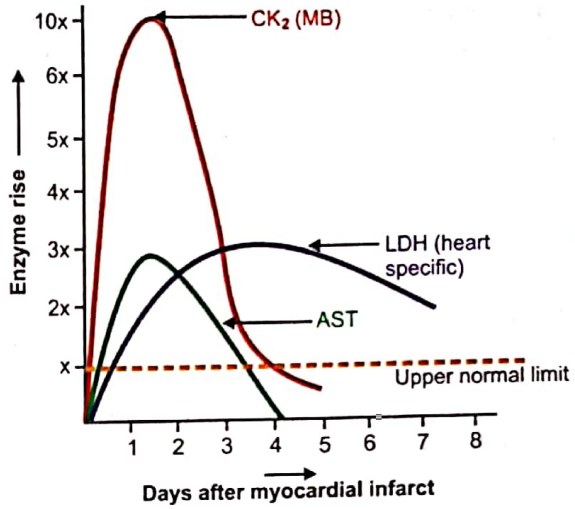


Figure 6.18: Typical rise in serum enzyme activities following a myocardial infarction

Therapeutic Use of Enzymes

Some enzymes are used in the treatment of some diseases of human being. For example:

- **Bacterial asparginase** is used in the treatment of some types of leukemia. Asparginase which hydrolyzes aspargine to aspartic acid. Aspargine is necessary for the formation of leukemic white cell.
- **Chymotrypsin:** Used for dissolving ligaments of the lens during the extraction of cataract.
- **Collagenase:** Used for debridement (cleaning of wound by removing dead tissue) of dermal ulcers and severe burns.
- **Fibrinolysin:** It is used in the venous thrombosis, pulmonary and arterial embolism (blood clot).
- **Hyaluronidase:** It is used to promote the rapid absorption of drugs injected subcutaneously. It acts by increasing tissue permeability. It is used in the treatment of traumatic or postoperative edema.
- **Lysozyme** (an antibiotic) found in human tears and egg white, is used in the infection of eye. It has antibacterial action, it acts on cellulose of bacteria.
- **Penicillinase** (bacterial enzyme) is used for the treatment of persons who are allergic to penicillin, penicillinase destroys penicillin.
- **Rennin** is used for the treatment of gastric achylia.
- **Streptokinase:** Streptokinase and urokinase are used in myocardial infarction to dissolve blood clot or purulent (containing pus) material. It causes fibrinolysis.
- **Trypsin:** It is a proteolytic enzyme and is used to clean the wounds by dissolving purulent material and in the treatment of acute thrombophlebitis to dissolve the blood clot.
- **Pepsin, trypsin peptidase, lipase, amylase elastase** and **cellulase** are used in gastrointestinal tract (GIT) disorders and chronic pancreatitis.

Analytical Use of Enzymes

- Enzymes can be used as ***reagents*** and ***labels.***
- In addition to measurement of serum enzyme activity for the diagnosis and management of disease, enzymes are widely used in the clinical laboratory as reagents for the estimation of serum constituents. Some examples are given in **Table 6.11.**
- Many enzymes have been used as the label in various immunoassays (enzyme linked immunosorption assay, ELISA) for determining the serum concentration of drugs, hormones or other compounds of interest. Commonly used label enzymes are:
  - Glucose-6-phosphate dehydrogenase
  - Alkaline phosphatase
  - β-galactosidase
  - Peroxidase.

Table 6.10: Enzymes in diagnosis of myocardial infarction and time course of plasma enzyme activity changes after myocardial infarction

Enzyme	*Abnormal activity detectable (hours)*	*Time for maximum rise (hours)*	*Time for return to normal (Days)*
CK_2 (MB-isoenzyme)	3-10	12-24	2-3
AST	6-12	24-48	4-6
LDH (heart specific)	8-16	48-72	7-12