ENZYMES

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Bsc; Msc

OCCURRENCE

- Produced by all living organisms including humans
- Present only in small amounts

MEDICAL AND BIOLOGICAL IMPORTANCE

- Chemical work horses of the body. Biological catalysts that speed up the pace of chemical reactions.

A chemical reaction without an enzyme is like a drive over a mountain. The enzyme bores a tunnel through it so that passage is far quicker and takes much less energy.

- Make life on earth possible, all biology from conception to the dissolution that follows death depends on enzymes.
- Regulate rate of physiological process. So, defects in enzyme function cause diseases.
- When cells are injured enzymes leak into plasma. Measurement of activity of such enzymes in plasma is an integral part of modern day medical diagnosis.

My Dear G.F Came BAck

- Used as drugs. Trypsin (in combination used to treat some inflammatory)
- Immobilized enzymes, which are enzymes attached to solid supports are used in clinical chemistry laboratories and in industry. For example glucose in blood or urine is detected by using immobilized glucose oxidase. In pharmaceutical industry, glucose isomerase is used to produce fructose from glucose.
- Enzymes are used as biosensors. Food industry-pesticides
- AIDS detection involves use of enzyme dependent ELISA technique.
- Enzymes are used as cleansing agents in detergent industry.

CHEMICAL NATURE OF ENZYMES (PROPERTIES)

- All the enzymes are proteins except ribozymes
- In 1878, Kuhne, introduced term ‘Enzyme’ to indicate biological catalyst.
- Enzymes cut big molecules apart and join small molecules to form big molecules.
- Most of the chemical reactions in the body are enzyme catalysed.

- The substance upon which an enzyme acts is called a substrate. By the action of an enzyme it is converted to a product. An enzyme-catalysed reaction consists of a substrate, enzyme and product as shown below.

Substrate + Enzyme→ Product

- Enzymes are big particles. Their molecular (size) weight ranges from few thousands to millions.
- Enzymes have enormous power of catalysis. They increase rate of reaction to 10^5 to 10^{10} folds. For example, carbonic anhydrase can hydrate to 10^6 molecules of CO_2 per second.

In the absence of this enzyme, hydration of CO_2 is 10^{-1} per second.

- Enzymes are far more efficient compared to non-enzyme (man made) catalysts.

Enzymes are not consumed in the overall reaction.

- Enzymes accelerate the rate of a reaction but do not alter the equilibrium constant (Keq).
- To know how enzymes work, physical chemistry of catalysts must be explored because enzymes are catalysts

Catalyst

- ✓Does not change the chemical reaction but it accelerates the reaction.
- ✓Not consumed in overall reaction.
- ✓It undergoes chemical or physical change during reaction and returns to original state at the end of reaction.
- ✓Transition state theory was proposed to explain action of catalyst.

For a chemical reaction A→ B to occur, energy is required. When enough energy is supplied, A undergoes to a transition state which is an unstable state.

Gets converted to product B which is more stable.

Amount of energy needed to convert a substance from ground state to transition state is called *activation energy*..

In presence of a catalyst, A goes to a transition state very fast and requires less energy (Fig.).

A catalyst accelerates the rate of a reaction by decreasing the energy of activation.

Likewise enzymes also speed up reactions by lowering energy of activation.

Activation energy is much less for a reaction in presence of enzyme than non-enzyme catalyst (Fig.). Therefore enzymes are more efficient than non-enzyme catalysts

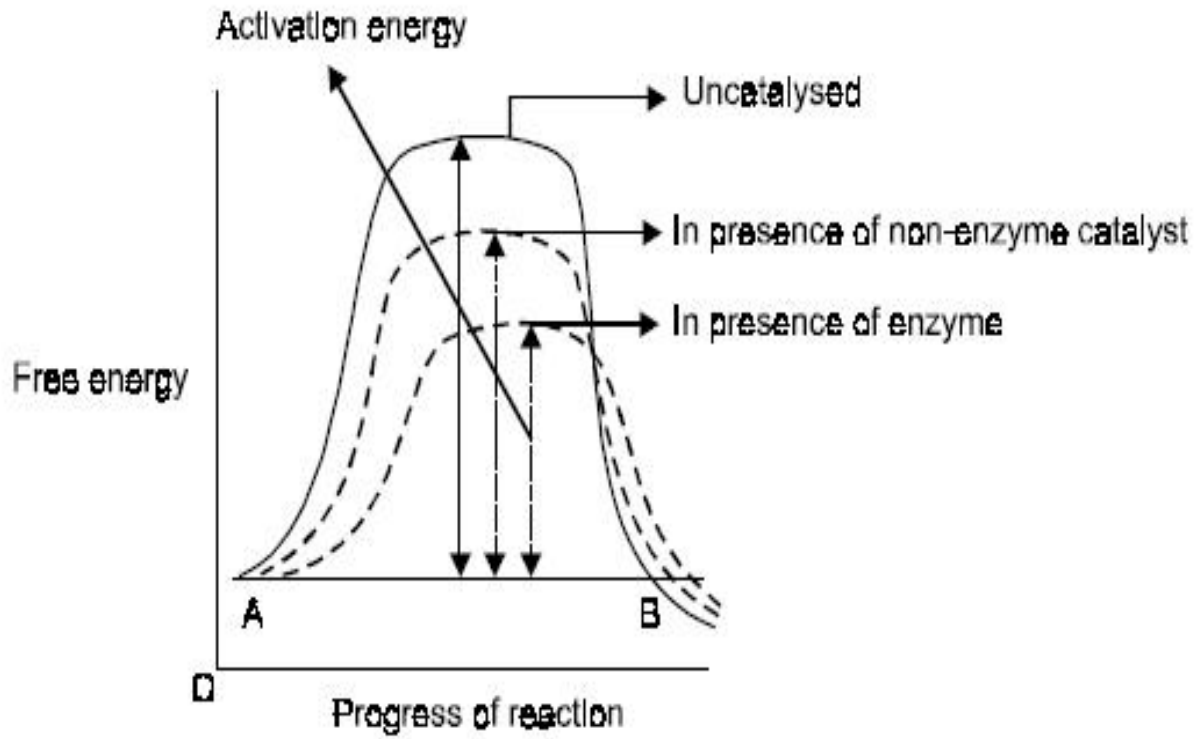
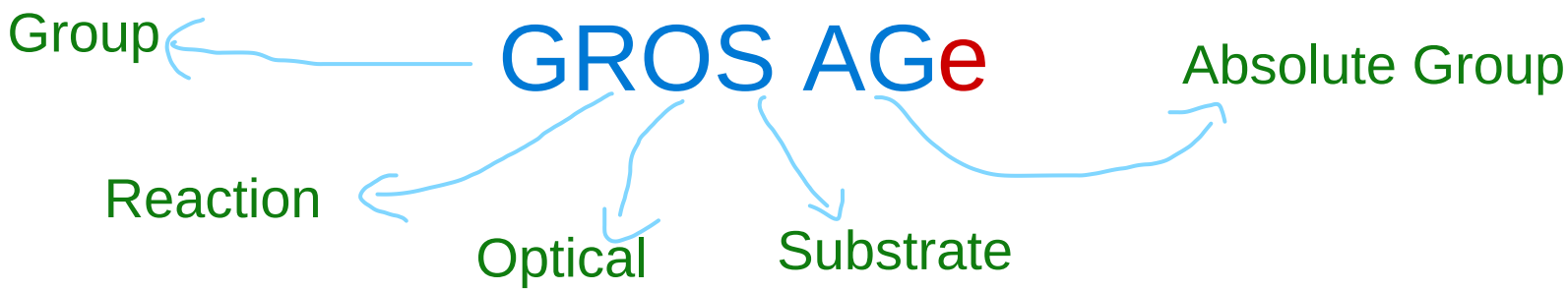


Fig: Energy of activation for uncatalysed, non-enzyme catalysed and enzyme catalysed reactions

ENZYME SPECIFICITY

Enzymes are highly specific compared to other catalysts. An enzyme catalyzes only a specific reaction. Some general types of enzyme specificity are:



Substrate Specificity

Enzymes are specific towards their substrates. For example, glucokinase catalyzes the transfer of phosphate from ATP to glucose. Galactokinase catalyzes transfer of phosphate from ATP to galactose.

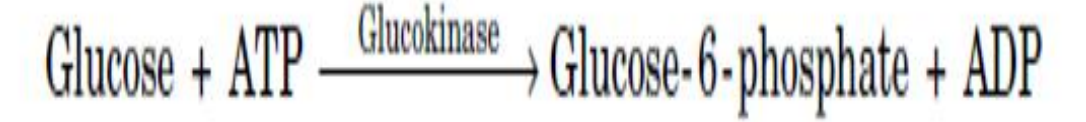
Both enzymes catalyze the transfer of phosphate from ATP, they act only on specific substrates.

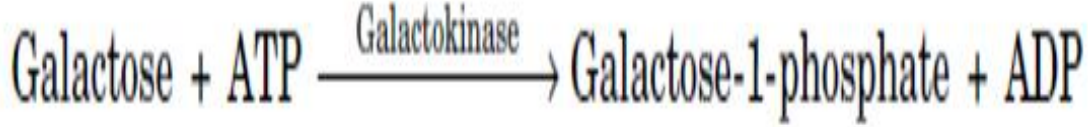
Transaminases which catalyze transfers of amino groups are specific to substrates.

Aspartate transaminase catalyzes the transfer of amino group from aspartate

Alanine transaminase catalyzes transfer of amino group from alanine only.

They are specific towards substrates


Glucose + ATP
Glucokinase
Glucose-6-phosphate + ADP


Galactose + ATP
Galactokinase
Galactose-1-phosphate + ADP

Reaction Specificity

A given enzyme catalyzes only one specific reaction.

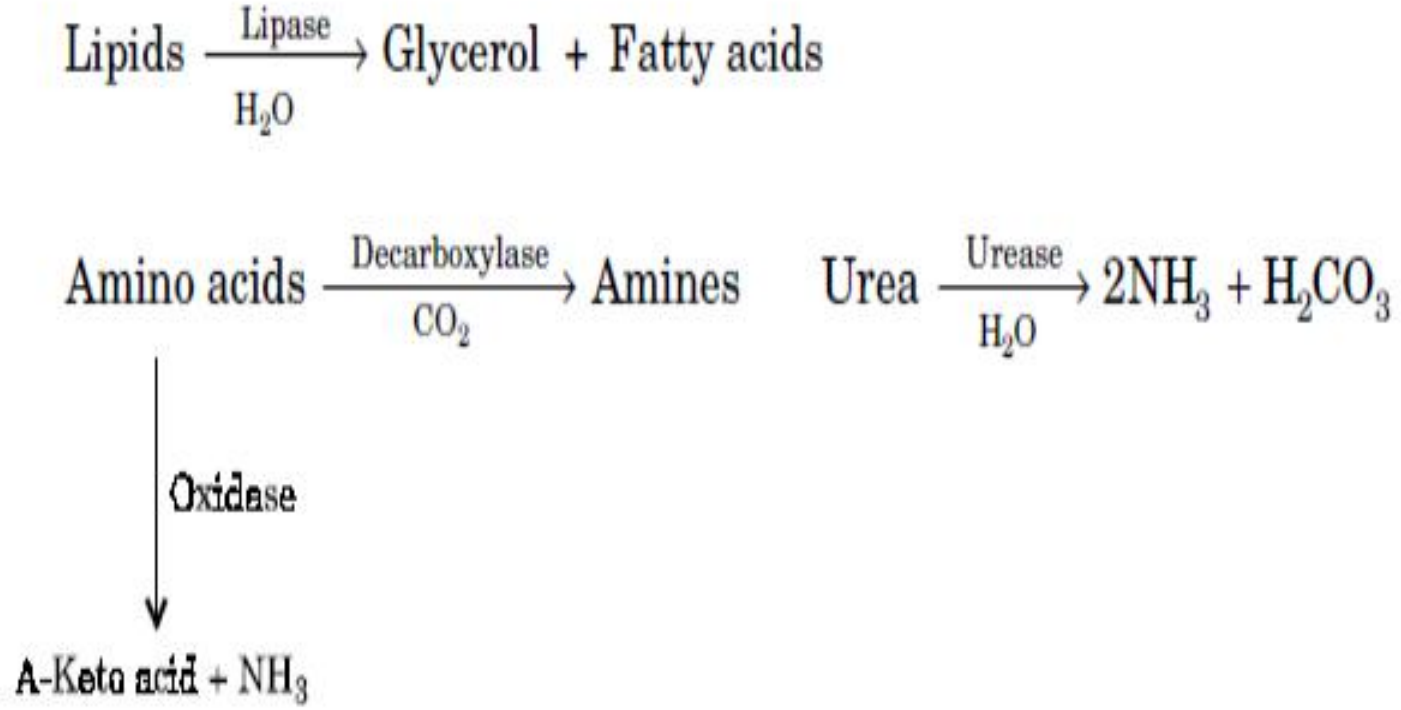
Lipases only hydrolyze lipids

Urease hydrolyzes urea.

Do not catalyze any other type of reaction.

Amino acid oxidase catalyzes oxidation of amino acids

Decarboxylase catalyzes only decarboxylation of amino acids


Lipids
Lipase
H_2O
Glycerol + Fatty acids
Amino acids
Decarboxylase
CO_2
Amines
Urea
Urease
H_2O
$2NH_3 + H_2CO_3$
Oxidase
A-Keto acid + NH_3

Group Specificity

Some lytic (hydrolases) enzymes act on specific groups.

Proteases specific for peptide groups

Glycosidases specific to glycosidic bonds.

$$\text{Proteins} \xrightarrow[H_2O]{\text{Protease}} \text{Amino acids} \qquad \text{Maltose} \xrightarrow[H_2O]{\text{Maltase}} \text{Glucose} + \text{Glucose}$$

$$\text{Ester} \xrightarrow[H_2O]{\text{Esterase}} \text{Acid} + \text{Alcohol}$$

Absolute Group Specificity

Certain lytic enzymes exhibit high order group specificity.

For example, chymotrypsin is a protein splitting enzyme *i.e.,* it hydrolyzes peptide bonds.

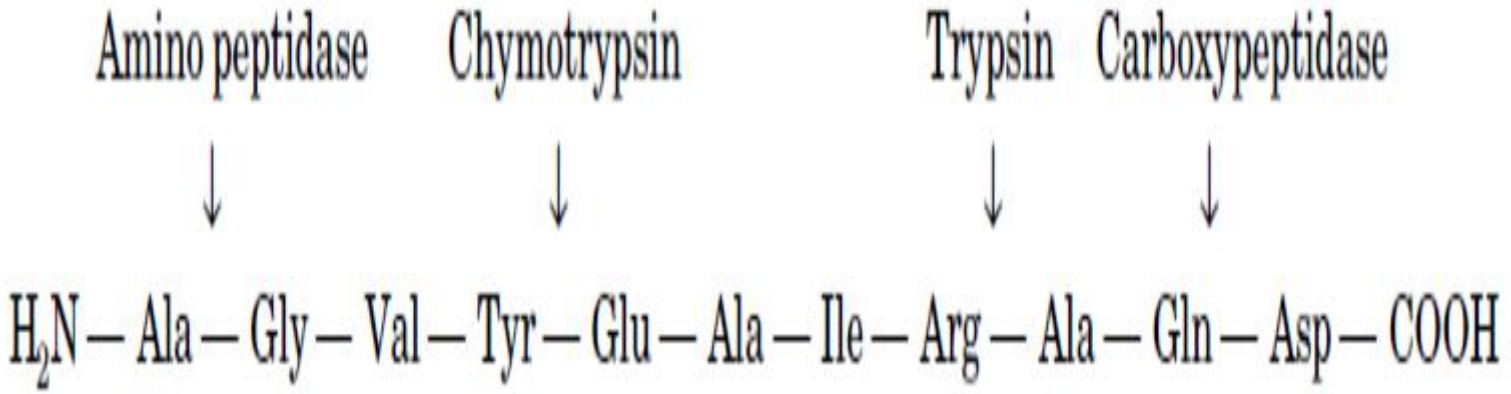
Preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by aromatic amino acids phenylalanine, tyrosine and tryptophan.

Trypsin, another peptide bond hydrolyzing enzyme preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by basic amino acids.

Carboxy peptidase removes one amino acid each time from carboxy terminus

Amino peptidase removes one amino acid each time from N-terminus.

Thrombin of blood clotting process is highly specific for Arg-Gly-bonds.


Amino peptidase
Chymotrypsin
Trypsin
Carboxypeptidase
H2N – Ala – Gly – Val – Tyr – Glu – Ala – Ile – Arg – Ala – Gln – Asp – COOH

Optical Specificity

Several enzymes exhibit optical specificity of substrate on which they act.

Enzymes able to recognise optical isomers of the substrate.

Enzymes of amino acid metabolism act only on L-isomers (L-amino acids) but not D-isomers (D-amino acids).

Likewise enzymes of carbohydrate metabolism act only on D-sugars but not on L-sugars.

Enzyme Classification and Nomenclature

International Union of Biochemistry classified all enzymes into six major classes based on the type of reaction they catalyze and reaction mechanism.

Nomenclature

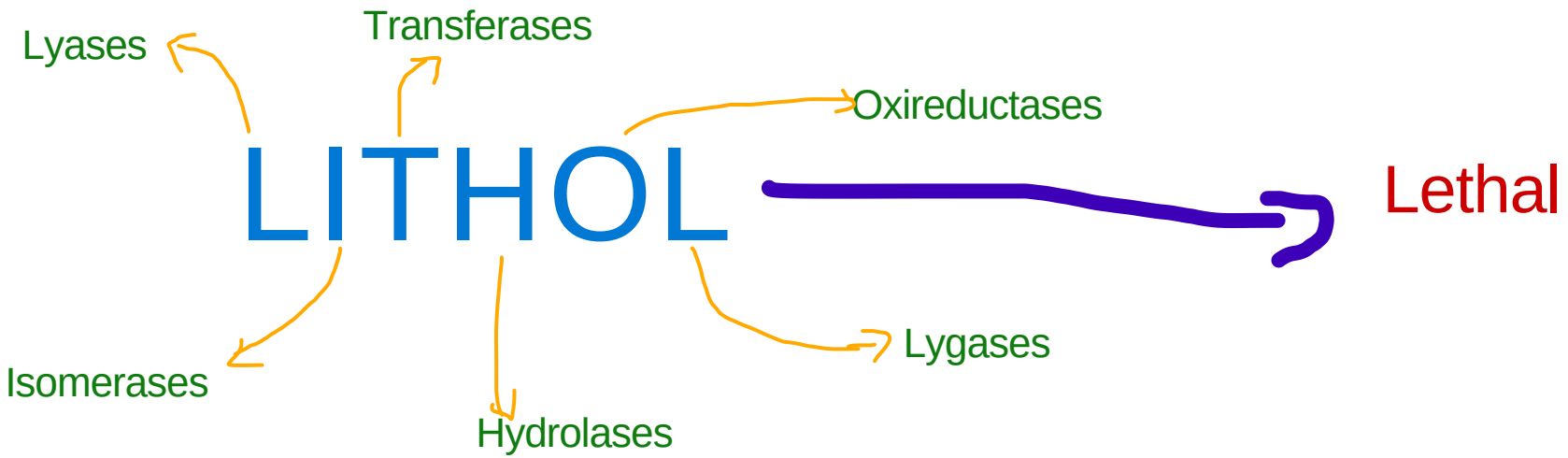
The name of an enzyme has two parts.

First part indicates name of its substrate and second part ending in ‘ase’ indicates the type of reaction it catalyzes.

Further, each enzyme has a code (EC) number. It is a four-digit number. The first digit indicates major class, second digit indicates sub class, third digit denotes sub sub class and final digit indicates specific enzyme.

Oxidoreductases

Catalyze oxidation and reduction reactions.



(a) Alcohol + NAD^+ $\xrightarrow{\text{Alcohol Dehydrogenase}}$ Aldehyde or ketone + NADH + H^+

(b) Glutamate $\xleftrightarrow[H_2O]{NAD^+}$ $\xrightarrow[NH_4]{NADH + H^+}$ α-ketoglutarate

Glutamate Dehydrogenase

Transferases

Catalyze transfer of groups

(a) $\text{Hexose} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{Hexokinase}} \text{Hexose-6-phosphate} + \text{ADP}$

(b) $\text{Acetyl-CoA} + \text{Choline} \xrightarrow[\text{Transferase}]{\text{Choline acyl}} \text{Acetyl choline} + \text{CoA}$

Hydrolases

Catalyze hydrolysis of peptide, ester, glycosyl etc. bonds.

(a) $\text{Casein} \xrightarrow[H_2O]{\text{Trypsin}} \text{Peptides}$

(b) $\text{Acetyl choline} \xrightarrow[\text{Esterase}]{\text{Choline, } H_2O} \text{Choline + acetic acid}$

Lyases

Catalyze removal of groups from substrates by mechanisms other than hydrolysis, forming double bonds.

Examples: (*a*)

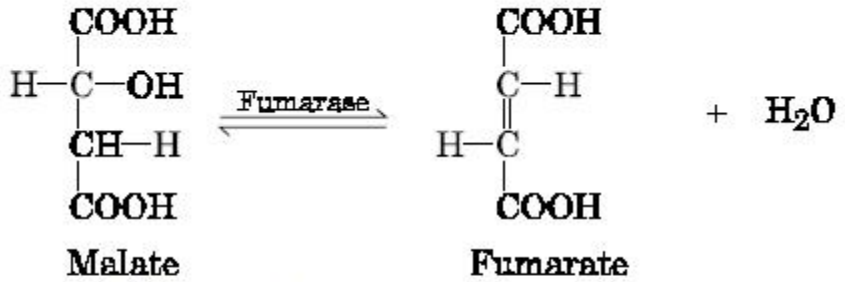


Fig. 4.2 Fumarase catalysed reaction

(*b*) Fructose-1, 6-biphosphate $\xrightarrow{\text{Aldolase}}$ Glyceraldehyde-3-phosphate + Dihydroxyacetone Phosphate

Isomerases

Catalyze interconversion of optical, functional and geometrical isomers.

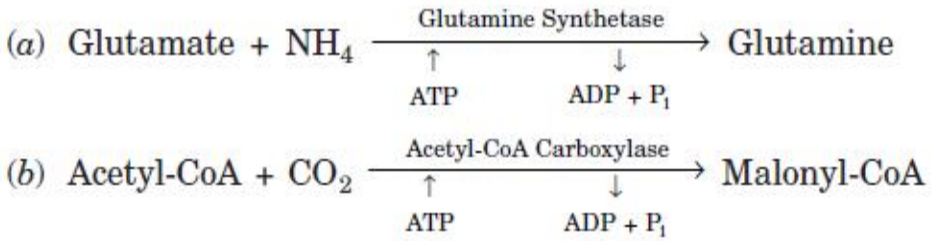
(*a*) Glyceraldehyde-3-Phosphate $\xrightarrow{\text{Triose phosphate isomerase}}$ Dihydroxyacetone phosphate

(*b*) L-Alanine $\xrightarrow{\text{Alanine Racemase}}$ D-Alanine

Ligases

Catalyze linking together of two compounds.

The linking is coupled to the breaking of phosphate from ATP.


(a) Glutamate + NH_4
Glutamine Synthetase
ATP
$ADP + P_i$
Glutamine
(b) Acetyl-CoA + CO_2
Acetyl-CoA Carboxylase
ATP
$ADP + P_i$
Malonyl-CoA

MECHANISM OF ENZYME ACTION

Mechanism of enzyme action deals with molecular events associated with conversion of a substrate to product in an enzymatic reaction.

Medical Importance

1. Some drugs are designed based on mechanism of enzyme action. For example, X-ray

crystallographic studies on mechanism of carboxy peptidase action led to the design of specific inhibitor to angiotensin converting enzyme like captopril which is used in treatment of hypertension.

2. Enzymes with specific properties can be designed based on mechanistic studies. They may be introduced into humans to correct specific abnormalities associated with disorders.

Larger size of an enzyme molecule relative to smaller size of its substrate always puzzled biochemists.

Ultimately, it led to the concept that small portion of enzyme is required for enzyme action. This part of the enzyme is known as active site.

CHARACTERISTICS OF AN ENZYME ACTIVE SITE

Consists of two parts:

(*a*) **Catalytic site.** Portion (part) of the enzyme that is responsible for catalysis.

Determines reaction specificity. Occasionally, catalytic site and active site are used synonymously.

(*b*) **Binding site.** Part of the enzyme that binds with substrate. Determines substrate specificity.

Active sites of enzymes are clefts within the enzyme molecule. For example, the active site of ribonuclease lies within cleft (Fig).

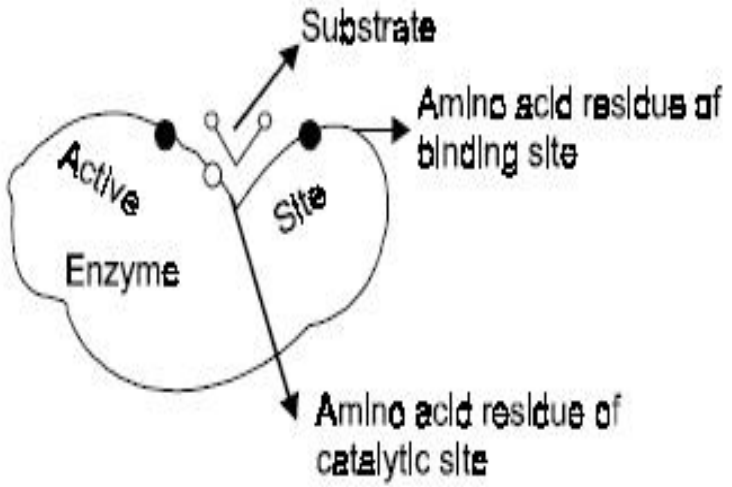


Fig. Schematic diagram showing an enzyme active site

- Active site consists of few amino acid residues only
- Active site is three dimensional
- Active site is contributed by amino acid residues that are far apart in the enzyme molecule. During catalysis, they are brought together
- Amino acids at the active site are arranged in a very precise manner so that only specific substrates can bind at the active site
- Usually serine, histidine, cysteine, aspartate or glutamate residues make up the active site. Enzymes are named according to the active site amino acid. For example, trypsin is a serine protease and papain is cysteine protease

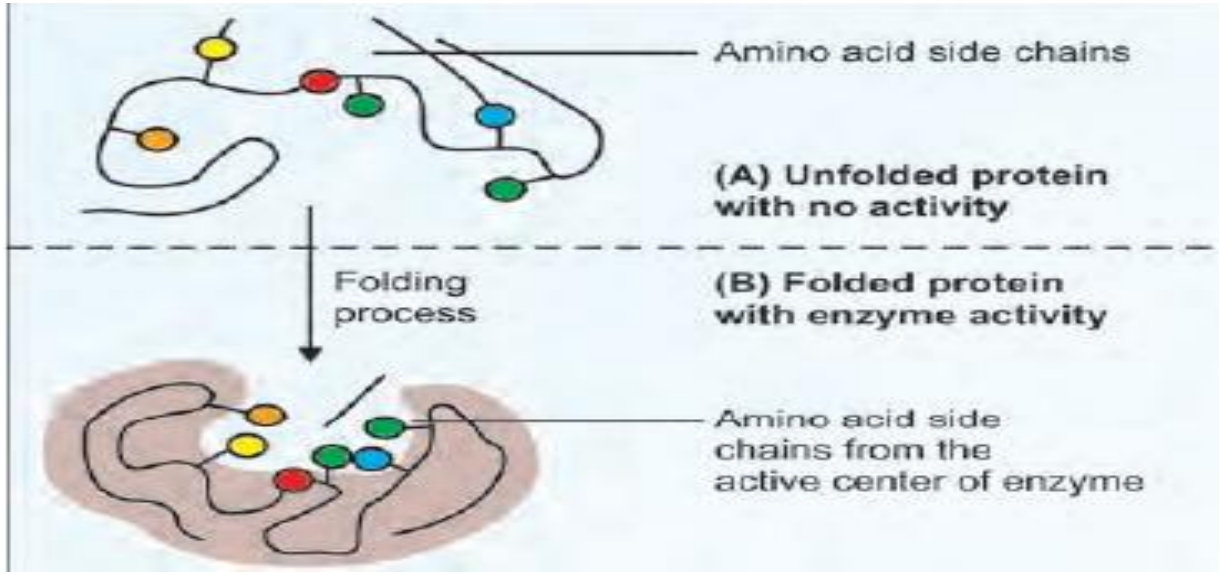


Fig. : Correct alignment of amino acids in the active center of the enzyme

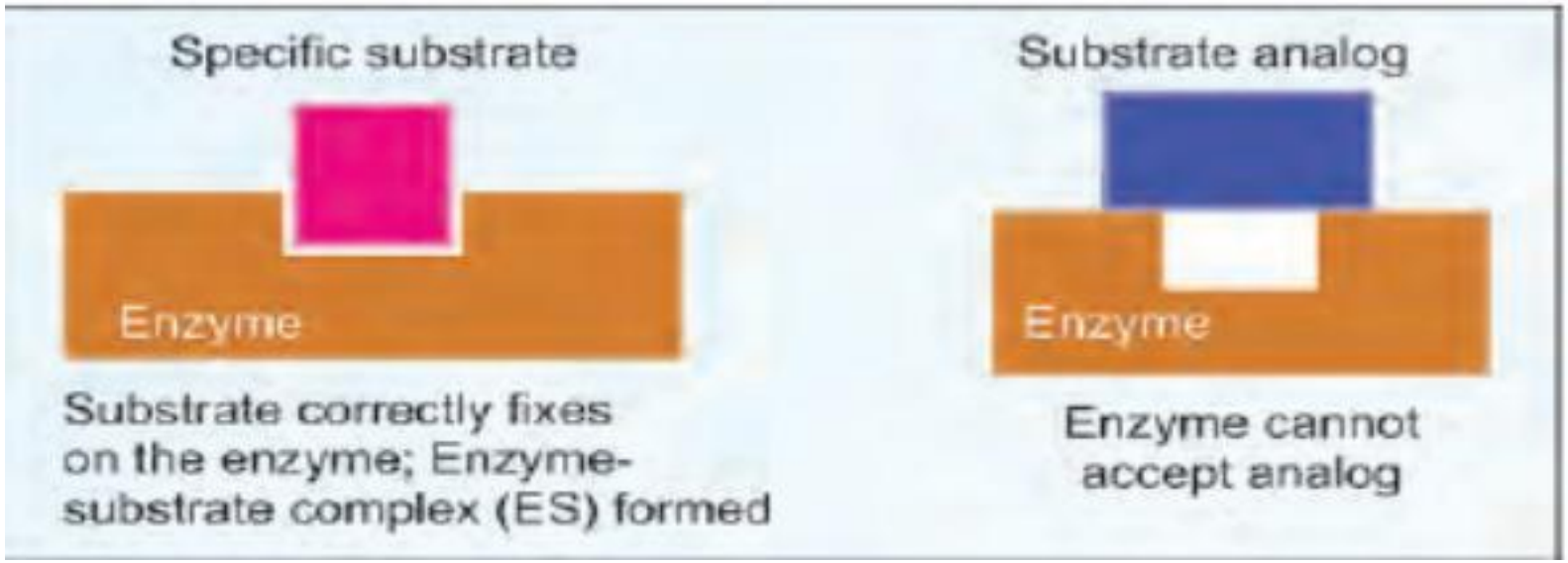


Fig: Fischer's template theory

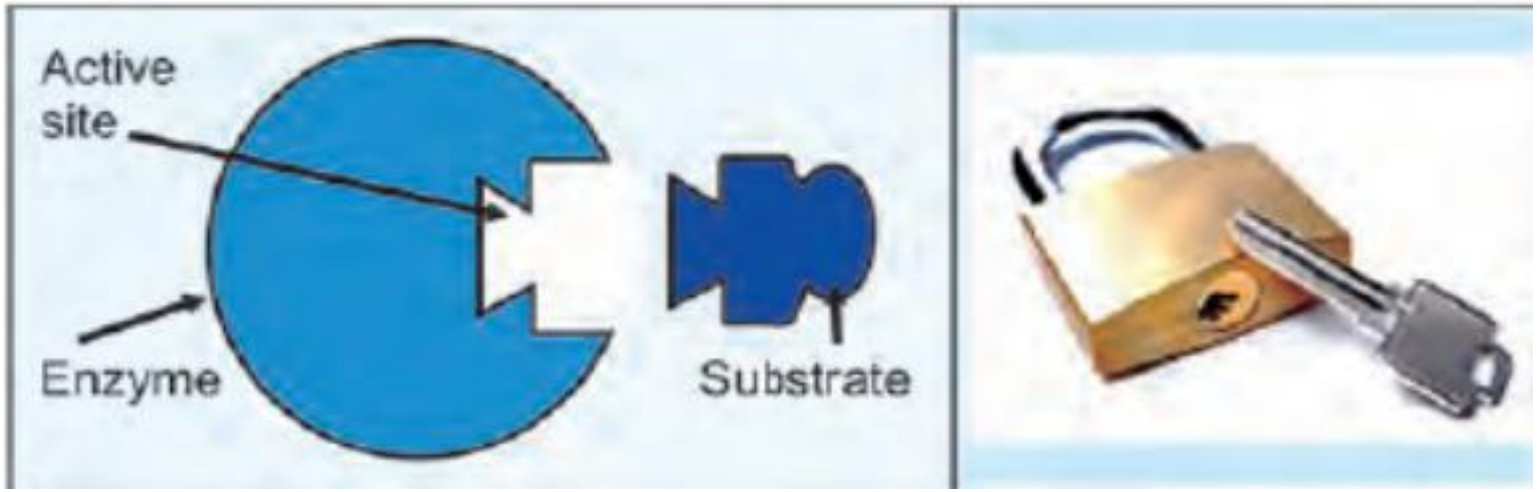


Fig: Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory)

MODELS OF ACTIVE SITE

Proposed active site models to explain enzyme specificity

A. Lock and Key Model

1. Active site is a rigid portion of the enzyme molecule and its shape is complementary to the substrate like lock and key.

2. Complimentary shape of substrate and active site favour tightly bound enzyme. Substrate complex formation followed by catalysis (Fig. *a*).

3. Model was unable to explain the possibility of rigid active site combining with the product to form substrate in reversible reaction.

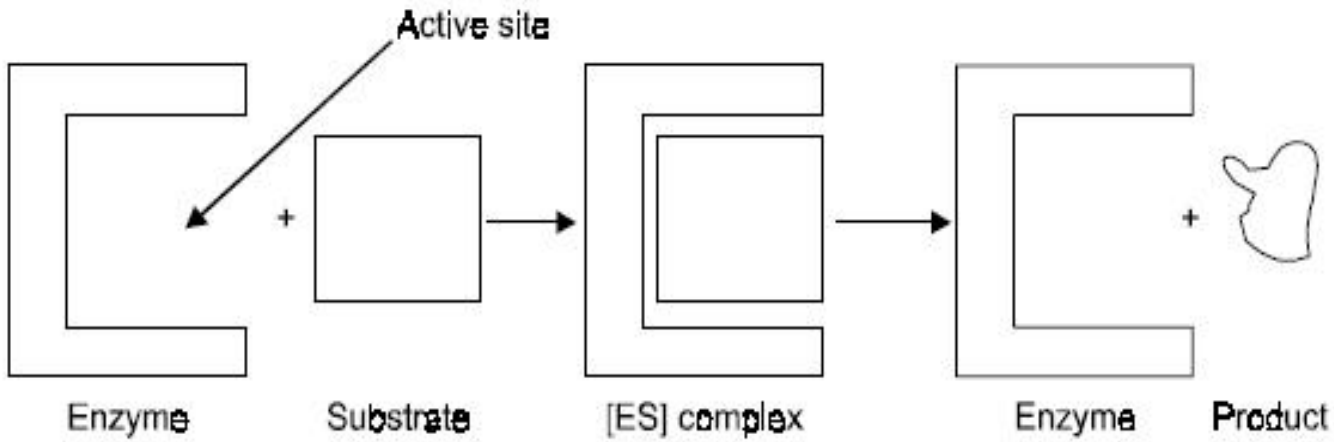


Fig (*a*): Lock and key model of an active site

B. Induced Fit Model

1. Active site is flexible unlike rigid type of the lock and key model.

2. In the enzyme molecule, amino acid residues that make up the active site are not oriented properly in the absence of substrate.

3. When substrate combines with enzyme, it induces conformational change in the enzyme molecule in such a way that amino acids that make the active site are shifted into correct orientation to favour tightly bound enzyme-substrate complex formation followed by catalysis.

4. The enzyme molecule is unstable in the induced conformation and returns to its native conformation in the absence of substrate (Fig. *b*).

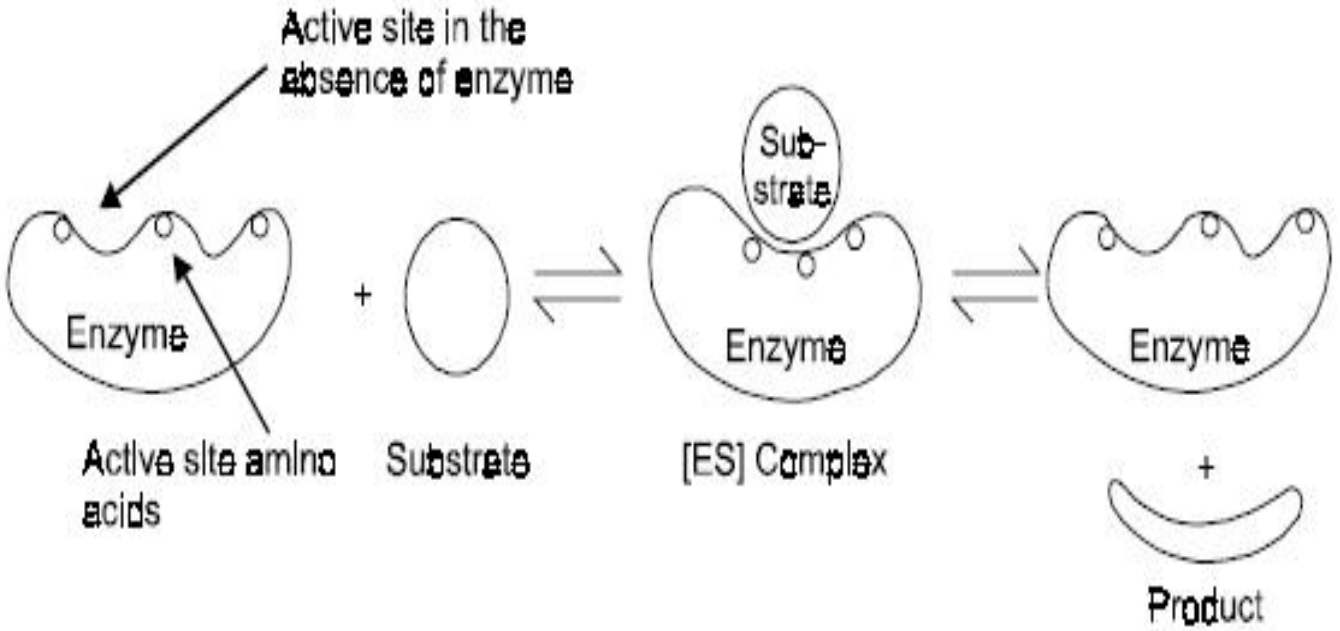


Fig. (*b*): Induced fit model of enzyme active site

FACTORS AFFECTING ENZYME ACTION

Rates of enzyme catalyzed reactions are affected by:

1. Enzyme concentration
2. Temperature
3. Hydrogen ion concentration or pH
4. Substrate concentration
5. Inhibitors and cofactors

MEDICAL AND BIOLOGICAL IMPORTANCE

1. For normal health, all enzymatic reactions must occur in the body and they must proceed at appropriate rates. Alterations in the rates of enzymatic reactions may disturb tissue homeostasis.

2. Any alteration in intracellular pH disturbs rates of enzyme reactions.

3. Organs for transplantation, blood and serum are preserved at low temperature as soon as they are removed from body because enzymatic reactions proceed at much lower rate at low temperature. Under such conditions, O_2 demand of cells decreases, so cells of the organs or fluids survive with available O_2 for sometime.

4. Rates of enzymatic reactions are altered in fever and hypothermia because temperature influences rate of enzyme reaction.

5. An understanding of factors affecting enzyme action is required for development of drugs. Many drugs act by decreasing rate of key metabolic reaction by blocking that particular enzyme. For example, AZT used in treatment of AIDS is an inhibitor of HIV enzyme. Lovastatin used in the treatment of atherosclerosis is an inhibitor of HMG CoA reductase, a cholesterol producing enzyme. Captopril, used in the treatment of hypertension is an inhibitor of angiotensin converting enzyme, an enzyme of blood pressure regulation.

6. Some poisons work by abolishing (affecting) essential enzymatic reactions

1. Enzyme concentration

The rate of an enzyme catalyzed reaction is directly proportional to the concentration of enzyme.

The plot of rate of catalysis versus enzyme concentration is a straight line (*a*).

- i. **Rate of a reaction or velocity (V) is directly proportional to the enzyme concentration,** when sufficient substrate is present. Velocity of reaction is increased proportionately with the concentration of enzyme, provided substrate concentration is unlimited (Fig. 5.12).
- ii. Hence, this property is made use of determining the level of particular enzyme in plasma, serum or tissues.
- iii. Known volume of serum is incubated with substrate for a fixed time, then reaction is stopped and product is quantitated (**end point method***)*. Since the product formed will be proportional to the enzyme concentration, the latter could be assayed.

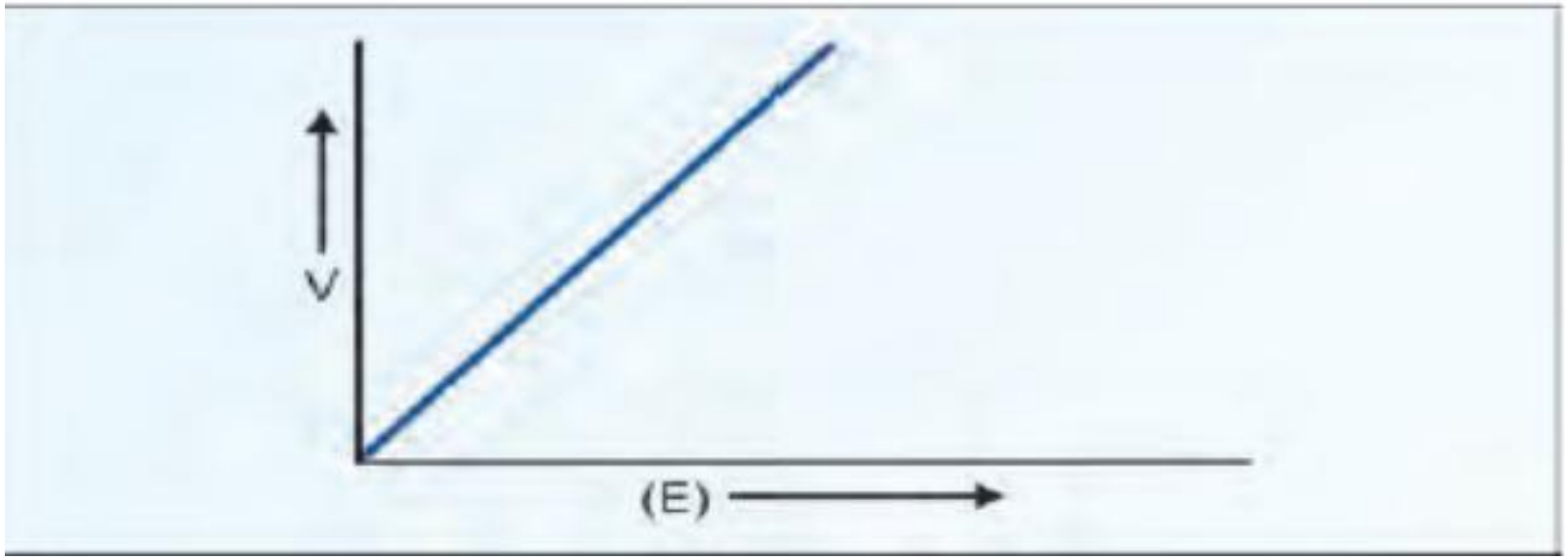


Fig: Effect of enzyme concentration

2. Temperature

Like any chemical reaction, enzyme activity increases with increase in temperature initially. After a critical temperature, the enzyme activity decreases with increase in the temperature. When the effect of temperature on enzyme activity is plotted, a cone-shaped curve is obtained (Fig. *b*).

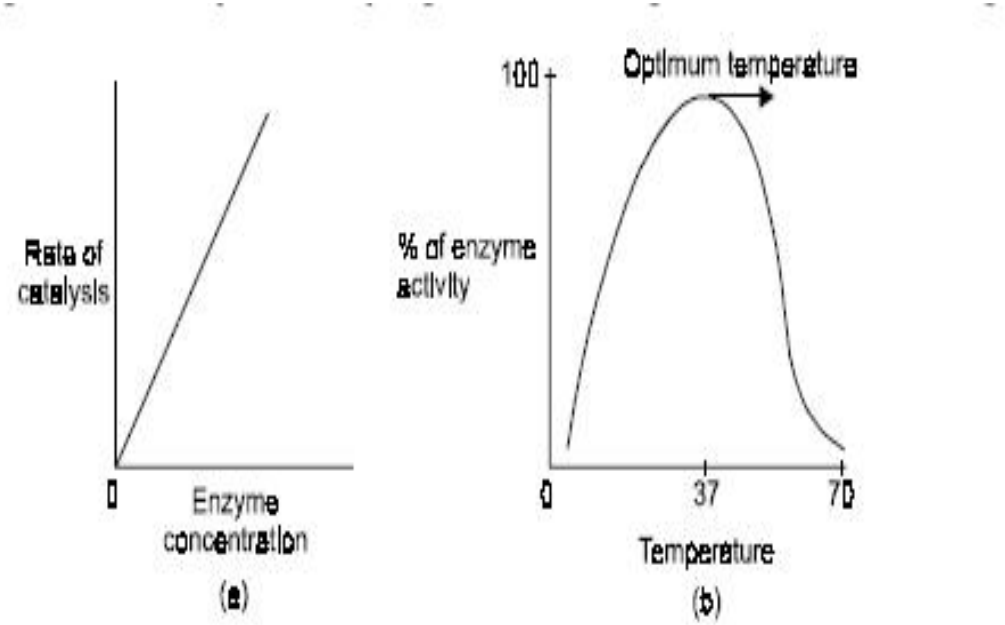


Fig. (*a*) Effect of enzyme concentration (*b*) Effect of temperature

The figure indicates that there is an optimal temperature at which the enzyme is optimally active. It is called an *optimum temperature*. For most of the enzymes, the optimum temperature is the temperature of the cell or body in which they occur. For example, for human trypsin the optimum temperature is 37°C which is the normal body temperature. The first half of the curve approaching the optimum temperature indicates that enzyme activity increases with increase in the temperature due to the increased kinetic energy of reacting molecules.

The other half which corresponds to decreased catalytic activity with increased temperature is due to denaturation of enzyme.

Enzymes of plants and micro-organisms growing in hot climates or hot springs may exhibit optimal temperature close to the boiling point of water. Examples are enzymes of thermophilic bacteria, snake venom phospholipase and urease (55 °C).

3. Effect of pH or hydrogen ion concentration

Most enzymes are not maximally active throughout pH scale (1-14). Several enzymes have optimum activity between pH of 5 to 9. When enzyme activity measured at several pH values is plotted a bell shaped curve is obtained (Fig. *a*).

Since enzymes are proteins pH changes affect.

1. Charged state of catalytic site.

2. Conformation of enzyme molecules.

In addition low or high pH cause denaturation of enzymes. It accounts for the less activity of enzymes at acidic or alkaline pH (Fig. *a*). For most of the enzymes, optimum pH is the pH of the blood or cell in which they occur. However, for some enzymes, optimum pH may not be in the neutral range. In the case of oligomeric enzymes, optimum pH is required for the association of protomers. When the pH is altered, the protomers dissociate with loss of biological activity

Name of the enzyme	Optimum pH
Trypsin	7.6
Pepsin	2-2.5
Acid phosphatase	5
Alkline phosphatase	9-10

4. Effect of substrate concentration

If the concentration of the substrate (S) is increased while other conditions are kept constant, the initial velocity v_0 (velocity measured when little substrate is reacted) increases proportionately in the beginning. As the substrate concentration continues to increase, the increase in v_0 slows down and reaches maximum velocity (V_{max}) and then there is no further increase (Fig. *b*). The plot of v_0 versus (S) is rectangular hyperbola. It is called *Michaelis plot*. To explain the reason for characteristic shape of the curve, Michaelis proposed that in an enzyme catalyzed reaction, the enzyme (E) combines with substrate (S) to form an enzyme-substrate (ES) complex which decomposes to form product (P) and free enzyme.

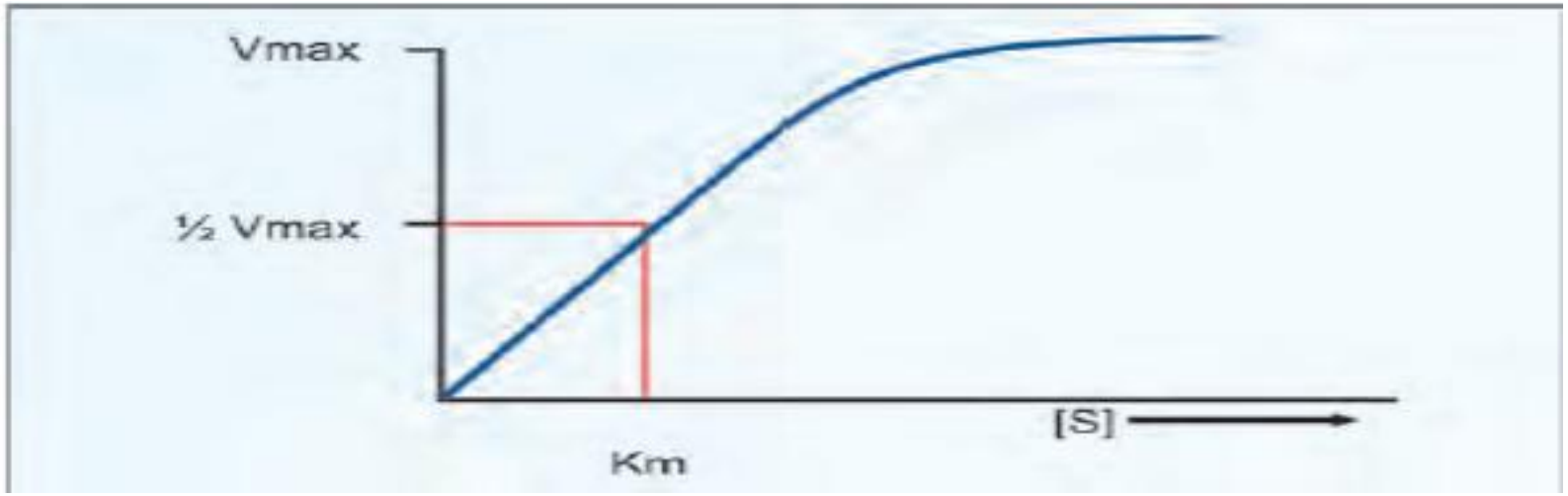


Fig: Effect of substrate concentration (substrate saturation curve)

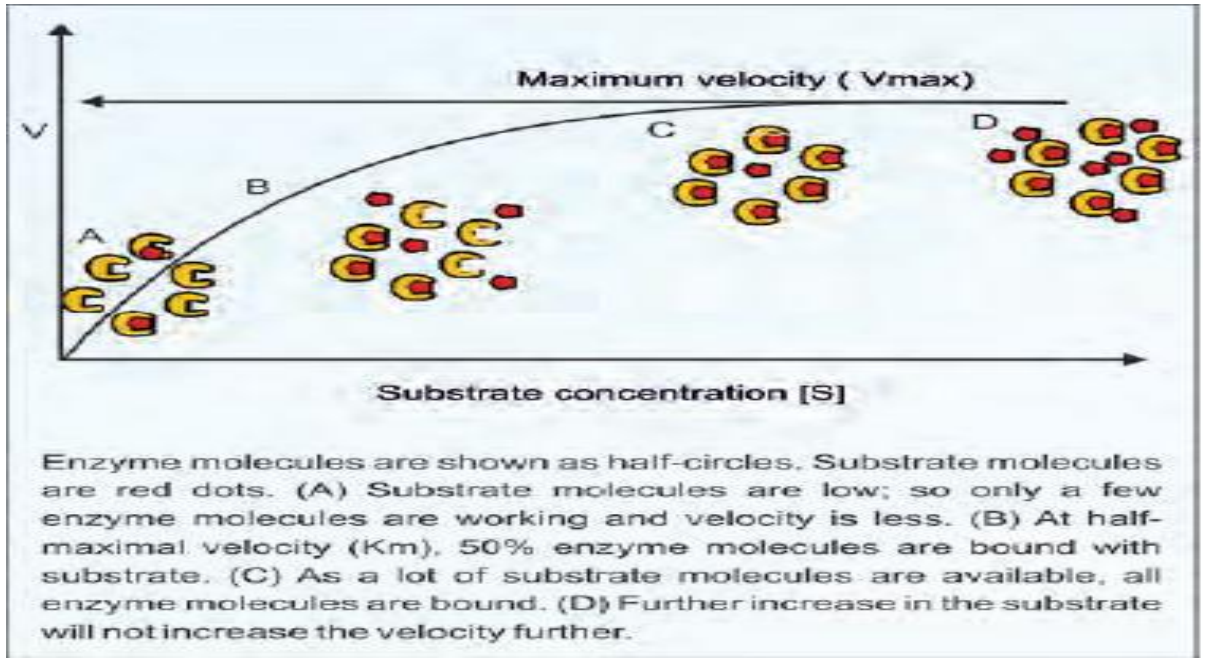
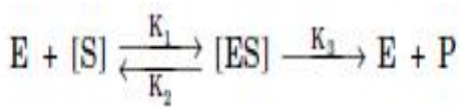


Fig: Effect of substrate concentration on enzyme activity


E + [S]
K1
K2
[ES]
K3
E + P

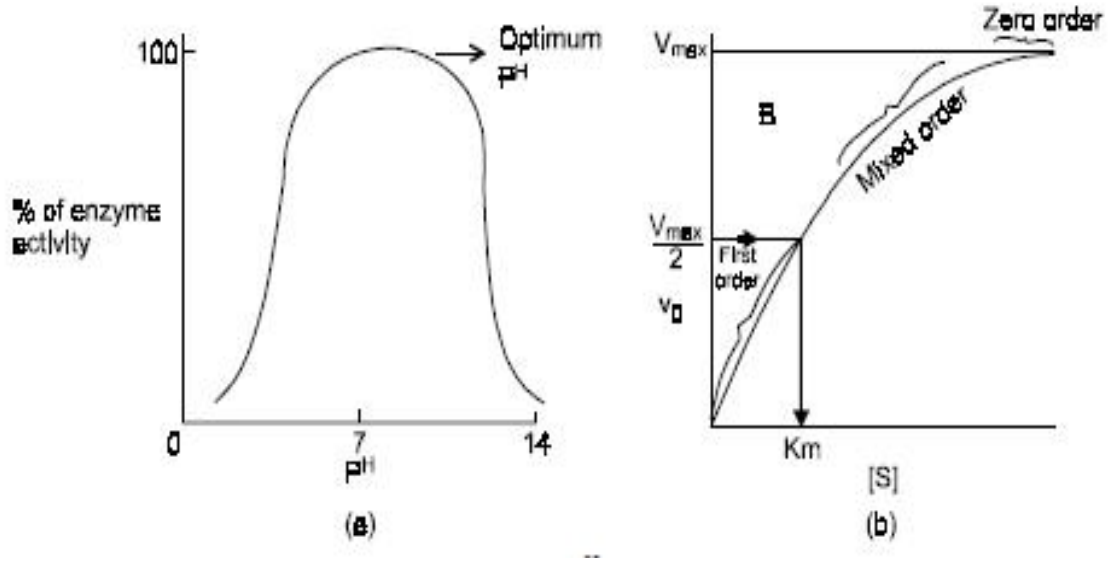


Fig.(*a*) Effect of PH on enzyme action

(*b*) Effect of substrate concentration

Based on this, reasons for the three phases of the curve can be interpreted.

1. In the first phase, substrate concentration is low and most of the enzyme molecules are free so they combine with the substrate molecules. Therefore, velocity is proportional to substrate concentration. At this state, enzymatic reaction shows first-order kinetics.

2. In the second phase, half of the enzyme molecules are bound to substrate, so the velocity is not proportional to substrate concentration. At this stage, enzymatic reaction shows mixed-order kinetics.

3. In the third phase, all the enzyme molecules are bound to substrate, so velocity remain unchanged because free enzyme is not available though the substrate is in excess. At this stage enzymatic reaction shows zero-order kinetics.

The Michaelis plot is used to determine Michaelis constant a characteristic of an enzyme (Fig. *b*) and type of enzyme inhibition.

Michaelis Constant or Km

The substrate concentration that produces half the maximal velocity (Vmax/2) is known as Michaelis constant. Apart from the graph, Km also can be determined from Michaelis-Menten equation. It is a simple equation and describes the dependence of initial velocity (v_0) on the concentration of the enzyme and substrate. It is the theoretical expression for the rectangular hyperbola.

$$v_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

when $v_0 = V_{max}/2$

The above equation is written as

$$V_{max}/2 = V_{max}\,[S]/K_m + [S]$$

i.e., $K_m + [S] = 2[S]$

$$K_m = 2[S] - [S] = [S]$$

$$K_m = [S]$$

when $v_0 = V_{max}/2\ [K_m = (K_2 + K_3)/K_1]$

Significance of Km

1. It is an enzyme kinetic constant.

2. It indicates the substrate concentration required for the enzyme to work efficiently.

3. Low Km indicates high affinity of enzyme towards substrate. High Km indicates low affinity of enzyme towards substrate. Hence, Km and affinity are inversely related.

(Km α 1/affinity)

Example: Hexokinase and glucokinase both phosphorylate glucose. However, hexokinase can phosphorylate glucose 2000 times more efficiently than glucokinase because Km of hexokinase is low (1×10^{-5} M) whereas Km of glucokinase is high (2.0×10^{-2} M).

4. Km is required when enzymes are used as drugs.

5. Use of enzymes in immunodiagnostics (ELISA) require Km of the enzyme.

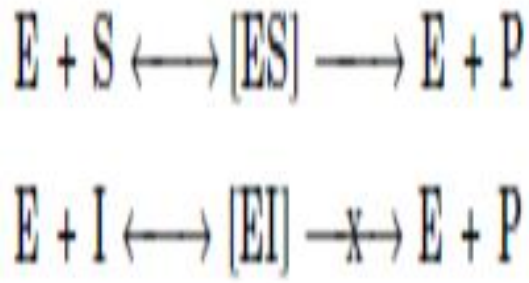
INHIBITORS

Substances that decrease catalytic activity of enzymes are called inhibitors. They may be protein or non-protein inhibitors. The decrease in enzyme activity is called inhibition.

More than two types of enzyme inhibition exist based on the mode of action of inhibitors

Competitive Inhibition

Competitive inhibition occurs at active site. Competitive inhibitor is structurally similar to that of substrate. Hence, it competes with substrate to bind at active site. Inhibition occurs when it binds at the active site of enzyme molecule. It is reversible. If the substrate concentration is increased then the competitive inhibition is relieved. Further, the rate of formation of product from (ES) complex is same as that of in the absence of inhibitor. So, velocity (V_{max}) is not altered in competitive inhibition but Km increases (affinity of enzyme towards substrate decreases) because of competition of substrate and inhibitor to bind at active site. The interaction of enzyme(E) substrate (S) and competitive inhibitor (I) is represented as equations in the next slide



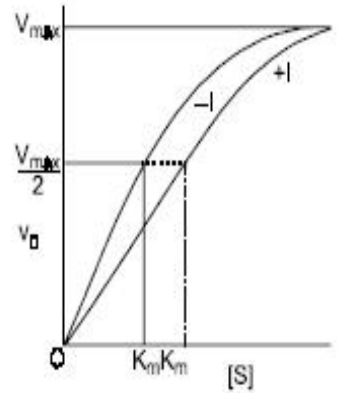


Fig. (*a*) Michaelis plot in presence (+I) and absence (–I) of competitive inhibitor

A classical example for reversible competitive inhibition is succinate dehydrogenase enzyme. Malonate competitively inhibits the enzyme because it is structurally similar to the substrate, succinate (Fig. *b*).

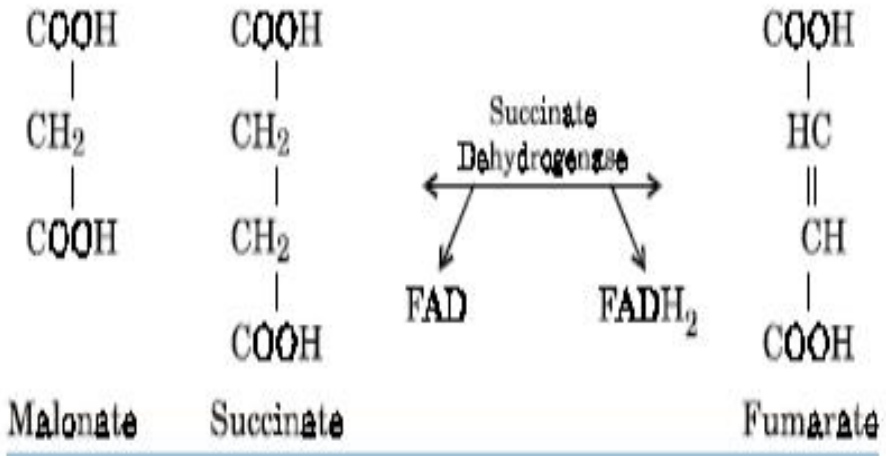


Fig. (*b*) Reaction catalyzed by succinate dehydrogenase and its competitive inhibitor malonate

Competitive Inhibitors as Chemotherapeutic Agents

When used in clinical situations, competitive inhibitors are called *antagonists* or *anti metabolites* of the substrate with which they compete. The use of anti-metabolites in the treatment of diseases is called *chemotherapy*. Therefore, competitive inhibitors are useful chemotherapeutic agents:

1. Antibiotics
2. Anti-cancer drugs
3. In the treatment of metabolic diseases like gout, atherosclerosis and hypertension

1. Sulfonamide antibiotics are used in the treatment of bacterial infections. Bacteria synthesize folic acid from p-amino benzoic acid (PABA). Since these sulfonamide drugs contain sulfanilamide, a structural analog of PABA (Fig. *c*), when used as a chemotherapeutic agent, sulfonamide blocks the synthesis of folic acid in bacteria. The lack of folic acid leads to death of bacteria. Sulfonamide acts as competitive inhibitor for the enzyme involved in the formation of folic acid using PABA as substrate.

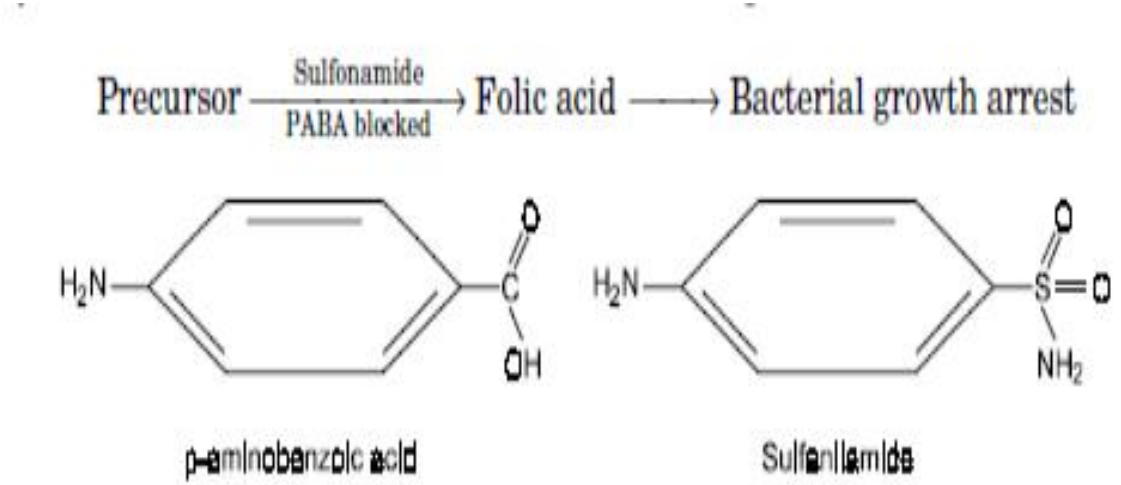
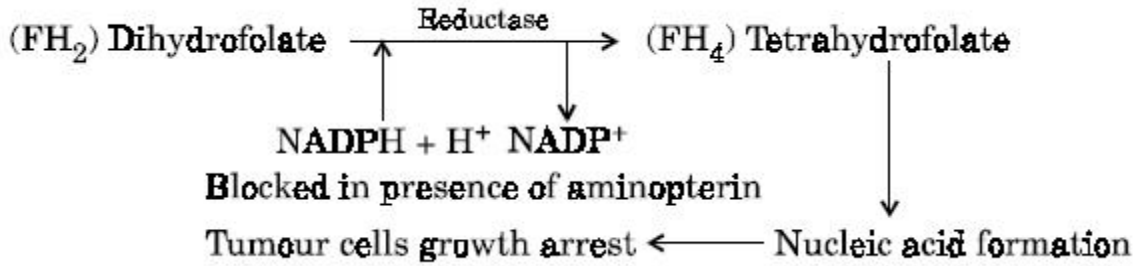


Fig. (*c*) Structure of p-aminobenzoic acid and sulfanilamide.

2. Competitive inhibitors used in the treatment of cancer are aminopterin and amethopterin (methotrexate). They are structural analogs of folic acid. They are competitive inhibitors for the enzyme dihydrofolate reductase. They are used in the treatment of leukaemia, a type of cancer.


(FH2) Dihydrofolate
Reductase
(FH4) Tetrahydrofolate
NADPH + H+
NADP+
Blocked in presence of aminopterin
Tumour cells growth arrest
Nucleic acid formation

When used, these drugs block formation of nucleic acids. For cell proliferation, nucleic acids are needed. So, lack of nucleic acids leads to arrest of tumour growth and advancement of cancer is prevented.

3. Allopurinol is a drug used in the treatment of gout. Gout is due to excessive production of uric acid. Xanthine oxidase is an enzyme involved in the formation of uric acid from hypoxanthine. Allopurinol is a structural analog of hypoxanthine and hence it is an antimetabolite of hypoxanthine. When it is used, it blocks formation of uric acid by inhibiting the enzyme xanthine oxidase.

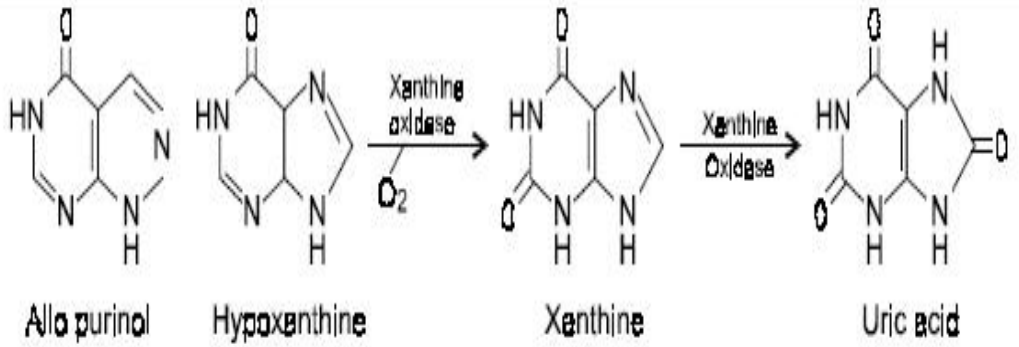


Fig. Reaction catalyzed by xanthine oxidase and its inhibitor

4. Lovastatin is a competitive inhibitor of the enzyme, HMG-CoA reductase. When used it blocks production of cholesterol. In atherosclerosis, cholesterol levels are elevated. Lovastatin reduces cholesterol formation thus arrests the advancement of atherosclerosis.

5. Competitive inhibitors used in the treatment of hypertension are captopril, lisinopril and enalapril. They competitively inhibit angiotensin converting enzyme, which is involved in regulation of blood pressure. When used they lower blood pressure by reducing activity of angiotensin converting enzyme.

Non-Competitive Inhibition

No competition occurs between substrate and inhibitor to bind at active site of enzyme. Inhibitor is not structurally related to substrate. In addition, the inhibitor binds to some other site of enzyme which is far off from the active site. The interaction of enzyme (E), substrate (S) and inhibitor (I) is shown in the next slide.

$$E + S \longrightarrow [ES] \xrightarrow{I} [ESI] \xrightarrow{\text{Slow}} E + P$$

$$E + I \longrightarrow [EI] \xrightarrow{S} [EIS] \xrightarrow{\text{Slow}} E + P$$

In non-competitive inhibition, the inhibitor can react with free enzyme as well as the enzyme-substrate complex, because its binding site is away from active site. In addition, the formation of product from enzyme substrate-inhibitor complex is not same as that, in the absence of the inhibitor. So, the V_{max} is decreased and Km (affinity) remains same because there is no competition of substrate and inhibitor in non-competitive inhibition. Michaelis plot also indicates same in the presence of non-competitive inhibitor Fig

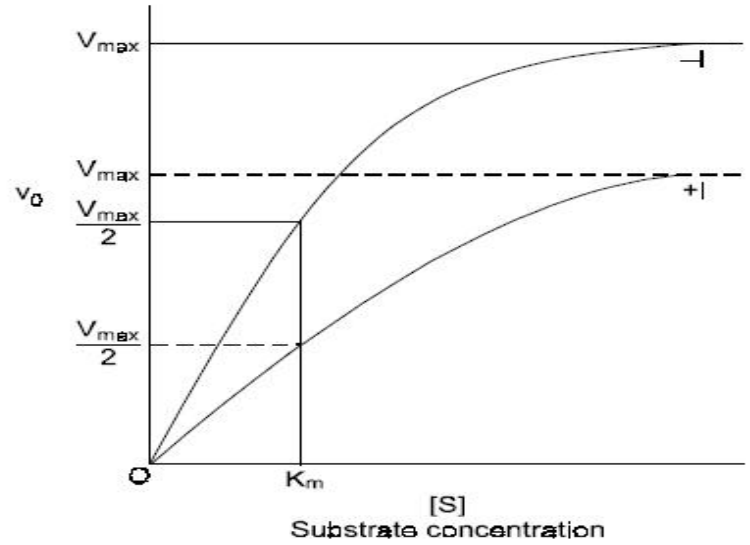


Fig. Michaelis plot in presence (+I) and absence (–I) of non-competitive inhibitor

Examples for Non-competitive Inhibition

Reversible non-competitive inhibitors are rare. Most of the known non-competitive inhibitors are irreversible. They are referred to as enzyme poisons.

1. Iodoacetate blocks the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate by inhibiting enzyme, glyceraldehyde-3-phosphate dehydrogenase

2. Fluoride blocks the action of enolase, which converts 2-phosphoglycerate to phosphoenol pyruvate.

3. Heavy metals like Hg^{2+} , Ag^{+} , Pb^{2+} and Arsenite are also enzyme poisons. They interact with –SH group of enzyme and activate it.

$E- SH + Hg^{2+} \rightarrow E - S - Hg + H^{+}$

Hg^{2+} inhibits –SH containing pyruvate dehydrogenase. Similarly, arsenite inhibits –SH containing α-ketoglutarate dehydrogenase.

4. Some non-competitive inhibitors are used as pesticides. DDT, melathion and parathion are inhibitors of enzyme choline esterase that catalyzes hydrolysis of acetylcholine.

5. Di-isopropyl fluro phosphate (DFP) is a non-competitive inhibitor used as nerve gas in World War II. It is an active site directed irreversible non-competitive inhibitor. It forms covalent linkage with -OH groups of serine residue of choline esterase. When used DFP causes constriction of larynx, pain in eyes and mental confusion.

6. CN^{-} inhibits activity of cytochrome oxidase, an enzyme of respiratory chain. Bitter almonds contain some cyanide.

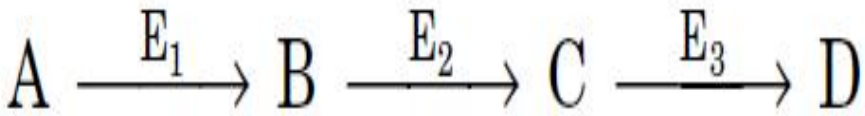
7. Ethylene diaminotetra acetic acid (EDTA) inhibits metalloenzymes by forming complexes with metal ions.

8. Tubers, bananas and beans contain inhibitors to trypsin, chymotrypsin and elestase.

FEEDBACK INHIBITION

Inhibition of activity of enzyme of a biosynthetic pathway by the end product of that pathway is called feedback inhibition.

For example, formation of a substance D from A is catalyzed by three enzymes E1, E2 and E3.



When enough D is formed it inhibits the activity of E_1. By inhibiting E_1, D regulates its own synthesis.

Examples:

1. Inhibition of aspartate trans carbamoylase by CTP.
2. Inhibition of HMG-CoA reductase by cholesterol.
3. Inhibition of ALA-synthase by heme.
4. Inhibition of anthranilate synthetase by tryptophan.

COFACTORS

1. Cofactors are non-protein molecules required for activity of some enzymes. They may be involved in catalysis or in structure maintenance.

There are two types of cofactors:

1. Organic cofactors

2. Inorganic cofactors.

The organic cofactors are further subdivided into.

1. Prosthetic groups

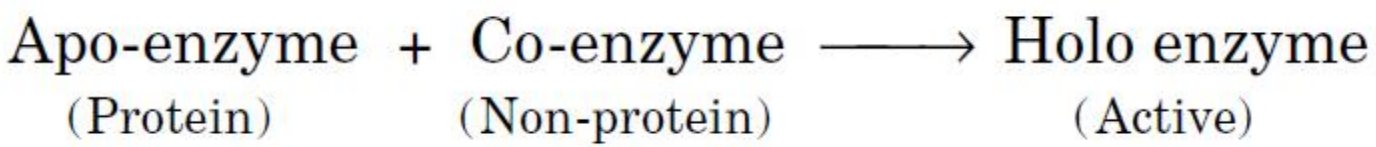
2. Co-enzymes

1. Prosthetic Groups

These organic molecules are covalently attached to the enzyme and they undergo change during catalysis but return to native state at the end of the reaction.

2. Co-enzymes

These organic molecules are loosely (non-covalent) attached to enzyme molecules. They undergo change during reaction. Since they undergo change along with substrate they are referred as co-substrates.

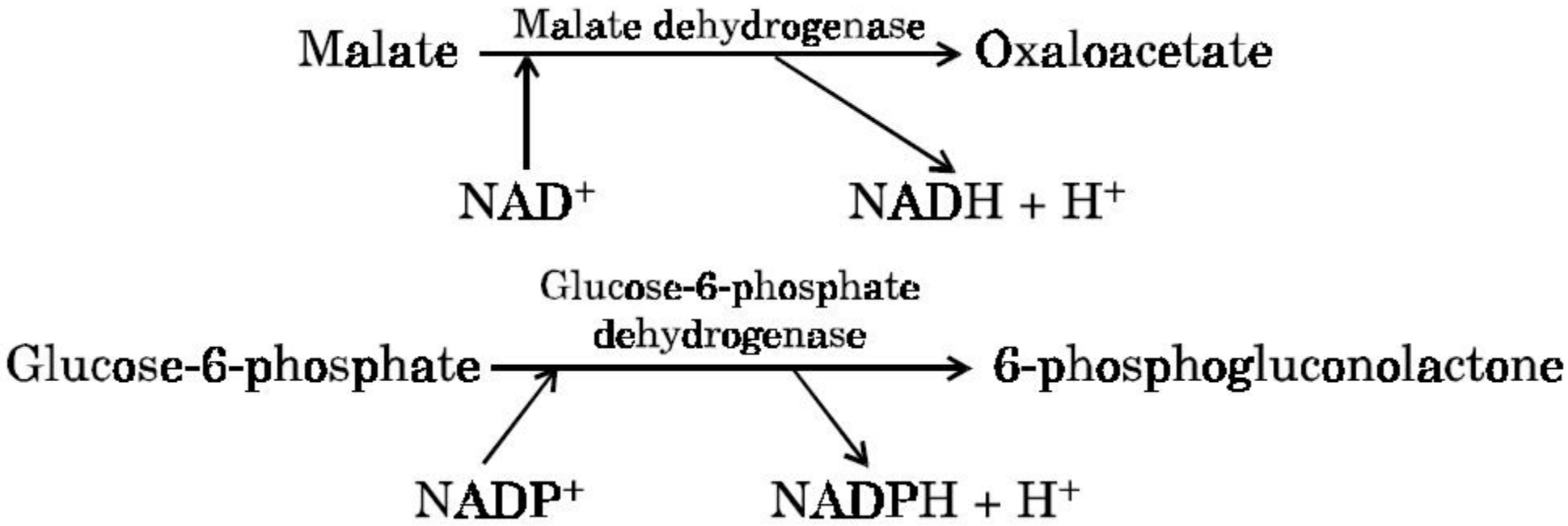

Apo-enzyme
(Protein)
+
Co-enzyme
(Non-protein)
Holo enzyme
(Active)

Examples for Organic Co-factors

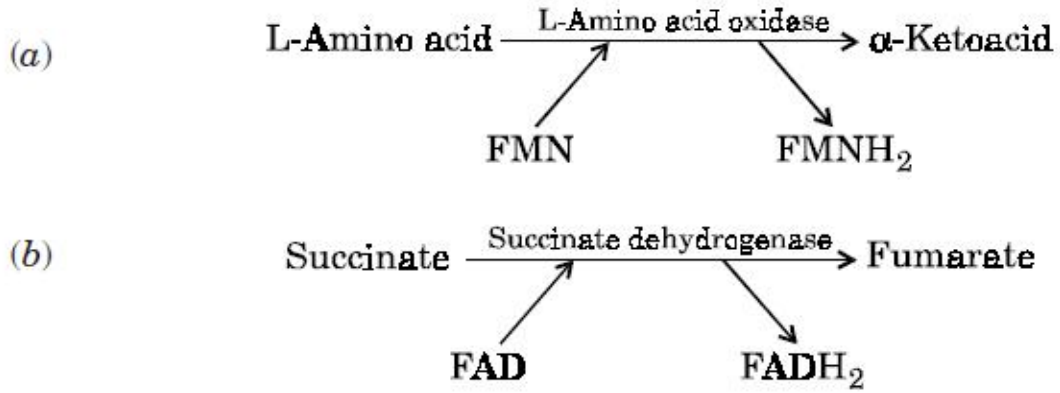
Major function of water soluble vitamins is to serve as co-factors, some of them serve as such, otherwise their derivatives serve as co-factors. They are divided on the basis of their function, in enzymatic reactions

1. Co-enzymes of oxidation-reduction reactions.

(a) Co-enzymes derived from niacin. They are NAD+, NADH + H+ and NADP+, NADPH+, H+. These co-enzymes are loosely bound to apo-enzymes. Reactions where they serve as co-enzymes are given in the next slide.

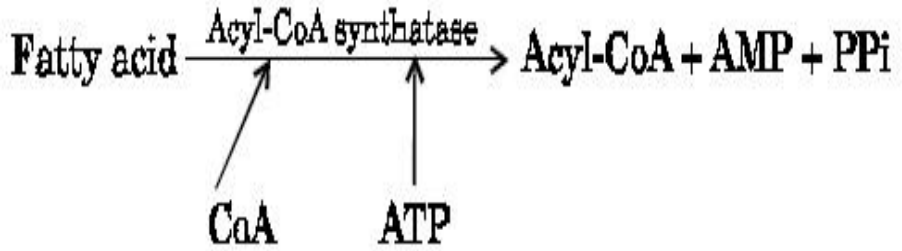

Malate
Malate dehydrogenase
Oxaloacetate
NAD+
NADH + H+
Glucose-6-phosphate
dehydrogenase
Glucose-6-phosphate
6-phosphogluconolactone
NADP+
NADPH + H+

Co-enzymes derived from riboflavin. They are FMN, $FMNH_2$ and FAD, $FADH_2$. They are covalently linked to apo-enzymes. So, they are prosthetic groups. Enzymes to which they are prosthetic groups are given below.

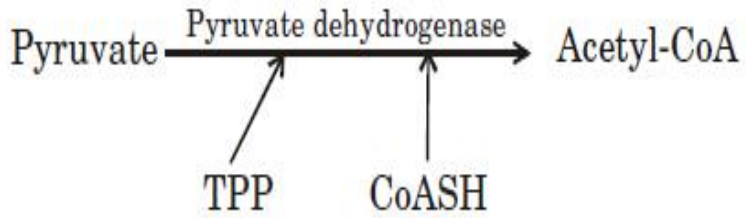


2. Coenzymes of group transfer reactions.

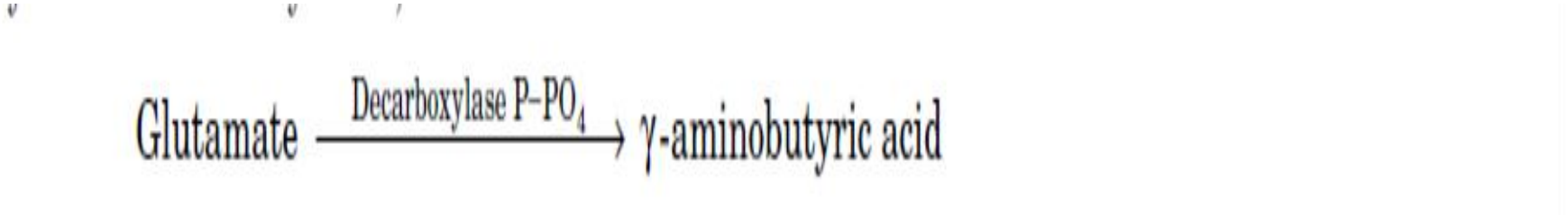
(*a*) Co-enzyme of pantothenic acid. It is co-enzyme of A (CoA, CoASH). It is involved in CoA transfer reaction.



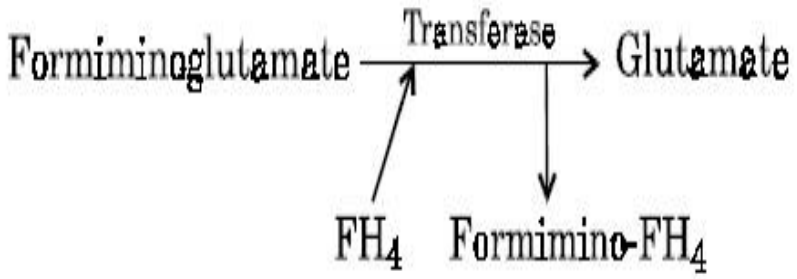
(*b*) Co-enzyme of thiamin. It is thiamin pyro(di)phosphate (TPP, TDP). It is a prosthetic group of several enzymes.



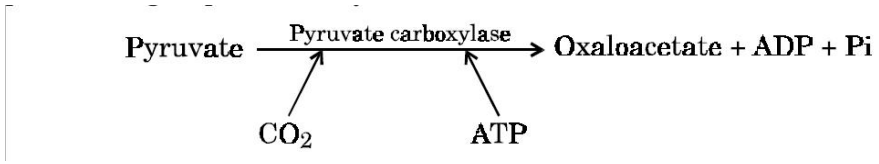
(*c*) Co-enzyme of pyridoxine. It is pyridoxal phosphate (P-PO4). It is prosthetic group of enzymes involved in amino group transfer. Other reactions where it serves as coenzyme are decarboxylation, transulfuration etc.



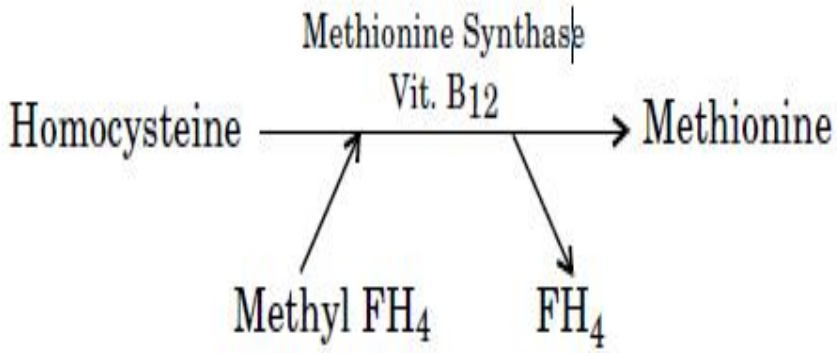
(*d*) Co-enzymes of folic acid. It is tetrahydrofolate (FH4). It participates in one carbon transfer reaction.



(e) Biotin is the only water-soluble vitamin that functions as coenzyme as such. It is the prosthetic group of carboxylases.



(*f*) Co-enzyme of vitamin B_{12} or cyanocobalamin. It is methylcobamide. It is involved in methyl transfer reactions



3. Many nucleotides also function as co-enzymes. They are adenosine triphosphate (ATP), cytidine diphosphate (CDP), uridine diphosphate (UDP), phosphoadenosine phosphosulfate (PAPS) and S-adenosyl methionine (SAM).

INORGANIC CO-FACTORS

Many enzymes require metal ions. They are required for maintenance of protein (enzyme) conformation and catalysis. Metal ions participate in enzymatic reactions in three ways.

1. Metallo Enzymes

- Metal is tightly bound to enzyme molecule and it is an integral part of enzyme molecule.
- Metals are attached to enzyme through coordinate bonds. They participate in catalysis

Examples:

(*a*) **Iron (Fe^{2+}):** It is required for cytochrome oxidase, catalase, xanthine oxidase, succinate dehydrogenase.

(*b*) **Copper (Cu^{2+}):** It is required for cytochrome oxidase, superoxide dismutase, lysyloxidase and ceruloplasmin.

(*c*) **Zinc (Zn^{2+}):** It is required for carbonic anhydrase, carboxy peptidase, alkaline phosphatase, alcohol dehydrogenase etc.

2. Metal-dependent Enzymes

Metal is loosely associated with enzyme molecule or it may be required for enzyme substrate complex formation. In the absence of metal, enzyme may not interact with substrate molecule or with co-enzyme molecule.

Examples:

(*a*) **Magnesium (Mg^{2+}):** It is needed by enzymes using ATP. Formation of Mg: ATP complex is essential. They include hexokinase, galactokinase, pyruvate kinase etc.

(*b*) **Calcium (Ca^{2+}):** It is required for the activity of calpain, a calcium-dependent protease.

Others are Na^{+}/K^{+}-ATPase and Ca^{2+} ATPase.

3. Metal-activated Enzymes

In presence of metals, some enzymes get activated *i.e.,* their activity increases many folds.

Examples:

(*a*) **Chloride (Cl^-):** It activates amylase and angiotensin converting enzyme.

(*b*) **Calcium (Ca^{2+}):** It activates trypsin.

ENZYME REGULATION

Metabolic pathways are controlled by regulating enzyme activity. If enzyme activity is not regulated, it can harm cellular activities and may lead to the development of diseases.

Alteration of enzyme regulation is one of the causes for cancer development. Over production of tyrosine kinase is associated with alteration of cell shape in tumour cells. Enzyme regulation can alter when drugs are used.

Enzyme regulation can be altered by environmental toxins or pollutants.

Enzyme activity can be regulated by:

(*a*) changing catalytic efficiency.

(*b*) altering the amount or quantity of enzyme in cell or body.

(*a*) Catalytic efficiency of enzymes can be regulated by:

1. subjecting enzyme to feedback inhibition
2. allosteric regulation or inhibition
3. covalent modification of enzyme molecule
4. synthesizing enzyme in inactive form

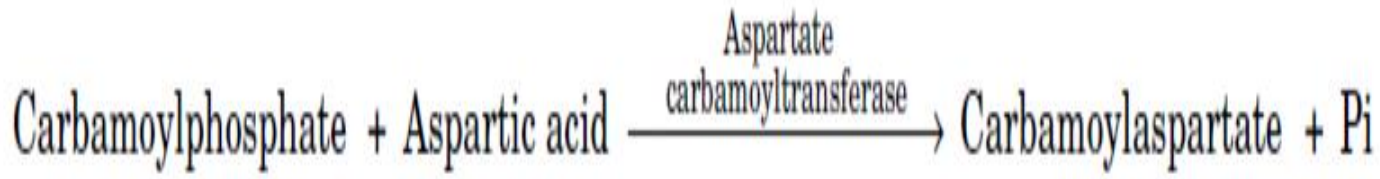
Allosteric Inhibition

Inhibition of activity of allosteric enzymes by allosteric inhibitor is called as allosteric inhibition.

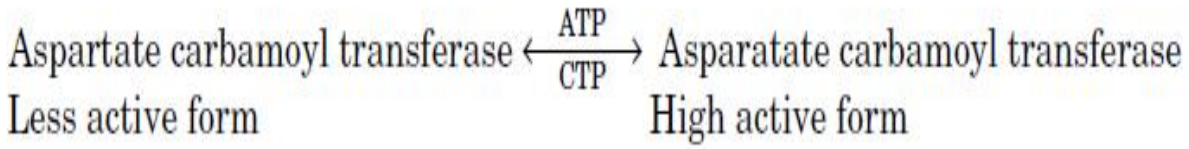
Allosteric inhibition is seen in pathways that are subject to regulation

Allosteric Inhibitors are not structurally similar to substrates of allosteric enzymes. They bind to enzyme at allosteric site which is different from active site. The activity of an allosteric enzyme is raised by allosteric activator. Most of the allosteric enzymes are oligomeric proteins *i.e.,* they consist of many subunits.

- The most extensively studied allosteric enzyme is aspartate transcarbamolyase (Aspartate carbamolytransferase). It catalyzes first reaction unique to pyrimidine nucleotide biosynthesis.



The enzyme consists of catalytic and regulatory subunits. It exists in less active form and high active form. Binding of CTP to regulatory subunit converts high active form to less active form. So CTP is called a negative effector or allosteric inhibitor. In contrast, binding of ATP to the regulatory subunit converts less active form to high active form. So ATP is called a positive effector or allosteric activator.



Kinetics of Allosteric Enzyme

1. These enzymes do not exhibit Michaelis-Menten Kinetics. A plot of velocity versus substrate concentration is sigmoidal or S shaped curve rather than rectangular hyperbola (Fig.).

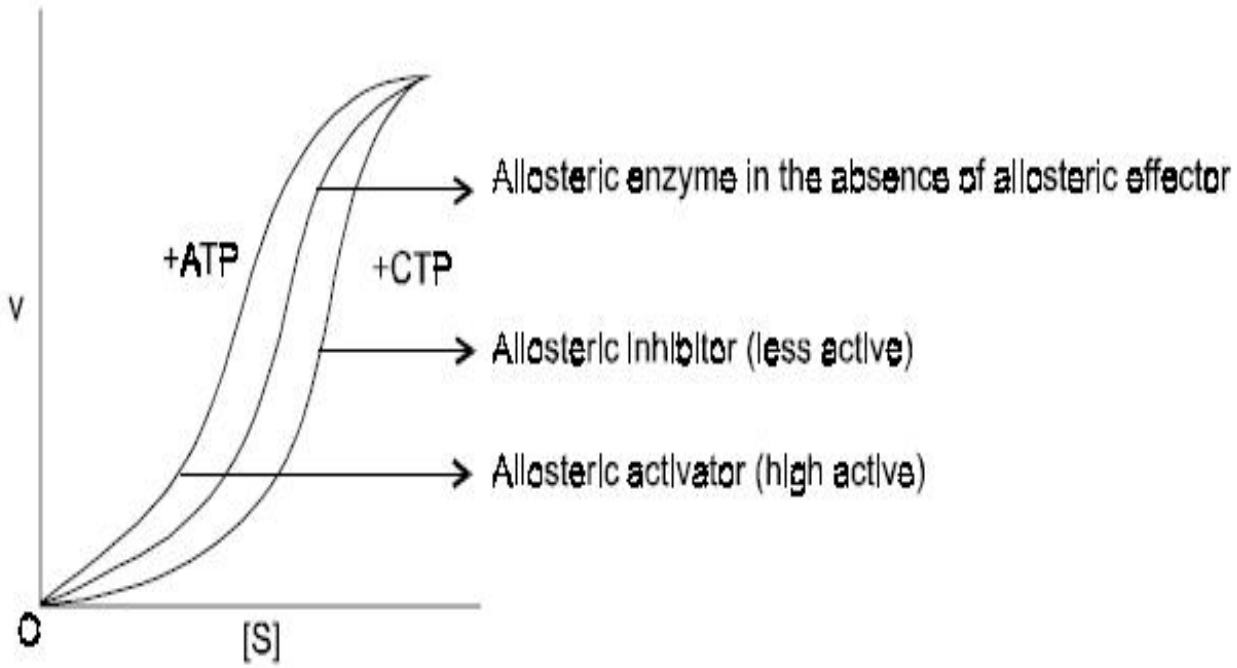


Fig. The plot of *v* versus [s] for an allosteric enzyme in presence (+) of allosteric effector and in the absence of allosteric effector

2. The sigmoidal curve indicates a rapid increase in velocity after a particular substrate concentration. It is due to phenomenon of co-operativity.

3. To explain co-operativity of allosteric enzymes ‘T’ and ‘R’ model was proposed. According to this model, the oligomer (allosteric enzyme) exists in two states. A tense (T) state and relaxed (R) state. Binding of substrate (ligand) to ‘T’ form which is initially slow, causes a conformational change (Fig.) in subunits resulting in ‘R’ form. Further binding of ligand (substrate) to the subunits is rapid.

4. Allosteric inhibitor stabilizes the enzyme in ‘T’ form, so the enzyme is less active. In contrast, allosteric activator stabilizes the enzyme in ‘R’ form, so the enzyme is highly active (Fig.).

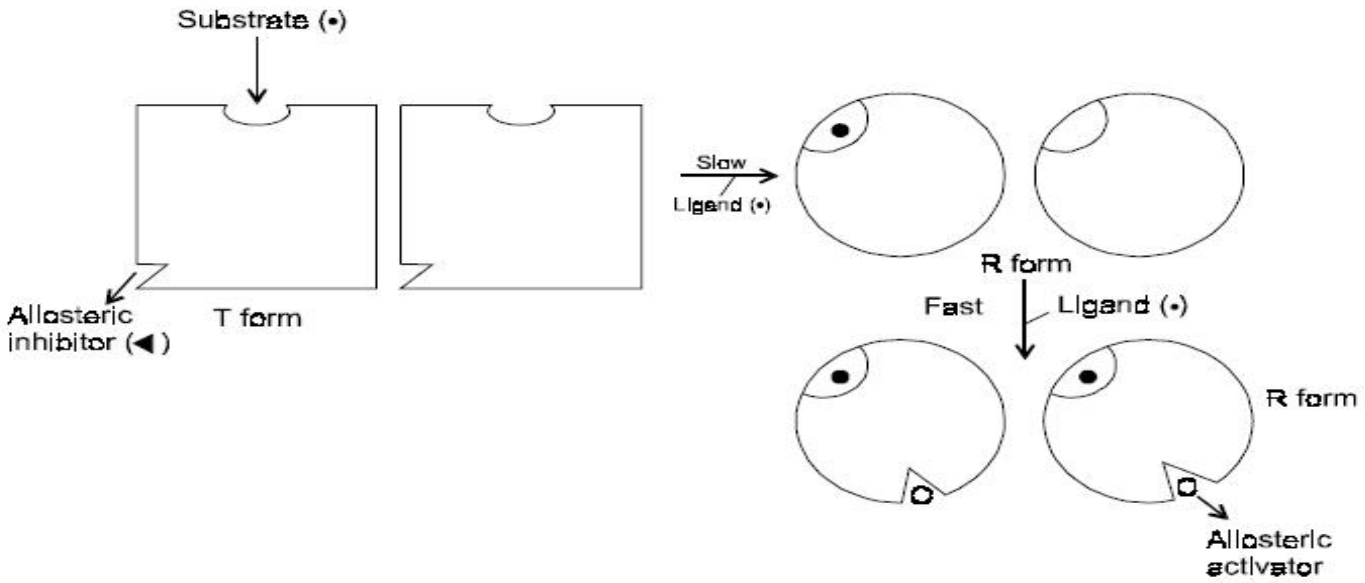


Fig. Model for cooperativity of allosteric enzyme.

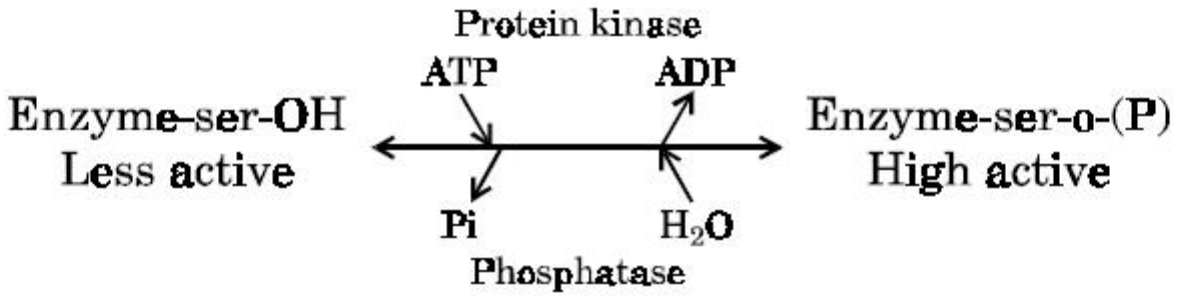
Enzyme Regulation by Covalent Modification

Enzyme activity is regulated by covalent attachment of a group to the enzyme molecule.

Phosphate group is most commonly used to modify enzyme activity. The other group involved in regulation of enzyme activity by covalent modification is nucleotide. Enzymes which undergo regulation by covalent modification exist in two forms, a less active and a high active form.

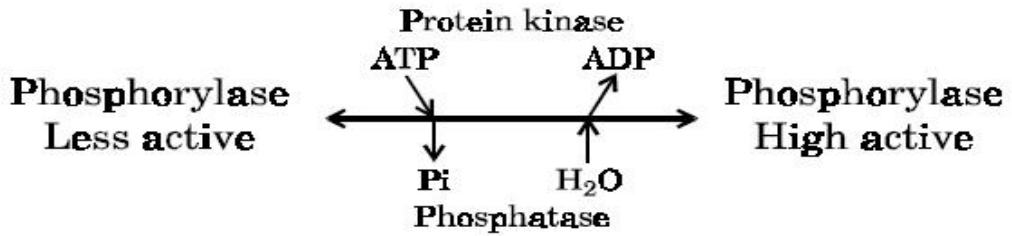
Depending on the enzyme, the phospho or de-phospho enzyme may be less or more active, respectively. The phosphorylation (attachment of phosphate) and de-phosphorylation are catalyzed by protein kinases and phosphatases, respectively. The -OH group of serine residue of the protein is the site of phosphorylation. ATP serves as donor of phosphate group.

Many hormones influence activities of protein kinases and phosphatases.

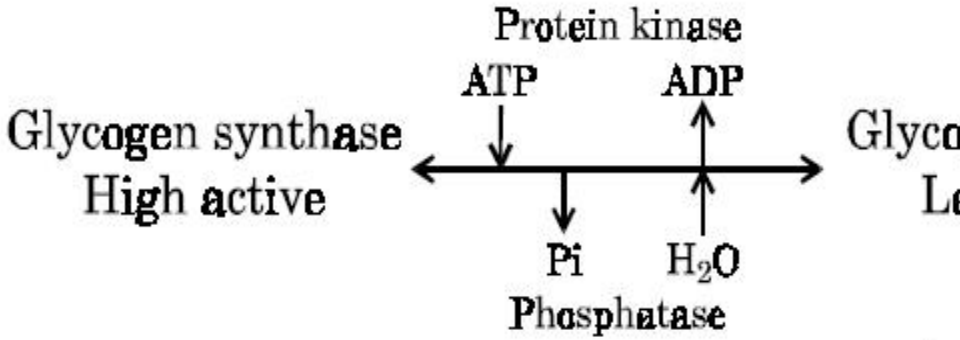

Protein kinase
ATP
ADP
Enzyme-ser-OH
Less active
Enzyme-ser-o-(P)
High active
Pi
H_2O
Phosphatase

Examples:

1. Phosphorylation of glycogen phosphorylase converts less active to highly active form. Dephosphorylation converts highly active to less active form.



2. Phosphorylation of glycogen synthase converts high active to less active form

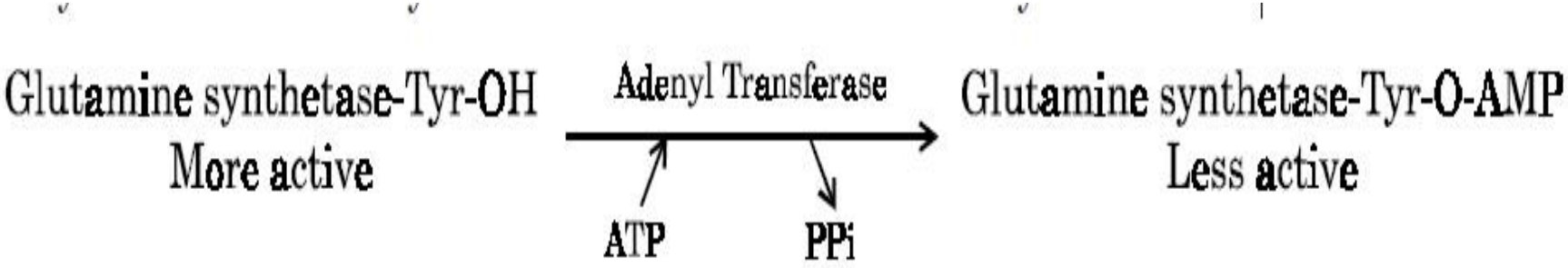


3. HMG-CoA reductase, hormone sensitive lipase and acetyl-CoA carboxylase are also regulated by phosphorylation and de-phosphorylation.

Enzyme Regulation by Covalent Attachment of Nucleotide

The activity of glutamine synthetase of *E. Coli* is regulated by covalent attachment of nucleotide to the enzyme molecule. The attachment of a nucleotide to the –OH group of tyrosine residue of enzyme molecule converts more active enzyme to less active enzyme.

Adenyl transferase catalyzes addition of nucleotide to enzyme molecule



PRO-ENZYMES

One way of regulating catalytic activity of an enzyme is synthesizing enzyme in inactive (precursor) form or pro-enzyme or zymogen. They are converted to active form later when need arises. The conversion of pro-enzyme to active enzyme involves limited proteolysis. Limited proteolysis removes few amino acids from proenzyme which results in conversion of inactive enzyme to active enzyme. So, conversion of pro-enzyme to active enzyme accompanies decrease in molecular weight of pro-enzyme due to removal of amino acids (peptides).

Most of the protein digesting enzymes of pancreas are synthesized in inactive forms to protect pancreatic cells from destructive action of proteases. Likewise, pepsin of stomach is also synthesized in pro-enzyme form to protect gastric mucosa from pepsin attack. Most of the blood clotting enzymes are also synthesized in inactive form. They are converted to active forms only at the time of blood coagulation.

Most of the protein digesting enzymes of pancreas are synthesized in inactive forms to protect pancreatic cells from destructive action of proteases. Likewise, pepsin of stomach is also synthesized in pro-enzyme form to protect gastric mucosa from pepsin attack. Most of the blood clotting enzymes are also synthesized in inactive form. They are converted to active forms only at the time of blood coagulation.

The protein splitting enzymes of pancreas are synthesized in inactive forms. They are trypsinogen, chymotrypsinogen, procarboxy peptidase and proelastase. A lipid digesting enzyme is also produced in pancreatic cells as a zymogen. It is prophospholipase. The conversion of these pro-enzymes to active enzymes is initiated by enterokinase produced by mucosal cells of duodenum. Enterokinase removes a hexapeptide from trypsinogen by hydrolysing-Lys-Ile bond.

The removal of hexapeptide converts trypsinogen to trypsin. When once few molecules of trypsin are formed it further catalyzes not only formation from trypsinogen but also the conversion of other proenzymes to active enzymes (Fig.). Since single molecule of trypsin can trigger the formation of battery of protein digesting enzymes, pancreas has another self protecting mechanism. It contains trypsin inhibitor in small amounts.

The formation of blood clot involves activation of (zymogens) blood clotting factors.

Prothrombin is converted to active thrombin by factor X and V. Thrombin in turn converts fibrinogen to fibrin (Fig.).

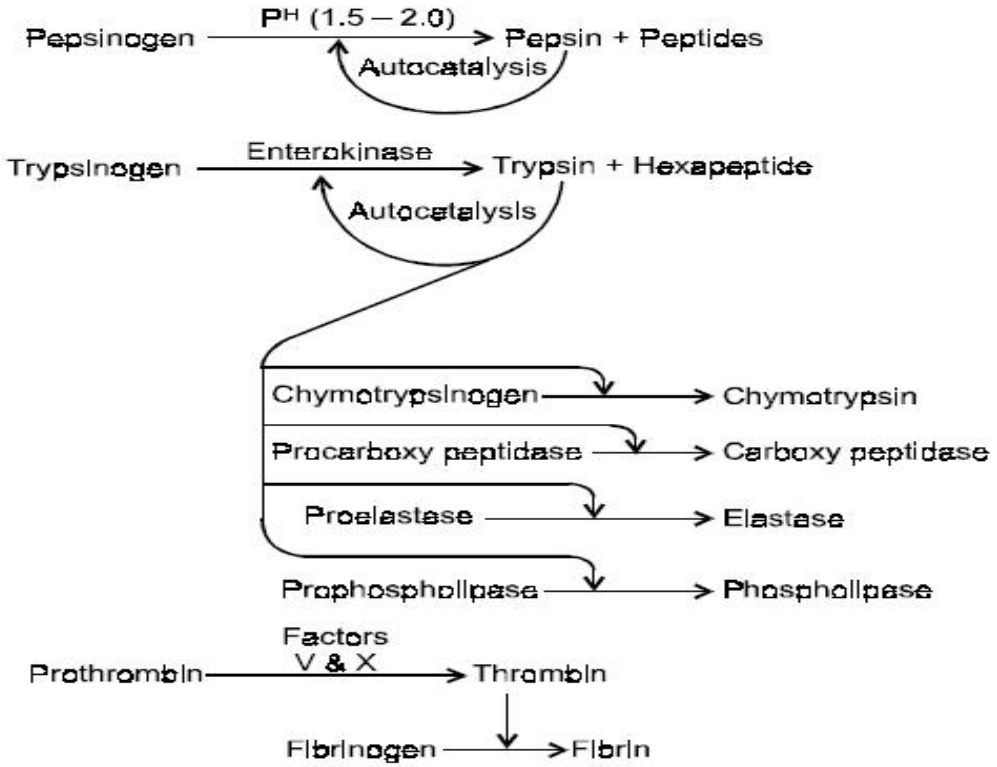


Fig. Conversion of pro-enzymes to enzymes

Medical Importance

Though there are two in-built defensive mechanisms in the pancreas to avoid activation of pro-enzymes, in acute pancreatitis the pro-enzymes get activated and cause damage to pancreas and severe abdominal pain.

The quantity of enzyme in cell or body is regulated by

1. Enzyme degradation
2. Enzyme induction and repression

Regulation of Enzyme Activity by Degradation

Enzymes produced as a part of development or enzymes produced to overcome certain environmental conditions or enzymes produced to remove toxins are not needed any more later. Their continued presence may be harmful to the body. So, if enzymes were immortal, then it leads to creation of unwanted side effects in the body. Hence, enzymes undergo turnover. They are synthesized and degraded. Individual enzymes have life spans. Some enzymes may last few seconds or minutes in the cell. However, some enzymes may last few days in the body. There are specific mechanisms for degradation of enzymes. Enzymes that control key metabolic events are degraded very fast. Likewise if a defective enzyme is produced, it is degraded very rapidly because it is not useful any more to the body.

Enzyme Regulation by Induction and Repression

The quantity of the enzyme can be increased by increasing its synthesis and quantity of the enzyme can be decreased by decreasing its synthesis. Depending on cell needs quantity of the enzyme increases or decreases. Enzymes which are regulated in this manner are called as *inducible enzymes*. It take place at nuclear level of the cell.

Inducible Enzymes

Normally these enzymes are present in small concentration but in presence of certain substance called inducer their quantity increases.

Induction

Increased synthesis of an inducible enzyme in response to inducer is known as induction.

Constitutive Enzymes

These are present in fixed quantities. They are not inducible.

Examples for enzyme induction: When *E. Coli* is grown on medium containing lactose, it produces more of β-galactosidase or lactase required for lactose utilization.

When the cells are transferred to medium free of lactose, formation of lactase decreases.

Thus, lactose induces the synthesis of lactase. So, in this case lactose is an inducer and lactase is an inducible enzyme

Repression

Certain substances block their own synthesis by decreasing synthesis of enzymes, which are required for their formation. This process is called as repression. Substances are called repressors.

Examples for repression: When histidine is added to the *S. Typhi*. containing medium, synthesis of all the enzymes required for histidine formation is blocked. In this case, histidine is a repressor molecule.

In humans also, induction and repression of enzymes takes place. They are called adaptable enzymes.

Examples:

1. Arginase, an enzyme of urea-cycle formation is more in starvation and on high protein diet.

2. Pyruvate carboxylase an enzyme of gluconeogenesis is induced by glucocorticoids and repressed by insulin.

3. Phenobarbitol, an anti-convulsive drug induces alkaline phosphatase.

ISO-ENZYMES OR ISOZYMES

1. They are multiple forms of enzymes.

2. The catalyze same reaction but differ in physiochemical properties. They occur in same species or in same individual.

3. They are tissue specific or species specific.

4. The are present in serum and other biological fluids and tissues.

5. Iso-enzymes for dehydrogenases, transaminases and phosphatases have been reported.

Separation of Iso-enzymes

Most commonly used technique for the separation of iso-enzymes is electrophoresis. The serum lactate dehydrogenase (LDH) iso-enzyme pattern is obtained by subjecting serum to electrophoresis at pH 8.6. On electrophoresis, iso-enzymes of lactate dehydrogenase separate into five bands. Each band exhibits same catalytic activity (Fig.). The five isoenzymes of LDH are LDH1, LDH2, LDH3, LDH4 and LDH5.

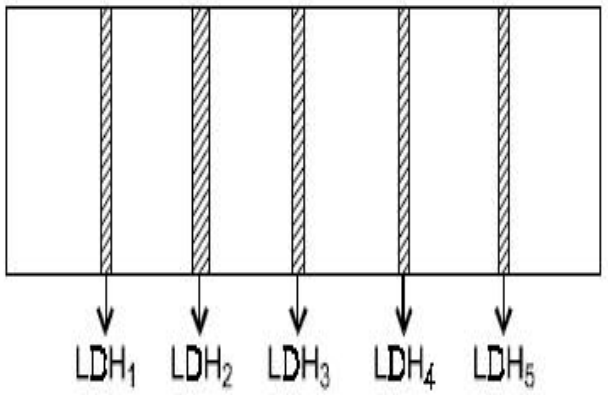


Fig. Lactate dehydrogenase isoenzyme pattern

Structure of LDH Iso-enzymes

Lactate dehydrogenase isoenzymes differ at the level of quaternary structure. The LDH consist of 4 subunits of two types. They are H and M subunits. The subunit composition of different LDH isoenzymes are shown in the next slide

Name of isoenzyme	Subunit composition
LDH_1	HHHH or H_4
LDH_2	HHHM or H_3M
LDH_3	HHMM or H_2M_2
LDH_4	HMMM or HM_3
LDH_5	MMMM or M_4

The synthesis of two subunits H and M is controlled by different genes. H is acidic and M is basic in nature. The molecule weight of each subunit is 35,000.

Alkaline Phosphatase Iso-enzymes

Electrophoresis is used for the separation of isoenzymes of alkaline phosphatase in serum. On electrophoresis, iso-enzymes of alkaline phosphatase separates into four bands. The four iso-enzymes of alkaline phosphatase are tissue specific. They differ in their carbohydrate content. The four isoenzymes originate from bone, liver, placenta and intestine.

Creatine Phosphokinase (CK) Iso-enzyme

CK iso-enzymes can be separated by electrophoresis. CK has three isoenzymic forms. They are CK1, CK2 and CK3. They differ in subunit composition. CK is a dimer. It consist of two subunits M and B. The subunit composition of three isoenzymes of CK are BB, MB and MM

for CK1, CK2 and CK3 respectively.

Carbonic Anhydrase Iso-enzymes

On electrophoresis carbonic anhydrase gives three bands. The three isoenzymes differ in amino acid composition.

CLINICAL ENZYMOLOGY

1. It deals with quantitative estimation of enzymes in body fluids in normal and diseased conditions.

2. Depending on pathological conditions different body fluids are used for enzyme measurement.

3. Serum and plasma are most commonly used.

4. Other body fluids used for enzyme measurement are cerebrospinal fluid, amniotic fluid, pleural fluid, peritonial fluid and synovial fluid.

5. Quantitative estimation of enzymes in serum is used to confirm the diagnosis which is made by observing clinical symptoms. Sometimes it is used to know the effectiveness of treatment *i.e.,* prognosis.

6. Hence measurement of serum enzyme levels is of both diagnostic and prognostic importance. Blood plasma contains several enzymes. Depending on their role they are divided into two groups.

A. Functional Enzymes

They are present in plasma at higher level than in most of tissues and they perform functions in plasma. They include lipoprotein lipase, choline esterase and enzymes of blood coagulation etc.

B. Non-functional Enzymes

The are present only at minimal concentrations in normal and have no known function in plasma. They mainly arise from normal destruction of various blood and tissues cells. So, they are mainly contributed by turnover of tissues. Increased concentration of these enzymes in plasma indicates increased tissue breakdown or damage to tissues due to disease or injury. If the plasma level of secretory enzyme is increased, it indicates block in the secretory pathway. Further distribution of enzymes among tissues varies from one organ to another. If an organ is rich in an enzyme, injury or damage to that organ leads to release of the enzyme into plasma in significant amounts. Some diseases or cancers of that organ also cause release of the enzyme into plasma. Quantitative measurement of the enzymes in plasma under such conditions serve as good index of disease of that organ. Further more, the amount of enzyme released is proportional to the mass of the affected tissue

Some of the clinically important enzymes which are routinely measured in clinical chemistry laboratory are:

1. Transaminases

Aspartate amino transferase (AST) and alanine amino transferase (ALT) are two transaminases most frequently measured. Normal levels are 3-20 U/L for AST and 4-20 U/L for ALT (Units-U). The former enzyme is also referred to as GOT (Glutamate oxalo acetate transaminase) and the latter is referred to as GPT (Glutamate Pyruvate Transaminase). These two enzymes differ in distribution. Heart is rich in AST where as liver contains both of them in almost equal amounts. Hence, AST estimation is most commonly done in diseases that affect the heart. AST level increases in plasma following heart attack or myocardial infarction. Since liver contains more of ALT, its elevation in plasma is specific indicator of liver damage. Plasma ALT level is more in liver diseases like alcoholic cirrhosis, biliary obstruction, cancer and toxic hepatitis.

Both the enzymes are elevated in acute infective hepatitis because liver contains both of them in significant amounts. After the onset of viral hepatitis, the levels of these enzymes reach the peak rapidly and come back to normal reference level within a week. Since the skeletal muscle contains appreciable amounts of ALT, its level is increased in muscle damage as in severe trauma and in muscular dystrophy. Serum transaminases are also elevated in lung disease.

2. Alkaline phosphatase

This enzyme catalyzes the hydrolysis of organic esters at alkaline pH 9.0, hence the name alkaline phosphatase. The normal level is 20-90 units/L. The level of the enzyme is elevated in rickets, obstructive jaundice, hyper para thyroidism, metastatic cancer, bone cancer and osteomalacia. In obstructive jaundice, its level is 10 times the normal level because its secretion is blocked due to obstruction. Its level also increases in some non-specific diseases like leukemia, lung and kidney damages and congestive heart failure, Hodgkin’s disease and intestinal disorders.

3. Acid phosphatase

This enzyme catalyzes the hydrolysis of organic esters at acidic pH (5.0) hence the name acid phosphatase. The normal level of the enzyme is 2.5-12.0 U/L. It is increased in prostrate cancer. Small increase is seen in bone disease and breast cancer.

4. γ-glutamyl transferase (GGT)

It is involved in the degradation of glutathione. Its level is increased in alcoholic cirrhosis.

The normal plasma level of GGT is less than 30 U/L. Since this enzyme is secreted into bile by liver, like alkaline phosphatase, γ-glutamyl transferase level increases in cholestatic or obstructive jaundice. It is also elevated in brain lesions

5. Creatine phosphokinase (CK)

The normal level of this enzyme in plasma is 12-60 U/l. Since skeletal muscle is rich in CK, serum CK level rises in diseases effecting skeletal muscle. Its level is elevated in muscular dystrophy, polymyositis, severe muscle exercise, muscle injury, hypothyroidism, epileptic seizures and in tetanus.

CK level is also elevated in diseases affecting cardiac muscle because of its high content in it. CK level is elevated in myocardial infarction

6. Lactate dehydrogenase (LDH)

The LDH normal level is 70-90 U/L. LDH levels are elevated in myocardial infarction.

The serum LDH level rises within 24 hours after infarction, reaches peak level around 2-3 days and returns to normal in a week. Serum LDH level is also elevated in pernicious anemia, megaloblastic anemia, acute hepatitis, blood cancer and in progressive muscular dystrophy.

7. Isocitrate dehydrogenase

The normal level of this enzyme in plasma is 1-5 U/L. Its level is elevated in inflammatory diseases of the liver, like infective hepatitis, toxic hepatitis. In obstructive jaundice, its level remains normal. This enzyme is found in cerebrospinal fluid. Measurement of enzyme in

C.S.F. is a valuable diagnostic aid in the cases of meningitis and brain tumors. In meningitis the level is elevated more than in cerebral tumors.

8. Amylase

The normal range of this enzyme in plasma is 800-1800 U/L. This enzyme is secreted by pancreas and salivary glands. So, its level is elevated mainly in acute pancreatitis and parotitis.

Its level is raised in other conditions like intestinal obstruction and in mumps.

9. Lipase

It is an enzyme produced by pancreas. It is secreted into duodenum through pancreatic duct.

The normal level of this enzyme is up to 150 U/L. It is mainly elevated in acute pancreatitis and pancreatic cancer. It is also elevated in patients with abdominal lesions, perforated peptic ulcer, intestinal obstruction and in acute peritonitis.

ISOENZYMES IN CLINICAL MEDICINE

1. In some cases, elevated serum enzyme level may not indicate severity and specific organ damaged, because the serum enzyme is derived from routine destruction of cells of various organs.

2. Since isoenzymes are organ specific, iso-enzyme determination gives an indication about the specific organ affected. Further, iso-enzyme distribution varies from one organ to the other. Hence, if an organ rich in a isoenzyme is damaged or diseased, more of that iso-enzyme enters plasma.

3. By measuring that isoenzyme level in serum, the specific organ diseased can be confirmed.

4. Therefore, iso-enzyme determination is useful in differential diagnosis

(a) LDH Isoenzymes

Serum LDH is the combination of five isoenzymes. Each iso-enzyme is derived from specific organ. LDH1 is derived from heart because heart is rich in LDH1. Similarly, LDH5 is derived from skeletal muscle because it is rich in LDH5. Liver also contains LDH2 to LDH5 isoenzymes in different amounts. LDH isoenzymes are present in different proportions. The proportions of LDH isoenzymes in normal serum are 25%, 35%, 27%, 8% and 5% for LDH1, LDH2, LDH3, LDH4 and LDH5, respectively.

When heart muscle is affected as in myocardial infarction, LDH1 level increases in plasma because of the release of LDH1 from damaged heart muscle. So measurement of LDH isoenzyme in serum in myocardial infraction is more sensitive index of myocardial necrosis than the measurement of total LDH activity. Similarly, elevated levels of LDH5 is more specific of muscle lesions and liver inflammation of hepatitis

(b) CK Isoenzymes

The normal serum CK is composed of CK1, CK2 and CK3. In normal persons, CK2 accounts only 2% of total CK but it accounts for 20% of CK in a patient within 4 hours after heart attack

(c) Alkaline Phosphatase Isoenzymes

The normal serum alkaline phosphatase is composed of 4 isoenzymes. They are derived from bone, liver, placenta and intestine. Measurement of isoenzymes of alkaline phosphatase is used to distinguish liver lesions from bone lesions in metastatic carcinoma.

SERUM ENZYME PROFILES

1. It involves estimations of different serum enzymes for few days following the onset of a disorder.

2. Multi enzyme determinations for a short span of time serve as good index of disorder.

More over determination of more than one enzyme in a particular disease is more useful in prognosis.

3. Several serum enzymes serve as diagnostic indices of myocardial infarction. Serum AST level starts increasing by 6 hours after heart attack, reaches peak value around one to second day and returns to normal by sixth day. CK level follows a pattern similar to AST. It contrast, LDH levels rise within 24 hours of heart attack, reach peak around 2-3 days and level, remain increased even after a week (Fig.).

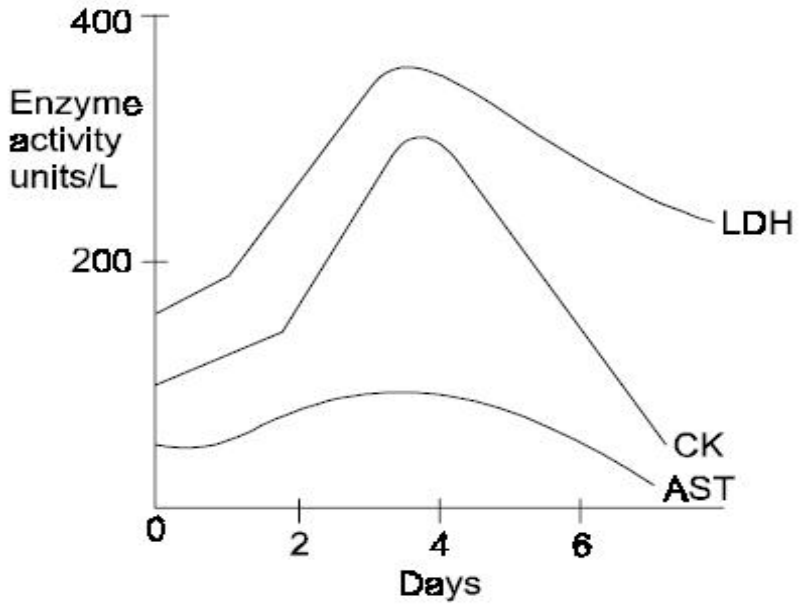


Fig. Serum enzyme profiles in myocardial infarction

Serum enzyme levels are also determined to detect inherited disorders associated with altered enzyme levels like galactosemia, glucose-6-phosphate dehydrogenase deficiency etc.

ENZYME-LINKED IMMUNO ADSORBENT ASSAY

It is popularly known as ELISA technique. The technique combines enzymology with immunology and photometry. It is used for detection and estimation of substances which are either antigens or antibodies. It is based on immune complex formation. The immune complex consists of an antibody, antigen and second antibody with bound enzyme (antibody antigen- antibody2-enzyme). Enzyme linked to second antibody has a crucial role in detection and estimation of antigen present in sample. When it reacts with substrate, color is produced.

Intensity of the color is proportional to amount of antigen present in sample.

Steps of this techniques are given below:

1. Antibodies specific to an antigen of interest are produced. They are fixed to support materials using coupling agent. The support materials are cellulose, plastic, polystyrene or glass. Plastic plates containing wells (depressions) which are coated with antibodies are commonly used.

2. Sample (serum) containing antigen is allowed to combine with antibody by placing sample in the well.

3. Unbound molecules of sample are removed by washing.

4. A second antibody linked to an enzyme is added. This also binds to antigen to form antibody-antigen-antibody2-enzyme complex. Thus, second antibody linked to enzyme is fixed to support material.

5. Unbound antibody2-enzyme complex is removed by washing.

6. In the final step, substrate is added. Enzyme linked to antibody2 convert substrate to colored product which is measured.

Medical Importance

1. Using this technique, antigens or antibodies that are present in very small amounts (picograms) in biological fluids are detected and estimated.
2. Several hormones like insulin, TSH, hCG, Calcitonin etc. are determined world wide using this technique.
3. Antibodies are detected using this technique by fixing antigen to support material.
4. Detection of highly infectious diseases like AIDS, Hepatitis, Malaria etc. World wide involves use of this technique.
5. Some tumor markers in biological fluids are detected and estimated using this technique.

COMMON TESTS IN MEDICAL BIOCHEMISTRY

Albumin, Alkaline Phosphatase, Acid Phosphatase, Total Bilirubin, Amylase, Calcium, Chloride, Cholesterol, Creatinine, C-Reactive protein (CRP), Creatine Phosphokinase (CPK), Gamma Glutamyl transferase (γ-GGT), Glucose, Aspartate aminotransferase (AST)/GOT, Alanine aminotransferase (ALT)/GPT, Inorganic Phosphate, Immunoglobulins (IgA, IgM, IgG), Iron (Total Iron Binding Capacity - TIBC), Lactate Dehydrogenase, Magnesium, Potassium, Total Protein, Triglycerides, Sodium, Uric Acid and Urea