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DEFINITION

- **Enzymes may be defined as biocatalysts synthesized by living cells.**

OR

- **Enzyme, a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.**
- **They are protein in nature (exception - RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.**

HISTORY

- Berzelius in 1836 coined the term catalysis (Greek: to dissolve)I.n 1878, Kuhne used the word enzyme (Creek: in yeast) to indicate the catalysis taking place in the biological systems.
- lsolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner.
- ln 1926, James Sumner first achieved the isolation and crystallization of the enzyme urease from jack bean and identified it as a protein.

NOMENCLATURE

- An enzyme will interact with only one type of substance or group of substances, called the substrate, to catalyze a certain kind of reaction. Because of this specificity, enzymes often have been named by adding the suffix "-**ase**" to the substrate's name (as in urease, which catalyzes the breakdown of urea).
- Not all enzymes have been named in this manner, however, and to ease the confusion surrounding enzyme nomenclature, a classification system has been developed based on the type of reaction the enzyme catalyzes.

PROPERTIES

- Proteins in nature
- Colloidal nature
- Substrate specificity
- Catalytic properties
- Sensitive to Temp, pH, Time and Light

CLASSIFICATION

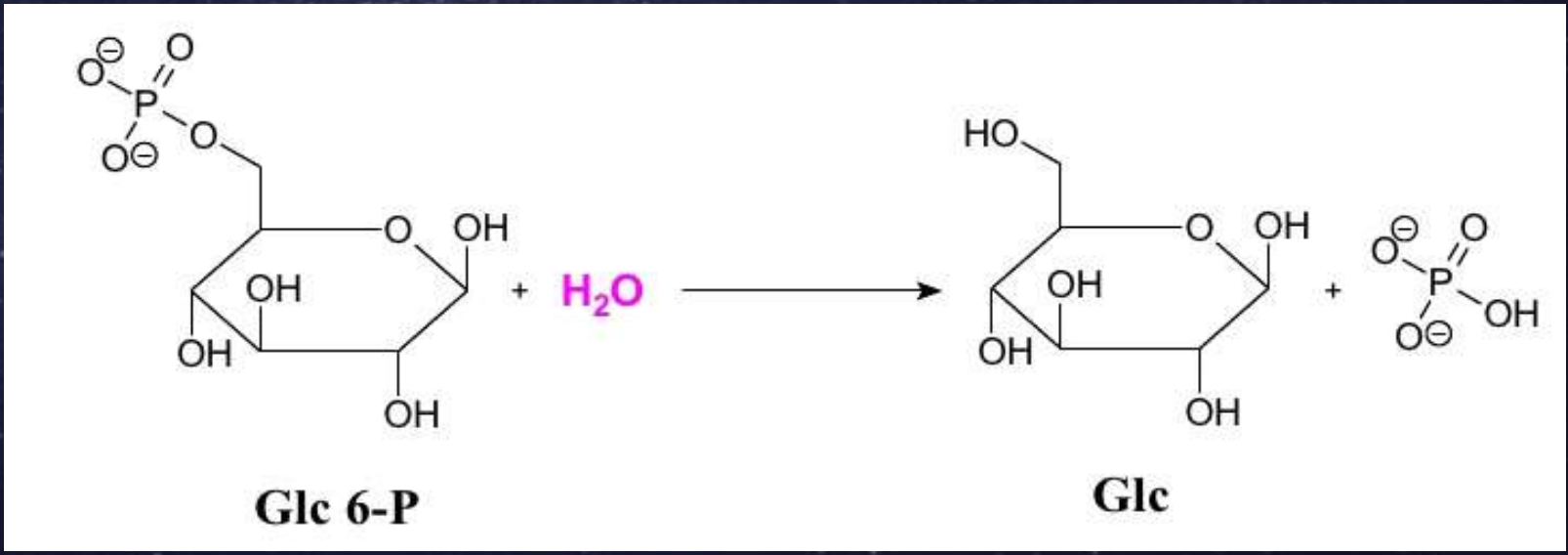
There are six principal categories and their reactions:

1. **OXIDOREDUCTASES:** which are involved in electron transfer or oxidation and reduction reactions. for example;

1. **TRANSFERASES:** which transfer a chemical or functional group from one substance to another. For example;

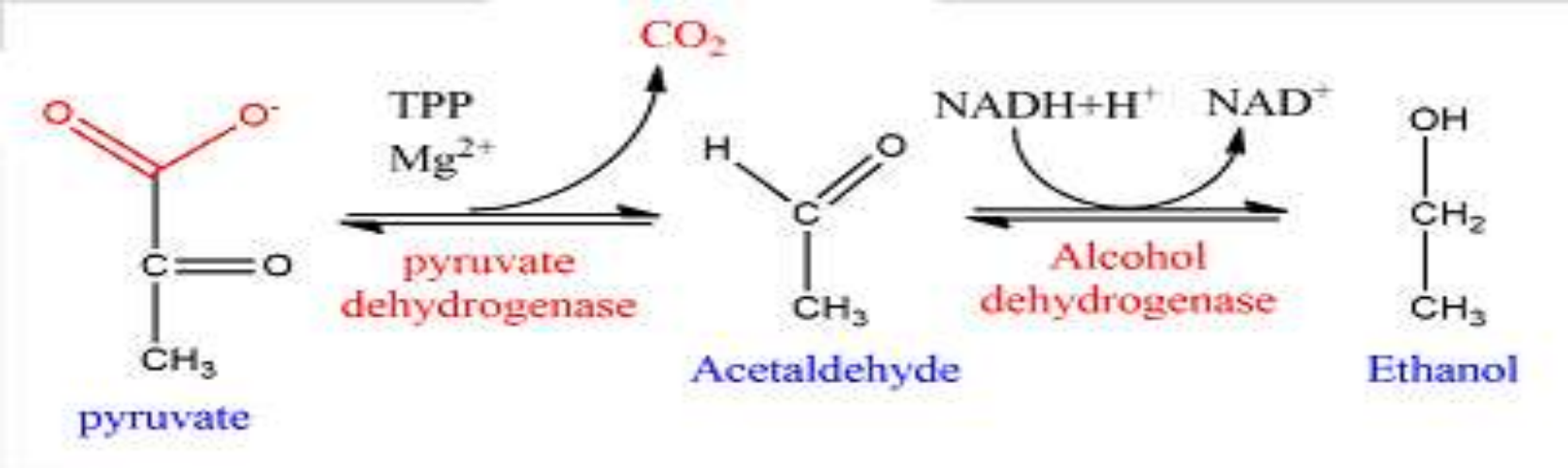
CLASSIFICATION

3. **HYDROLASES:** which cleave the substrate by uptake or addition of a water molecule (hydrolysis). For example glucose 6 phosphatase.



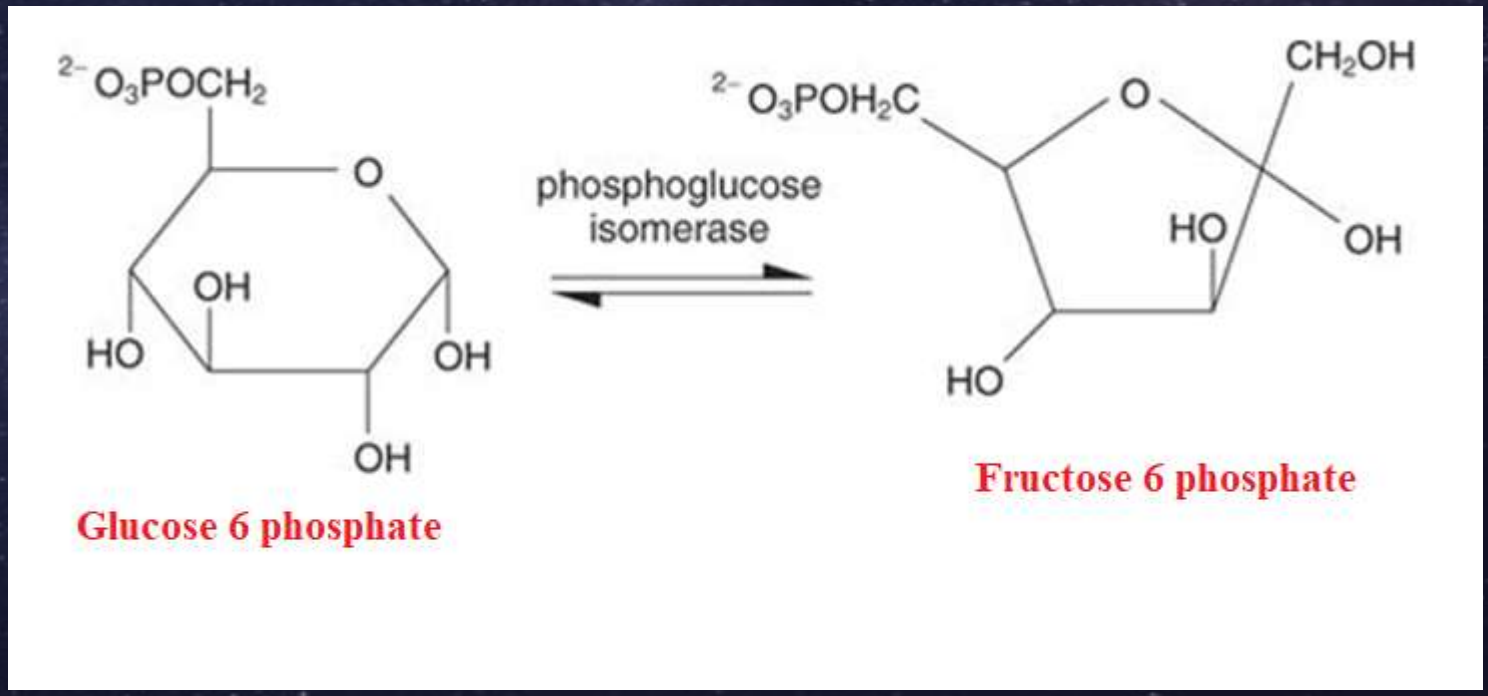
CLASSIFICATION

- **LYASES:** which catalyze cleavage and form bonds by adding or removing a chemical group without involving water molecule such as CO_2 , NH_4^+ etc.



CLASSIFICATION

5) ISOMERASES: which transfer a group within a molecule to form an isomer. For example phosphoglucose isomerase.



CLASSIFICATION

6) **LIGASES:** which couple the formation of various chemical bonds to the breakdown of a pyrophosphate bond in adenosine tri-phosphate or a similar nucleotide.

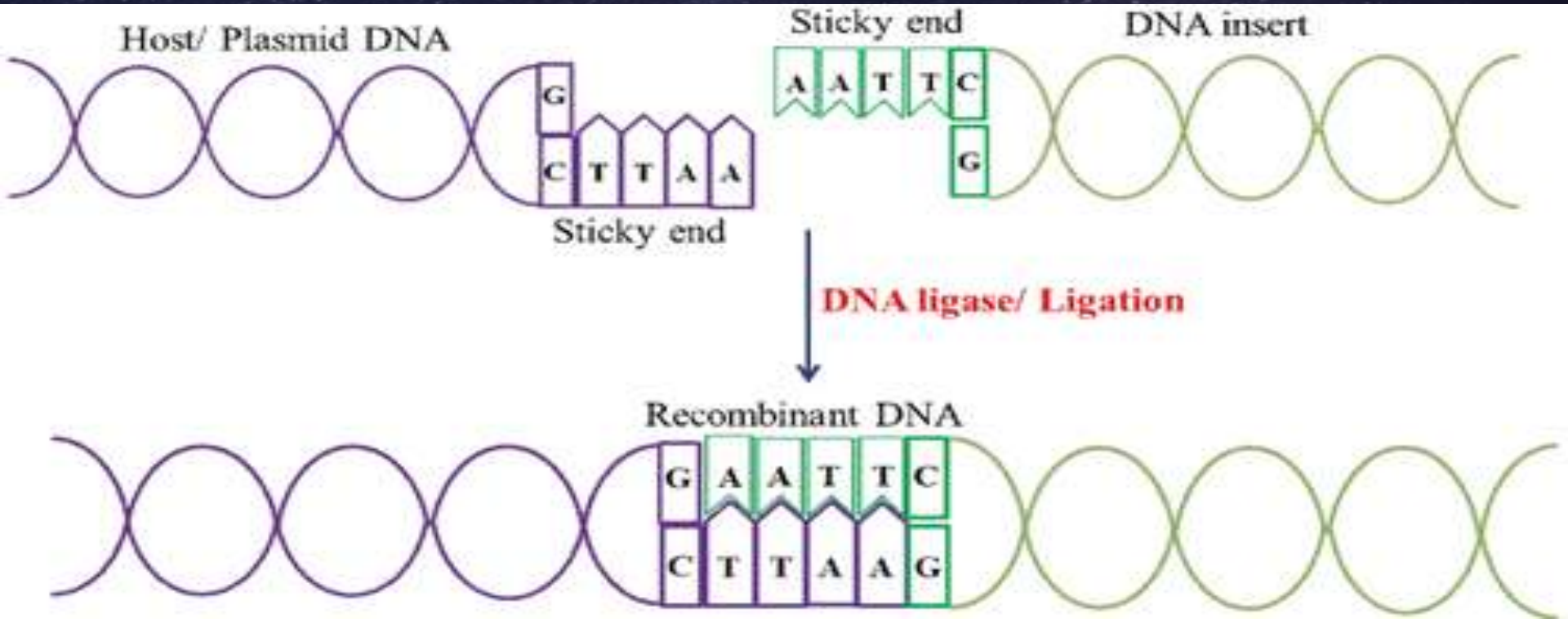


Table 6.1 Classification of enzymes

*Enzyme class with examples**	*Reaction catalysed*
1. **Oxidoreductases**	
Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation $\longrightarrow$ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. **Transferases**	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. **Hydrolases**	
Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. **Lyases**	
Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition $\longrightarrow$ Elimination $A - B + \dot{X} - \acute{Y} \longrightarrow A\dot{X} - B\acute{Y}$
5. **Isomerases**	
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. **Ligases**	
Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP \quad ADP + Pi]{} A - B$

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

CO-ENZYMES OR CO-FACTORS

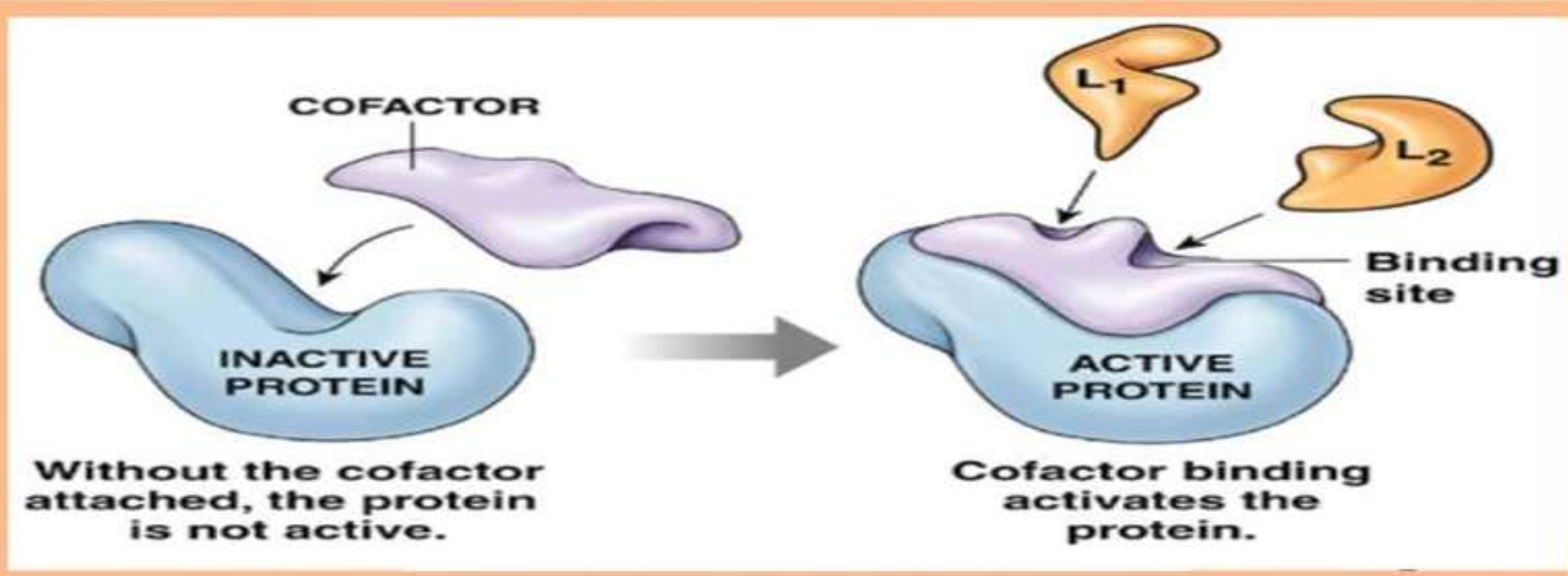
- The non-protein, organic, low molecular weight and dialysable substances associated with enzyme function is known as coenzymes.
- The activator is referred to the non-protein, inorganic molecule is known as cofactor like Ca^{2+} Mg^{2+} etc.
- The functional enzyme is referred to as ***holoenzyme*** which is made up of a protein part (**apoenzyme**) and non protein part (**coenzyme**).

FUNCTIONAL ENZYME OR HOLOENZYME

HOLOENZYME

APOENZYME

CO-ENZYME



CO-ENZYMES

Co-enzymes participate in various reactions involving transfer of atoms or groups like Hydrogen, aldehyde, keto, amino, acyl, methyl etc.

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups*

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals (Vitamins)
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Common Cofactors Used in Catalysis

Table 7.1 REPRESENTATIVE METAL-ION COFACTORS IN ENZYMES

Cofactor	Representative enzymes	Role in catalysis
Fe^{2+}	Cytochrome oxidase	Oxidation–reduction
Mg^{2+}	Hexokinase	Helps bind ATP
Mn^{2+}	Ribonucleotide reductase	Oxidation–reduction
Cu^{2+}	Nitrite reductase	Oxidation–reduction
Zn^{2+}	Alcohol dehydrogenase	Helps bind the substrate
Ni^{2+}	Urease	Required in the catalytic site
K^{+}	Pyruvate kinase	Increases enzyme activity
Se	Glutathione peroxidase	Oxidation–reduction
Mo	Xanthine oxidase	Oxidation–reduction

ENZYME STRUCTURE

- Enzymes are **proteins**
- They have a **globular** Shape
- A complex **3-D** structure

ACTIVE SITE

- The ***active site*** of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.
- Active site has a ***specific shape*** due to tertiary structure of protein.
- Active site can be further divided into:

It chooses the substrate and binds it to active site.

It performs the catalytic action of enzyme.

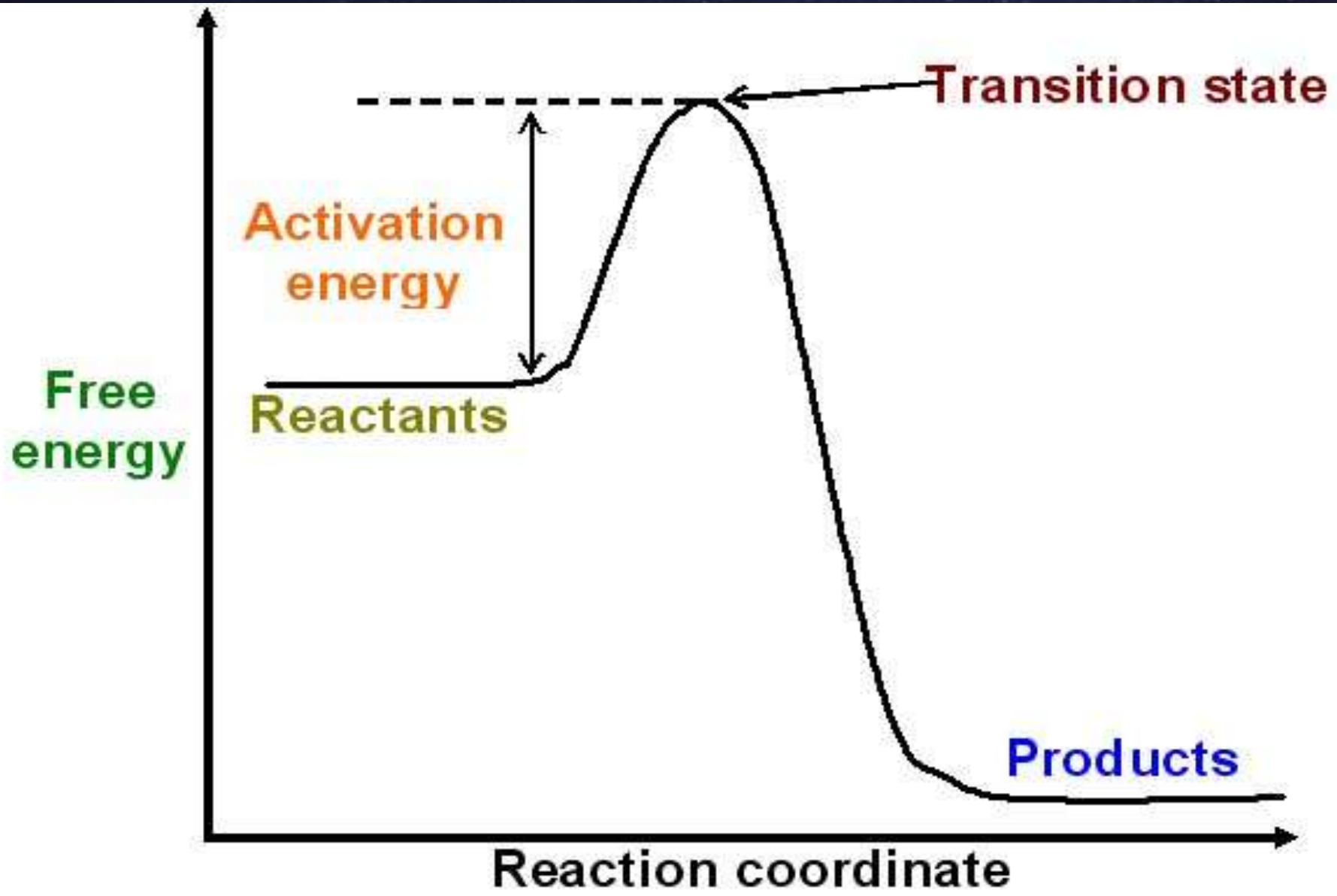
MECHANISM OF ENZYME ACTION

THE ACTIVATION ENERGY FOR REACTION

❖Chemical reactions need an initial input of energy = **THE ACTIVATION ENERGY**

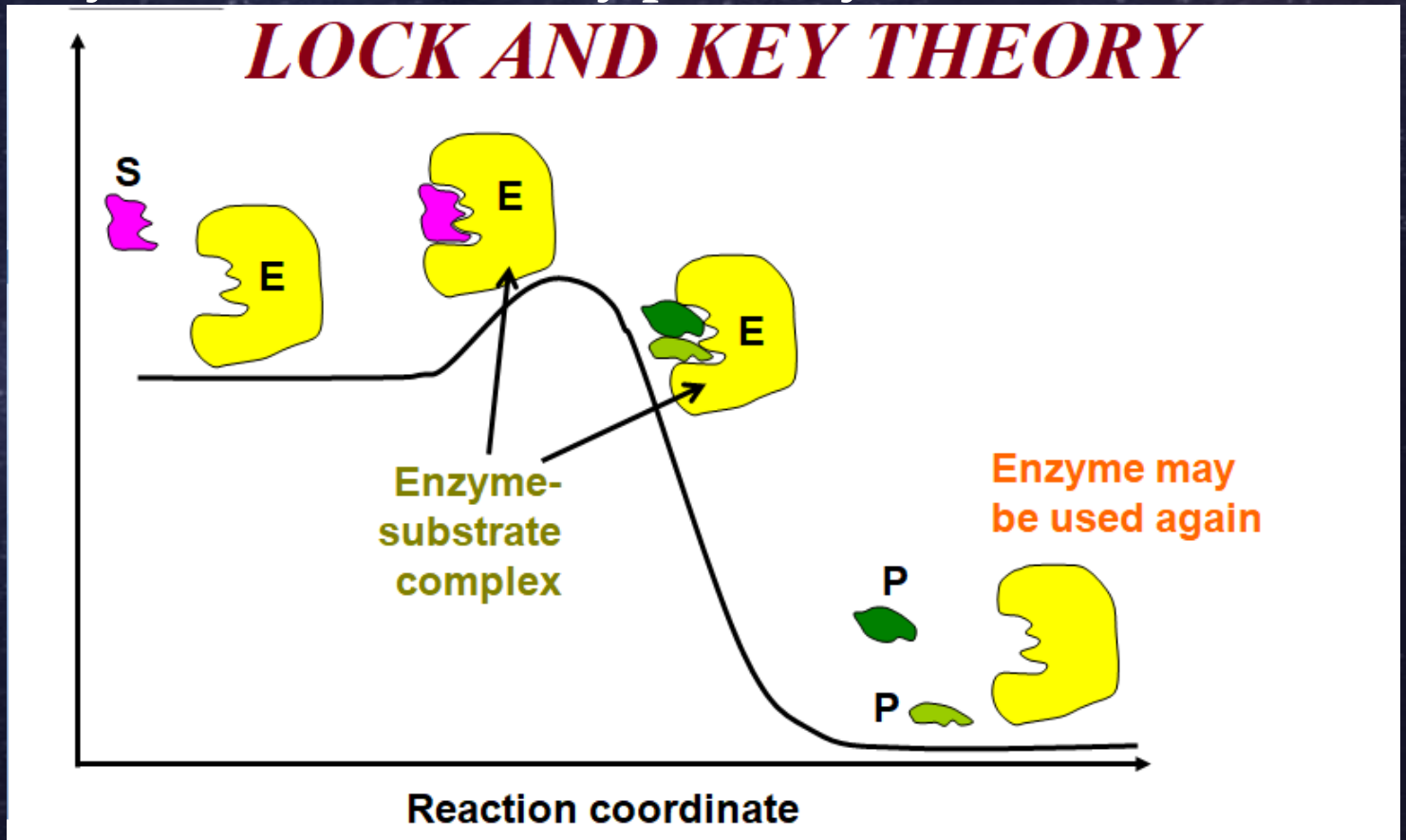
❖During this part of the reaction the molecules are said to be in a **transition state**.

RÉACTION PATHWAY



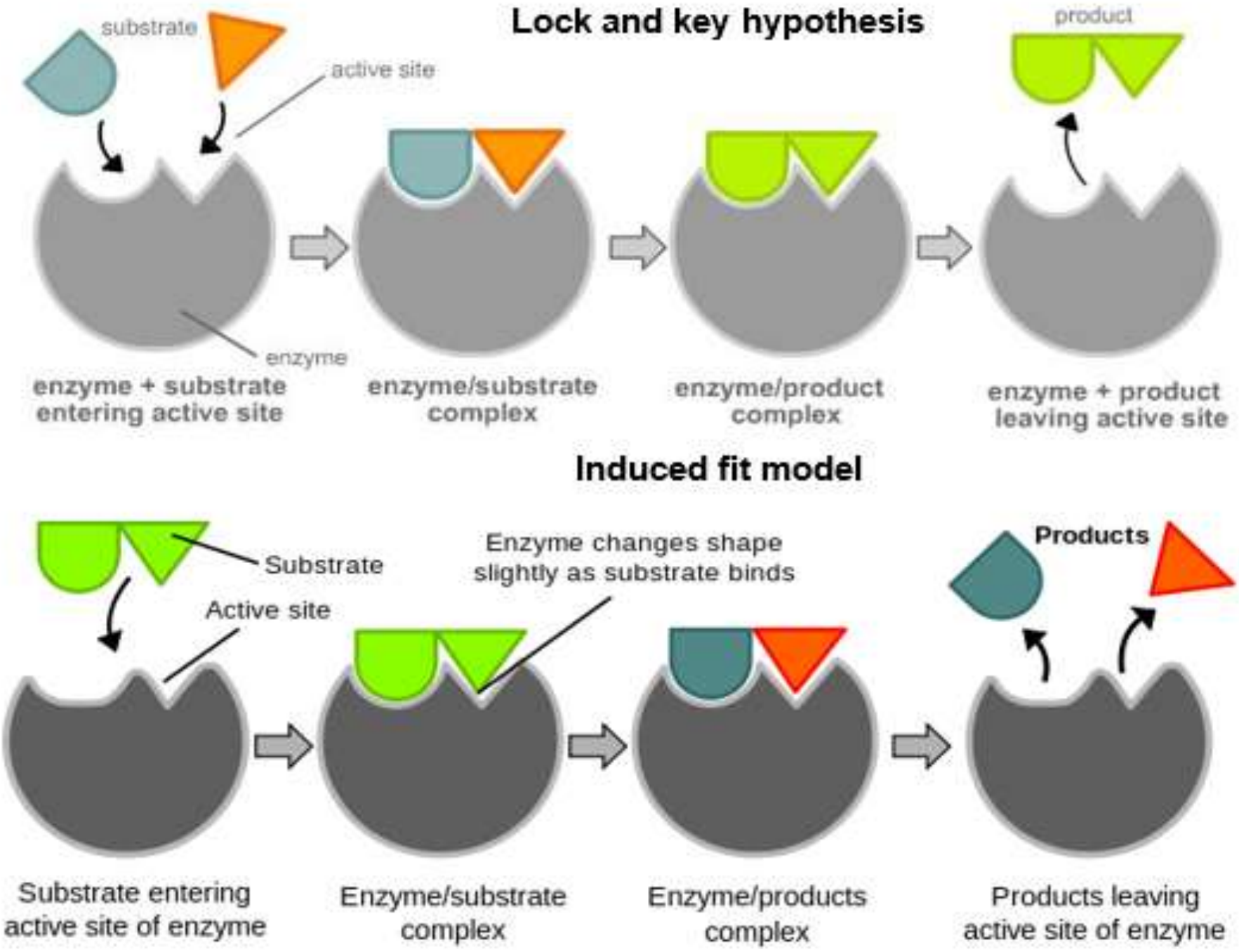
LOCK AND KEY MODEL

- ✓ Proposed by EMIL FISCHER in 1894.
- ✓ Fit between the substrate and the active site of the enzyme is exact
- ✓ Like a key fits into a lock very precisely.



INDUCED FIT MODEL

- More recent studies have revealed that the process is much more likely to involve an induced fit model(proposed by DANIAL KOSH LAND in 1958).
- When a substrate combines with an enzyme, it induces a change in the enzyme's conformation
- The active site is then moulded into a precise conformation
- Making the chemical environment suitable for the reaction
- The bonds of the substrate are stretched to make the reaction easier (lowers activation energy)


Lock and key hypothesis
substrate
active site
enzyme
product
enzyme + substrate entering active site
enzyme/substrate complex
enzyme/product complex
enzyme + product leaving active site
Induced fit model
Substrate
Active site
Enzyme changes shape slightly as substrate binds
Products
Substrate entering active site of enzyme
Enzyme/substrate complex
Enzyme/products complex
Products leaving active site of enzyme

FACTORS AFFECTING

- **The several factors are:**

  (1) Concentration of Enzyme

  (2) Concentration of Substrate

  (3) Effect of Temperature

  (4) Effect of pH

  (5) Effect of Product Concentration and

  (6) Effect of Activators.

  (7)Effect of time

  (8)Effect of light and radiation

- **Factor # 1. Concentration of Enzyme:**
- As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases. In fact, this property of enzyme is made use in determining the activities of serum enzymes for diagnosis of diseases.

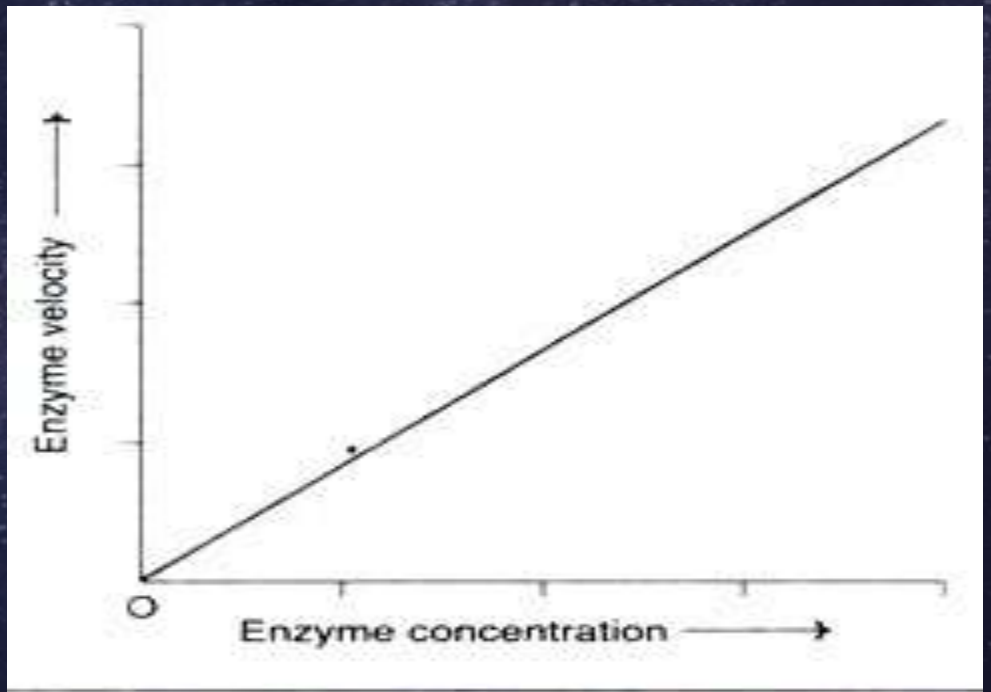


Fig. 66.1 : Effect of enzyme concentration on enzyme velocity.

- **Factor # 2. Concentration of Substrate:**
- Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration. Three distinct phases of the reaction are observed in the graph.

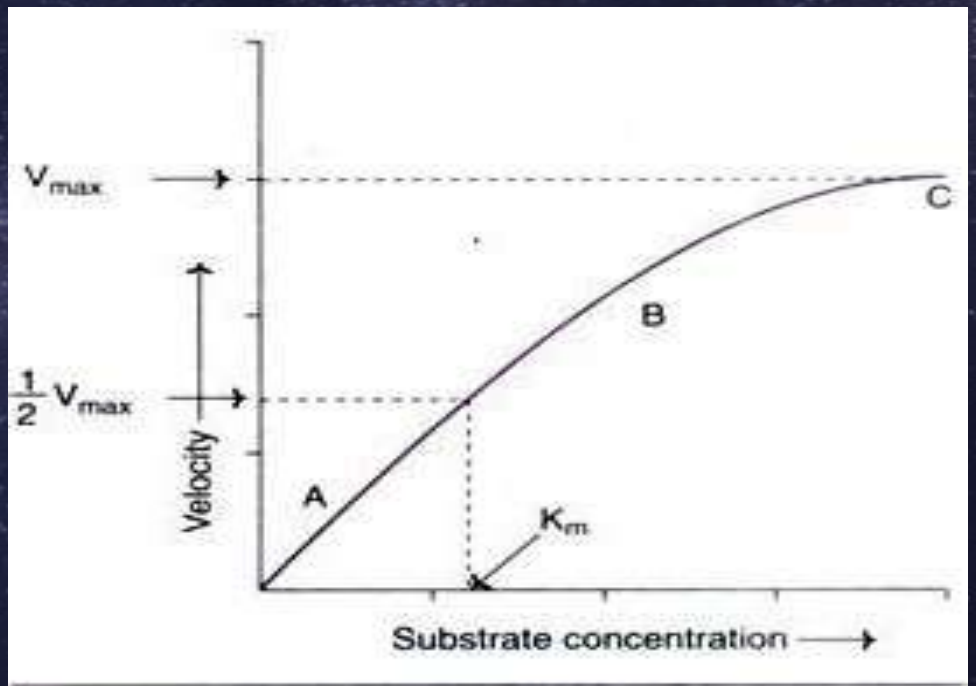


Fig. 66.2 : Effect of substrate concentration on enzyme velocity (A–linear; B–curve; C–almost unchanged).

Enzyme kinetics and K_m value:

- The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

1

MICHAELIS-MENTEN EQUATION & BRIG'S HALDANE'S MODIFICATION

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Where, $k_1 + k_2$ for forward reaction

$k_{-1} + k_{-2}$ for backward Reaction

During initial Period →

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Rate of formation of ES complex = Rate of Breakdown of ES complex.

$$k_1 [E][S] = k_{-1} [ES]$$ "According to Michaelis menten"

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} \quad \left(\text{if } \frac{k_{-1}}{k_1} = k_s\right)$$

$$\frac{[E]\cdot[S]}{[ES]} = k_s$$ ← Ks is dissociation constant of [ES] complex → Rec^n (1)

Total Enzyme $= [E_0]$

$[E_0] = [E] + [ES]$

$[E] = [E_0] - [ES]$

Put the [E] value into equation 1

Then,

$$\frac{([E_0] - [ES])[S]}{[ES]} = k_s \rightarrow \frac{[E_0][S] - [ES][S]}{[ES]} = k_s$$

$$\rightarrow [E_0][S] - [ES][S] = k_s[ES]$$

$$\rightarrow [E_0][S] = k_s[ES] + ES[S]$$

$$\rightarrow [E_0][S] = (k_s + [S])ES$$

$$\rightarrow \frac{[E_0][S]}{(k_s + [S])} = [ES] \quad \text{——} \quad \textbf{equation 2}$$

Velocity of reaction during the breakdown of [ES] complex and formation of product

$$ES \xrightarrow{k_2} E + P$$

$$\left\{ \because \frac{[E_0][S]}{k_2 + [S]} = [ES] \right\}$$

$$V_0 = k_2 [ES]$$

$$V_0 = \frac{k_2 [E_0][S]}{k_s + [S]}$$

Maximum Velocity $V_{max} = k_2 [E_0]$

So, $$\boxed{V_0 = \frac{V_{max}[S]}{k_s + [S]}}$$ [Michaelis equation]

BRIG & HALDANE'S MODIFICATION:

According to Brig and Haldane's, the reaction can be back after the formation of product

$$\text{so, } E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$\rightarrow k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$\rightarrow \frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = k_m$$

$$\rightarrow \frac{[E][S]}{[ES]} = k_m \quad \text{equation 4}$$

(MICHAELIS-MENTEN CONSTANT)

Total enzyme [Eo]

$$[E_o] = [E] + [ES]$$

$$[E] = [E_o] - [ES]$$

so, put the value of [E] into equation 4

$$\Rightarrow \frac{([E_o] - [ES])[S]}{[ES]} = k_m$$

$$\Rightarrow [E_o][S] = k_m[ES] + [S][ES]$$

$$= \frac{[E_o][S]}{(k_m + [S])} = [ES]$$

$\because V_o = k_2[ES]$

so Put the [ES] value into velocity equation then,

$$\Rightarrow V_o = \frac{k_2[E_o][S]}{k_m + [S]}$$

maximum velocity
Vmax=K2 [Eo]

$$V_o = \frac{V_{max}[S]}{k_m + [S]}$$

MICHAELIS-MENTEN & BRIG & HALDANE'S CONSTANT

- The following equation is obtained after suitable algebraic manipulation.
  - where V or Vo = Measured velocity,
  - V_{max} = Maximum velocity,
  - S = Substrate concentration,
  - K_m = Michaelis-Menten constant.

According to graph of substrate concentration.

$$V = \frac{V_{max}}{2}$$

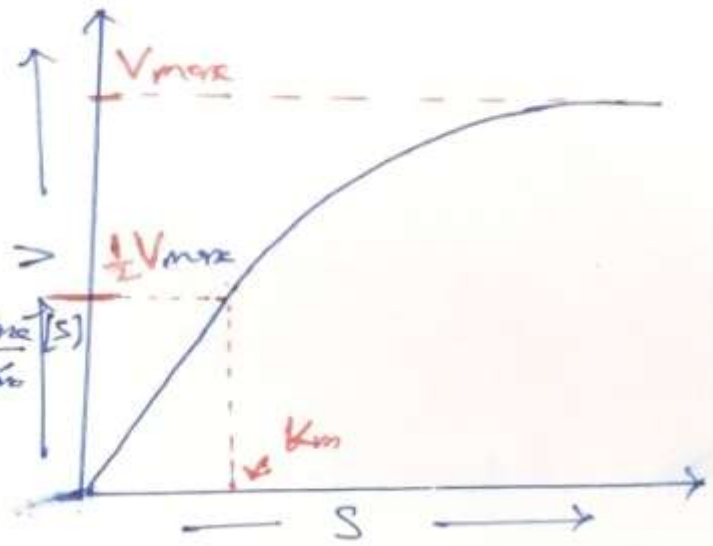
So,

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \rightarrow K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$\rightarrow K_m + [S] = 2[S]$$

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

$$\boxed{K_m = [S]}$$



ENZYME KINETICS AND K_M VALUE

- K_m value is a constant and a characteristic feature of a given enzyme. It is a representative for measuring the strength of ES complex. A low K_m value indicates a strong affinity between enzyme and substrate, whereas a high K_m value reflects a weak affinity between them.
- K_m or the Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/lit) to produce half-maximum velocity in an enzyme catalyzed reaction. It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the K_m value.

FACTOR # 3. EFFECT OF TEMPERATURE:

- Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed.
- The optimum temperature for most of the enzymes is between 40°C-45°C. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100°C.

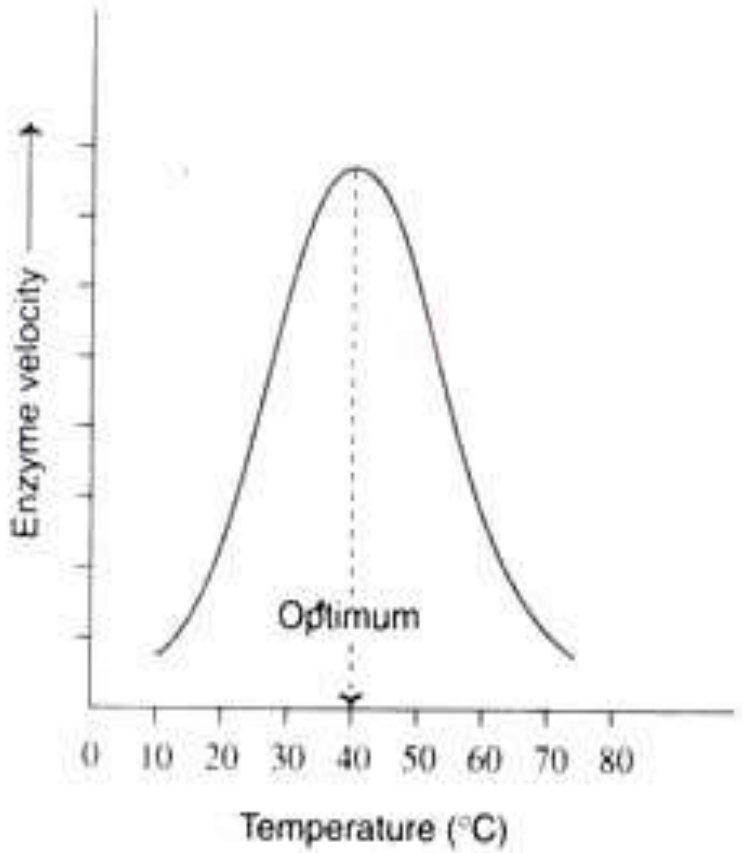


Fig. 66.4 : Effect of temperature on enzyme velocity.

FACTOR # 4. EFFECT OF PH

- Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell-shaped curve is normally obtained. Each enzyme has an optimum pH at which the velocity is maximum.
- Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11) for optimum pH.

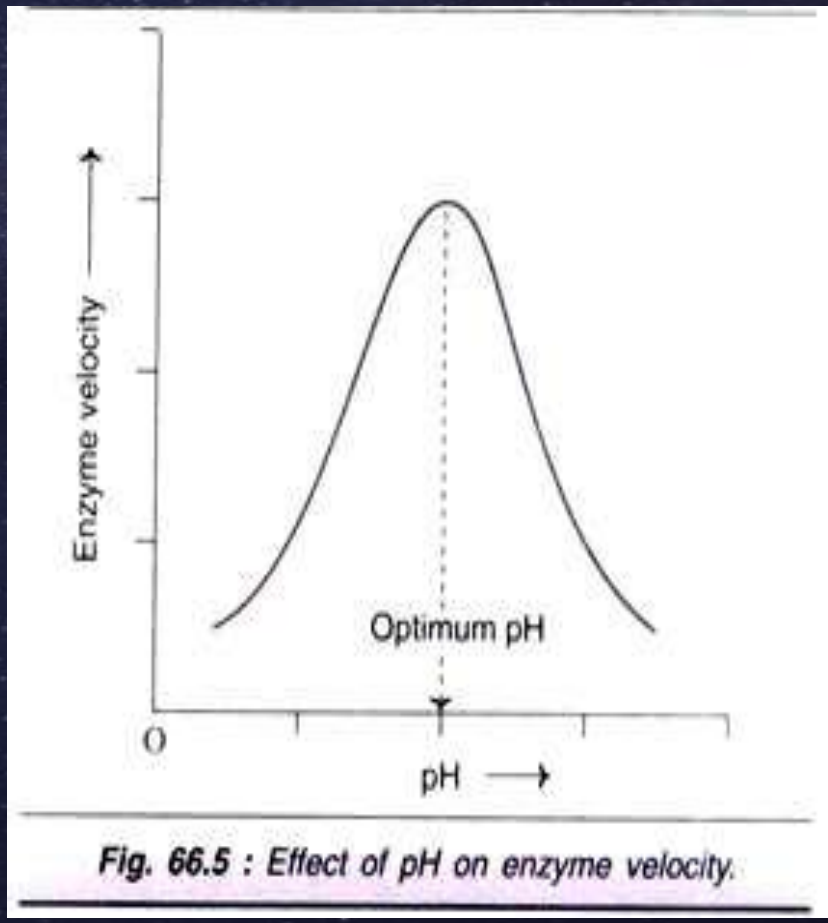


Fig. 66.5 : Effect of pH on enzyme velocity.

FACTOR # 5. EFFECT OF PRODUCT CONCENTRATION

The accumulation of reaction products generally decreases the enzyme velocity. For certain' enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity.

FACTOR # 6. EFFECT OF ACTIVATORS

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

FACTOR # 7. EFFECT OF TIME

Under ideal and optimal conditions (like pH, temperature etc.), the time required for an enzyme reaction is less. Variations in the time of the reaction are generally related to the alterations in pH and temperature

FACTOR # 8. EFFECT OF LIGHT AND RADIATION

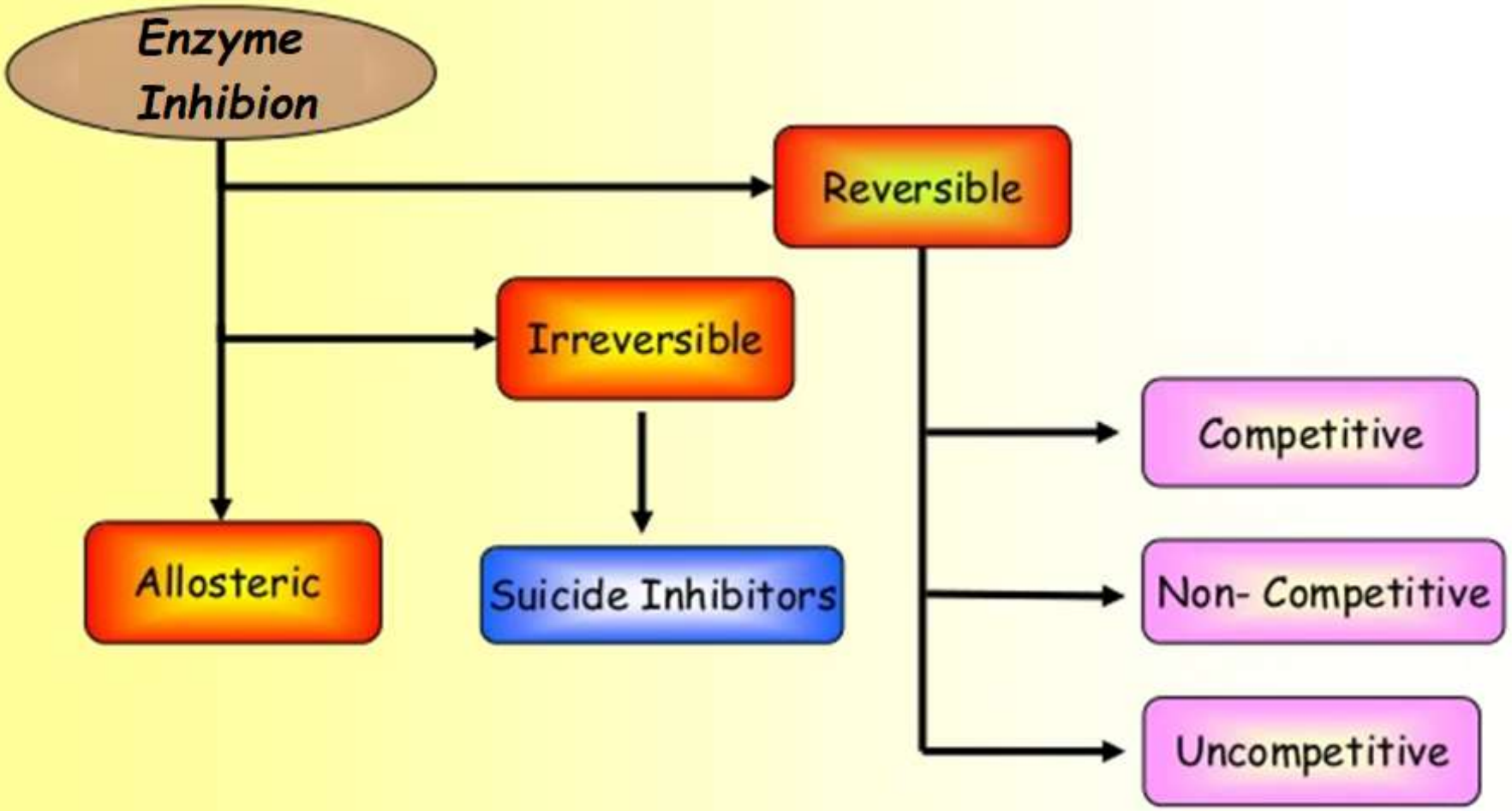
Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. e.g. UV rays inhibit salivary amylase activity.

ENZYME INHIBITION

INHIBITORS:

- Inhibitors are chemicals substances that reduce the enzyme activity and this processes is known as enzyme inhibition.
- The are usually specific and they work at low concentrations.
- They block the enzyme activity but they do not usually destroy it.
- Many drugs and poisons are inhibitors of enzymes in the nervous system.

TYPES OF ENZYME INHIBITION



REVERSIBLE INHIBITION

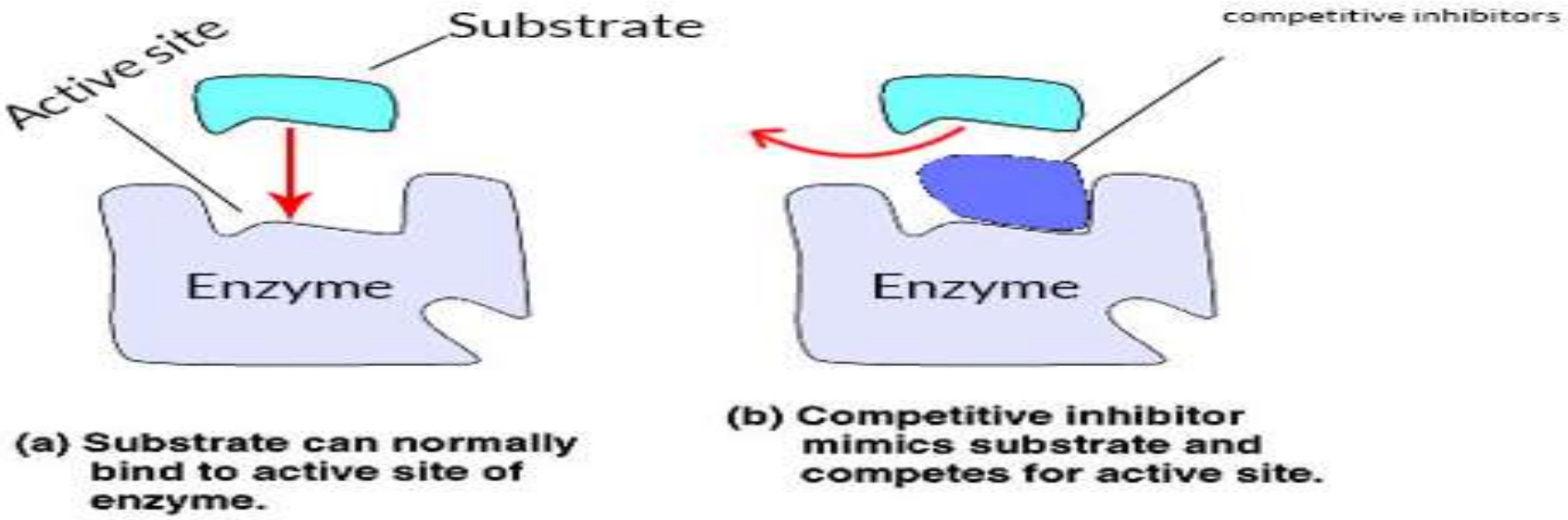
The inhibitor bindes non covalentently with enzyme and enzyme inhibition can be reversed if inhibitor is removed.

TYPES OF REVERSIBLE INHIBITION

There are two types:

- Competitive inhibition.
- Non-competitive inhibition.

REVERSIBLE INHIBITIONCOMPETITIVE INHIBITION



(a) Substrate can normally bind to active site of enzyme.

(b) Competitive inhibitor mimics substrate and competes for active site.

1. **Succinic acid dehydrogenase**
has as substrate **succinic acid** and is inhibited by **Malonic acid**.

2. **Acetylcholin esterase**
has as substrate **acetylcholin** and is inhibited by **Neostigmin**. Note that obviously only the charged $N(CH_3)_3^+$-group is active.

3. **Sulfonamide(antibiotica)**
block as competitive inhibitors the production of DNA, Since they are used by the enzyme instead of the vitamine precursor **p-Aminobenzoesäure**.

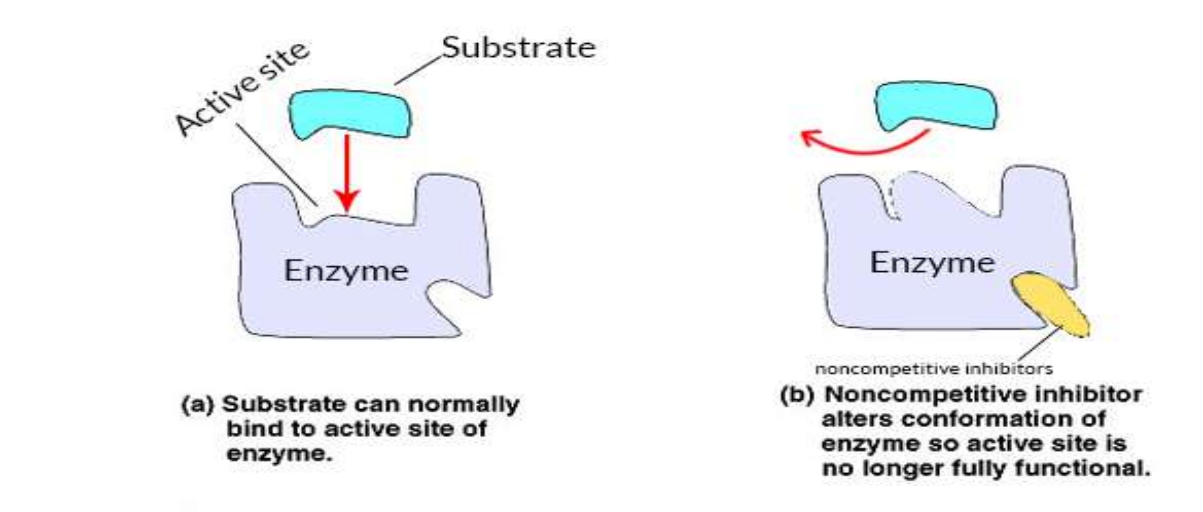
Competitive inhibitors-Therapeutic uses

Inhibitor (drug)	Enzyme inhibited	Disease treated
Allopurinol	**Xanthine Oxidase**	**Gout**
Epidrene (MAO INHIBITOR)	Mono Amino Oxidase	Psychiatric Treatment
Succinyl CoA	Acetyl CoA	Anesthesia
Dicumarol	VITAMIN K EPIOXIDE REDUCTASE	ANTICOAGULANT
Lovastatin	HMG CoA reductase	Reducing Cholesterol levels
INH (Isonicotinic acid hydrazide)	Pyridoxal phosphate	Tuberculosis
Neostigmine	Acetyl Choline Esterase	Myasthenia Gravis
Alpha Methyl Dopa	Dopa Carboxylase	Myasthenia Gravis

Inhibitor (drug)	Enzyme inhibited	Disease treated
Penicillin	Trans peptidase	bactericidal
Sulphonamide (analog PABA)	Steroid synthatase	Bactericidal
Trimethoprim	FH2 reductase	bactericidal
Pyri methamine	FH2 reductase	bactericidal
Methotrexate	FH2 reductase	Leukemia
6 –Mercapto purine	Adenylo Succinate Synthtase	Cancer
5 –Fluoro Uracil	Thymidylate Synthtase	Cancer
Azo Serine	Phospho Ribosyl Amido Transferase	Cancer
Cytosine Arabinoside	DNA Polymerase	Cancer
ACYCLOVIR	DNA Polymerase	Cancer

REVERSIBLE INHIBITION

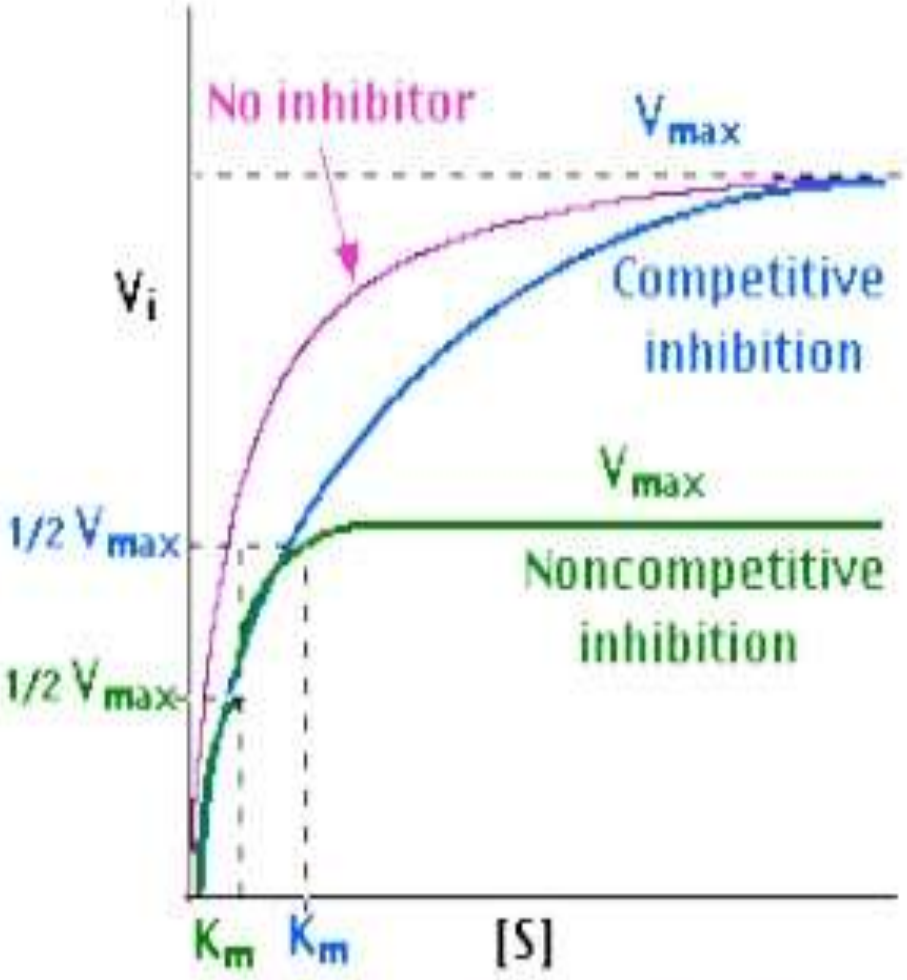
Non-COMPETITIVE INHIBITION

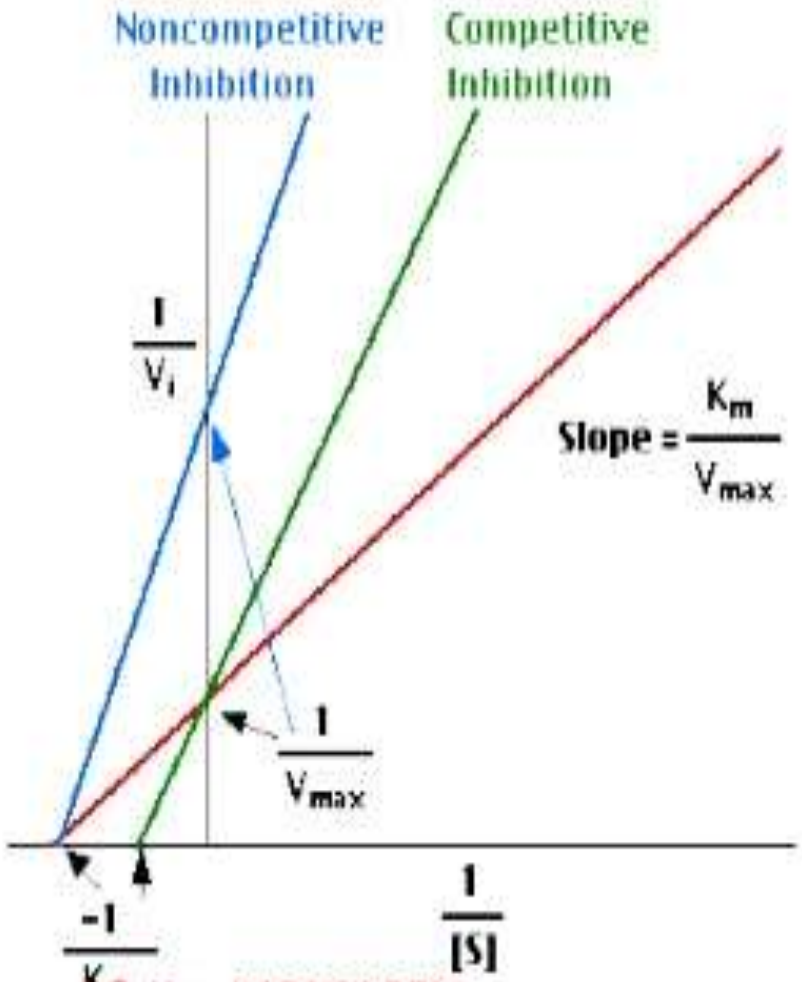


Some examples of non competitive inhibition;

1. Cyanide inhibits cytochrome oxidase.
2. Fluoride will remove magnesium and its ions and so will inhibit the enolase enzyme of glycolysis.
3. BAL (British Anti Lewisite;Dimercaprol) is used as an antidote for heavy metal poisoning.

Comparison between competitive & Non competitive inhibition





Competition inhibition –Vmax unchanged & Km INCREASED

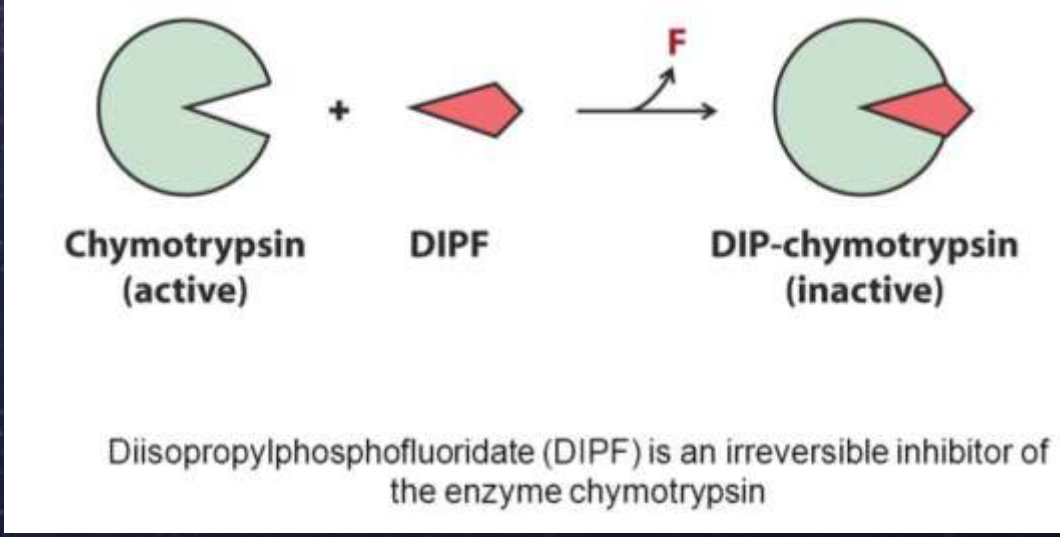
Non competitive inhibition –Vmax decreased & Km not altered

IRREVERSIBLE INHIBITION

- This type of inhibition involves the *covalent attachment* of the inhibitor to the enzyme.
- The *catalytic activity* of enzyme is completely lost.

Examples;

1. ***Aspirin*** which targets and covalently modifies a key enzyme involved in inflammation is an irreversible inhibitor.



Diisopropylphosphofluoridate (DIPF) is an irreversible inhibitor of the enzyme chymotrypsin

2. **Penicillin** antibiotics acts as irreversible inhibitors for serine containing enzymes and block the bacterial cell wall synthesis.

SUICIDE INHIBITION

It is an unusual type of irreversible inhibition where the enzyme converts the inhibitor into a reactive or more potent form in its active site.

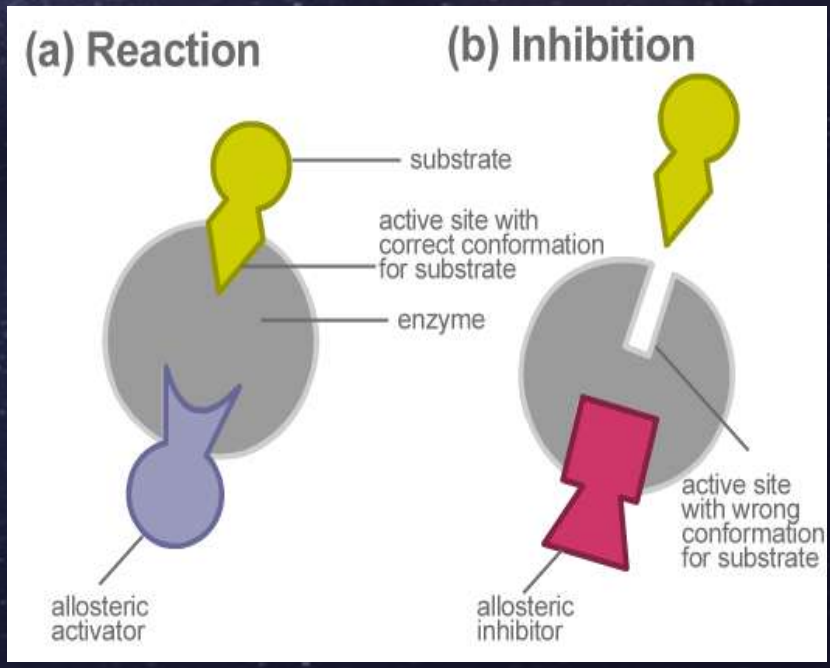
For example;

Allopurinol (used in treatment of GOUT), an inhibitor of xanthine oxidase, gets converted to alloxanthine a more effective inhibitor of this enzyme.

ALLOSTERIC INHIBITION

A non-competitive inhibitor which attaches to the enzyme at allosteric site i.e. any place on enzyme except active site, is called allosteric inhibitor.

For example: ATP act as allosteric inhibitor of enzyme pyruvate kinase during glycolysis. Pyruvate kinase speeds up the last step of glycolysis by transferring the phosphate from phosphoenol pyruvate(PEP) to ADP to form ATP. The formed ATP itself distorts the shape of the active site by binding to the allosteric site of pyruvate kinase. Hence it actually act as allosteric inhibitor in this case.



APPLICATIONS OF INHIBITORS

- **Negative feedback**: end point or end product inhibition
- **Medicine** antibiotics, sulphonamides, sedatives and stimulants

ISOENZYMES

- Isoenzymes or isozymes are multiple forms of same enzyme that catalyze the same chemical reaction
- Different chemical and physical properties:
- Electrophoretic mobility
- Kinetic properties
- Amino acid sequence
- Amino acid composition

ISOENZYMES

1. Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms.

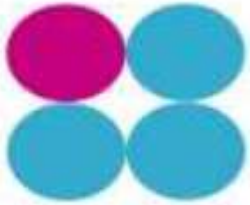
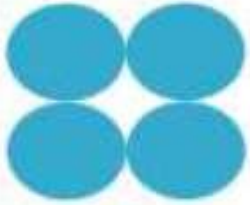
- Lactate dehydrogenase, reversibly converts lactate to pyruvate, in different tissues.
- LDH consists of 5 iso-enzymes – LDH1,LDH2,LDH3,LDH4 & LDH5
- These isoenzymes are separated by cellulose acetate electrophoresis at pH 8.6
- Normal values:

Serum -100 -200 U/L

CSF - 7 -30 U/L

Urine - 40 -100 U/L

LDH isoforms

Isoenzymes of lactate dehydrogenase	Highest levels found in the following:	Isoenzymes of lactate dehydrogenase	Highest levels found in the following:
		 H_2M_2 (LDH_3)	Brain, lung, white blood cells
 H_4 (LDH_1)	Heart, kidneys	 HM_3 (LDH_4)	Lung, skeletal muscle
 H_3M (LDH_2)	Red blood cells, heart, kidney, brain	 M_4 (LDH_5)	Skeletal muscle, liver

LDH isoforms

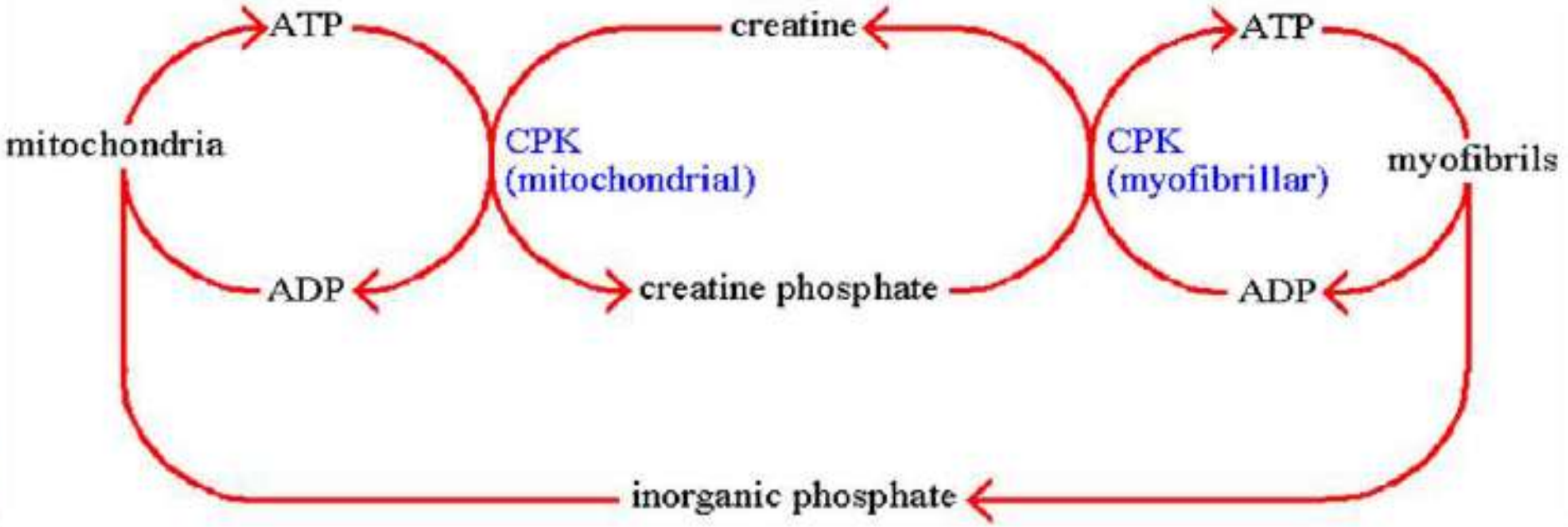
Isoenzyme name	Composition	Electrophoretic migration	Present in	Elevated in
LDH 1 Heat resistant	(H_4)	Fastest moving	Myocardium, RBC, kidney	myocardial infarction
LDH2 Heat resistant	(H_3M_1)		Myocardium, RBC, kidney	Kidney disease, megaloblastic anemia
LDH3	(H_2M_2)		brain	Leukemia, malignancy
LDH4 Heat labile	(H_1M_3)		Lung, spleen	Pulmonary infarction
LDH5 Heat labile Inhibited by urea	(M_4)	Slowest moving	Skeletal muscle, Liver	Skeletal muscle and liver diseases

ISOENZYMES

2. Creatinine phosphokinase (CPK)

- It catalyses creatine to creatine phosphate.
- Normal serum value:
- 15-100 U/L for males & 10-80 U/L for females.
- CPK consists of 3 isoenzymes.
- Each isoenzyme of CK is a dimer;
- Molecular weight of 40 kD.
- The subunits are called B for brain (chromosome -14) & M for muscle (chromosome -19)

Creatine Phosphokinase (CPK)



- It is an important enzyme in energy metabolism.
- Immediate source of ATP in contracting muscle.

Creatine phosphokinase isoenzymes

ISOENZYMES	SUB-UNIT	TISSUE	% IN SERUM
CK1 Fast moving	BB	Brain	1
CK2 2% of total	MB	Heart	5
CK3 Slow moving	MM	Skeletal muscle	80

ISOENZYMES

3. Alkaline Phosphatase (ALP)

- ALP is nonspecific enzyme.
- It hydrolyses aliphatic, aromatic or heterocyclic compounds.
- Optimum pH-9 &10 & it is activated by Mg2+ & Mn.
- Zn is a constituent of ALP.
- It is produced by osteoblasts of bone, and is associated with the calcification process.
- It is localised in cell membranes -ecto-enzyme.
- It is associated with transport mechanisms in liver, kidney & intestinal mucosa.
- Normal range-40-125 U/L.
- In children, Increased levels are seen, due to increased osteoblastic activity.

Isoenzymes of ALP

- Alpha-1 ALP moves in alpha-1 position, it is synthesized by epithelial cells of biliary canaliculi.
- It is about 10% of total activity and is increased in obstructive jaundice.
- Alpha-2 heat labile ALP is stable at 56°C; but loses its activity when kept at 65°C for 30 minutes.
- It is produced by hepatic cells.
- This liver iso-enzyme forms about 25% of total ALP.

- Alpha-2 heat stable ALP will not be destroyed at 65°C, but is inhibited by phenylalanine.
- It is of placental origin, which is found in blood in normal pregnancy.
- An isoenzyme closely resembling the placental form is characteristically seen in circulation in about 15% cases of carcinoma of lung, liver and gut and named as Regan iso-enzyme or carcinoplacental iso-enzyme.
- Normal level is only 1% of the total ALP.

- Pre-beta ALP is of bone origin and elevated levels are seen in bone diseases.
- This is heat labile (destroyed at 56°C, 10 min).
- This constitutes about 50% of normal ALP activity.
- Heat labile bone iso-enzyme of alkaline phosphatase (BAP) is a marker of bone disease.
- Gamma-ALP is inhibited by phenylalanine and originates from intestinal cells.
- It is increased in ulcerative colitis.
- About 10% of plasma ALP are of intestinal origin.
- The leukocyte alkaline phosphatase (LAP) is significantly decreased in chronic myeloid leukemia & it is increased in lymphomas.

DIAGNOSTIC ENZYMES

- Assay of enzymes present in blood plasma or serum have been routinely carried out in clinical chemistry laboratories
- Diagnostic enzymes refers to the enzymes that are used directly or as components of the assay system for the determination of number of substances.
- Changes in the concentrations of various biomolecules are indications of abnormal metabolic activities, infections, infectious and non-infectious diseases and inflammatory conditions.
- As labels in enzyme immuno assay (EIA) system •
- There are many alternative techniques which are routinely used for the diagnosis by clinical laboratories and include Electrophoresis, chromatographic techniques,isoelectric focusing etc.

Serum Enzymes used for diagnostics

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

Thank You!