(BDS) **Enzymes**

Definition

- Enzymes are biological catalysts produced by living tissues. They are proteins (except ribozymes) which accelerate specific chemical reactions without being consumed in the process.

Classification

- Each enzyme is assigned a four digit systematic code called Enzyme commission number (EC number).

Example – E.C. 1.1.1.1 is the enzyme code for alcohol dehydrogenase.

- The first digit of the code denotes the main class of the enzyme (which is based on the general type of chemical reaction the enzyme catalyses).

- The second digit denotes the subclass (which is based on the nature of the chemical group removed or transferred or any bond formed or spliced).

- The third digit denotes sub sub class (which describes sub division of each sub class).

- The fourth or last digit indicates the serial number of the particular enzyme in the sub sub class.

- Enzyme commission classified enzymes into six classes namely:

E.C 1 – Oxidoreductase

- Catalyses oxidation and/or reduction reactions.

- Dehydrogenase, oxidase, reductase and peroxidase are the sub classes.

- Examples are lactate dehydrogenase, glucose-6-phosphate dehydrogenase, cytochrome oxidase etc.

E.C 2 – Transferase

- Catalyses transfer of a particular group from one substrate to another.

- Transaldolase, phosphotransferase and phosphomutase are the subclasses.

- Examples are aspartate transaminase, alanine transaminase, hexokinase etc.

E.C 3 – Hydrolase

- Catalyses the hydrolysis of a substrate with the addition of a molecule of water.

- Esterase, glycosidase, peptidase and phosphatase are the sub classes.

- Examples are lipase, amylase, trypsin, lactase, sucrase, pepsin etc.

E.C 4 – Lyase

- Catalyses the removal of a small molecule from substrate without addition of water.

- Decarboxylase, aldolase and dehydratase are the subclasses.

- Examples are fumarase, pyruvate decarboxylase, histidase etc.

E.C 5 – Isomerase

- Catalyses the isomerisation of the substrate.

- Racemase, epimerase and isomerase are the subclasses.

- Examples are phosphohexose isomerase, phosphoglucomutase, alanine racemase etc.

E.C 6 – Ligase

- Catalyses synthesis of a molecule from two substrates.

- Synthetase and carboxylase are the subclasses.

- Examples are glutamine synthetase, pyruvate carboxylase, DNA ligase etc.

Factors influencing enzyme activity

- The factors which influence enzyme activity are:

1. Temperature 2. pH 3. Enzyme concentration 4. Substrate concentration 5. Product concentration 6. Oxidation of enzymes 7. Enzyme inhibitors

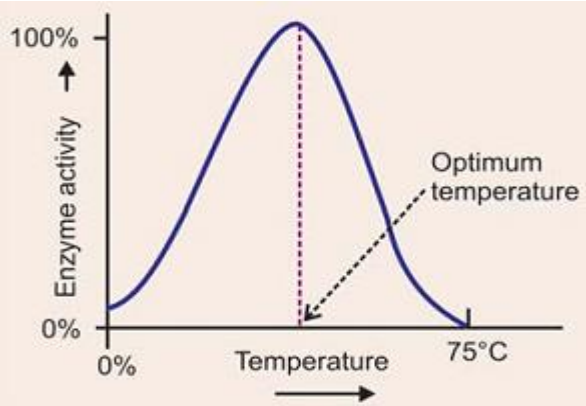
(competitive and non-competitive) 8. Enzyme inactivators 9. Antienzymes 10. Light 11. Rays.

1. Temperature

- Increase in temperature increases the rate of enzyme activity.

- Maximum activity is seen at optimum temperature (37°C).

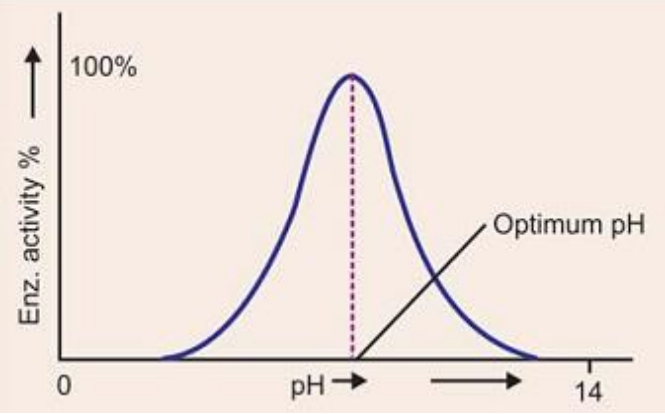
- Beyond optimum temperature, enzyme becomes denatured and loses activity.



2. pH (Hydrogen ion concentration)

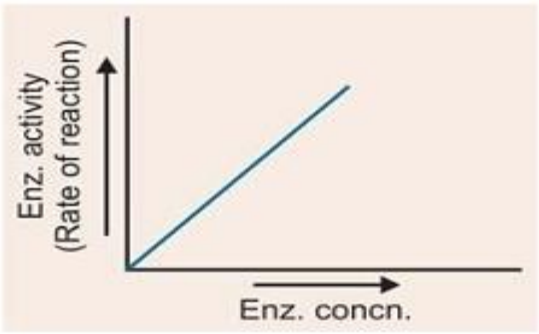
- Enzymes show maximum activity at their own optimum pH.

- Pepsin shows maximum activity at pH 1.5 and alkaline phosphatase shows maximum activity at pH 8.4.



3. Enzyme concentration

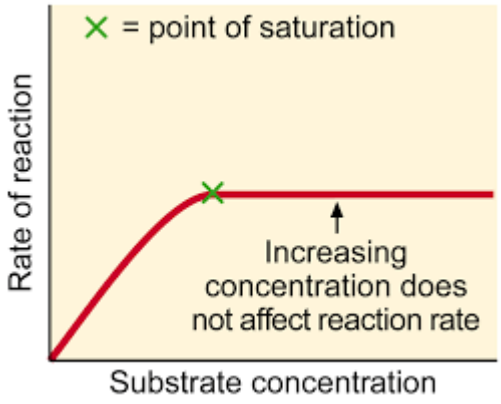
- Reaction velocity is directly proportional to the enzyme concentration.



4. Substrate concentration

- At a fixed enzyme concentration, reaction velocity increases with rise in substrate concentration until a maximum velocity is reached.

- Most enzymes show *hyperbolic curve* when the reaction velocity is plotted against substrate concentration.



5. Product concentration

- Accumulation of products in excess amount causes a decrease in reaction velocity.

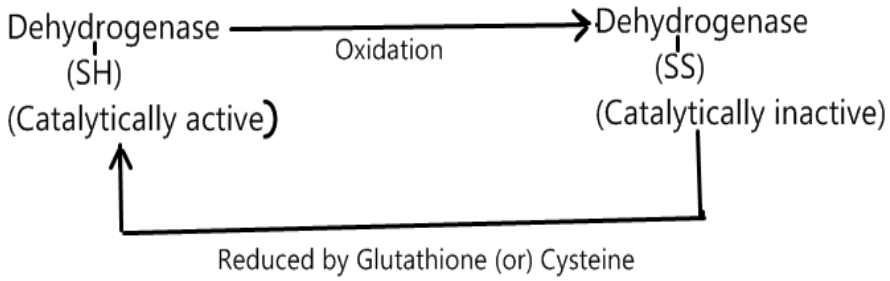
6. Light

- Yellow light denatures the enzymes.

7. Rays

- Rays denature the proteins.

8. Oxidation of enzymes



9. Enzyme inactivators

- Heavy metals like arsenic, mercury, lead etc inactivate the enzymes.

10. Antienzymes

- They are produced by intestinal worms (Example – Ascaris).

- They act against the proteolytic enzymes of the gut.

- Deficiency or absence of proteolytic enzymes causes problems of indigestion and malabsorption.

- Protein malnutrition and malabsorption lead to growth retardation.

11. Enzyme inhibitors

- They are chemical substances which check or destroy enzyme activity.

- They are of two types namely:

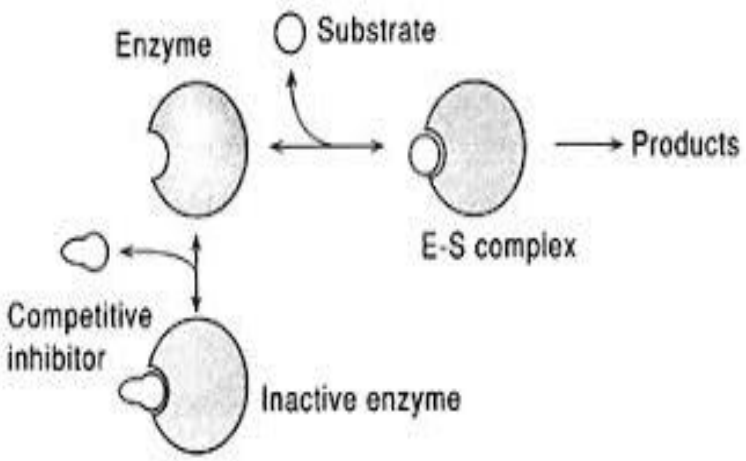
a) Competitive enzyme inhibitors

- They are structurally (three dimensionally) similar to normal substrate.

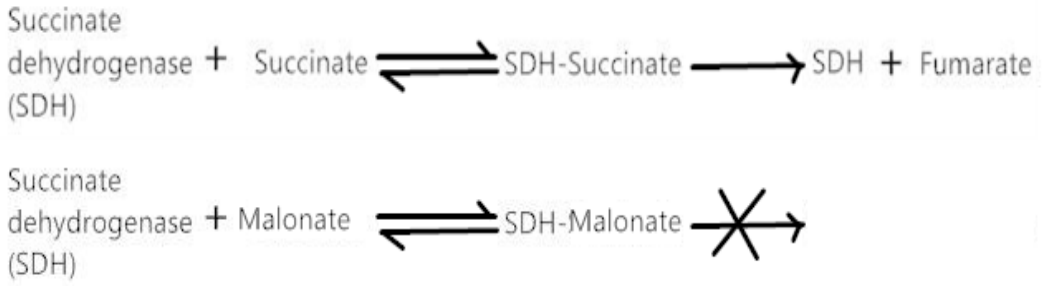
- Should be more in concentration than normal substrate.

- Their inhibition is reversible.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$
$$+$$
$$I$$
$$\Updownarrow$$
$$EI$$



- Examples of competitive enzyme inhibitors are malonate, oxalate, allopurinol, sulfonamide, methotrexate etc.



- Some chemicals, which act as competitive inhibitors, are used as drugs to treat diseases.

Enzyme		Competitive Inhibitor	Use
1.	Pteroate synthase	Sulfonamide	Antibacterial agent
2.	Dihydrofolate reductase	Methotrexate	Antibacterial agent
3.	Xanthine oxidase	Allopurinol	Treating gout disease
4.	Vit K epoxide reductase	Dicoumarol	Anticoagulant

b) Non – competitive enzyme inhibitors

- It causes complete destruction of the enzyme activity.

- Its concentration can be normal or low or more compared to the substrate.

- It may or may not be structurally similar to the substrate.

- It is irreversible.

- Poisons like iodoacetate, heavy metals like lead, mercury etc are non-competitive enzyme inhibitors.

- Examples of non-competitive enzyme inhibition are :

1. Cyanide inhibition of cytochrome C oxidase enzyme.

2. Fluoride inhibition of enolase enzyme in glycolysis.

3. Iodoacetate inhibits enzymes having sulfhydryl (-SH) group.

4. Lead inhibits heme synthesizing enzymes like ALA dehydratase and causes anemia.

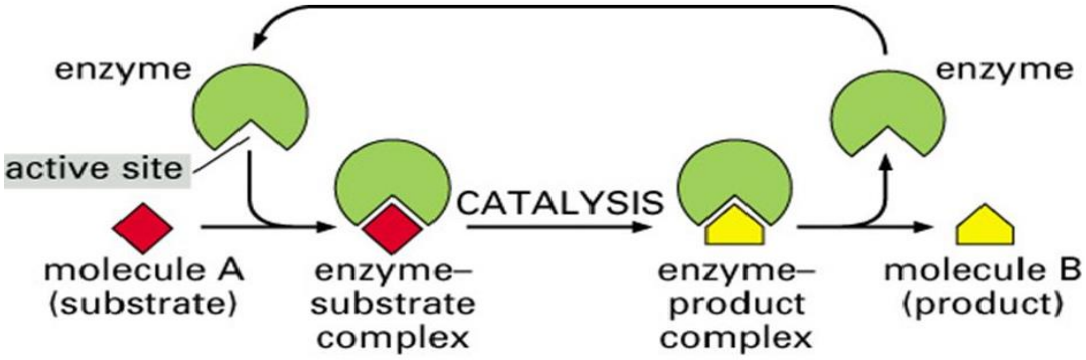
5. Mercury inhibits cholinesterase, lipase, amylase etc.

Mechanism of enzyme action

Catalytic site –

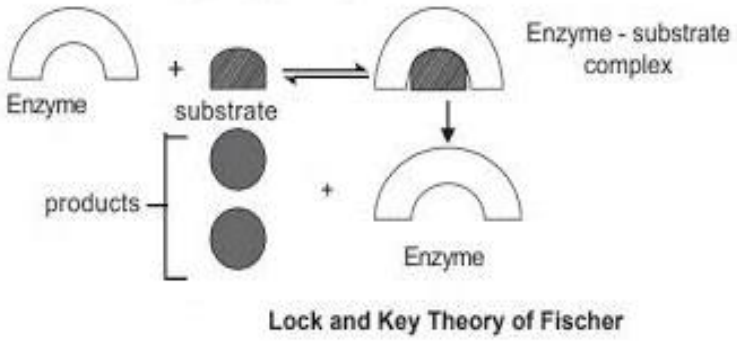
- Each enzyme (E) possesses a special site known as catalytic or active site where the substrate binds to form enzyme-substrate (ES) complex. This ES complex undergoes catalytic process to form enzyme-product (EP) complex. This complex then dissociates into enzyme (E) and product (P).

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



Enzyme substrate complex formation

Fisher's Lock and Key Model



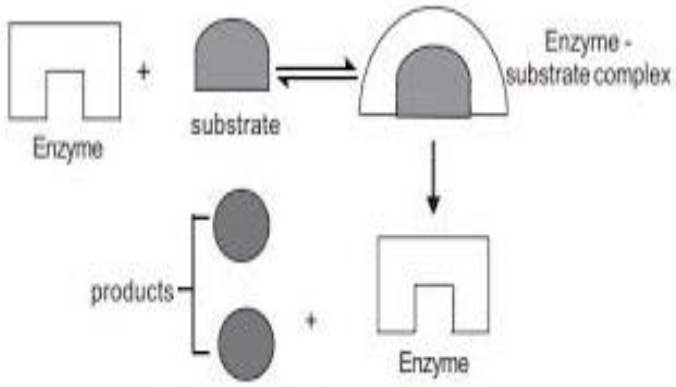
Lock and Key Theory of Fischer

- A substrate binds to pre-shaped active site of a specific enzyme as like a key fitting into pre-shaped key hole of a specific lock.

- This model was rejected as active site of allosteric enzyme was found to undergo conformational change during allosteric modulation.

Koshland's Induced Fit Model

- Before a substrate binds to an enzyme, it induces conformational change in three dimensional structure of the active site. This results in formation of binding site with structure complementary to that of the substrate.



Induced Fit Model of Koshland

Michaelis and Menten equation

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

Where

V = Velocity of the reaction

V_{max} = Maximum velocity of the reaction

[S] = Substrate concentration

K_m = Michaelis constant

- Michaelis constant (K_m) is the substrate concentration at half the maximum velocity of the reaction ($K_m = ½\ V_{max}$). It denotes that 50% of enzyme molecules are bound with substrate molecules at that particular concentration.

- K_m denotes the affinity of the enzyme for substrate. The lesser the numerical value of K_m, the affinity of the enzyme for the substrate is more.

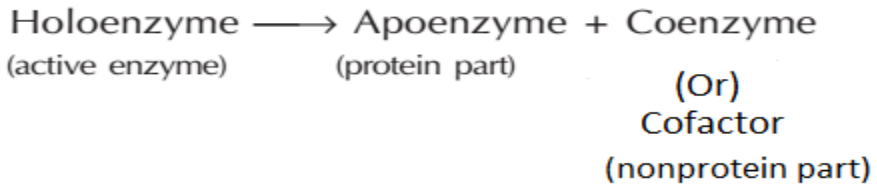
Co-enzyme

- Co-enzyme is an organic substance required as prosthetic group by an enzyme for its catalytic activity. If the prosthetic group is an inorganic substance like metal ion, then it is called as co-factor.

- The co-enzyme or co-factor may be an integral part of the enzyme molecule or its presence may be required during the reaction.

- The protein portion of the enzyme that requires co-enzyme or co-factor is called apo-enzyme.

- The apo-enzyme combines with the co-enzyme or co-factor to form holoenzyme which is involved in catalysis.



Examples of co-enzymes –

- Derivatives of vitamin B complex like TPP, FAD, NAD, co-enzyme A etc.

Examples of co-factors –

- Metal ions like Mg^{2+} Zn^{2+} etc.

Co-enzymes

TPP → Pyruvate dehydrogenase complex.

FAD → Succinate dehydrogenase complex.

Co-factors (Metalloenzymes)

Cu^{2+} → Cytochrome C oxidase and Ceruloplasmin.

Fe^{2+} → Cytochrome C oxidase and Glutathione peroxidase.

Se → Glutathione peroxidase.

Ni^{2+} → Urease.

Co-factors (Metal activated enzymes)

Cl^{-} → Salivary amylase.

Mg^{2+} → Enolase.

K^{+} → Pyruvate kinase.

Isoenzymes (Isozymes)

- Isoenzymes are physically distinct forms of the same enzyme and catalyses the same reaction. They differ from each other structurally, electrophoretically and immunologically.

Examples –

1) Lactate dehydrogenase (LDH) (60 to 250 IU/L)

- Made up of 4 polypeptide chains of 2 types (H type and M type).

LDH1 – HHHH (or) H4 (Heart)

LDH2 – HHHM (or) H3M

LDH3 – HHMM (or) H2M2

LDH4 – HMMM (or) HM3

LDH5 – MMMM (or) M4 (Muscle)

- LDH1 and LDH2 are increased in myocardial infarction, megaloblastic anemia and renal disorders.

- LDH2 and LDH3 are increased in leukemia and various cancers.

- LDH4 and LDH5 are increased in liver disorders and muscle damage.

2) Creatine kinase (CK) (20 to 80 IU/L)

- Made up of two polypeptide chains of two types (B type and M type).

CK1 – BB (Brain)

CK2 – MB (Heart)

CK3 – MM (Muscle)

- CK2, present in small amount in serum, is raised in myocardial infarction.

- CK3 activity increases in muscular dystrophies and muscular injuries.

- CK1 activity increases in brain tumours and cerebrovascular diseases.

3) Alkaline phosphatase (ALP) (40 to 50 IU/L)

- Exists in six forms.

- Isoenzymes differ in amount of sialic acid groups (prosthetic group).

- The bone isozyme is raised in plasma in bone diseases.

- The liver isozyme is raised in liver diseases like obstructive jaundice.

- The other examples of isozymes are acid phosphatase (prostate, erythrocyte, platelet, liver, spleen, kidney and bone marrow), amylase (salivary and pancreatic) and hexokinase (liver and muscle).

- Isoenzymes can be differentiated by

1. Agar/ PAGE electrophoresis

2. Heat stability

3. Sensitivity to inhibitor

4. Tissue localization

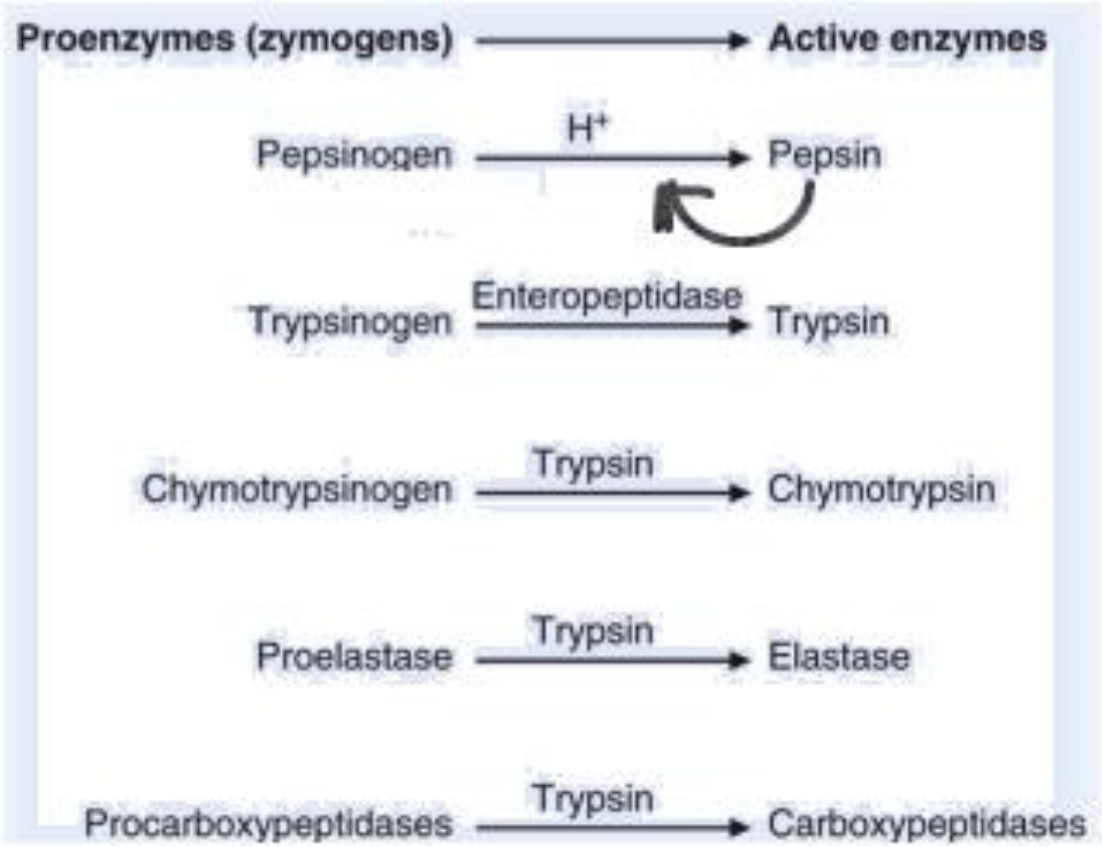
5. Immunologically by preparing antibodies for an enzyme.

Proenzyme (zymogen)

- Some enzymes are synthesized and secreted in their catalytically inactive form. Such catalytically inactive forms of enzymes are termed as zymogens or proenzymes.

Examples – Pepsinogen, trypsinogen and chymotrypsinogen are the proenzyme forms of gastrointestinal enzymes like pepsin, trypsin and chymotrypsin respectively.

- The proenzyme forms are converted to their active forms before they act on substrates.



Allosteric Regulation

- An allosteric enzyme is an oligomeric enzyme possessing both catalytic and regulatory sites (also called allosteric sites).

- The activity of an allosteric enzyme is regulated by non-covalent binding of certain substances (allosteric modulators) to its allosteric site.

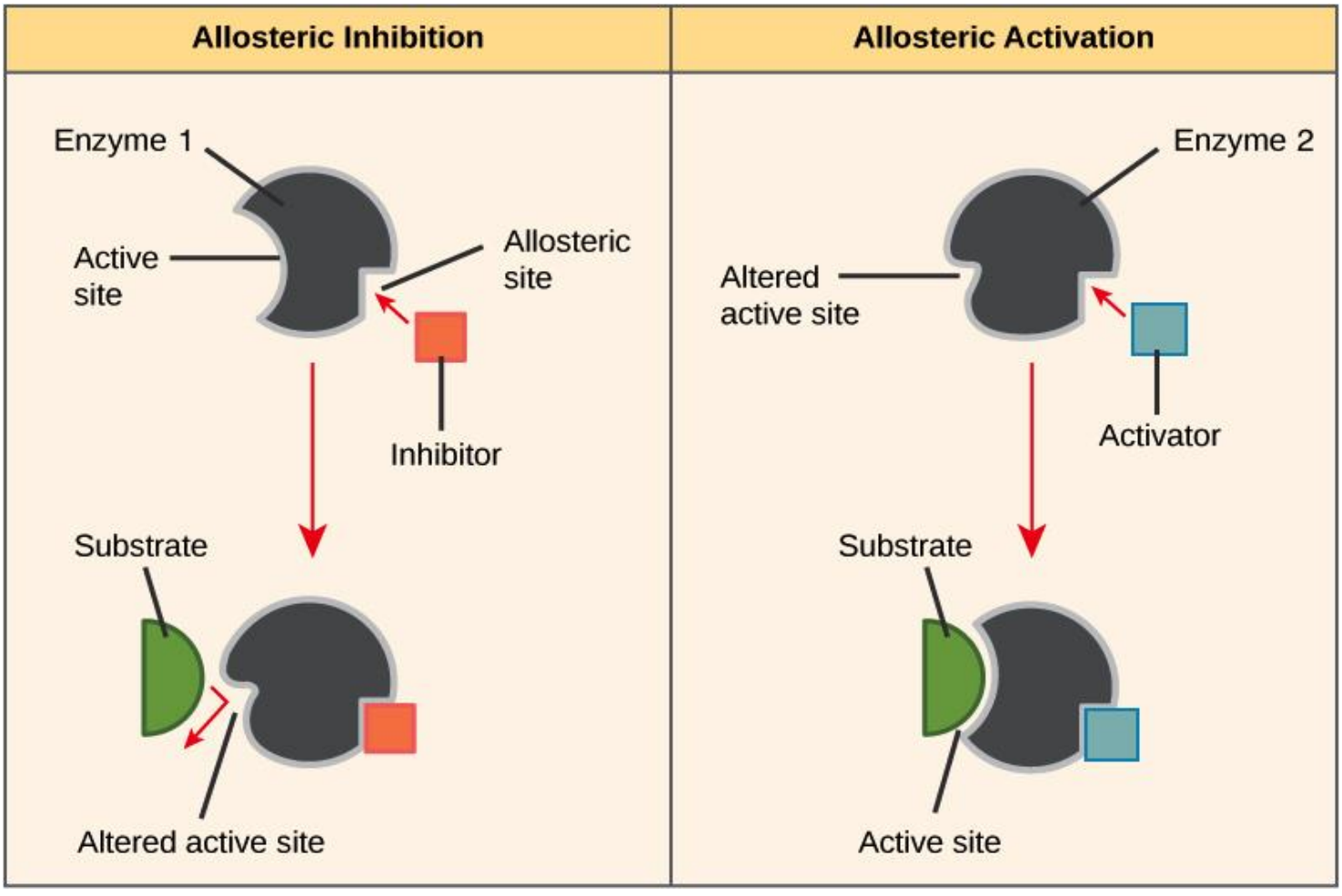
- The non-covalent binding of allosteric modulator to the allosteric site induces conformational change in catalytic site of the enzyme.

- The binding of positive modulator to the allosteric site stimulates the enzyme activity. Binding of negative modulators inhibits the enzyme activity.

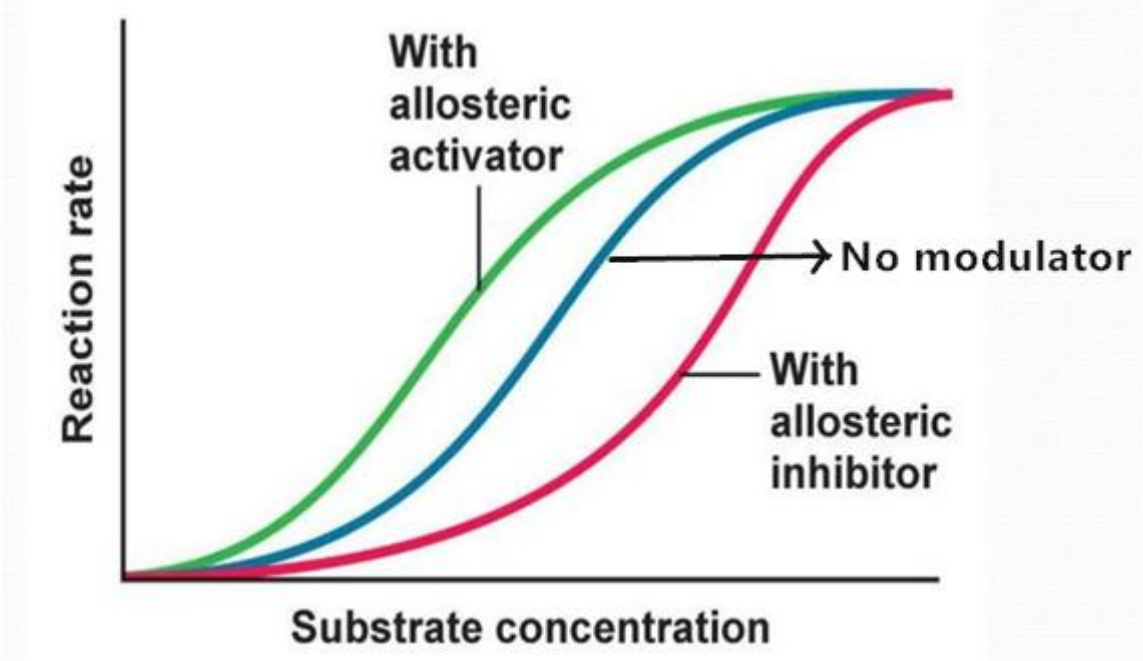
- An enzyme can have more than one allosteric site.

- If substrate itself serves as allosteric modulator, then the modulator is called homotropic allosteric modulation. If the allosteric modulation is by substances other than substrate, then it is called heterotropic allosteric modulation.

S.No	Allosteric Enzyme	Positive Modulator	Negative Modulator
1.	Hexokinase	ADP	Glu-6-PO_4, ATP
2.	Pyruvate carboxylase	Acetyl CoA	ADP
3.	Phosphofructokinase	AMP, ATP	ATP
4.	Isocitrate dehydrogenase	ADP, NAD	ATP



Effect of activator/ inhibitor on allosteric enzyme



Covalent modification of enzymes

- Activities of many enzymes (pyruvate kinase, glycogen phosphorylase, pyruvate dehydrogenase etc) are covalently modified through phosphorylation and dephosphorylation. The phosphate group may be attached to serine, threonine or tyrosine residues.

- Phosphorylation and dephosphorylation of enzymes are catalysed by cAMP dependent protein kinases and phosphoprotein phosphatases respectively.

- Several hormones (epinephrine, glucagon, growth hormone, thyroxine etc) regulate the activities of these protein kinases and phosphatases.

- Depending on enzymes, the phosphorylated and dephosphorylated forms of enzymes may be active or inactive.

No.	Enzyme	Phosphorylated Form	Dephosphorylated Form
1.	Pyruvate dehydrogenase	Inactive	Active
2.	Pyruvate kinase	Inactive	Active
3.	Glycogen phosphorylase	Active	Inactive
4.	Glycogen synthase	Inactive	Active
5.	Triglyceride lipase	Active	Inactive

Regulation of enzyme activity by induction or repression of enzyme synthesis

- Cells can regulate the amount of enzyme by altering the rate of enzyme synthesis.

- The regulation of enzyme activity by increasing (induction) or decreasing (repression) the synthesis of that enzyme is brought about by hormones through induction and repression of gene transcription.

- Enzymes that are needed at only one stage of the development or under selected physiological conditions are regulated in this way.

- For example, insulin stimulates and glucagon inhibits the rate of glycolysis by enhancing and suppressing the synthesis of regulatory enzymes (glucokinase, phosphofructokinase – 1 and pyruvate kinase) respectively.

- The synthesis of regulatory enzymes of glycolysis is regulated by insulin and glucagon through induction and repression of genes (transcription process) encoding the enzymes.

Clinical Enzymology

(i) Normal values

1) Amylase – 30 to 100 IU/L (or) 80 to 180 Somogyi units/dL

2) GOT (AST) – 10 to 40 IU/L

3) GPT (ALT) – 5 to 40 IU/L

4) Creatine kinase (CK) – 20 to 80 IU/L

5) Acid phosphatase (ACP) – 2.5 to 12 IU/L

6) Alkaline phosphatase (ALP) – 40 to 120 IU/L

7) Lactate dehydrogenase (LDH) – 50 to 250 IU/L

8) Lipase – 0.5 to 1.5 IU/L

9) 5' Nucleotidase (NTP) – 2 to 15 IU/L

10) Gama glutamyl transferase (γ GGT) – 10 to 30 IU/L

(ii) Marker enzymes

1) Myocardial infarction (MI)

- Creatine kinase (CK_2 – MB)
- Aspartate transaminase (AST)
- Lactate dehydrogenase (LDH_1 – HHHH)

2) Muscle diseases

- Creatine kinase (CK_3 – MM)
- Aspartate transaminase (AST)
- Aldolase (ALD)

3) Hepatic diseases

- Alanine transaminase (ALT) (In parenchymal liver disease)
- Alkaline phosphatase (ALP) (In obstructive jaundice)

- Gama glutamyl transferase (GGT) (In obstructive jaundice and alcoholic liver disease)

- 5' Nucleotidase (or nucleotide phosphatase) (NTP) (In liver dysfunction and cholestasis).

4. Bone disorders

- Alkaline phosphatase (In rickets and Paget's disease)

5. Prostate cancer

- Acid phosphatase

6. Acute pancreatitis

- Lipase

- Amylase

- Activity of glucose -6- PO_4 dehydrogenase is reduced in hemolytic anemia.

(iii) Therapeutic enzymes

1) Asparaginase for acute leukemia.

2) Streptokinase to lyse intravascular clot.

3) Urokinase to lyse intravascular clot.

4) Pancreatin for acute pancreatitis.

5) Papain as anti-inflammatory agent.

6) Hyaluronidase as local anesthetic.

(iv) Enzymes as reagents

1) Urease – Urea estimation.

2) Uricase – Uric acid estimation.

3) Glucose oxidase – Glucose estimation.

4) Peroxidase – Glucose and cholesterol estimation.

5) Hexokinase – Glucose estimation.

6) Cholesterol oxidase – Cholesterol estimation.

7) Lipase – Triglycerides estimation.

8) Horse radish peroxidase – ELISA

9) Alkaline phosphatase – ELISA

10) Restriction endonuclease – Southern blot.

11) Reverse transcriptase – Polymerase chain reaction (PCR).
