Clinical Enzymology

University Of Fallujah
College Of Medicine

Lecture : Clinical Enzymology
Stage : second

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Department: Chemistry and Biochemistry

Date:

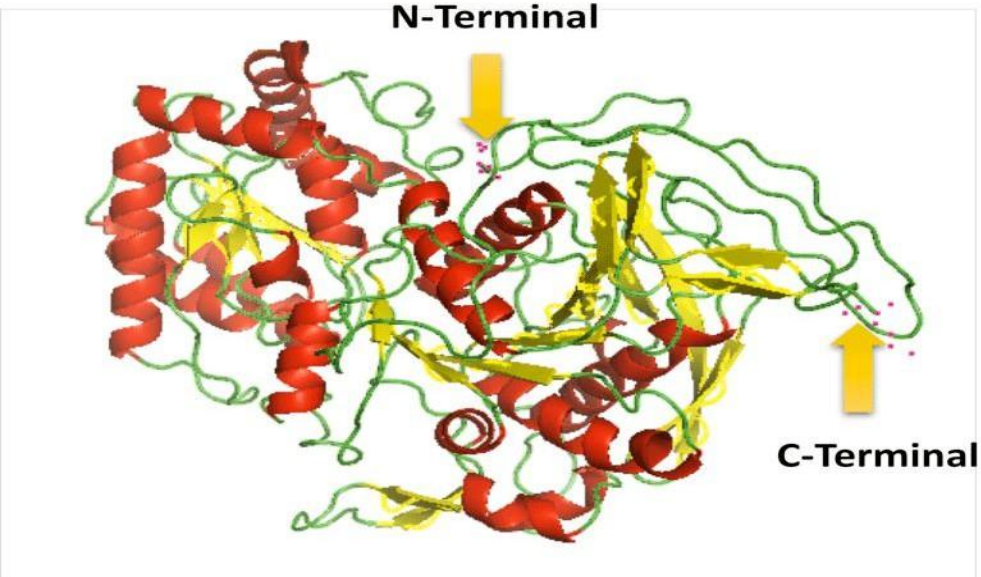
A learning objective

- **At the end of this Lecture you should be able to:**

1. Explain the basic concept and biological role of enzymes as catalysts.
2. Describe enzyme structure, including apoenzyme, holoenzyme, cofactors, and coenzymes.
3. Illustrate enzyme kinetics including **Km and Vmax**.
4. Explain factors affecting enzyme activity (temperature, pH, substrate concentration).
5. Differentiate between types of enzyme inhibition (competitive and non-competitive).
6. Discuss the clinical applications of enzymes in diagnosis and treatment of diseases.
7. Interpret the role of isoenzymes in identifying tissue damage.

Clinical Enzymology

- Is the application of the science of enzymes to the diagnosis and treatment of diseases.
- Nature of enzymes: are protein catalysts the chemical reactions that occur in the body
- Biological catalysts.



❑ Application of clinical enzymology

- Determination of the level of enzymes in biological fluids to provide information for diagnosis, Monitoring Treatment of diseases
- Provide evidence of a disease as enzymes can act as diagnostic markers of underlying diseases.
- Locate the anatomical site of a disease/disorder by: detecting & localizing tissue cell damage or proliferation
- Differential diagnosis and prognosis of diseases

❑ Effect of Enzyme on Chemical Reaction

- Enzymes are the most significant and highly specialized proteins,
- They have a high degree of specificity for their substrates, they accelerate chemical reactions greatly, and they function in aqueous solutions under very mild conditions of temperature and pH.

❑Enzyme Nomenclature

- Enzymes are named by the type of reaction that they catalyse. Usually this means adding the suffix –ase to the name of their
- substrate or reaction that they catalyse e.g. Lactase hydrolyses lactose into glucose and galactose

Six Major Classes of Enzymes

TABLE 6-3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Properties of enzymes

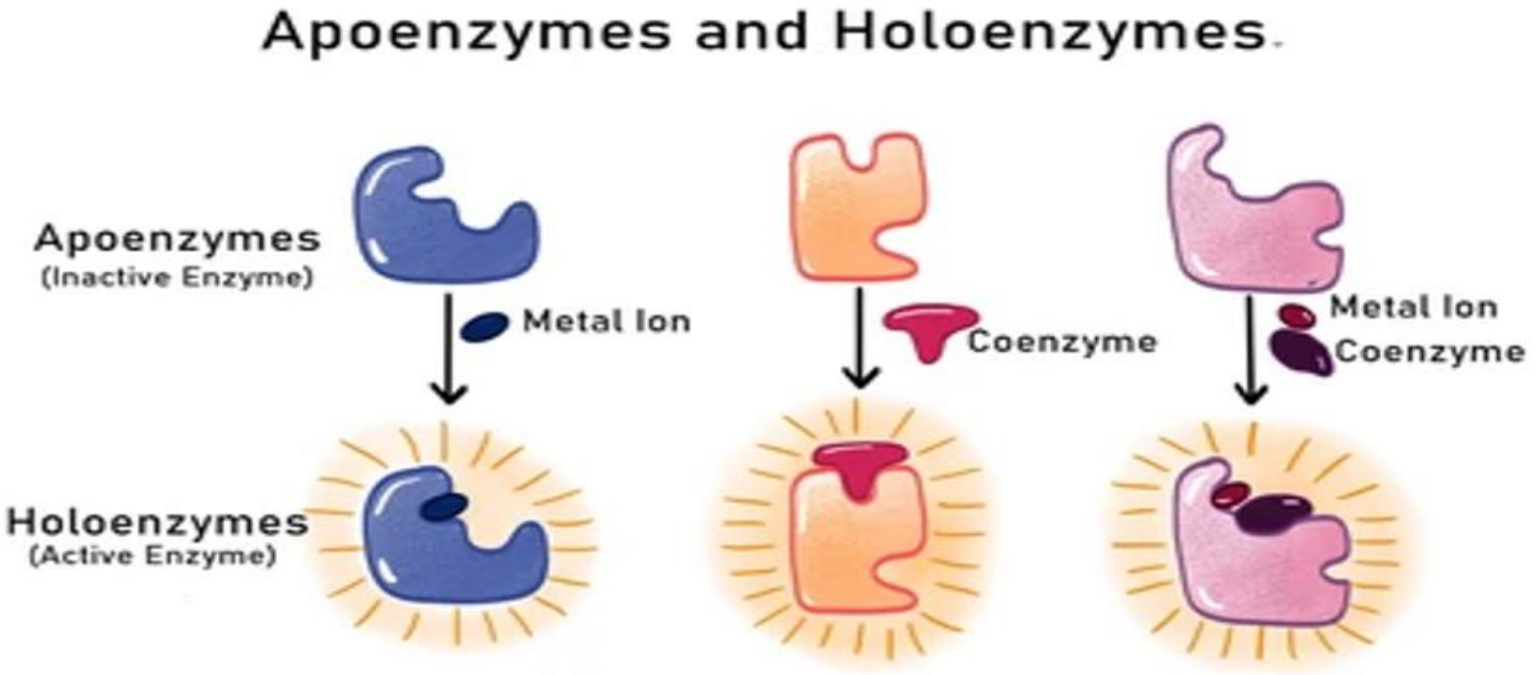
- 1. Enzyme are protein and require the presence of additional chemical components to catalyse reactions.

- **Cofactors** are inorganic ions such as zinc , Mn2+etc.

- **Coenzymes : Coenzymes are organic compounds required by many enzymes for catalytic activity.**
- **They are often vitamins, or derivatives of** that act as temporary carriers of groups in the reaction e.g. nicotinamide adenine dinucletide (NAD), **Coenzyme A (CoA).**

❑Properties of enzymes

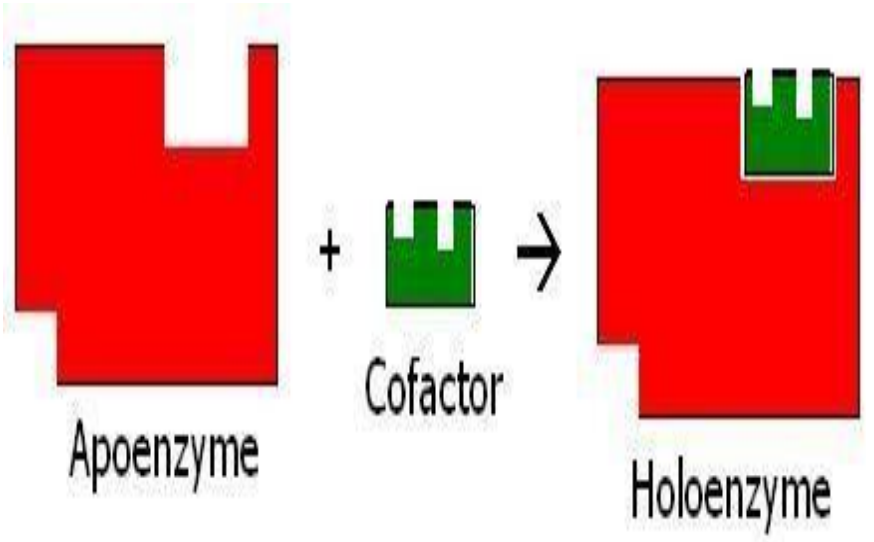
- The protein part of such an enzyme is called the <u>apoenzyme or apoprotein</u>
- A complete, catalytically active enzyme together with its bound coenzyme and/or metal ion is called a <u>holoenzyme</u>

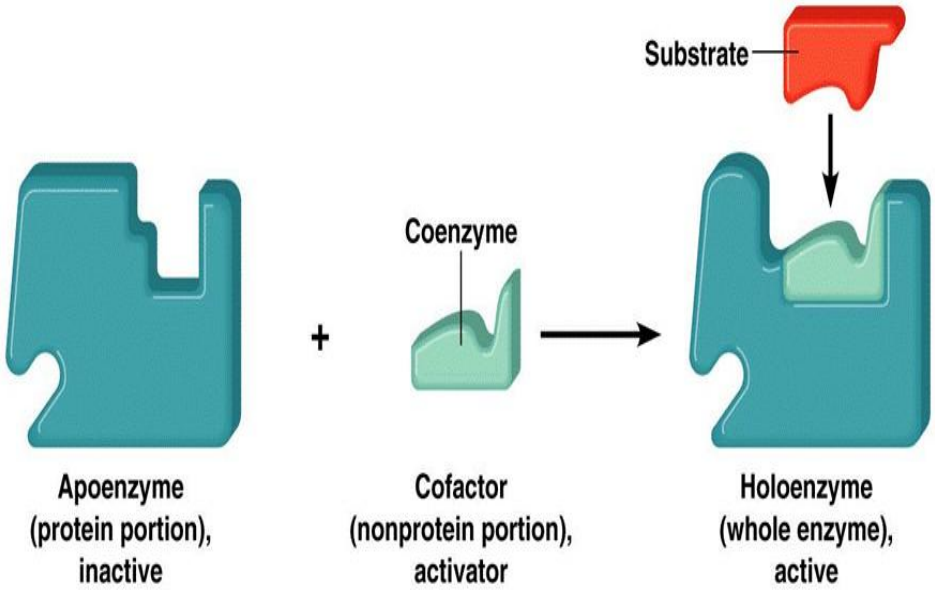


- **Holoenzymes:**

Some enzymes require molecules other than proteins for enzymatic activity.

- The term holoenzyme refers to the active enzyme with its non-protein component.
- The term apoenzyme is inactive enzyme without its non-protein part.
- If the non-protein part is a metal ion such as Zn 2+ or Fe2+, it is called **a cofactor**.
- If it is a small organic molecule, it is termed a **coenzyme.**

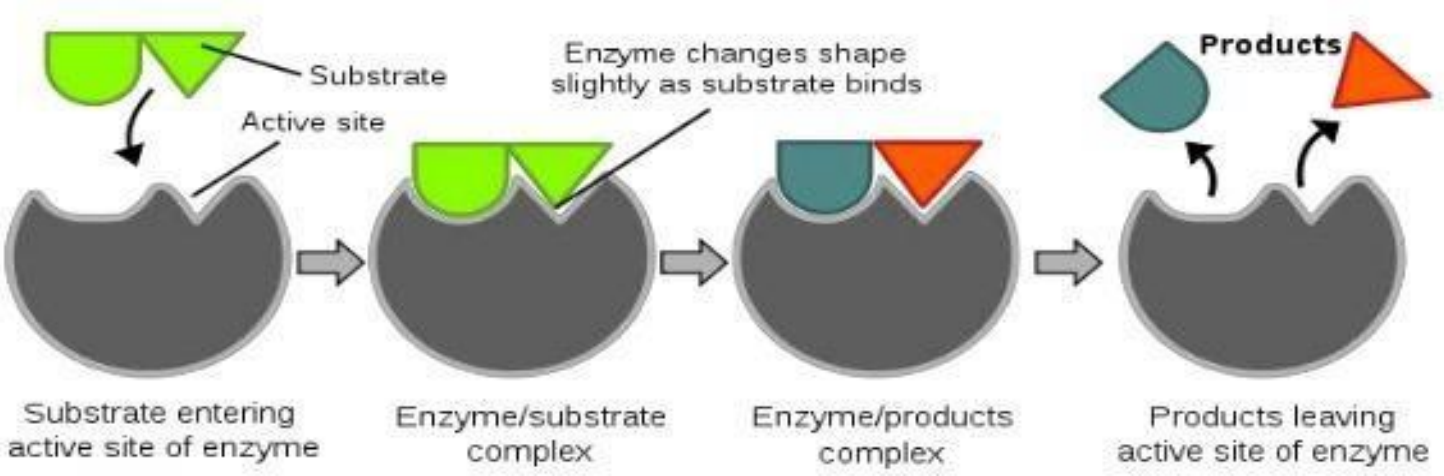




How do Enzymes Work?

- Enzyme-catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (an ES complex).
- Substrate binding occurs in a pocket on the enzyme called the active site.

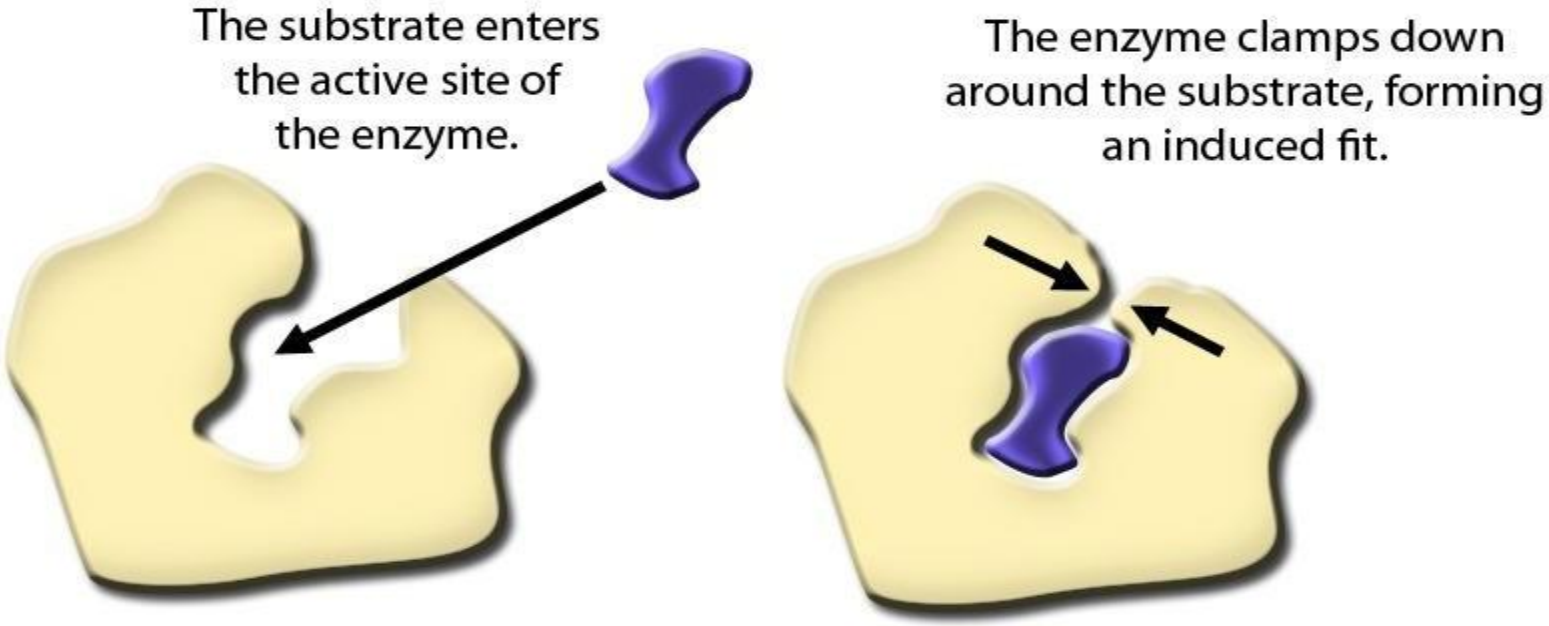
$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



❑Enzyme Active Sites

- **Enzyme-catalyzed reactions take place within the confines of a pocket on the enzyme called the active site. The reactant molecule is referred to as the substrate.**
- The enzyme's active site is the site at which the enzyme binds to the substrates and increases their chances of reacting.

The surface of the active site is lined with amino acid residues



- **Reaction Rates as a Function of Enzyme and Substrate Conc.**

The relationship between [S] and Vo has general shape for most enzymes (hyperbolic), which can be expressed algebraically by the Michaelis-Menten equation:

WhereV0= initial reaction velocity

[S] = substrate concentration

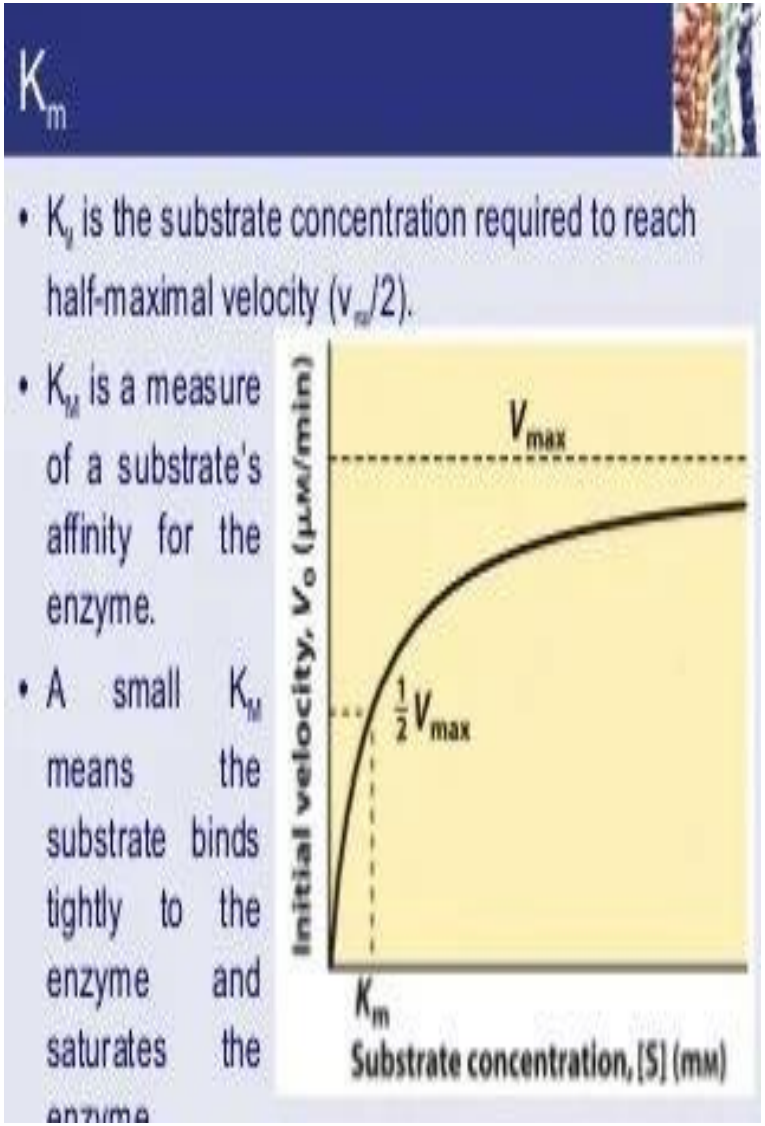
Vmax = maximal velocity

Km = Michaelis constant

The Michaelis-Menten constant (Km), the concentration of substrate ([S]) providing half of enzyme maximal activity,

Low Km means high affinity of the enzyme to the substrate.

- High Km means low affinity of the enzyme to the substrate.



The Michaelis-Menten Equation

- The basic equation derived by Michaelis and Menten to explain enzyme-catlyzed reactions is

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

K_m - Michaelis constant;

V_o - initial velocity caused by substrate concentration, [S];

V_{max} - maximum velocity

Clinical Importance :

1. Disease Diagnosis
2. Drug Action .
3. Laboratory Interpretation.
4. Toxicology.
5. Vitamin Deficiency

❑Factors Affecting enzyme activity

1. **Temperature**

- **Clinical relevance:**
- Fever may alter enzyme activity
- Improper sample storage → false lab results

2. **pH**

Clinical relevance:

- Acidosis/alkalosis affects enzyme function
- Example: pepsin (acidic), trypsin (alkaline)

3. Substrate Concentration

Clinical relevance:

- High glucose → affects enzyme kinetics in diabetes
- Important in lab assay standardization

- **4. Enzyme Concentration**

Clinical relevance:

- ↑ ALT, AST → liver damage
- ↑ CK → muscle or cardiac injury

5. Inhibitors / Drugs

Clinical relevance:

- Statins → inhibit cholesterol synthesis
- Cyanide → inhibits cellular respiration

6. Cofactors / Coenzymes

Clinical relevance:

- Vitamin deficiency → ↓ enzyme activity
- Example: B vitamins (coenzymes)

❑International unit of Enzyme Activity

- Unit of enzyme1 U or IU is defined as the enzyme quantity that liberates 1 micromole of product per ml of the reaction mixture per minute (usually at 25 °C).

- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses

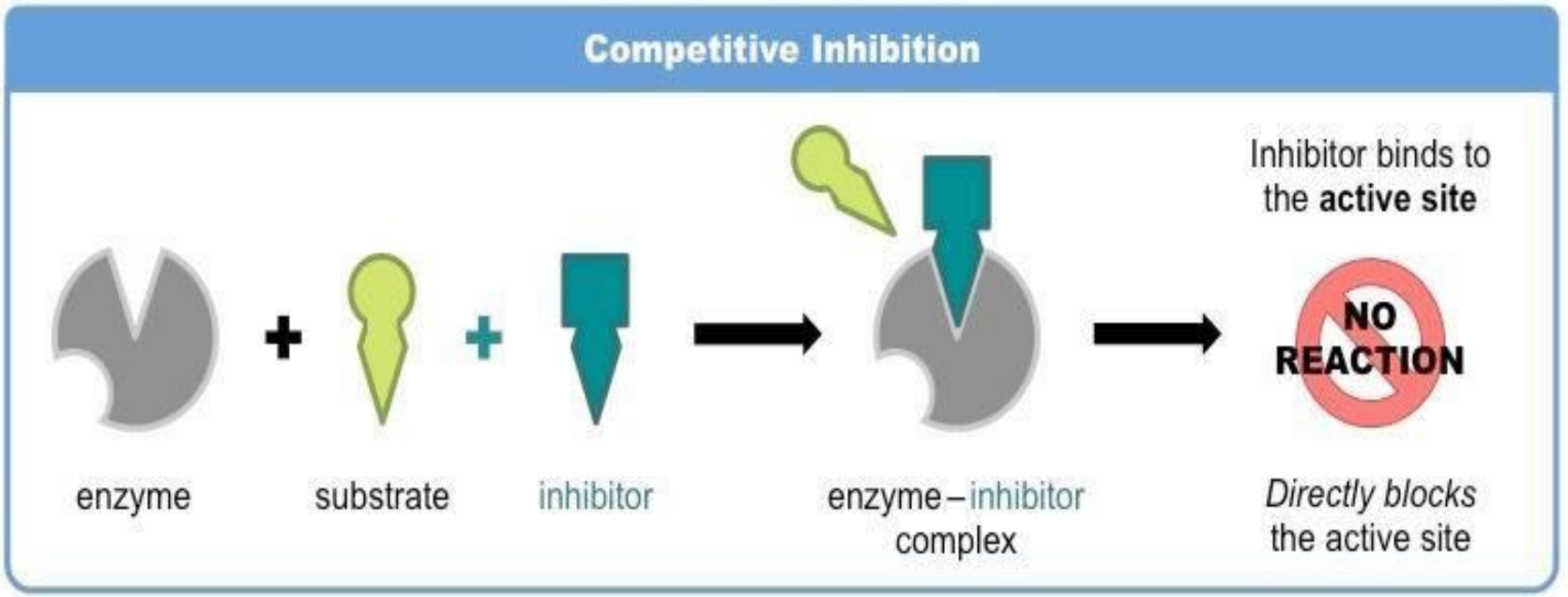
Enzyme inhibition

- In a tissue and cell different chemical agents (**metabolites, substrate analogs, toxins, drugs, metal complexes** etc.) can inhibit the enzyme activity

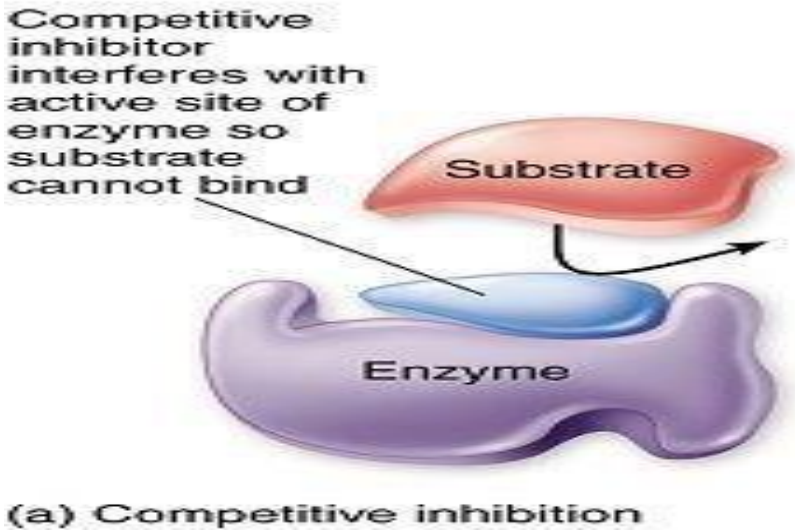
- **Inhibitor** (**I**) binds to an enzyme and prevents the formation of ES complex or breakdown it to E + P
- **Competitive inhibitors:** - Binds at the active site
- Affects Km not Vmax , Can be overcome by increasing the substrate concentration Enzyme is inactive only when bound to inhibitor

❑Reversible and irreversible inhibitors

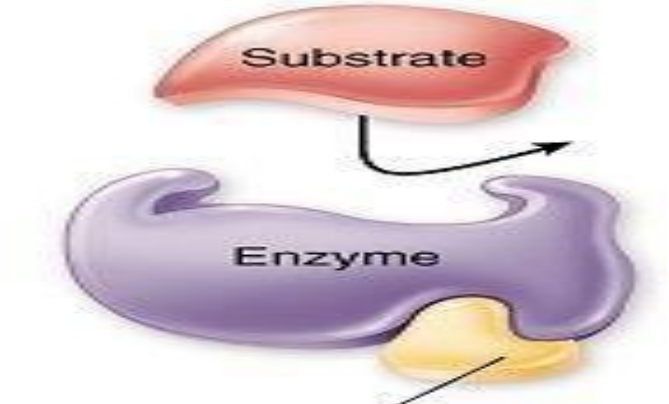
- **<u>Reversible inhibitors</u>**
- **Competitive inhibitors:** -Binds at the active site , Affects Km not Vmax Can be overcome by increasing the substrate concentration
- Enzyme is inactive only when bound to inhibitor



- ii) **Non-competitive inhibitors :** Binds at a site other than the active site
- Affects Vmax not Km
- Cannot be overcome by increasing the substrate concentration.



(a) Competitive inhibition

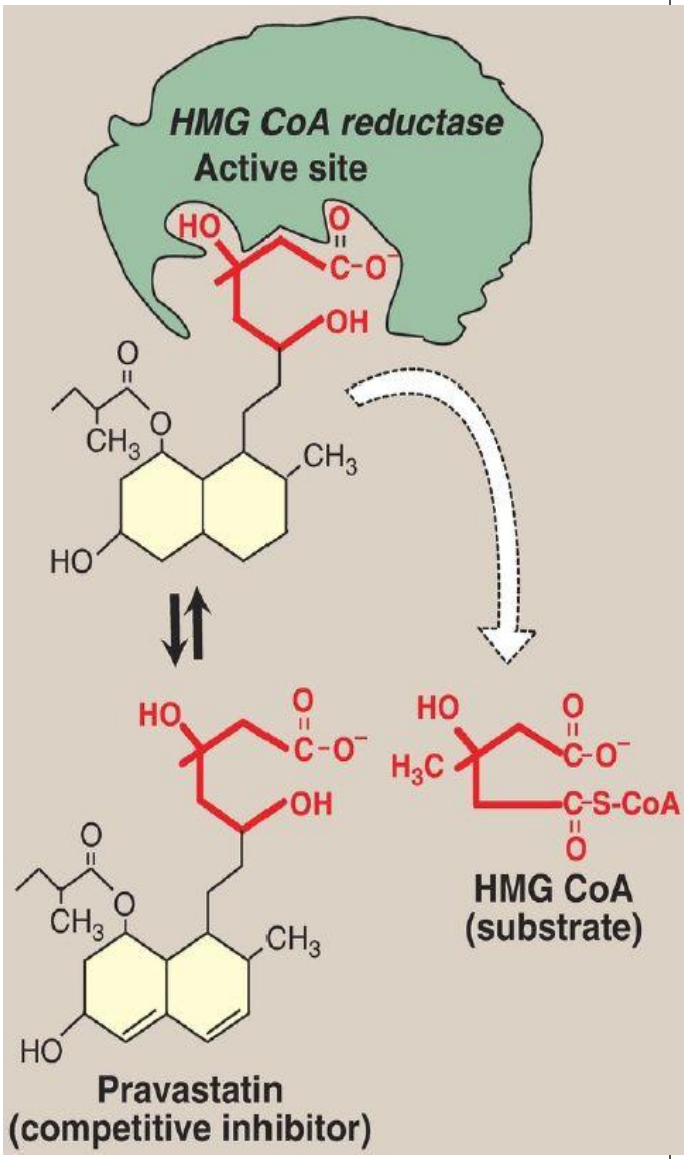


(b) Noncompetitive inhibition

❑Statin drugs as examples of competitive inhibitors

- **:** Antihyperlipidemic agents competitively inhibits the rate- limiting (slowest) step in cholesterol biosynthesis.

- Statins, such as atorvastatin (Lipitor) are structural analogs of the natural substrate for this enzyme :



Enzyme inhibitors as drug

- **β-lactam** antibiotics, such as penicillin and amoxicillin, act by inhibiting •
- enzymes involved in bacterial cell wall synthesis
- **Angiotensin-converting enzyme** (ACE) inhibitors.
- They lower blood pressure. by blocking. the enzyme that cleaves angiotensin I to form the potent vasoconstrictor, angiotensinThese drugs, which include captopril
- **Aspirin,,**
- irreversibly inhibits for enzyme required for prostaglandin and thromboxane synthesis

❑ENZYMES IN CLINICAL DIAGNOSIS

- In general, each enzyme of clinical significance is found in many tissues of the body, and in healthy individuals, these enzyme exhibit very low levels in serum.

- In certain disease states or with cell injury, these intracellular enzymes are released into the blood and are indicative of the presence of a pathological condition.

- Quantification of enzyme levels in serum is useful in determining the presence of disease.

- Q/ Cases : A 58-year-old man presents with severe chest pain radiating to the left arm. Blood tests show elevated **CK-MB and LDH-1**.
- **Question:**
  Which enzyme is most specific for myocardial injury?

- Q A 48-year-old woman presents with fatigue and jaundice.
  Laboratory findings:
- ALT: markedly elevated
- AST: elevated
- ALP: slightly elevated
- **Questions:**
- What type of liver injury is suggested?
- Why is ALT more specific than AST for liver damage?
- What is the mechanism of enzyme release into blood?

A. Plasma Enzymes as diagnostic tools

- Diseases that cause tissue damage result in increased release of intracellular enzymes into the plasma.
- Determination of the level of these enzymes is used for diagnosis of heart, liver, skeletal muscle, etc.
- The level of these enzymes in plasma correlates with the extent of tissue damage.

❑ Enzymes are helpful as diagnostic in different .

- diseases, namely:
- Myocardial infarction .
- Liver diseases .
- Muscle diseases.
- Bone diseases .
- Cancers .

Table 7–2. Principal Serum Enzymes Used in Clinical Diagnosis

	Serum Enzyme	Major Diagnostic Use
	Aminotransferases	
	Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction, but also in Liver
*	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 transferase	Various liver diseases
*	Lactate dehydrogenase isozyme 5	Liver diseases
*	Lipase	Acute pancreatitis
*	Phosphatase, acid	Metastatic carcinoma of the prostate
*	Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

Note: Many of the above enzymes are not specific to the disease listed.

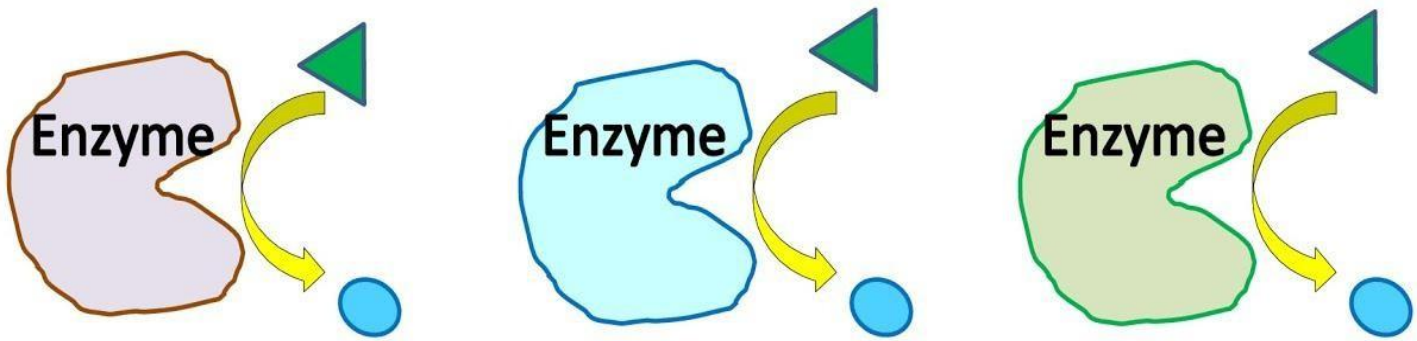
Know the enzymes marked by * and their principal use.

❑Isoenzymes

- Forms of the same enzyme
- Catalyse the same chemical reaction Different in terms of physical forms

Quickly understand

Isoenzymes



❑Isoenzymes and Diseases

- The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage.
- For example, Increase in total LDH level is seen in **hemolytic anemias, hepatocellular damage, muscular dystrophy, carcinomas, and leukemias.**
- The study of isoenzymes of LDH is of more significance.
- **LDH enzyme is a tetramer with 4 subunits.**
- But the subunit may be either **H (heart)** or **M (muscle)** polypeptide chains.
- Although both of them have the same molecular weight (32 kD), there are minor amino acid variations. So combinations of H and M chains are possible; H4, H3M, H2M2, M3H and M4, forming 5 iso- enzymes **(LDH1, LDH2, LDH3, LDH4, LDH5).**
- All these 5 forms are seen in all persons. M4 form is seen in skeletal muscles while H4 form is seen in heart.

- **Isoenzymes are multiple enzyme, isomers of enzyme. • There are five isoenzymes of LDH.**

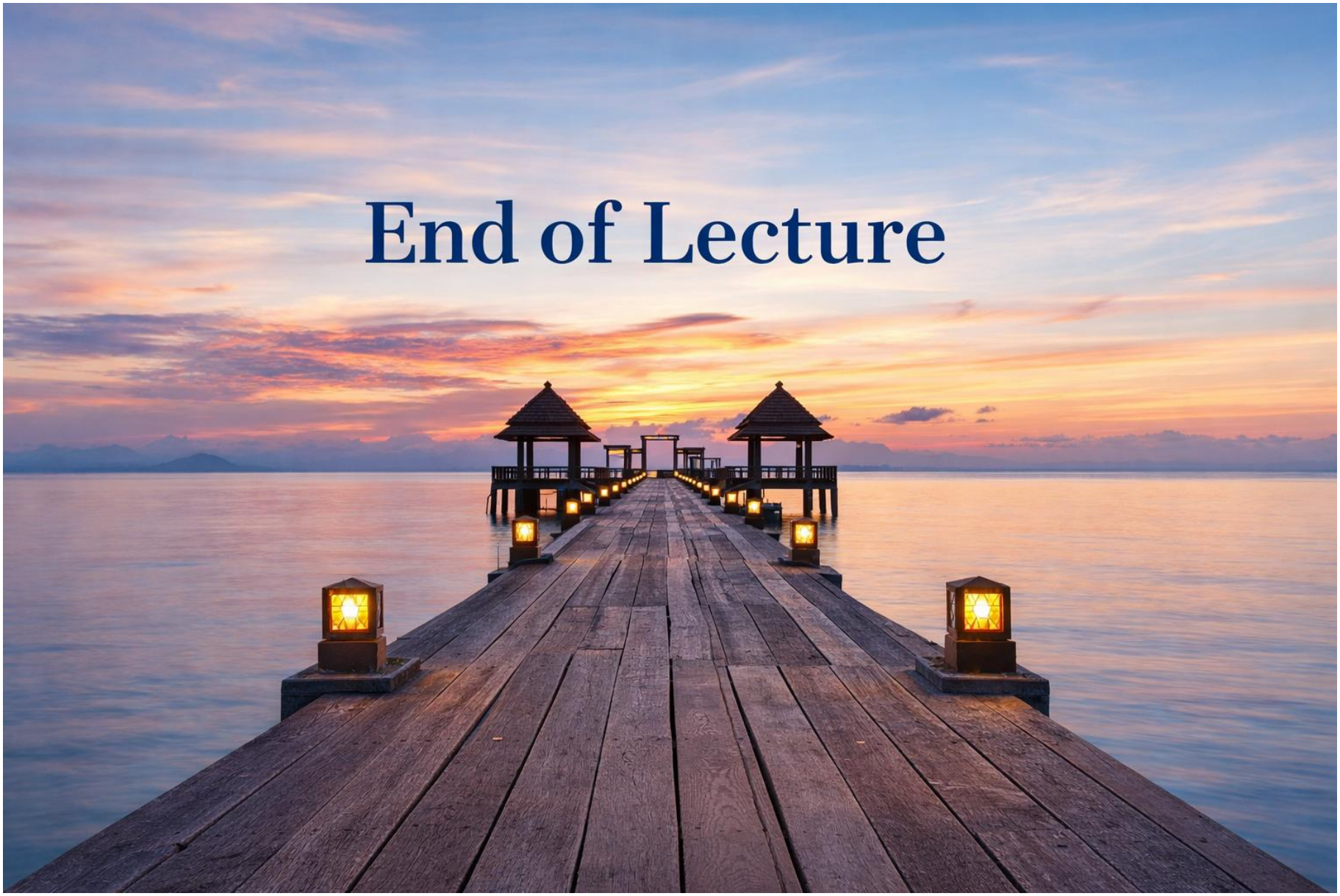
- LDH-1 found in heart and in RBC as well as in brain.
- LDH-2 found in the reticuloendothelial system.
- LDH-3 found in the lungs.
- LDH-4 found in the kidneys,placenta and pancrease.
- LDH-5 found in the liver and striated muscle

❑Enzymes as Therapeutic Agents

- Streptokinase (from Streptococcus) or Urokinase (from urine) can lyse intravascular clots and are therefore used in myocardial infarction.

- Pepsin and trypsin are given to patients with defective digestion.

- Asparaginase is used as an anticancer drug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