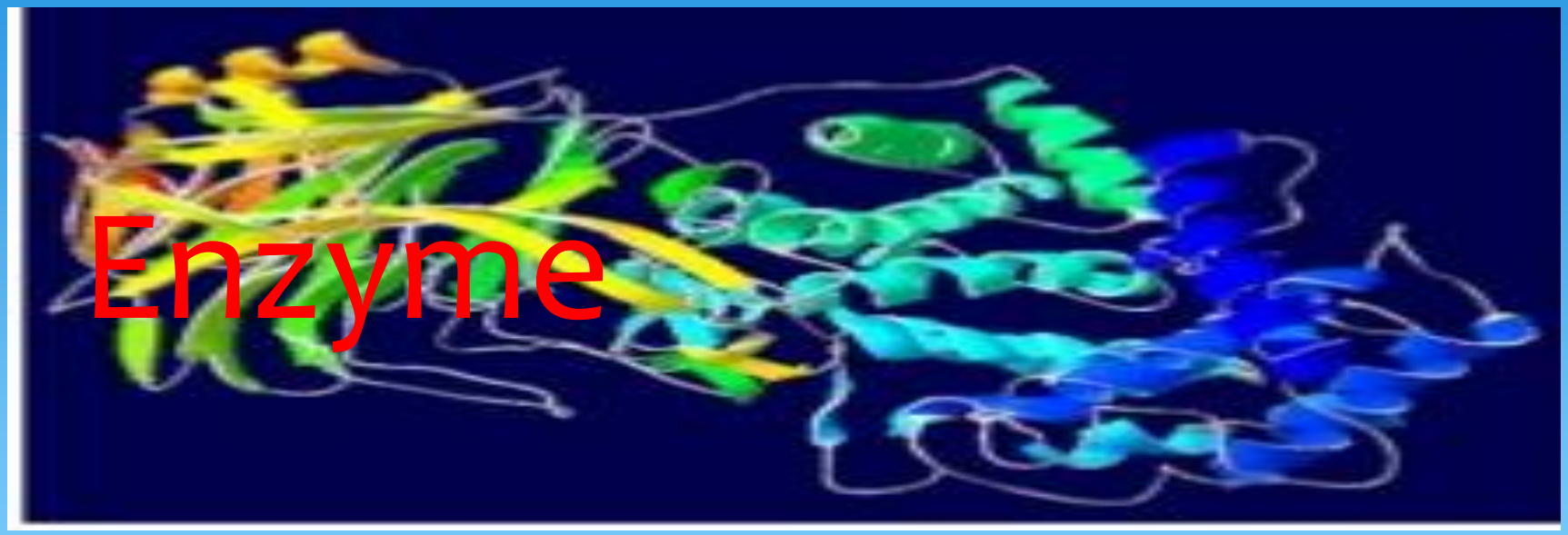
كلية طب الاسنان
college of dentistry

جامعة البصرة



Wisal abdulrhman Althamiry
M.SC.Ch (Biochemistry)

- Definition
- Terminology
- Model of enzyme substrate binding
- Enzyme nomenclature
- Classification
- Factors affecting enzyme activity
- properties of enzyme
- Specificity of enzyme action
- Mechanism of enzyme catalysis
- Enzyme regulation
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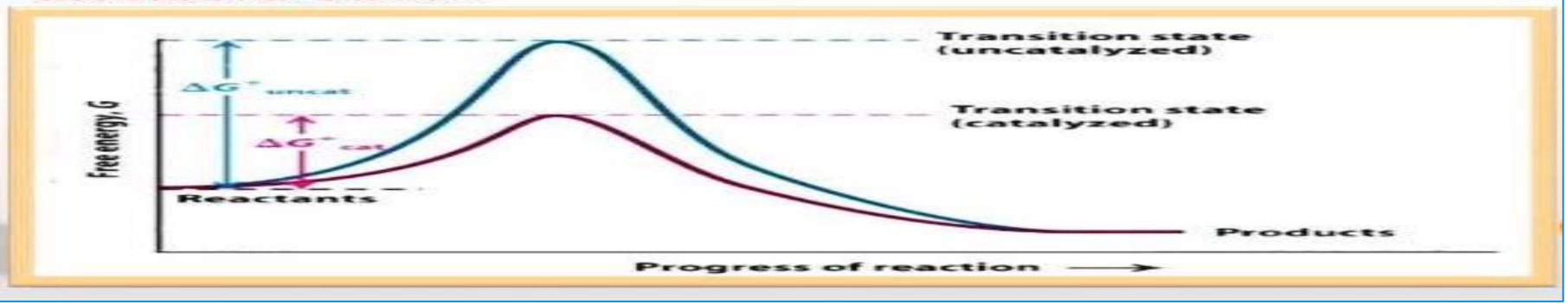
❑Definition

Enzyme:

- Are protein that function act as catalysts to speed up biological reaction by lowering activation energy.
- Enzyme undergo physical change during the reaction but revert to their original state when reaction is complete.
- They are not consumed during chemical reaction.

THERMODYNAMIC CHANGES

- All chemical reactions have energy barriers between reactants and products.
- The difference in transitional state and substrate is called *activational barrier*.



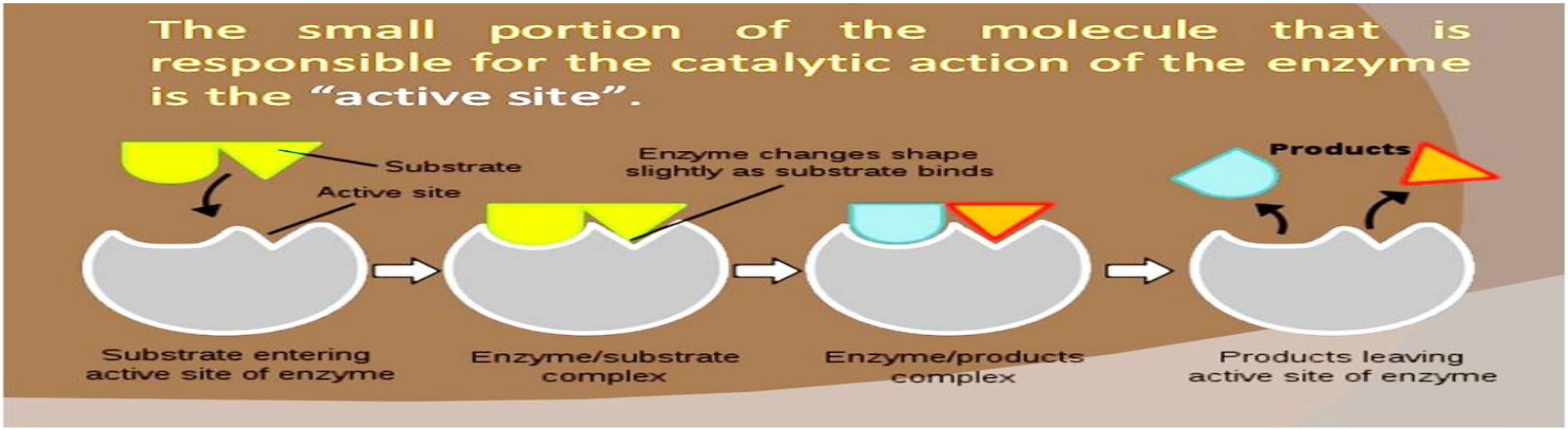
❑ Terminology

Substrate : The compound on which an enzyme acts converting it into product

Active site : Is the site that bind to the substrate .

Its the area in the enzyme structure involved in its catalytic activity.

- It is three dimensional entity.
- It take small portion of the enzyme molecule



Some enzyme do not need additional components to show full activity . Other require non-protein molecules for catalytic activity called **cofactor.** Cofactor can be either :

- Inorganic (mate ions Zn, Mg, Fe)
- Organic (NADH,FADH2)) it is called **Coenzyme.**
- Prosthetic groups (Heme group of cytochrome, Biotin of acetyal CoA carboxylase).

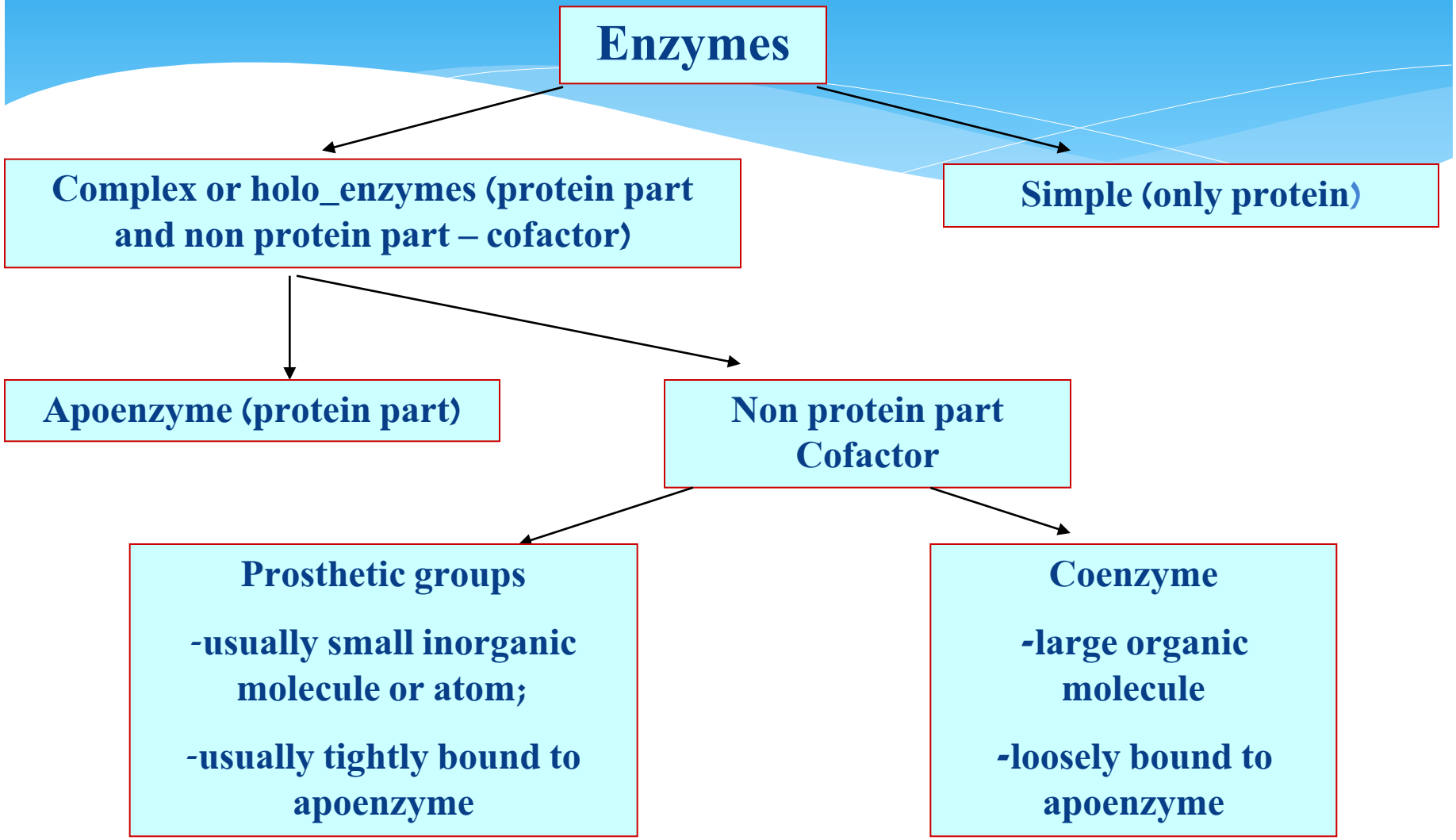
The protein part in non-active enzymes are called **apoenzymes** .

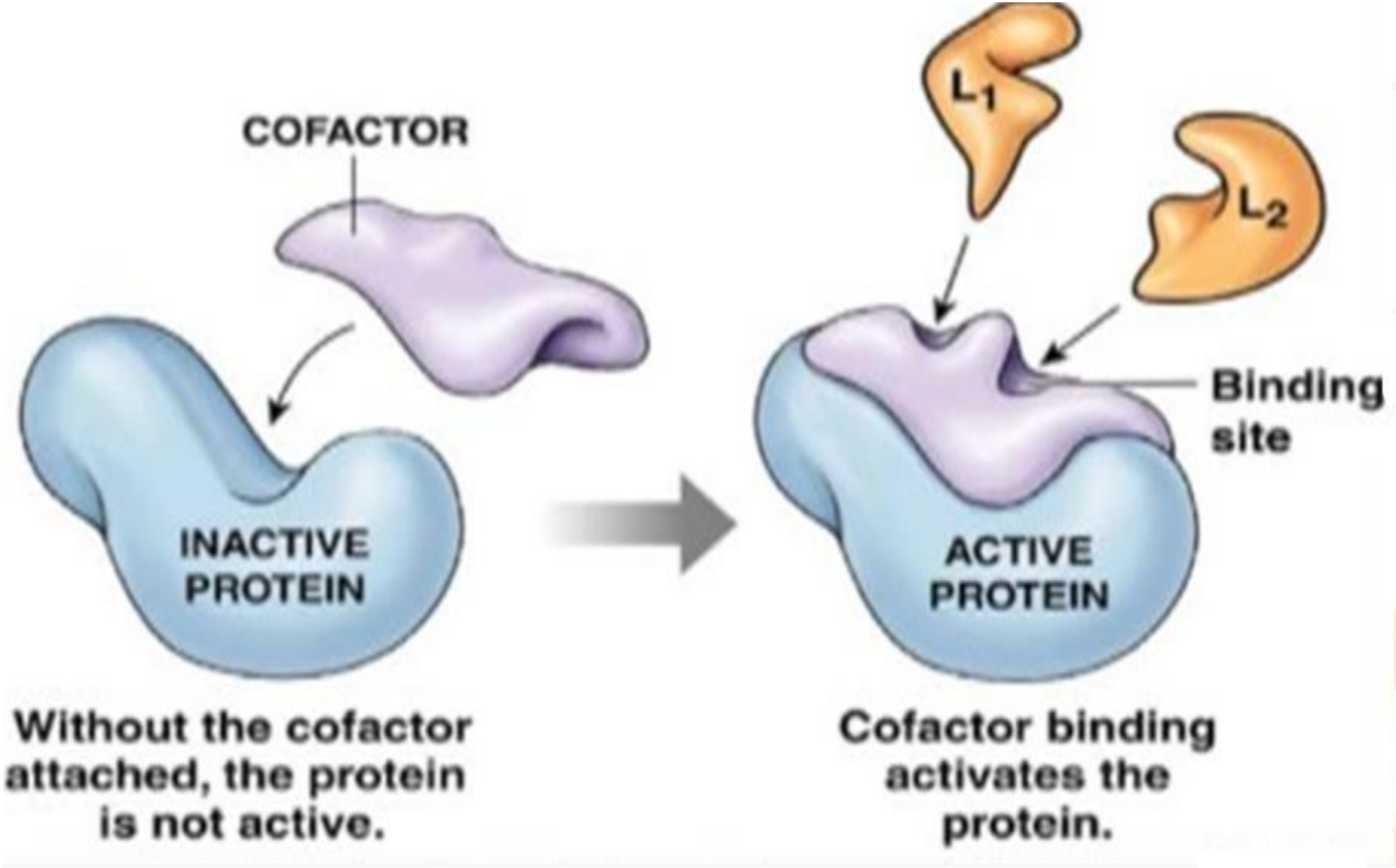
An enzyme together with coenzyme or cofacter required for activity is called a **holoenzyme**.

Apoenzyme + Coenzyme ⟶ holoenzyme

protein part non-protein part complete catalytic system

Structure of enzymes




COFACTOR
INACTIVE PROTEIN
Without the cofactor attached, the protein is not active.
L1
L2
Binding site
ACTIVE PROTEIN
Cofactor binding activates the protein.

Apoprotein – responsible for the reaction.

Cofactors – responsible for :

a. Bond formation between enzyme and substrate

b. Transfer of functional group.

c. Takes place in the formation of tertiary structure of protein part.

Coenzyme organic molecule derived from the vitamin B which participate directly in enzymatic reactions. Enzymes that require a metal in their composition known as **metalloenzymes**.

Difference between enzyme catalysts and chemical catalysts

enzyme catalysts	chemical catalysts
• Protein in nature • Catalyses a specific reaction • Catalysis occur via active site of enzymes. • The enzyme does not return to their original state after a biochemical reaction. • Generally produced by living cells and acts inside living cells.	• Non-protein in nature • Catalyze different reactions • Catalysis takes part as a whole. • Catalyst always return to its original state. • Reacts outside living cells.

Model of enzyme substrate binding(Mechanisms of enzyme action)

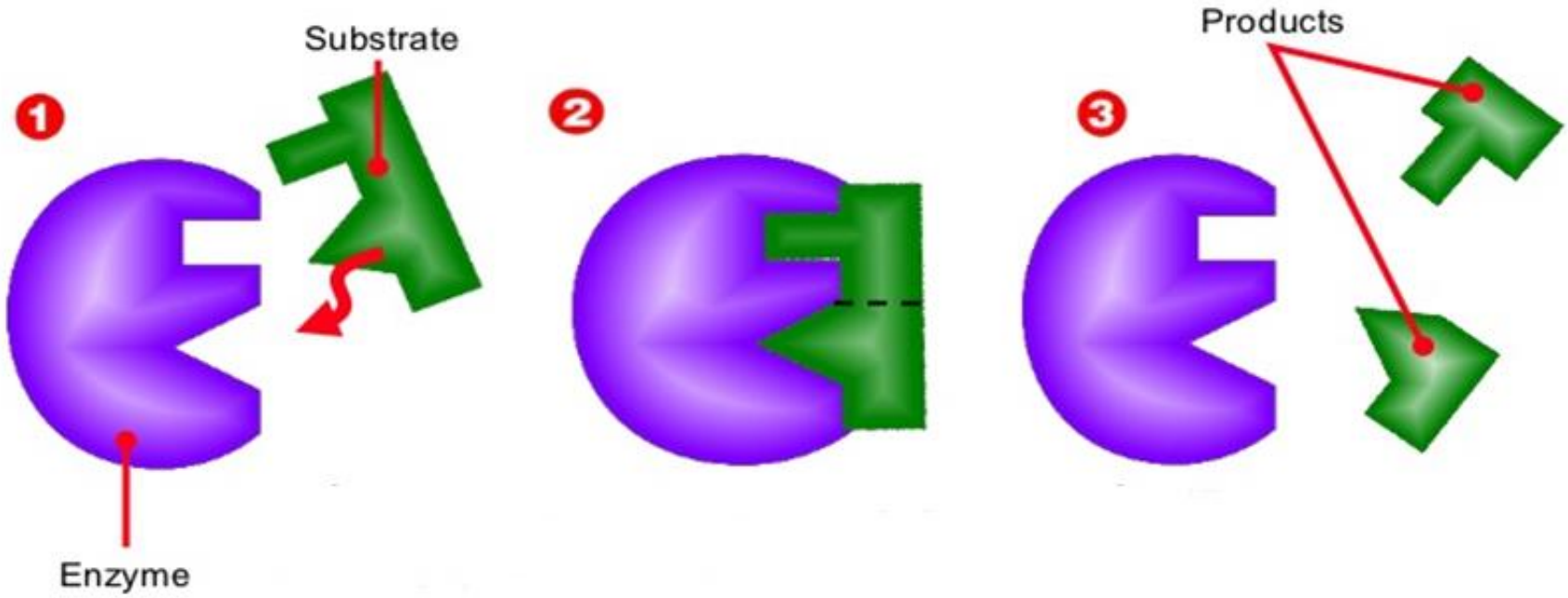
Two theories includes the reaction between the substrate and active site to form the enzyme- substrate complex :

1. Lock and key model
2. Induced - fit model

1.Lock and key model of enzyme action

proposed earlier this century , proposed that substrate must have a matching shape to fit into the active site of the enzyme, the enzyme can be visualized as containing grooves with fixed dimensions that permit only compounds with specific shape to be inserted in .

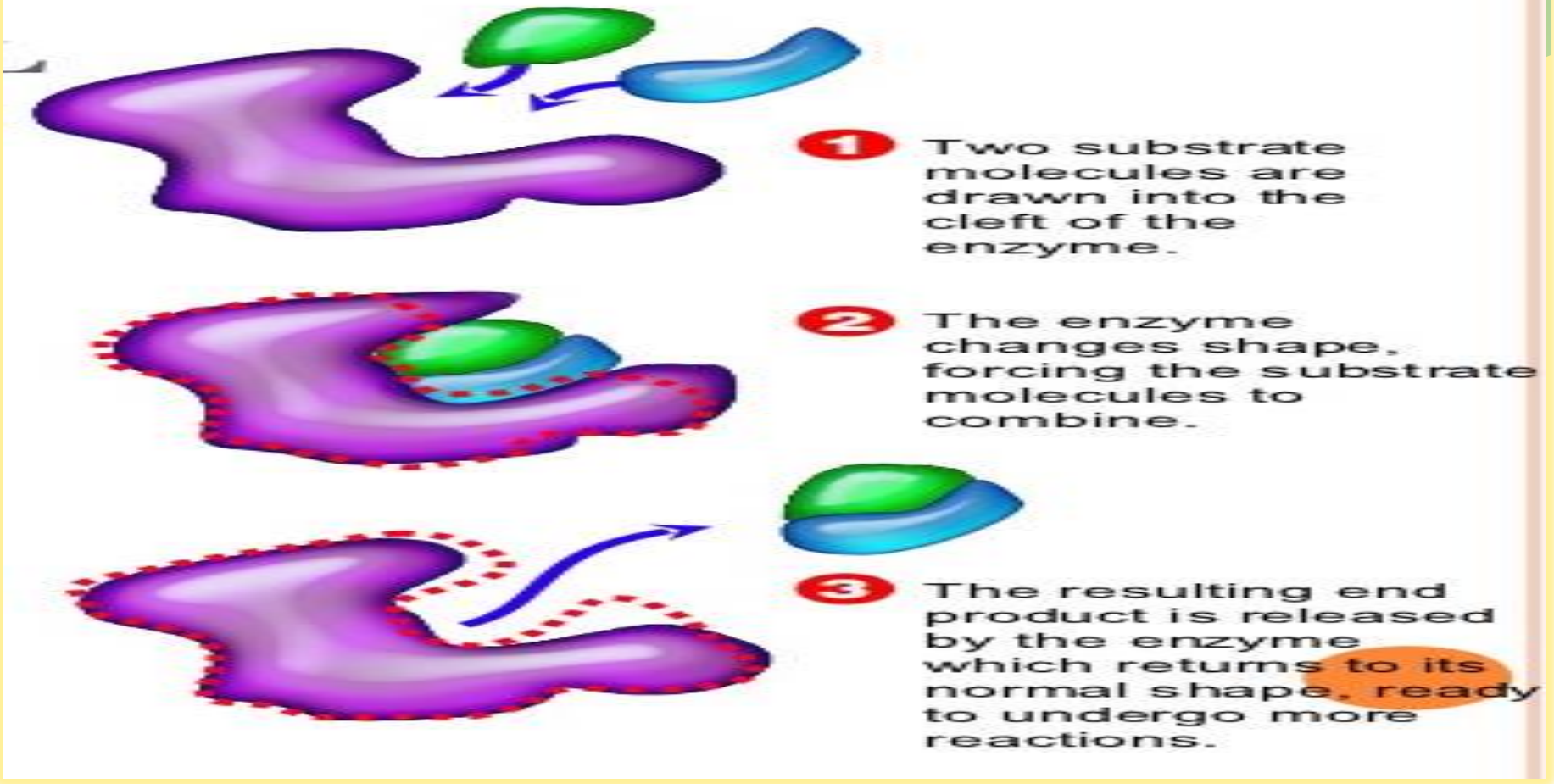
Lock and key theory implies rigidity of the active site.



2.Induced - fit model

More recent studies have revealed that the process is much more likely to involve an induced – fit .
The enzyme allow an initial superficial attachment of the substrate .
This causes conformational changes that provide a more perfect apposition between the active site of the enzyme and the substrate .So, the shape of the active site is modified by substrate binding , and the active site has a shape which is complementary to that of the substrate only after the substrate is bound.

Induced - fit model implies flexibility of the active site.



Lock-and key Model

Wherein the substrate must "fit" into the active site of the enzyme – hence the specifity of the enzyme.

Induced-Fit Model

Suggests that the active site is not rigid as the Lock-and-Key Model, but flexible. That is, the site changes in conformation upon binding to a substrate in order to yield an enzyme-substrate fit.

❑ Enzyme nomenclature

Two systems:

A. IUB system : International union of Biochemistry

The enzyme name consists to part

The 1st part : the name of substrate(s).

The 2nd part : the type of reaction (ending in – ase).

Examples:

Aspartate aminotransferase

Glutamate dehydrogenase.

B. By adding "ase" to the name of substrate.

Examples:

Urea is called Urase

Arginine is called arginase

❑ Classification

There are six major classes

1. **Oxidoreedutase** - are enzymes that catalyzes oxidation – reduction . The enzymes that catalyze oxidation – reduction in the body are important because these reaction responsible for production of heat and energy .
2. **Transferase** - are enzymes that catalyzes the transfer of particular group from one molecule to another like one carbon , amino and phosphate groups.
3. **Hydrolase** – hydrolytic enzymes catalyze the hydrolysis of ester, carbohydrates and protein.
4. **Lyase** - are enzymes that catalyzes the removal of groups from substrates by means other than hydrolysis , usually with the formation of double bonds (catalyzes the joining of spical molecules or group by a double bond).

5. **Isomerase** - are enzymes that catalyzes the interconvertion of cis- trans isomer.

6. **Ligase or Synthetase** - are enzymes that catalyzes the coupling of two compounds with breaking of pyrophosphate bond in ATP or similar compound .

ENZYME CLASS	REACTION TYPE	EXAMPLES
Oxidoreductases	Reduction-oxidation (redox)	Lactate dehydrogenase
Transferases	Move chemical group	Hexokinase
Hydrolases	Hydrolysis; bond cleavage with transfer of functional group of water	Lysozyme
Lysases	Non-hydrolytic bond cleavage	Fumarase
Isomerases	Intramolecular group transfer (isomerization)	Triose phosphate isomerase
Ligases	Synthesis of new covalent bond between substrates, using ATP hydrolysis	RNA polymerase

Coenzymes usually functions as intermediate carriers of functional groups or specific atoms or electrons are transferred in the over all enzymatic reactions.

Enzymes classes requires coenzymes

Classes : 1 , 2 , 5 , 6.

Enzymes classes **does not** requires coenzymes

Classes : 3 , 4.

Factors affecting enzyme activity

1. TEMPERATURE
2. PH ACIDITY AND BASICITY
3. ENZYME CONCENTRATION
4. SUBSTRATE CONCENTRATION
5. PRODUCT CONCENTRATION
6. INHIBITORS AND ACTIVATORS

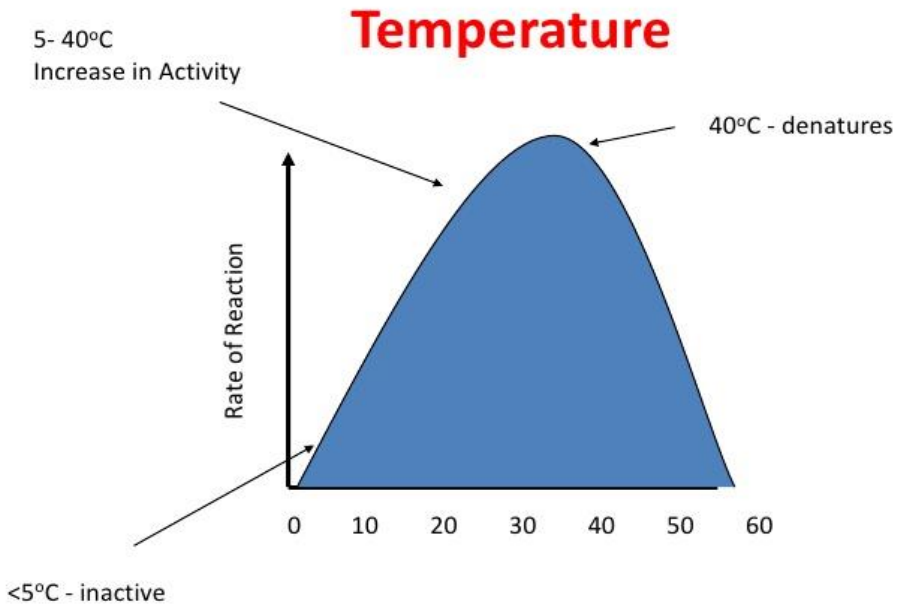
1. TEMPERATURE

Over a limited temp. range , the velocity of enzyme – catalyzed reactions increased as the temp. increases. The velocity doubles with a 10C rise in temp. and is halved if temp. is decreased by 10 C .

However, since enzymes are proteins which are rapidly denatured at high temp. So an excessive increase in temp has deactivating effect on the enzyme.

Optimal temperature: is the temp. at which the enzyme activity is the highest and the enzyme molecule is stable. The optimal temp. for most enzymes is 37 C.

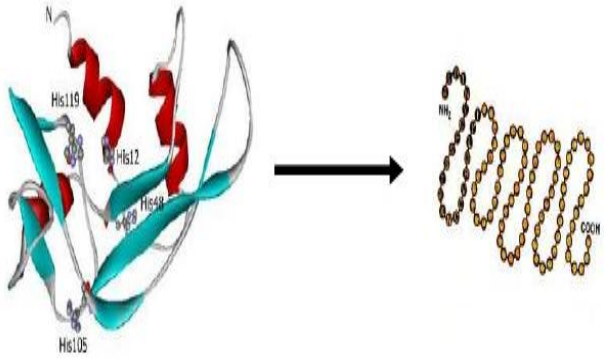
Effect of Temp.

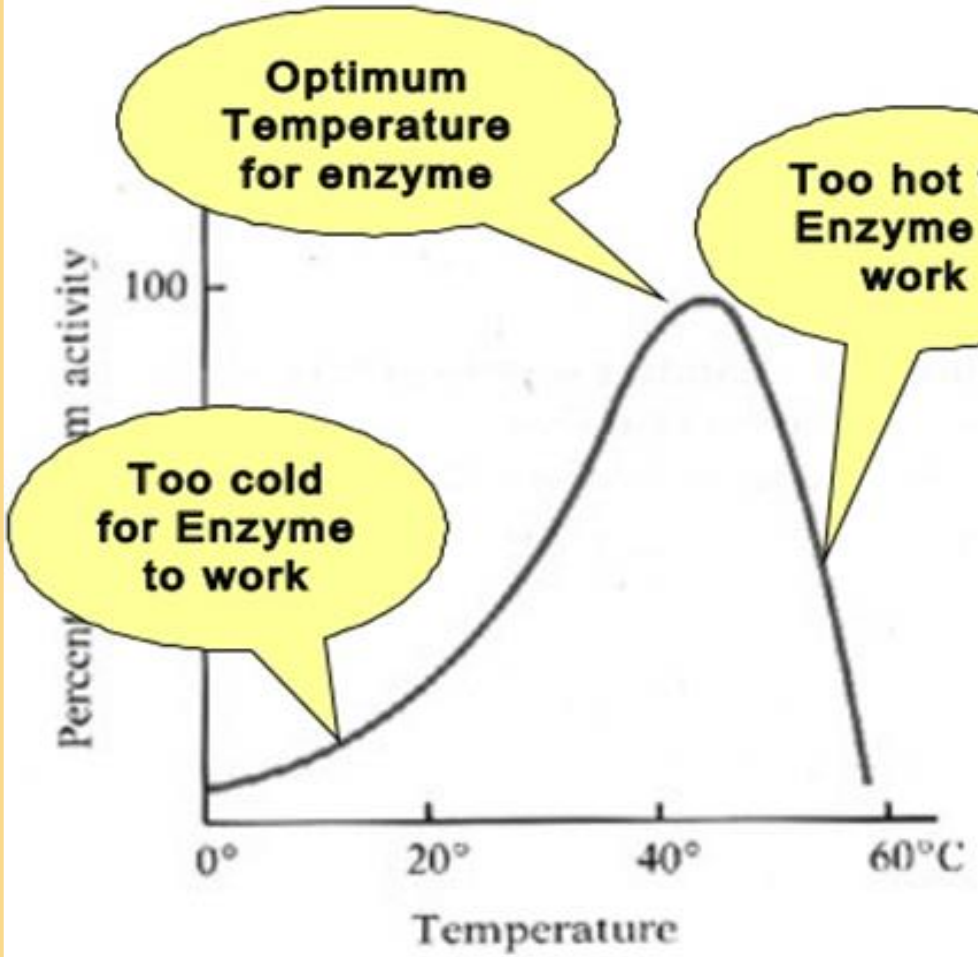


If you heat the protein above its **optimal temperature**

bonds break

meaning the protein loses it secondary and tertiary structure





- Speeds up all reactions, but the rate of denaturation of enzymes also increases at higher temperatures.
- High temperatures break the disulphide bonds holding the tertiary structure of the enzyme together thus changing the shape of the enzyme.
- This destroys the active sites & therefore makes the enzyme non – functional.

2.PH ACIDITY AND BASICITY

The pH -scale measures how acidic or alkaline a substance is:

The chemical properties of many solutions enable them to be divided into three groups:

1- Neutral: solutions a pH of 7.

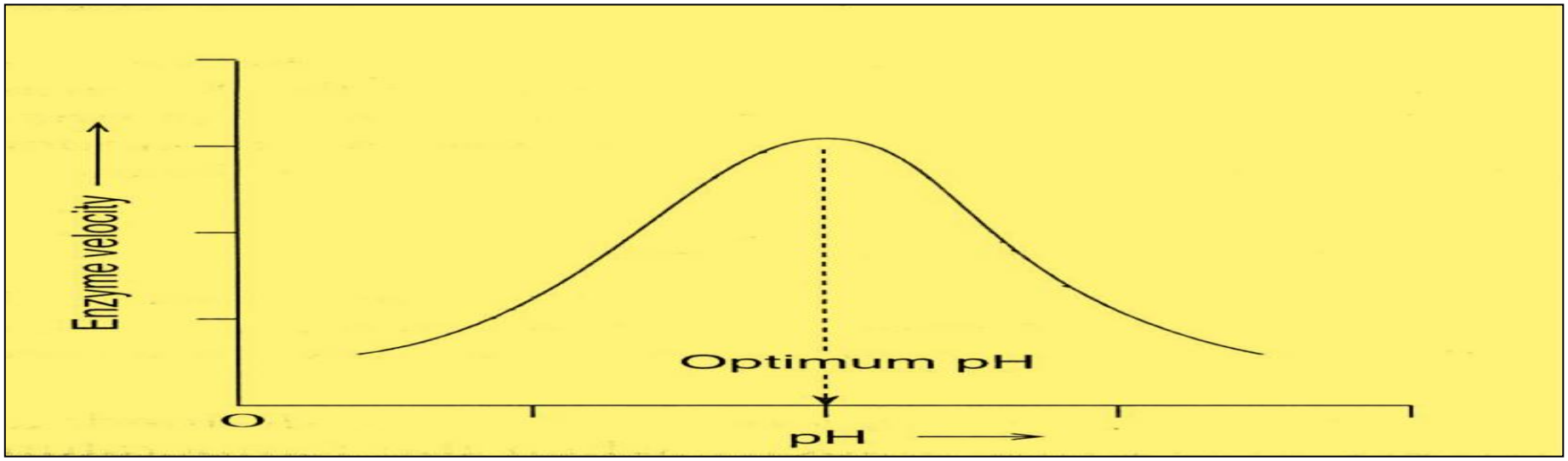
2- Alkaline: solutions with a pH greater than 7.

3- Acidic: solutions with a pH less than 7.

The net effect of pH on enzyme activity may be the result of the following factors :-

1. The H^+ ion conc. Of the medium determine whether certain groups are ionized or remain in an undissociated form

2. The effect of PH on the degree of S. ionization.



Optimal PH : the pH at which the enzyme is most active.

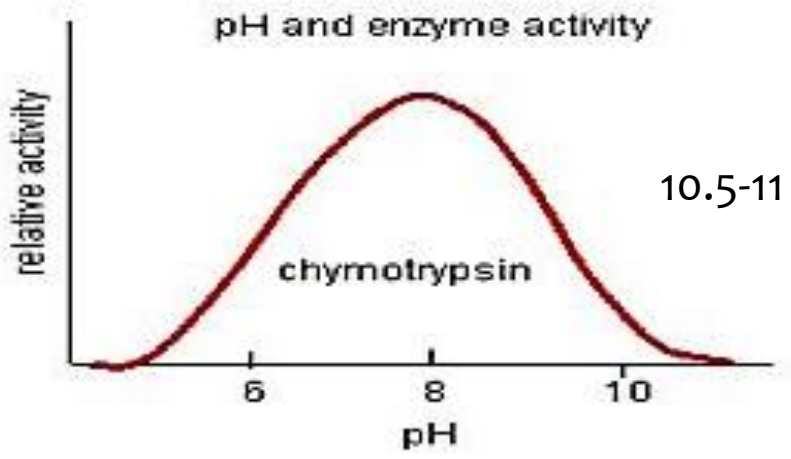
However, So enzymes have their activities below or above there pH values.

Like all protein, enzymes are denatured by extremes PH(acidity or alkalinity).

Each enzyme has own optimum pH at which the velocity maximum.

Most of the enzyme showed optimum activity around neutral pH , pH(6-8).

Different enzymes have different optimum pH



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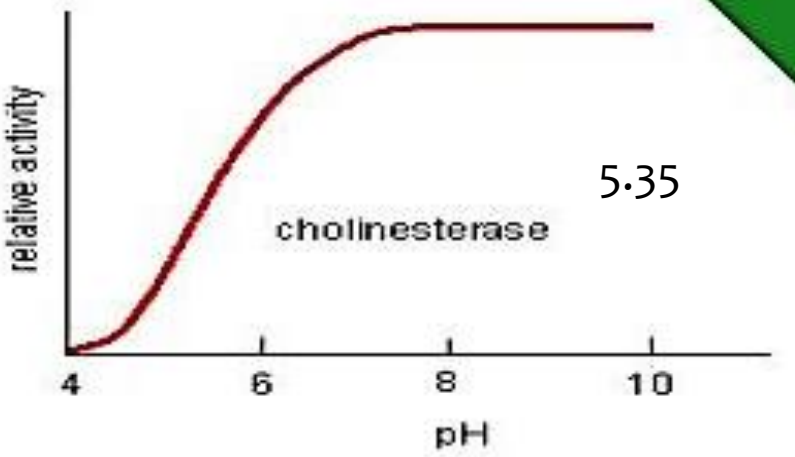

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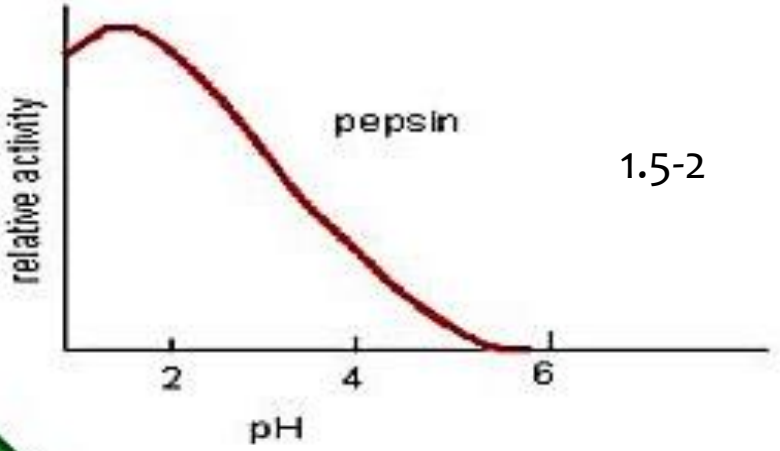

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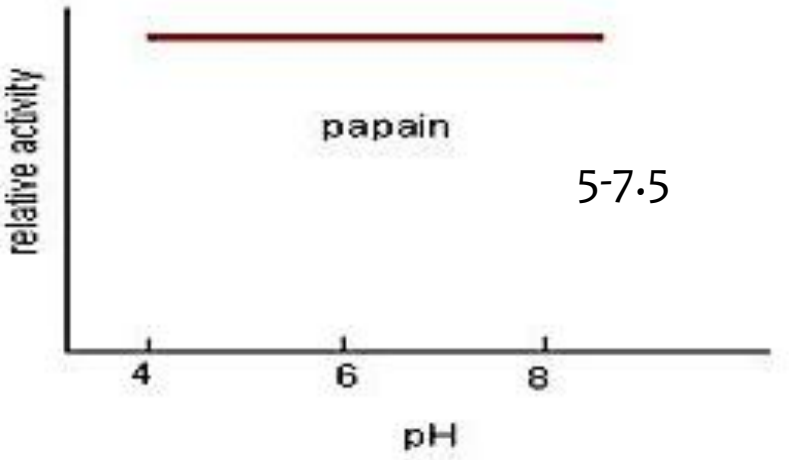
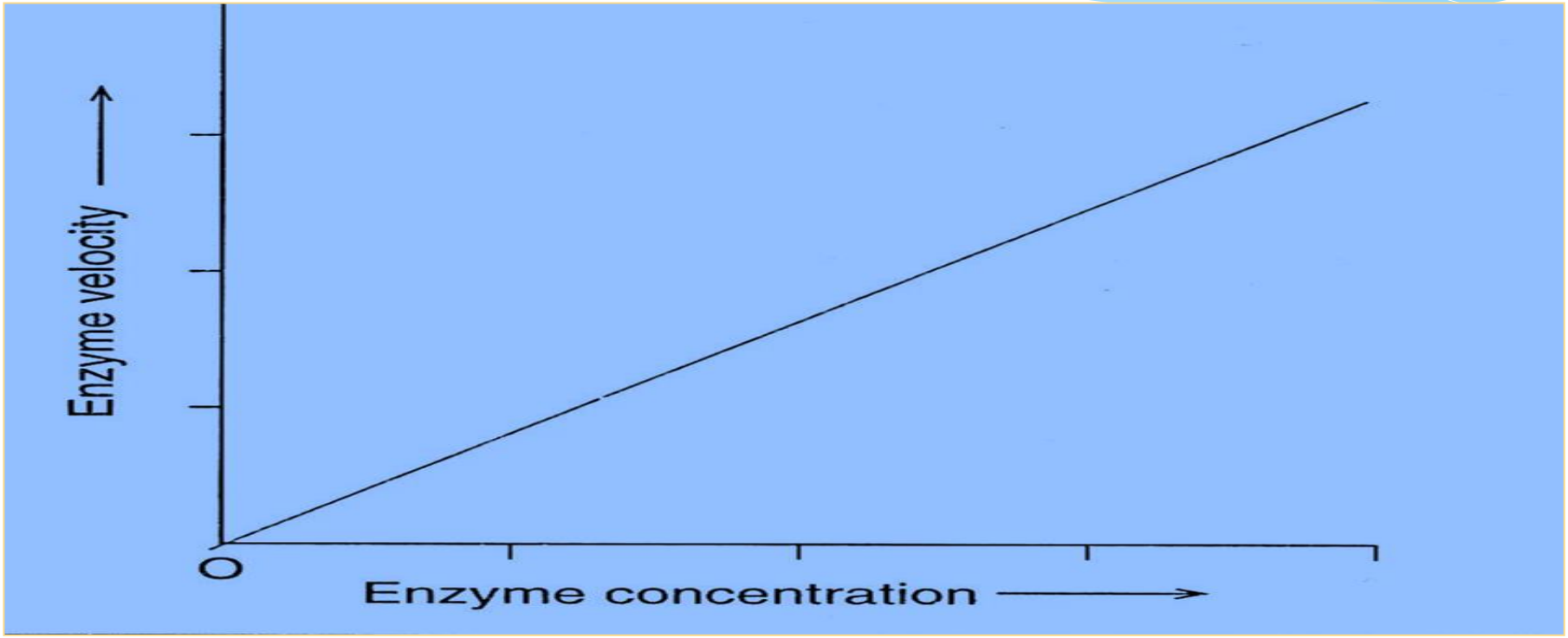


5-7.5

3.ENZYME CONCENTRATION

as the conc. Of enzyme is increased ,the velocity of the reaction is increased.



4.SUBSTRATE CONCENTRATION

- **At low S. conc.** or phase A **:-**

The reaction velocity is directly proportional to the S. conc. (First order kinetics).

- **At intermediate S. conc. (or phase B) :-**

The effect of S. conc. On initial velocity diminishes.

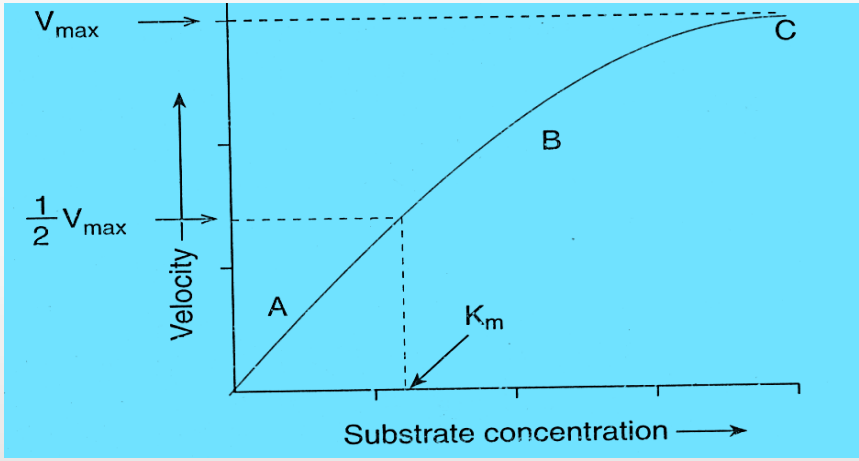
- **At high S. conc :- In the phase C**

the velocity reaches a maximum and cannot by increased by increasing S. conc., this velocity is called maximal velocity (**V max**) , and at which the enzyme is fully saturated & reached it's maximal catalytic effect (Zero order kinetics).

Michaelis-Menten Equation:

"It is an equation which describes how reaction velocity varies with substrate concentration."

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



- Where
- V_o is the initial reaction velocity.
- V_{max} is the maximum velocity.
- K_m is the Michaelis constant = $(k_{-1}+k_2)/k_1$.
- [S] is the substrate concentration.

* In biochemistry, **Michaelis–Menten kinetics** is one of the best-known models of enzyme kinetics. It is named after German biochemist Michaelis and Canadian physician Menten. The model takes the form of an equation describing the rate of enzymatic reactions, by relating reaction rate to , the concentration of a substrate .

- Michaelis-Menten Model:

"According to this model the enzyme reversibly combines with substrate to form an ES complex that subsequently yields product, regenerating the free enzyme."

where:

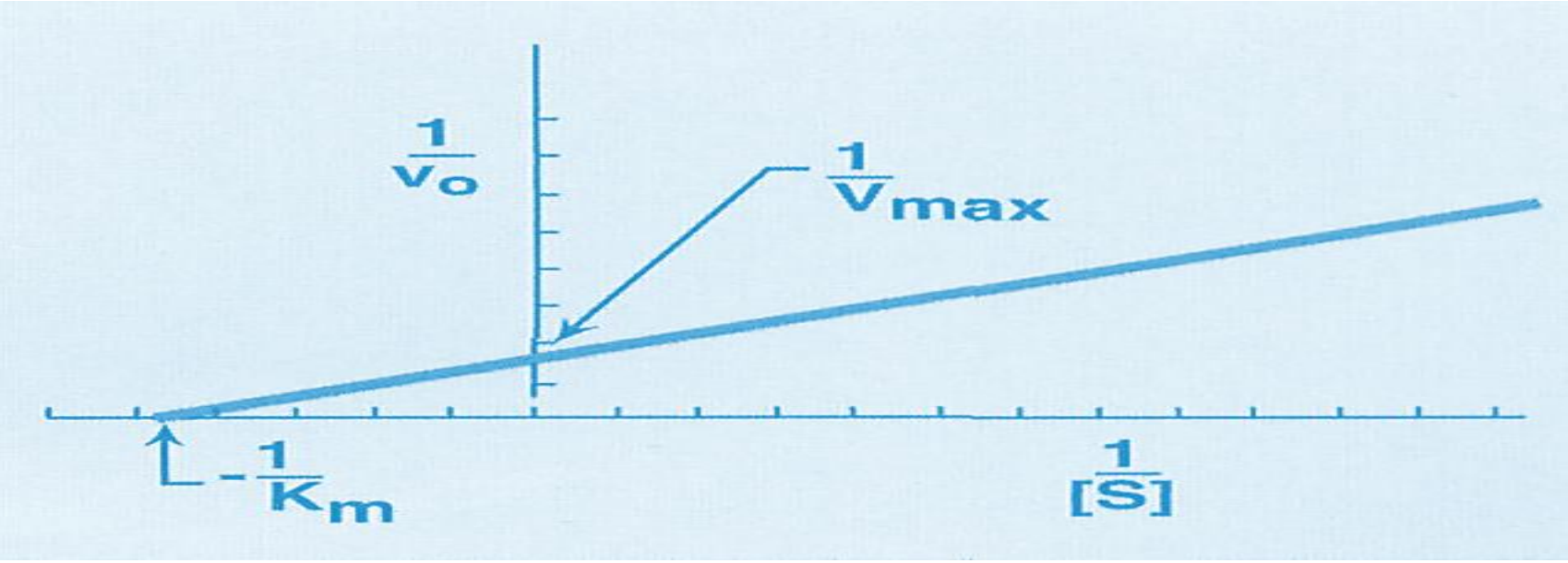
- S is the substrate
- E is the enzyme
- ES-is the enzyme substrate complex
- P is the product
- K1,K-1 and K2 are rate constants

Michaelis – menten equation is modified by taking the reciprocal of both sides of the equation.

The new plot is called **Lineweaver – Burk plot.**

Advantages of Lineweaver - Burk plot:-

1. **Allow accurate determination of Vmax & Km.**
2. **Give valuable information on enzyme inhibition.**



Use:

The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software. The x -intercept of such a graph is equivalent to the inverse of V_{max}; the x-intercept of the graph represents $-1/K_m$. It also gives a quick, visual impression of the different forms of enzyme inhibition.

5.PRODUCT CONCENTRATION

the accumulation of reactive product generally decrease the enzyme velocity .

For certain enzymes , the product combine with active site of the enzyme and form a loose complex and this inhibit the enzyme activity.

6.INHIBITORS AND ACTIVATORS

Activators : substances which increases enzyme activity

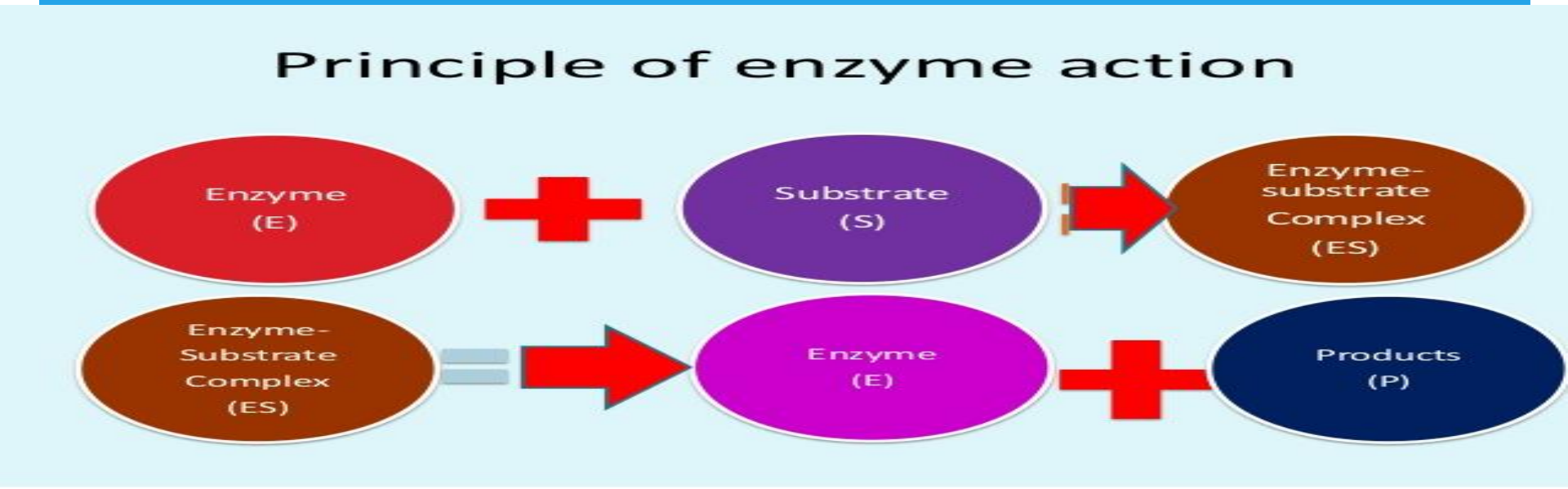
Ex: Cl^- act as activator of salivary amylase .

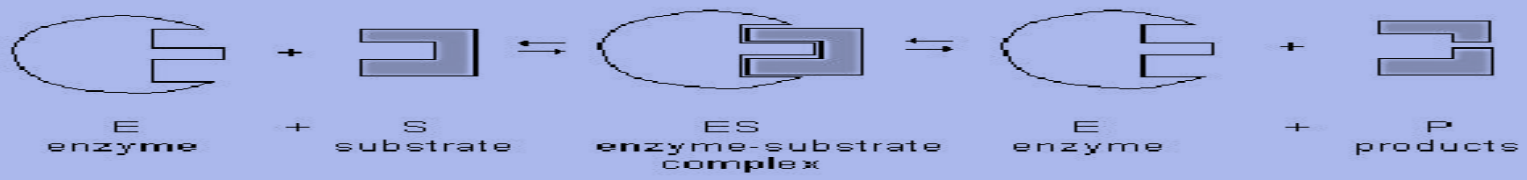
Inhibitors: substances which reduces or abolishes enzyme activity.

❑ properties of enzyme

- Enzymes can act on only one type of substrate (specific).
- They always produce the same products
- Although they take part in the reaction ,they are not used up
- Enzymes have enormous catalytic power .
- Because enzymes are proteins they are denatured by heat or some chemicals.
- Most enzymes are soluble in water
- Enzymes are colloidal in nature
- Small quantity is required for enzyme action

❑ Mechanism of enzyme catalysis





- Enzyme regulation

1. Inhibition
2. Covalent modification and Genetic control

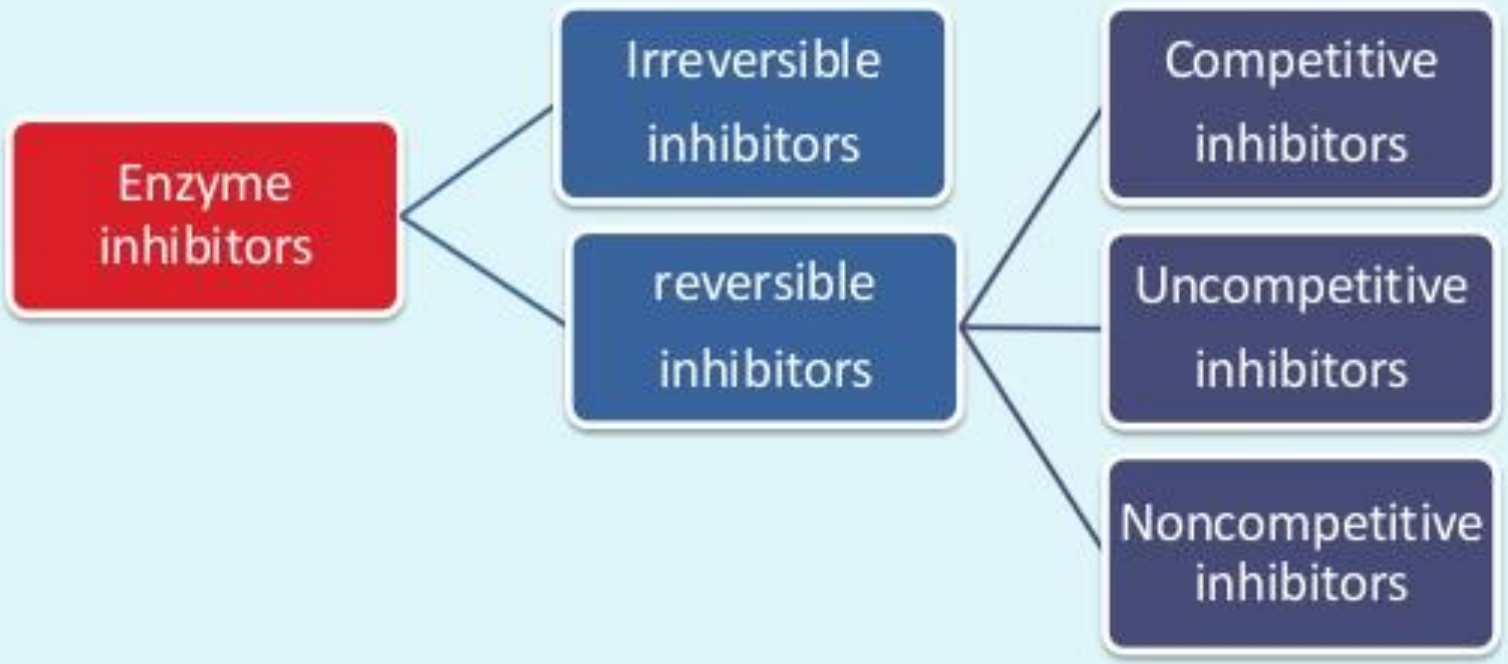
1. Inhibition :

The prevention of an enzyme process as a result of interaction of inhibitors with enzyme.

Inhibitors:

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called inhibitor.

Enzyme inhibitors



The nature of enzyme inhibitors :

Irreversible inhibitors : Inhibition of enzyme activity by combining with active site.

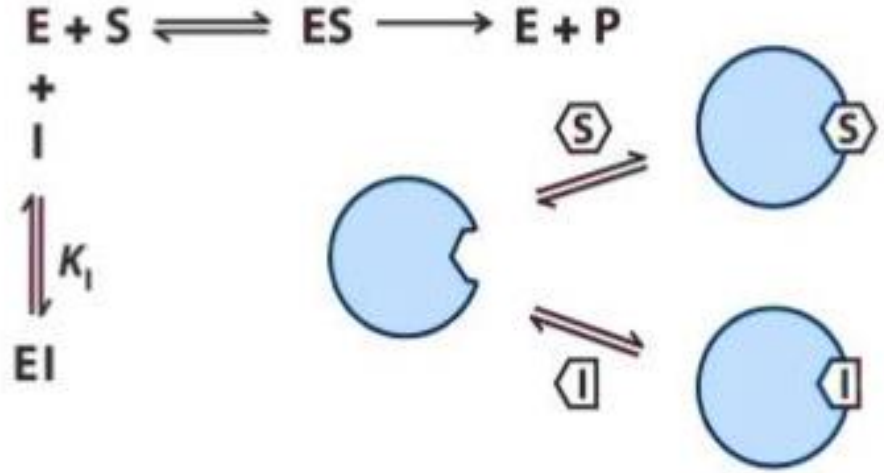
Reversible inhibitors : Inhibitors binds non-covalently with the enzyme and the enzyme can be reversed if the inhibitor is removed.

a-Competitive inhibitors : Inhibition of enzyme activity by competing with active site .

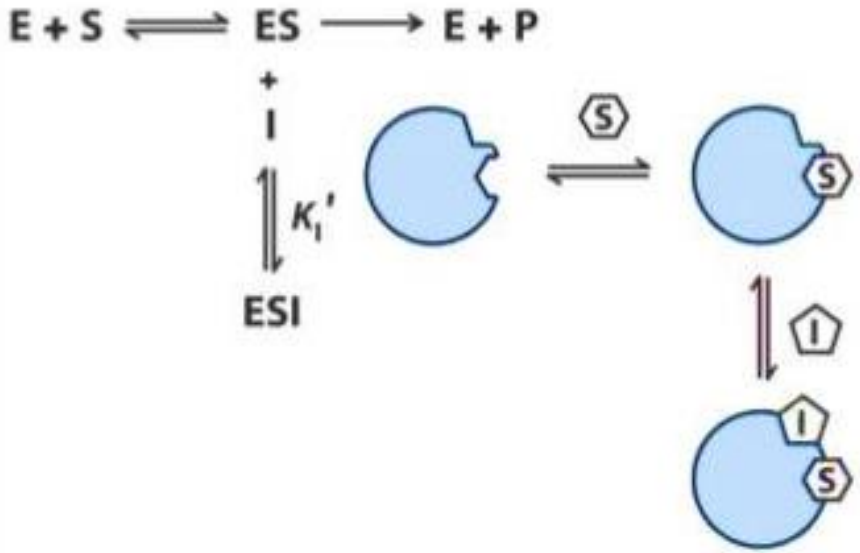
b-Un Competitive inhibitors : Inhibition of enzyme activity by combining with allosteric site.

c-Non Competitive inhibitors : Inhibition of enzyme activity by combining with both to free enzyme and ES at allosteric site.

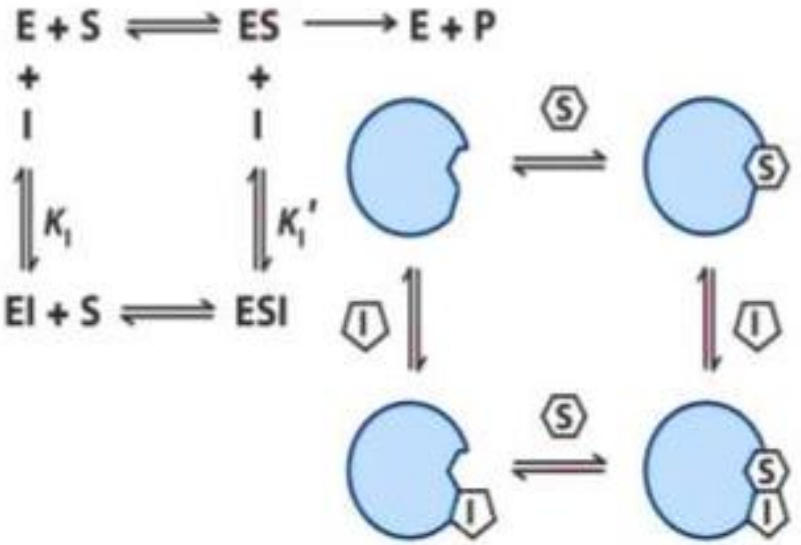
(a) Competitive inhibition



(b) Unccompetitive inhibition



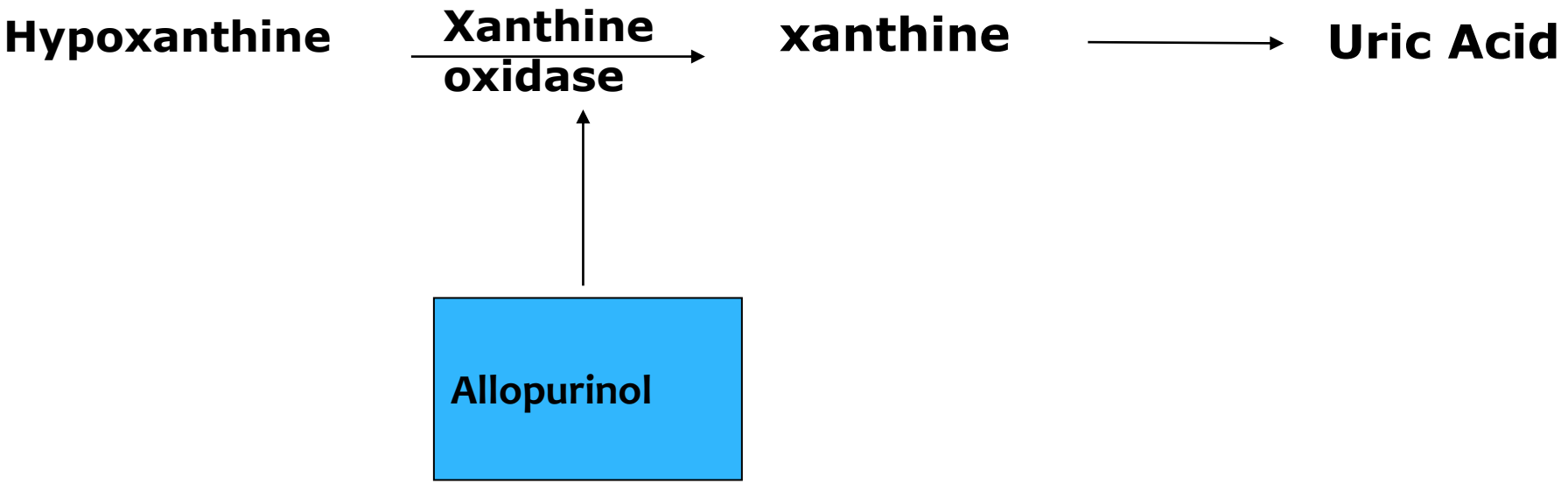
(c) Mixed inhibition



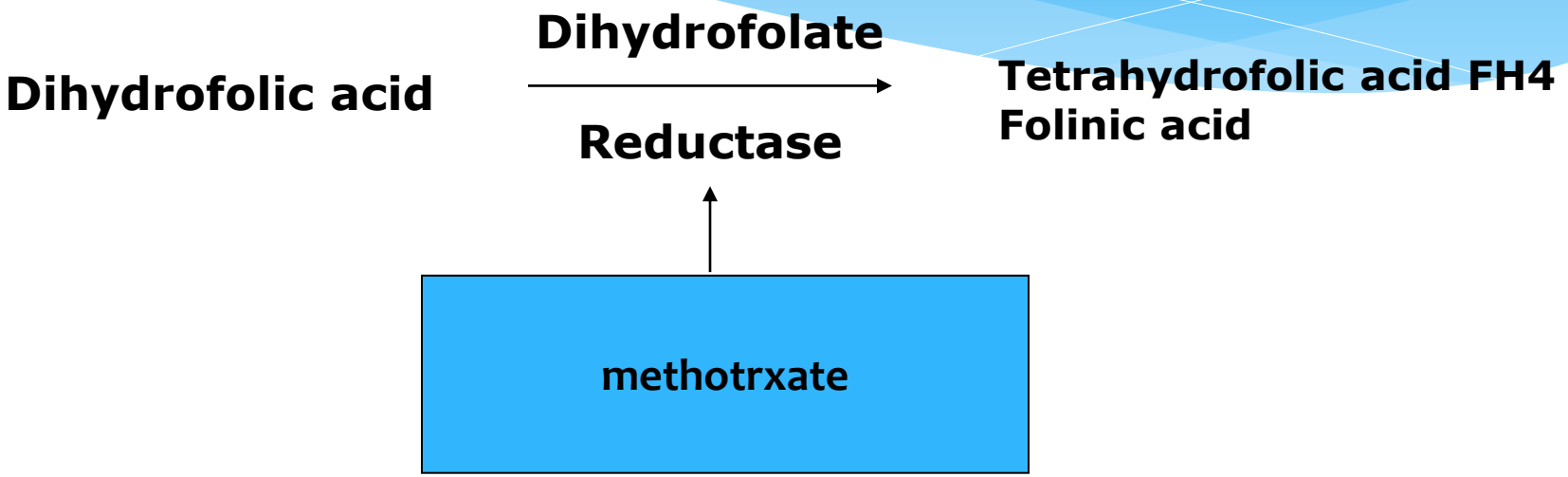
a. Competative inhibition:

The inhibitor is closely resemble the substrate {S} substrate analogue.

The inhibitor competes with the substrate and binds at the active site of the enzyme but doesn't undergo any catalysis.
Ex. Of clinical &pharmacological inhibition.



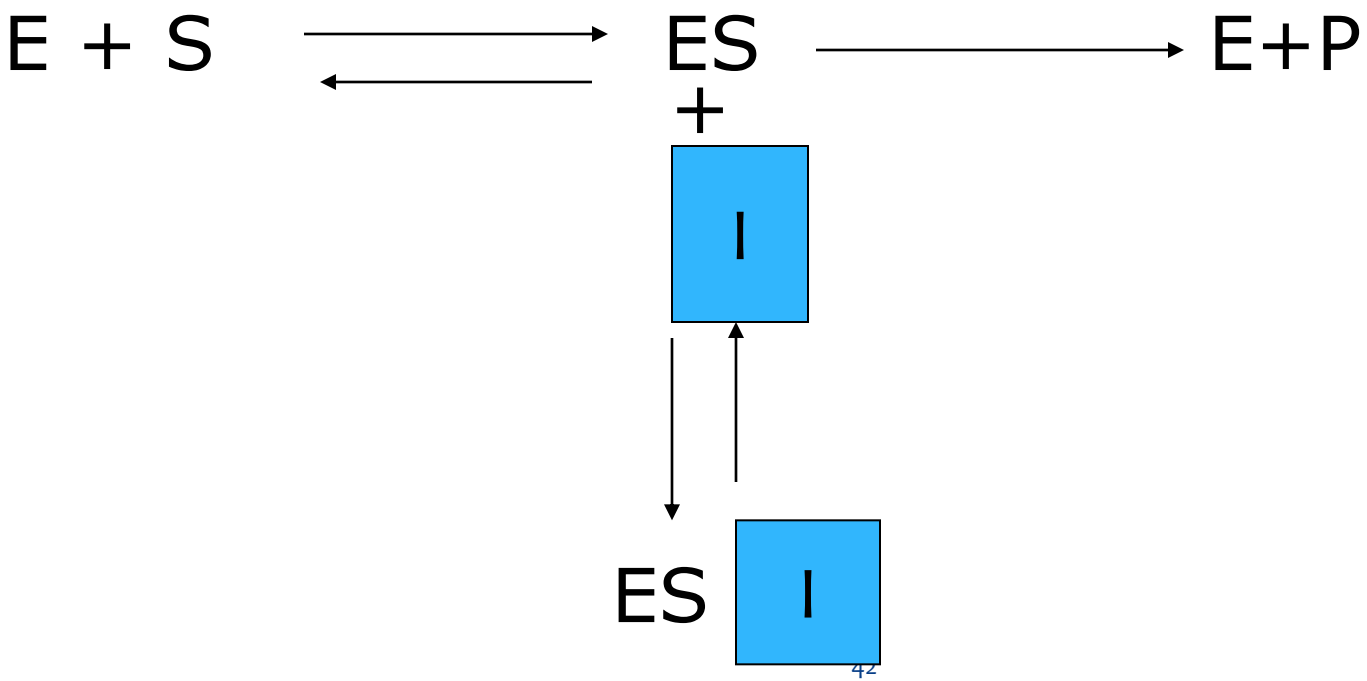
Allopurinol acts as competitive inhibitor to xanthine oxidase , it competes with enzyme xanthine oxidase and prevent or block the reaction.
so control Gout (decrease uric acid production).



Antimetabolites : are chemical compound that block the reaction by their inhibitor effect on the enzyme .
Antimetabolite are structural analogues of substrate and they are a competitive inhibitor .
They are used for cancer therapy

b. Un-competitive inhibition:

Reversible inhibition, not very common ,the inhibitor doesn't bind with the enzyme but only bind with enzyme-substrate complex



C-Non-competitive Inhibitor:

The inhibitor binds at the site other than the active site on the enzyme surface & impair the enzyme function with strong affinity for the inhibitor to bind and prevent the catalysis possibly due to in the enzyme conformation.

Ex: heavy metal ions $\longrightarrow$ $Ag^{+2}, Pb^{+2}, Hg^{+2}$

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

Ex. Hg^{+2} heavy metal ion as non competitive inhibitor.

2.Irreversable Inhibition:

The inhibitors bind covalently with the enzymes and inactivate them which is irreversible and the inhibition is usually toxic substance.

Ex : Di isopropyl flurophosphate (DFP) is a nerve gas irreversibly bind with enzyme at the active site.

Organophosphorous compound (insecticides) block the activity of Ach.esterase essential for nerve conduction resulting in paralysis of vital body function.

Diagnostic importance of Enzymes:

Measurement of enzyme activities in biological fluid (plasma/serum) is of great clinical importance.

Enzymes in the circulation is divided into two groups:

- ✓ **plasma specific or plasma functional enzymes:**

Certain enzymes are normally present in the plasma and they have specific function.

Generally these enzymes activities are higher in plasma than in tissues. They are mostly synthesized in the liver and enter the circulation.

Ex: lipoprotein lipase plasmin, choline esterase.

Impairment of liver function or genetic disorders leads to fall in the activities of plasma function enzyme.

- ✓ **Non –plasma specific:** these enzymes are either totally absent or present at a low concentration in plasma compared to their level found in the tissues.

All GIT enzymes ,amylase, pepsin , trypsin present in the plasma are known as secretary enzymes.

Measurement of the activities of non plasma specific is important in the diagnosis and prognosis of several diseases

Serum enzyme (elevated)	*Disease (most important)*
Amylase	Acute pancreatitis
Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
Alkaline phosphatase	Rickets, obstructive jaundice
Acid phosphatase	Cancer of prostate gland
Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
Creatine phosphokinase (CPK)	Myocardial infarction (early marker)
Aldolase	Muscular dystrophy
5′-Nucleotidase	Hepatitis
γ-Glutamyl transpeptidase (GGT)	Alcoholism

Specificity of enzyme action

Narrow Enzyme specificity	• Maltase hydrolyzes only maltose • Urease acts on urea
Broad Enzyme specificity	• Proteases hydrolyze peptide linkages • Exopeptidases hydrolyze terminal of protein chain. • Endopeptidases hydrolyze within protein chain
stereo specificity	• L-amino acid oxidase acts on L-amino acids. • D-amino acid oxidase acts on D-amino acids

Isozymes or Isoenzymes

are enzymes with the same function but slightly different structural features. The reason for their existence is not unknown, but they are made use of clinically. Lactate dehydrogenase (LDH), creatine kinase, and alkaline phosphatase all occur in isoenzyme form and are diagnostic value. LDH has five forms.

CLINICAL SIGNIFICANCE OF RELATIVE AMOUNT OF LDH

Condition	Isoenzyme Pattern
Myocardial Infarction	Moderate elevation of LDH_1; Slight elevation of LDH_2
Acute Hepatitis	Large elevation of LDH_5; Moderate elevation of LDH_4
Muscular Dystrophy	Elevation of LDH_1, LDH_2, LDH_3
Megaloblastic Anemia	Large elevation of LDH_1
Sickle-cell Anemia	Moderate elevation of LDH_1, LDH_2
Arthritis with Joint effusions	Elevation of LDH_5

Allosteric regulation

is the regulation of an enzyme or other protein by binding an effector molecule at the enzyme's allosteric site (that is, a site other than the active site).

Effectors that enhance the protein's activity are referred to as *allosteric enzymes*, whereas those that decrease the protein's activity are called *noncompetitive inhibitors*.

Allosteric regulation

This control of key enzymes is utmost importance to ensure that biologic processes remain coordinated at all times to meet the immediate metabolic needs of the cells.

Thank you