

BIOCHEMISTRY

UNIT 5 NOTES

ENZYMES

- NOMENCLATURE & IUB CLASSIFICATION
- ENZYME INHIBITORS
- REGULATION OF ENZYMES
- COENZYMES



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ENZYMES

- An Enzyme is a type of protein that acts as a biological catalyst. In living organism.
- Catalyst are substances that accelerates chemical reactions without being consumed or permanently altered in the process.
- Enzymes are essential for life because they regulate the speed of biochemical reactions within cell and tissues, allowing them to occur at rates necessary for metabolism, growth & survival.

Properties / Characteristics OF Enzymes

- Enzymes increases rate of reaction without being consumed in the reaction.
- Enzymes are specific for their substrate
- They are generally Heat sensitive
- Enzymes are generally reusable & remain unchanged after reaction.
- The temperature & pH in which they exhibit maximum catalytic activity is known as Optimal Temperature & pH
- Enzymes are protein with high molecular weight.
- All enzymes are protein in nature except group of catalytic of RNA.

Factors Affecting Enzyme Activity

- Concentration of Substrate
- Concentration of Enzymes
- Temperature
- pH
- Inhibitors
- Activators
- Coenzymes

NOMENCLATURE & IUB CLASSIFICATION OF ENZYMES

- In early days, enzymes were given name by their discoverers in an arbitrary manner, that convey no information about the function of enzyme.
- The nomenclature of few enzymes was named according to their sources by adding suffix - 'in' like :
 - Pepsin
 - Trypsin etc.
- The molecule upon which enzyme act is known as Substrate.
- Most of the enzymes were named by adding suffix 'ase' in the name of substrate on which they act such as
 - Hydrolases (catalyzing hydrolysis)
 - Maltase (act on Maltose)
 - Tyrosinase (act on Tyrosine)
 - Sucrase (Act on Sucrose)

IUB CLASSIFICATION

- The International Union Of Biochemistry (IUB) appointed an Enzyme Commission in 1961.
 - The committee made a thorough study of existing enzymes and devised some basic principle for classification & nomenclature of enzymes.
 - Since 1964, the IUB system of enzyme classification has been in force.
 - According to this classification enzymes are divided into six major classes.
 - These are as follows.
- ① Oxidoreductases
 - ② Transferases
 - ③ Hydrolases
 - ④ Lyases
 - ⑤ Isomerases
 - ⑥ Ligases

OXIDOREDUCTASES

- These are the enzymes that involves the oxidation - reduction reactions, involving transfer of electrons between substrates.
- Example : Alcohol Dehydrogenase, Cytochrome C Oxidase

TRANSFERASES

- These are the enzymes that are involved in the transfer of functional groups b/w molecules.
- Example : Hexokinase, Transaminases.

HYDROLASES

- They catalyzes the hydrolysis (breakdown) of various bonds by adding water molecules.
- Example : Lipase , Protease, Urase etc.

LVASES

- It catalyzes the addition or removal of groups to form double bonds or reverse.
- Example : Decarboxylase , Aldolase etc.

ISOMERASES

- These are the enzymes that catalyzes the rearrangement of atoms within a molecule to form isomers.
- Example : Isomerase , Epimerase

LIGASES

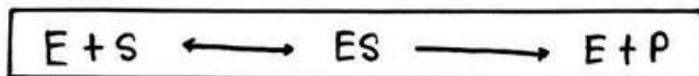
- These are the enzymes that catalyzes the synthesis reactions, where two molecules joined together using ATP.
- Example : DNA Ligase.

MECHANISM OF ENZYME ACTION

- Mechanism of enzyme action involves several key steps that facilitate the catalysis of biochemical reactions.
- Enzymes are biological catalyst that accelerate the rate of reaction through which substrate converts into product.
- It involves following steps
 - ① Substrate Binding
 - ② Formation of Enzyme Substrate Complex
 - ③ Catalysis
 - ④ Product Formation
 - ⑤ Enzyme Regeneration

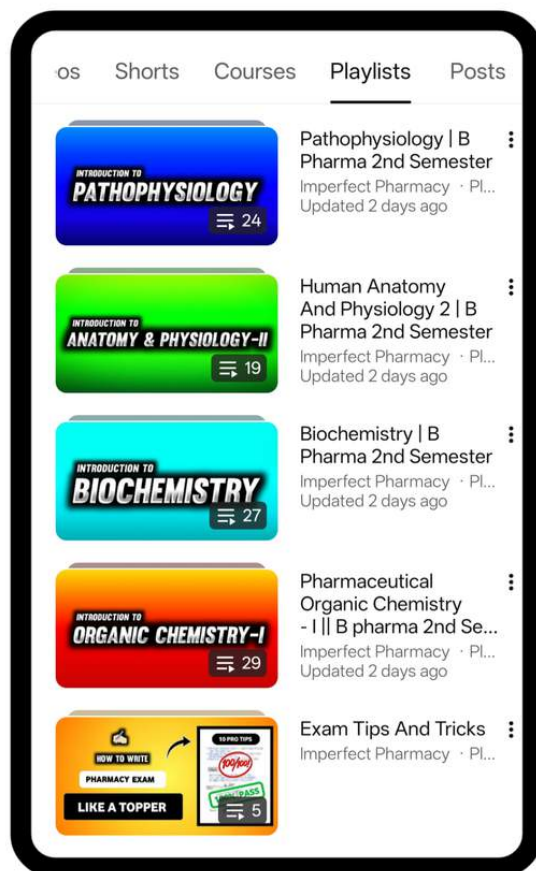
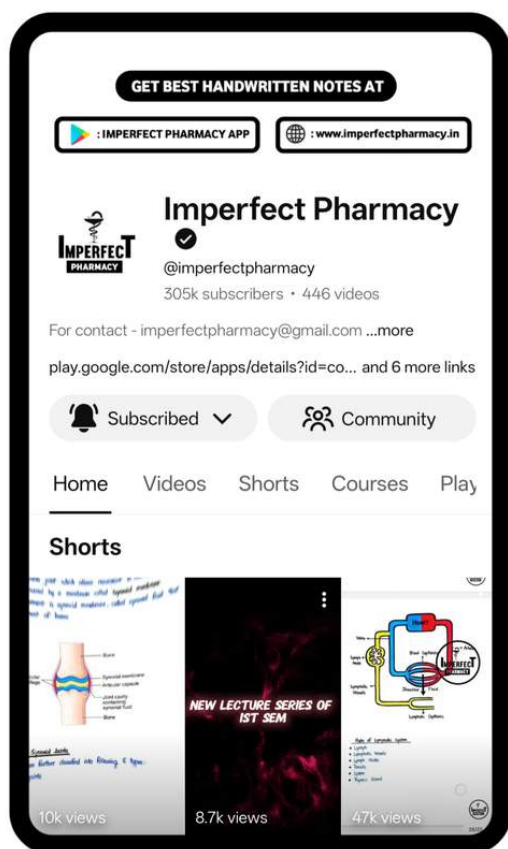
Enzyme Substrate Complex Formation

- The prime requirement for enzyme catalysis is that substrate must combine with the enzyme at active site to form **Enzyme Substrate Complex** that ultimately results in product formation.

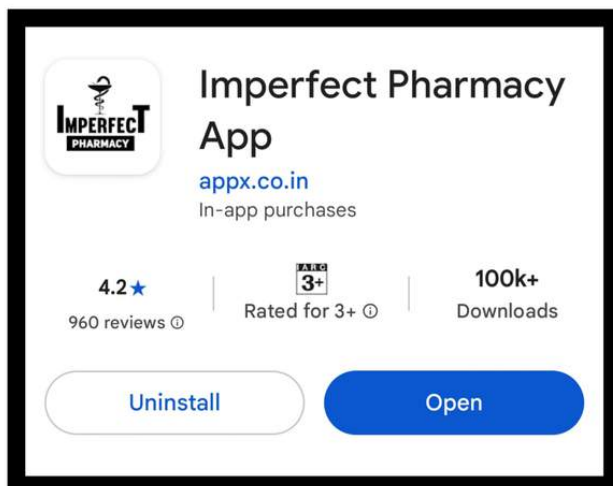


- A few theories have been put to explain the mechanism of Enzyme-Substrate Complex Formation.
- Lock & key Model is one of them & most acceptable one.

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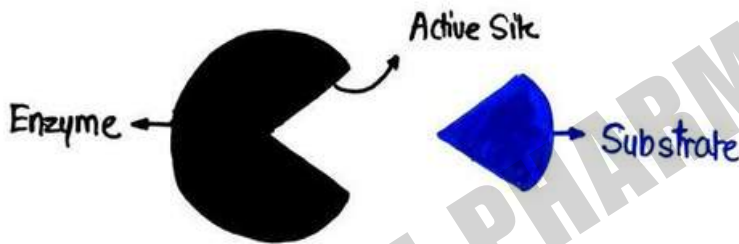


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Lock & key Model

- This theory was proposed by a German biochemist, Emil Fischer
- This the very first model proposed to explain an enzyme catalysed reaction.
- According to this model structure of enzyme is rigid & fix.
- The substrate fits to the binding site just as key fits into the proper lock.
- The active site of an enzyme is rigid where only a specific substrate can bind.



ENZME KINETICS

- Enzyme kinetics is study of rates at which enzyme catalyzes chemical reactions.
- It defines how fast chemical reactions occur when catalyzed by enzymes.
- It is mainly studied through Michaelis - Menten Equation.

Michaelis - Menten Equation

- The Michaelis - Menten equation describes the rate of enzymatic reactions as a function of substrate concentration.
- It is given as :

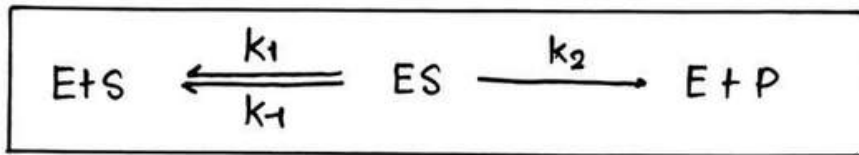
$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

- Here ,
 - V_0 = Initial reaction velocity
 - $V_{\max}$ = Maximum reaction velocity
 - $[S]$ = Substrate Concentration
 - K_m = The Michaelis - Menten Constant
- The Michaelis Menten equation assumes steady state conditions where rate of formation of enzyme - substrate complex equals the rate of its breakdown to form product.

Derivation

- Michaelis - Menten equation derived on the basis of STEADY STATE ASSUMPTION.
- According to this :

Rate of ES Formation = Rate of ES Breakdown



① Rate of E-S Formation

$$\frac{d_1[ES]}{dt} = k_1 [E][S]$$

② Rate of E-S Breakdown

$$\frac{d_2[ES]}{dt} = k_{-1}[ES] + k_2[ES]$$

Now, Rate of Formation = Rate of Breakdown

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

$$k_1[E][S] = [ES](k_{-1} + k_2)$$

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

$$\text{Now } \frac{k_{-1} + k_2}{k_1} = k_m$$

So, we get $\frac{[E][S]}{[ES]} = k_m$ ——— ①

Now Total Enzyme $[E^0] = [E] + [ES]$

$$[E] = [E^0] - [ES] \text{ ——— ②}$$

Now putting the value of $[E]$ from ② to ①

$$\frac{([E^0] - [ES])[S]}{[ES]} = k_m$$

$$([E^0] - [ES])[S] = k_m [ES]$$

$$[E^0][S] - [ES][S] = k_m [ES]$$

$$[E^0][S] = k_m [ES] + [ES][S]$$

$$[E^0][S] = [ES] (k_m + [S])$$

$$[ES] = \frac{[E^0][S]}{k_m + [S]} \text{ ——— ③}$$

② Rate of Product Formation

$$\frac{dP}{dt} = k_2 [ES]$$

$$V_0 = k_2 [ES] \quad \text{--- ④}$$

putting value of ③ in ④

$$V_0 = \frac{k_2 [E^0] [S]}{k_m + [S]}$$

$$\text{Now } k_2 [E^0] = V_{\max}$$

$$V_0 = \frac{V_{\max} [S]}{k_m + [S]}$$

ENZYME INHIBITION

- Enzyme Inhibition refers to the process where a molecule (Inhibitor) binds to an enzyme and decreases or completely stops its activity.
- Enzyme Inhibition is performed by Enzyme Inhibitors.
- It can either prevent the formation of Enzyme-Substrate Complex or can prevent ES breakdown to Enzyme + Product.
- Inhibitors can be either organic or inorganic in nature.

Types Of Enzyme Inhibition

There are mainly 3 types of enzyme inhibition :

- ① Reversible Inhibition
- ② Irreversible Inhibition
- ③ Allosteric Inhibition

REVERSIBLE INHIBITION

- In this, inhibitors bind to with Enzyme through Non-Covalent Bond.
 - This enzyme inhibition is reversed, if inhibitor is removed.
 - It can be further subdivided into 2 types :
- ① Competitive Inhibition
 - ② Non-Competitive Inhibition

① Competitive Inhibition

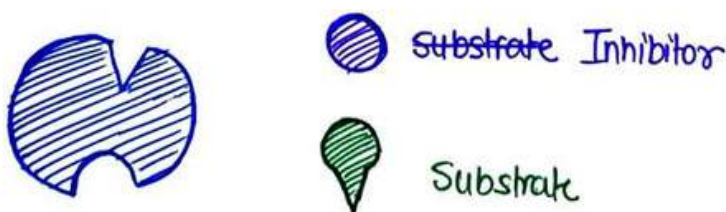
- In this, inhibitor closely resembles the real substrate.
- The inhibitor competes with substrate molecule for binding on the active site.
- As long as inhibitor holds the active site, the enzyme is not available for substrate to bind.

② Non-Competitive Inhibition

- In this inhibitor binds at a site other than active site on enzyme surface.
- The inhibitor has no structural resemblance with the substrate.



Competitive Inhibition



Non-Competitive Inhibition

IRREVERSIBLE INHIBITION

- Irreversible Inhibition refers to a type of enzyme inhibition where inhibitor forms a stable covalent bond with the enzyme, permanently inactivating its catalytic activity.
- They permanently deactivate the enzyme.
- They generally destroy the functional group on the enzyme that is essential for its activity.

ALLOSTERIC REGULATION

- Allosteric Regulation is a mechanism of enzyme activity modulation where a molecule binds to Allosteric site of enzyme distinct from active site, leading to a conformational change in enzyme's structure.
- It is seen only in some specific enzymes.
- The conformational change alters the enzyme's catalytic activity, either enhancing or inhibiting its function.

Properties Of Allosteric Regulation

- Allosteric Enzymes have two binding site.
 - (1) Active Site
 - (2) Allosteric Site.
- Allosteric effect can be either activating or inhibiting.
- It leads to conformational change in enzyme's tertiary or quaternary structure.
- Allosteric regulation is crucial for regulating metabolic pathways & maintaining cellular Homeostasis.

COENZYMES

- The enzymes, sometimes are not always sufficient to show their catalytic activity.
- Many enzymes requires certain non-protein additional cofactors to show their activity.
- The non protein, organic, low molecular weight substance that is required for some enzymes to show their catalytic activity is known as Coenzyme.

Properties Of Coenzymes

- Coenzymes cannot function alone but can be reused several times when paired with an enzyme.
- Enzyme without a coenzyme is known as Apoenzyme.
- Enzyme along with a coenzyme is known as Holoenzyme.
- Coenzymes undergo alterations during enzymatic reactions.
- They participate in various reactions involving transfer of atoms or groups like Hydrogen, Aldehyde, keto, Amino Carbon - Di - Oxide etc.
- Coenzymes are usually recycled or regenerated during enzymatic reactions.
- They are specific for particular enzyme or classes of enzymes.

Example Of Coenzymes

Certain most useful example of coenzymes are as follow :

- ~~NAD~~ NAD⁺ (Nicotinamide Adenine Dinucleotide)
- FAD (Flavin Adenine Dinucleotide)
- CoA (Coenzyme A)
- TPP (Thiamine Pyrophosphate)
- THF (Tetrahydrofolate)
- ATP (Adenosine Triphosphate)

Functions Of Coenzymes

- The function of coenzyme is to transport groups between enzymes .
- Chemical Groups inside hydride ions are carried by coenzymes such as NAD⁺
- Some coenzymes also acts as Allosteric Regulators .
- They regulate various metabolic pathways.

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