BIOCHEMISTRY

UNIT 5 NOTES

IMPORTANT TOPICS

Rx Pharma Education

IMPORTANT QUESTIONS OF UNIT-5

① Define Enzymes.

- Enzymes are the biocatalyst : the catalyst of life.
- A catalyst is defined as a chemical substance that increases the rate of chemical reaction without doing any change in the overall process.
- Enzymes are synthesized by living cells.
- The word Enzyme was first used by Frederick W. kuhne in 1878.

Properties Of Enzymes

- All the enzymes are proteins except group of catalytic RNAs
- The molecular weight of enzymes ranges from 12,000 to 1 million or more.
- As we know enzymes are catalyst in nature & this catalytic activity of enzymes is due the their primary, secondary, teotiary & quaternary structures of protein & their specific conformation.
- Most of the enzymes needs only amino acid to show their effect but some enzymes needs cofactor & conenzymes foo their activity.
- Enzymes only affect the rate of biochemical reaction not the direction of biochemical reaction.
- Enzymes are specific to substrate of a reaction.
- Enzymes remain unchanged after a reaction & therefore can work again.
- Enzymes are also specific to pH

Nomenclature & Classification of Enzymes

- In early days enzymes were named by adding the suffix - 'ase' to substrate like urase enzymes catalyses urea etc.
- Some enzymes were also named on the basis of function they were performing like **Pepsin** is a digestive enzyme, derived from Greek word 'pepsis' means '**digestion**'.
- As the time passed several new enzymes were discovered.
- as a result, some uncertanties started occuring in their naming.
- To overcome these uncertainities **IUB** (**International Union of Biochemistry**) classified the enzymes into six major classes on the basis of type of reaction they catalyse.

① Oxidoreductases

Enzymes involved in oxidation- reduction reactions.

② Transferases

Enzymes that catalyses the transfer of functional groups.

③ Hydrolases

Enzymes that brings about hydrolysis of various compounds.

④ Lyases

Enzymes specialised in the addition or removal of water, ammonia & CO_2 etc.

⑤ Isomerases

Enzymes involved in all the isomerization reactions.

⑥ Ligases

Enzymes catalysing the synthetic reactions.

Mechanism of enzyme action

① Enzyme - Substrate Complex Formation

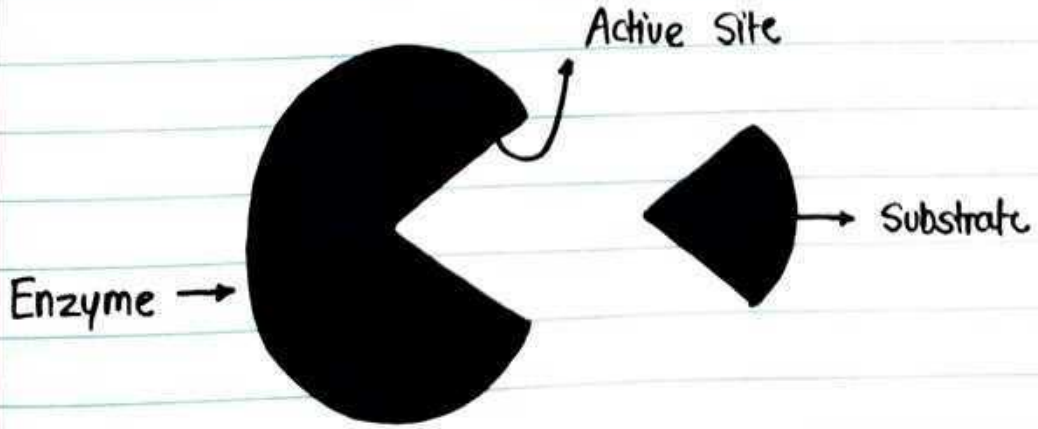
The prime requirment for enzyme catalysis is that the substrate must combine with the enzyme at the active site to form Enzyme - substrate complex that ultimately results in product formation.

$$E + S \longleftrightarrow ES \longrightarrow E + P$$

A few theories have been put to explain the mechanism of enzyme - substrate complex formation.

Lock And Key Model

- This theory was proposed by a German biochemist, Emil Fischer
- This is the very frost model proposed to explain an enzyme catalysed reaction.
- According to this model, the structure of enzyme is rigid.
- 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.



Factors Affecting Enzyme Catalyzed Reactions

- Effect of substrate concentration.
- Effect of Inhibitors.
- Effect of pH
- Effect of Temperature
- Effect of Pressure
- Effect of water

Application of Enzyme

- Enzymes are used for aiding digestion. example : Amylases, Lipase etc.
- They are used as deworming Agent. e.g. Popain.
- They act as anti-clotting agents like fibrinolytic & thrombolytic.
- They act to treat atherosclerosis
- They are used to treat wounds & swelling.
- They are used as surface disinfectants.
- They are also used in the diagnosis purpose. Example : Glucose Oxidase along with peroxidase to detect the level of glucose.
- Liver Disease : gama glutamyl transpephidase
- Heart Attacks : Aspartate Aminotransferase
- Uric acid : Uricase

② Define Coenzymes

- The enzymes sometimes are not always adequate to show their catalytic activity.
- Many enzymes required certain non-protein additional factors to show their activity.
- The non-protein, organic, low molecular weight & dialysable 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 called as Apoenzyme.
- Without coenzyme, enzymes cannot catalyze reactions effectively.
- Coenzymes undergo alterations during the enzymatic reactions.
- Coenzymes participates in various reactions involving transfer of atoms or groups like hydrogen, aldehyde, keto, amino carbon-di-oxide etc.
- Coenzymes like enzymes can be reused & recyled without changing reaction rate or effectiveness.
- When enzyme is denatured by extreme temperature or pH, the coenzyme can no longer attach to the site.

Coenzyme forms B-Complex Vitamins

Most of the coenzymes are derivatives of water soluble B-complex vitamins.

example
- Thiamine Pyrophosphate derived from Thiamine (Vit B_1)
- Flavin Mononucleotide derived from Riboflavin (Vit B_2)

Non- Vitamin Coenzymes

Not all coenzymes are vitamin derivatives.

example
- Cytidine Diphosphate
- Uridine Diphosphate

Biochemical Functions Of Coenzymes

- The function of coenzymes is to transport groups between enzymes.
- Chemical groups inside hydride ions are carried by coenzymes such as NAD.
- A coenzyme is a low molecular weight organic substance without which enzyme cannot exhibit any reaction.
- A coenzymes prepares the active site for catalytic activity.
- It is necessary helper for enzymes that assist in biochemical transformations.

- Michaelis Menten Equation -

- Enzyme is said to increase the rate of reac^n
- However to measure the rate of reac^n is not possible.
- Michaelis & Menten derived an equation to determine 'Vo' - rate of reaction.

Overall Reaction of product forma^n is :-

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P \quad -(1)$$

Michaelis & Menten derived equa^n on the basis of :-
"STEADY STATE ASSUMPTION".

Initially, at the start of reac^n ⇒ Product is Negligible

∴ Rate of reaction (Vo) is determined by the breakdown of ES to form product;

$$V_o = K_2 [ES] \quad — \quad (2)$$

As ES cannot be easily measured experimentally an alternative term is introduced.

$[E_T]$: Total enzyme Conc. (Sum of free & substrate bound enzyme)

$[E_T] - [ES]$: Free or Unbound enzyme

$[S] >>> [E_T]$

Conc. Conc.

Step 1: K_1: Rate constant for formation of ES
$K_{-1} + K_2$: Rate constants for breakdown of ES

∴ Rate of ES Formation = $K_1 [E][S]$

$= K_1 \{[E_T] - [ES]\}[S]$ — (4)

Rate of ES Breakdown = $K_{-1}[ES] + K_2[ES]$ — (5)

Step 2: An Assumption that a steady state is seen initially in the reac^n i.e. rate of formation of ES is EQUAL to rate of breakdown of ES (Rate of fwd reac^n = Rate of bkward r^n)

Hence, equating eq^n (4) & (5)

$$K_1 \{[E_T] - [ES]\}[S] = K_{-1}[ES] + K_2[ES]$$

$$K_1[E_T][S] - K_1[ES][S] = (K_{-1} + K_2)[ES] \quad — (6)$$

Step 3:

$$K_1[E_T][S] = (K_1[S] + K_{-1} + K_2)[ES] \quad - (7)$$

Solving eq^n for [ES]:-

$$[ES] = \frac{K_1[E_T][S]}{K_1[S] + K_{-1} + K_2}$$

$$[ES] = \frac{[E_T][S]}{[S] + (K_{-1} + K_2)/K_1} \quad — (8)$$

$\left(\frac{K_{-1} + K_2}{K_1}\right)$ is defined as the Michaelis Constant ⇒ Km

$$K_m = K_{-1} + K_2 / K_1 \quad — (9)$$

Eq^n (8) can be substituted c̄ Km & we get

$$[ES] = \frac{[E_T][S]}{[S] + K_m} \quad — (10)$$

Maximum Velocity (Vmax) occurs when enzyme is saturated i.e. $[ES] = [E_T]$ — (11)

From Eq^n (2) $V_0 = K_2[ES]$ — (2)

$$V_{max} = K_2[E_T] \quad — (12)$$

Step 4:- Substituting eq^n (10) i.e [ES] in eq^n (2)

$$V_0 = \frac{K_2 [E_T][S]}{[S] + K_m} \quad — (13)$$

Substituting value of Vmax from (12) in eq^n (13)

$$V_0 = \frac{V_{max}[S]}{[K_m + [S]]} \quad — (14)$$

This is c/as Michaelis-Menten equation.