Enzymes

Presented By;-

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Specialization:- Pharmaceutical Chemistry

INTRODUCTION

- **Enzymes are biological catalysts, mostly proteins, that accelerate chemical reactions within living organisms without being consumed in the process.**
- **They are essential for various metabolic processes, including digestion, respiration, and muscle function.**

PROPERTIES OF ENZYMES

1) **Catalytic nature**: Enzymes speed up chemical reactions without being consumed.
2) **Specificity**: Each enzyme is specific to a particular substrate or type of reaction.
3) **Efficiency**: Enzymes are highly efficient and can catalyze thousands of reactions per second.
4) **Reversibility**: Many enzymes can catalyze reactions in both forward and reverse directions.
5) **Saturation**: Enzyme activity increases with substrate concentration up to a certain point, after which it levels off.
6) **Regulation**: Enzymes can be activated or inhibited by other molecules, allowing control of metabolic pathways.
7) **Reusable**: Enzymes are not altered permanently during the reaction and can be used repeatedly.
8) **Protein nature**: Most enzymes are proteins (except ribozymes), and their function depends on their 3D structure.
9) **Sensitivity to temperature and pH**: Enzymes work best at an optimal temperature and pH; extremes can denature them.

IUBMB CLASSIFICATION OF ENZYMES

The IUBMB classification system classifies enzymes based on the type of chemical reaction they catalyze and assigns them a unique EC number. This system, maintained by the *International Union of Biochemistry and Molecular Biology (IUBMB)*

The six original classes are:-

1) **Oxidoreductases (EC 1):** Catalyze oxidation-reduction reactions, involving the transfer of electrons or hydrogen atoms.
2) **Transferases (EC 2):** Transfer a functional group between two molecules.
3) **Hydrolases (EC 3):** Catalyze hydrolysis reactions, where bonds are broken by the addition of water.
4) **Lyases (EC 4):** Catalyze the breaking of bonds by means other than hydrolysis or oxidation.
5) **Isomerases (EC 5):** Catalyze the conversion of one isomer into another.
6) **Ligases (EC 6):** Catalyze the formation of bonds between molecules, often with the expenditure of energy.

A seventh class, Translocases (EC 7), has been added:-

7) **Translocases (EC 7):** Catalyze the movement of ions or molecules across membranes or within membranes.

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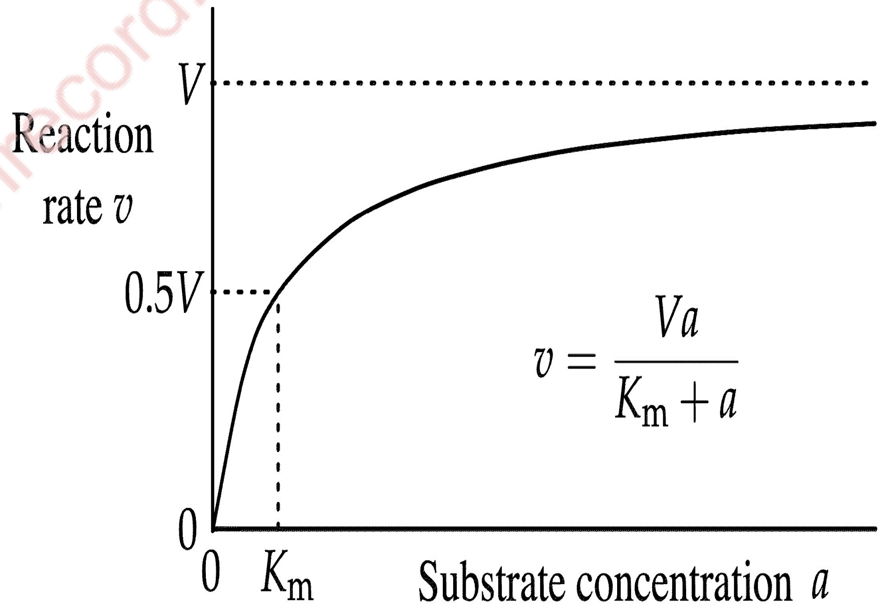
ENZYME KINETICS

- Enzyme kinetics is the study of how enzymes catalyze chemical reactions and how these reactions are affected by factors like substrate concentration, temperature, and inhibitors.
- It helps understand enzyme mechanisms, metabolic pathways, and how drugs or modifiers influence enzyme activity

MICHAELIS-MENTEN PLOT

- The **Michaelis-Menten plot** is a graphical representation of the relationship between the **rate of an enzyme-catalyzed reaction (V_0)** and the **substrate concentration [S]**.
- It's a *hyperbolic curve* that shows *how the reaction rate initially increases rapidly with increasing substrate concentration, then plateaus as the enzyme becomes saturated.*
- The plot is a visual representation of the Michaelis-Menten equation, which describes the kinetics of enzyme-substrate reactions.



MICHAELIS-MENTEN EQUATION

Michaelis-Menten Equation:-

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

Key Terms:

1. **V_0 (Initial velocity)**: Reaction rate at the beginning before the substrate is significantly depleted.
2. **V_{max} (Maximum velocity)**: The highest rate of reaction when the enzyme is saturated with substrate.
3. **K_m (Michaelis constant)**:
   - Substrate concentration at which $V_0 = \frac{1}{2}V_{max}$
   - Indicates enzyme's **affinity** for its substrate:
     - Low K_m = high affinity
     - High K_m = low affinity

MICHAELIS - MENTEN EQUATION DERIVATION

The **Michaelis-Menten equation** describes the rate of enzymatic reactions by relating reaction rate (**v**) to substrate concentration (**[S]**). Here's a clear step-by-step derivation:-

Step 1: The Basic Reaction

An enzyme (E) binds a substrate (S) to form an enzyme-substrate complex (ES), which can either dissociate back or form product (P):-

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Step 2: Assumptions

1.Steady-State Assumption:

The concentration of the ES complex remains constant over time:- $$\frac{d[ES]}{dt} = 0$$

2.Initial Velocity (v_0):

Measure the rate before significant product accumulates:- $$v = \frac{d[P]}{dt} = k_2[ES]$$

3.Total Enzyme Concentration

$$[E]_T = [E] + [ES]$$

Step 3: Express [ES]

From the steady-state assumption:-

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

Solve for [ES]:

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

Define:

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

Now, express [E] in terms of total enzyme:

$$[E] = [E]_T - [ES]$$

Substitute back:

$$[ES] = \frac{([E]_T - [ES])[S]}{K_m}$$

Solve for [ES]:

$$[ES](K_m + [S]) = [E]_T[S] \Rightarrow [ES] = \frac{[E]_T[S]}{K_m + [S]}$$

Step 4: Final Equation

Now plug [ES] into the velocity equation:

$$v = k_2[ES] = k_2 \cdot \frac{[E]_T[S]}{K_m + [S]}$$

Define:

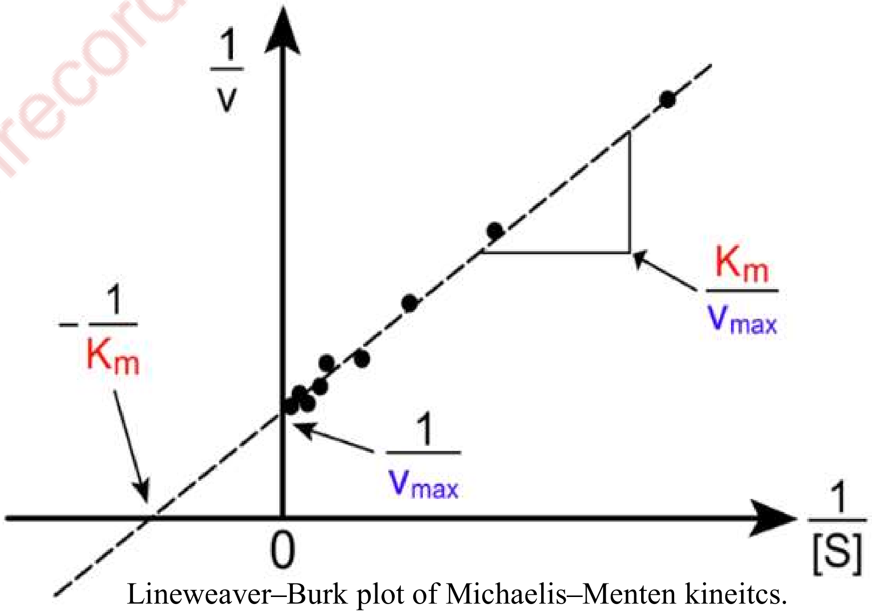
$$V_{\max} = k_2[E]_T$$

So the **Michaelis-Menten Equation** is:

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

LINE-WEAVE BURKE PLOT

- The Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.
- The Lineweaver-Burk plot ***results*** in a straight line with the slope equal to **$K_M/k_2[E]_0$** and y-intercept equal to **$1/k_2[E]_0$** which is **$1/V_{max}$** via Equation.



Lineweaver–Burk plot of Michaelis–Menten kineitcs.

LINE-WEAVE BURKE EQUATION

The plot provides a useful graphical method for analysis of the Michaelis–Menten equation:

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

Taking the reciprocal gives

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

where

- **V** is the reaction velocity (the reaction rate),
- **Km** is the Michaelis–Menten constant,
- **Vmax** is the maximum reaction velocity, and
- **[S]** is the substrate concentration.

The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as Km and Vmax, before the wide availability of powerful computers and non-linear regression software.

ENZYME INHIBITORS

Inhibitor Name	Target Enzyme
Methotrexate	Dihydrofolate reductase
Aspirin	Cyclooxygenase (COX-1 and COX-2)
Penicillin	Transpeptidase (bacterial cell wall synthesis)
Allopurinol	Xanthine oxidase
Atorvastatin	HMG-CoA reductase
Neostigmine	Acetylcholinesterase
Captopril	Angiotensin-converting enzyme (ACE)
Sildenafil	Phosphodiesterase type 5 (PDE5)
Disulfiram	Aldehyde dehydrogenase
Ritonavir	HIV protease
Fluoxetine	Serotonin reuptake transporter (indirectly inhibits degradation enzymes)
Cyanide	Cytochrome c oxidase (in mitochondrial ETC)
Arsenic	Pyruvate dehydrogenase

ENZYME INDUCTION AND REPERSSION

- **Induction:** **Increases** enzyme synthesis in response to a specific stimulus (e.g., substrate, inducer), leading to higher enzyme levels.
- **Repression:** **Decreases** enzyme synthesis in response to a specific stimulus (e.g., end product, corepressor), leading to lower enzyme levels.

COENZYME

- A coenzyme is defined as an organic molecule that binds to the active sites of certain enzymes to *assist in the catalysis of a reaction*.
- Coenzymes often act as carriers of electrons, atoms, or functional groups transferred in the reaction.

COENZYMES AND THEIR BIOLOGICAL ROLES

Coenzyme	Derived From	Role in Enzyme Function
NAD^+ (Nicotinamide adenine dinucleotide)	Niacin (Vitamin B3)	Electron carrier in redox reactions
FAD (Flavin adenine dinucleotide)	Riboflavin (Vitamin B2)	Electron carrier in redox reactions
Coenzyme A (CoA)	Pantothenic acid (Vitamin B5)	Carries acyl groups (e.g., acetyl group)
TPP (Thiamine pyrophosphate)	Thiamine (Vitamin B1)	Transfers aldehyde groups; key in decarboxylation reactions
Biotin	Biotin (Vitamin B7)	Carries CO_2 in carboxylation reactions
Pyridoxal phosphate (PLP)	Pyridoxine (Vitamin B6)	Amino group transfer in transamination reactions
Tetrahydrofolate (THF)	Folic acid (Vitamin B9)	Transfers one-carbon units (e.g., methyl, methylene)
Cobalamin (Vitamin B12)	Vitamin B12	Involved in rearrangement reactions and methyl group transfers
Lipoic acid	Not vitamin-derived	Carries acyl groups and electrons in oxidative decarboxylation

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THERAPEUTIC AND DIAGNOSTIC APPLICATION OF ENZYME AND ISOENZYME

Therapeutic Applications:

1) **Leukemia:** Certain types of leukemia can be treated with bacterial asparaginase.
2) **Lens Extraction:** Chymotrypsin is used to dissolve ligaments between the lens and cornea during lens extraction.
3) **Dermal Ulcers/Burns:** Collagenase can clean dermal ulcers and severe burns by removing dead tissue.
4) **Drug Delivery:** Hyaluronidase facilitates the subcutaneous injection of drugs.
5) **Allergy Treatment:** Penicillinase is used to treat penicillin allergies.
6) **Blood Clot Dissolution:** Streptokinase and urokinase dissolve blood clots, such as those caused by myocardial infarctions.
7) **Digestive Disorders:** Enzymes like pepsin, lipase, amylase, and trypsin peptidase are used to treat digestive problems and chronic pancreatitis.
8) **Enzyme Replacement Therapy:** Treating enzyme deficiencies, such as in cystic fibrosis, helps with digestion and overall well-being.

THERAPEUTIC AND DIAGNOSTIC APPLICATION OF ENZYME AND ISOENZYME

Diagnostic Applications:

1) **Heart Attacks:** Enzymes like lactate dehydrogenase (LDH) and creatine kinase (CK) are measured to detect heart attacks.
2) **Liver Damage:** Alanine transaminase (ALT) levels are elevated in acute liver damage.
3) **Bone Diseases:** Alkaline phosphatase (ALP) is elevated in bone diseases.
4) **Prostate Cancer:** Acid phosphatase can be a marker for prostate cancer.
5) **Other Diseases:** Enzymes like ceruloplasmin and lipase are used to diagnose liver function, while creatine kinase and lactate dehydrogenase can indicate tissue damage.
6) **Metabolic Disorders:** Measuring specific enzymes can help diagnose and manage metabolic disorders.
7) **Cancer Detection:** Enzymes like cathepsin-D and cysteine cathepsins are associated with breast cancer and other cancers.

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