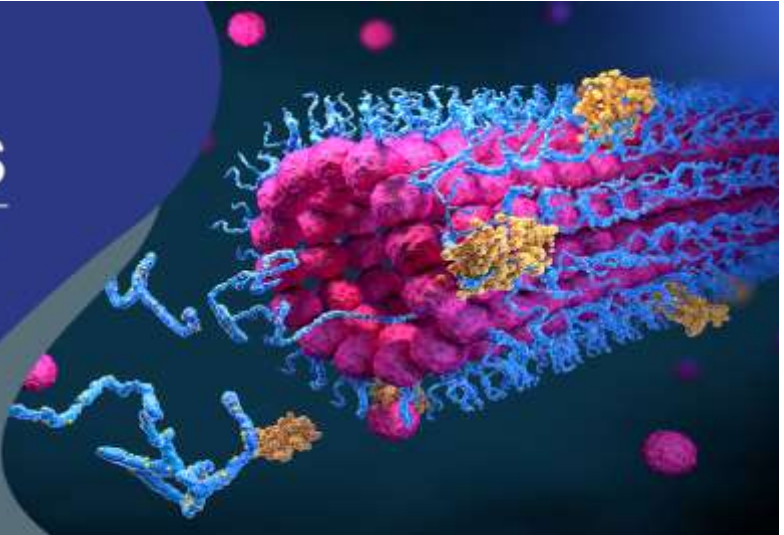


DEPARTMENT OF BIOTECHNOLOGY

ENZYMES

Essential catalyst

.....



Ms. Payal Talekar

CONTENTS

- Chemistry
- Classification
- Mechanism of Enzyme Action
- Enzyme Kinetics
- Inhibition
- Activation
- Specificity



Introduction

- Enzymes are *biological catalysts* that speed up the rate of the biochemical reaction.
- Most enzymes are three dimensional *globular proteins* (tertiary and quaternary structure).
- Some special RNA species also act as enzymes and are called *Ribozymes* e.g. hammerhead ribozyme.



Hammerhead enzyme

STRUCTURE OF ENZYMES

- The *active site* of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.
- Active sites generally occupy less than 5% of the total surface area of enzyme.
- Active site has a *specific shape* due to tertiary structure of protein.
- A change in the shape of protein affects the shape of active site and function of the enzyme.



ACTIVE SITE

- Active site can be further divided into:



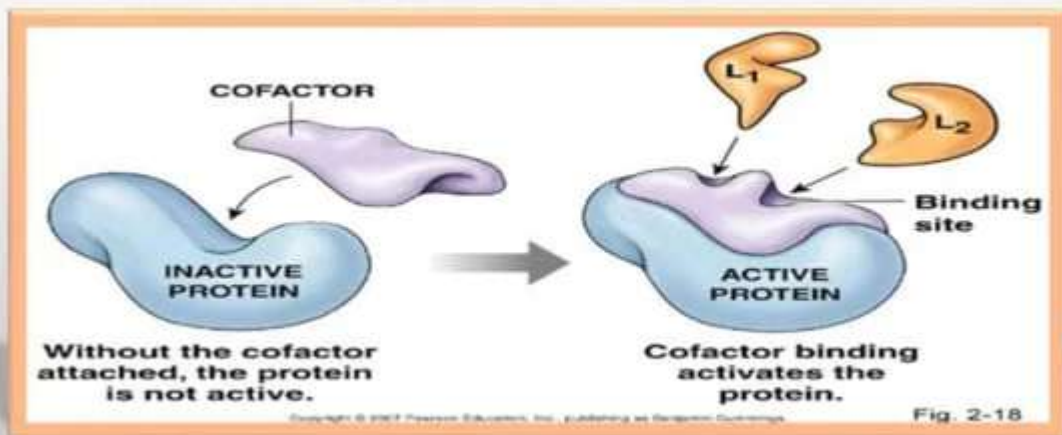
It chooses the substrate and binds it to active site.

It performs the catalytic action of enzyme.



CO-FACTORS

- Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.
- Co-factors are of two types:
 - Organic co-factors
 - Inorganic cofactors



INORGANIC CO-FACTORS

- These are the inorganic molecules required for the proper activity of enzymes.

Examples:

- Enzyme carbonic anhydrase requires Zn^{++} for its activity.
- Hexokinase has co-factor Mg^{++}

ORGANIC CO-FACTORS

- These are the organic molecules required for the proper activity of enzymes.

Example:

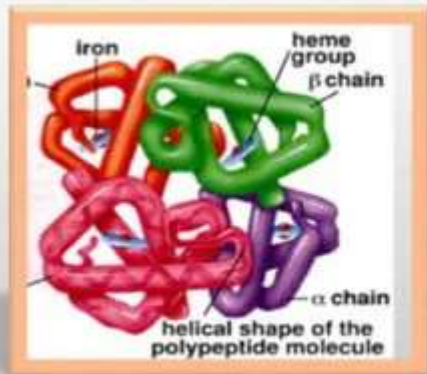
- Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate.



TYPES OF ORGANIC CO-FACTORS

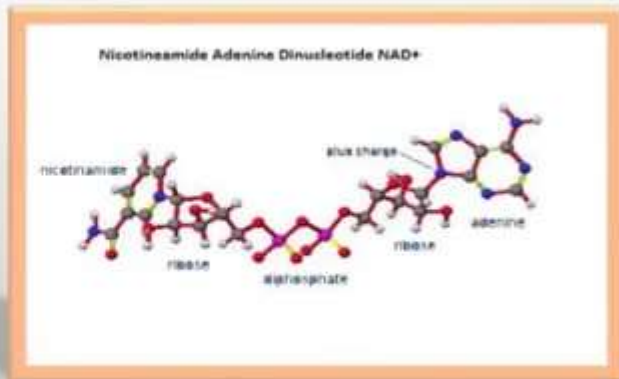
Prosthetic Group

- A prosthetic group is a tightly bound organic co-factor e.g. Flavins, heme groups and biotin.

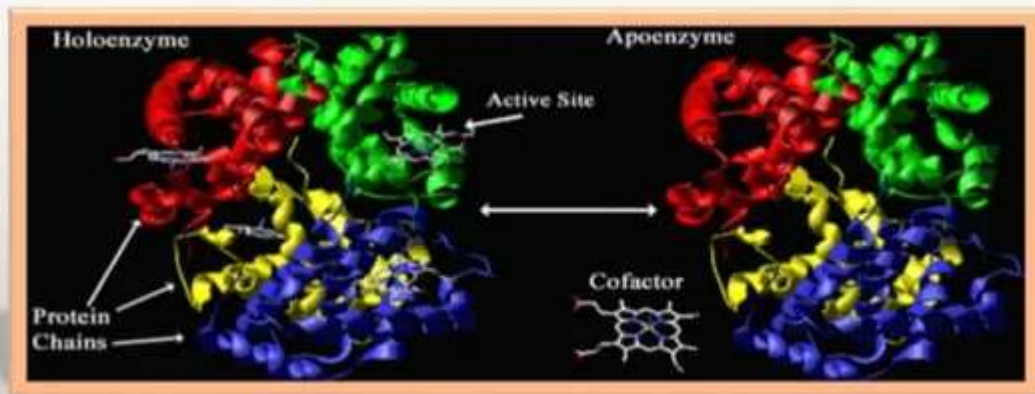


Coenzyme

- A coenzyme is loosely bound organic co-factor. E.g. NAD⁺



- An enzyme with its co-factor removed is designated as *apoenzyme*.
- The complete complex of a protein with all necessary small organic molecules, metal ions and other components is termed as *holoenzyme* or *holoprotein*.



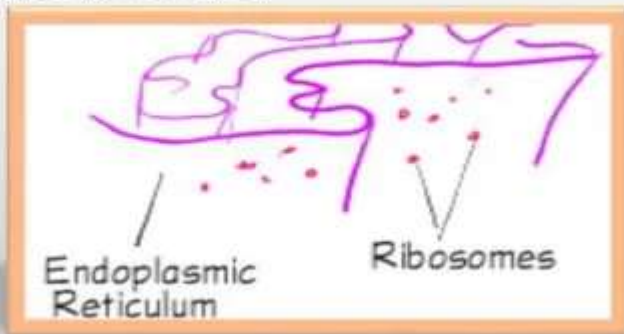
SUBSTRATE

- The reactant in biochemical reaction is termed as **substrate**.
- When a substrate binds to an enzyme it forms an **enzyme-substrate complex**.



SITES OF ENZYME SYNTHESIS

- Enzymes are synthesized by *ribosomes* which are attached to the rough endoplasmic reticulum.
- Information for the synthesis of enzyme is *carried by DNA*.
- Amino acids are bonded together to form specific enzyme according to the DNA's codes.



INTRACELLULAR AND EXTRACELLULAR ENZYMES

- **Intracellular** enzymes are *synthesized and retained in the cell for the use of cell itself.*
- They are found in the cytoplasm, nucleus, mitochondria and chloroplast.

Example :

- Oxydoreductase catalyses biological oxidation.
- Enzymes involved in reduction in the mitochondria.

- **Extracellular** enzymes are *synthesized in the cell but secreted from the cell to work externally.*

Example :

- Digestive enzyme produced by the pancreas, are not used by the cells in the pancreas but are transported to the duodenum.



CHARACTERISTICS

- Enzymes *speed up* the reaction by lowering the activation energy of the reaction.
- Their presence *does not effect* the nature and properties of *end product*.
- They are *highly specific* in their action that is each enzyme can catalyze one kind of substrate.
- Small amount of enzymes can accelerate chemical reactions.
- Enzymes are *sensitive* to change in pH, temperature and substrate concentration.
- *Turnover number* is defined as the number of substrate molecules transformed per minute by one enzyme molecule.

Catalase turnover number = $6 \times 10^6/\text{min}$



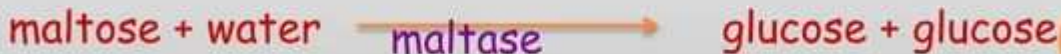
NOMENCLATURE OF ENZYMES

- An enzyme is named according to the name of the substrate it catalyses.
- Some enzymes were named before a systematic way of naming enzyme was formed.

Example: pepsin, trypsin and rennin

- By adding suffix **-ase** at the end of the name of the substrate, enzymes are named.
- Enzyme for catalyzing the hydrolysis is termed as hydrolase.

Example :



EXAMPLES

substrate	enzymes	products
lactose	lactase	glucose + galactose
maltose	maltase	Glucose
cellulose	cellulase	Glucose
lipid	lipase	Glycerol + fatty acid
starch	amylase	Maltose
protein	protease	Peptides + polypeptide





CLASSIFICATION

CLASSIFICATION OF ENZYMES

- A systematic classification of enzymes has been developed by *International Enzyme Commission*.
- This classification is based on the type of reactions catalyzed by enzymes.
- There are *six* major classes.
- Each class is further divided into sub classes, sub sub-classes and so on, to describe the huge number of different enzyme-catalyzed reactions.



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





MECHANISM OF ENZYME ACTION

MECHANISM OF ENZYME ACTION

- The catalytic efficiency of enzymes is explained by two perspectives:

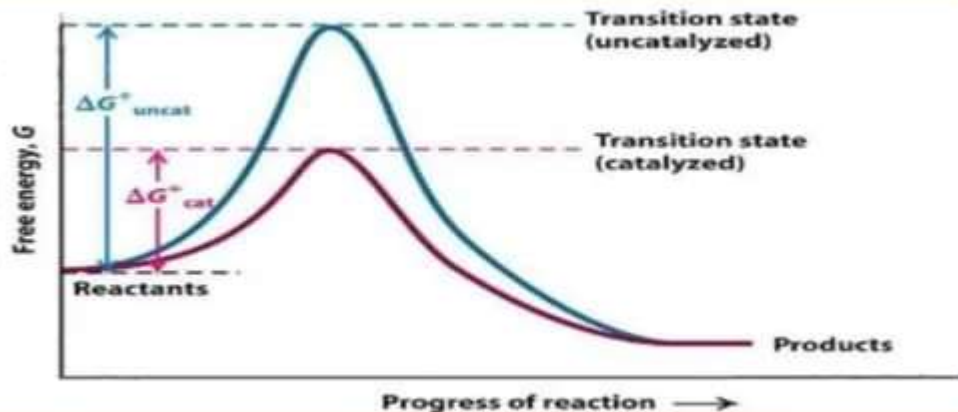
Thermodynamic
changes

Processes at the
active site



THERMODYNAMIC CHANGES

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



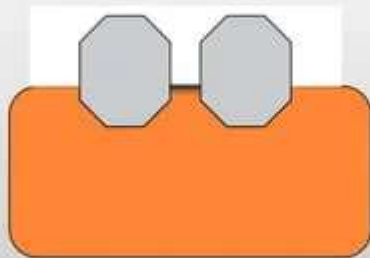
THERMODYNAMIC CHANGES

- Only a few substances cross the activation barrier and change into products.
- That is why rate of uncatalyzed reactions is much slow.
- Enzymes provide an alternate pathway for conversion of substrate into products.
- Enzymes accelerate reaction rates by forming transitional state having low activation energy.
- Hence, the reaction rate is increased many folds in the presence of enzymes.
- The total energy of the system remains the same and equilibrium state is not disturbed.



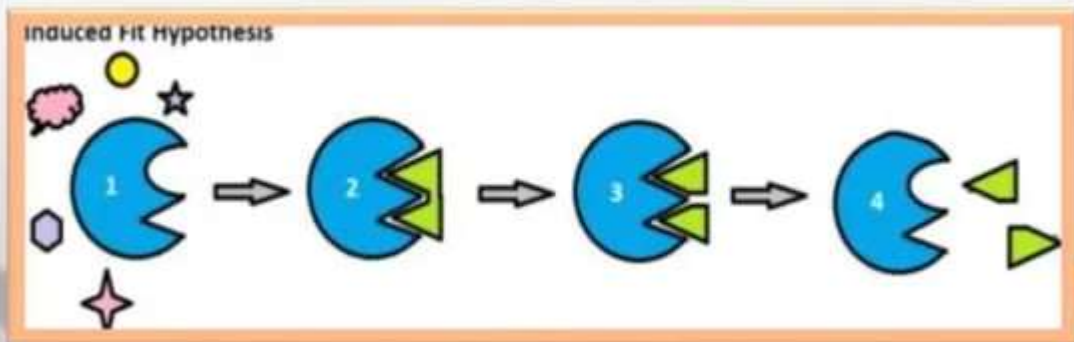
LOCK AND KEY MODEL

- Proposed by EMIL FISCHER in 1894.
- Lock and key hypothesis assumes the active site of an enzymes are rigid in its shape.
- There is no change in the active site before and after a chemical reaction.

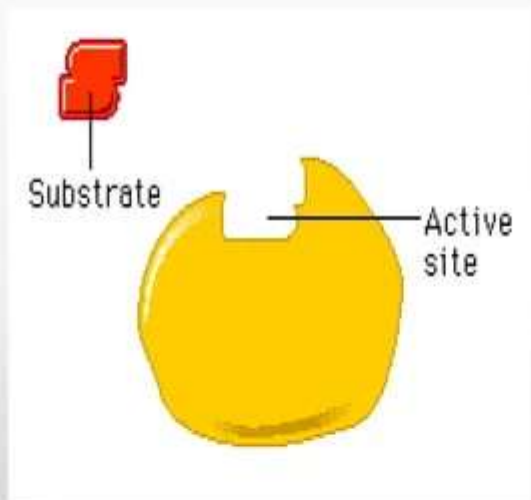


INDUCED FIT MODEL

- More recent studies have revealed that the process is much more likely to involve an induced fit model (proposed by DANIAL KOSH LAND in 1958).
- According to this exposure of an enzyme to substrate cause a change in enzyme, which causes the active site to change it's shape to allow enzyme and substrate to bind.



INDUCED FIT MODEL





ENZYMES KINETICS

INTRODUCTION

"It is a branch of biochemistry in which we study *the rate of enzyme catalyzed reactions.*"

- Kinetic analysis reveals the number and order of the individual steps by which enzymes transform substrate into products
- Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of that enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme



RATES OF REACTION AND THEIR DEPENDENCE ON ACTIVATION ENERGY

- Activation Energy (E_a):

“The least amount of energy needed for a chemical reaction to take place.”

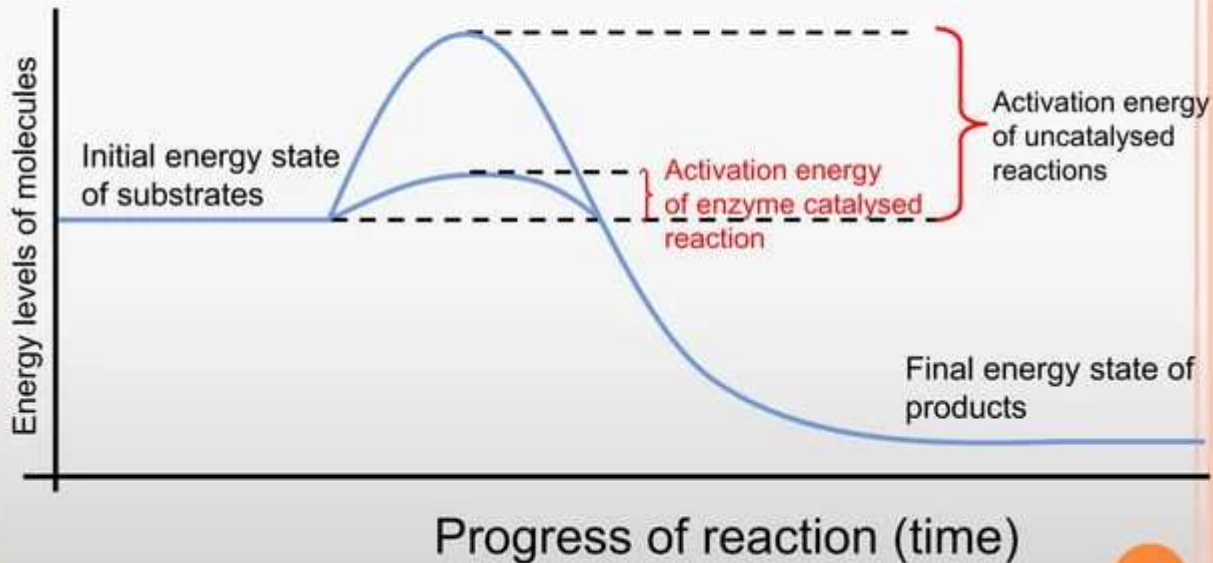
- Enzyme (as a catalyst) acts on substrate in such a way that they lower the activation energy by changing the route of the reaction.
- The reduction of activation energy (E_a) increases the amount of reactant molecules that achieve a sufficient level of energy, so that they reach the activation energy and form the product.

Example:

- *Carbonic anhydrase* catalyses the hydration of 10^6 CO_2 molecules per second which is 10^7 x faster than spontaneous hydration.



ENZYMES LOWER THE ACTIVATION ENERGY OF A REACTION



KINETICS OF ENZYMES CATALYSIS

- Enzymes catalysis:

“ It is an increase in the rate of reaction with the help of enzyme(as catalyst).”

- Catalysis by enzymes that proceed via unique reaction mechanism, typically occurs when the transition state intermediate forms a covalent bond with the enzyme(covalent catalysis).
- During the process of catalysis enzymes always emerge unchanged at the completion of the reaction.




FACTORS AFFECTING RATE OF ENZYME CATALYZED REACTIONS

- Temperature
- Hydrogen ion concentration(pH)
- Substrate concentration

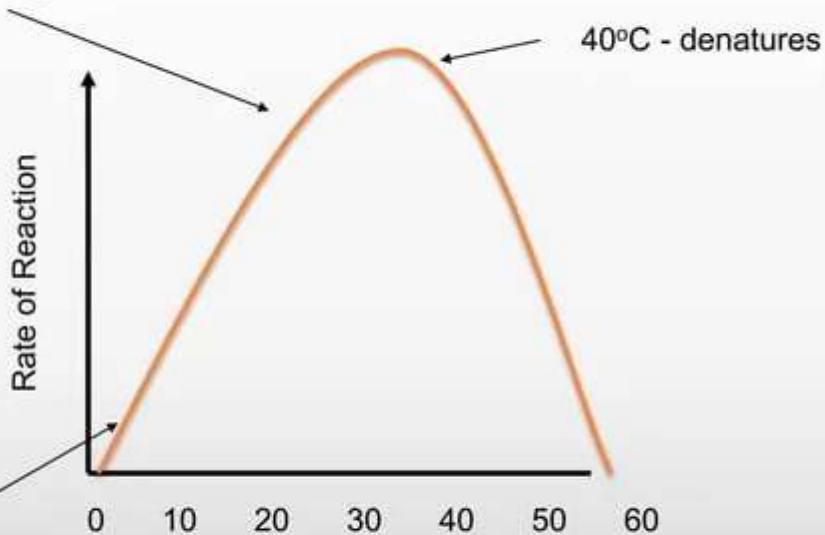


EFFECT OF TEMPERATURE

- Raising the temperature increases the rate of enzyme catalyzed reaction by increasing kinetic energy of reacting molecules.
 - Enzymes work maximum over a particular temperature known as *optimum temperature*. Enzymes for humans generally exhibit stability temperature up to 35-45 °C.
 - The temperature coefficient is a factor Q_{10} by which the rate of biological processes increases for a 10 °C increase in temperature.
 - For most biological processes $Q_{10} = 2$.
 - However some times heat energy can also increase kinetic energy to a point that exceed the energy barrier which results in denaturing of enzymes.
- 

Temperature

5- 40°C
Increase in Activity

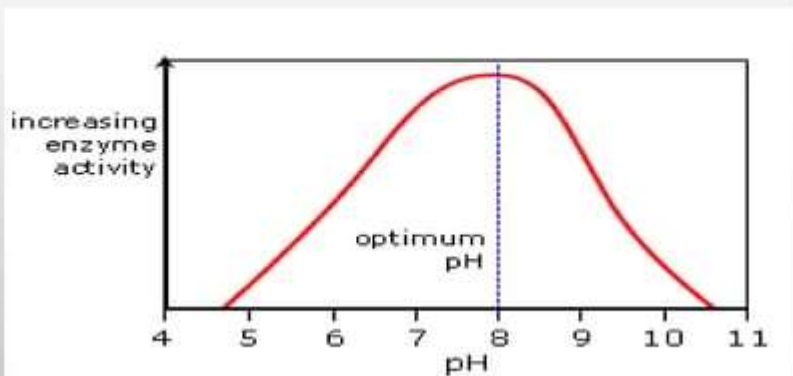


<5°C - inactive

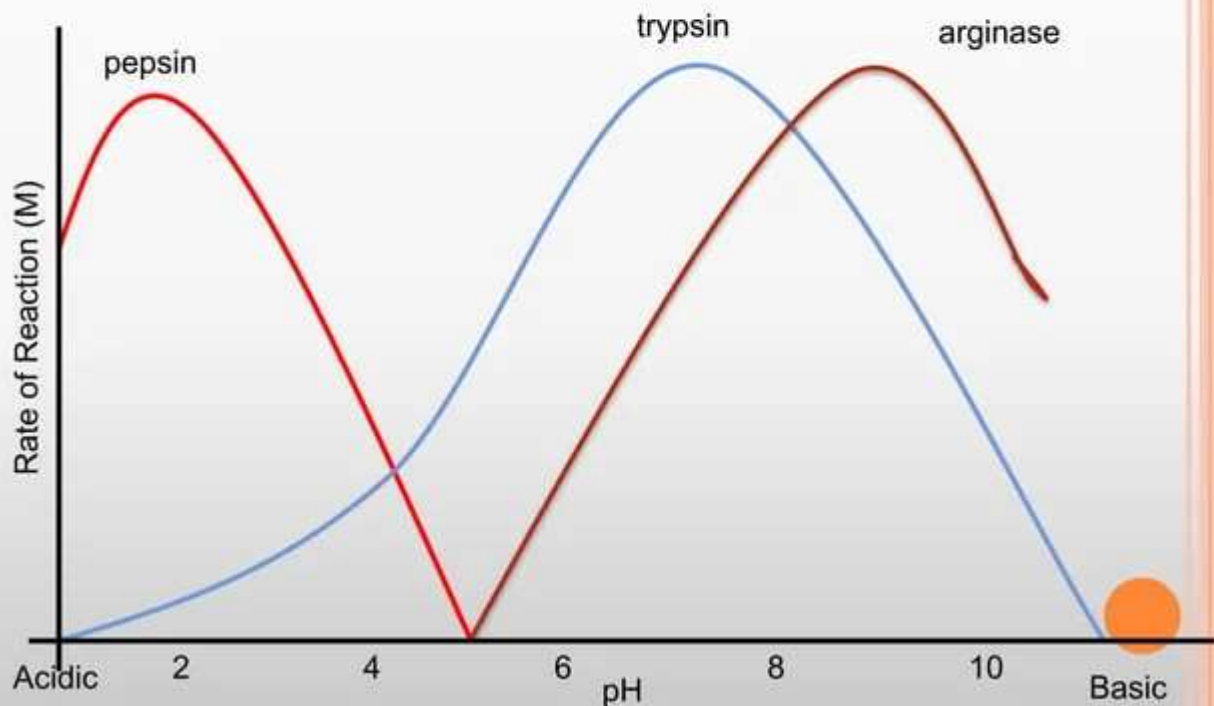


EFFECT OF pH

- Rate of almost all enzymes catalyzed reactions depends on pH
- Most enzymes exhibit optimal activity at pH value between 5 and 9
- High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme



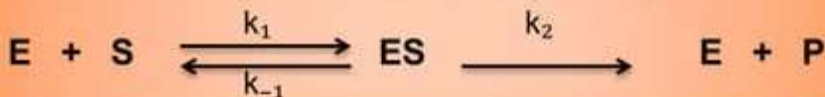
PH AFFECTS THE FORMATION OF HYDROGEN BONDS AND SULPHUR BRIDGES IN PROTEINS AND SO AFFECTS SHAPE.



MICHAELIS-MENTEN MODEL & EFFECTS OF SUBSTRATE CONCENTRATION

○ 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

- 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.

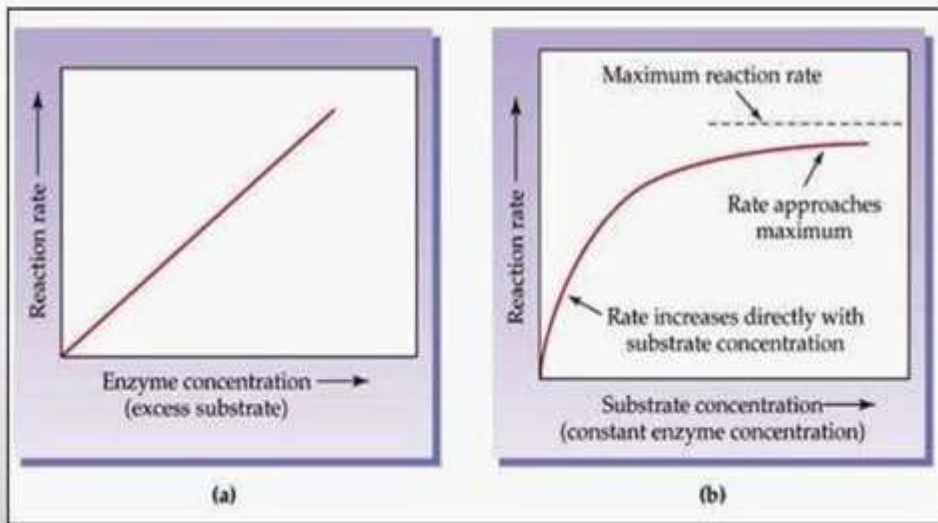


ASSUMPTIONS FOR MICHAELIS-MENTEN EQUATION

- Following assumptions are made in deriving the Michaelis-Menten equation:
 - Relative concentrations of E and S.
 - Steady-State assumptions
 - Initial Velocity



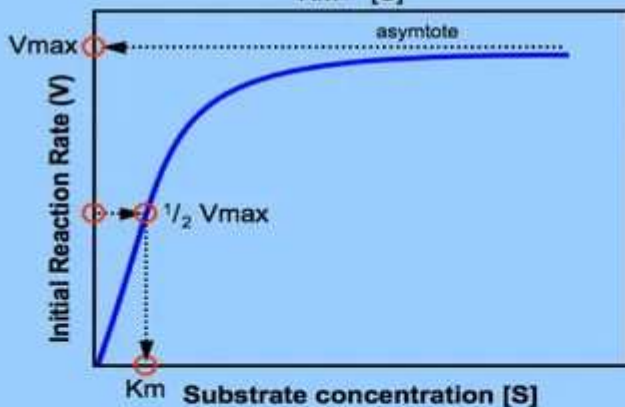
SUBSTRATE CONCENTRATION



SUBSTRATE CONCENTRATION

Michaelis Menten Plot

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

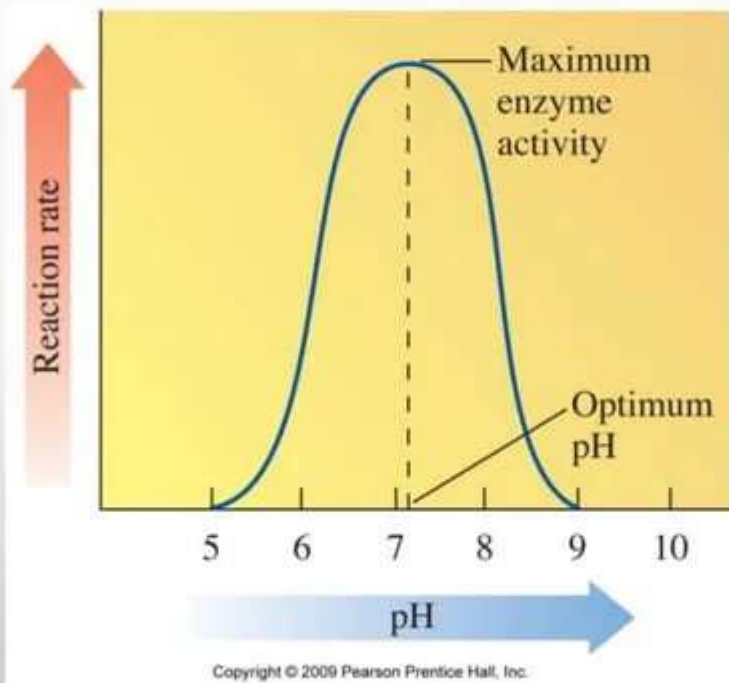


PHARMACEUTICAL IMPORTANCE

- Enzymes are virtually involved in all physiological processes which makes them the *targets of choice for drugs* that cure or ameliorate human disease.
- Applied enzyme kinetics represents the *principal tool* by which scientist identify and characterize therapeutic agents that selectively inhibit the rates of specific enzymes catalyzed processes.
- Enzymes kinetics thus play a critical role in *drug discovery* as well as elaborating the mode of action of drugs.



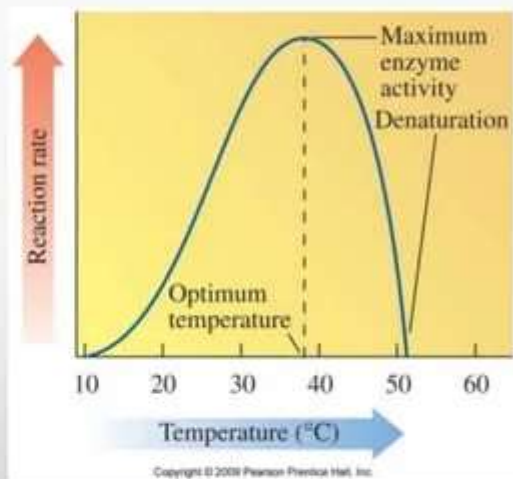
FACTORS AFFECTING ENZYME ACTIVITY



TEMPERATURE AND ENZYME ACTION

Enzymes

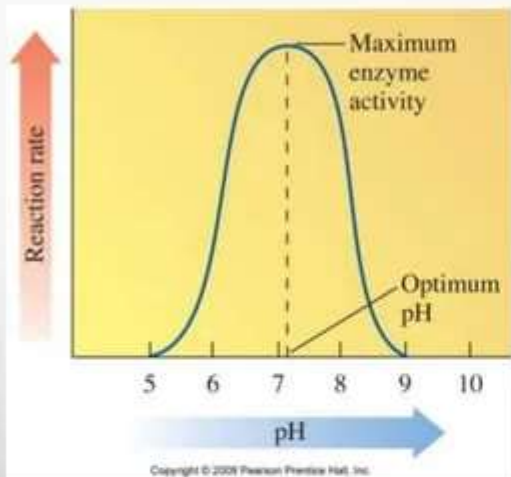
- are most active at an optimum temperature (usually 37 °C in humans).
- show little activity at low temperatures.
- lose activity at high temperatures as denaturation occurs



PH AND ENZYME ACTION

Enzymes

- are most active at optimum pH.
- contain R groups of amino acids with proper charges at optimum pH.
- lose activity in low or high pH as tertiary structure is disrupted.

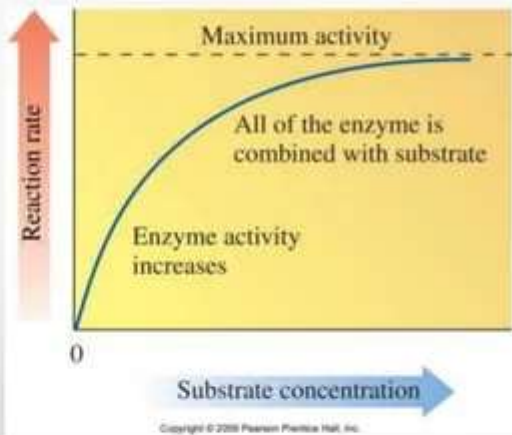


SUBSTRATE CONCENTRATION

As **substrate concentration**

increases,

- the **rate of reaction** increases (at constant enzyme concentration).
- the enzyme eventually becomes saturated, giving maximum activity.



DIAGNOSTIC SIGNIFICANCES OF ENZYMES

Some clinically important enzymes

Enzymes and their Concentration	Concentration increases in
Lactate dehydrogenase (LDH) – 60-12 IU/litre	Myocardial infarction, myopathy or muscle disorder. Also in leukemias, acute hepatitis, carcinomatitis.
Transaminases—	
(a) Aspartyl transaminase (AST) or Serum glutamyl oxaloacetate transaminase (SGOT) – 5-20 IU/litre	Myocardial infarction
(b) Alanine transaminase (ALT) or Serum glutamyl pyruvate transaminase (SGPT) – 5-15 IU/litre	Liver disorders
Creatine phosphokinase (CPK) – 10-60 IU/litre	Myocardial infarction, myopathy
Alkaline phosphatase – 4-17 King Armstrong (KA) units/100 ml	Bone disorders, obstructive jaundice, hyperparathyroidism
Acid phosphatase	Prostate carcinoma
Isocitrate dehydrogenase	Brain tumor and meningitis, liver diseases
Amylase	Pancreatitis, parotitis (inflammation of parotid gland) intestinal obstruction, diabetes
Lipase	Pancreatitis or carcinoma of pancreas
Gamma glutamyl transpeptidase (g-GT)	Liver damage (indicator of alcoholism)

DIAGNOSTIC SIGNIFICANCES OF ISOENZYMES

Iso Enzyme	Present in	Elevated in
LDH 1 Heat resistant	Myocardium, RBC Kidney	MI
LDH 2 Heat resistant	Myocardium, RBC Kidney	Kidney disease and Megaoblastic Anemia
LDH 3	Brain	Leukemia and Malignancy
LDH 4 Heat labile	Lung and Spleen	Pulmonary infarction
LDH 5 Heat labile limited by urea	Skeletal muscle, Liver	Skeletal muscles & Liver Diseases

Allosteric Enzyme

- Allosteric Enzyme are those enzymes possess additional sites, known as allosteric sites besides the active site.
- The allosteric sites are unique places on the enzyme molecules; allosteric enzymes have one or more allosteric site.
- The term allosteric has been introduced by the two Noble laureates, **MONOD AND JACOB**, to denote an enzyme site, different from the active site ,which non competitively binds molecule other than the substrate and may influence the enzyme activity.

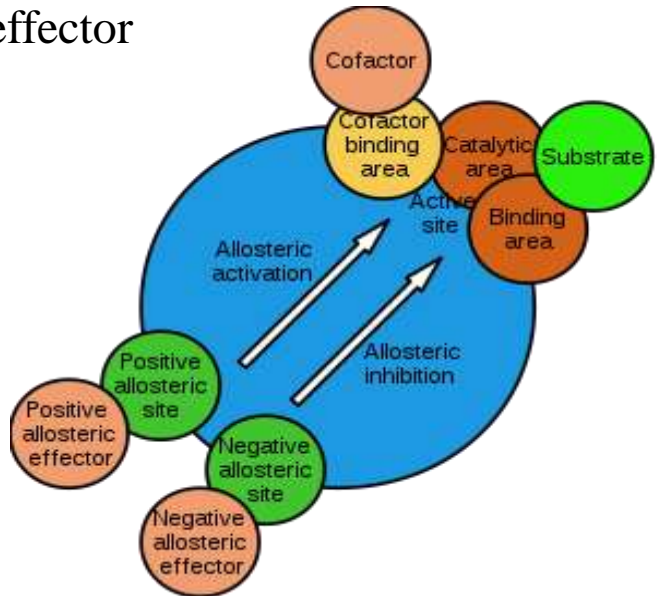
PROPERTIES OF ALLOSTERIC ENZYME.

- Allosteric enzyme have one or more allosteric sites
- Allosteric sites are binding sites distinct from an enzyme active site or substrate binding site
- Molecule that bind to allosteric sites are called effector **or modulator**
- Effector may be positive or negative, this effector regulate the enzyme activity. The enzyme activity is increased when a positive allosteric effector binds at the allosteric site known as **activator site**. On the other hand, negative allosteric effector bind at the allosteric site called **inhibitor site** and inhibit the enzyme activity.
- Binding to allosteric sites alter the activity of the enzyme, this is called cooperative binding. Allosteric enzymes display sigmoidal plot of V_0 vs[S].

ALLOSTERIC EFFECTOR

Allosteric effectors are of two types:

- Positive allosteric effector
- Negative allosteric effector



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