Topic- Enzymes
B.Sc. Zoology
Sem-II

Introduction to Enzymes

The living cell is the site of tremendous biochemical activity called metabolism.

"Enzymes can be defined as biological polymers that catalyze biochemical reactions."

Eduard Buchner submitted his first paper on the study of yeast extracts in 1897.

An overview of Enzymes

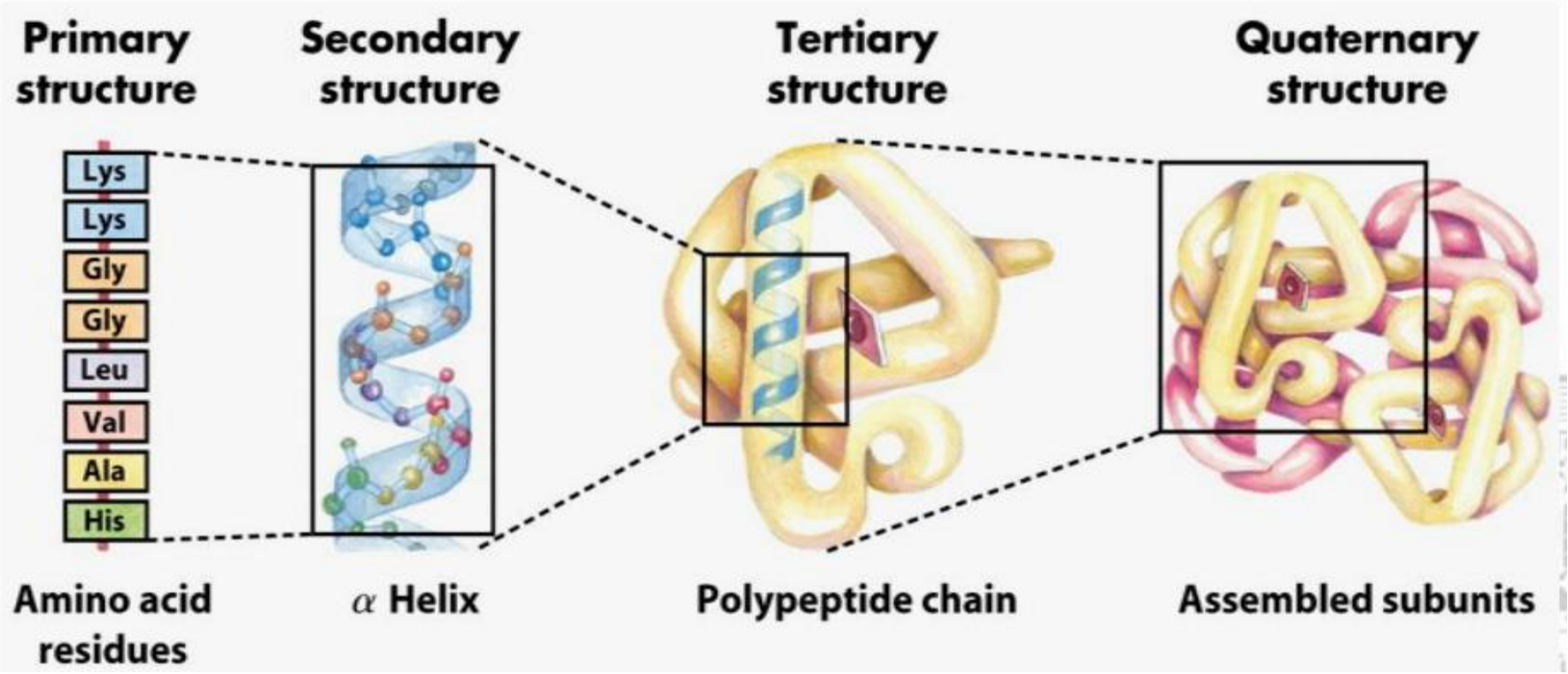
- Enzymes are **biological catalysts**
- Enzymes are specialized proteins (Except – Ribozymes)
- Chemical reactions in the cell are catalyzed by the enzymes
- Enzymes have extraordinary catalytic power
- Enzymes accelerate reactions up to **1014 to 1020 times**
- Enzyme **reduce the activation energy** of reaction
- High degree of **specificity** for **substrates** and **reactants**
- Function in aqueous solution
- Work under mild condition of **temperature** and **pH**

Properties of Enzymes

1. Catalytic property
2. Specificity
3. Reversibility
4. Sensitiveness to heat and temperature
5. Specific to pH

Enzymes 3D structure

Enzymes are proteins and their activities depends on the 3D structure of the amino acids that compose them (note: also some RNAs have catalytic activity but they won't be covered in this course)

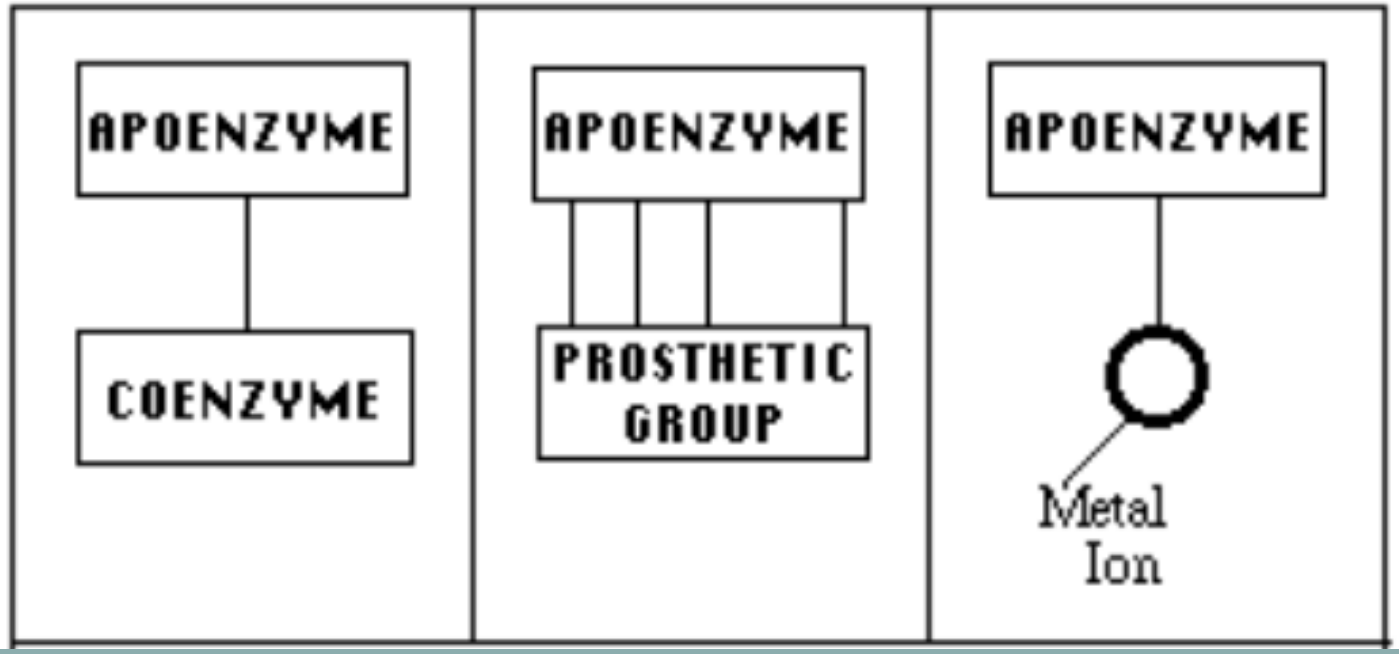


Structure of enzymes

- Enzymes are a linear chain of amino acids that generate the three-dimensional structure.

- The sequence of amino acids enumerates the structure, which in turn identifies the catalytic activity of the enzyme.

- The structure of the enzyme denatures when heated, leading to loss of enzyme activity, which is typically connected to the temperature.



Cofactors

Cofactors are non-proteinous substances that associate with enzymes. A cofactor is essential for the functioning of an enzyme.
An enzyme without a cofactor is called an apoenzyme. An apoenzyme and its cofactor together constitute the **holoenzyme**.

There are three kinds of cofactors present in enzymes:

1. **Prosthetic groups**: These are cofactors tightly bound to an enzyme at all times. A fad is a prosthetic group present in many enzymes.

2. Coenzyme: A coenzyme is bound to an enzyme only during catalysis. At all other times, it is detached from the enzyme. NAD^+ is a common coenzyme.

3. Metal ions: For the catalysis of certain enzymes, a metal ion is required at the active site to form coordinate bonds. Zn^{2+} is a metal ion cofactor used by a number of enzymes.

INORGANIC CO-FACTORS

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

Examples:

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

ORGANIC CO-FACTORS

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

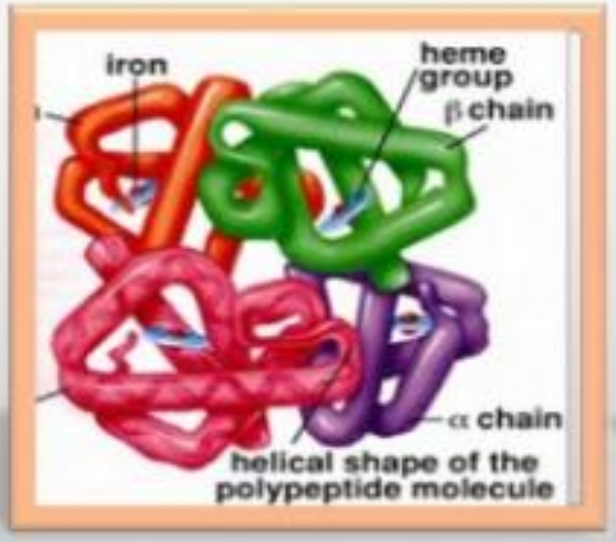
Example:

- Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate.

TYPES OF ORGANIC CO-FACTORS

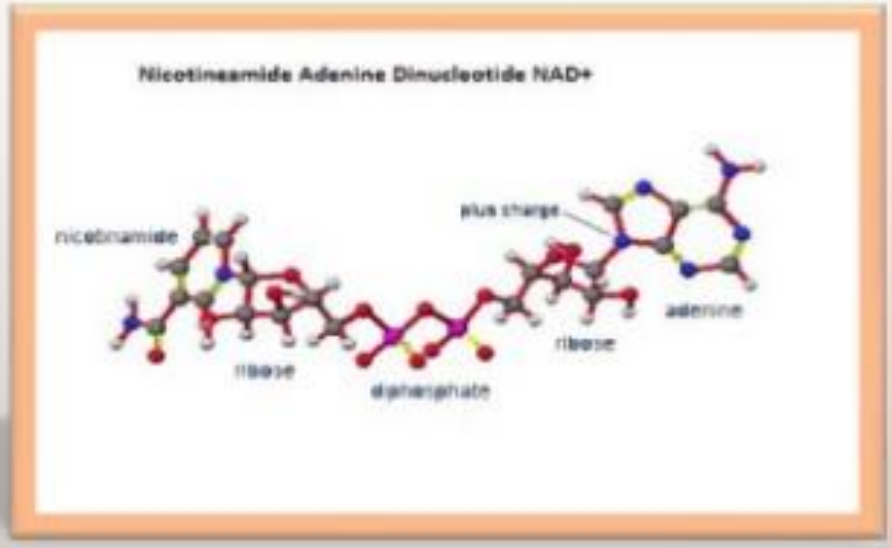
Prosthetic Group

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

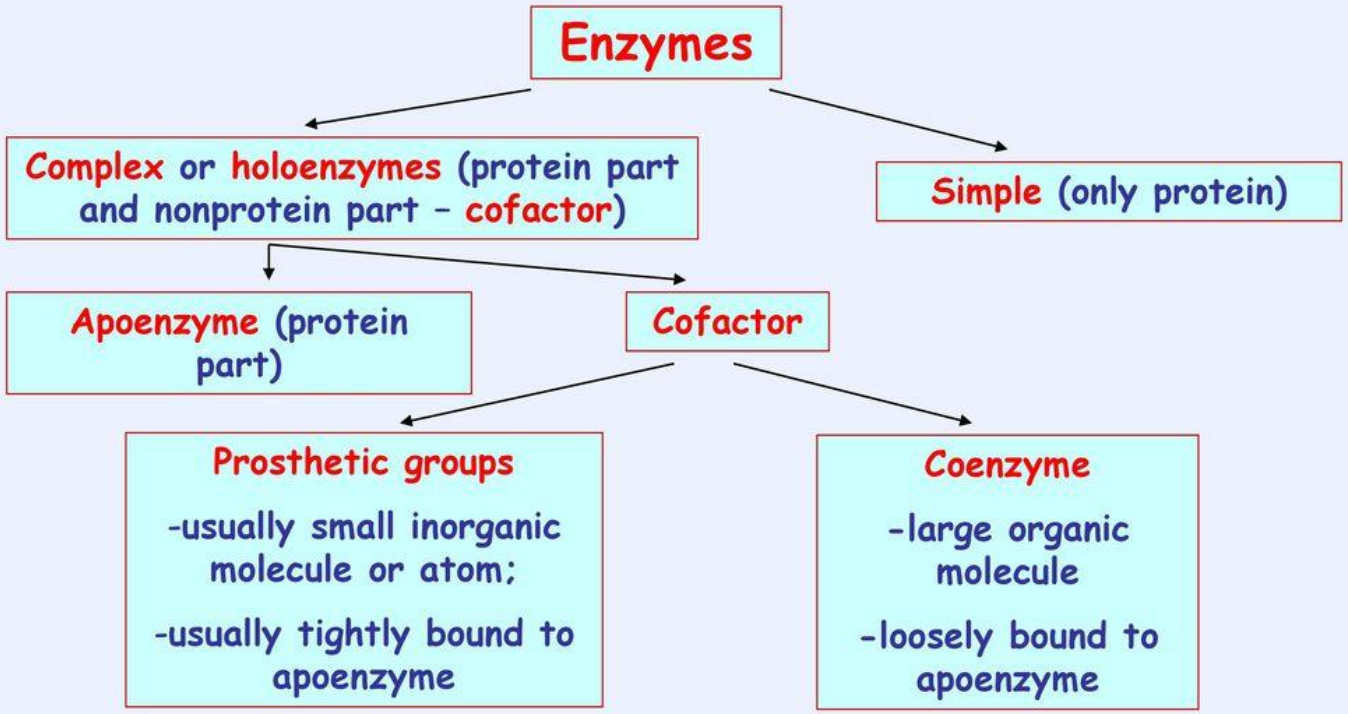


Coenzyme

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

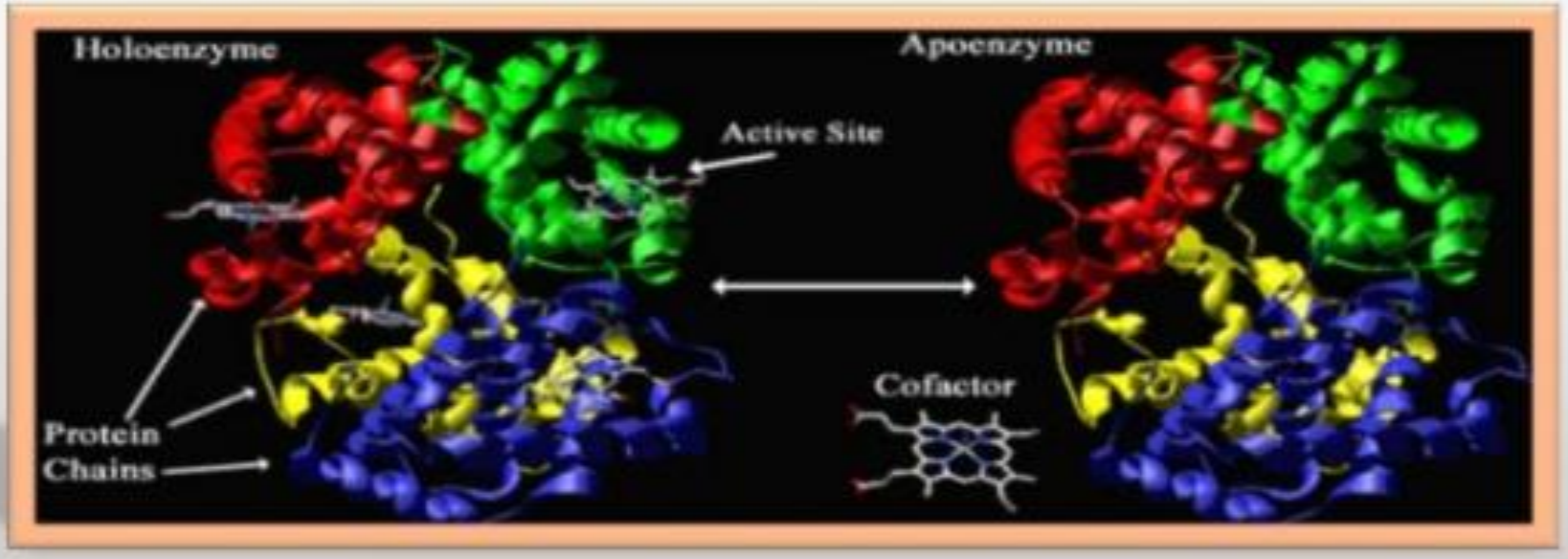


Structure of enzymes



Types of co-factors Continued…

- An enzyme with it's 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* of *holoprotein*.



Coenzymes

Coenzyme	Reaction Mediated
Biotin	Carboxylation
Cobalamin (B_{12}) coenzymes	Alkylation
Coenzyme A	Acyl transfer
Flavin coenzymes	Oxidation–reduction
Lipoic acid	Acyl transfer
Nicotinamide coenzymes	Oxidation–reduction
Pyridoxal phosphate	Amino group transfer
Tetrahydrofolate	One-carbon group transfer
Thiamine pyrophosphate	Aldehyde transfer

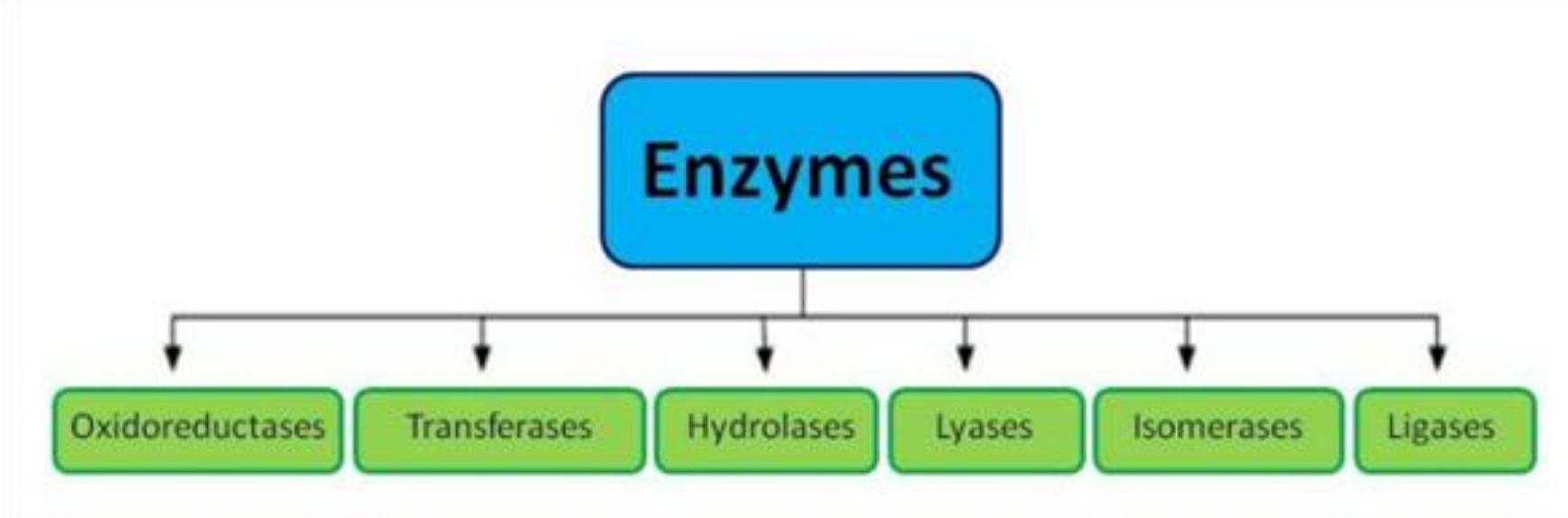
Many Vitamins Are Coenzyme Precursors

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

Coenzymes and the reaction catalyzed

Co-enzyme	Entity transferred
Thyamin pyrophosphate	Aldehydes
Tetrahydrofolate	Other one carbon groups
Pyridoxal phosphate	Amino groups
Nicotinamide adenine dinucleotide	Hydrogen atoms (electrons)
Flavin adenine dinucleotide	Hydrogen atoms (electrons)
Co-enzyme A	Acyl groups
Biocytin	CO_2
3′-deoxyadenorylcohalamine (co-enzyme B12)	H atoms and alkyl groups

Classification of Enzymes

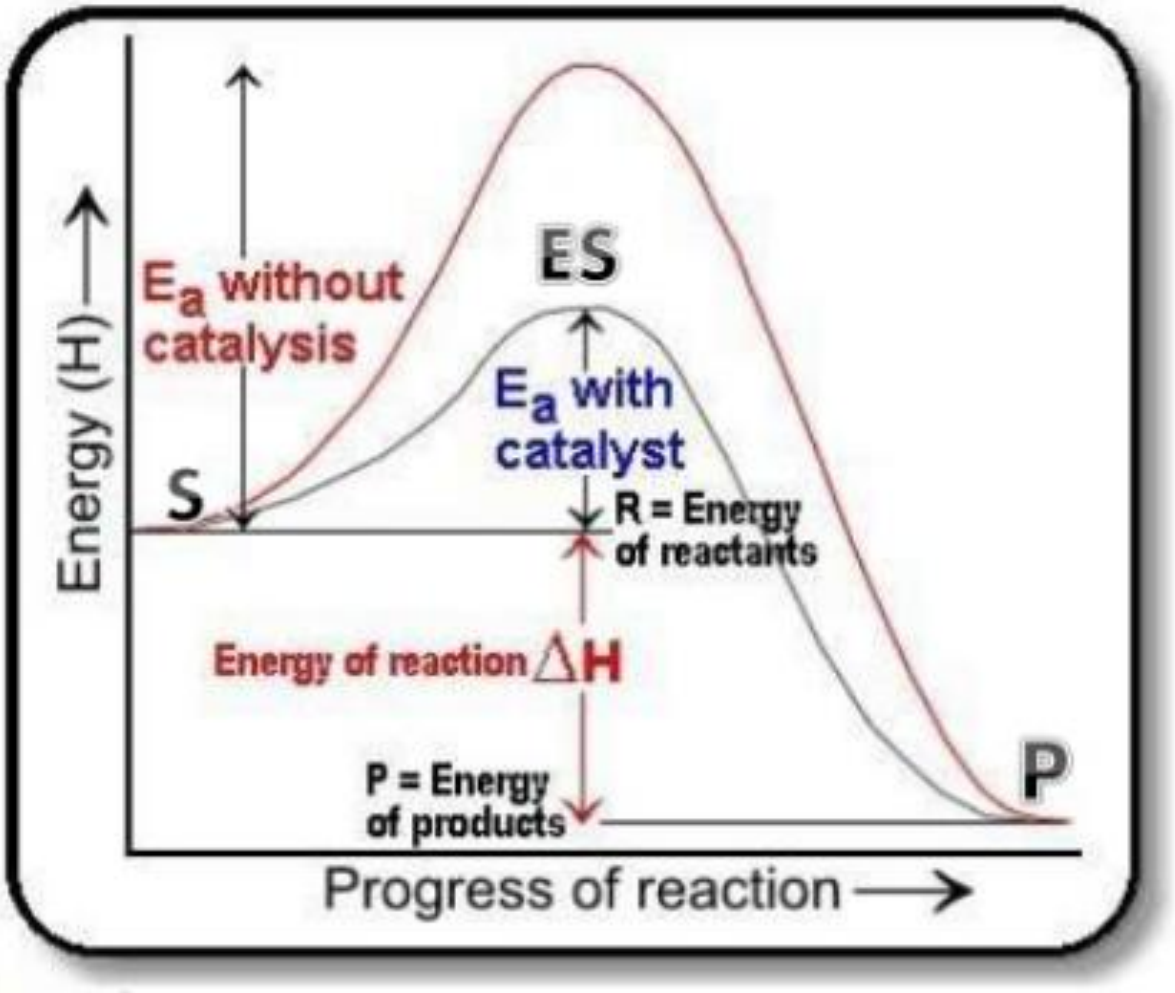


Classification of Enzymes..... Contd

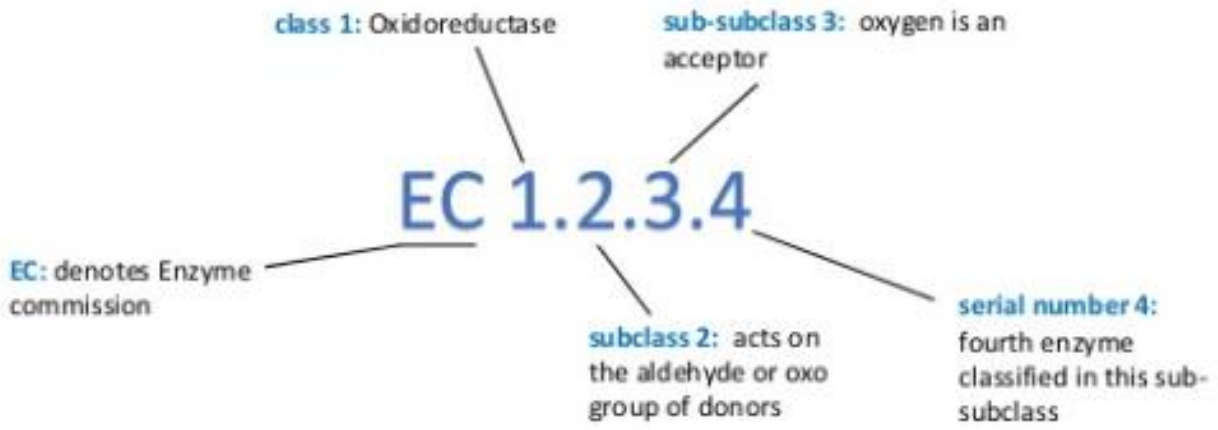
Types	Biochemical Property
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donors molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

What Enzymes do?

Lower the activation energy of the reaction



Nomenclature



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:

Properties of Active Site

1. The active site occupies a relatively small portion of the enzyme molecule
2. The active site is neither a point nor a line or even a plane but is a 3- dimensional entity. It is made up of groups that come from different parts of the linear amino acid sequence. For example lysozyme has 6 subsites in the active site. The amino acid residues located at the active site are 35, 52, 59, 62, 63 and 107
3. Usually the arrangement of atoms in the active site is well defined, resulting in a marked specificity of the enzymes. Although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in structure from its own substrate

Properties of Active Site

4. The active site binds the substrate molecule by relatively weak forces
5. The active sites in the enzyme molecules are grooves or crevices from which water is largely excluded. It contains amino acids such as aspartic acid, glutamic acid, lysine serine etc. The side chain groups like -- COOH, –NH2, --CH2OH etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which certain polar residues acquire special properties which are essential for catalysis

Coenzymes

- Enzymes functional groups takes part in acid-base reaction, to form transient bonds with substrate or take part in charge – charge interactions. They are less stable for catalyzing Redox reaction or transfer of a chemical group.

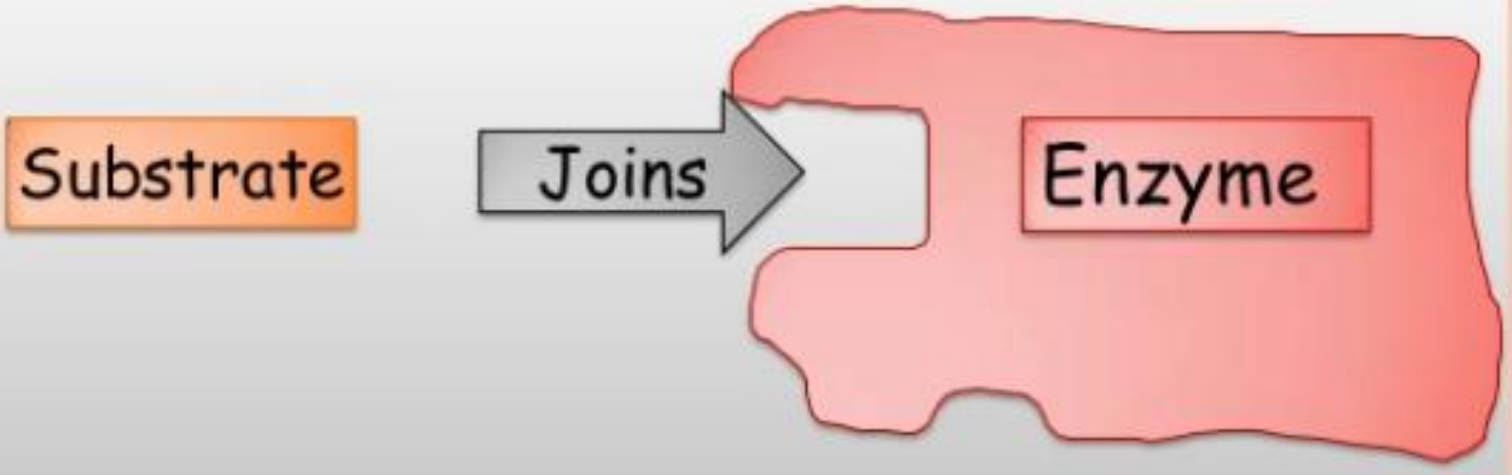
- Such reactions are carried out by small molecules called ***cofactors***

- **Cofactors** may be metal ions, such as the Zn2+ required for the catalytic activity of carboxypeptidase A, or organic molecules known as **coenzymes**, such as the NAD+ in YADH

	Cofactor	Coenzyme
Binding strength	It is loosely or tightly bound that is covalently bound to an enzyme.	It is loosely bound to an enzyme.
Action	They act on catalyst to increase the speed of the reaction.	They act as carries to the enzymes.
Role	They increase the rate of the reaction that is catalyzed by the relevant enzyme.	They serve as carriers to the enzymes.
Removal	They can only be removed by denaturing the enzyme.	They can be removed from the enzyme easily since they are loosely bound to the enzyme.

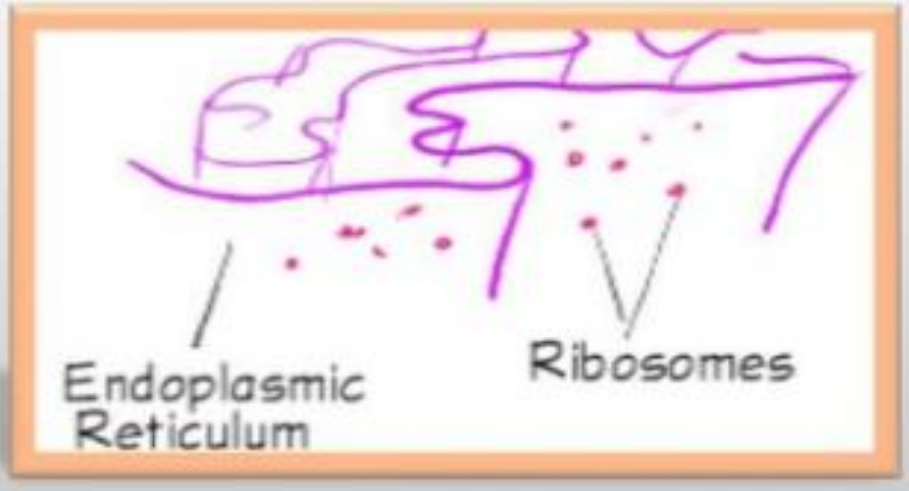
SUBSTRATE

- 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.

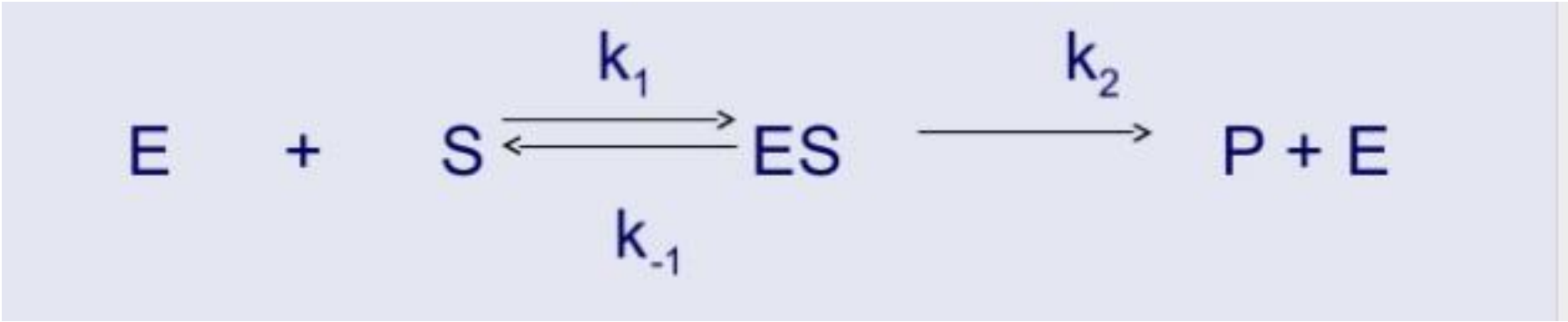


Enzyme kinetics

❖Enzyme kinetics is the quantitative measurement of the rates of enzyme catalyzed reactions.

❖Started with Brown's proposal- overall reaction is composed of two elementary reactions in which substrate forms a complex with the enzyme which is decomposed to products and enzymes.

Here E,S, ES and P symbolizes the enzyme, substrate, enzyme- substrate complex and products.



Michaelis- Menten Kinetics

The rapid equilibrium assumption:

- Assumes a rapid equilibrium between the enzyme and substrate to form an [ES] complex.

$$E+S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

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

- The equilibrium constant K_m can be expressed by the following equation in a dilute system.

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

Assumptions for Michaelis- Menten Equation

1. Relative concentration of E and S
2. Steady state assumptions
3. Initial velocity

- Since the enzyme is not consumed, the conservation equation on the enzyme yields

$$[E] = [E_0] - [ES]$$

- Then rearrange the equilibrium constant equation

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

- Substituting [E] in the above equation with enzyme mass conservation equation

$$[ES] = = \frac{([E_0] - [ES])[S]}{K_m}$$

$$[ES] = \frac{([E_0]-[ES])[S]}{K_m}$$

$$[ES]K_m = [E_0][S]-[ES][S]$$

$$[ES]K_m + [ES][S] = [E_0][S]$$

$$[ES](K_m + [S]) = [E_0][S]$$

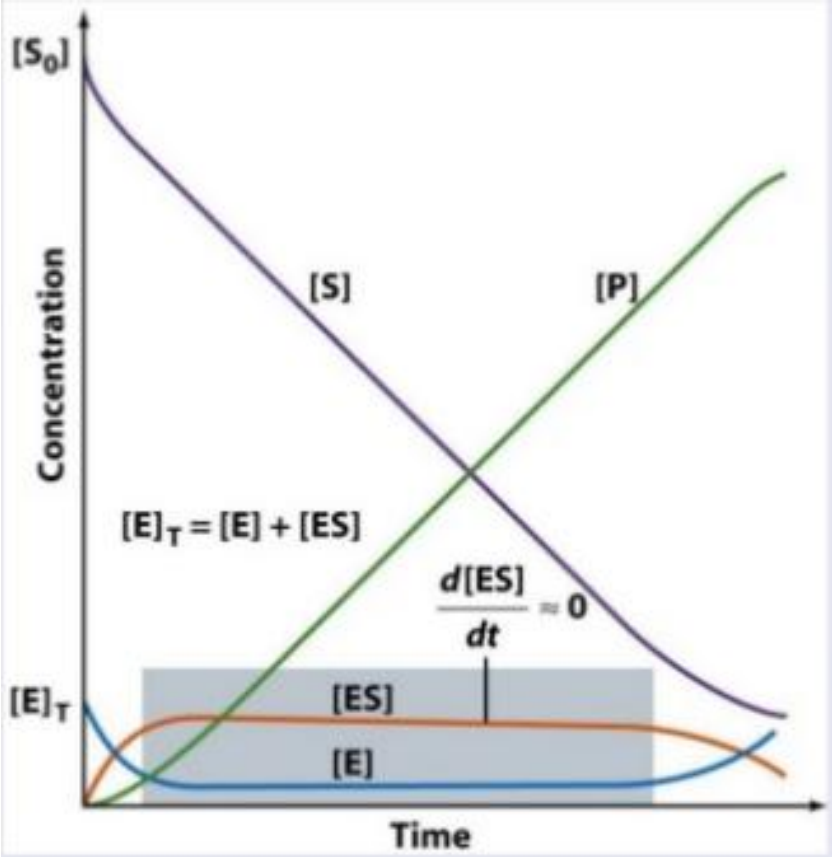
$$[ES] = \frac{[E_0][S]}{K_m + [S]}$$

- Then the rate of production formation v can be expressed in terms of [S]

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- Where $V_{max} = k_2[E_0]$

Steady- State Assumptions



- Progress curve for the components of a simple michaelis-Menten reaction
- Except the transition phase of the reaction (before shaded block) [ES] remains constant until the substrate is nearly exhausted.
- Hence synthesis of ES must equals to its consumption over the course of reaction i.e. ES maintain **steady state**

SSA leads to the following equation of Michaelis-Menten

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

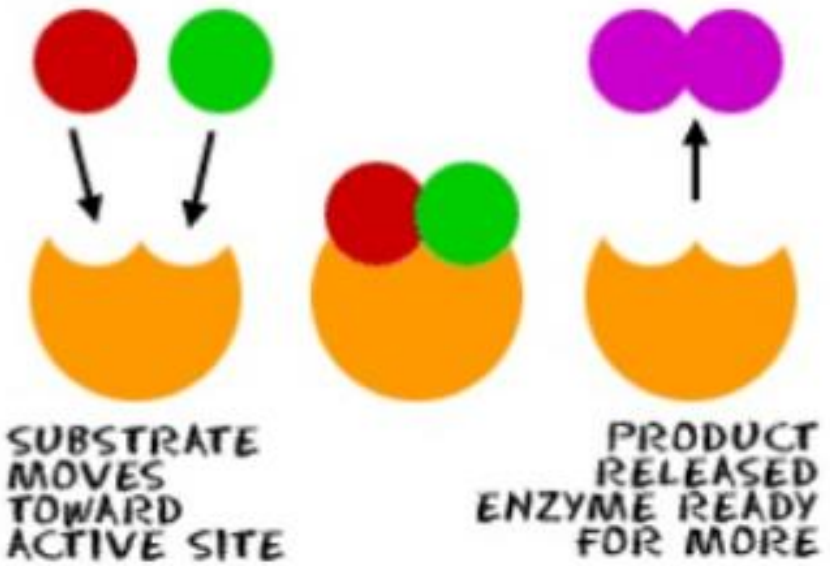
- Where $V_{max} = k_2[E_0]$

- Michaelis Menten Equation

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

Significance of Km

- K_M is the concentration of substrate at which half the active sites are filled. *It* provides a measure of the substrate concentration required for significant catalysis to occur.



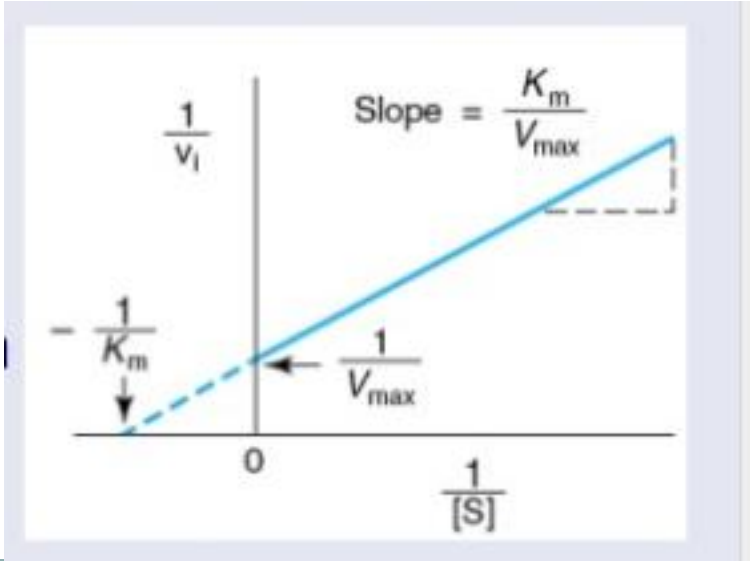
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k2} E + P$$

- Assumes the formation of Enzyme substrate complex
- Assumes that the ES complex is in rapid equilibrium with free enzyme
- Breakdown of ES to form products assumed to be slower than
  1. Formation of ES and
  2. Breakdown of ES to reform E and S

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver -Burk plot

- Starting with the MM equation $$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$
- Reciprocal of MM equation $$\frac{1}{v_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$
- Lineweaver-Burk Equation $$\frac{1}{v_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$
- Equation is the equation for a straight line, $y = ax + b$, where $y = 1/v_0$ and $x = 1/[S]$.



Reference books for further study:

1. Lehninger
2. Voet and Voet
3. Stryer