ENZYMES

General Aspects

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LEARNING OUTCOMES

- History of enzyme research
- Properties of enzymes
- Classification and Nomenclature of enzymes
- Structure and composition of enzymes
- Models for enzyme -substrate complex formation
- Active sites
- Allosteric sites

HISTORY OF ENZYME RESEARCH

- While studying the fermentation of sugar to alcohol by yeast, **Louis Pasteur (1850)** concluded that fermentation was caused by vital force contained within the yeast cells called “ferments” that function only in living yeast cells.

- **Wilhelm Kuhne (1878)** proposed the name “enzyme” which in Greek means “in yeast”.

HISTORY OF ENZYME RESEARCH

- **Eduard Buchner(1897)** found that sugar was fermented by yeast extracts even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sugar “zymase”.

In 1907, he received Nobel prize in chemistry for the discovery of cell-free fermentation.

- **James Sumner(1926, 37)** isolated enzyme urease and Catalase in pure crystalline form and found them to be made of protein.

HISTORY OF ENZYME RESEARCH

- **John Howard Northrop and Wendell Meredith Stanley** crystallized digestive enzymes pepsin, trypsin and chymotrypsin and found them to be pure proteins.

- **Northrop and Stanley shared the 1947 Nobel prize with Sumner**. They **established the protein nature of enzymes** . The precipitation technique devised by them has been used to crystallize several enzymes.

HISTORY OF ENZYME RESEARCH

- **David Chilton Phillips et al (1965)** resolved the structure of lysozyme through X- ray crystallography .

- **Emil Fischer (1899)** carried out systematic studies on enzyme specificity and gave " lock and key mechanism" for enzyme – substrate complex formation.

- **Daniel Koshland (1958)** proposed " induced fit model".

HISTORY OF ENZYME RESEARCH

- **Leonor Michaelis and Maud Menten (1913)** investigated enzyme kinetics of invertase (hydrolyses sucrose into glucose and fructose).

They proposed a mathematical model in the form of an equation describing the rate of enzymatic reaction. Michaelis- Menten kinetics is best known models of enzyme kinetics .

PROPERTIES OF ENZYMES

- Enzymes are biological catalysts and are functional units of cell metabolism.
- Enzymes are highly specialized proteins with catalytic activity. (Exception : Ribozymes are ribonucleoproteins and have catalytic activity in RNA part and these catalyse reactions on phosphodiester bonds of other RNAs).
- Enzymes possess all the properties of proteins (such as large molecular weight, colloidal nature, amphoteric nature).

PROPERTIES OF ENZYMES

- Enzymes have high degree of specificity for their substrates.
- Enzymes accelerate specific chemical reactions without the formation of by-products.
- Enzymes function in dilute aqueous solutions under very mild conditions of temperature and pH.

PROPERTIES OF ENZYMES

- Enzymes get denatured (loses its catalytic activity) when subjected to elevated temperature or extremes of pH or non-physiological concentrations of salt, organic solvents, urea or other chemical agents.
- Enzymes increase the rate of reaction but do not alter the equilibrium.

PROPERTIES OF ENZYMES

- Enzymes catalyse reactions in both directions (forward and reverse) when free energy change in reaction is small.
- During enzymatic catalysis, substrate binds at the active site of the enzyme protein transiently (for a brief time) and after catalysis, product is released.

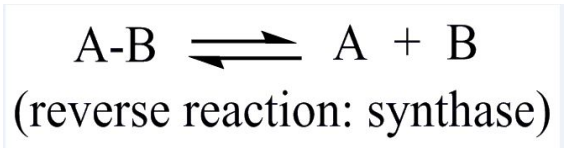
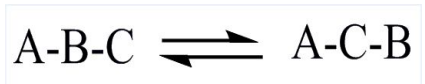
NOMENCLATURE AND CLASSIFICATION OF ENZYMES

According to **International Union of Biochemistry and Molecular Biology (IUBM),** nomenclature and classification of enzymes is based on the

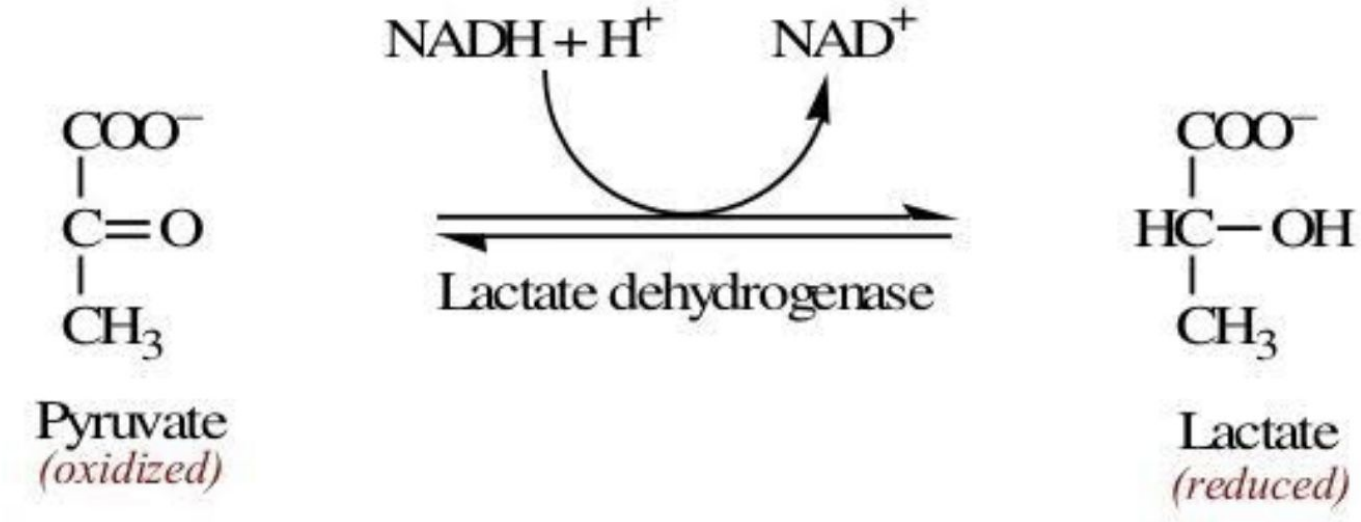
- Reactions they catalyse
- Substrates transformed &
- The products formed by the enzyme

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

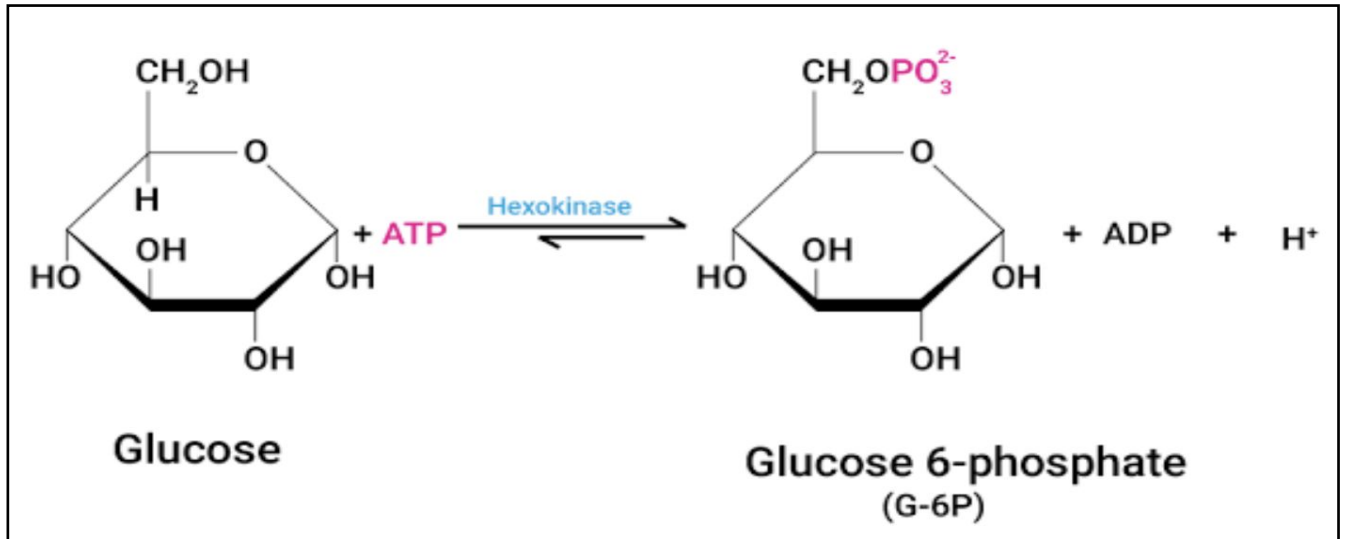
- Enzymes were divided into **6 major classes** according to type of reaction catalysed and a **seventh class Translocases was added in 2018.**
- Currently there are **6476 enzymes** assigned to seven classes and the number keeps updating.
- Enzymes are identified by 4 digit EC (Enzyme Commission) numbers.

Enzyme class	Reaction type	Description	Enzyme count
EC 1 Oxidoreductases		Catalyze redox reaction and can be categorized into oxidase and reductase.	1908
EC 2 Transferases		Catalyze the transfer or exchange of certain groups among some substrates	1929
EC 3 Hydrolases		Accelerate the hydrolysis of substrates	1314
EC 4 Lyases		Promote the removal of a group from the substrate to leave a double bond reaction or catalyze its reverse reaction	708
EC 5 Isomerases		Facilitate the conversion of isomers.	304
EC 6 Ligases		Catalyze the synthesis of two molecular substrates into one molecular compound with the release energy	223
EC 7 Translocases		Catalyze the movement of ions or molecules across membranes or their separation within membranes	90
	All classes		6476

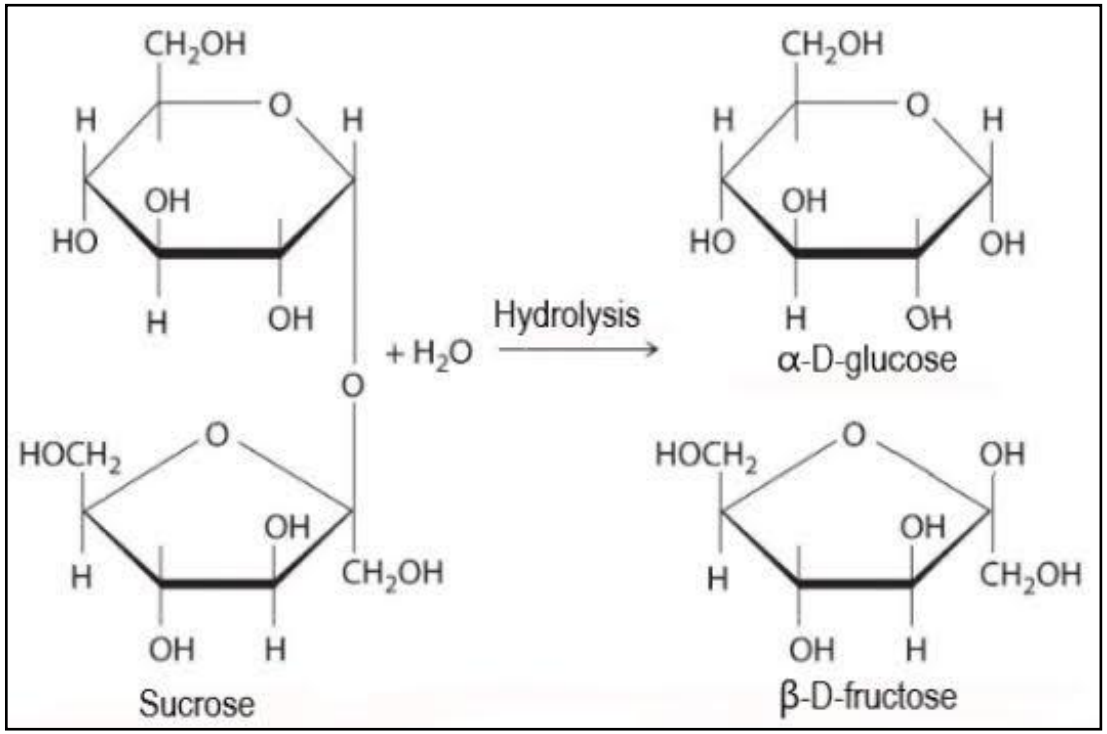
1.**OXIDOREDUCTASE**



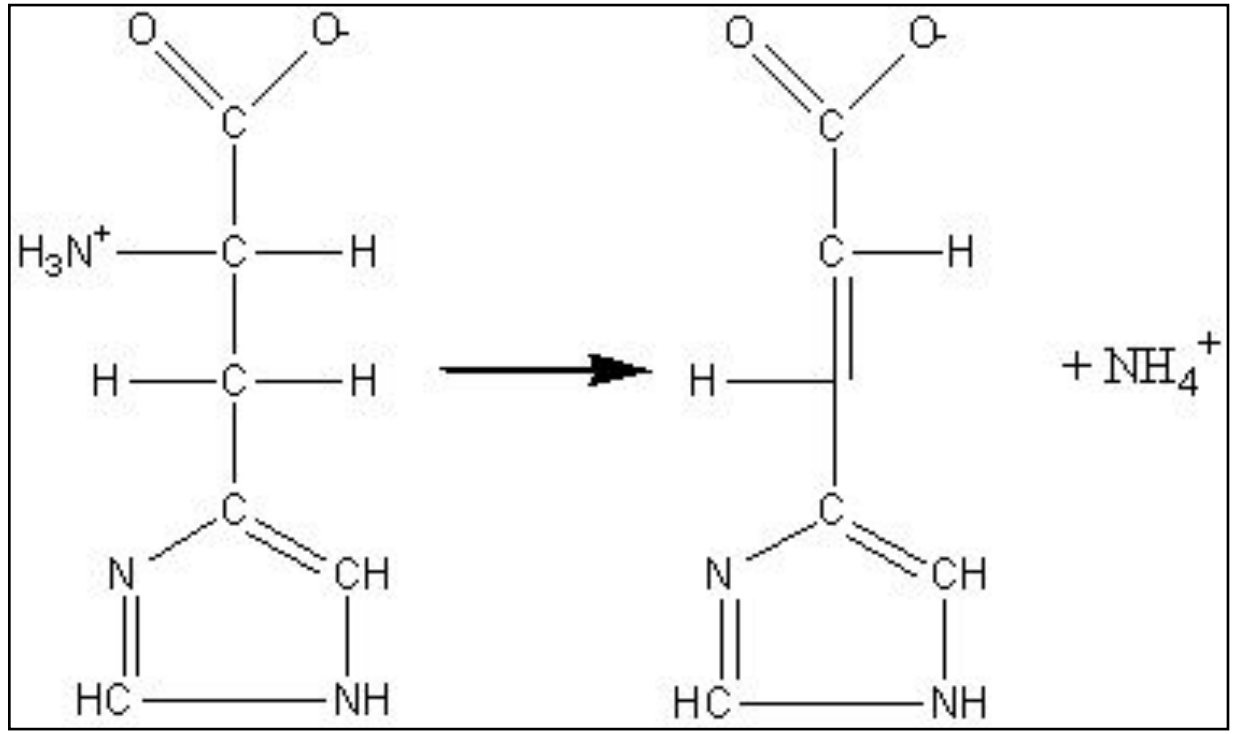
2. **TRANSFERASES**



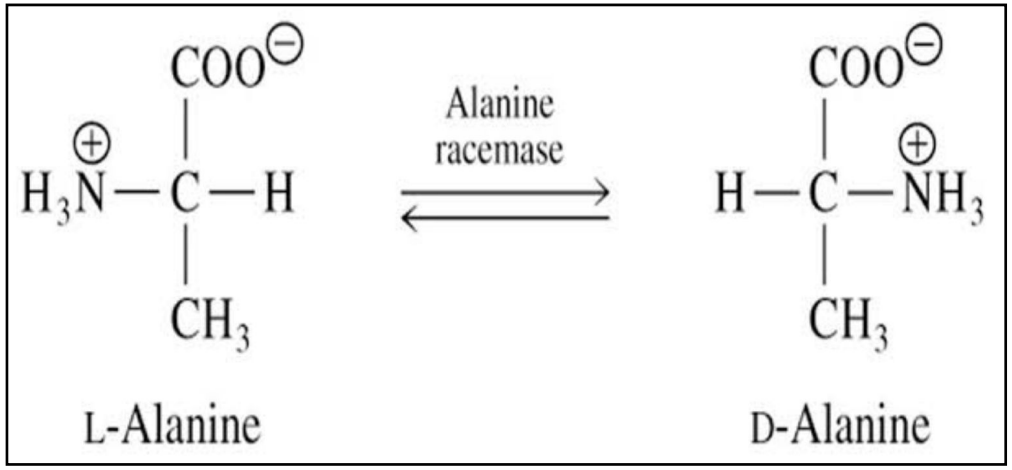
3. **HYDROLASES**



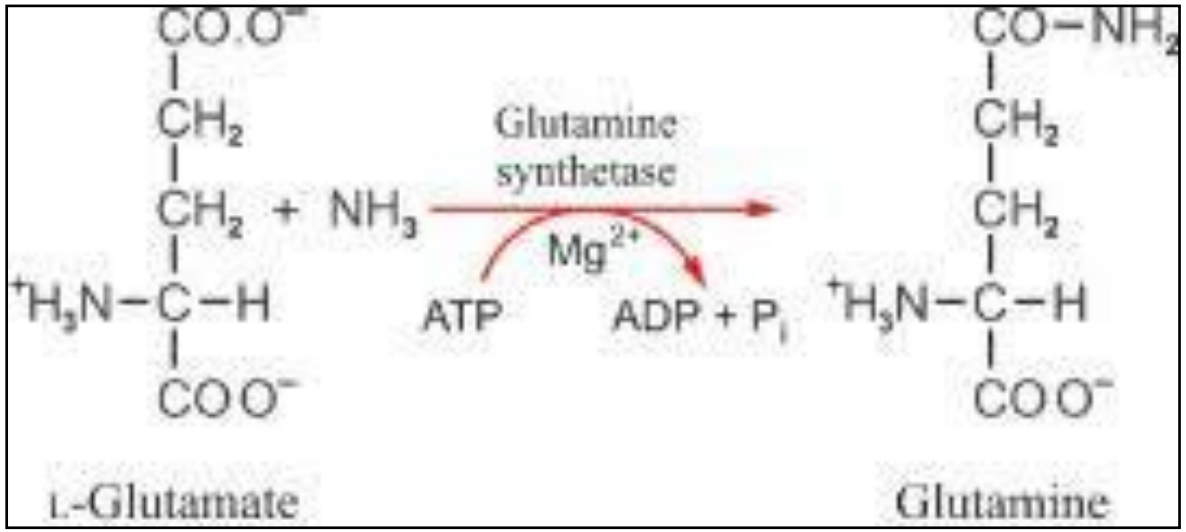
4. LYASES

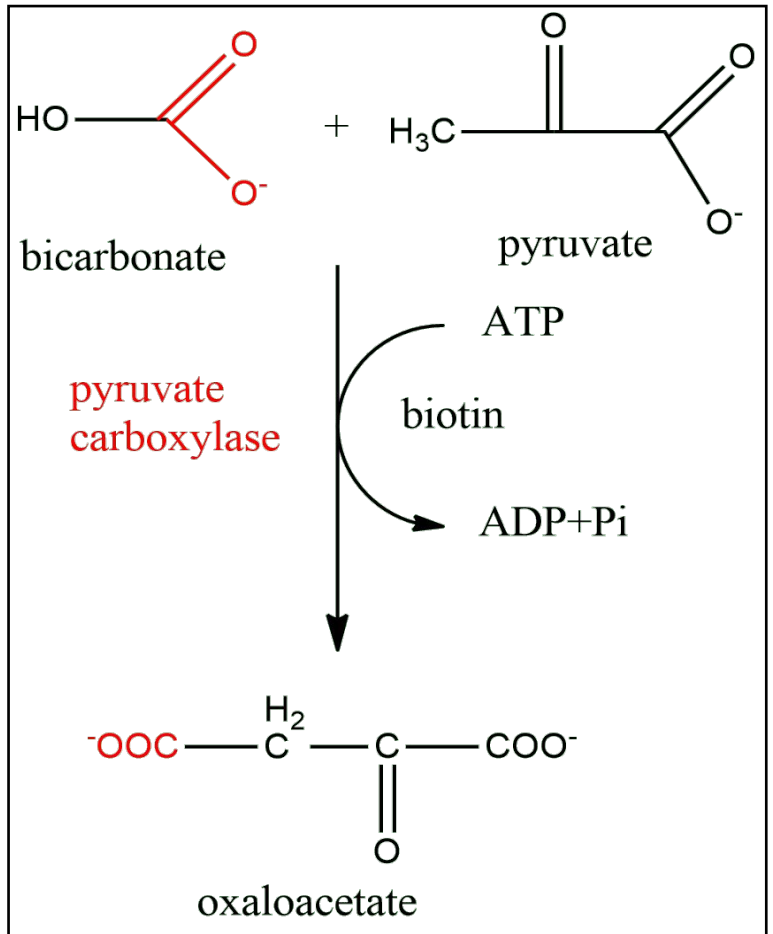


5. **ISOMERASES**

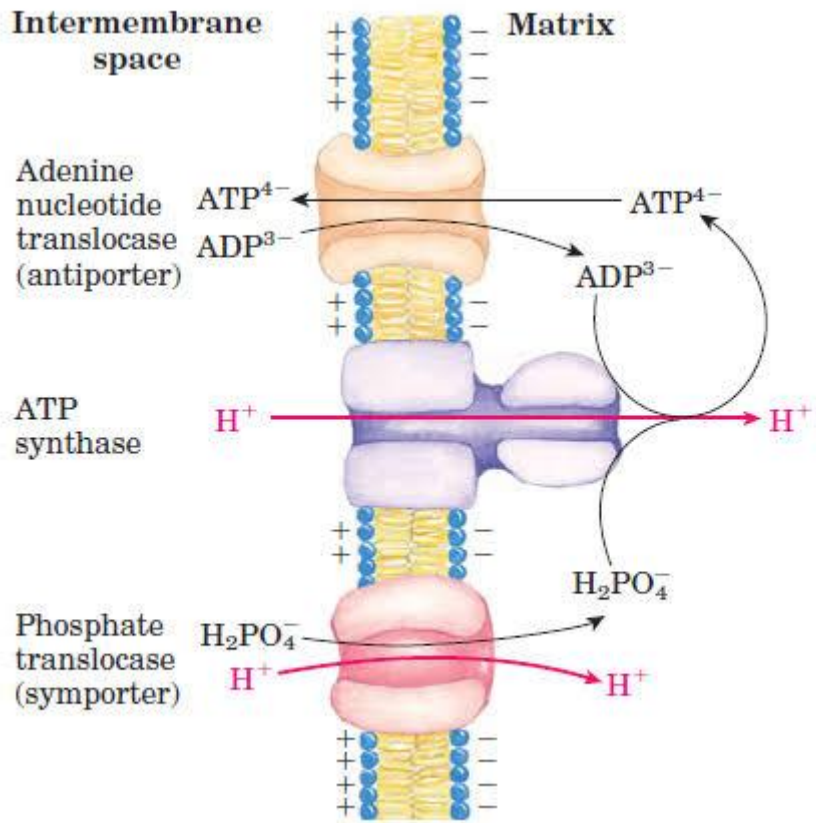


6. **LIGASES/ SYNTHETASES**





7. **TRANSLOCASES**



https://microbiologynote.com/mitochondrial-shuttles-and-transporter-proteins/

NOMENCLATURE OF ENZYMES

EC 1.11.1.6 Catalase

- **1**. Oxidoreductases
- 1.**11** Acting on a peroxide as acceptor
- 1.11.**1** Peroxidases
- 1.11.1.**6** Catalase
- **Accepted name:** catalase-peroxidase
- **Reaction:** (1) donor + H_2O_2 = oxidized donor + **2** H_2O
  (2) H_2O_2 + H_2O_2 = O_2 + **2** H_2O
- **Systematic name:** donor:hydrogen-peroxide oxidoreductase

NOMENCLATURE OF ENZYMES

EC 1.18.6.1 - nitrogenase

- 1. Oxidoreductases
- 1.18 Acting on iron-sulfur proteins as donors
- 1.18.6 With dinitrogen as acceptor
- 1.18.6.1 nitrogenase
- **Accepted name:** nitrogenase
- **Reaction:** **8** reduced ferredoxin + **8** H^+ + N_2 + **16** ATP + **16** H_2O = **8** oxidized ferredoxin + H_2 + **2** NH_3 + **16** ADP + **16** phosphate
- **Systematic name:** ferredoxin:dinitrogen oxidoreductase (ATP-hydrolysing, molybdenum-dependent)

STRUCTURE AND COMPOSITION OF ENZYMES

On the basis of chemical nature, enzymes are of 2 types:

- **(i) Simple enzymes:**

They consist of only proteins, e.g. urease, lysozyme, pepsin, trypsin etc.

- **(ii) Holoenzyme or Conjugate enzyme:**

Holoenzyme = Apoenzyme (proteinaceous part) + Prosthetic group/ Co-factor/ Co-enzyme non- protein part)

COFACTORS, COENZYMES, PROSTHETIC GROUPS

According to IUPAC (International Union of Pure and Applied Chemistry),

Cofactors are organic molecules (coenzymes) or ions (usually metal ions) that are required by an enzyme for its activity and are attached loosely to the enzyme.

If attached tightly to enzyme protein it is called a prosthetic group.

A cofactor binds with its associated protein (apoenzymes), which is functionally inactive, to form the active enzyme (holoenzyme).

Co factors

Cofactors can be divided into two types:

- Inorganic ions such as **Mg^{2+}, Cu^{+}, Mn^{2+}** and **iron-sulfur clusters**

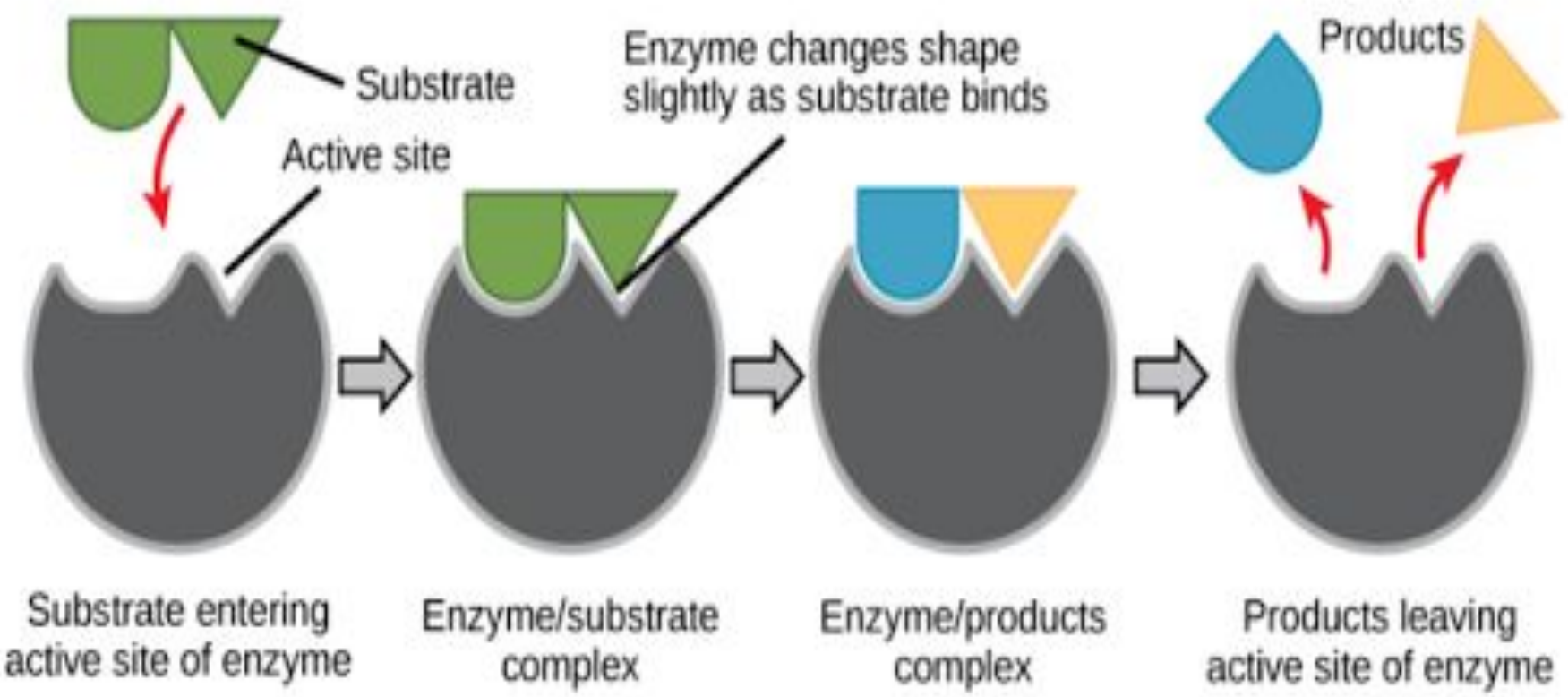
- Coenzymes

Coenzymes are mostly derived from vitamins and other organic essential nutrients in small amounts, examples of coenzymes are - **thiamine pyrophosphate (TPP), biotin, and lipoamide**. Many coenzymes also contain a nucleotide, such as the electron carriers **NAD and FAD, and coenzyme A.**

ENZYME – SUBSTRATE COMPLEX

- The formation of enzyme – substrate complex during enzyme catalysis was postulated by Michaelis and Menten to explain the mechanism of enzyme action.

$$E + S \rightleftharpoons ES \rightleftharpoons P + E$$



https://cdn.kastatic.org/googleusercontent

LOCK AND KEY MODEL/ TEMPLATE MODEL :

This model was proposed by **Emil Fischer (1898)**.

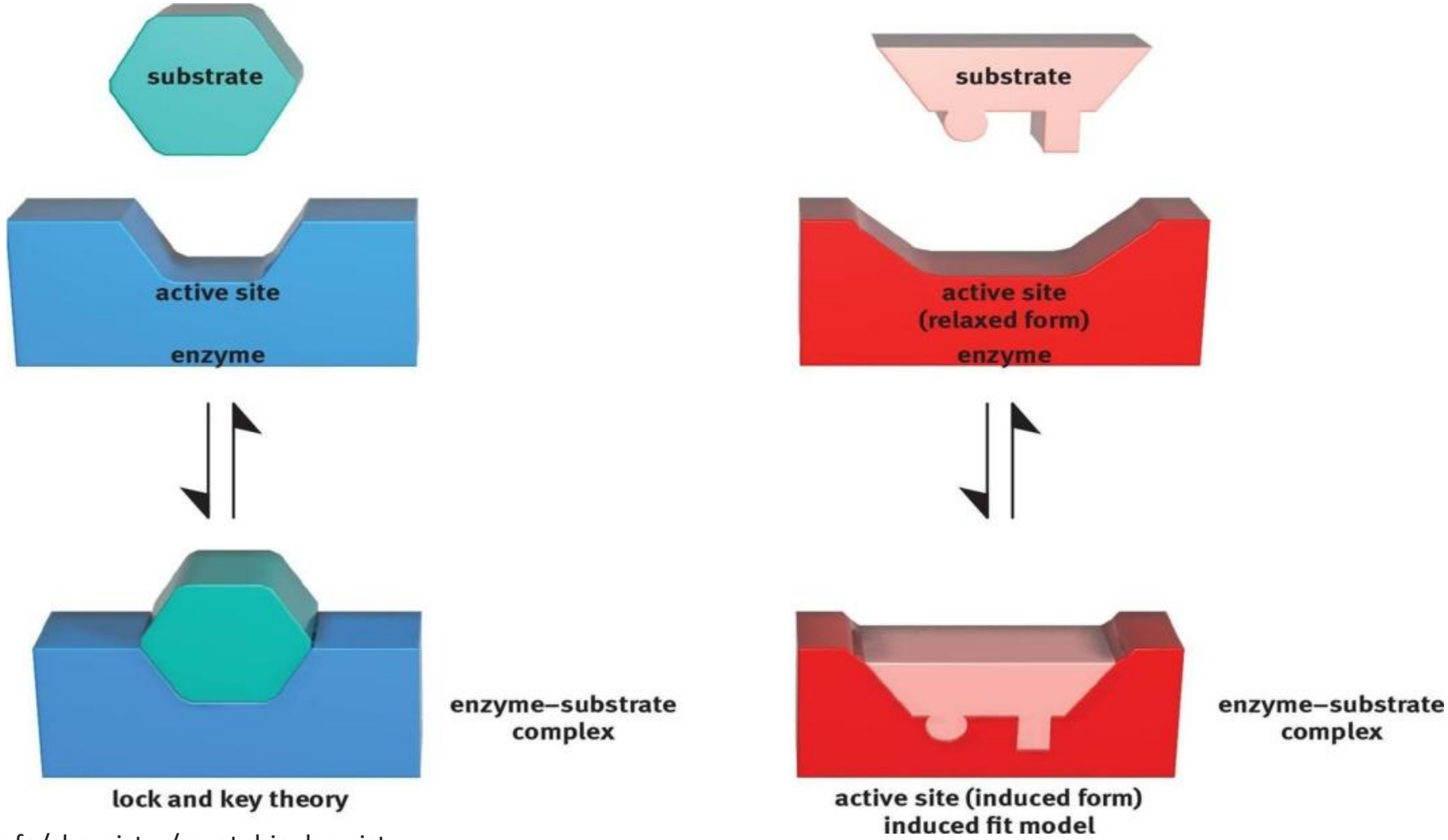
- According to this model, the union between enzyme and substrate takes place at the active site in a manner in which a key fits a lock and results in the formation of enzyme – substrate (ES) complex.

- This hypothesis is also known as "concept of intermolecular fit". In Emil Fischer's model , the active site is presumed to be rigid and pre- shaped to fit the substrate.

INDUCED FIT MODEL :

Daniel Koshland (1958) presumed that the enzyme molecule does not retain its original shape and structure.

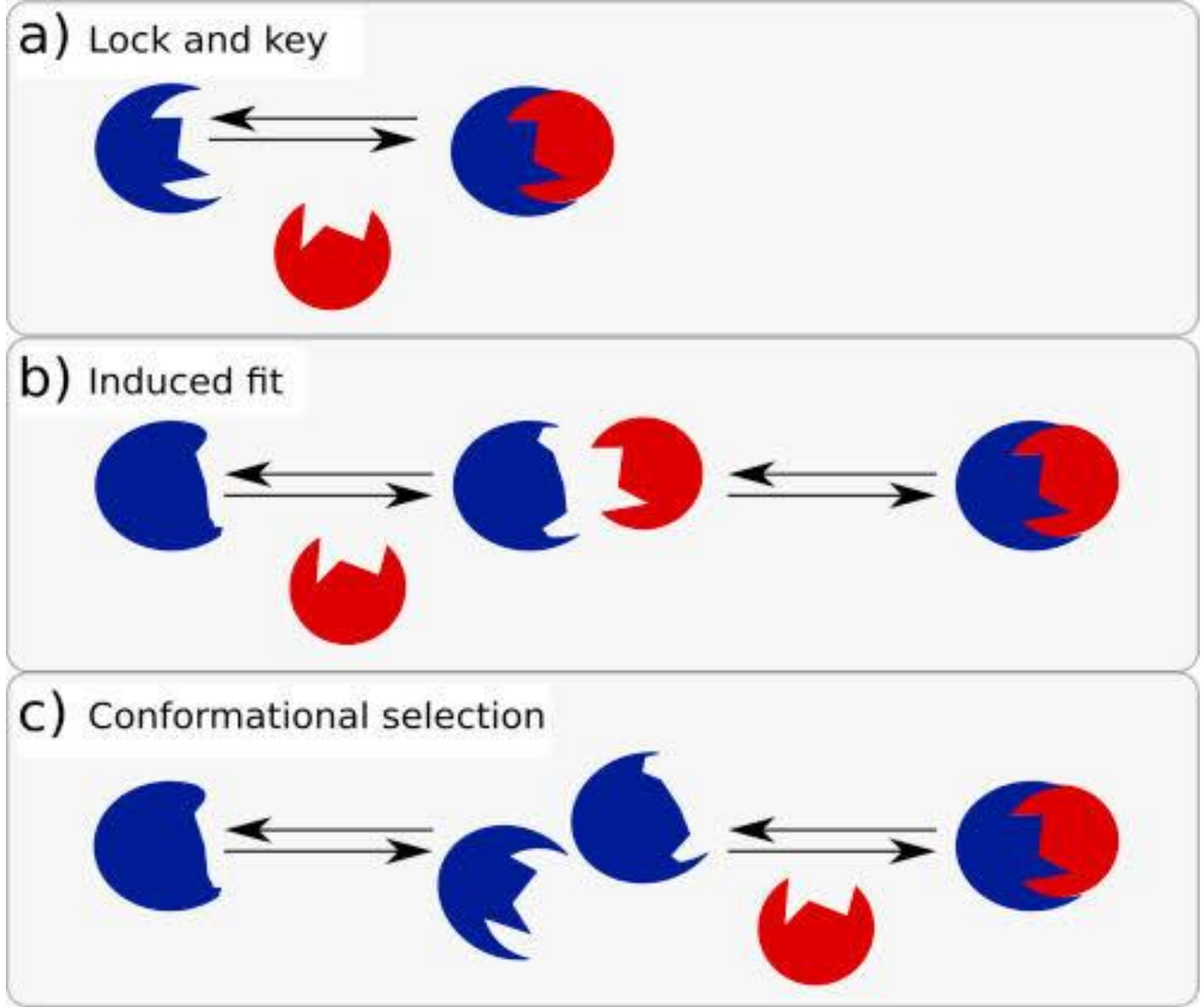
- The contact of the substrate induces some configurational or geometrical changes in the active site of the enzyme molecule and consequently the enzyme molds itself to the shape of the substrate molecule.
- Koshland's model has gained experimental support with various enzymes such as Phosphoglucomutase, Creatine kinase, Carboxypeptidase etc.



https://schoolbag.info/chemistry/mcat_biochemistry

Conformational selection hypothesis

- This model suggests that enzymes exist in a variety of conformations, only some of which are capable of binding to a substrate.



ScienceDirect.com

Induced Fit Model - an overview | ScienceDirect Topics

ACTIVE OR CATALYTIC SITE

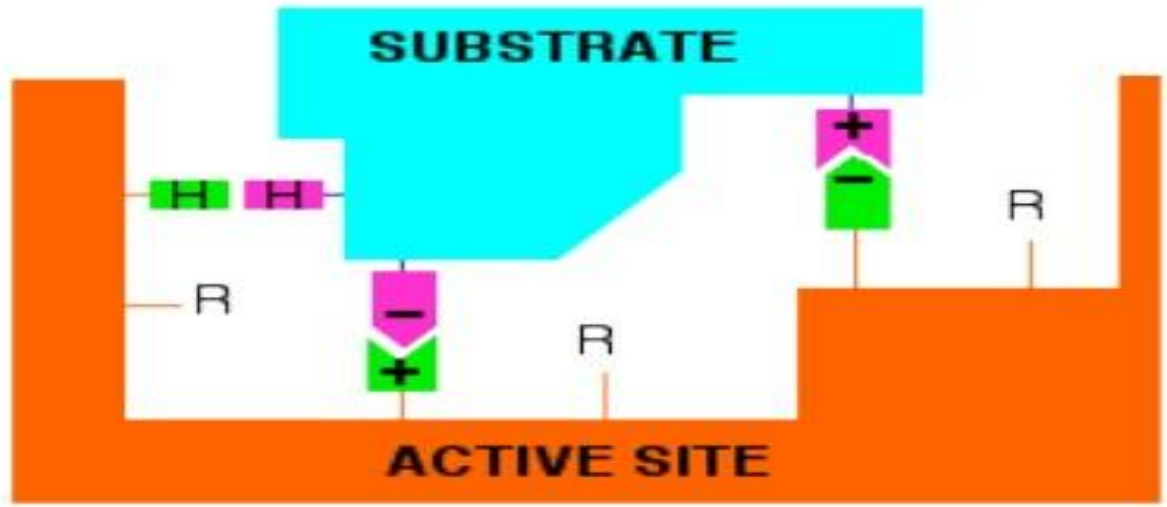
- The **active or catalytic site** is the region (groove/ pocket) of an enzyme where substrate molecules bind and undergo chemical reaction to form products.
- Active site (substrate binding site) occupies only ~10–20% of the volume of an enzyme and usually consists of 3-4 amino acid residues.
- The proper fit between the substrate and the active site is required for the reaction catalyzed by an enzyme to occur.

ACTIVE OR CATALYTIC SITE

- The specificity of active site is determined by the arrangement of amino acids within the active site and the structure of the substrates.
- The interaction between the active site and the substrate is non-covalent and transient.
- There are four important types of interaction that hold the substrate in a defined orientation and form an enzyme-substrate complex (ES complex):

  hydrogen bonds,
  van der Waals interactions,
  hydrophobic interactions and
  electrostatic force interactions.

Active Site



- The substrate is held in the active site by a variety of bonds, such as hydrogen bonds and electrostatic interactions

REGULATORY OR ALLOSTERIC SITE

- Besides the presence of an active or catalytic site, the allosteric enzyme have one or more **regulatory or allosteric site** (allos = other, stereos = space/ site) for the binding of modulator/ effector.
- A modulator alters the kinetic characteristics of an enzyme.
- The modulator may be either an inhibitor or an activator.
- A stimulator is often the substrate itself.
- The regulatory enzymes for which substrate and modulator are identical are called homotropic. When the modulator has a structure different then the substrate, the enzyme is called heterotropic.

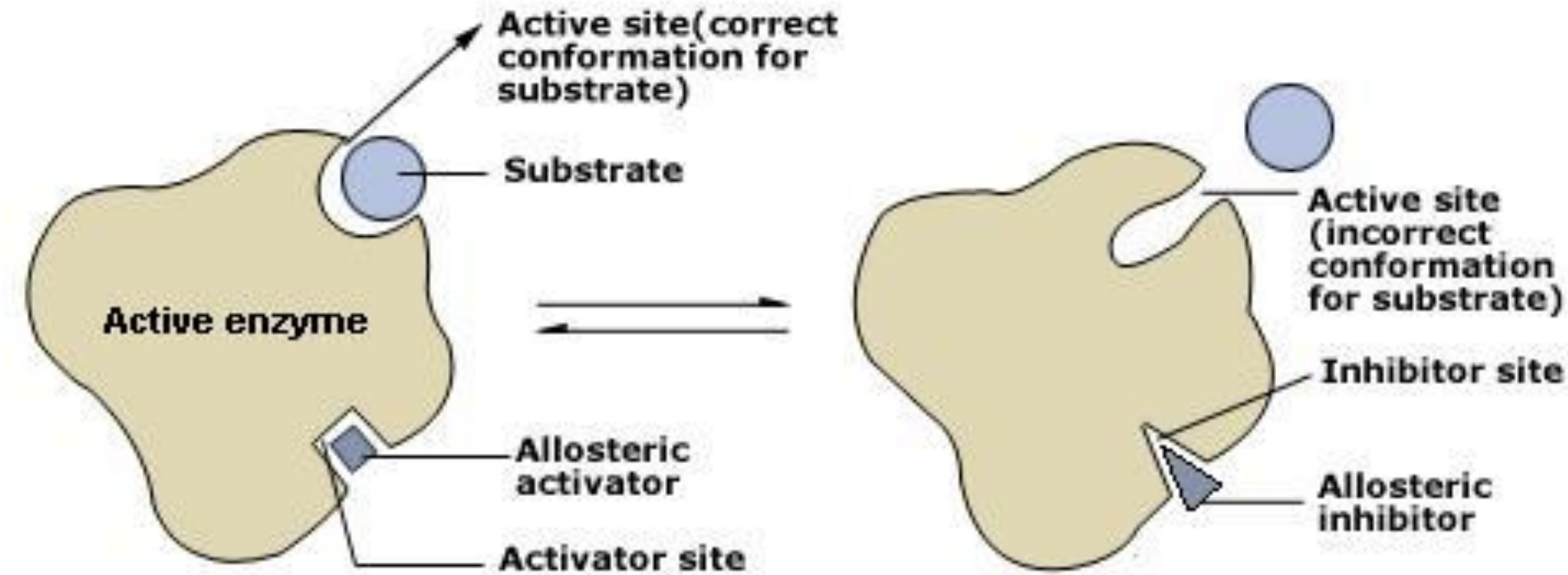
REGULATORY OR ALLOSTERIC SITE

- **In most of the cases** , the allosteric inhibitors are the end products of the metabolic pathway in which that particular enzyme is participating. This kind of enzyme inhibition is also called **feedback/ end product/retro inhibition**.
- The effect of allosteric modulators is reversible, when they are withdrawn, the enzyme resumes the original activity.
- It is suggested that binding of modulator changes the physical configuration of the enzyme molecule.

REGULATORY OR ALLOSTERIC SITE

- Interaction of modulator at the regulatory sites on the enzyme, cause changes in the shape of the enzyme protein.

- The changes profoundly affect the catalytic properties of the enzyme, either inhibiting or stimulating the rate of the reaction.



Schematic representation of allosteric enzyme activity

https://biology.stackexchange.com/questions/42725/difference-between-negative-allosteric-regulation-and-non-competitive-inhibition

Let's Revise:

Q.1 Describe the properties of the enzymes.

Q.2 Differentiate between cofactor, coenzyme and prosthetic group.

Q.3 Write a note on enzyme classification.

Q.4 Describe the characteristics of an active site.

Q.5 What is an allosteric/ regulatory site?

Q.6 Give various theories of enzyme - substrate complex formation.