بسم الله الرحمن الرحيم

Basic Principles and Perspectives in Medical Chemistry and Biochemistry

Enzymes

Part 1

7th Medical and Biochemistry (BIQC-101) Lecture
Second Semester

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Enzymes

Learning Objectives

1. Define enzyme and explain basic functions of enzymes
2. Explain basic properties of enzymes
3. Discover and defines the enzyme components
4. Express localization of enzymes in the cell
5. Defines the active site and catalytic activity of enzyme
6. Discuss working principle of enzymes
7. Express the relationship between enzyme and substrate

Introduction

- **Enzymes** are biological catalysts which speed up the rate of a chemical reaction without being itself changed in the process.
- With the exception of some biocatalysts RNA molecules, called **ribozymes** that catalyze their own splicing, all enzymes are proteins.
- Enzymes are known to catalyze more than 5,000 biochemical reaction types.
- Enzymes can increase the rate of a reaction by a factor of up to 10^{20} over an uncatalyzed reaction.
- Chemically, enzymes are like any catalyst and are not consumed in chemical reactions, nor do they alter the equilibrium of a reaction. Enzymes differ from most other catalysts by being much more specific

Naming Enzymes

Enzymes names can be formed in Four ways:-

1. **Depend of the reacting substance (substrate)**

   Usually ends in *–ase add to the substrate*

   Ex: *sucrase* catalyzes the hydrolysis of sucrose

2. **Depend on the function (reaction) of the enzyme**

   Ex: *oxidases* catalyze oxidation reactions.

2. **Depend on both the substrate and the function**

   Ex: *alcohol dehydrogenase* oxides ethanol

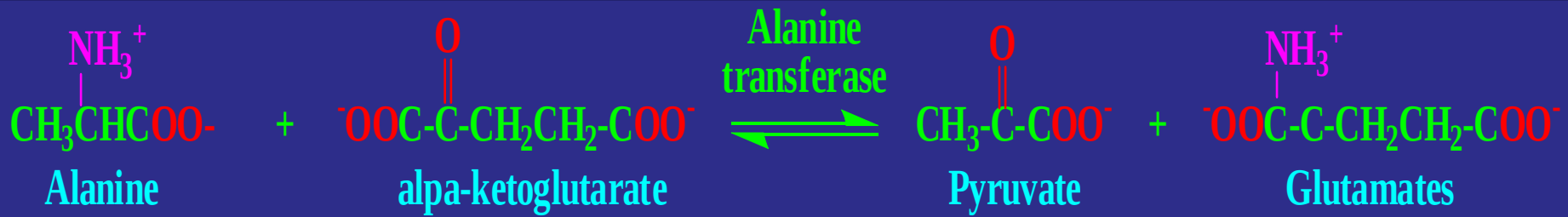
1. **Sometimes common names are used**, particularly for the digestion enzymes such as *pepsin* and *trypsin*

Classification of Enzymes

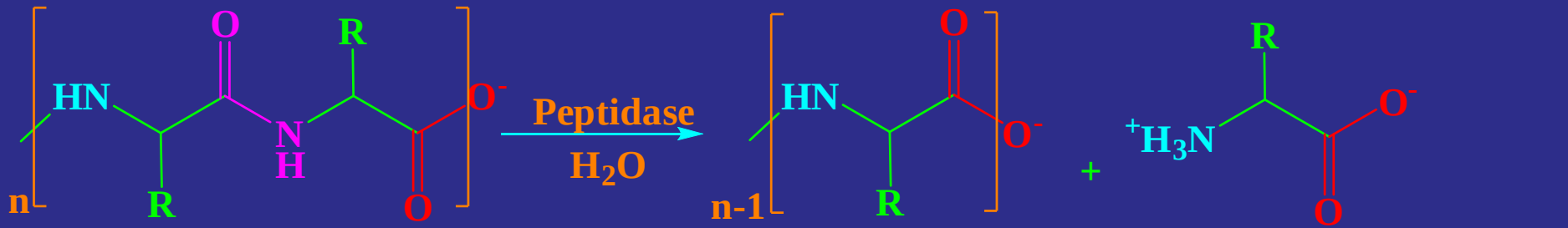
Enzymes are classified according to the type of reaction they catalyze:

	Class	General reaction Catalyzed	Typical subclasses	Functions
1	Oxidoreductase	Oxidation-reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Dehydrogenation

2	Transferase	Transfer functional groups	Transaminase Kinase	Transfer amine group Transfer phosphate group

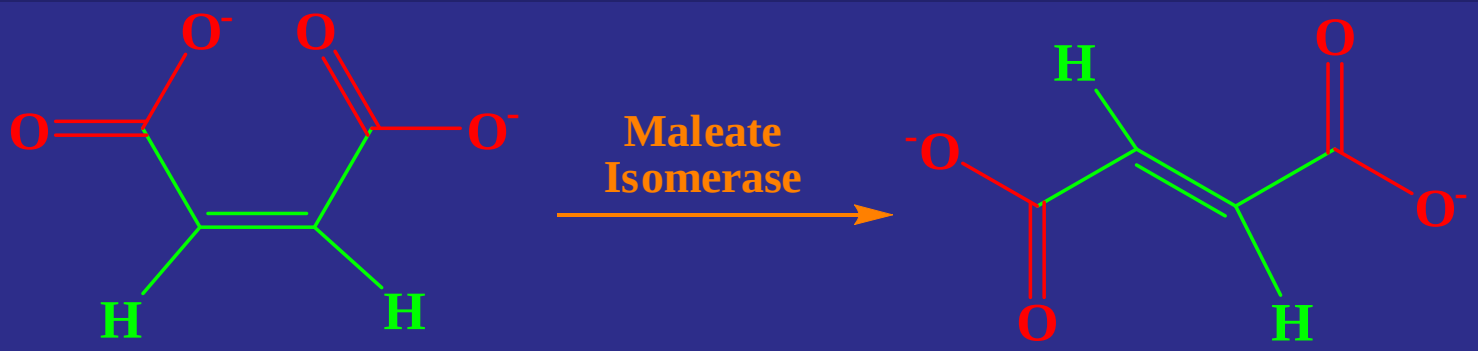


	Class	General reaction Catalyzed	Typical subclasses	Functions
3	Hydrolases	Hydrolysis reactions	Peptidase Lipases Amylases	Hydrolysis peptide pond Hydrolysis ester bond in lipids Hydrolysis 1,4-glycoside bond in amylose



4	Lyases	Addition or removal group from double bond without hydrolysis	Decarboxylase Dehydrase Deaminase	Remove CO_2 Remove H_2O Remove NH_2

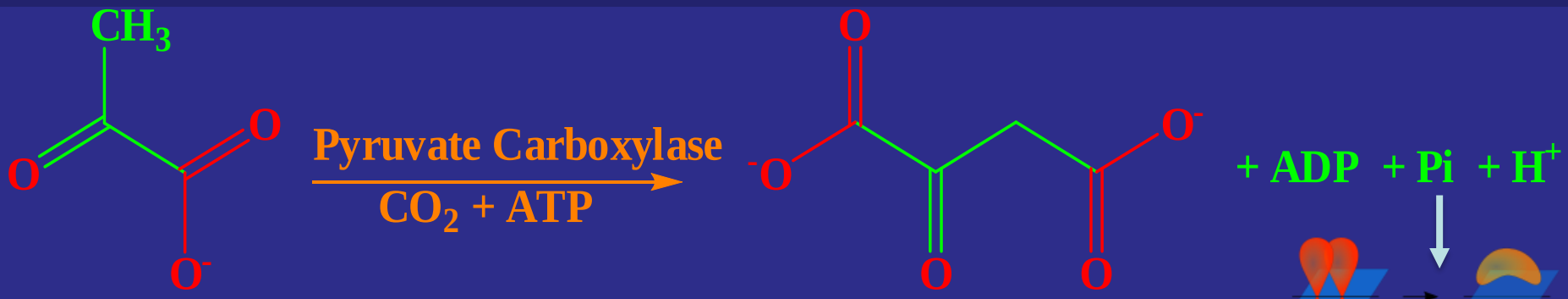
	Class	General reaction Catalyzed	Typical subclasses	Functions
5	Isomerase	Rearrangement of atoms to form isomers	Isomerase Epimerase	Convert cis and trans Convert D and L



Cis Maleate

Trans Maleate

6	Ligases	Bonding of molecules using ATP energy	Synthetase Carboxylases	Combine molecules Add CO_2



Pyruvate

Oxaloacetate

Two p-orbitals forming a π-bond

Types of enzymes

1. Endoenzymes

Enzymes that function within the cells. Most of the enzymes are these types. Eg. metabolic enzymes cytochrome oxidase found mitochondria of eukaryotes (catalyzes the reduction of molecular oxygen to water).

2. Exoenzymes

Enzymes that are liberated by cells and catalyse reactions outside the cell. Eg. digestive enzymes amylase, lipase, protease (catalyzes proteolysis, the breakdown of proteins into smaller polypeptides or single amino acids

Enzyme Structure

1. **Simple enzymes:** only protein structure
2. **Complex enzymes (holoenzymes):** Protein structure (Apoenzyme) + cofactor

Cofactors are nonprotein compounds. Cofactor can be:

1) **Inorganic element**: Zn^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}.

2) **Organic molecule:-**

a) Coenzymes:

- Small non-proteins **slightly** bound to the enzyme
- Undergo a chemical change and are released.
- Ex: Coenzyme A (acyl transfer), Flavins (redox reaction) NAD^+ ($NADP^+$)(redox reactions), Vitamins: derivatives of B vitamins (B1, B2, B6, B12), niacin, folic acid, riboflavin

b) **Prosthetic groups:**

- Large complex **tightly** bound to the enzyme.
- Remain associated with enzyme during reaction: heme, …

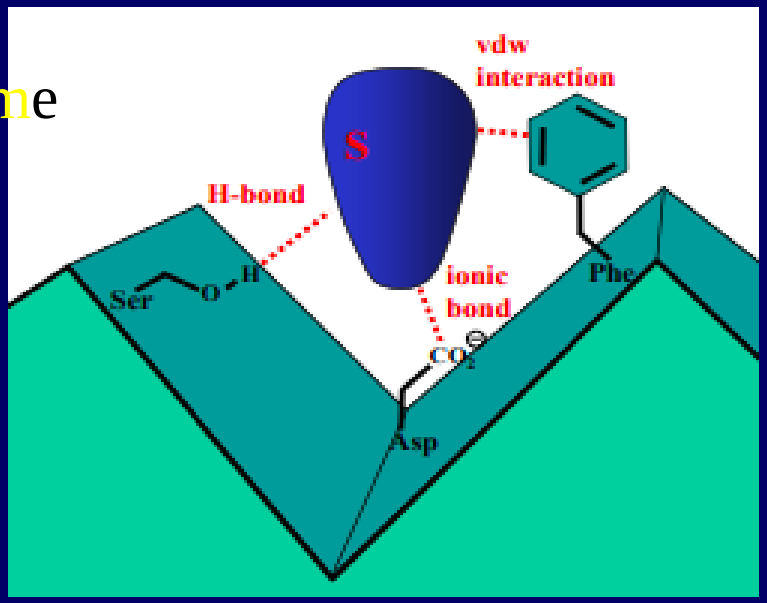
Enzyme Active Site and Substrate Specificity

- **Substrate:** A reactant in a chemical reaction is called a substrate when acted upon by an enzyme.
- **Active site:** The active site is the part of an enzyme to which **substrates bind** and where a **reaction is catalyzed**. Since enzymes are proteins, this site is composed of a unique combination of amino acid residues. Size of amino acids (large or small), properties of amino (acids weakly acidic or basic; hydrophilic or hydrophobic; and positively-charged, negatively-charged, or neutral in addition to the positions, sequences, structures, of these residues create a very specific chemical environment within the active site specific with unique substrate.

Substrates binding: A specific chemical substrate matches this and makes the enzyme specific to its substrate.

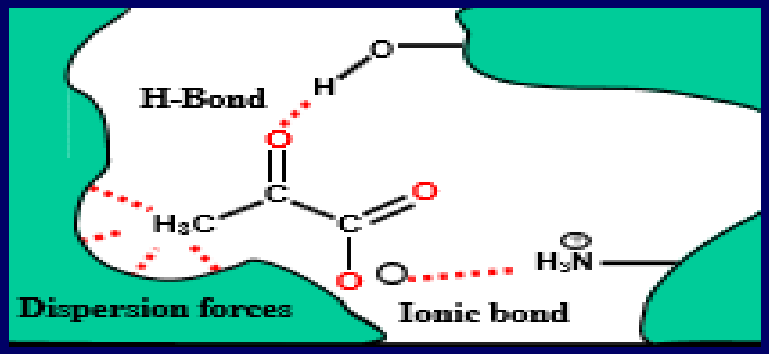
Substrate binding to active site using the usual forces of interaction.

1. Ionic
2. H-bonding
3. Dispersion forces (van der wale VDW)
4. Dipole -dipole
5. Covalent bonds
6. Pi stacking (noncovalent interactions between aromatic rings)



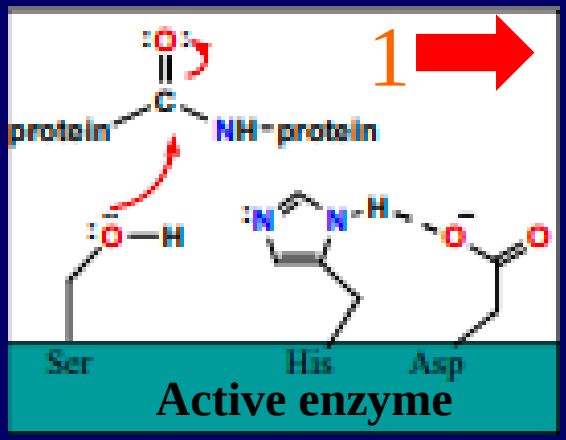
Ex: Binding of pyruvic acid in LDH (lactic dehydrogenase enzyme)

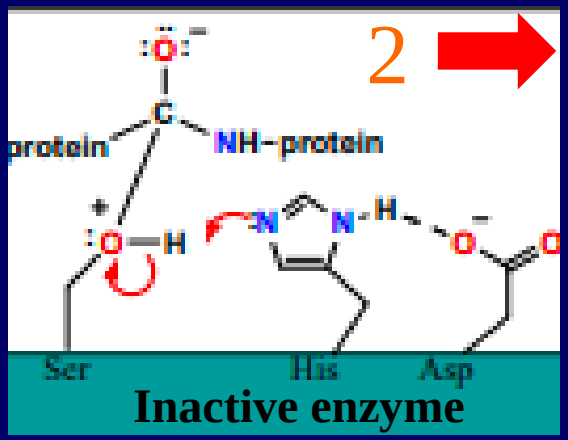
Ionic bonding, H-bonding, dispersion forces

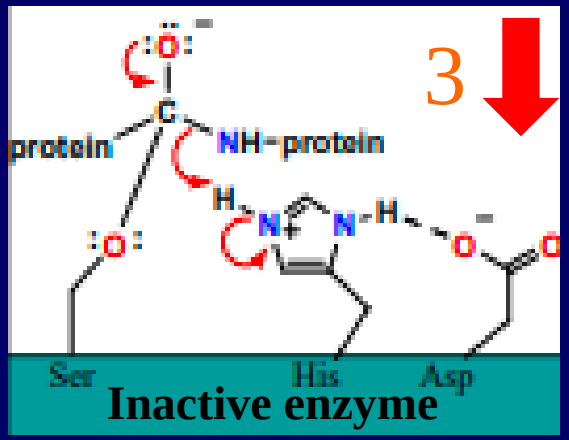


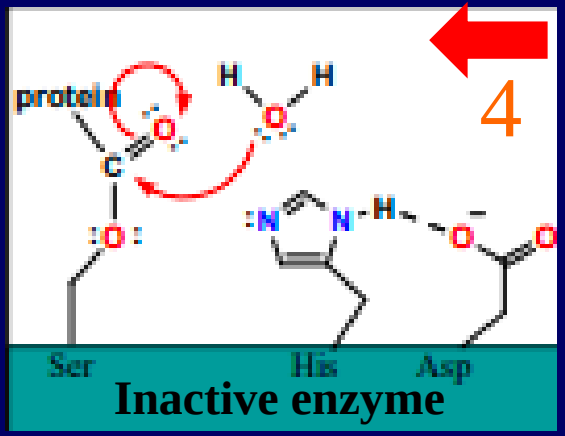
Reaction catalyzed: Chymotrypsin is a digestive enzyme belonging to a super family of enzymes called **serine proteases**. It uses an active serine residue to perform hydrolysis on the C-terminus of the aromatic amino acids of other proteins.

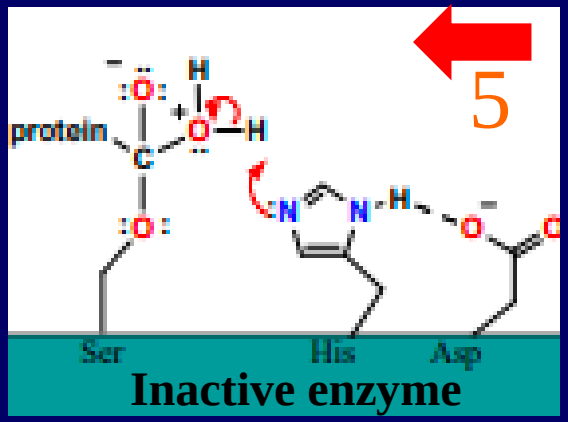
Mechanism of Chymotrypsin catalysis reaction

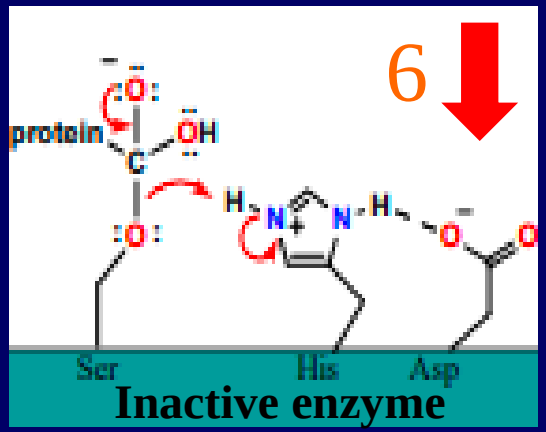


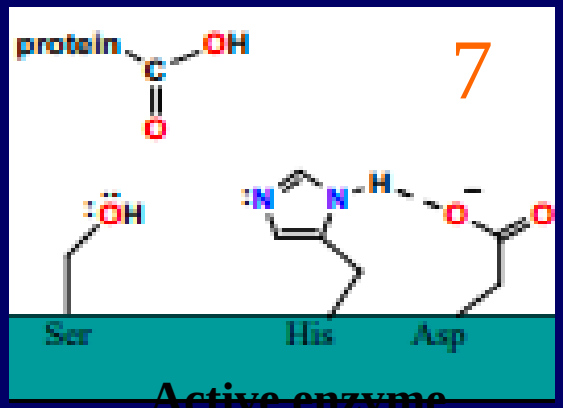












Chymotrypsin is a **protease enzyme** that cleaves on the C-terminal phenylalanine (F), tryptophan (W), and tyrosine (Y) on peptide chains. It shows specificity for aromatic amino acids because of its hydrophobic pocket.

Chemical Reactions Proceed

- All chemical reactions proceed through one or more transition-state intermediates whose content of free energy is greater than that of either the reactants or the products.
- For the simple reaction R (reactants) ⇌ P (products), we can write

Where:

S: The reaction intermediate with the highest free energy.

***K*‡:** The equilibrium constant for the reaction R ⇌ S, the conversion of the reactant to the high-energy intermediate S

***V*:** The rate constant for conversion of S into the product P.

The rate *V* of the overall reaction R ⟶ S will be proportional to the rate constant *v* and to the number of molecules in the transition state S, that is, the concentration of the transition-state intermediate, **[S]**: $V \propto [S]$

$$V = v\,[S]$$

But since S is in equilibrium with R, the reactant, we can write

$$K^{\ddagger} = \frac{[S]}{[R]} \qquad \text{Or} \qquad [S] = [R]\,K^{\ddagger}$$

As with all equilibrium constants, K and ΔG are related as shown by the following equation:- $\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$

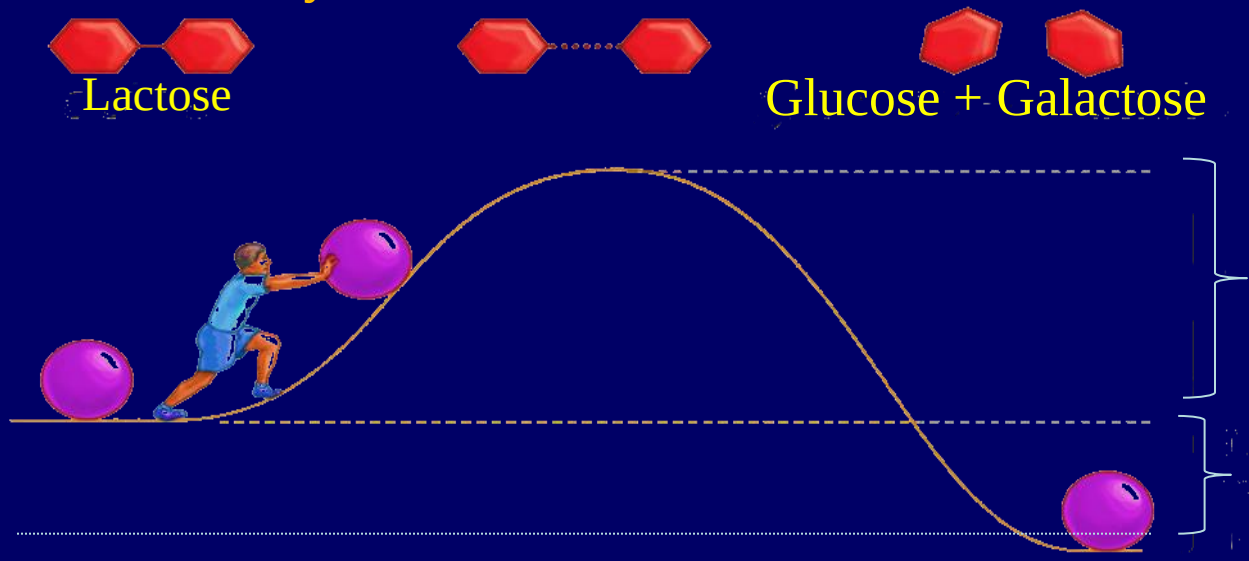
$$V = v\,[R] \times 10^{-(\Delta G^{\ddagger}/2.3RT)}$$

From this equation, we can see that lowering the activation energy that is, decreasing the free energy of the transition state ΔG leads to an acceleration of the overall reaction rate V by increasing the concentration of S. A reduction in ΔG of 1.36 kcal/mol leads to a tenfold increase in the concentration of S, and thus a tenfold increase in the rate of the reaction

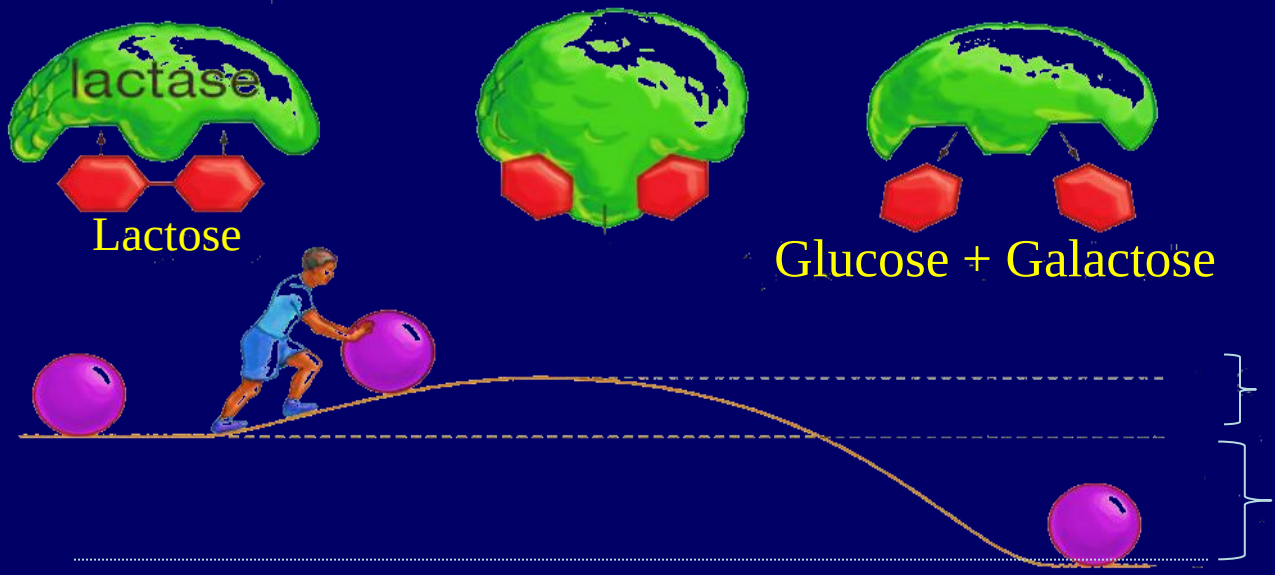
How enzyme catalyze the reaction?

- Enzymes are catalyst that accelerate the rate of biochemical reaction by **decreasing the energy of activation.**
- Every chemical reaction have energy barrier that must be crossed by the reactant molecules in order to convert itself into the product.
- The amount of energy supplied to reactant molecules in order to cross the energy barrier to from product is known as **energy of activation**.
- If energy of activation is higher, rate of reaction is slower and if it is lower, the rate of reaction is faster.
- The role of enzyme in biochemical reaction is to reduce the amount of energy of activation such that the rate of reaction increases.

A: Without Enzyme

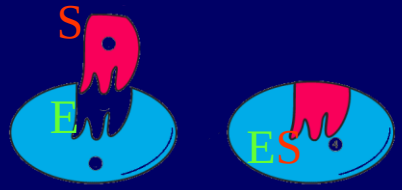


B: With Enzyme



During enzyme catalysis, active site of enzyme binds with substrate molecules to form Enzyme-substrate (ES) complex.

$$E + S \leftrightarrows ES$$



During this binding some binding energy is released which is utilized to activate the substrate (reactant) molecules to form product.

$$ES \rightarrow E + P$$

Thus the requirement of the amount of activation energy is decreased such that rate of reaction increases.

The amount of activation decrease is equal to the amount of binding energy released during binding of enzyme and substrate

$$E + S \leftrightarrows ES \rightarrow E + P$$

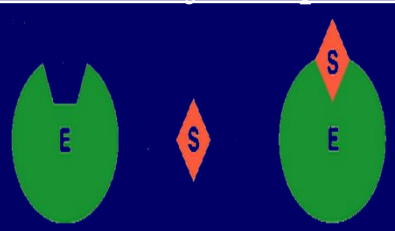
Mechanism of enzyme action

There are two hypothesis:-

1. Lock and Key model:(Fischer in 1890)

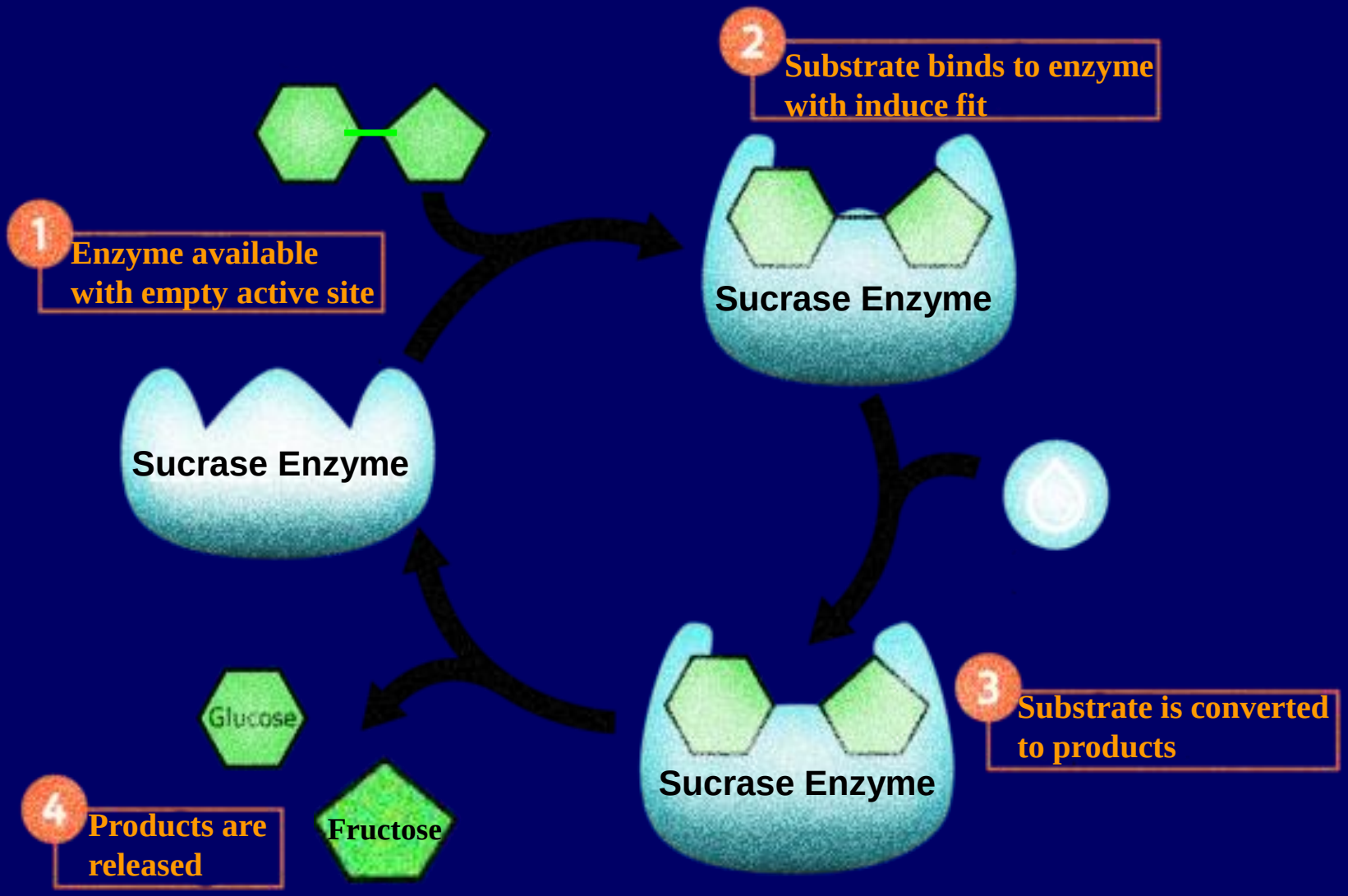
- According to this model, shape of active site of enzyme is complementary to the shape of substrate molecules. The substrate is like a key whose shape is complementary to the enzyme which is supposed to be lock and they fit perfectly.
- Enzymes catalyze only those substrates which fit perfectly on the active site of that enzyme.
- Most enzymes are far larger than the substrates molecules that act on and the active site is usually a very small portion of the enzyme, between 3 and 12 amino acids. The remaining amino acids which make the bulk of the enzyme, function to maintain the correct globular shape of the enzyme.
- Once the product is formed, they no longer fit into the active site and escape into surrounding medium.

2. Induced fit model: (1959, Koshland)



- A modified to the 'Lock and Key' hypothesis .
- Working from evidence that some enzymes and their active site are more flexible.so, this hypothesis proposed that the active site can modify its shape as the substrate interact with the enzyme.
- The amino acids which make up the active site are moulded into precise shape which enable the enzyme to perform its catalytic function most efficiently.
- For instance, a suitable analogy to describe induced fit model would be that of a hand changing the shape of the glove as the individual put on the glove. Therefore in this case, glove is the active site of enzyme and the hand is substrate.
- However, in some cases, the substrate molecules changes slightly as it enters the active site before binding.

The Catalytic Cycle of an Enzyme



Factors affecting enzyme activity

1. **Temperature:** Raising temperature generally speeds up a reaction, and lowering temperature slows down a **reaction**. However, extreme high temperatures can cause an enzyme to lose its shape (denature) and stop working.
2. **pH:** Each enzyme has an optimum pH range. Changing the pH outside of this range will slow enzyme activity. Extreme pH values can cause enzymes to denature.
3. **Enzyme concentration:** Increasing enzyme concentration will speed up the reaction, as long as there is substrate available to bind to.
4. **Substrate concentration:** Increasing substrate concentration also increases the rate of reaction to a certain point.

$$Vo = \frac{Vmax\,[S]}{Km + [S\}}$$

The Michaelis-Menten Equation

Km = Michaelis constant , Vo = initial velocity caused by substrate concentration, [S]

V_{max} = maximum velocity

thank you!