4

CHAPTER 4

BIOCATALYSIS

4.0 BIOCATALYSIS

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4.1: Properties of enzymes and mechanisms of actions

4.2 : Cofactor

4.3 : Inhibitors

Amazing fact

Did you know that enzymes help to fight disease?

Fungi, bacteria and parasites that invade our bodies and cause sickness are made up of proteins.

Even a virus is protected by a protein based shell.

Although enzymes are themselves proteins, there is one called protease which breaks down other proteins.

High levels of protease in the blood can help to break do wn the disease causing vectors in our body

So remember…enzymes are not only for digestion…..

4.1 : Properties of enzyme and mechanism of actions

Learning outcome :

At the end of this subtopic, students should able to :

a) State the **properties** of enzymes.

b) State the **six classes of enzyme** according to IUB classification.

c) Explain how enzyme lowering activation energy

d) Illustrate to explain the mechanism of enzyme action based on **Induced Fit Model**

e) State the factor that affect the enzymatic reaction

Introduction

Enzymes

An **organic catalyst** (usually a protein)

- that **speed up/accelerates** a **specific** chemical reaction
- by **lowering activation energy (E_A)** required for that reactions
- without itself being affected by the reaction.

Properties of enzyme

Required Only in Small Amount.

Not altered during reaction

Highly specific

Not damaged or destroyed by reactions it catalyzes.

No effect on the thermodynamics of the reaction.

Denatured by high temperature and pH.

Speed up the chemical reactions

Amazing fact

Since an enzyme is a protein, then it means that it can be dissolved in water
and can be diluted with the use of a salt solution…

THE CLASSIFICATION OF ENZYMES

- A systematic nomenclature for enzymes was recommended by **the International Union of Biochemistry and Molecular Biology (IUBMB).**

- Each enzymes was allocated a trivial name:
  - The name of the substrate act upon the enzyme
  - The type of reaction catalysed
  - The suffix –ase
  - E.g: urase, sucrase, protease.

THE CLASSIFICATION OF ENZYMES

Oxidoreductase	Transferase	Hydrolase
Transfer of O and H atoms between substances.	Transfer of a functional group from one substance to another.	Hydrolysis reactions
All oxidation-reduction reactions.	E.g :Kinase	E.g :
E.g:		Peptidase
Dehydrogenase		Lipase
		Amylase

THE CLASSIFICATION OF ENZYMES

Lyases

Addition or removal of a functional group without addition of water

E.g :Pyruvate decarboxylase

Isomerase

Catalyse geometric or structural rearrangement within one molecule

E.g :

Phosphoglucomutase

Ligase

Formation of bonds between two molecules using energy derived from the breakdown of ATP

E.g :

Synthetase

4.0 BIOCATALYSIS

Share & Remember

H	Hydrolase
O	Oxidoreductase
T	Transferase
I	Isomerase
L	Lyases
L	Ligase

What is activation energy ?

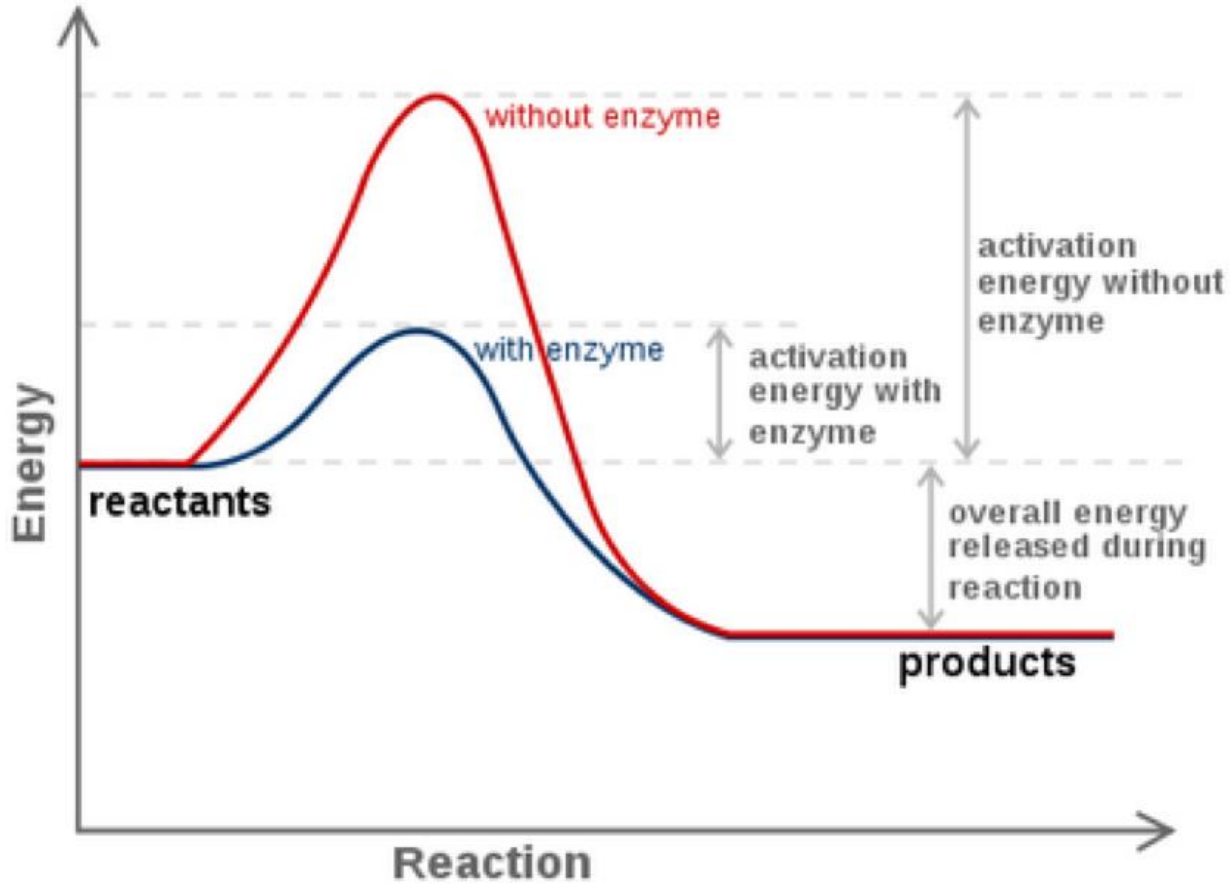
- Every chemical reaction between molecules involves bond breaking and bond forming.

- The initial energy needed to start a chemical reaction is called the **free energy of activation** or **activation energy (E_A)**.

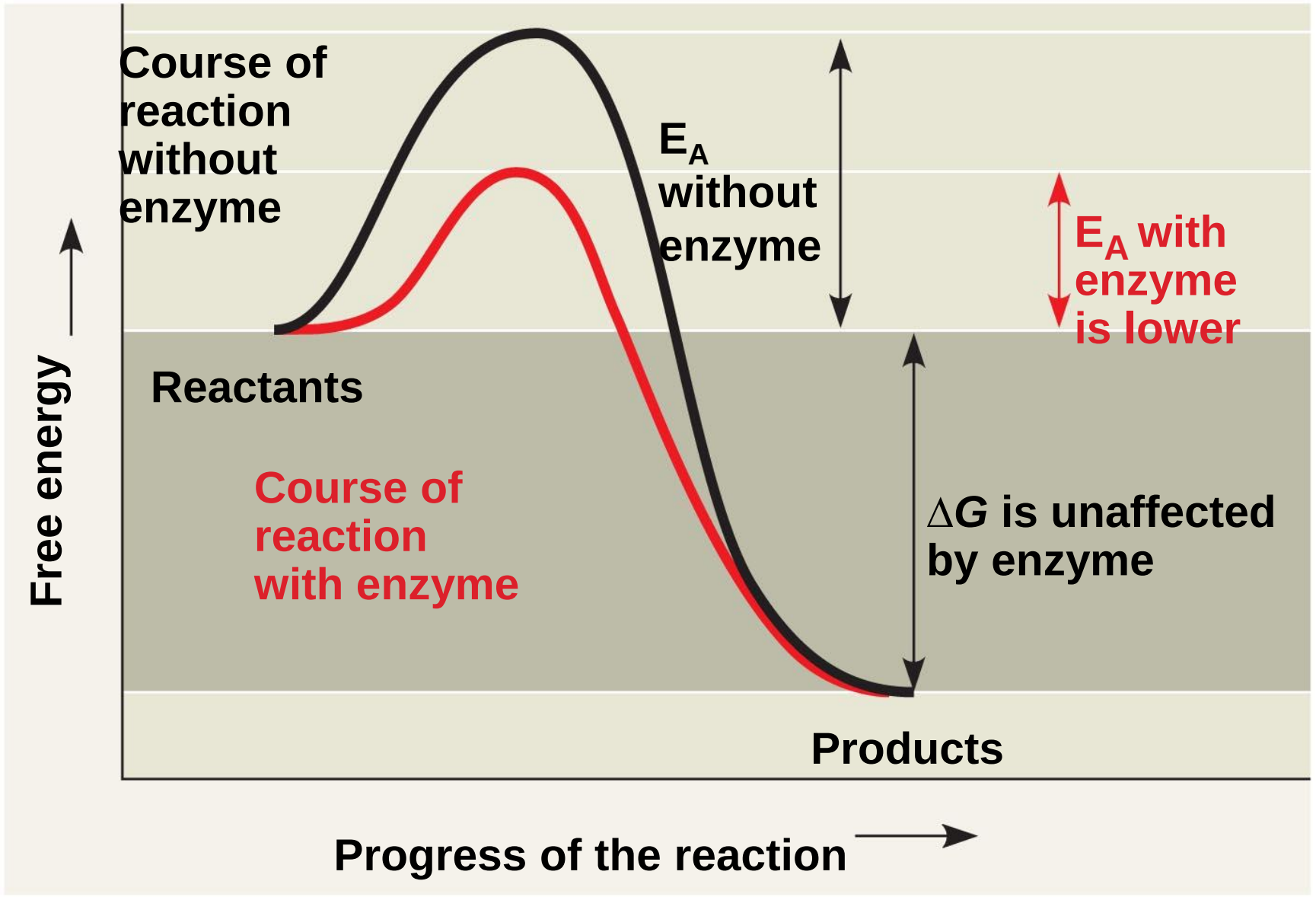
- Activation energy is often supplied in the form of heat from the surroundings.

How do enzymes work?

- Lowering the activation energy (E_A) barrier necessary to initiate a chemical reaction

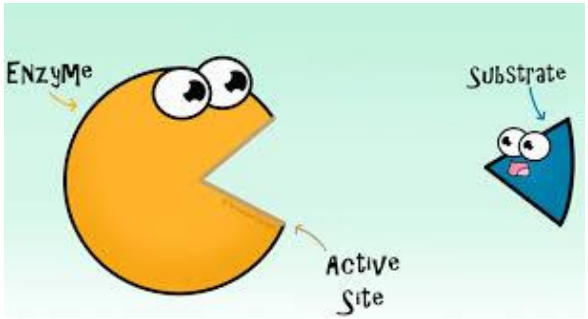


How do enzymes work?

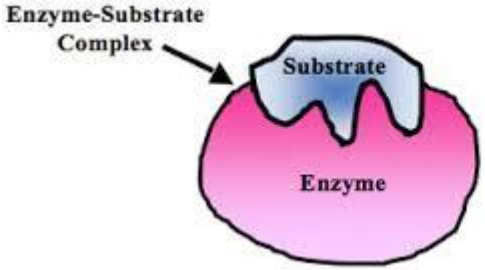


MECHANISM OF ENZYME ACTION

- The reactant that an enzyme acts on is called the **substrate.**
- The **active site** is the region on the enzyme where the substrate binds.
- In an enzymatic reaction, the substrate binds to the active site of the enzyme



- Forming an **enzyme-substrate complex.**



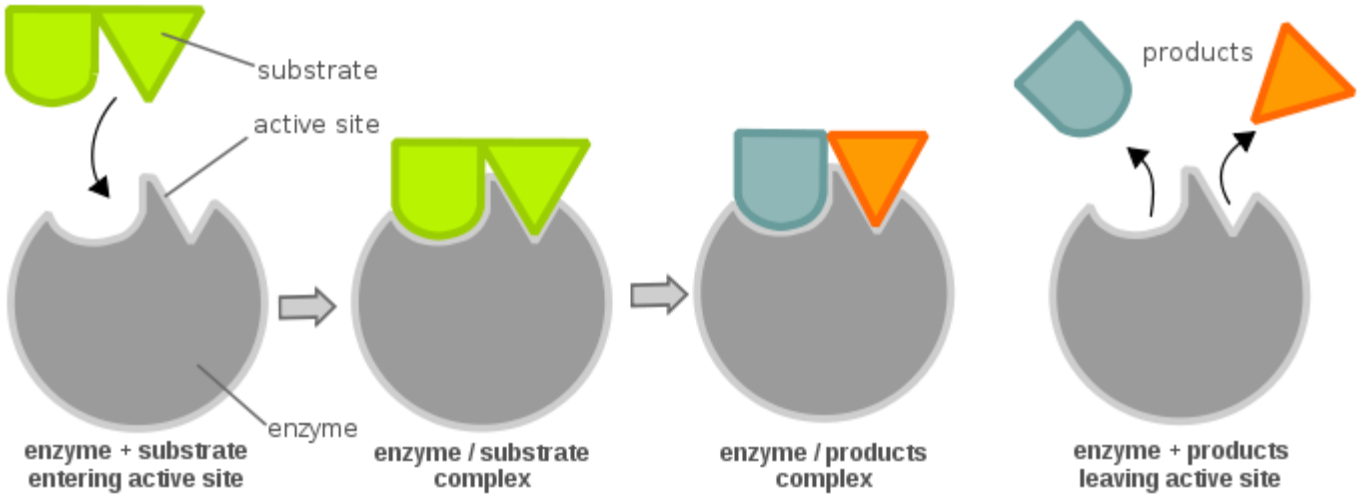
Catalysis in the Enzyme's Active Site

- In an enzymatic reaction, the substrate binds to the active site of the enzyme.

- The active site can lower an E_A barrier by :
  - Orienting substrates correctly
  - Straining substrate bonds
  - Providing a favorable microenvironment
  - Covalently bonding to the substrate

(Campbell. Biology 9th edition. International edition. Page 200 – 201)

Mechanism of Enzyme Action



'Induced Fit' Model

Active site (enzyme) is able to change its shape (flexible) to enfold a substrate molecule.

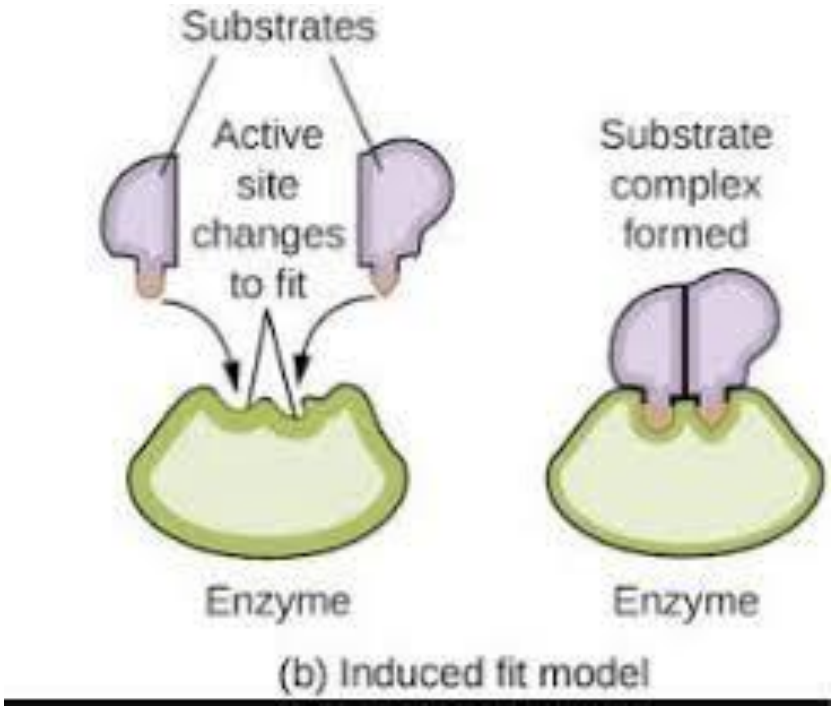
- Distorted enzyme molecule in turn distorts the substrate molecule.
- Straining or twisting the bonds.
- Substrates less stable and thus lowering E_A.

https://www.khanacademy.org/test-prep/mcat/biomolecules/enzyme-structure-and-function/v/the-induced-fit-model-of-enzyme-catalysis

‘Induced Fit’ Model

When products are formed

- Enzyme returns to its original shape.
- Ready to bind the next substrate molecule.



DID YOU KNOW?

Enzyme production decreases with age. As we age, our bodies produce less protease, lipase and amylase, which means digestion of protein, fats and carbohydrates can be impaired as we get older...

FACTORS THAT AFFECT THE ENZYMATIC REACTION

❖The rate of an enzyme-catalyzed reaction is affected by :

❖Chemical and Physical Factor that alters the enzyme's 3-D shape :

pH
Temperature
Present of Regulatory molecules

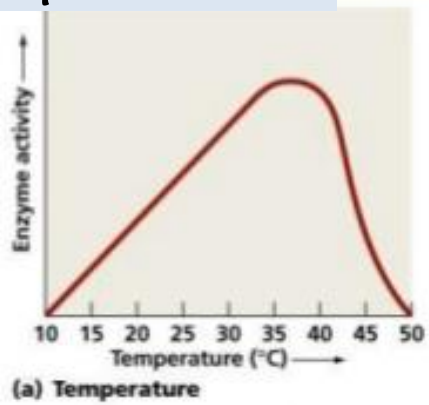
❖ Concentration of **substrate** and **enzyme**.

❖ Chemicals that specifically influence the enzyme.
Cofactors

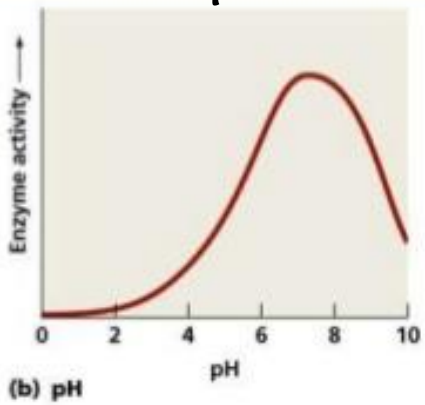
Four factors affecting enzyme reaction rate

Temperature

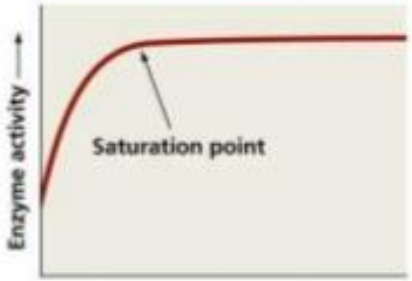
pH



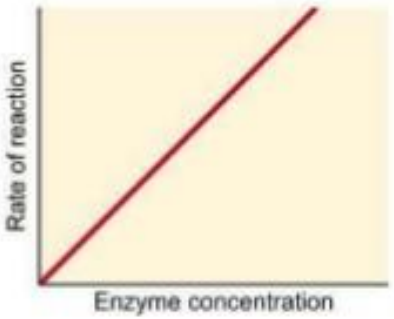
(a) Temperature



(b) pH



(c) Substrate concentration



Enzyme Concentration

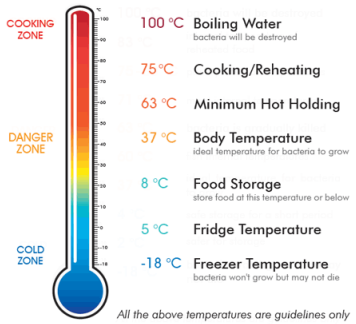
Substrate Concentration

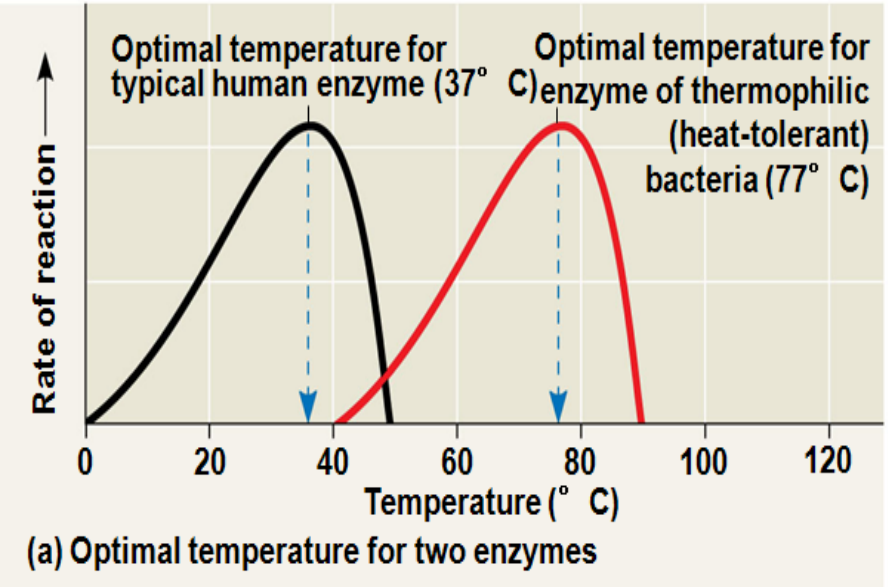
1. Temperature

- Each enzyme has an optimal temperature.

- **Optimal temperature**: at which an enzyme produces the highest production rate.

- Optimal conditions favor the most active shape for the enzyme.



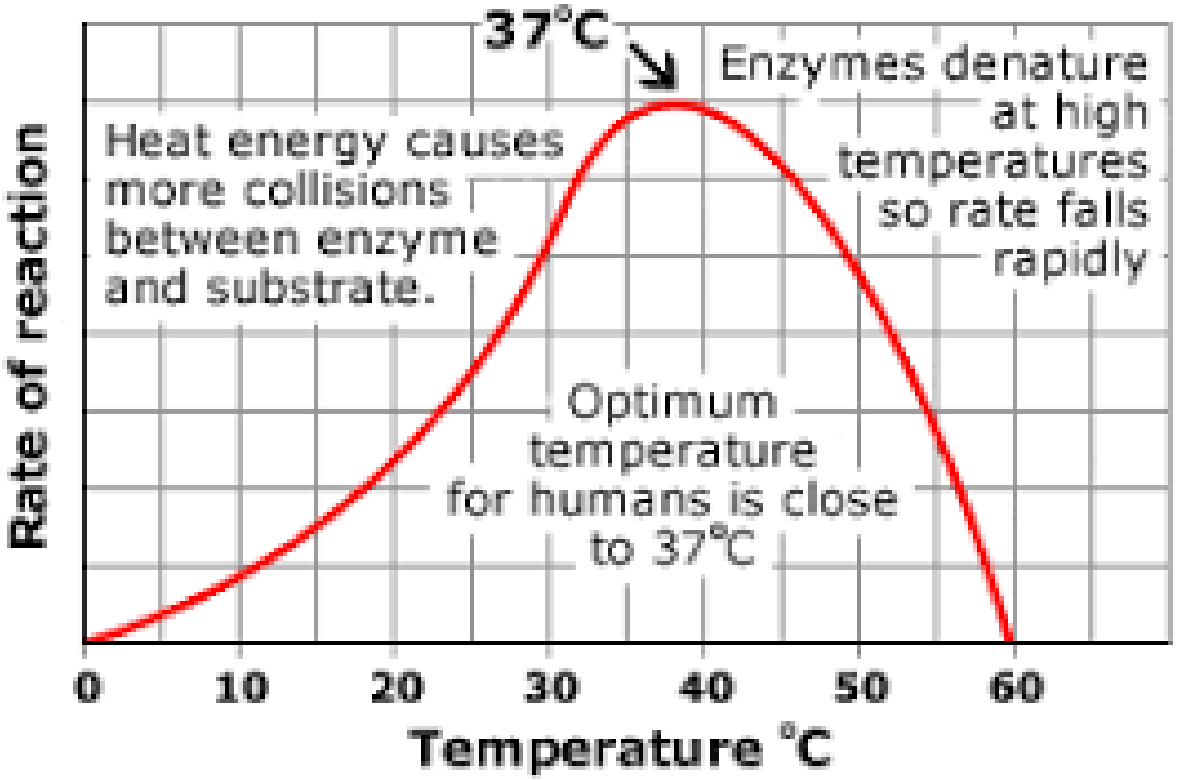


(a) Optimal temperature for two enzymes

Temperature

- Effects the movement of enzyme.
- Increase temperature : The rate of an enzymatic reactions increase.
- Substrates **collide** with active sites more frequently.
- Above optimal temperature:
  Enzymes denatured
  Rate of reactions drop sharply

Rate of reaction of an enzyme reaction changes at different temperatures



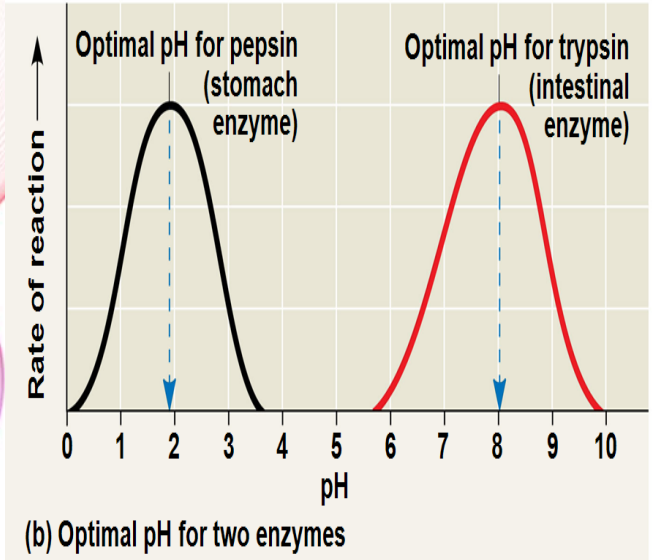
2. pH

- Affects the charge of amino acids side chains with carboxyl or amino groups (active site).

- Maximum rate of reaction occurs at optimum pH (most enzyme pH 6-8)

Different enzymes, different optimum pH.

Example : Pepsin and Trypsin

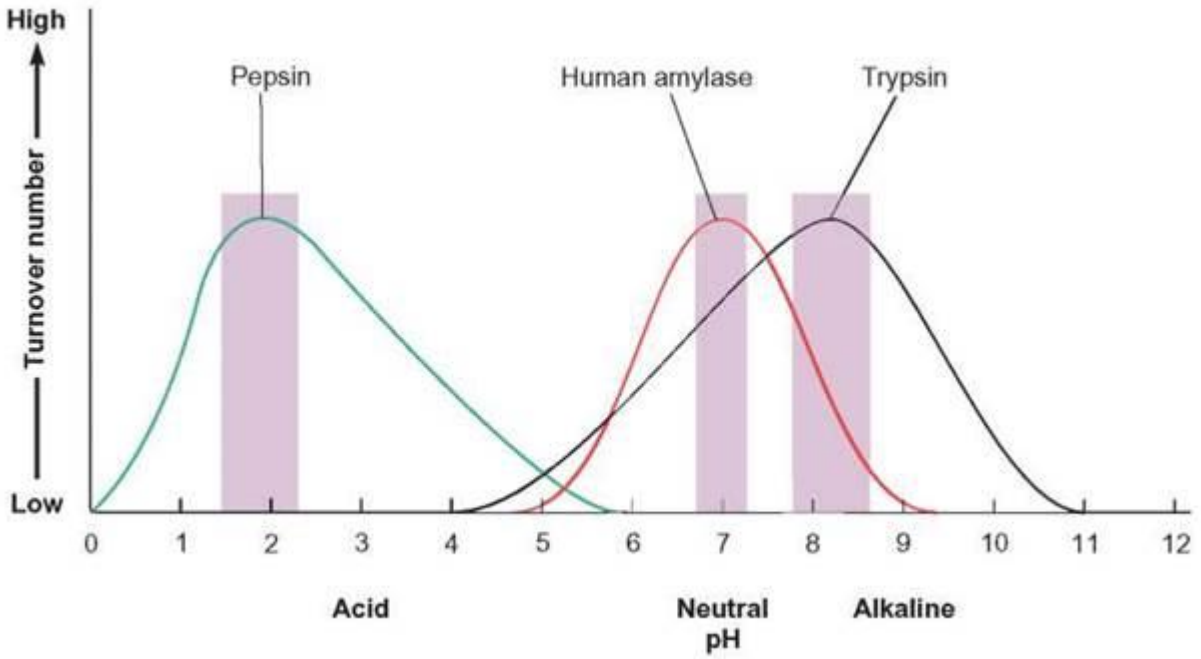


(b) Optimal pH for two enzymes

The activity and shape of enzymes is also affected by pH

Enzymes prefer to work at an optimum pH.

Outside of its pH range the enzyme is denatured.



How does pH affect enzyme activity?

- Ionic interactions hold enzymes together.
- Deviation from **optimal pH** range : lead to excess H^+/ OH^- .

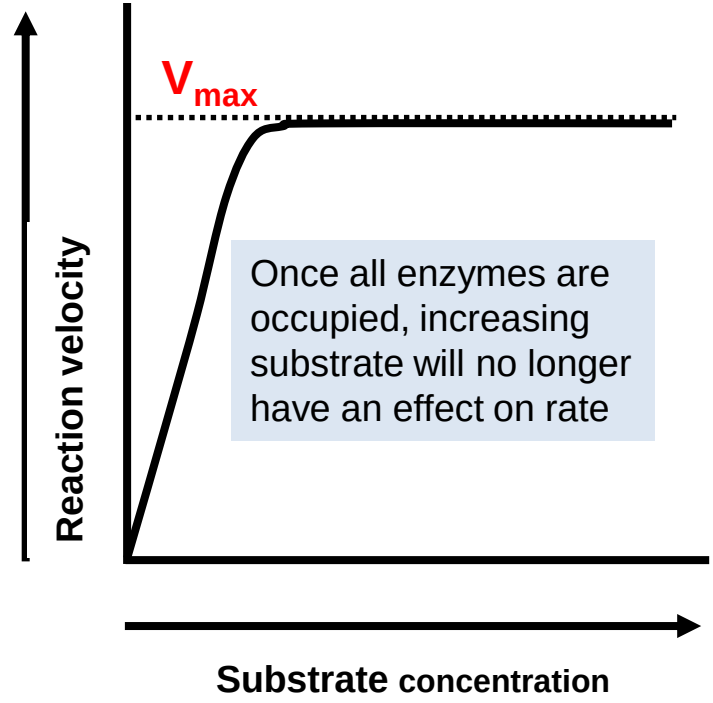
- Alters the acidic/ basic/ functional group/side chain of amino acid in enzyme.

- Shifts the balance between positively and negatively charged amino acid residues.
- Change in the bonds and tertiary structure breakdown.

3. Substrate Concentration

- The amount of substrate will affect the rate of reaction.
- Substrate concentration increases, rate of reaction will **increase proportionally**
- Then it will level off.
- All the enzyme molecules (active sites) are occupied.

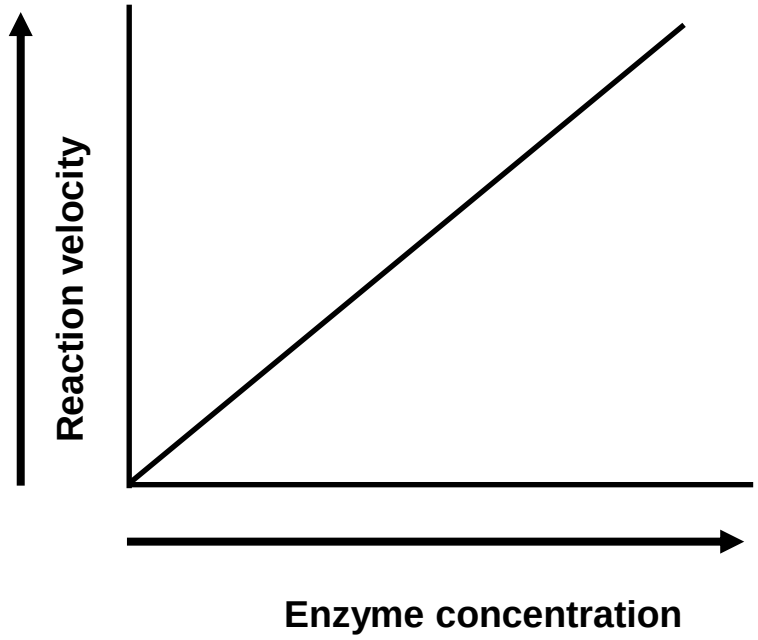
Graph showing effect of increasing substrate concentration



*Constant enzyme concentration

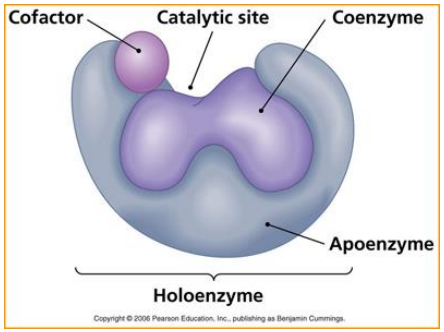
4. Enzyme Concentration

- At low enzyme concentration, the rate of reaction is low.
- Great competition for the active sites.
- As the enzyme concentration increases, the reaction can proceed at a faster rate.
- more active sites.
- Substrate as a limiting factor.



*Constant substrate concentration

4.2 COFACTOR



Learning outcome

a) Define cofactor

b) Explain the three types of cofactor and functions of :

i. Metal ion activators (e.g: Mg^{2+})

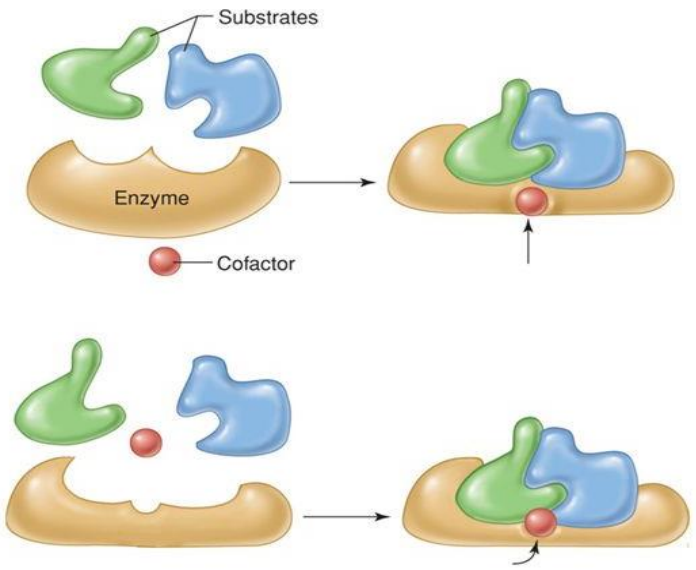
ii. Coenzyme (e.g: NAD^{+})

iii. Prosthetic group (e.g: haem group)

COFACTORS

- One or more **non protein** components required by enzymes in order to function efficiently.
- Atom or molecules that are not part of the enzyme's primary structure.

- Cofactor binding changes conformation of active site
  - and aids in temporary bonding between enzyme and substrates

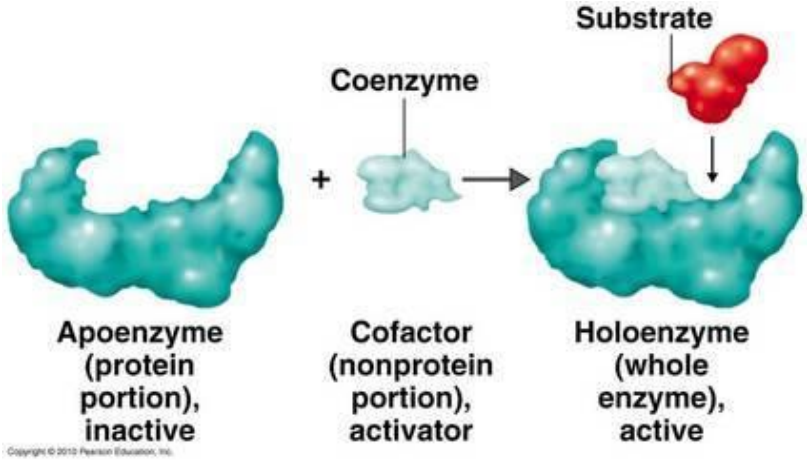


FUNCTION OF COFACTOR

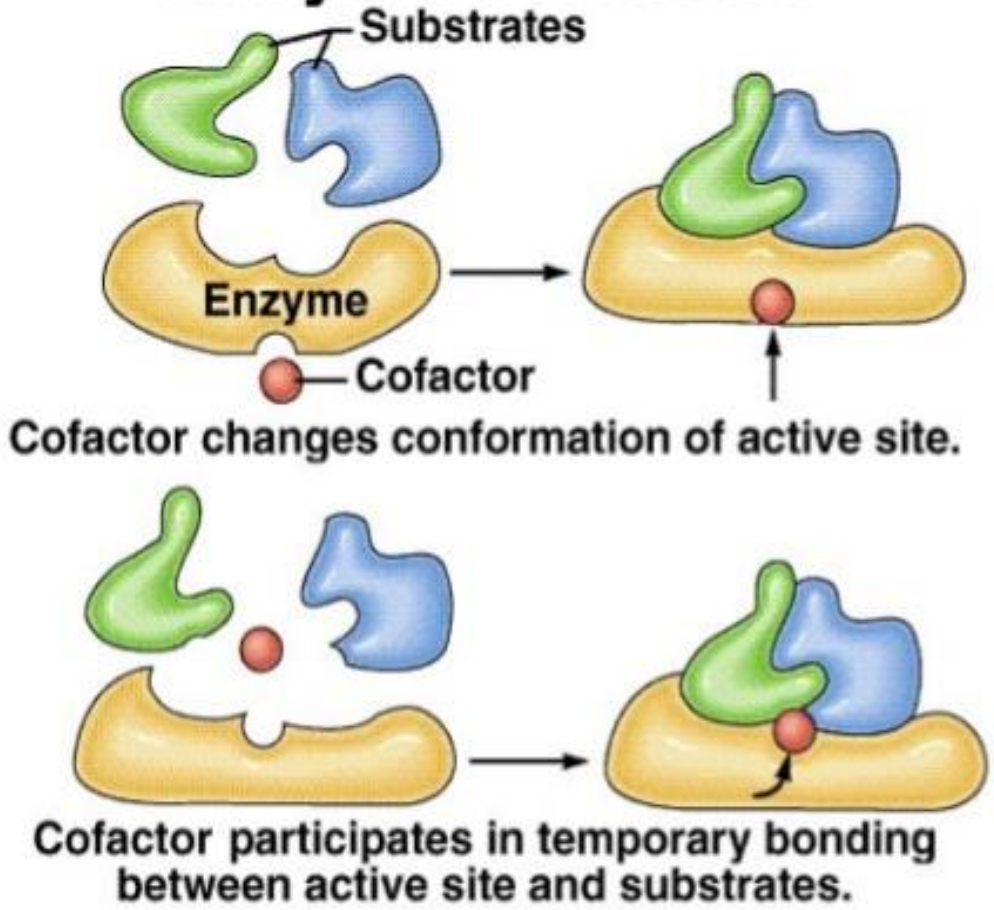
- Assist the formation the enzyme-substrate complex by altering the active site into a more suitable shape.

- May function to withdraw electrons from the substrate.

- May involve in transfer of electrons / atoms / chemical groups in enzyme reactions.



Roles of Cofactors in Enzyme Function



3 types of Cofactors:

Metal Ions/Activator

e.g Mg^{2+}

Coenzymes

e.g NAD^{+}

Prosthetic group

e.g haem group

https://study.com/academy/lesson/coenzymes-cofactors-prosthetic-groups-function-and-interactions.html

1. Metal ion activators

- ✓ Non-protein inorganic substances.
- ✓ Assist in forming ES complex by **bind temporarily**
- ✓ Eg: Mg^{2+},Ca^{2+}, Zn^{2+},Cl^{-}

Metal Ions

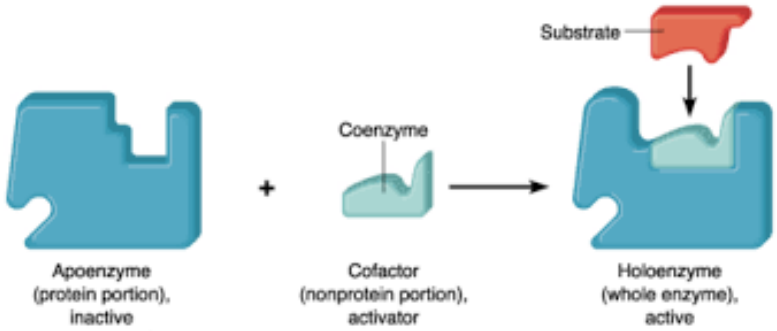
TABLE 6–1	Some Inorganic Ions That Serve as Cofactors for Enzymes
Ions	**Enzymes**
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

2. Coenzymes

- Non-protein organic or organometallic substances.
- Tightly or weakly bound to the enzyme.
- To remove functional group from one substrate and add it to another substrate.
- E.g: Vitamin B_6 and B_{12}

- nicotinamide adenine dinucleotide (NAD)

NAD act to dehydrogenases by acting as hydrogen acceptor.

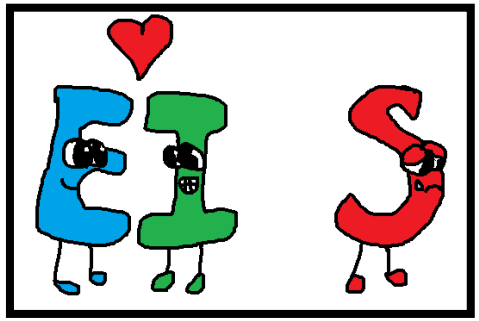


3.Prosthetic group

- A metal or coenzymes that are covalently or non covalently bound very tightly to th eir enzyme.

- E.g:
  - prosthetic group of electron carrier cytochrome and enzyme catalase.
  - FAD (riboflavin) - prosthetic group of the electron carrier cytochrome. It take part in oxidation-reduction reactions.

4.3 Inhibitors



Learning outcome

a) Define inhibitors

b) Explain the roles and types of inhibitors:

i. competitive inhibitors

ii. non competitive inhibitors.

c) Analysed graph related to competitive and non competitive inhibition.

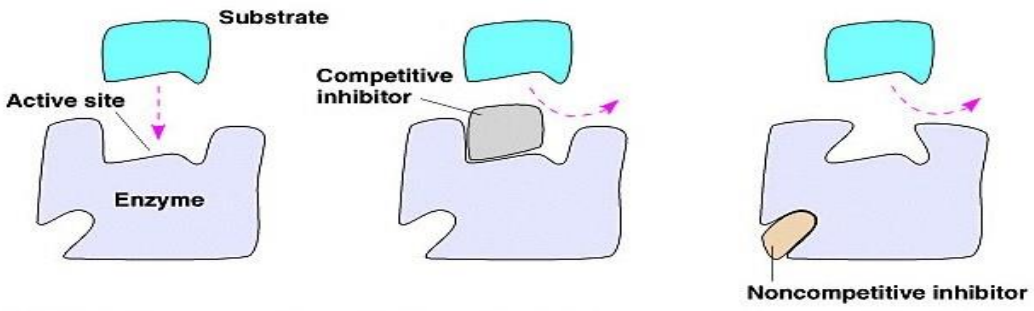
What is inhibitors?

- A substance that binds to an enzyme and decreases its activity.

(*Raven and Johnson. Biology 9th edition. McGrawHill*)

- Any molecule which acts directly on an enzyme to lower its catalytic activity.

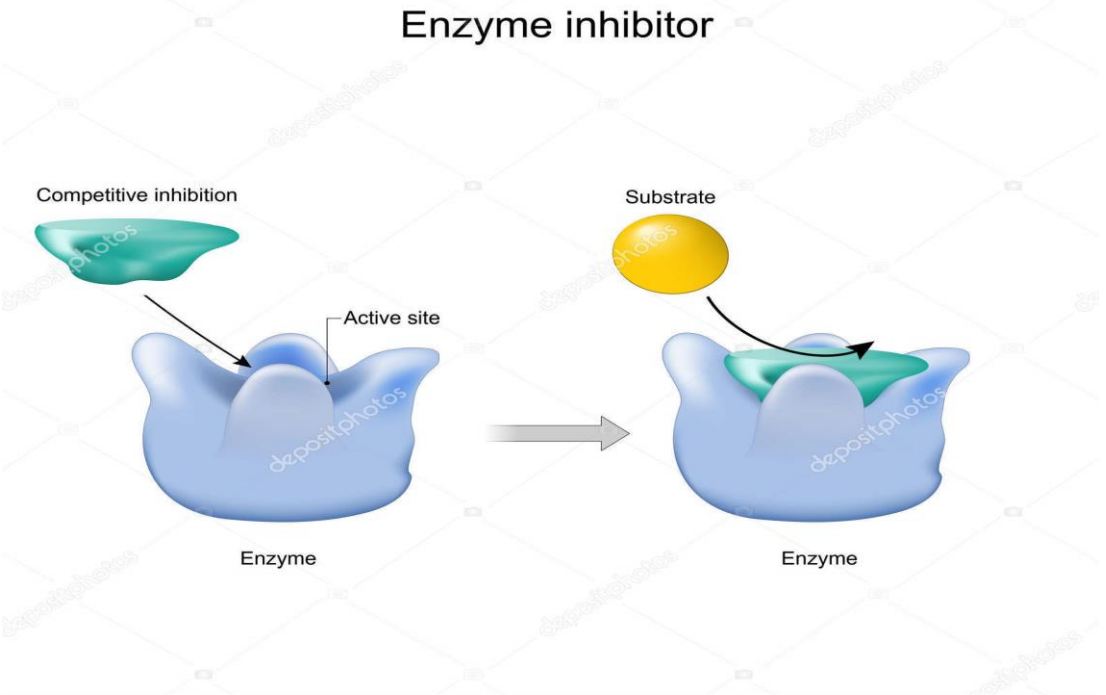
(*David Hames and Nigel Hooper. Biochemistry 3rd edition.*)



Inhibitors

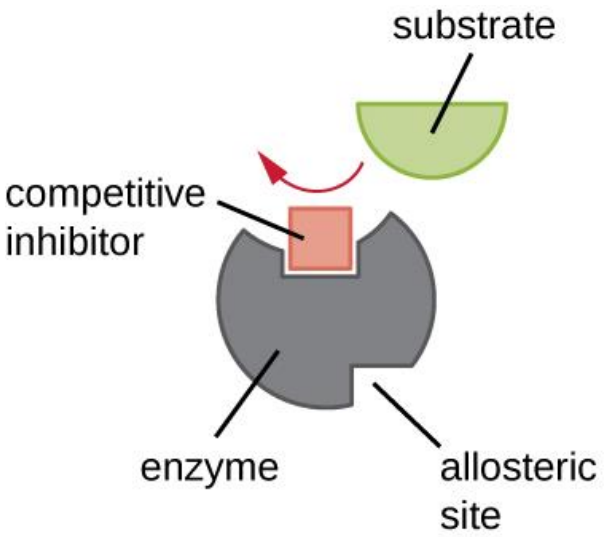
- ✓ Usually specific.
- ✓ Work at low concentrations.
- ✓ They block the enzyme but they do not usually destroy it.
- ✓ Many drugs and poisons are inhibitors of enzymes in the nervous system.

  E.g Nerve gase

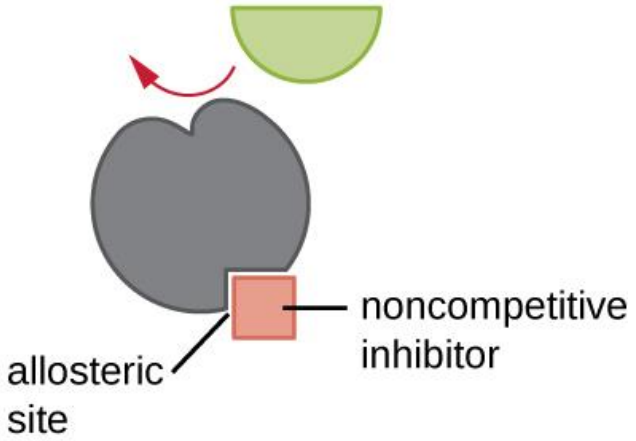


Types of Inhibitors

1. Competitive Inhibitor
2. Noncompetitive Inhibitor

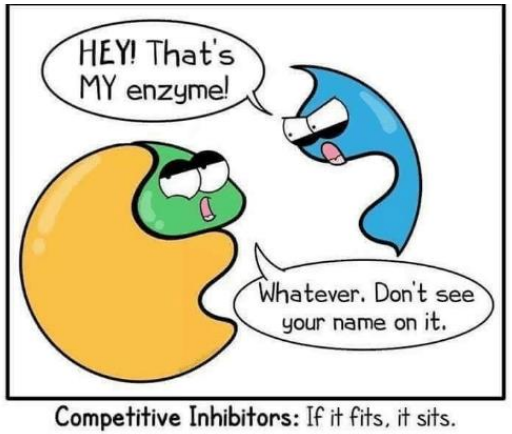


Competitive inhibition

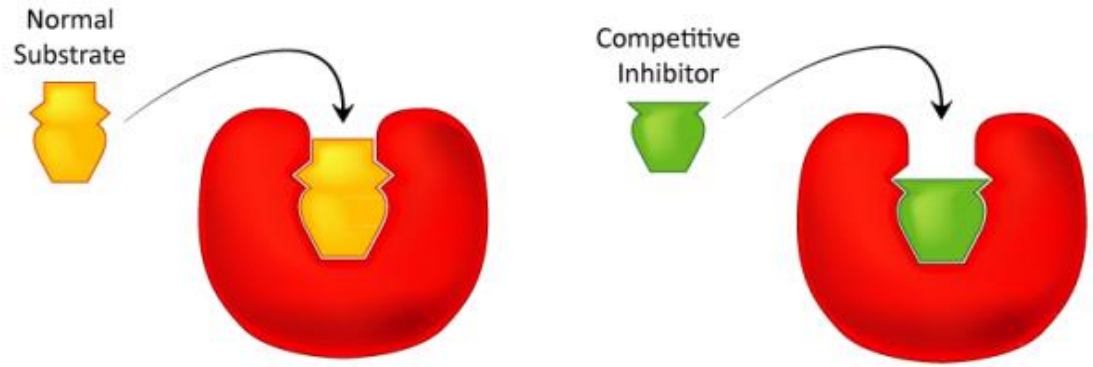


Noncompetitive inhibition

Competitive Inhibitor



- ✓ Has close structural **similarities** to the normal substrate.
- ✓ Inhibitor & substrate "**compete**" for active site.
- ✓ Bind loosely to the active site

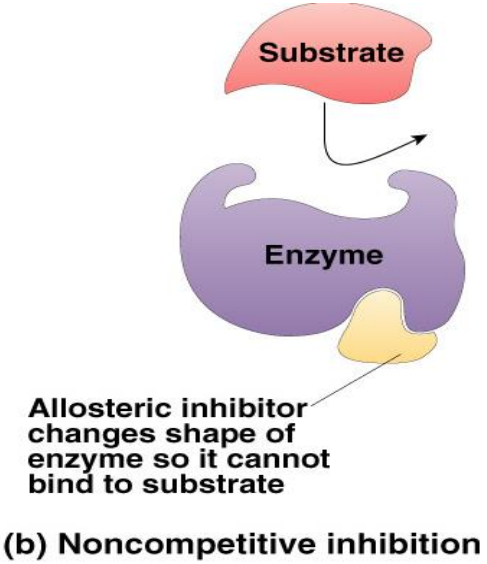


- ✓ Effect is **temporarily** to the enzyme.
- ✓ Enzyme can be **reused** again (do not destroy and do not denatured).
- ✓ Causes **No Permanent Damage** to the enzyme .

https://www.khanacademy.org/science/biology/energy-and-enzymes/enzyme-regulation/v/competitive-inhibition

Noncompetitive Inhibitor

- ✓ Shape of inhibitor is **NOT SIMILAR** to the shape of substrate.
- ✓ Inhibitor will **DO NOT** directly compete with the substrate to bind to the enzyme at the active site.

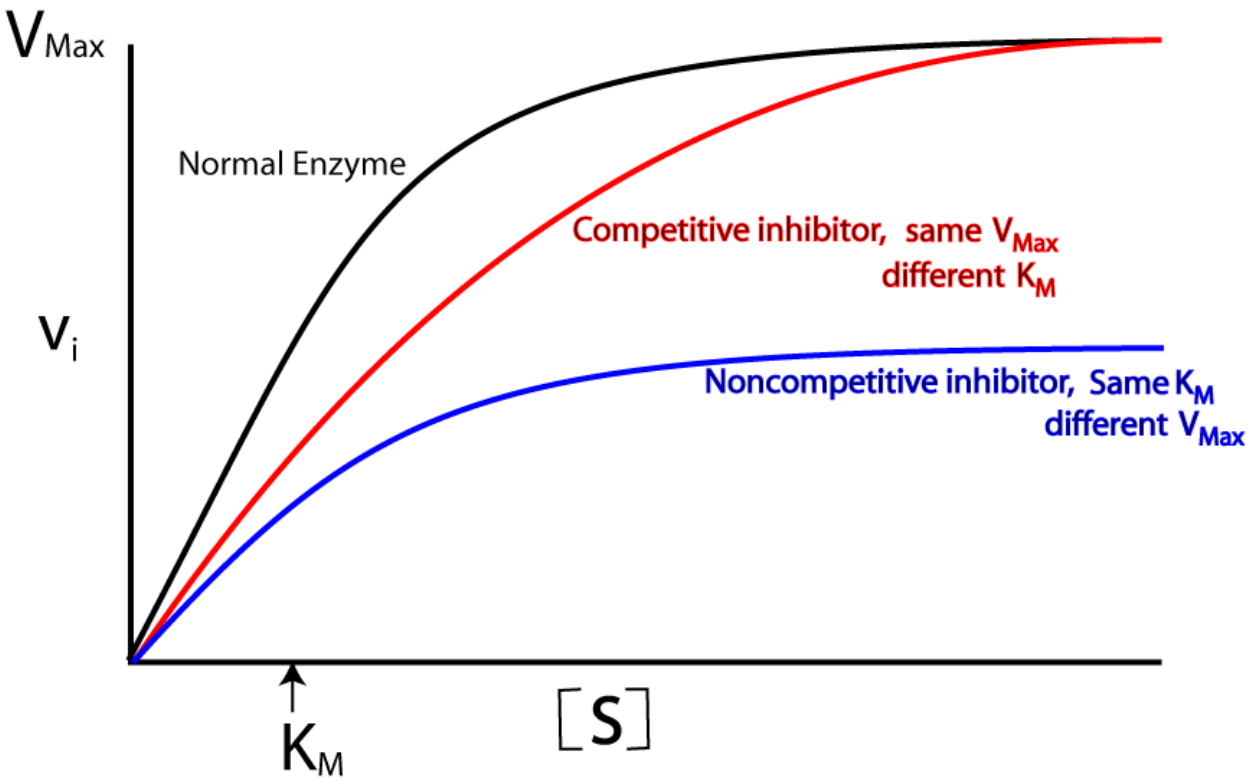


https://www.khanacademy.org/science/biology/energy-and-enzymes/enzyme-regulation/v/noncompetitive-inhibition

- ✓Noncompetitive inhibitor bind to **allosteric site** .
- ✓Causes enzyme active site to change shape.

  (Conformational change).
- ✓The substrate cannot bind with the enzyme.
- ✓ When the inhibitor concentration diminishes the enzyme's conformation changes back to its active form.

Graph related to competitive and noncompetitive inhibition.



✓ Rate of reaction with competitive inhibitor is higher than rate of reaction with non competitive inhibitor.