Etege Menen Girls' Boarding secondary School

Biology Department

Grade 11



Prepared by; Genene Teshome

Unit 3 Enzymes

3.1 Nature of enzymes

- Properties of enzymes
- naming of enzymes
- Classification of enzymes
- Industrial uses of enzyme technology

3.2 Functions of enzymes

- models of enzyme action
- Why do some enzymes need cofactors?

3.3 Factors affecting the functions of enzymes

- Temperature
- pH
- Substrate concentration
- Inhibitors

End-product inhibition (Allosteric inhibition)

UNIT 3: ENZYMES

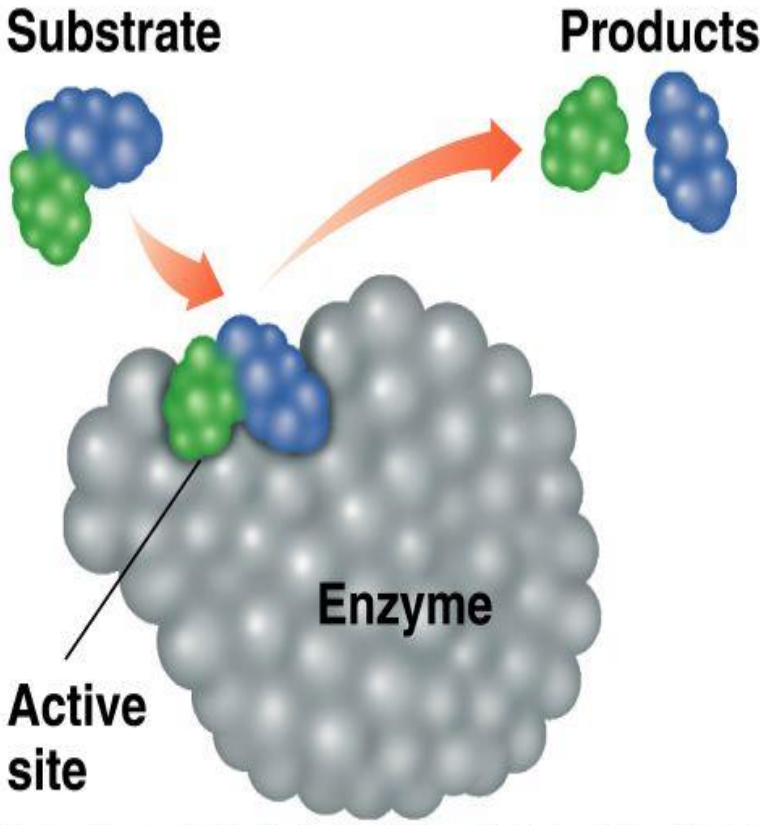
- Enzymes are biological catalyst that speed up a reaction in the cell without being altered in the process
- Enzyme are a biological catalyst because they are made up of globular proteins
- So, enzyme have unique three-dimensional shapes with a specific function

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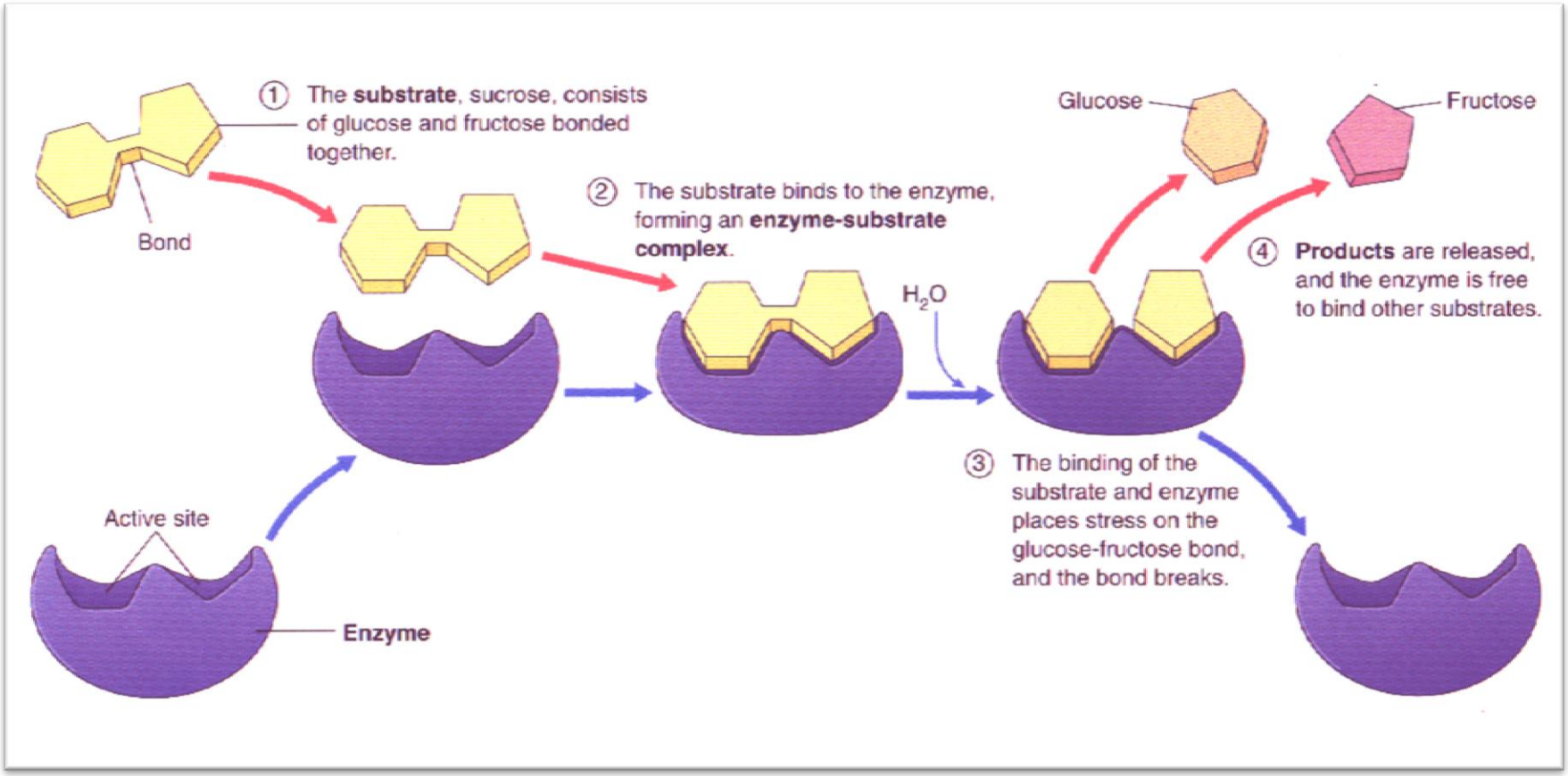
- Enzyme have an **active site** that binds with its substrate
- **Substrate (reactants) a** substance which an enzyme acts in a biochemical reaction
- Substrates bind to the active site to form **enzyme–substrate complex (ES).**
- **The active site of an enzyme used for:**

✓ binding with a particular substrate only

✓ Slow down energy that a reaction requires.



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Turnover number; The numbers of molecules of reactant forms enzyme–substrate complexes with each molecule of an enzyme, per second.

Enzyme catalyzed reaction

- When a substrate (S) fits properly in an active
- site, an **enzyme-substrate (ES) complex** is formed:
- Within the active site of the ES complex convert substrate to product (P):
- The products are then released, allowing another substrate molecule to bind the enzyme
- The overall reaction for the conversion of substrate to product can be written as follows:

$$\mathbf{E + S \leftrightarrows ES \rightarrow E + P}$$

Properties of enzymes

1. Enzymes are **globular proteins**
2. Enzymes are **biological catalyst** that speed up a reaction
3. Enzymes are **not used up during the reaction** they catalyzed. So they can be used over and over again.
4. **Not consumed during chemical reaction**; Enzyme are not changed, destroyed by the reaction they catalyze. So, enzymes can be reused again and again
5. They are **specific**: they catalyse one reaction only.
6. Because of the conformation of the active site only a certain substrate or combination of substrates can bind with it.
7. Enzyme bring about a **change in a large amount of substrate**.

Affected by pH and temperature.

- Enzymes are **affected by pH and temperature**.
- **Temperature;** enzyme work actively at optimum temperature
- Higher temperatures destroy the active site the enzyme and it can no longer bind with substrate. (the process known as **denaturation**)
- **pH** that is too low (too acid) or too high (too alkaline) changes the charges on the active site that cause the active site to lose its conformation

Common nomenclature (naming of enzymes)

- Most commonly enzymes are named by ends in ***– ase*** *to* the substrate.
  - E.g. **lipase**-lipid hydrolyzing enzyme
  - **Sucrase** - sucrose hydrolysing enzyme
- Sometimes the enzymes are **named on the basis of the reaction** they catalyse.
  - E.g. **polymerase**-aids in **polymerization**; joining similar units together),
  - **Dehydrogenase** -**removal of hydrogen** atoms or ions).

Contd.

- Some enzymes **named based on the source** from which they were first identified.
  - For example**, papayin** from **papaya.**
- Others are **named according to where they act**.
  - for example, **intestinal protease** acts on proteins in the intestine.
- Some enzymes end with **‘in’,** indicating that they are **basically proteins**.
  - for example, pepsin, trypsin, etc

Classification of enzymes

- **Enzyme Commission (EC)** produces a systematic way of naming enzymes. **Enzymes are classified into six functional classes based on the type of reactions they catalyse.**

1. **Oxidoreductases (EC 1);** transfer of hydrogen and oxygen atoms or electrons from one substrate to another
   - **AH** + **B** ⟶ **BH** + **A** (reduced)
     Dehydrogenases
   - **A** + **O** ⟶ **AO** (oxidized) **Oxidases**
2. **Transferases (EC 2):** Catalyze the `transfer of a specific group from one substrate to another
   - **Transaminase; transfer of amino group**
   - **Kinases**; **transfer of phosphate group**

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3. Hydrolases (EC 3); Catalyze hydrolysis reaction

- Esterases, Digestive enzymes (Lipase, amylase, peptidase)

4. Isomerases (EC 4);

- Catalyze internal rearrangement of the substrate molecule
- **Phosphoglucoisomerase;** rearranges glucose-6-phosphate into Fructose-6-phosphate

5. Lyases (EC 5);

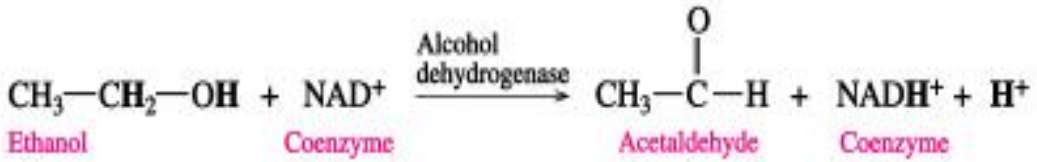
- Catalyze a non-hydrolytic removal of a group or addition of a group to a substrate E.g. **Decarboxylases, Aldolases**

6. Ligases (EC 6):

- Catalyze the joining of two molecules by the formation of new bonds. **E.g. Citric acid synthetase**

Oxidoreductases, Transferases and Hydrolases

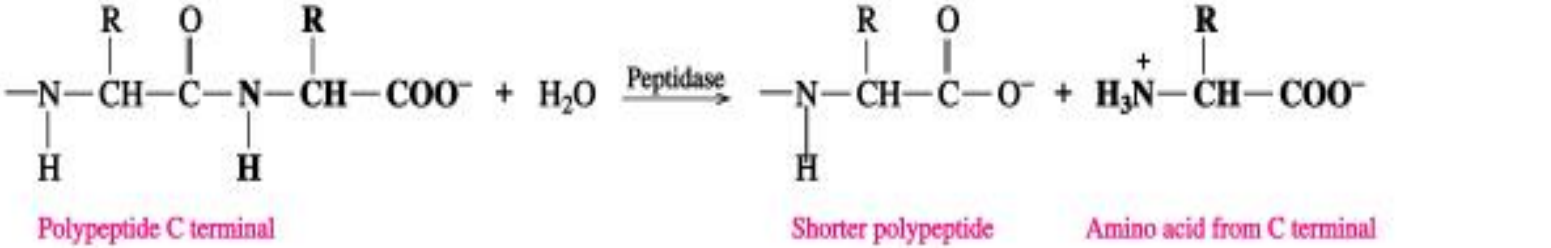
Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds



2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups



3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose

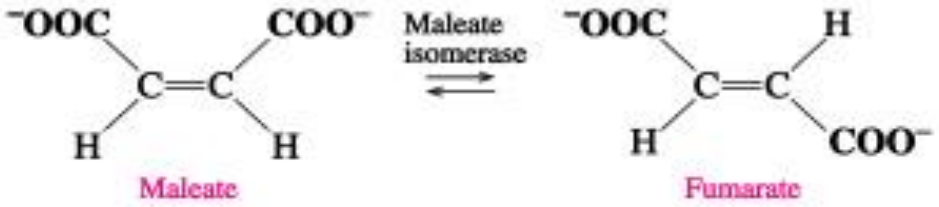


Lyases, Isomerases and Ligases

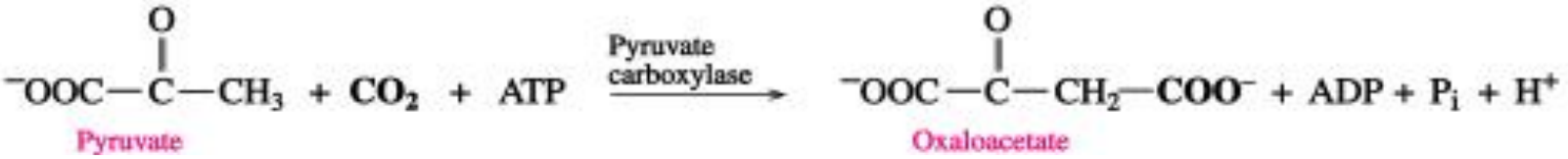
Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3



5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers



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



International naming of enzymes

- So, in the systematic naming of enzymes **consisting of four digits:**
- E.g. **EC: (2.7.1.1) Hexokinase**
- **EC: (2.7.1.1)** these components indicate:
  - **2.** Is **class (transferase)**
  - **7.** Is **subclass (transfer of phosphate)**
  - **1.** Is **sub-sub class (alcohol is phosphate acceptor)**
  - **1. Specific name ATP,D-hexose-6-phosphotransferase (hexokinase)**

Some industrial uses of enzyme technology

1. Dairy industry

- **Biochymosin;** produce cheese
- **Lactase**; produce lactose-free milk
- **Lipase;** ripening of Danish blue cheese,
- **Renine:** Coagulate milk protein

2. Detergents

- **proteases, lipases and amylases** in biological washing powders
- **proteases** and **amylases** in dish washer detergents

3. Textiles.

- **Proteases** to remove hair , **lipases** to degrease animal hides
- **cellulase** to 'bio-polish' cotton fabrics

4. Food processing

- **Pectinase** to process fruit juice,
- **Inverase** to produce liquid chocolates

Contd.

5. Pulp and paper

- **Amylases** improves quality of paper by reduces the quantity of starch
- **Xylanase** Produces a whiter paper by pre bleaching the pulp
- **Esterases** improving the quality of the paper by controlling of 'stickies'

6. Medicine

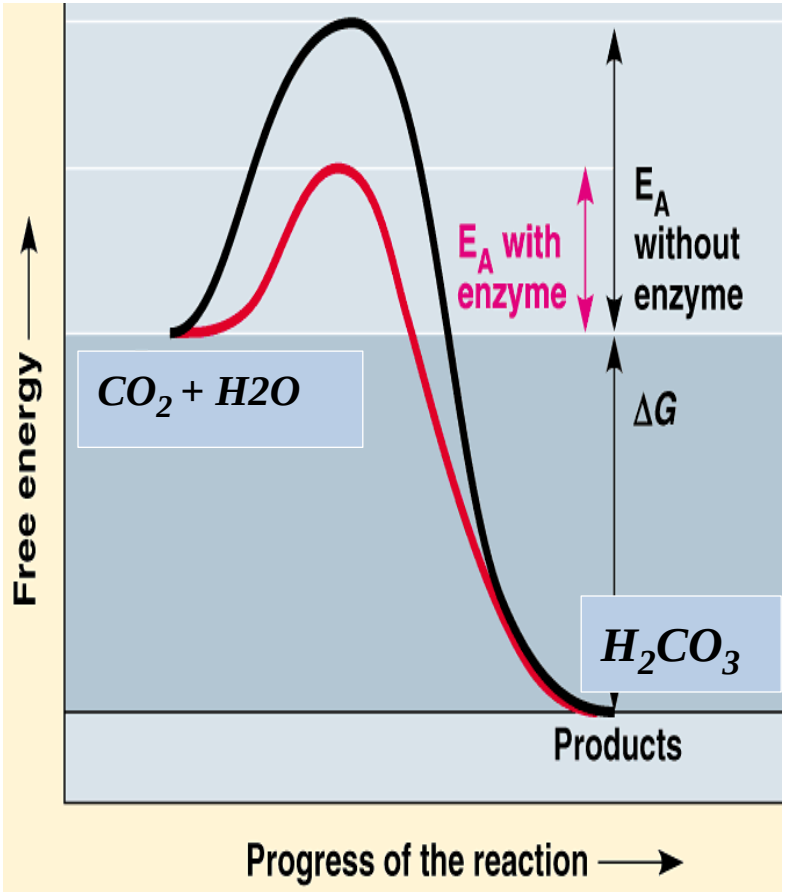
- **Glucose oxidase;** Allows easy diagnosis of diabetes by testing urine
- **Pulmozyme**; treat cystic fibrosis by reducing viscosity (stickiness) of mucus

7. Pharmaceutical

- **Streptokinase** Restores blood supply to area of heart muscle
- **Abacavir sulphate** is an important anti-AIDS drug

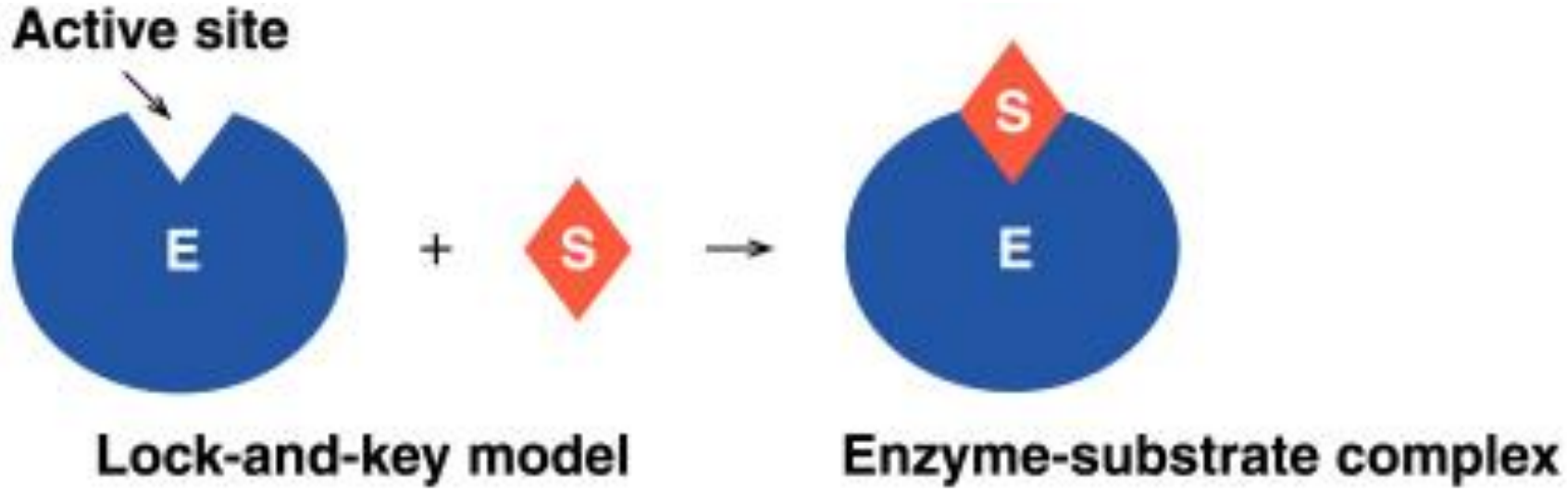
3.2 Functions of enzymes

- Enzymes are proteins that increase the rate of reaction by **lowering the activation energy (E_A)**
- Activation energy the energy required to start a chemical reaction
- There are **two models of enzyme action;** suggest that the enzyme catalyses the reaction by lowering the activation energy.
- **Lock-and-key model**
- **Induced-fit model**



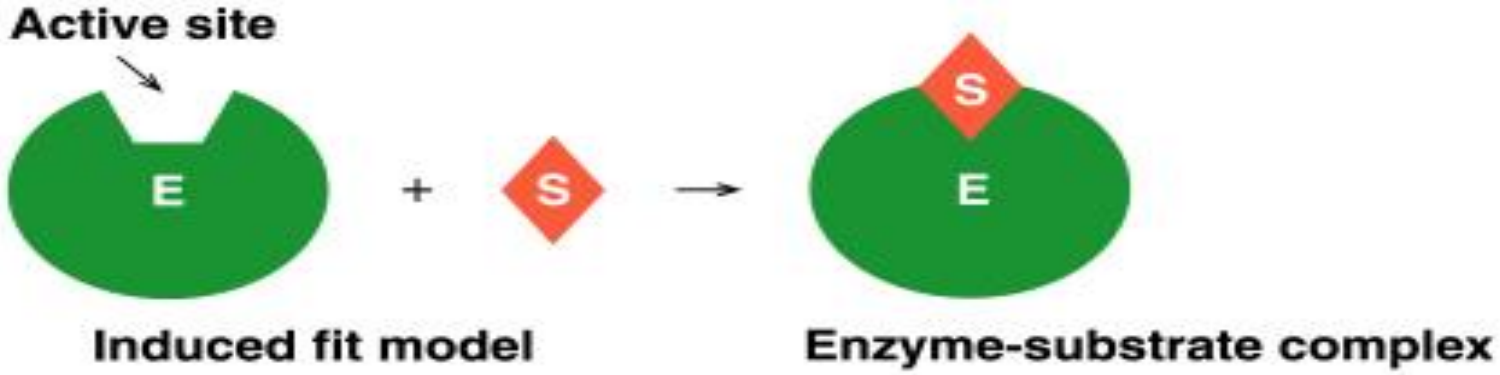
Lock-and-key model

- The shapes of the substrate molecules are complementary to that of the active site
- Explain **enzyme specificity** **(one lock - one key)**
- the substrate is a key that fits the lock of the active site
- This is an older model, however, and does not work for all



Induced-fit model

- The active site and the substrate **aren't naturally complementary in shape**
- **In the induced-fit model of enzyme action:**
  - the active **site is flexible,** not rigid
  - Active site **change to a precise conformation**
  - Have a **greater range of substrate specificity**
- This model is more consistent with a wider range of enzymes



Why do some enzymes need cofactors?

- **Holoenzyme** is an active enzyme with its protein (Apoenzyme) and non-protein (cofactor) component
  - **Cofactor** – a small non-protein particle essential for the activity of some enzymes.
  - A protein that combines with a cofactor is termed as **apoenzyme** and it is **inactive.**
- **Type of cofactors**
  1. **Coenzymes**
  2. **Mineral ions**
  3. **Prosthetic group**

Coenzymes are organic molecules and many are derived from vitamins. They bind with the enzyme to give catalytic activity.

- E.g. Nicotinamide adenine Dinucleotide **(NAD)**
- Flavin adenine dinucleotide **(FAD)**

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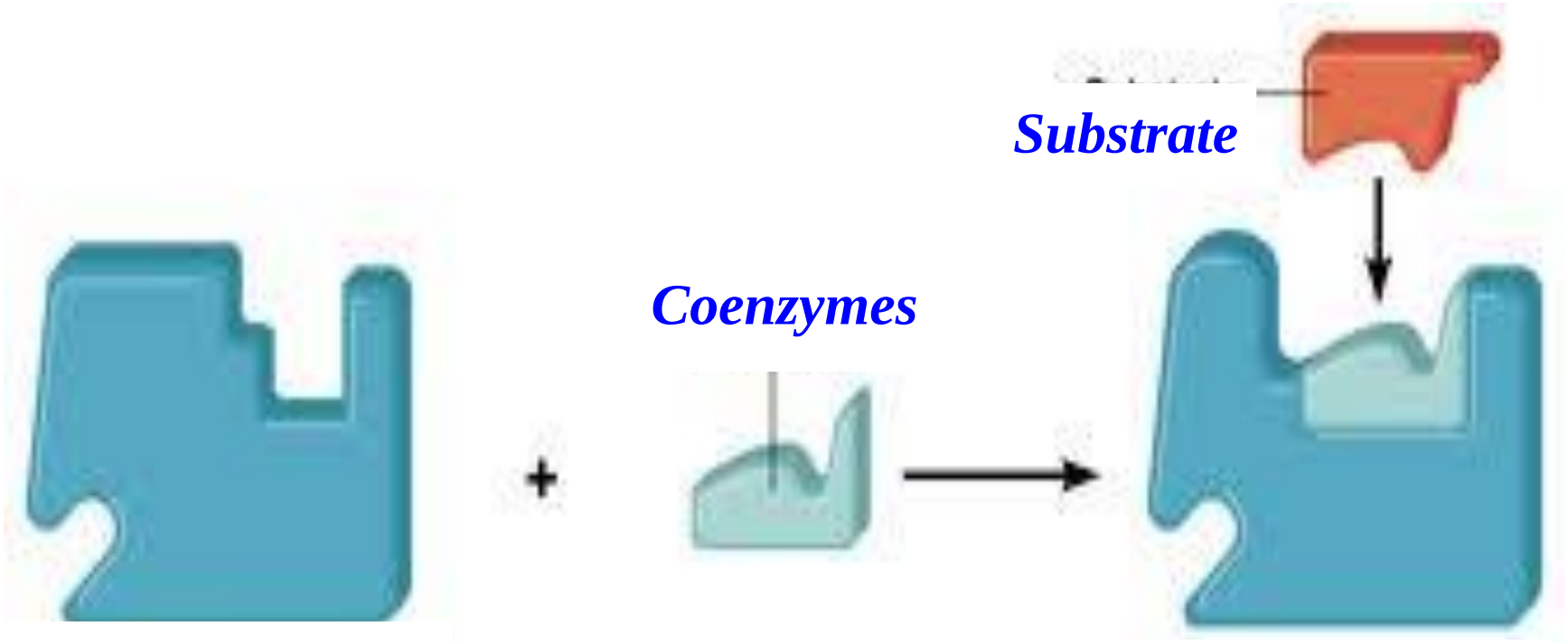
Mineral ions

- **Zinc ions (Zn++)**
  - Carbonic anhydrase
    - Causes CO2 to react with water to form hydrogen carbonate
  - **Alcohol dehydrogenase;** Oxidises alcohol
- **Cytochrome oxidase**
  - Copper ions (Cu++ or Cu+);
  - Transfers electrons to oxygen during respiration

Prosthetic group

- The **non-protein component,** tightly bound to the apoenzyme on a permanent basis by covalent bonds is called a Prosthetic group.

The apoenzyme and the cofactor make the Holoenzyme.



Apoenzymes

(Protein portion)

Inactive

Cofactor

Non protein portion

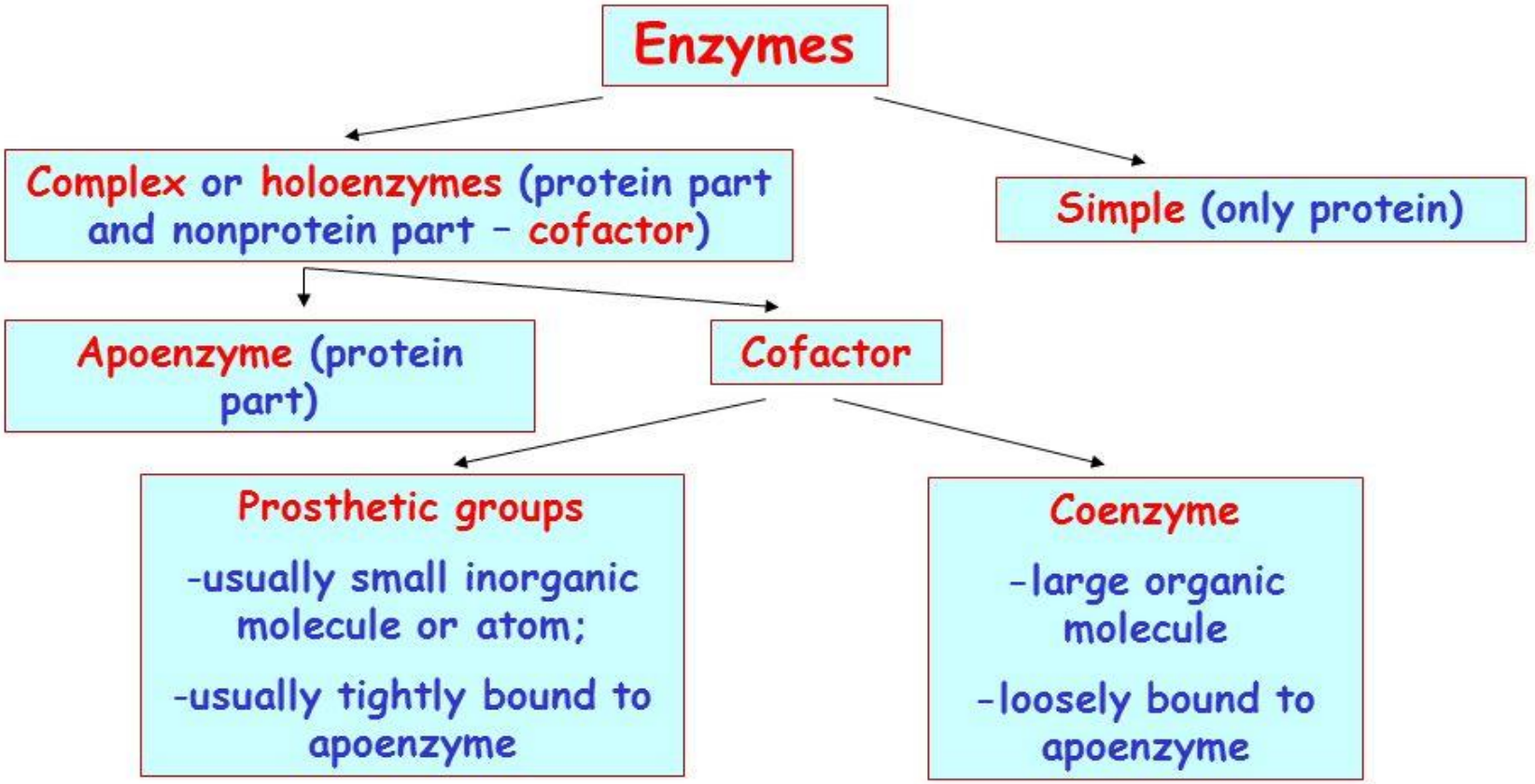
Activator

Holoenzyme

Whole enzymes

Active

Structure of enzymes



- **Prosthetic groups can be organic or inorganic metal ions. They are often attached to protein by a covalent bond,**

3.3 Factors affecting the functions of enzymes

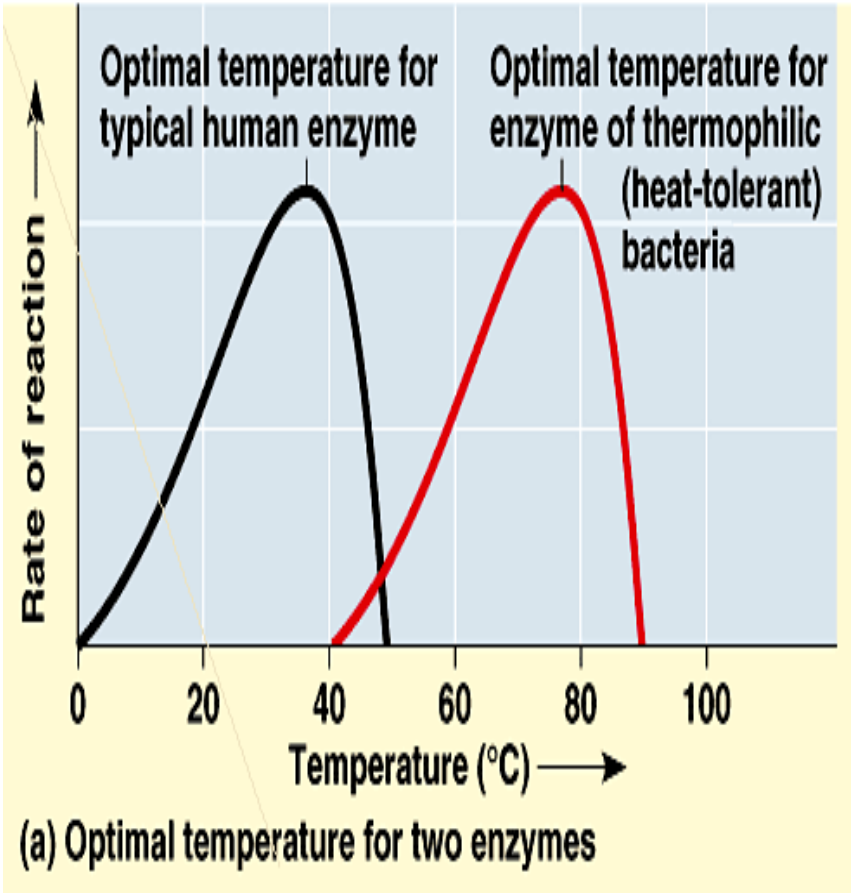
- The proper functions of enzyme are affected by external factor. Such as:
  - **Temperature**
  - **pH**
  - **Substrate concentration**
  - **Inhibitors**

TEMPERATURE

- Optimum temperature the temp at which enzymatic reaction occur fastest
- **Enzymes in human body** required optimum temperature around **37 °C**
- **Bacteria** that live in hot springs **(thermophilic bacteria)** may be over 90 °C.
- Effect temperature on rates of enzyme activity
- **Optimum temperature**

✓ Greatest number of collisions between enzyme & substrate

✓ Increase the rate of enzyme activity because more molecules have sufficient energy to overcome activation energy



(a) Optimal temperature for two enzymes

Con't

Raise temperature (boiling);

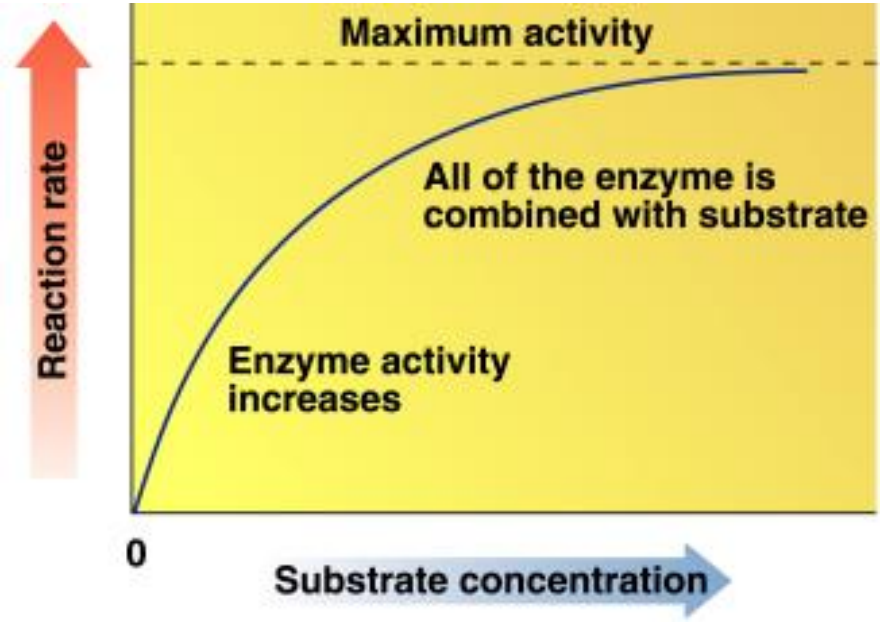
- **Denature** of enzyme, changing its conformation and lose shape
- The rate decrease as more molecule denature
- **Denaturation;** changing the structural properties of protein that result in the loss of its property

Lower temperature T°

- molecules move slower
- **fewer collisions** between enzyme & substrate
- The rate decrease as molecules do not have sufficient energy to overcome activation energy

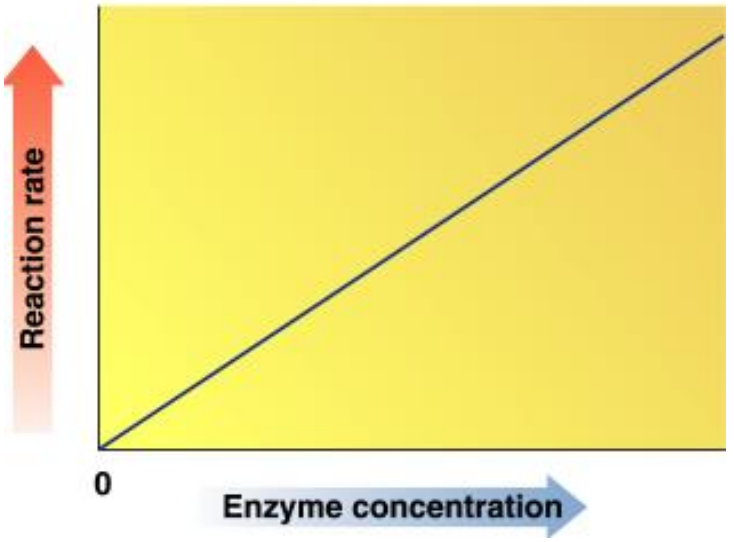
Substrate concentration

- The **rate of enzyme action increase with increasing substrate** concentration
- Maximum activity occurs when the enzyme **is saturated**
- So, Increasing the substrate concentration
- Beyond point will have no effect on the activity of the enzyme because all the active sites are occupied.



Enzyme concentration

- **Under high substrate concentration** and constant pH and temperature the rate of reaction is directly proportional to substrate concentration.
- However at a very high enzyme concentration, the substrate concentration may become limiting factor so the rate of reaction will likely to be fairly constant.

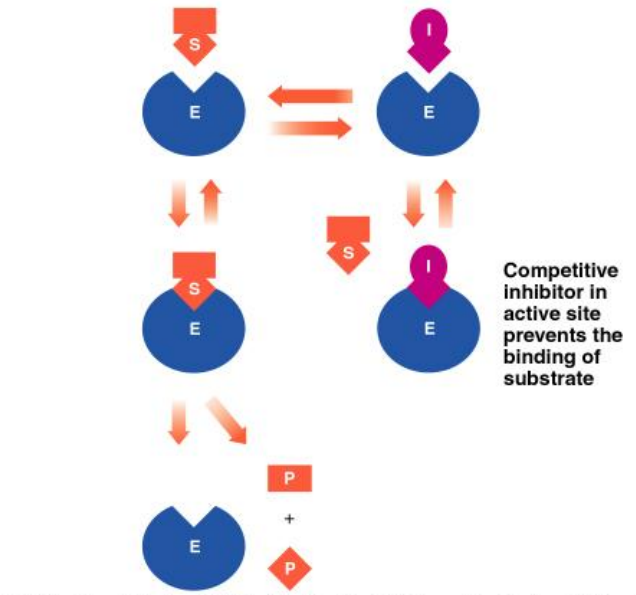


Inhibitors

- **Inhibitors (I)** are substances that bind to enzymes and prevent them from forming enzyme–substrate complexes (ES) and, as a result, stop, or slow down, the reaction.
  - **E** + **S** ⇆ **ES** → **E** + **P**
  - **E** + I ⇆ **E**I → **no P formed**
- There are two main types of inhibitors:
  - **Irreversible inhibitors** bind strongly to enzymes, usually by a covalent bond, permanently altering the structure of the enzyme.
  - **Reversible inhibitors** bind to enzymes only weakly and the bond that holds them breaks easily releasing the inhibitor
- There are two main kinds of reversible inhibitors:
  - **Competitive inhibitors in active site prevent the binding of substrate**
  - **Competitive inhibitors**, and **Non-competitive inhibitors**

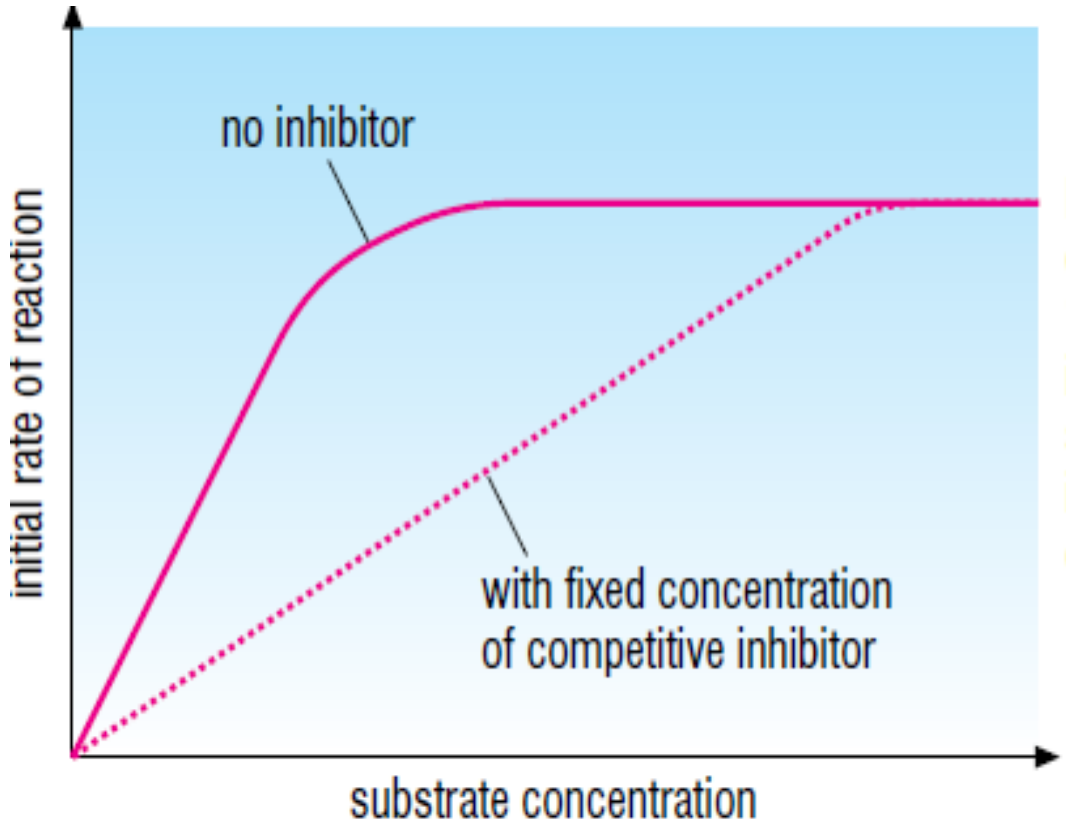
Competitive inhibitor

- A **competitive inhibitor** is reversible and has a structure like the substrate
  - it competes with the substrate for the active site
  - its effect is reversed by increasing substrate concentration



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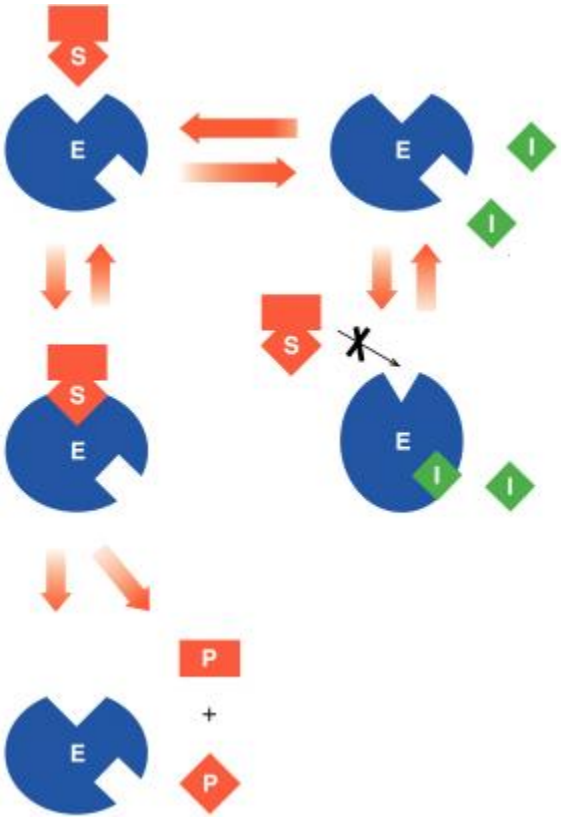
Effect of substrate concentration on inhibition by a competitive inhibitor



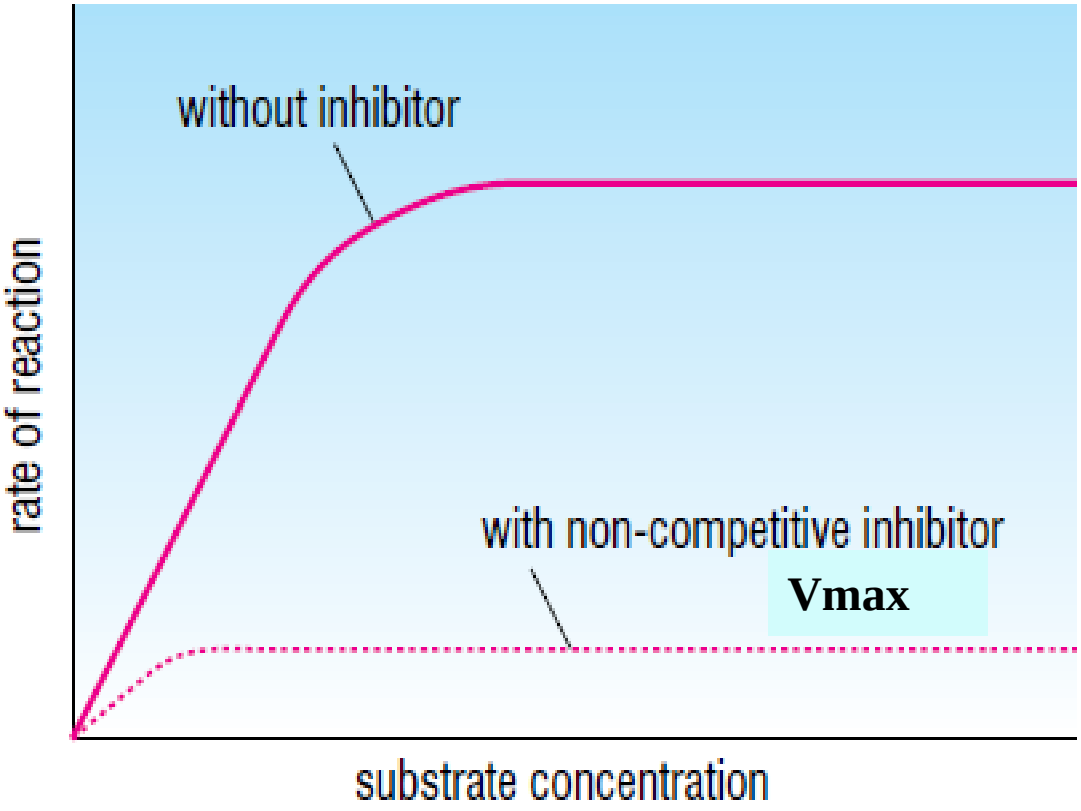
- Increasing substrate concentration effectively reduces the effect of the ***competitive*** inhibitor. If enough substrate is added, the inhibitor is unlikely to collide with the enzyme
- **Vmax** (maximum rate of reaction) the same in the presence of competitive inhibitors

Noncompetitive inhibitor

- **Noncompetitive inhibitor** has a structure that is different than the substrate
  - **Does not compete for the active site**
  - it **binds with the allosteric site** rather than to the active site
  - it **distorts the shape of the enzyme**, which alters the shape of the active site and
  - **Non-Competitive inhibitors change enzyme conformation**
  - prevents the binding of the substrate **cannot be effected adding substrate concentration**

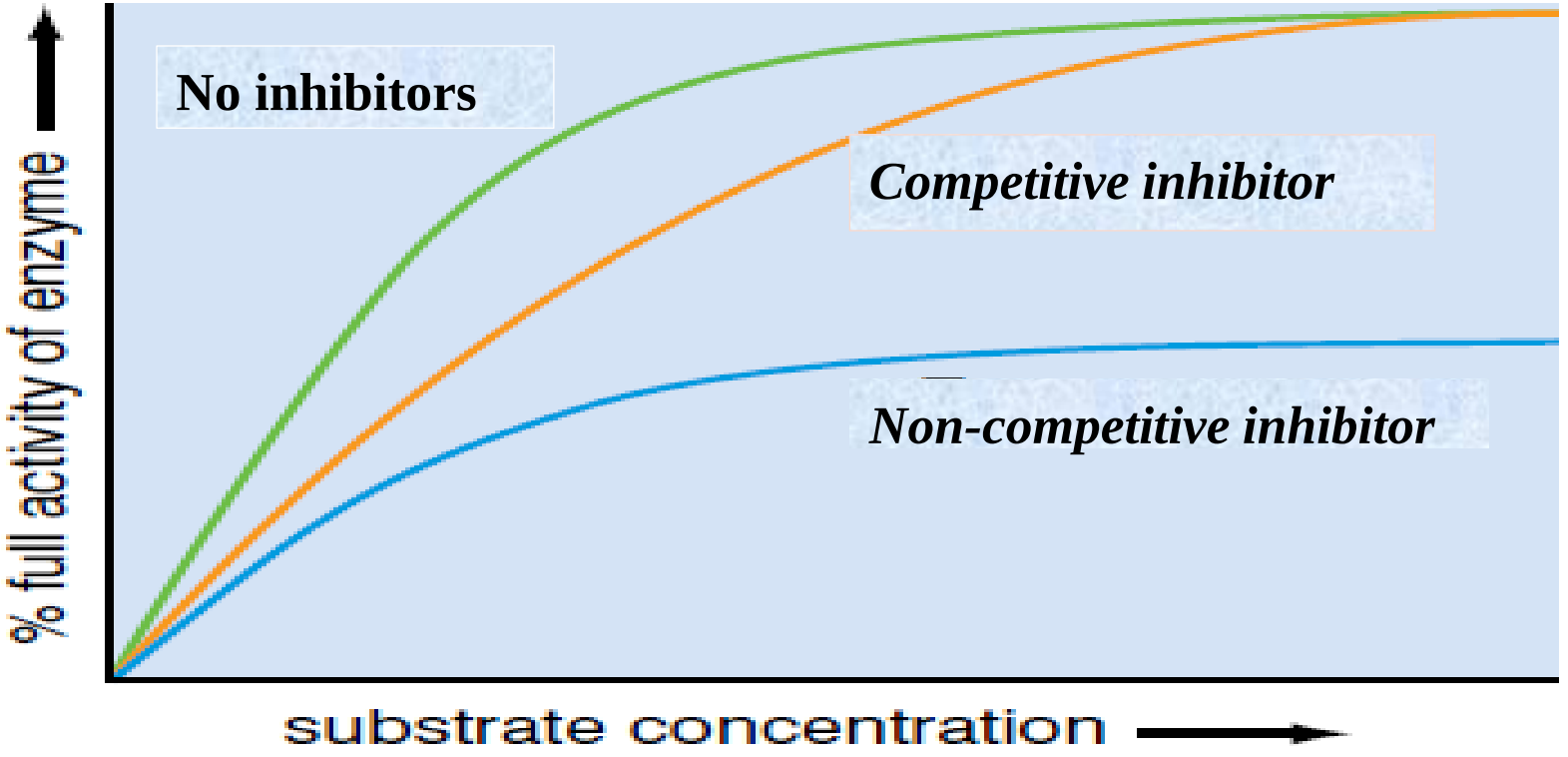


The effect of substrate concentration on a non-competitive inhibitor



- Vmax decrease in the presence of non-competitive inhibitor
- Maximum rate of reaction is controlled by the number of inhibitor molecules present

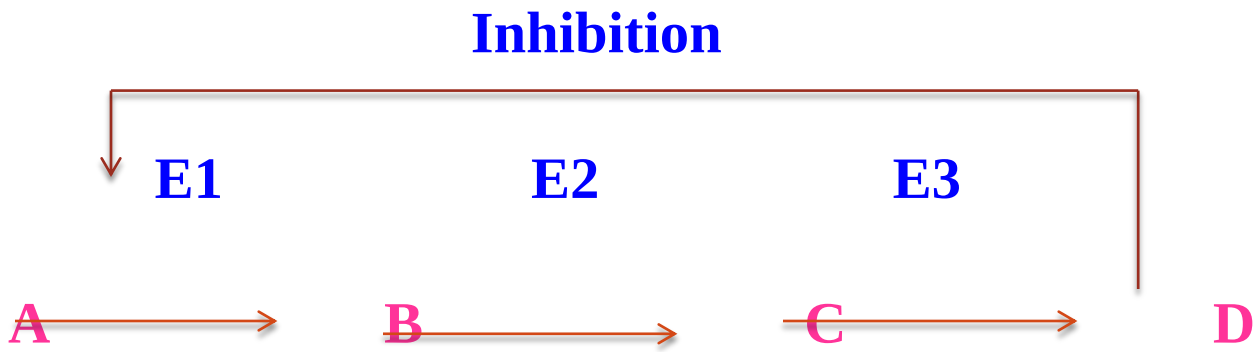
Activity of an enzyme at different substrate concentrations under three conditions:



End-product inhibition

- **End-product inhibition (Allosteric inhibition)** is mechanisms cells use to regulate enzyme catalyzed reactions in metabolic path way
- **End product inhibits** the enzyme controlling the first stage of a reaction sequence
- The final product of a series of reactions inhibits the enzyme controlling the first reaction in the series
- **Enzymes are inactive** in a cell by an **inhibitor** and are activated by a specific **activator** only when they are required.
- **Activator** a substance that removes an inhibitor

End-product inhibition



- The **end product (D) inhibits** the enzyme controlling the first stage of the reaction sequence.
- **(D)** acts as a **non-competitive inhibitor**, which prevents the enzyme **E1** from catalysing the reaction that converts **A to B.**
- As a result, the entire reaction sequence is halted.
- When the inhibition is removed, the metabolic pathway can start again.