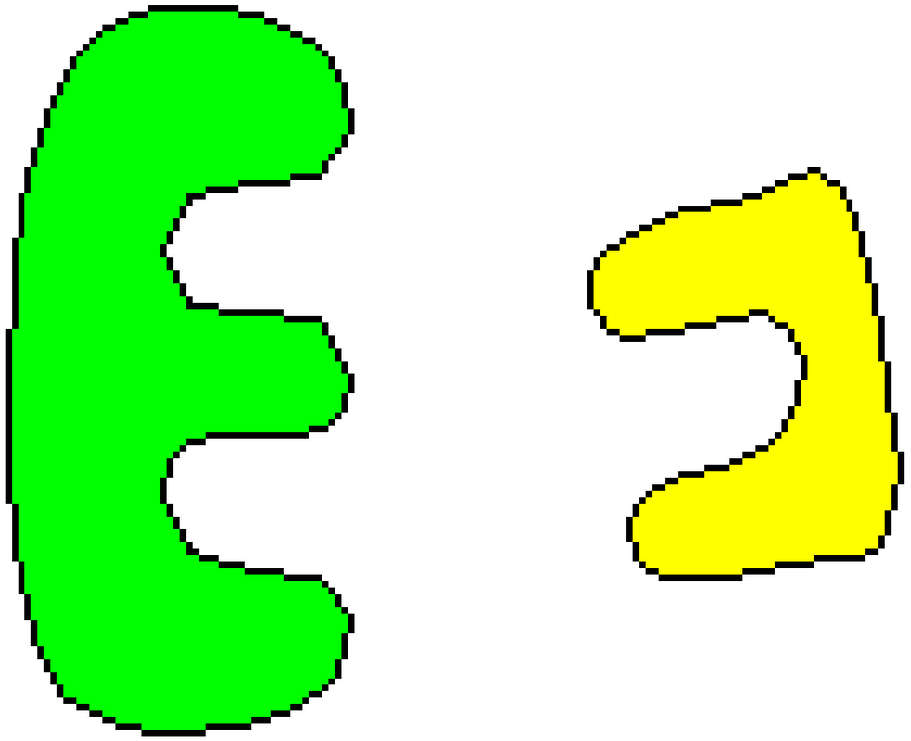
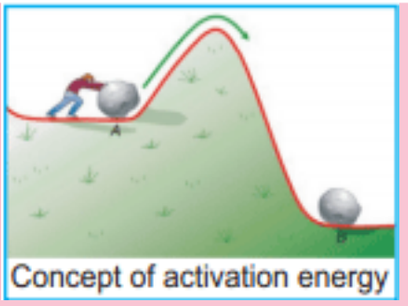
CHAPTER

6 Enzymes



Animation 6: Enzymes
Source & Credit : greydefence

The life of living organisms is a reflection of what is going on in their bodies. Metabolism is the set of biochemical reactions that occur in living organisms in order to maintain life.These processes allow organisms to grow and reproduce maintain their structures, and respond to their environments. Anabolism includes the biochemical reactions in which larger molecules are synthesized while catabolism includes the biochemical reactions in which larger molecules are broken down. Usually, energy is released in catabolism and it is utilized in anabolism. In this way the biochemical reactions are actually energy transfers. During metabolism, chemicals are transformed from one form to the other by enzymes. Enzymes are crucial to metabolism because they act as **biocatalysts** and speed up and regulate metabolic pathways.



Concept of activation energy

The term metabolism is derived from a Greek word meaning “change . The concept of metabolism was first of all given by Ibn-e-Nafees, who stated that “the body and its parts are always undergoing change.

Enzymes are proteins that catalyze (i.e. speed up) biochemical reactions and are not changed during the reaction. The molecules at which enzymes act are called **substrates,** and enzyme converts them into different molecules, called **products**.

All chemical reactions require **activation energy.** It is defined as minimum energy required to start a reaction. The need for activation energy acts as a barrier to the beginning of reaction as symbolized in the diagram). Enzymes lower such barriers by decreasing the requirement of activation energy. Thus, in the presence of enzymes, reactions proceed at a faster rate (Figure 6.1)

Enzymes lower the activation energy in several ways. They may alter the shape of substrate and reduce the requirement of energy for this change. Some enzymes do so by disrupting the charge distribution on substrates. Enzymes may also lower activation energy by bringing substrates in the correct orientation to react.

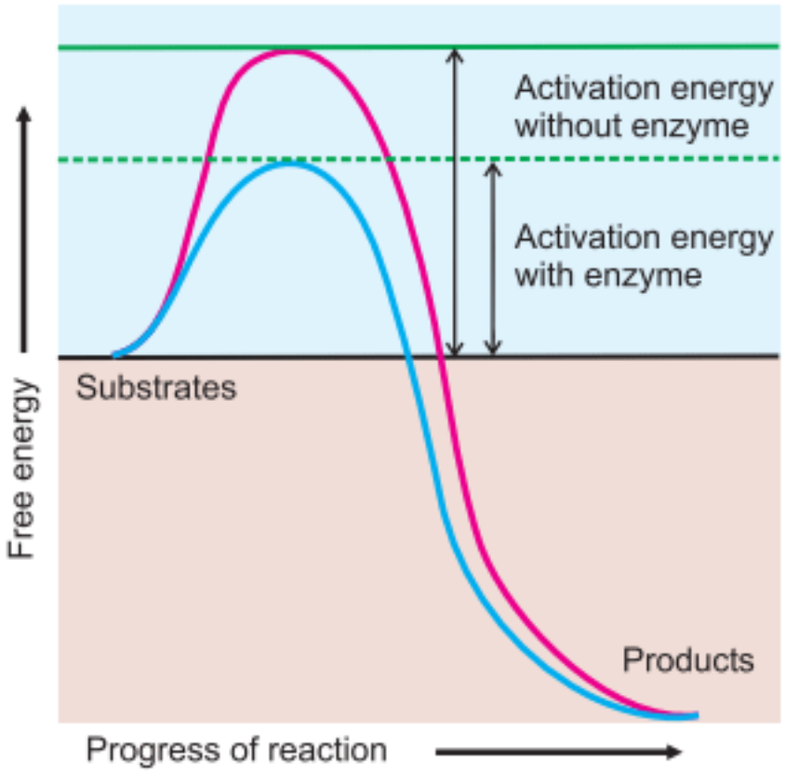


Figure 6.1: Enzymes lower the activation energy

Enzymes can be categorized on the basis of the site where they work i.e. they may be **intracellular enzymes** (e.g. enzymes of glycolysis working in the cytoplasm) or may be **extracellular enzymes** (e.g. pepsin enzyme working in the stomach cavity)

? statement 1: All enzymes are catalysts.Statement 2: All catalysts are enzymes.Which one is correct?

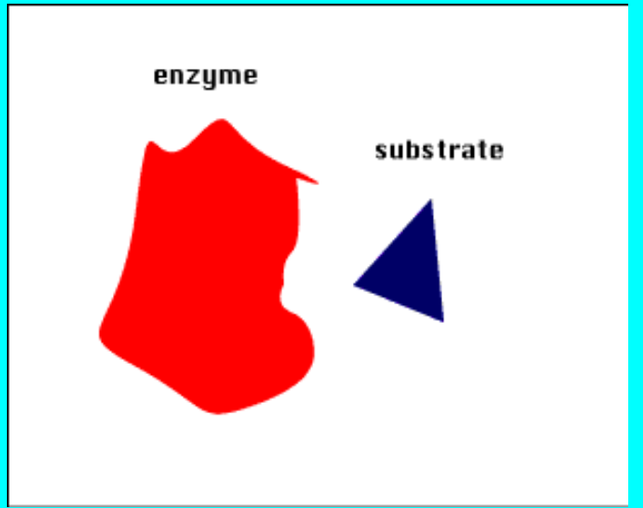
Statement 1

All biochemical catalysts are not proteins for example some RNA molecules also catalyze reactions.

6.1 Characteristics Of Enzymes

In 1878, German physiologist **Winhelm Kuhne** first used the term enzyme. Enzymes are globular proteins Like all proteins, enzymes are made of long linear chains of amino acids that fold to produce a three-dimensional molecule.

- Almost all enzymes are proteins i.e. they are made of amino acids.
- Most enzyme reaction rates are millions of times faster than those of comparable uncatalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze.
- Enzymes are usually very **specific** for the type of reaction and for the nature of their substrates.
- Only a small portion of enzyme molecule is directly involved in catalysis. This catalytic region is known as **active site** It recognizes and binds substrate and then carries out reaction.
- Enzyme production can be enhanced or diminished by a cell according to needs. Enzyme activity can also be regulated by **inhibitors** and **activators.**
- Some enzymes do not need any additional component to work. However, others require non-protein molecules or ions called **cofactors**. Cofactors can be either inorganic (e.g. metal ions) or organic (e.g. flavin and heme).If organic cofactors are tightly bound to enzyme, they are called **prosthetic groups**. If organic cofactors are loosely attached with enzyme, they are called **co-enzymes**. Coenzymes transport chemical groups from one enzyme to another. Some important vitamins (e.g. riboflavin, thiamine and folic acid) act as coenzymes.
- Several enzymes can work together in a specific order, creating **metabolic pathways** In a metabolic pathway**,** one enzyme takes the product of another enzyme as a substrate. After the reaction, the product is passed on to the next enzyme.



Animation 6.1: Enzyme
Source & Credit: dynamicscience

Uses of enzymes

Enzymes are extensively used in different industries for fast chemical reactions. For example;

1. **Food industry:** Enzymes that break starch into simple sugars are used in the production of white bread, buns etc.
2. **Brewing industry:** Enzymes break starch and proteins. The products are used by yeast for fermentation (to produce alcohol).
3. **Paper industry:** Enzymes break starch to lower its viscosity that aids in making paper.
4. **Biological detergent:** Protease enzymes are used for the removal of protein stains from clothes. Amylase enzymes are used in dish washing to remove resistant starch residues.

6.1.1 Factors Affecting The Rate Of Enzyme Action

Enzymes are very sensitive to the environment in which they work. Any factor that can change the chemistry or shape of enzyme molecule, can affect its activity. Some of the factors that can affect the rate of enzyme action are being discussed next.

Temperature

Increase in temperature speeds up the rate of enzyme catalyzed reactions, but only to a point (Figure 6.2). Every enzyme works at its maximum rate at a specific temperature called as the **optimum temperature** for that enzyme.

When temperature rises to a certain limit, heat adds in the activation energy and also provides kinetic energy for the reaction. So reactions are accelerated. But when temperature is raised well above the optimum temperature, heat energy increases the vibrations of atoms of enzyme and the globular structure of enzyme is lost. This is known as the **denaturation** of enzyme. It results in a rapid decrease in rate of enzyme action and it may be blocked completely.

The optimum temperature for the maximum working speed of human enzymes is 37°C.

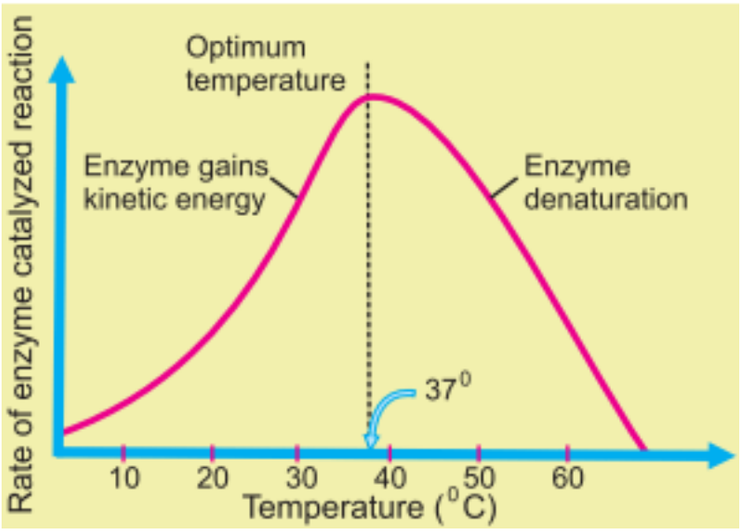


Figure 6.2: Effect of temperature on enzyme activity

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Birds have higher body temperature than mammals.What would happen to the activity of a bird's enzyme if it is given temperature of 37° C?

Reaction will slow down

Substrate concentration

If enzyme molecules are available in a reaction, increase in substrate concentration increases the rate of reaction. If enzyme concentration is kept constant and amount of substrate is increased, a point is reached where any further increase in substrate does not increase the rate of reaction any more. When the active sites of all enzymes are occupied (at high substrate concentration), any more substrate molecules do not find free active sites. This state is called **saturation** of active sites and reaction rate does not increase (Figure 6.3).

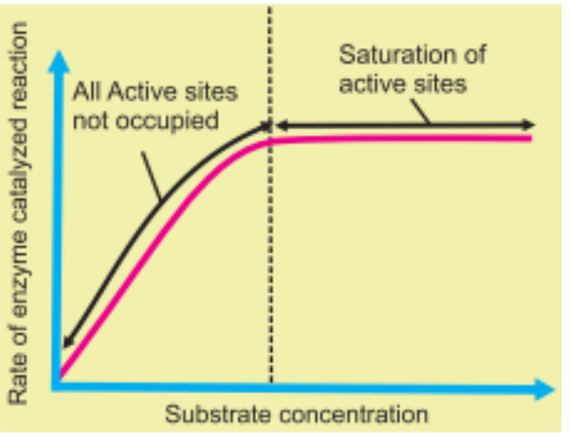

Figure 6.3: Effect of substrate concentration on enzyme activity

pH

All enzymes work at their maximum rate at a narrow range of pH, called as the **optimum pH** (Figure 6.4). A slight change in this pH causes retardation in enzyme activity or blocks it completely. Every enzyme has its specific optimum pH value. For example pepsin (working in stomach) is active in acidic medium (low pH) while trypsin (working in small intestine) shows its activity in alkaline medium (high pH). Change in pH can affect the ionization of the amino acids at the active site.

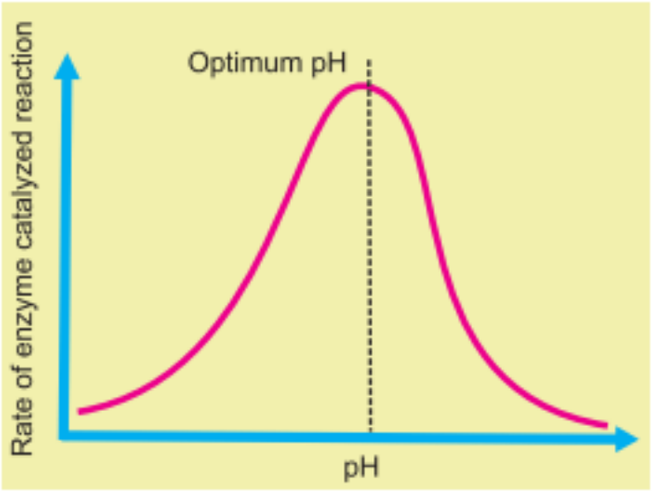

Figure 6.4: Effect of pH on enzyme activity

6.2 Mechanism Of Enzyme Action

When enzyme attaches with substrate, a temporary enzyme-substrate (ES) complex is formed. Enzyme catalyzes the reaction and substrate is transformed into product. After it, the ES complex breaks and enzyme and product are released.

$$E + S \longrightarrow ES\ complex \longrightarrow E + P$$

In order to explain the mechanism of enzyme action a German chemist **Emil Fischer**, in 1894, proposed **lock and key model**. According to this model, both enzyme and substrate possess specific shapes that fit exactly into one another. This model explains enzyme specificity (Figure 6.5).

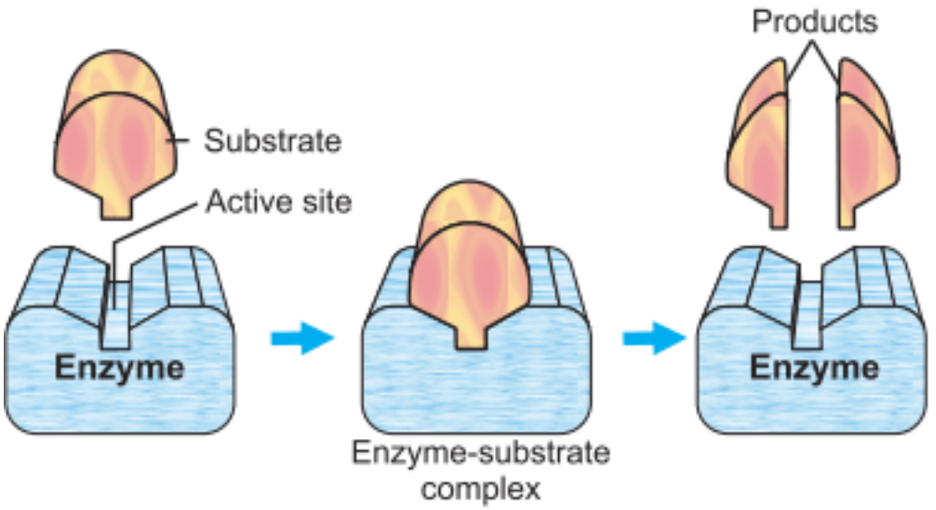

Figure 6.5: Lock and key model of enzyme action

In 1958, an American biologist **Daniel Koshland** suggested a modification to lock and key model and proposed **induced-fit model.** According to this model, active site is not a rigid structure rather it is molded into the required shape to perform its function. Induced fit model is more acceptable than "lock and key" model of enzyme action.

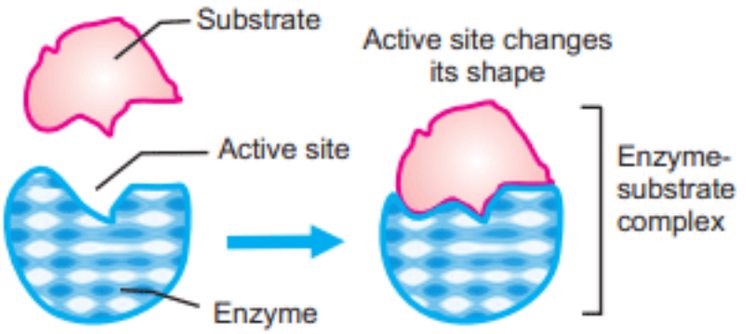

Figure 6.6: Induced-Fit model of enzyme action

6.3 Specificity Of Enzymes

There are over 2000 known enzymes, each of which is involved in one specific chemical reaction. Enzymes are also substrate specific. The enzyme protease (which breaks peptide bonds in proteins) will not work on starch (which is broken down by an enzyme amylase . Similarly lipase enzyme acts only on lipids and digests them into fatty acids and glycerol. Specificity of different enzymes is determined by the shapes of their active sites. Active sites possess specific geometric shapes that fit with specific substrates.

See in Figure 6.7 how the geometric shape of active site of enzyme determines its specificity for substrate **(point out which substrate can exactly fit in the active site).**

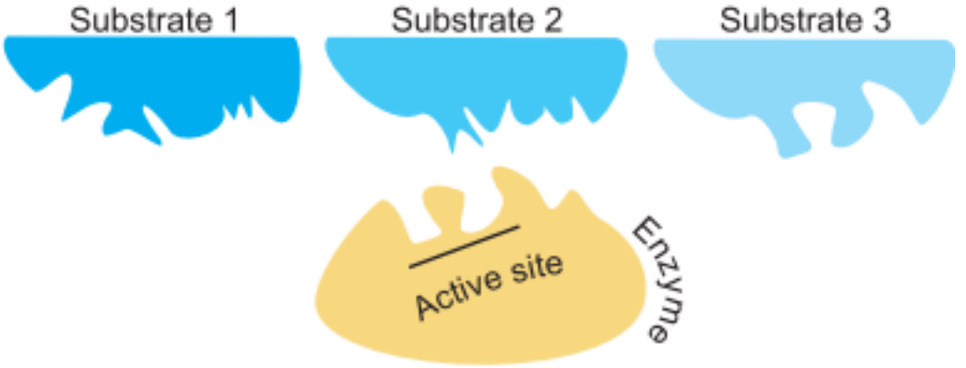

Figure 6.7: Specificity of enzyme due to the geometric shape of active site

Practical Work:

Perform an experiment to show the working of an enzyme in vitro

Enzymes can catalyze in-vivo and in-vitro reactions. We can design an experiment to observe the in-vitro enzyme activity. For this purpose we will select meat proteins as substrate and pepsin as the protein digesting enzyme.

Problem: Can pepsin digest the proteins present in meat?

Apparatus required: Meat, Test tube, pepsin solution, HCl, Biuret reagent

Background information:

- In-vitro means outside living body (in artificial environment) while in-vivo means inside living body
- Animal flesh (meat) contains lot of proteins.
- Pepsin enzyme is produced in stomach (in its inactive form pepsinogen). It acts on protein molecules and digests them to peptides.

Procedure:

- Take a small piece of meat in two test tubes and pour 15 ml of pepsin in one of them the and pour 15 ml water in the second tube (for comparison).
- Add 10 drops of HCl in both test tubes and place them at 37° C in incubator.

Observation: Observe the piece of meat after four hours. Perform the Biuret test to confirm the presence of proteins in both tubes. Go to chapter 8 (section 8.2) for the procedure of Biuret test.

Results: The Biuret test gives negative results in the tube in which pepsin was added. It confirms that no proteins are present in this test tube and all have been digested by the enzyme pepsin.

Evaluation:

- What effect did pH have on pepsin activity?
- What is the optimum pH for pepsin?
- An organism lives in a hot springs. What will be the effect on its enzymes if it is placed in cold water?

Practical Work:

Perform an experiment to show the working of amylase in vitro

Amylase is an enzyme that catalyses the breakdown of the polysaccharide starch to the disaccharide maltose. It is present in saliva, plant tissues and also in seeds. To observe the in-vitro enzyme activity we can select starch as substrate and amylase as the starch digesting enzyme.

Problem: Can amylase digest starch?

Apparatus required: Meat, Test tube, pepsin solution, HCl, Biuret reagent

Background information:

- Starch turns iodine solution dark purple/black while disaccharides do not react with the iodine.

Procedure:

1. Prepare 1% solution of amylase and put some of it in a test tube.

2. Add 2 ml of starch solution in the test tube.

3. Incubate the test tube at 37^0 C for 15 minutes.

Observation: Observe the test tube after 15 minutes. Perform iodine test to confirm the presence of starch. This can be done by putting few drops of iodine solution in the test tube. Observe the color change in the test tube.

Results: Iodine test gives negative results. There was no color change. It confirms that no starch is present in the test tube and all have been digested into disaccharides.

Evaluation:

i. What color appears when iodine test is positive?

ii. Why was the experimental test tube incubated at 37^0 C?

iii. If we perform the iodine test on starch solution before putting it in amylase, what would be the results?

UNDERSTANDING THE CONCEPTS

1. How would you define enzymes? Describe their characteristics.
2. What do you mean by activation energy and why it is referred in the definition of enzymes?
3. In a range of 0-35°C, the rate of reaction of an enzyme is proportional to temperature. Above 35°C and below 0°C, enzyme activity slows down and eventually stops. Explain why?
4. How does pH affect enzyme activity?
5. What characteristic of enzymes makes them specific for substrates?
6. Briefly describe the factors that affect the activity of enzymes.
7. Describe the lock and key mechanism of enzyme action.

Short Questions

- Define cofactor and coenzyme.
- What is the main use of enzymes in paper industry?

THE TERMS TO KNOW		
Activation energy Active site Amylase Anabolism Biocatalyst, Enzyme Catabolism Catalyst	Coenzyme Cofactor Denaturation Optimum pH Optimum temperature Enzyme-substrate	Lipase Lock-and-key model Metabolism Product Saturation Substrate

Initiating And Planning

- Draw graphs showing the effects of temperature, pH, and concentration of substrate on the rate of enzyme-catalyzed reactions.
- Illustrate through a diagram, the lowering of activation energy by enzyme.

Activities

1. Perform experiment to show the in-vitro working (in test tube) of pepsin on meat.
2. Perform experiment to show the in-vitro working (in test tube) of amylase on starch.

Science, Technology And Society

1. List the uses of enzymes in different industries.

ON-LINE LEARNING

en.wikipedia.org/wiki/Enzyme
www.biology-online.org/dictionary/Enzyme
encarta.msn.com/encyclopedia_761575875/enzyme.html
www.brooklyn.cuny.edu/bc/ahp/BioWeb/