

CHAPTER

3

Enzymes

Animation 3.1: Enzyme
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Enzymes are the most important group of proteins which are biologically active. They tremendously increase the efficiency of a biochemical reaction and are specific for each type of reaction. Without these enzymes the reaction would proceed at a very slow speed making life impossible.

Enzymes are composed of hundreds of amino acids joined together and coiled upon themselves to form a globular structure. The catalytic activity is restricted to a small portion of the structure known as the active site. The reactant called substrate is attached to the active site consisting of only a few amino acids, while rest of the bulk of the amino acids maintains the globular structure of the enzyme.

Some enzymes consist solely of proteins. Others also have a non-protein part known as a **co-factor**, which is essential for the proper functioning of the enzymes. The cofactor usually acts as “bridge” between the enzyme and its substrate, often it contributes directly to the chemical reactions which bring about catalysis. Sometimes the co-factor provides a source of chemical energy, helping to drive reactions which would otherwise be difficult or impossible. Some enzymes use metal ions as co-factors like Mg^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+} etc. The detachable co-factor is known as an activator if it is an inorganic ion (Fig. 3.1).

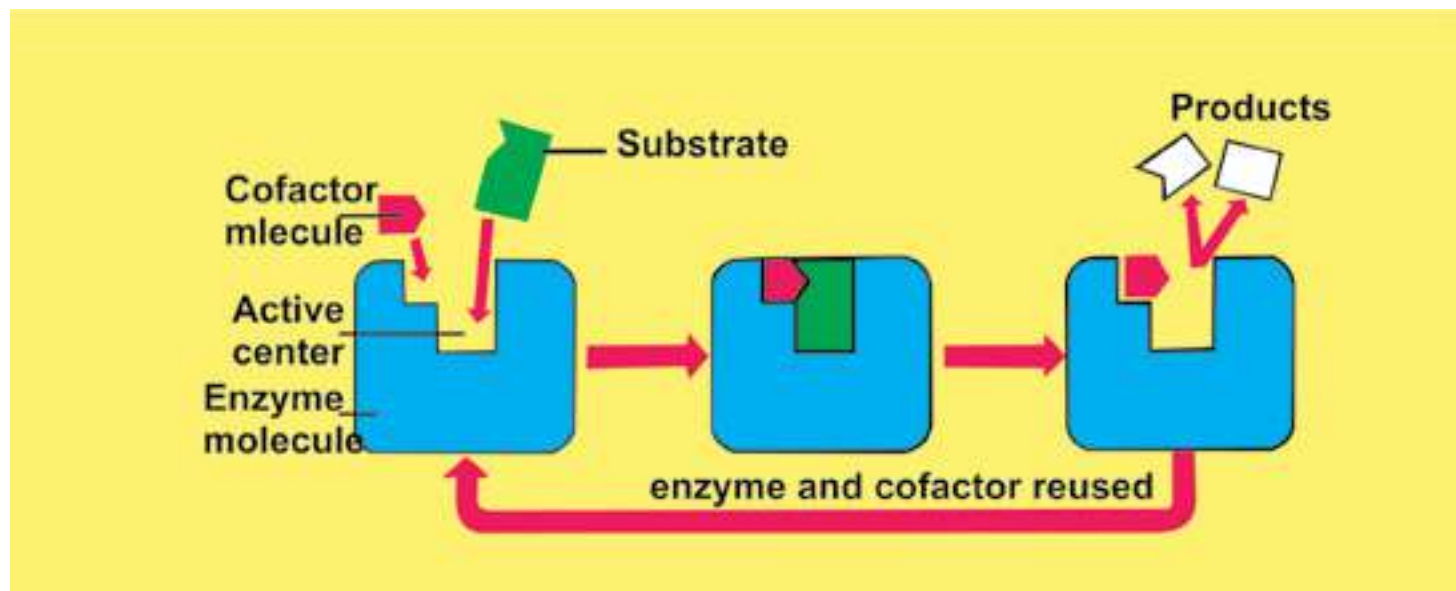
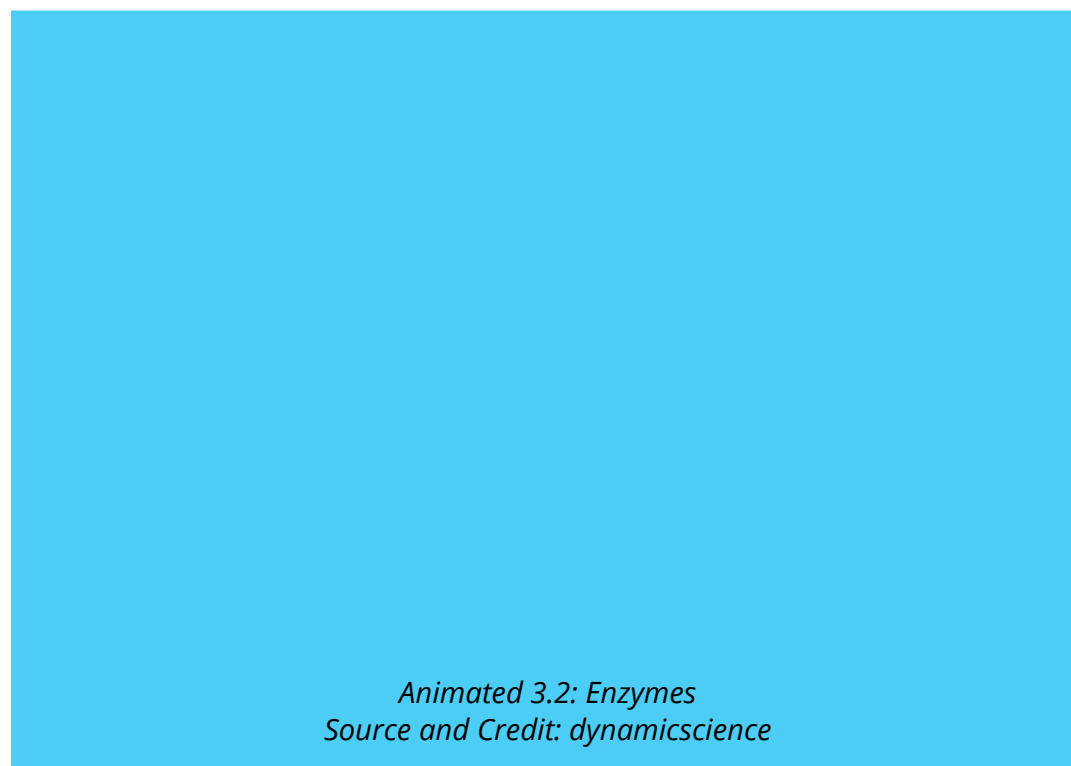


Fig. 3.1 Substrate molecules will not fit correctly at the active centre and there will be no catalytic action unless the cofactor molecule is also present

If the non-protein part is covalently bonded, it is known as a **prosthetic group**. If it is loosely attached to the protein part, it is known as **coenzyme**. It is closely related to vitamins, which represent the essential raw materials from which coenzymes are made. Only small quantities of vitamins are

needed because, like enzymes, co-enzyme can be used again and again. An enzyme with its coenzyme, or prosthetic group, removed is designated as **apoenzyme**. Adding the correct concentrated coenzyme to the apoenzyme will restore enzyme activity. An activated enzyme consisting of polypeptide chain and a cofactor is known as **holoenzyme**.

Many enzymes are simply dissolved in the cytoplasm. Other enzymes are tightly bound to certain subcellular organelles. They are produced by living cells for use in or near the site of their production. The enzymes important in photosynthesis are found in the chloroplasts and enzymes involved in cellular respiration are found in the mitochondria. Some of the enzymes which are involved in the synthesis of proteins are integral part of ribosomes.



CHARACTERISTICS OF ENZYMES

Enzymes, the biochemical catalysts possess the following important characteristics.

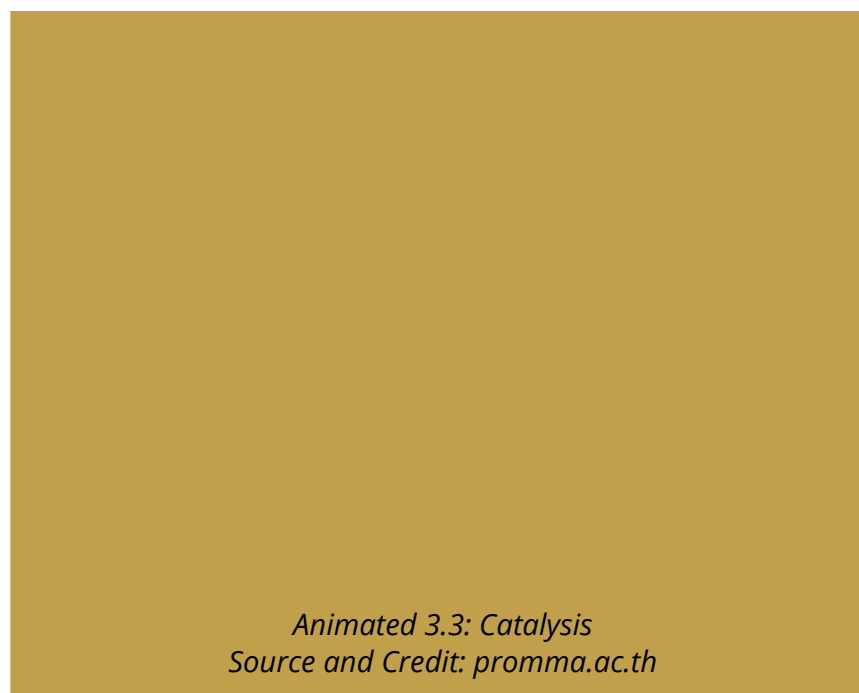
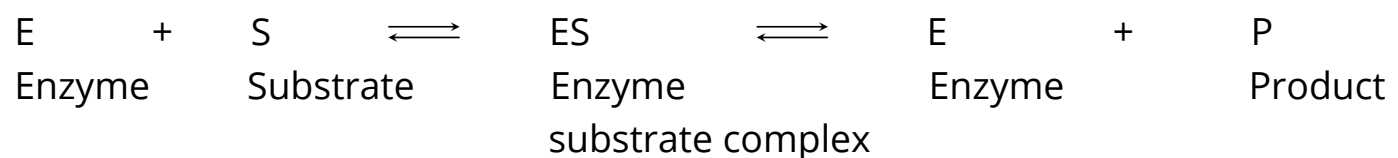
1. All enzymes are globular proteins.
2. They increase the rate of reaction without themselves being used up.
3. Their presence does not affect the nature or properties of end products.
4. Small amounts of an enzyme can accelerate chemical reactions.

5. They are very specific in their action; a single enzyme catalyzes only a single chemical reaction or a group of related reactions.
6. They are sensitive to even a minor change in pH, temperature and substrate concentration.
7. Some enzymes require a co-factor for their proper functioning.
8. They lower the activation energy of the reactions.

Some enzymes are potentially damaging if they are manufactured in their active form. For example, **pepsin** is a powerful protein - digesting enzyme and is quite capable of destroying cell's internal structure and thus is produced in inactive **pepsinogen** form by the cell. It is converted in its active form only in the digestive tract where it is required to be active.

MECHANISM OF ENZYME ACTION (CATALYSIS)

An enzyme is a three dimensional globular protein that has specific chemical composition due to its component amino acids and a specific shape. Every enzyme by virtue of its specificity recognizes and reacts with a special chemical substance called substrate. Any enzyme, therefore, reacts only with its specific substrate and transforms it into product(s). It is then released unaltered and thus can be used again and again.



In certain cases enzymes act in a series of chemical reactions in a particular order to complete a metabolic pathway such as respiration or photosynthesis. The successive enzymes containing these reactions are normally present together in a precise order of reaction such that substrate molecules can be literally handed on from one enzyme to another forming an enzyme to enzyme chain. In this way, the products from one step in pathway are transferred to the enzyme catalyzing the next step.

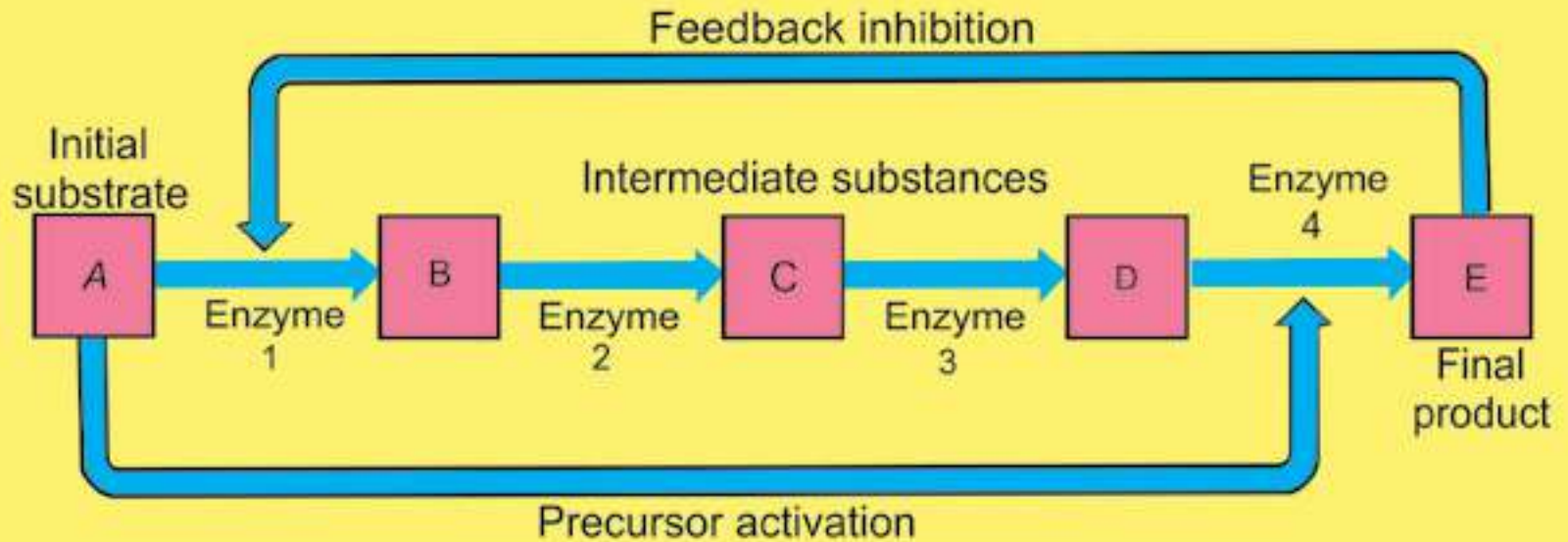


Fig. 3.2 Enzyme to enzyme chain (association)

Animated 3.4: Enzyme to enzyme chain
(association)
Source and Credit: faculty.ccbcmd

An enzyme and its substrate react with each other through a definite charge-bearing site of an enzyme called active site. The charge and shape of the active site is formed by some amino acids present in the polypeptide chain of the active site of the enzyme. These amino acids are brought closer and are arranged in a specific way by coiling and folding of the polypeptide chain within the globular symmetry of the enzyme (Fig. 3.3).

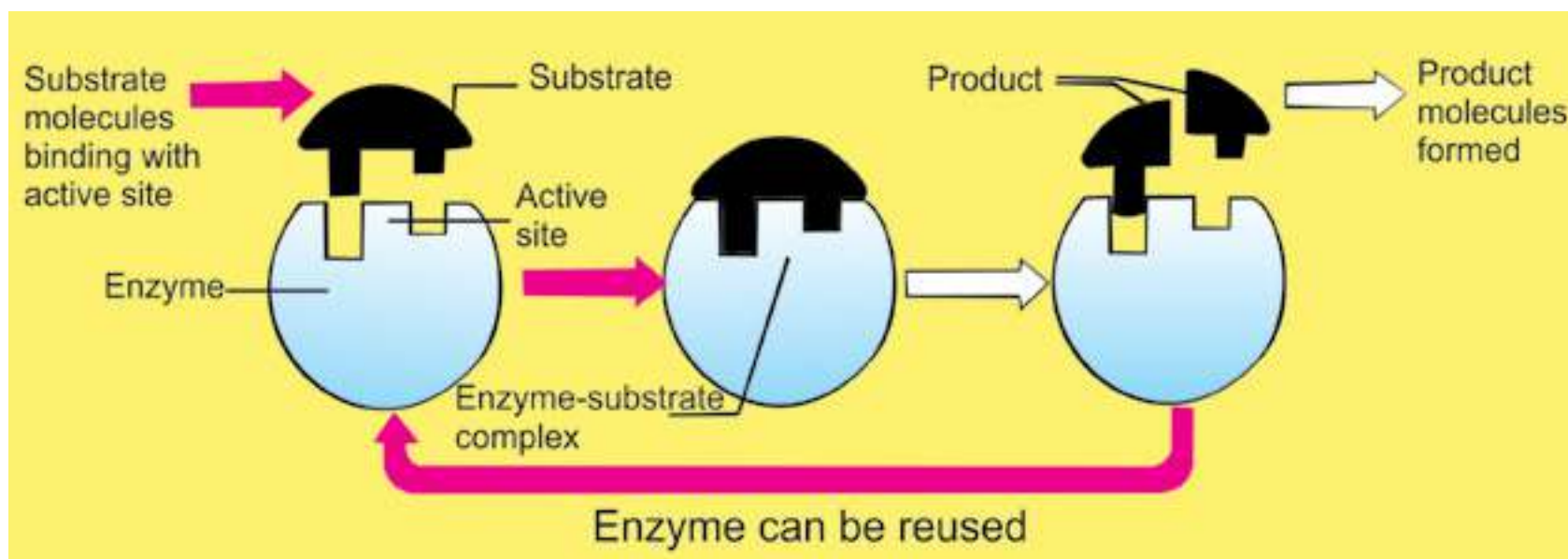


Fig. 3.3 Diagrammatic representation of an enzyme-substrate reaction (Lock and Key Model)

The active site of the enzyme is made up of two definite regions i.e the binding site and the catalytic site. The binding site helps the enzyme in the recognition and binding of a proper substrate to produce an ES complex. This reaction activates the catalytic site. Activated catalytic site catalyzes the transformation of the substrate into product(s). Thus the enzyme after catalysis detaches itself from the products unchanged. Enzyme requires aqueous medium for its activity.

Most enzymes do not float about in a kind of cytoplasmic soup' but are attached to membrane systems inside the cell in specific and orderly arrangements. Mitochondria and chloroplasts are good examples of this.

Emil Fischer (1890) proposed a Lock and Key model to visualize substrate and enzyme interaction. According to this model, as one specific key can open only a specific lock, in the same manner a specific enzyme can transform only one substrate into products(s).

According to Lock and Key Model the active site is a rigid structure. There is no modification or flexibility in the active site before, during or after the enzyme action and it is used only as a template. Later studies did not support this model in all reactions.

On the basis of new evidences Koshland (1959) proposed its modified form. This is known as Induce Fit Model.

He argued that when a substrate combines with an enzyme, it induces changes in the enzyme structure. The change in structure enables the enzyme to perform its catalytic activity more effectively.

FACTORS AFFECTING THE RATE OF ENZYME ACTION

The functional specificity of every enzyme is the consequence of its specific chemistry and configuration. Any factor that can alter the chemistry and shape of an enzyme can affect its rate of catalysis. Some of the important factors that can affect the rate of enzyme action are: concentration of enzyme, concentration of substrate, temperature, and pH of the medium.

1. Enzyme Concentration

The rate of reaction depends directly on the amount of enzyme present at a specific time at unlimited substrate concentration. If the amount of enzyme is increased by two fold the reaction rate is doubled.

By increasing the enzyme molecules an increase in the number of active sites takes place. More active sites will convert the substrate molecules into product(s), in the given period of time. After a certain limiting concentration, the rate of reaction will no longer depend upon this increase.

2. Substrate Concentration

At low concentration of substrate the reaction rate is directly proportional to the substrate available.

If the enzyme concentration is kept constant and the amount of substrate is increased, a point is reached when a further increase in the substrate does not increase the rate of the reaction any more (Fig.3.4). This is because at high substrate level all the active sites of the enzyme are occupied and further increase in the substrate does not increase the reaction rate.

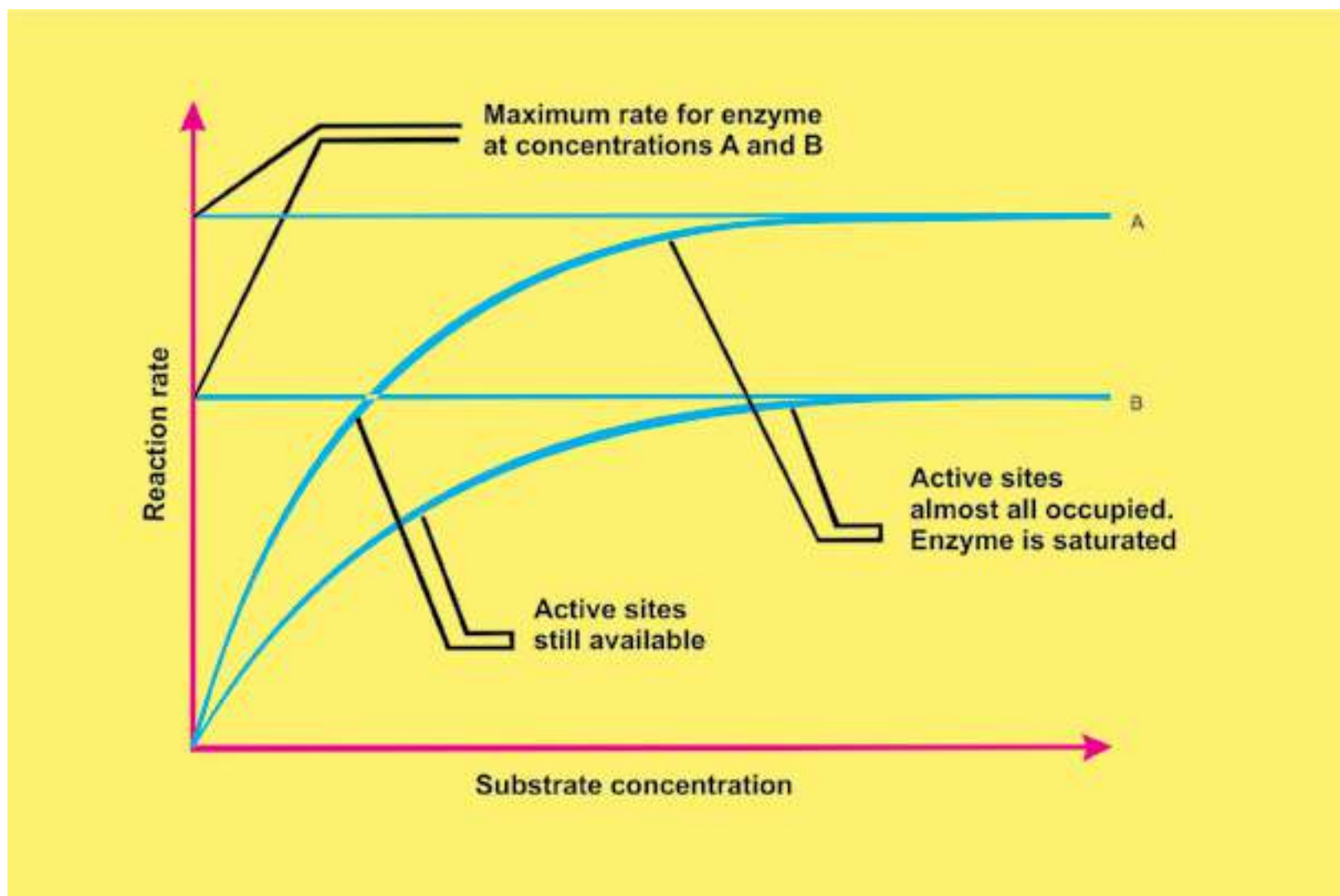


Fig. 3.4.1 Effect of substrate concentration on the rate of an enzyme catalyzed reaction.

3. Temperature

The rate of enzyme controlled reaction may increase with increase in temperature but up to a certain limit. All enzymes can work at their maximum rate at a specific temperature called as optimum temperature. For enzymes of human body 37°C is the optimum temperature (Fig.3.5).

Heat provides activation energy and therefore, chemical reactions are accelerated at high temperatures. Heat also supplies kinetic energy to the reacting molecules, causing them to move rapidly. Thus the reactants move more quickly and chances of their collision with each other are increased. However, further increase in heat energy also increases the vibrations of atoms which make up the enzyme molecule. If the vibrations become too violent, globular structure essential for enzyme activity is lost and the enzyme is said to be denatured.

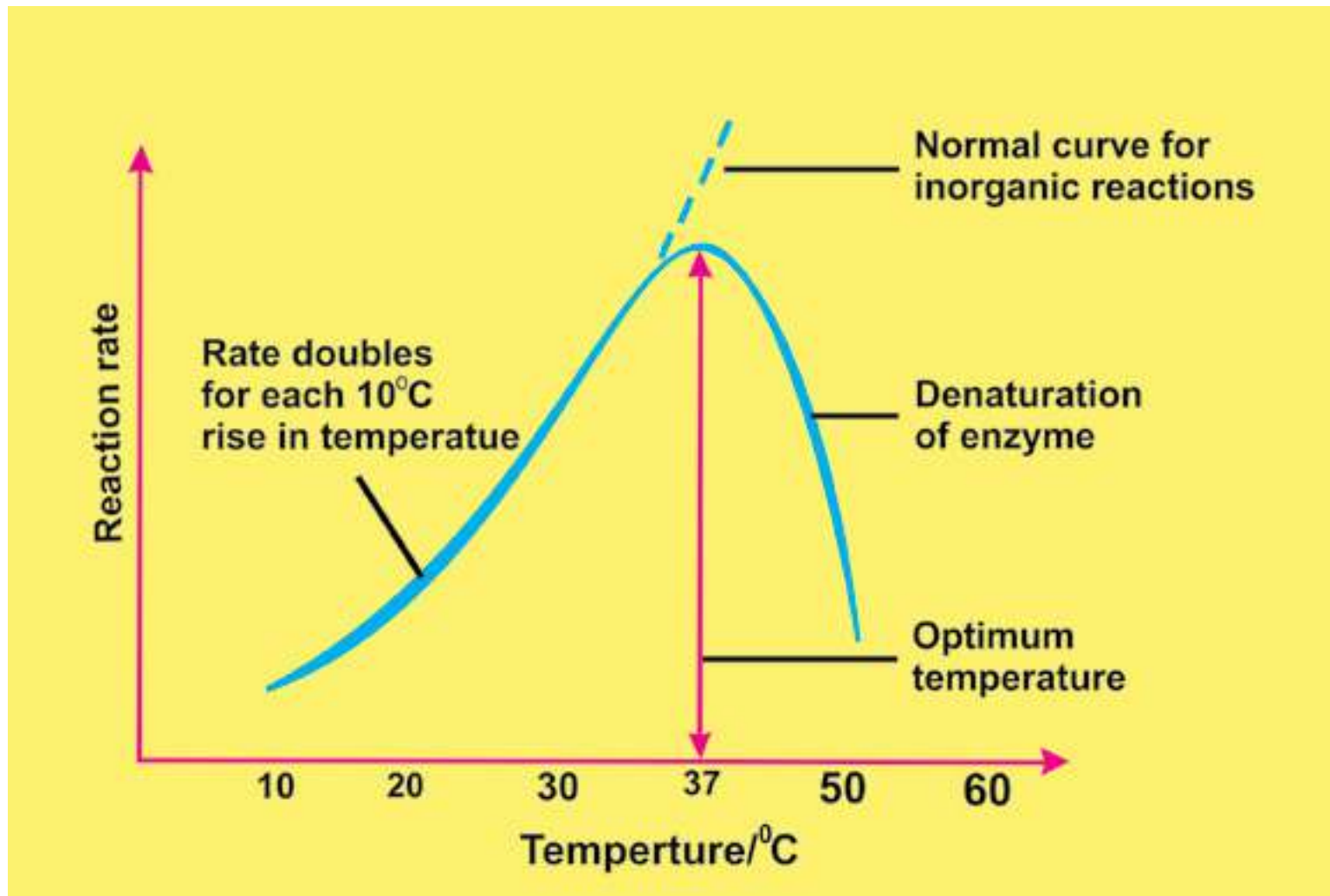


fig.3.5. Effect of temperature on the rate of an enzyme catalyzed reaction

4. pH Value

Every enzyme functions most effectively over a narrow range of pH known as the **optimum pH** as shown in Table 4.1.

A slight change in pH can change the ionization of the amino acids at the active site. Moreover, it may affect the ionization of the substrates. Under these changed conditions enzyme activity is either retarded or blocked completely.

Extreme changes in pH cause the bonds in the enzyme to break, resulting in the enzyme denaturation.

Table 4.1 Optimum pH values for some enzymes

Enzyme	Optimum pH
Pepsin	2.00
Sucrase	4.50
Enterokinase	5.50
Salivary amylase	6.80
Catalase	7.60
Chymotrypsin	7.00-8.00
Pancreatic lipase	9.00
Arginase	9.70

I. Inhibitors

An inhibitor is a chemical substance which can react (in place of substrate) with the enzyme but is not transformed into product(s) and thus blocks the active site temporarily or permanently, for example poisons, like cyanide;, antibiotics, anti-metabolites and some drugs.

Inhibitors can be divided into two types: (i) Irreversible (ii) Reversible

Irreversible Inhibitors

They check the reaction rate by occupying the active sites or destroying the globular structure. They occupy the active sites by forming covalent bonds or they may physically block the active sites.

Reversible Inhibitors

They form weak linkages with the enzyme. Their effect can be neutralized completely or partly by an increase in the concentration of the substrate.

They are further divided into two major types: A. Competitive B. Non-competitive

A. Competitive Inhibitors

Because of the structural similarity with the substrate they may be selected by the binding sites, but are not able to activate the catalytic sites. Thus product(s) are not formed (Fig.3.6).

B. Non-competitive Inhibitors

They form enzyme inhibitor complex at a point other than the active site. They alter the structure of the enzyme in such a way that even if genuine substrate binds the active site, catalysis fails to take place.

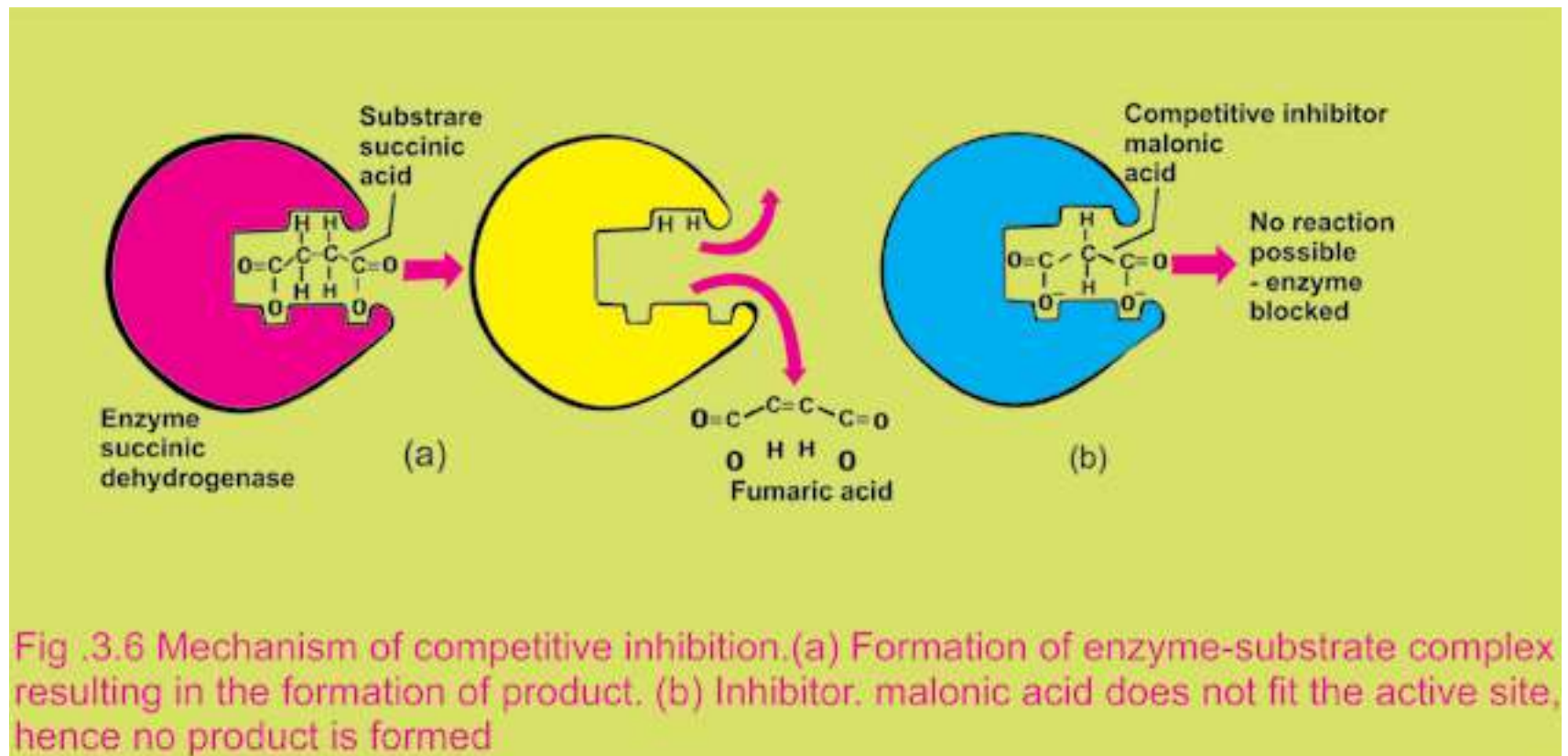


Fig .3.6 Mechanism of competitive inhibition. (a) Formation of enzyme-substrate complex resulting in the formation of product. (b) Inhibitor, malonic acid does not fit the active site, hence no product is formed

Animated 3.5: Non-competitive Inhibitors
Source and Credit: academic.brooklyn

Exercise**Q.1. Fill in the blank.**

- (i) Enzymes are composed of hundreds of _____
- (ii) Some enzymes consist of a non-protein part known as a _____
- (iii) Many enzymes require non-protein component called _____ for their proper functioning.
- (iv) Enzymes are highly _____ in nature.
- (v) The enzymes which carry out the synthesis of _____ are integral parts of ribosomes.

Q.2. Write whether the statement is 'true' or 'false' and write the correct statement if it is false.

- (i) The enzymes important in photosynthesis are found in tire mitochondria
- (ii) Large amounts-of an enzyme can accelerate chemical reactions.
- (iii) Calvin Malvin proposed Lock and Key model for enzyme action.
- (iv) The active site of an enzyme is composed of four regions.
- (v) Structure of an enzyme has no specific importance.

Q.3. Short Questions

- (i) List two conditions that destroy enzymatic activity by disrupting bonds between the atoms in an enzyme.
- (ii) How do low and high temperature, affect an enzyme activity?
- (iii) What is a prosthetic group?
- (iv) Define inhibitors of enzyme.
- (v) How does an enzyme accelerate a metabolic reaction?

Q.4. Extensive questions.

1. Describe in detail the mechanism of enzyme action.
2. Give the effect of pH and temperature on the efficiency of an enzyme action.
3. Write a note on inhibitors of enzymes.
4. What is the importance of enzymes in life?