

Chapter 3
Enzymes

3.1 ENZYMES

All those biological molecules (proteins), which catalyze biological reactions and remain unchanged after completion of reaction, are called enzymes.

CHEMICAL COMPOSITION

1) Amino Acids

Enzymes are composed of hundreds of amino acids joined together and coiled upon them to form 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 maintain the globular structure of enzyme.

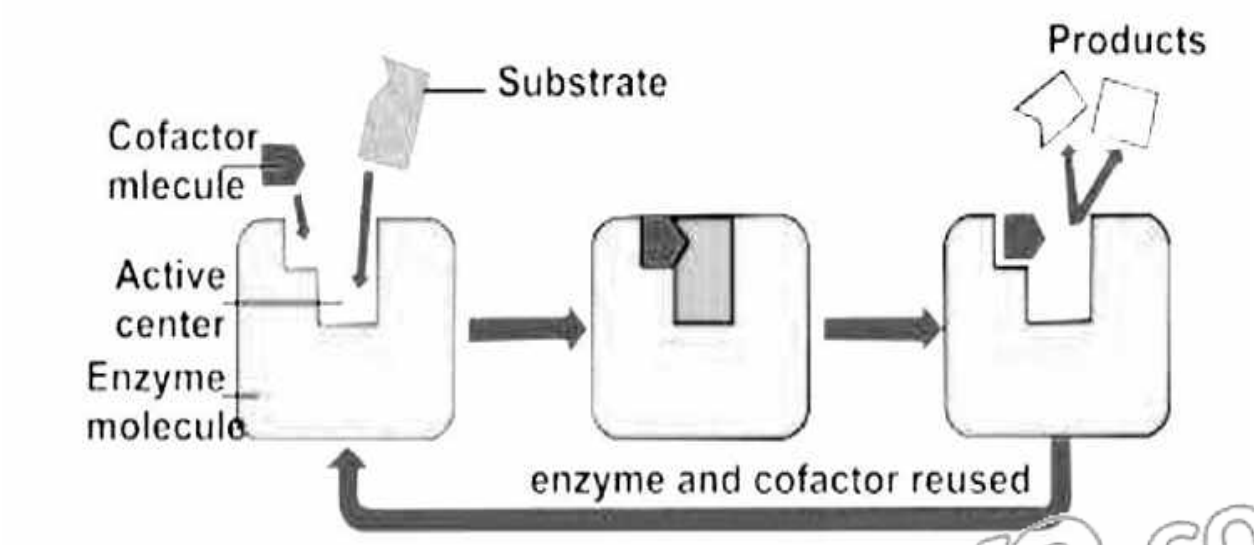


Fig. 3.1 Substrate molecules will not fit correctly at the active centre and there will be no catalytic action unless the cofactor molecule is also present.

2) Co-Factor

- Some enzymes consist solely of proteins.
- Others also have a non-protein part known as **co-factor.**

A co-factor,

i) Is essential for the proper functioning of the enzymes.
ii) They usually act as a bridge between the enzyme and its substrate.
iii) Often it contributes directly to the chemical reactions, which brings about catalysis.
iv) Sometimes the co-factor provides a source of chemical energy, helping to drive reactions which would otherwise be difficult or impossible.
v) The detachable co-factor is also known as an ***activator*** if it is an inorganic ion. Some enzymes use metal ions as co-factors like Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+} etc.

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3) Coenzyme, Apoenzyme and Holoenzyme

- If the non-protein part is covalently bonded, it is known as ***prosthetic group***.
- If non-protein part is loosely attached to the protein part, it is known as ***coenzyme*** and 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 enzyme, co-enzyme can be used again and again.
- An enzyme from which its coenzyme or prosthetic group has been removed is called as ***apoenzyme***.
- An activated enzyme consisting of polypeptide chain and a cofactor is known as ***holoenzyme***.

Location and Production of Enzymes

➢ As related to their ***location*** in cell

- Many are simply dissolved in the ***cytoplasm***.
- Some are tightly bound to certain ***subcellular organelles***. For example, enzymes for photosynthesis are found in chloroplast and enzymes involved in cellular respiration are found in mitochondria. Those, which are involved in the synthesis of proteins, are integral part of ribosomes.

➢ As related to their ***production***, they are produced by living cells for use in or near the site of their production.

- Enzymes secreted inside the cell are called ***intracellular*** enzymes.
- Enzymes secreted outside the cell in cavity e.g. gut, are called ***extracellular*** enzymes.

QUESTION RELATED TO ABOVE ARTICLE

What is Enzyme? Explain the following terms.
(a) Substrate (b) active site
Explain following terms.
(a) Apoenzyme (b) Coenzyme (c) Prosthetic group
(d) Holoenzyme (e) Co-factor (f) Activator
What is the importance of enzymes in life? (Exercise Question iv)

3.2 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. Even a ***small amount*** of an enzyme can bring about the ***change in a large amount*** of the substrate.
5. They are very ***specific*** in their action; generally a single enzyme catalyzes only a single substrate or a group of related substrates.
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 reactants.
9. Some enzymes are ***potentially damaging*** if they become active in the wrong place. 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 form, e.g; **pepsinogen** formed by the cell in membrane-bounded bodies called lysosomes.
10. Enzymes ***require aqueous medium*** for their activity.

QUESTION RELATED TO ABOVE ARTICLE

Explain the properties of enzymes.

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3.3 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.

Specificity of Enzymes

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 products. It is then released and thus can be used again and again.

Simple Mechanism of Enzyme Action

An enzyme and its substrate react with each other through definite charge bearing sites called ***active sites***.

i) The ***active site*** of an enzyme is a three-dimensional cavity bearing a specific charge by which the enzyme reacts with its substrate. The charge and shape of the active site is formed by some amino acids present in the polypeptide chain 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.

ii) 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 products.

Enzyme, after catalysis detaches itself from the products unchanged. Enzyme requires aqueous medium for its activity.

Mechanism in Complex Reactions

In certain cases enzymes act in a series of chemical reactions in a particular order to complete 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 molecule can be literally 'handed on' from one enzyme to another forming an enzyme coenzyme chain. In this way, the products from one step in pathway are transferred to the enzyme catalyzing the next step.

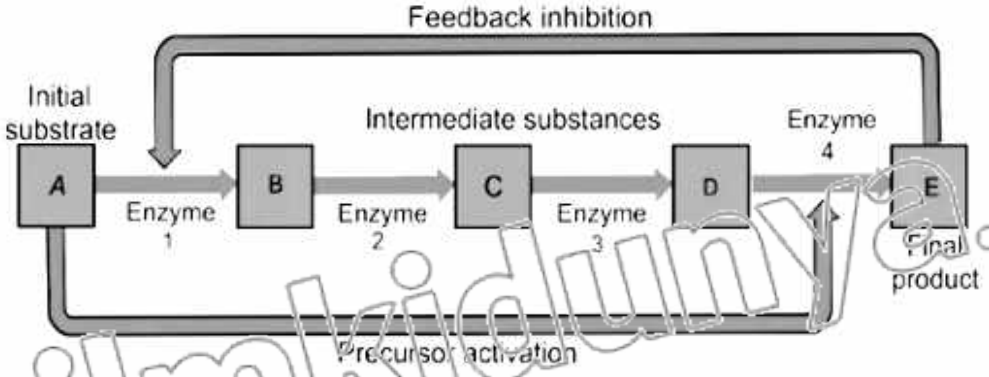


Fig. 3.2 Enzyme to enzyme chain (association)

Lock and Key Model of Enzyme Action

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.

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 is used only as a template. Later studies did not support this model in all reactions.

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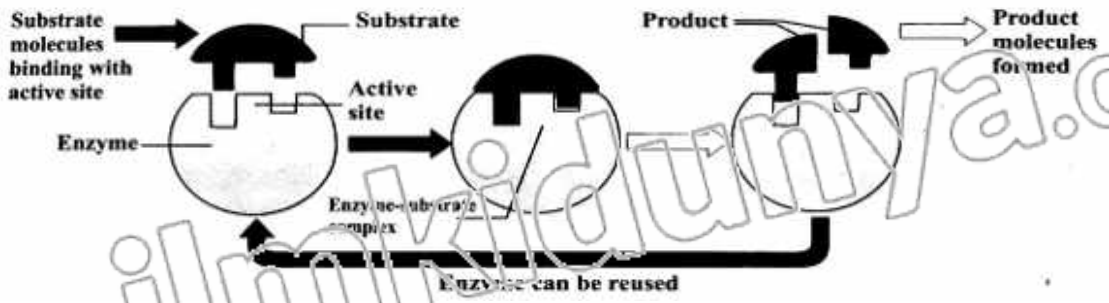


Fig. 3.3 Diagrammatic representation of an enzyme-substrate reaction (Lock and Key Model)

Induce Fit Model of Enzyme Action

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.

QUESTION RELATED TO ABOVE ARTICLE

Explain mechanism of enzyme with different models.

Describe the mechanism of enzyme action. Explain your answer with the help of diagram.

What are enzymes? Describe in detail the mechanism of enzyme action.

Describe in detail the mechanism of enzyme action. (Exercise Question i)

3.4 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 given below.

i. Enzyme Concentration.
ii. Substrate Concentration.
iii. Temperature.
iv. pH Value.

3.4.1) 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 number of active sites takes place. More active sites will convert the substrate molecules into products, in the given period of time
- When concentration of substrate is increased to a limit, then rate of reaction no longer depends on the increase in enzyme concentration.

3.4.2) Substrate concentration

The rate of reaction depends directly on the **amount of substrate** present at a specific time at **unlimited enzyme 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. This is because at high substrate level all the active sites of the enzyme are occupied **(saturated)** and further increase in the substrate does not increase the reaction rate.

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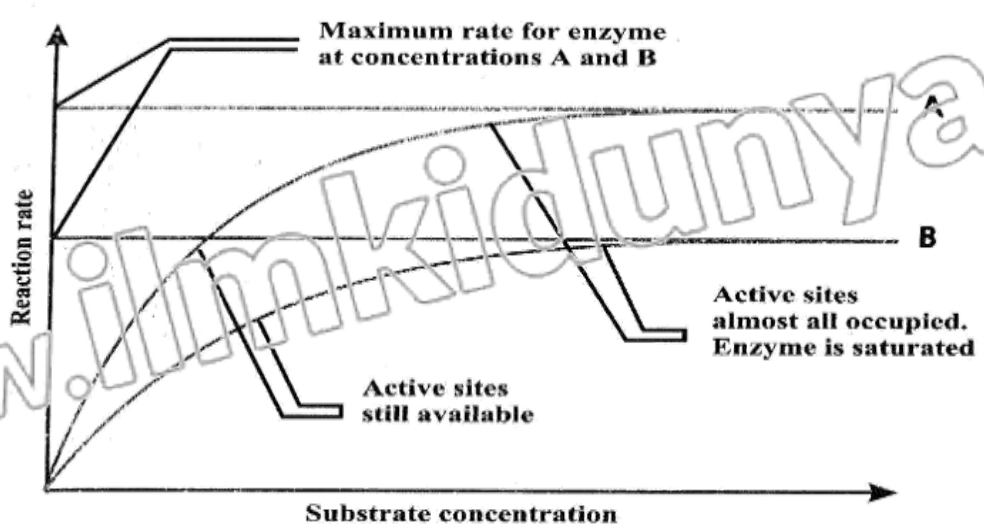


Fig. 3.4 Effect of substrate concentration on the rate of an enzyme catalyzed reaction.

3.4.3) Temperature

The rate of enzyme controlled reaction may increase with increase in temperature but ***up to a certain limit.***

Optimum Temperature

All enzymes can work at their maximum rate at a specific temperature called as ***optimum temperature***.

For example, for enzymes of human body **37 *ºC*** is the optimum temperature.

Effect of Alterations

- 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.
- If heat is increased too much, then it causes increase in vibration of atoms, by which enzyme activity is lost and the enzyme is said to be denatured.

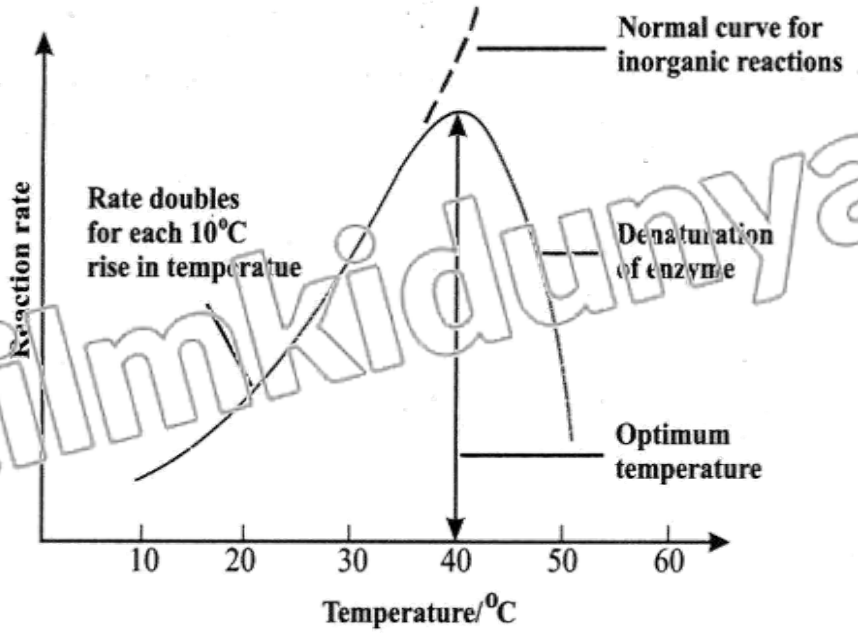


Fig. 3.5 Effect of temperature on the rate of an enzyme catalyzed reaction

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3.4.4) pH value

Optimum pH

Every enzyme functions most effectively over a narrow range of pH known as the optimum pH.

Effect of Alterations

- 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.

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

QUESTION RELATED TO ABOVE ARTICLE

Describe the factors affecting the enzyme action.

Give the effect of pH and temperature on the efficiency of an enzyme action.

(Exercise Question ii)

3.5 INHIBITORS

An inhibitor is a chemical substance, which can react (in place of substrate) with enzyme but is not transformed into products and thus blocks the active site temporarily or permanently.

Examples

Poisons like ***cyanide***, ***antibodies***, ***anti-metabolites*** and ***some drugs***.

Types

Inhibitors can be divided into two types:

(i) Irreversible

(ii) Reversible

1) 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 e.g. cyanide and some drugs.

2) 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 subdivided into two major types: competitive and non-competitive.

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A. Competitive Inhibitors

They have structural similarity with substrate, so selected by binding sites, but are unable to activate the catalytic sites. Thus products are not formed. Example is malonic acid.

B. Non-Competitive Inhibitors

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

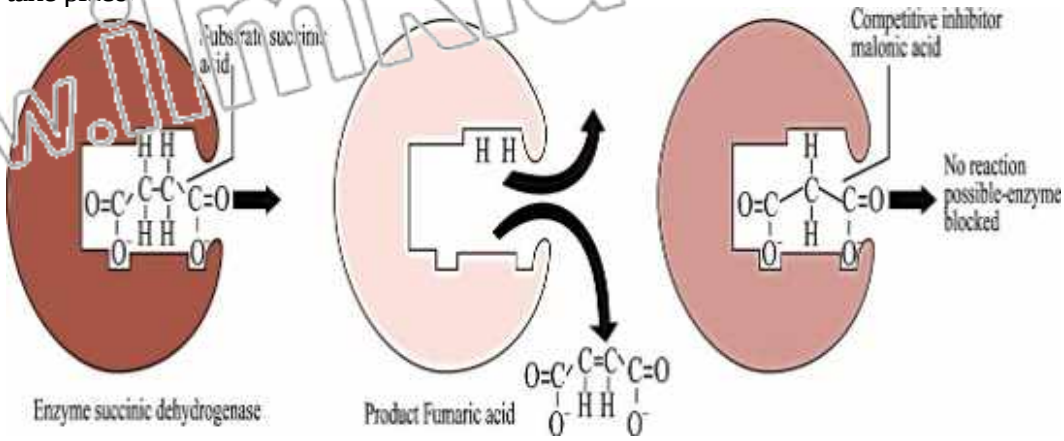


Fig. 3.6 Mechanism of competitive inhibition. (a) Formation of enzyme-substrate complex resulting in the formation of product. (b) Inhibitor malonic acid does not fit the active site, hence no product is formed.

QUESTION RELATED TO ABOVE ARTICLE

What are inhibitors? Give their types and role.
Define inhibitors. Write their types.
Write a note on inhibitors of enzymes. (Exercise Question iii)

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KEY POINTS

Activation Energy

The amount of minimum energy required to start a reaction is called activation energy. Enzyme reduces the amount of activation of energy and increases the rate of reaction.

pH value: (may be called, power of Hydrogen)

The antilog of the Hydrogen ion concentration is called pH. It means it is opposite of the hydrogen ions. If there are more H ions (more acidic) the pH will be less. On the other hand, if there are less H ions and more OH ions (Alkaline), there will be more pH. So acidic medium has les pH and alkaline medium as more pH.

pH from 0 to 7 = acidic
pH from 7 to 7.2 = n eutral
pH from 7.2 to 14 = alkaline

Difference between prosthetic group and coenzyme

Coenzyme

Any organic molecule which does not bind tightly with the enzyme is called coenzyme. They do not form covalent bonds with the enzymes. They attach with enzyme with weak bonds like dipole interaction. Such bond can easily be broken Coenzymes are mostly vitamins or derived from vitamins. Example: **NAD** (Nicotine amide dinucleotide).

Prosthetic group

Any organic molecule which binds tightly with the enzyme is called prosthetic group. It forms covalent bond with the enzyme. So it cannot be easily separated from the enzymes. In some cases they remain permanently with the enzyme. Some coenzyme can from covalent bonds with certain enzymes. Such coenzyme may be called prosthetic group. For example: **FAD** (Flavin adenine dinucleotide) is a coenzyme of certain enzyme. But with succinic dehydrogenase, it forms covalent bond. So it is prosthetic cofactor of the enzyme.

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EXERCISE

Q.1.

Fill in The Blanks.

i) Enzymes are composed of hundreds of _____.

ii) If the non-protein part is covalently bonded, it is known as _____.

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 part of ribosomes.

Ans:

i) Amino acids ii) Prosthetic group
iii) Co-factor iv) Catalytic
v) Proteins

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

i) Ligases catalyze the breakdown of complex substances into simple ones.

(False)

Hydrolases catalyze the breakdown of complex substances into simple ones.

ii) Oxidases catalyze the transfer of hydrogen atoms to oxygen.

(True)

iii) Calvin Malvin proposed Lock and Key model for enzyme action.

(False)

iv) Emil Fisher proposed Lock and Key model for enzyme action.

(True)

v) The active site of an enzyme is composed of four regions.

(False)

vi) The active site of an enzyme is composed of two regions.

(True)

vii) Structure of an enzyme has no specific importance.

(False)

Q.3. Each question has four options. Encircle correct answer.

i) If more substrate to an already occurring enzymatic reaction is added, more enzyme activity is seen because:

(a) Then is probably more substrate present than there is enzyme

(b) Then is probably more enzyme available than there is substrate

(c) Then is probably more product present than there in either substrate or enzyme

(d) The enzyme substrate complex is probably failing to form during the reaction

ii) If you add more substrate to already occurring enzymatic reaction and it has no effect on the rate of reaction? What is the form given for this situation

(a) Saturation

(b) Denaturation

(c) Composition

(d) Inhibition

iii) The rate of an enzyme-catalyzed reaction:

(a) Is constant under all conditions

(b) Decreases as substrate concentration increases

(c) Cannot be measured

(d) Can be reduced by inhibitors

iv) The active site of an enzyme:

(a) Never changes

(b) Forms no chemical bond with substrate

(c) Determines, by its structure, the specificity of the enzyme

(d) Looks like a lump projecting from the surface of an enzyme

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v) Which statement about enzyme is not true?

(a) They consist of proteins, with or without a non-protein part
(b) They change the rate of catalyzed reaction
(c) They are sensitive to heat
(d) They are non-specific in their action

Answer key	
i	a
ii	a
iii	d
iv	c
v	d

Q.4. Short Questions

i) List two conditions that destroy enzymatic activity by disrupting bonds between the atoms in an enzyme.

Ans:

(1) Temperature:
If heat is increased too much, then it causes increase in vibration of atoms by which enzyme activity is lost and enzyme is said to be lost.

(2) pH:
Extreme changes in pH cause the bonds in the enzyme to break, resulting in the enzyme denaturation.

ii) How do low and high temperature, respectively effect an enzyme activity.

Ans: Effect of Low & High Temperature:
Low temperature decreases while the high temperature increases the enzyme activity (rate doubles for each 10°C rise in temperature) but within certain limits.

iii) What is prosthetic group?

Ans: In an enzyme, if the non-protein part is covalently bonded, it is known as prosthetic group.
For example TPP, NAD^+ etc.

iv) Define inhibitors of enzyme.

Ans: An inhibitor is a chemical substance, which can react (in place of substrate) with the enzyme but is not transformed into products and blocks the active site temporarily or permanently.
For example cyanide, antibiotics, anti-metabolites etc.

v) How an enzyme does accelerates a metabolic reaction?

Ans: Enzyme acts as a catalytic agent and accelerates a metabolic reaction by converting a compound into its fragments (products) without itself being changes. It lowers activation energy.

Q.5. Extensive Questions.

i) Describe in detail the mechanism of enzyme action.

Ans: (See article 3.3)

ii) Give the effect of pH and temperature on the efficiency of an enzyme action.

Ans: (See article 3.4.3 & 3.4.4)

iii) Write a note on inhibitors of enzymes.

Ans: (See article 3.5)

iv) What is the importance of enzymes in life?

Ans: (See article 3.1)

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