

Dr. Bbosa Science

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Enzymes

Specific objectives

The learner should be able to:

- Describe the criteria for naming enzymes using type of substrate and type of reaction
- Explain the characteristics/properties of enzymes
- State factors that affect enzyme action (pH, temperature, inhibitor, substrate concentration)
- Explain the mechanism of enzyme action using the lock and key mechanism and induced fit
- The role of enzyme in the organism's life.

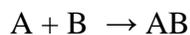
Practical

- Demonstrate properties of enzymes action in specific temperature, pH range, substrate concentrations
- Identify enzymes in different parts of the gut based on their different actions on different food substances

The chemical reaction that occur in cells constitute metabolism and the participating molecules are called metabolites.

Types of metabolism

- Anabolism [or synthetic reaction]. Here single molecules are linked together by chemical bonds to form more complex compounds.



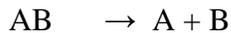
A and B are substrate molecules or reaction and AB represent the product.

Generally anabolic reactions require [absorb] energy and are therefore endogenic reaction.

These reactions are concerned with building up structures, storage compounds and complex metabolites in the cell. Starch, glycogen, lipids and proteins are all products of anabolic pathway.

Plants and other autotrophic organisms synthesize these complex organic molecules from simple inorganic sources such as carbon dioxide and water. They have much greater synthetic power, and their metabolic pathways are therefore more extensive than heterotrophs.

2. Catabolism [or breakdown reaction] The complex compounds are split into simple molecules



In this case AB is the substrate and A and B are products.

Generally catabolic reactions release energy and are therefore exergonic reactions. These reactions are mainly concerned with mobilizing food stores and making energy available in cells. Energy is required for 3 main purposes.

- (a) For synthesis e.g. the synthesis of proteins, storage compounds
- (b) For work e.g., contraction of muscles
- (c) for maintenance; such as the constant body temperature.

Small steps gentle reactions

Metabolites are not converted into products in single large reactions. Instead they are converted gradually, step by step, through a series of small reactions which together comprise a metabolic pathway, each reaction though small, brings the raw materials closer to the end product.

There are five main reasons why metabolism proceeds in small steps.

- (i) Large catabolic reactions would create unfavorable conditions, such as very high temperature, that would be incompatible with life.
- (ii) Energy can be obtained from small catabolic reactions in a usable form.
- (iii) Substances can be partially broken down so as to provide raw materials for others.
- (iv) It is not possible to synthesize, in one step, a complex organic compound from simple small raw materials in the gentle conditions prevailing in cells.
- (v) Having small steps in an anabolic pathway increases the cell's ability to control what products are made.

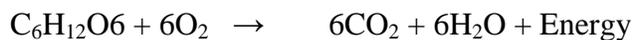
Living cells have the unique ability to perform numerous individual reactions in dilute aqueous solution at low temperatures and with a narrow range of pH.

However, the sheer number of reactions requires a fantastic degree of organization in the cell. Much of this organization is achieved by enzymes which catalyze individual reactions.

Energy

All living organisms may be regarded as working machines which require a continuous supply of energy in order to keep working and so to stay alive.

Energy is defined as the ability [capacity] to do work. The main source of metabolic energy is the cell in the oxidative breakdown of glucose [respiration] given by the following overall simplified reaction



Activation energy

The speed of biological reaction.

It's affected the following factors

- The concentration of the substrate molecule. The higher the concentration the higher the rate and reaction due to high rate of collision.
-
- The temperature of the reaction mixture: Temperature speeds up biological reaction by supplying the activation energy for the reaction and increase the rate of collision of the substrate molecules.
- Enzymes are biological catalyst i.e. a catalyst is a substance that increase the rate of the chemical reaction without taking part in a chemical reaction through lowering the activation energy.

Difference between enzyme and catalysts.

	Enzymes	Catalysts
1.	proteins in nature	inorganic chemicals e.g. Pt
2.	catalyze specific reactions Such as hydrolysis of starch	may catalyze more than one reaction e.g. platinum catalyze decomposition of H_2O_2 and reduction of benzene
3.	Denatured by heat above 45°C	Usually are not affected by heat
4.	Very sensitive to pH	Not sensitive to pH
5.	Initiate reaction	Do not initiate reaction

Enzyme classification

Enzymes are placed into six groups according to the general type of reaction which they catalyze Each enzyme is given a systematic name, accurately describing the reaction it catalysis.

However, since many of these names are very long and complicated, each enzyme as allocated a trivial name of the substrate acted on by the enzyme

- (i) the name of the substrate acted on by the enzyme
- (ii) the type of reaction catalyzed.
- (iii) the suffix-ase.

The categories of enzyme are;

1. **Oxidoreductase**; are involved in biological oxidation and reduction reaction. They include dehydrogenase which catalyze removal of hydrogen atoms from a substrate and oxidase which formation of water e.g., in respiration.
2. **Hydrolase**; catalyze the addition of water to or the removal from a substrate.eg protease.
3. **Transferase**; These catalyze transfer of chemical groups or atoms from one substrate to another. Those that transfer amino groups $[\text{NH}_2]$ are called transaminase.

4. **Lyase**; break chemical bonds other than hydrolysis this creating double bond. They include carboxylase which remove carboxyl group [COOH] from intermediates in respiratory pathways.
5. **Isomerase**; These enzymes catalyze the transfer of an atom from one part of a molecule to another.
6. **Ligase or synthetase**; catalyze joining together of two molecules coupled with the breakdown of ATP e.g., phosphokinase which catalyze the addition of phosphate group to a compound.

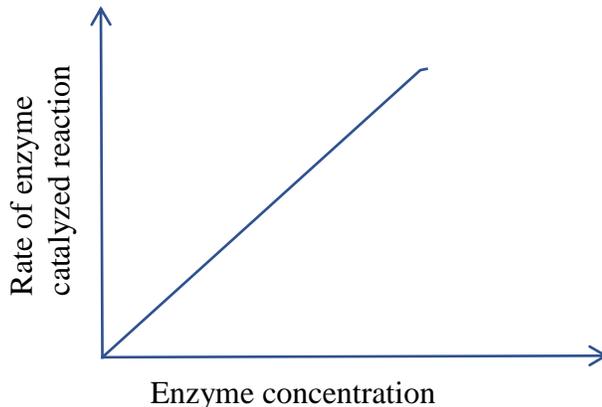
The properties of enzymes.

1. They catalyze the rate of biological reactions.
2. They are not destroyed by the reaction in which they catalyze.
3. They work in either direction i.e., catalyze both forward and backward reaction.
4. They are inactivated by high temperatures
5. They are sensitive to pH changes
6. They are usually specific to particular reactions

FACTORS AFFECTING THE RATE OF ENZYME REACTION

- (a) **Enzymes concentration**; provided that the substrate concentration is maintained at a high level, and other conditions such as pH and temperature are kept constant, the rate of reaction is proportional to enzyme concentration.

A graph showing relationship between enzyme concentration and the rate of enzyme- controlled reaction

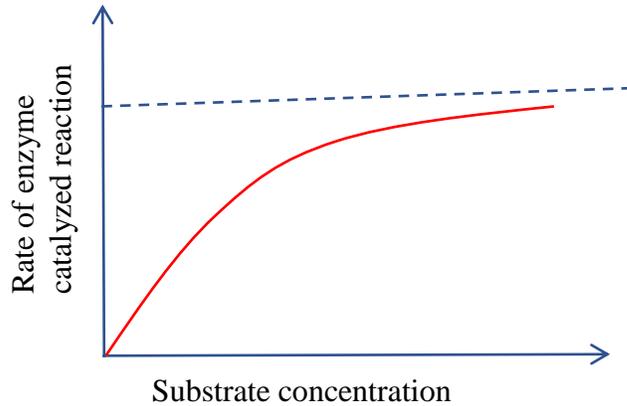


Question; How would you design an experiment to show this?

(b) Substrate concentration.

For a given enzyme concentration, the rate of an enzyme reaction increases with increasing substrate concentration. The theoretical maximum rate [V_{max}] is never quite obtained, but there comes a point when any further increase in substrate concentration produces no significant change in reaction rate. This is because at high substrate concentration the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. Thus, any extra

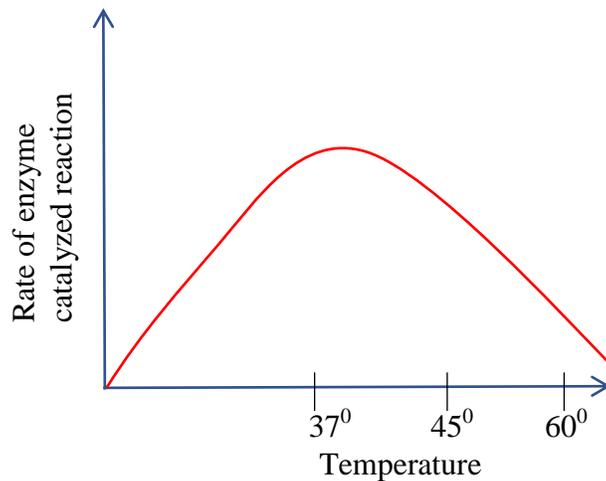
substrate has to wait until the enzyme/ substrate complex has dissociate into product and free enzyme before it may itself complex with the enzyme.



(c) Temperature

Up to 40°C , the rate of enzyme -controlled reaction increase smoothly with temperature, a ten degree rise in temperature being accompanied by approximately doubling the of reaction. Above that 40°C the rate begins to fall off and then decline rapidly, ceasing at about 60°C , because the enzyme is denatured.

Graph showing the effect of temperature on the activating of enzyme as salivary amylase.



An experiment to investigate the rate of action of amylase

Materials.

- 1% starch solution
- 40 water bath
- White tile
- Test tubes
- Glass rod
- Iodine solution

Method

1. Prepare 3 test tubes each with 2ml of starch solution and 2ml of 1% amylase solution.

Test tube 1; in beaker containing ice

Test tube 2; in a water bath at 40°C water bath.

Test tube 3; in a beaker of boiling water
And simultaneously start the stop clock.

2. Every after 1 minute withdraw a few drops from every test tube, place them on a white tile and add a drop of iodine solution.

3. Note the time taken for starch to disappear from each test tube.

NB; The rate of enzyme reaction is inversely proportion to this time.

(b) pH

Under conditions of constant temperature, every enzyme function most efficiently over a narrow pH range e.g. pepsin at pH= 2, Amylase (salivary) at pH 6.8.

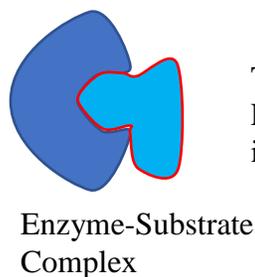
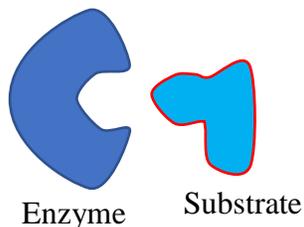
Mechanism of enzyme action.

The action of enzymes are specific to a given substrate and this specificity can be explained by two hypothesis.

1. Lock and key hypothesis

Enzyme are very specific to the substrate they act on because they have particular shape / configuration into which substrate with complementary shape fit in exactly as the key fit into the lock, thus the lock (enzyme) and key (substrate) hypothesis.

When an enzyme / substrate complex is formed, the substrate activated into forming the product of the reaction. Once formed, the product no longer fit into the active site and escape into the surrounding medium leaving the active site free to receive other substrate molecule.



The Substrate fit exactly into Enzyme active site like a key in the lock

2. Induced fit hypothesis.

This hypothesis claim that enzyme and their active site are physically rather more flexible structure than this described by the lock and key hypothesis, and that the active site of the enzyme is molded into a precise configuration in presence of a substrate to enable it perform its catalytic functions more effectively.

Inhibitors of enzymes

Certain substrate inhibit enzyme, thereby slowing down or stopping enzyme – controlled reactions.

These enzyme inhibitors are of specific interest for the following reasons.

- a. They give us important information about the shape and properties of active site of enzyme.
- b. They can be used to block particular reactions thereby enabling biochemist to reconstruct metabolic pathways.
- c. They have important medical and agricultural use as for example drugs and pesticides.

Type of inhibitors

- (i) Competitive reversible inhibitor;

Here a compound is structurally similar to that of the usual substrate associates with the enzymes active site but it's unable to react with it. While it remains there, it prevents access of any molecules of the substrate. This type of inhibition depends on the concentration of the substrate and that of an inhibitor. At high concentration of the substrate, inhibition is overcome.

This knowledge of competitive inhibitors has been utilized in chemotherapy. Sulphonamides drugs and antibiotic such as penicillin are competitive inhibitors of essential metabolites for enzymes in bacteria.

- (ii) Noncompetitive reversible inhibitors.

This is a type of inhibition in which the inhibitors attach themselves outside the active site thereby preventing the enzyme normal catalytic reaction by changing the shape of the enzyme or allosteric effect. It may be reversible when the inhibitor forms loose attachment to the enzyme that may be detached when circumstances permit e.g. cyanide or irreversible noncompetitive inhibition when the inhibitor permanently disorganizes the structure of the enzyme that it may no longer react with the substrate, e.g., mercury.

Allosteric enzymes and their inhibition

Allosteric enzymes are enzymes which exist in two different form, one active and the other inactive. The activity of these enzyme is regulated by compounds which are not their substrate and these substances (**allosteric effectors**) binds the enzyme at specific sites well away from the active site. They modify enzyme activity by causing a reversible change in the structure of the enzyme active site. Allosteric activators speed up enzyme catalyze reaction whereas or allosteric inhibitor slow down the action of allosteric enzymes.

Enzyme cofactors.

These are non-protein components required by enzymes to function efficiently. Cofactor may vary from simple inorganic ions to complex organic molecules, and may either remain unchanged to the end of a reaction or be regenerated by the later processes. The enzyme-cofactor

complex is called a **holo enzyme** whilst the enzyme portion without its cofactor is called an **apoenzyme**.

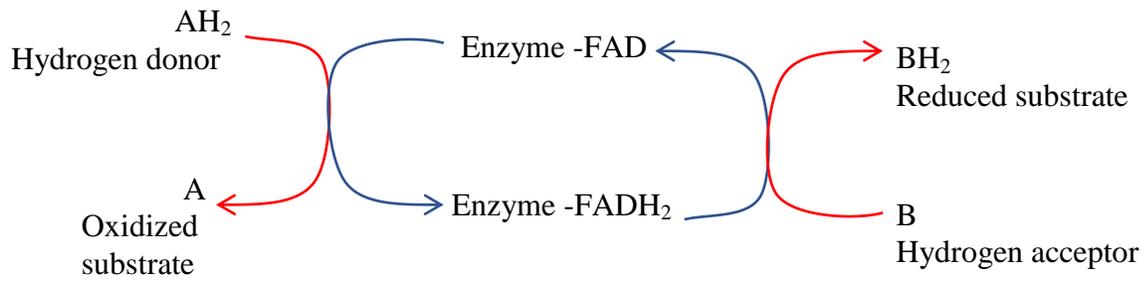
There are three recognized cofactors.

(i) Inorganic ions (activator)

These are thought to modify either the enzyme or the substrate such that an enzyme-substrate complex can be formed, hence increasing the rate of reaction catalyzed by that particular enzyme, salivary amylase activity is increased in the presence of chloride ions.

(ii) Prosthetic group (for example FAD, Biotin, Haem)

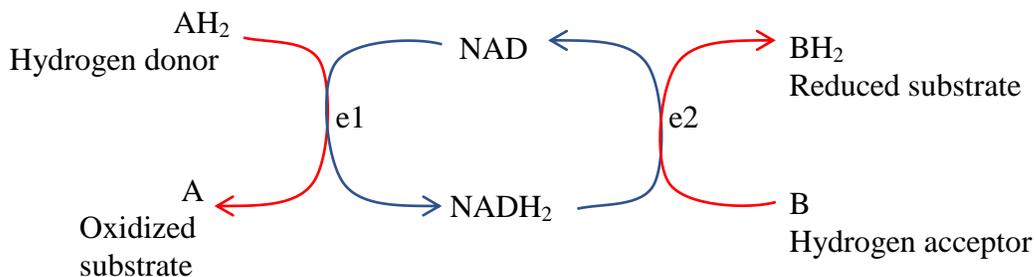
The organic molecule is integrated in such a way that it effectively assists the catalytic function of its enzyme, as in Flavin adenine dinucleotide [FAD]. This contains riboflavin [vitamin B₂] which is the hydrogen accepting part of FAD. It's concerned with cell oxidative pathways such as part of the respiratory chain in respiration.



Net effect; 2H are transferred from A to B. one holo enzyme acts as a link between A and B.

(iii) Co enzyme (NAD, NADP, Co enzyme A, ATP]

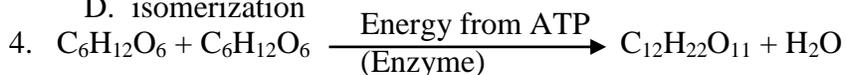
Nicotinamide adenine dinucleotide (NAD) is derived from the vitamin nicotinic acid and can exist in both a reduced and an oxidized form. In the oxidized state, it functions in catalysis as a hydrogen acceptor.



Where e1 and e2 are two different dehydrogenase enzyme.

Exercise

1. Chloride ions are vital for efficient functioning of salivary amylase because ion
 - A. Activator
 - B. Are coenzyme
 - C. Are co factor
 - D. For alkaline medium
2. Enzyme that catalyze the removal of water molecules from a substrate are known as
 - A. Reductase
 - B. Dehydrase
 - C. Hydrolase
 - D. hydrase
3. What name is given to a chemical reaction in which two or more hexose sugars combine to form larger units
 - A. condensation
 - B. hydrolysis
 - C. dehydrogenation
 - D. isomerization

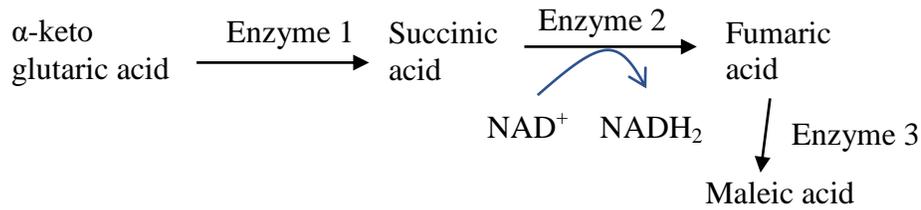


Glucose Fructose

What is the above called?

- A. Dehydration synthesis
 - B. Hydrolysis
 - C. Dehydration process
 - D. Condensation reaction
-
5. The activity of an enzyme in a
 - A. Molecular weight of the enzyme
 - B. Protein nature of the enzyme
 - C. Activation energy of the enzyme
 - D. Surface configuration of the enzyme
 6. The reaction rate of salivary amylase with starch decrease as the concentration of chloride ions.
 - A. Co enzymes
 - B. Competitive inhibitor
 - C. Co factor
 - D. Allosteric inhibitor
 7. Which one of the following characteristic of enzyme distinguishes than from inorganic catalyst
 - A. Initiation and speed up the rate of reaction
 - B. Remain the same at the end of the reaction
 - C. May promote reversible reaction
 - D. Exert their effect when present even in small quantities

8. Use the figure below, the part of reaction of the Krebs cycle to answer 8 and 9



Succinic acid accumulates when malonic acid is added to the reaction medium. Which one of the following statements best describes the role of malonic acid?

- Malonic acid is an inhibitor of enzyme 1
 - Malonic acid reacts with α -keto glutaric acid to form succinic acid
 - Malonic acid is an inhibitor of enzyme 2
 - Malonic acid acts as coenzyme of enzyme 1
9. Enzyme 2 is a
- Dehydrogenase
 - Decarboxylase
 - Dehydrase
 - Reductase
10. An enzyme which catalyzes the conversion of dipeptide into separate amino acid is an example of
- Dehydrogenase
 - Hydrolase
 - Transferase
 - Oxidase
11. An enzymatic reaction of the type $\text{ATP} + \text{hexose} \longrightarrow \text{ADP} + \text{hexose-6-phosphate}$ is an example of
- A hydrolysis
 - An isomerase
 - Transfer
 - A synthesis
12. When a piece of liver is dropped into a solution containing H_2O_2 , there is a vigorous reaction. This is due to the enzyme
- Catalase
 - Amylase
 - Trypsin
 - Carbonic anhydrase
13. In the lock and key hypothesis for the mechanism of enzyme action, how does an inhibitor substance stop enzyme action? By
- Raising activation energy
 - Distorting substrate molecule
 - Destroying coenzyme
 - Occupying active sites on substrate and enzyme

14. When the extent of inhibition in an enzyme controlled reaction depends entirely on the concentration of the inhibitor, it indicates that the inhibition is
- Competitive
 - Reversible
 - Non-competitive
 - Irreversible.
15. Which **one** of the following is not true about the lock and key theory in enzyme-catalyzed reaction?
- A small change in the active site alter the enzyme
 - The substrate and active site are complementary
 - Enzyme catalyzed action go through the enzyme-substrate complex stage
 - A molecule which fits in the active site is a substrate
16. Which one of the following does not have an effect on a non-competitive inhibition?
- Temperature change
 - pH change
 - enzyme concentration
 - substrate concentration
17. Which one of the following describes the turnover number of an enzyme?
- Number of molecules affected by the enzyme
 - Number of substrate molecules turned into its product per minute
 - Number of product molecules formed
 - Number of substrate molecules catalyzed per minute
18. Which of the following is true about non-competitive inhibition of enzyme catalyzed reaction?
- The degree of inhibition decreases with increase in substrate concentration
 - The inhibitor has a similar structure and chemical composition with the substrate
 - The degree of inhibition is independent of the substrate concentration
 - The shape of the enzyme is not affected by the inhibitor
19. When the extent of inhibition of an enzyme-controlled reaction depends entirely on the concentration of the inhibitor, it indicates that the inhibition is
- Competitive
 - Reversible
 - Non-competitive
 - irreversible
20. Chloride ion are vital for efficient functioning of salivary amylase because the ions
- are activator
 - are co-enzyme
 - are co-factors
 - form alkaline medium
21. The activity of an enzyme in a chemical reaction depends on the
- Molecular weight of the enzyme
 - Protein nature of the enzyme
 - Activation energy of the enzyme
 - Surface configuration of the enzyme

22. Which one of the following correctly represents the effect of increasing substrate concentration on the degree of inhibition in a competitive and noncompetitive inhibition reaction?

	competitive	noncompetitive
A	Decrease	increased
B	Decrease	No change
C	increased	Decrease
D	No change	

23.



If an excess X controls the metabolic pathway of the reaction, the control mechanism is known as,

- A. Multi-enzyme control
- B. Excess inhibition
- C. End product inhibition
- D. Negative feedback

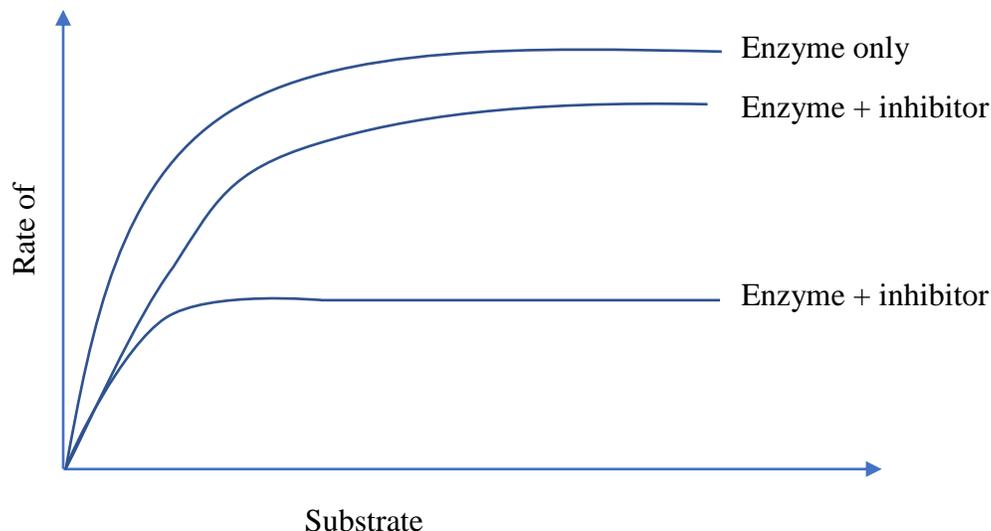
24. An adaptation by plant to obtain nitrogen include all the following except.

- A. Mycorrhiza on plant roots
- B. Bacteria in root nodules
- C. Possession of aerial roots
- D. Being insectivorous

25. Which one of the following environmental factors has a direct effect on all organisms?

- A. Light.
- B. Humidity.
- C. Temperature.
- D. Rainfall.

26. Figure 4 show the effect of increasing the concentration of a substrate on the rate of an enzyme controlled reaction in presence of inhibitors A and B, in relation to the control experiment without an inhibitor.



(a) Describe the effect of each inhibitor on the rate of reaction.

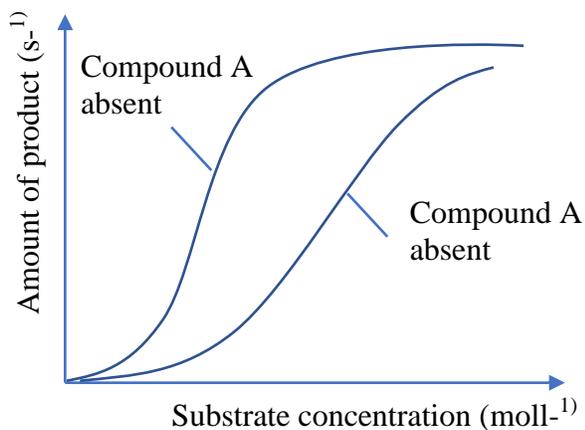
- (i) Inhibitor A
- (ii) Inhibitor B

(b) Explain the difference in the effect of inhibitors A and B on the rate of reaction

27. Explain the following

- (i) Competitive inhibition
- (b) In what way do enzymes differ from catalyst?
- (c) Briefly describe the lock and key hypothesis of enzyme action

28. The figure below shows the effect of varying substrate concentration on an enzyme catalyzed reaction, in absence and presence of compound A



- (a) Explain the relationship between the reaction rate and substrate concentration
- (i) In absence of compound A (3marks)
 - (ii) In presence of compound A(4marks)
- (b) State two factors which would have kept constant in this experiment (1mark)
- (c) What would be the effect of increasing the concentration of compound A in this experiment? (2marks)

29. (a) Explain the difference between coenzyme and activators
- (b) Giving example explain what is meant by enzyme inhibitors
 - (c) How do enzyme bring about their effects.

30. (a) Describe the working of an enzyme using the Lock and Key hypothesis (11mark)
- (b) Explain the
 - (i) Effect of excessive heat on the enzyme action (3marks)
 - (ii) Non-competitive inhibition in an enzyme controlled reaction (6mark)

31. (a) Classifies enzymes basing on the type of reaction they catalyze. (06marks)
- (b) Explain how competitive and noncompetitive inhibitions of enzymes occur. (10marks)
 - (c) What is the importance of enzyme in enzyme catalyzed reaction?

Suggested answers

1	A	6	C	11	D	16	D	21	D
2	C	7	A	12	A	17	B	22	B
3	A	8	C	13	D	18	C	23	D
4	D	9	D	14	B	19	A	24	C
5	D	10	B	15	D	20	A	25	C

26. Solution:

(a) Inhibitors A reduced of reaction initially but as substrate concentration increase the rate of reaction also increase at a lower rate than the control. It attains a lower maximum but at a higher substrate concentration than the control.

Inhibitors B greatly reduces rate of reaction, however, rate increase gradually with substrate concentration to a very low maximum at a lowest substrate concentration than all.

(b) Inhibitor A is reversible competitive inhibitors of the enzyme. It competes with the substrate to bind to the active site of the enzyme, being structurally similar to the substrate.

At low substrate concentration, the inhibitors out compete the substrate for the active site. Fewer than normal substrate molecules combine with the enzyme and the reaction is slower than the control.

As substrate concentration increase, the substrate competes more favorable for the active site and the reaction increase with the substrate concentration up to saturation.

Saturation is slightly lower than control since some enzyme active sites still occupied by inhibitors A molecules.

Inhibitor B is a non-competitive inhibitor. It binds with the enzyme permanently and prevents the substrate from binding to the enzyme. This reduces effective enzyme molecules and the reaction proceeds as it would occur if the enzyme concentration was lower than the control.

27. (a)(i) competitive inhibition

- Substances structurally similar to enzyme substrate compete with enzyme substrate for active site on the enzyme molecule where both reversibly bind.

- fewer true substrate bind to the enzyme and so the rate is reduced or inhibited.

- this is a competitive inhibition whose degree depends on the relative amount of the substrate and inhibitor.

(ii) Noncompetitive inhibitor

- A substance with the structure different from that of the substrate irreversibly binds on the enzyme at a point different from the active site stops or reduces the action of the enzyme. The degree of inhibition depends on the concentration of inhibitor and not the substrate.

(b) Differences between enzyme and catalysts

	Enzymes	Catalysts
1.	proteins in nature	inorganic chemicals e.g. Pt
2.	catalyze specific reactions Such as hydrolysis of starch	may catalyze more than one reaction e.g. platinum catalyze decomposition of H ₂ O ₂ and reduction of benzene
3.	Denatured by heat above 45 ⁰ C	Usually are not affected by heat
4.	Very sensitive to pH	Not sensitive to pH
5.	Initiate reaction	Do not initiate reaction

(c) Lock and key hypothesis

- The enzyme's active site and its substrate have complementary structure.
- The enzyme randomly binds with the substrate to form enzyme-substrate complex like a key fits in the lock.
- The enzyme acts on the substrate to form products.
- The products do not fit in the active site, leave the active site for another substrate.

28. Solution

(a) (i) Reaction rate increase rapidly with substance concentration. Rate then increase gradually before it becomes constant at higher substrate concentration

Explanation

Initially, the number of enzymes molecules available for reaction is higher than the number of substrate molecules; any available substance will react to form a product.

As the substrate concentration increase, the number of enzymes molecules occupied by substrate at a given time increase, hence more amount of product is formed per unit time.

This continues until all the enzymes molecules are occupied so that a substrate has to wait for the active site of the enzymes to be freed before it can bind the enzymes, in order to react. the rate of formation of products therefore becomes constant.

(ii). Relationship

Rate of reaction increase slowly with substrate concentration and reaches a lower maximum at a higher substrate concentration.

Explanation:

Compound A competes with the substrate for the active sites of the enzyme molecules.

As a result, the number of enzymes molecules available for binding the substrate at a given time is lower.

As the substrate concentration increase, the substrate (out-competes compound A and the reaction rate increase, through at a lower rate. He enzymes molecules become saturated at a higher substrate concentration. However, at this point, the output is lower because some enzyme molecules are occupied by compound A and cannot react with substrate.

(b)

Temperature of the reaction medium

PH of the reaction medium

Others:

The amount of the enzyme.

(c) The rate of reaction would decrease with increase in amount of compound A added until the reaction stops altogether at higher concentrations of compound A.

29. (a) **Cofactors/activators** serve the same purpose as **coenzymes**, as they regulate, control, and adjust how fast these chemical reactions would respond and take

effect **in** our body. The big **difference** is that **coenzymes** are organic substances, while **cofactors/activators** are inorganic.

(b) **Enzyme inhibitors** are molecules that interact in **some** way with the **enzyme** to prevent it from working in the normal manner. There are a variety of types of **inhibitors** including: nonspecific, irreversible, reversible - competitive and noncompetitive. Poisons and drugs are **examples of enzyme inhibitors**.

(i) Competitive reversible inhibitor;

Here a compound is structurally similar to that of the usual substrate associates with the enzymes active site but it's unable to react with it. While it remains there, it prevents access of any molecules of the substrate. This type of inhibition depends on the concentration of the substrate and that of an inhibitor. At high concentration of the substrate, inhibition is overcome.

This knowledge of competitive inhibitors has been utilized in chemotherapy. Sulphonamides drugs and antibiotic such as penicillin are competitive inhibitors of essential metabolites for enzymes in bacteria.

(ii) Noncompetitive reversible inhibitors.

This is a type of inhibition in which the inhibitors attach themselves outside the active site thereby preventing the enzyme normal catalytic reaction by changing the shape of the enzyme or allosteric effect. It may be reversible when the inhibitor forms loose attachment to the enzyme that may be detached when circumstances permit e.g. cyanide or irreversible noncompetitive inhibition when the inhibitor permanently disorganizes the structure of the enzyme that it may no longer react with the substrate, e.g., mercury.

Chemical or substance added or applied to another substance, to slowdown a reaction or to prevent an unwanted chemical change. For example, anti-oxidants are added as **inhibitors** to food to retard its spoilage from exposure to air (oxygen