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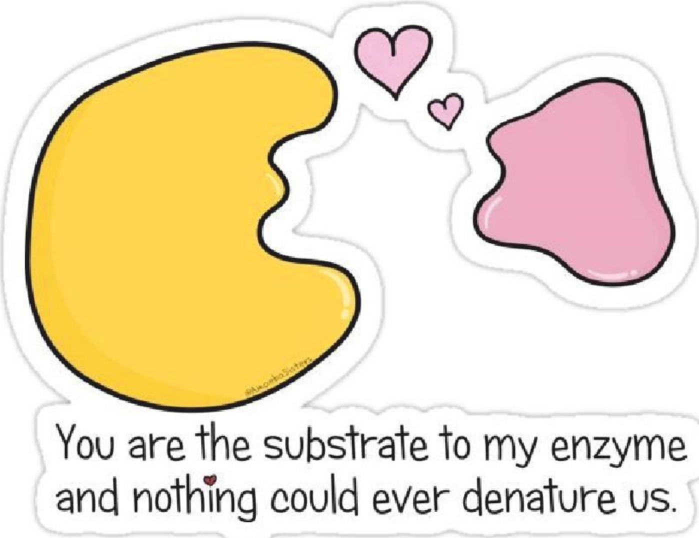

UNIT 02

Enzymes

2.4.0 : Energy Relationships

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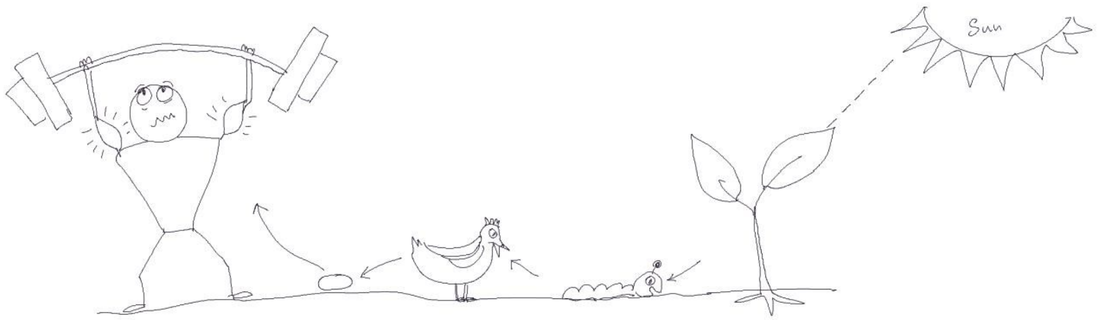


1. (Most) enzymes are globular proteins.
2. They are biological catalysts.
3. They increase the rate of reactions by
4. lowering the activation energy of the reactions.
5. (Most) enzymes are heat labile/sensitive.
6. They do not alter the nature/properties of the end products.
7. They are (highly) specific to the substrate/substrate specific.
8. Most/Some catalyzed reactions are reversible.
9. The rate of enzymatic reaction is affected by (pH, temperature, Inhibitors) and substrate/enzyme
10. concentrations.
11. They are not used up during the reaction/ They can be reused/ remain unchanged.
12. They possess (specific) active sites where the reactions take place/ enzyme binds with substrate.
13. Some enzymes need non-proteinous components/ cofactors (to catalyze the reactions/ for their activity).

(b) (i) Explain how pH and temperature affect the rate of enzyme activity.

1. pH
2. Enzymes function most efficiently within a certain pH range.
3. The pH at which the highest rate of reaction occurs is the optimum pH of the enzyme.
4. The alteration in pH above
5. or below the optimum pH
6. may lead to decline in enzyme activity.
7. This is due to the alteration of chemical bonds
8. involving in formation of enzyme substrate complex.
9. In most enzymes optimum pH range is 6—8
10. Pepsin works best at pH 2 and optimum pH for Trypsin is 8.
11. Temperature
12. Increase in temperature increases molecular motion.
13. Therefore the speed of the moving molecules of both enzymes as well as the substrate will be accelerated.
14. This will enhance the colliding probability
15. for both enzyme active sites and substrate molecules.
16. More collision between the enzyme active sites and substrate molecules generate greater chances for the reaction to occur.
17. This can continue up to a certain point, after which there is a rapid decline in enzyme activity.
18. This point is referred to as optimum temperature.
19. This may vary from organism to organism.
20. Optimum temperature of bacteria in hot springs is about 70˚C.
21. When the temperature increases beyond the optimum temperature, the hydrogen bonds,
22. ionic and
23. other weak chemical bonds
24. of enzyme active sites may be disrupted.
25. This will result a change in the shape of the active site of enzyme
26. which will alter the complementary nature of the active site of enzyme molecules.
27. Therefore, the complementary binding of enzyme active sites and substrate molecules will be prevented.
28. The above event is called as denaturation of enzyme molecules.
29. Therefore the rate of enzyme catalyzed reaction will start to decline when the temperature increases beyond the optimum temperature
30. and stops completely at certain temperature, although rate of collision will keep on increasing.

(ii) Explain the action of competitive and non competitive inhibitors in enzyme reaction.

1. Competitive inhibitors are reversible inhibitors.
2. These chemicals resemble the shape and nature of the substrate.
3. Therefore they compete with the substrate selectively for the active site of certain enzymes.
4. As a result of the above, the number of active sites available for the enzymes may decline and therefore reduces the rate of enzyme catalyzed reactions.
5. The above situation may be reversed by increasing the substrate concentration.
6. Eg. Protease inhibitor of drugs against HIV.
7. Non-competitive inhibitors do not compete with substrate molecules.
8. They interrupt enzymatic reaction by binding to a part of the enzyme other than the active site.
9. This causes the enzyme molecule to change its shape
10. in such a way that the active site becomes less effective for the formation of enzyme substrate complex.

Model

1. **((a) Describe the general characters of enzymes and mechanism of enzyme action.**

Answer

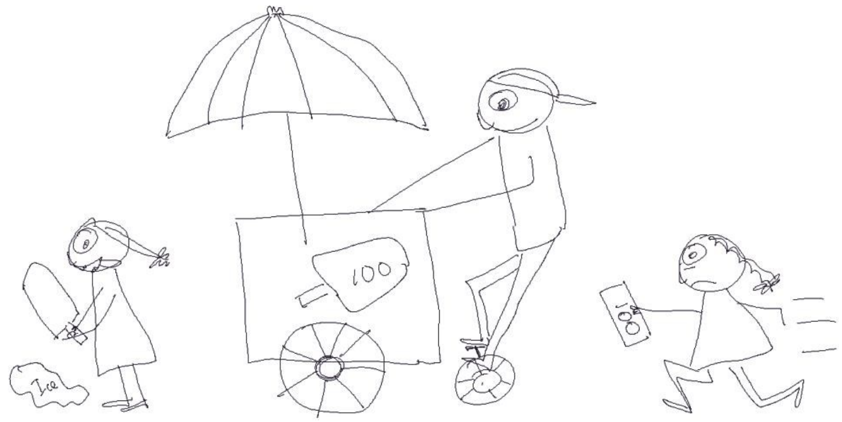
1. An enzyme is a macromolecule, which acts as a biological catalyst
2. Enzymes are produced in living cells.
3. Most of the enzymes are globular proteins.
4. Enzymes are biological catalysts.
5. They lower the activation energy of the reaction they catalyze (increases the rate of reaction).
6. Most enzymes are heat labile/sensitive
7. Their presence does not alter the nature or properties of the end products of any reaction.
8. Enzymes are highly specific to the substrate (substrate specific)
9. Most enzyme catalyzed reactions are reversible.
10. The rate of enzyme activity is affected by pH, temperature, substrate concentrations and inhibitors.
11. They are not being used up during the reaction.
12. Enzymes possess active sites where the reaction takes place.
13. The reactant on which the enzyme acts on is referred to as the substrate.
14. The enzyme binds to its substrate forming enzyme-substrate complex.
15. While enzyme and substrate form their complex, catalytic action of the enzyme converts the substrate to the product.
17. The reaction catalyzed by each enzyme is very specific.
18. The specificity of an enzyme results from its shape.
19. The substrate binds to a specific region of the enzyme.
20. This region is called the active site.
21. The active site is formed by only a few amino acids.
22. Other amino acids are needed to maintain the shape of the enzyme molecule.
23. The shape of the active site of the enzyme is complementary to the shape of its specific substrate.
24. The shape of the active site of an enzyme is not always fully complementary to its substrate.
25. As enzymes are not rigid structures,
26. the interactions between substrate and active site may slightly change the shape of the active site,
27. so that the substrate and the active site become complementary to each other.
28. This is called induced fit mechanism.
29. Thereafter. the product departs from the active site of the enzyme.
30. The enzyme is then free to take another substrate molecule into its active site.
31. Some enzymes need non-proteinous components to catalyse the reaction which are known as cofactors.
32. Non-proteinuos components which are essential for the catalytic activities of certain
33. enzymes are called cofactors.
34. These cofactors bind to the enzymes in two ways.
35. Some tightly bind and remain
36. permanently and others loosely bind temporarily. Loosely bound cofactors are
37. reversible under certain circumstances.
38. Organic cofactors are called co-enzymes. eg. derivatives of vitamins e.g. NAD‘, FAD‘
39. and biotin
40. Inorganic co-factors — e.g. Zn^{+2}, Fe^{+2}, Cu^{2+}
41. Factors affecting the rate of enzymatic reactions
42. Temperature
43. pH
44. Substrate concentration
45. Inhibitors

2019 AL

(a) Briefly describe the general characteristic s of enzyme

(b) (i) Explain how pH and temperature affect the rate of enzyme activity.

(ii) Explain the action of competitive and non competitive inhibitors in enzyme reaction.



2.4.1 : Analyses the energy relationship in metabolic processes

The energy relationships in metabolic processes

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Catabolism is breaking down of complex molecules into simple molecules by releasing free energy. Therefore it is an ………………….. reaction. Anabolism is making complex molecules from the simple molecules by absorbing free energy. Hence it is an ……………….. reaction. ATP acts as the energy carrier in all living organism including the simplest bacteria. Therefore the ATP is known as the universal currency of energy transactions. Energy can be defined as the capacity to do work. All living organisms require energy for their living process in many ways. Such processes are;

- …………………………………………………………………………………………
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Overall idea of the energy relations of living system on biosphere is composed of following steps.

- Energy flows into biological systems from the environment through solar radiation. (Primary energy source is the Sun)
- Light energy is captured in the cells having photosynthetic pigments (chlorophyll) by the process of photosynthesis and stored as chemical energy in the organic compounds such as carbohydrates
- Captured energy in organic food is transformed into chemical energy in ATP by a process called cellular respiration.
- The energy stored in ATP is utilized in various energy requiring processes.

ATP (Adenosine Tri Phosphate) : ATP is a nucleotide, consisting of,

- Ribose- sugar
- Adenine - nitrogenous base
- A chain of three phosphate groups.

(iii) State five factors affecting rate of enzyme reactions.

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(iv) Explain the term “specificity” in relation to enzymes.

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(v) Name substrate and products of the reactions catalyzed by the following enzymes.

Enzyme Substrate Product

Catalase

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Lipase

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Invertase

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AL/2009

1. (i) What is the active site of an enzyme?

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(ii) What is co- enzyme

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Essay AID

Model

1. **(a) Describe the general characters of enzymes and mechanism of enzyme action.**

2019 AL

2. (a) Briefly describe the general characteristic s of enzyme
   (b) (i) Explain how pH and temperature affect the rate of enzyme activity.
   (ii) Explain the action of competitive and non competitive inhibitors in enzyme reaction.

2. You are provided with 3 solution labeled as A, B, and C. One of the solutions contains amylase and the other 2 contain 0.1% w/v and 0.5% w/v starch solutions. If you are provided with following items, then explain how you identify.

(i) Amylase solution (ii) 0.5% w/v starch solution

Items: White porcelain tile, Measuring cylinder, stop watch, water, Iodine solution, test tubes, test tube rack, glass tubes and glass rods.

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AL/2007

1. (i) What is the basic function of an enzyme in the catalysis of biological reactions?

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(ii) Some enzymes require co-factors for their efficient activity. Name three such co -factors and indicate one function of each of them.

Co- factor Function

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During the hydrolysis of ATP, ADP and Pi are produced due to the ………………….. of ……………. phosphate. As a result, a very ……………………….. energy is released. This is because the reactants (ATP and water) contain more energy in comparison to products (ADP and Pi). Therefore it yields energy and is an exergonic reaction.

When ATP is hydrolyzed, the free energy yield is -30.5kJ/mo1.

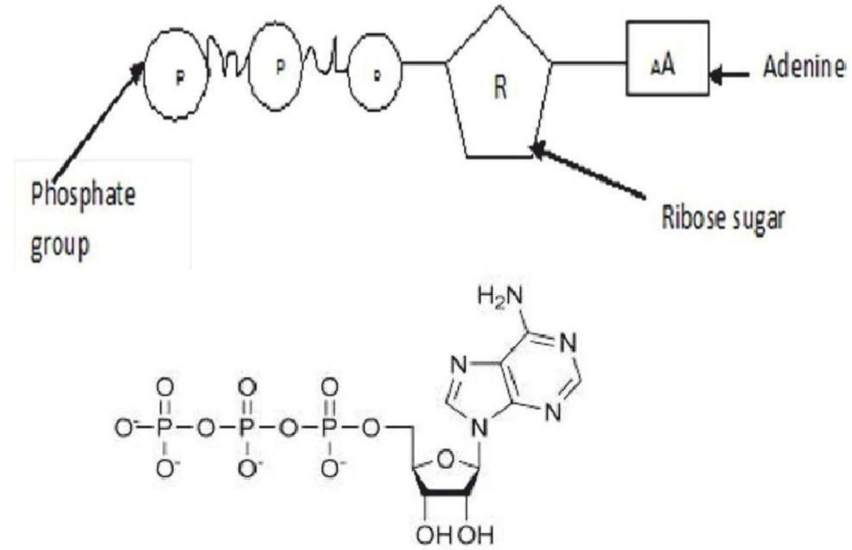


Fig 2.29: Chemical structure of ATP molecule (need not be memorized)

Most biological reactions use the energy released during breaking of the terminal phosphate bond. ATP is mobile. Therefore it can carry energy to anywhere in the cell, for any energy consuming reaction.

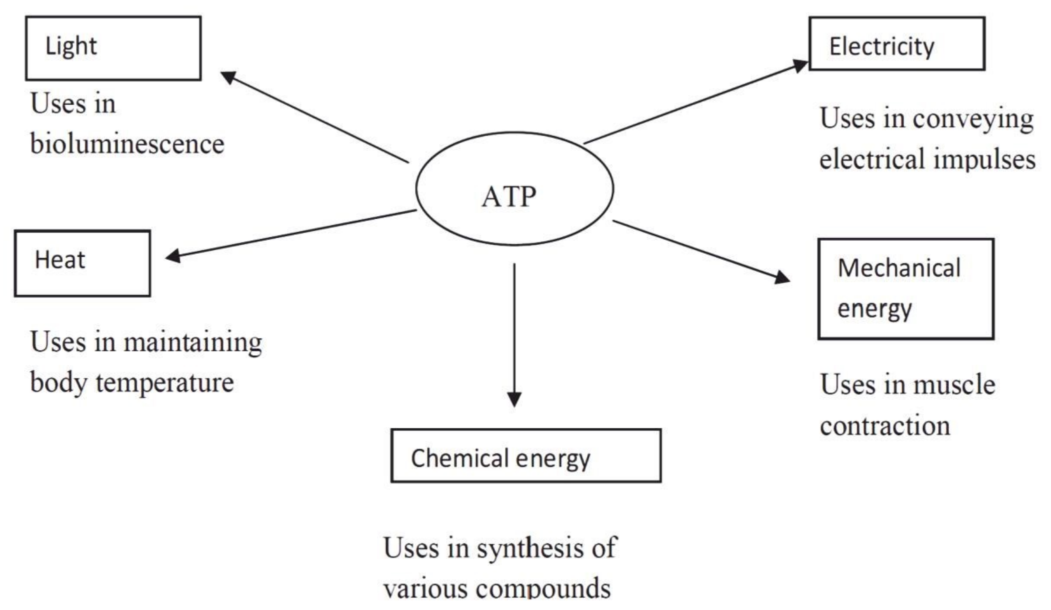
ATP can be produced within living cells within a short period of time, using ADP, inorganic phosphate (Pi) and energy. Production of ATP within cells is called phosphorylation. According to the energy source phosphorylation is divided as;

i. ………………………………… synthesis of ATP using solar energy in photosynthesis

ii. ………………………………… synthesis of ATP using energy released by the breaking down of complex molecules into simple ones.

iii. ………………………………… synthesis of ATP using energy released as a result of respiration.

In living cells energy in ATP is transformed in to various energy forms which are used for different functions.



The role of enzymes in regulating metabolic reactions

An enzyme is a macromolecule, which acts as a biological catalyst Enzymes are produced in living cells/

General characteristics of an enzyme:

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9. Which of the following statements regarding enzymes is correct?
(1) Activators affect the function of enzymes by binding to active sites through covalent bonds.
(2) Shape of the active sites of enzymes change due to temperatures higher than the optimum level.
(3) Many competitive inhibitors hind to active sites of enzymes irreversibly and change their shape.
(4) Toxins bind to enzymes reversibly through covalent bonds.
(5) Co-enzymes arc proteinous components which are permanently or temporarily bound to enzymes. 2022/5

10. In allosteric regulation of enzymes
(1) regulatory molecules bind reversibly to the active site of enzyme.
(2) regulatory molecules bind to the enzyme via non-covalent interactions.
(3) an activator molecule that binds to a particular sub unit will affect the active site of that sub unit only.
(4) inhibitory molecules affect the function of the enzyme but not the shape.
(5) ATP functions as an allosteric activator. 2023 AL/5

Structured Essay

G.C.E. A/L 2002

1. What are enzymes?

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2. Give the names of the substrate and products of following enzymes.

Enzyme	Substrate	Product
Amylase complex		
Catalase		
Trypsin		

GCE A/L 2005

1. Explain why enzymes lose their activity at high temperatures.

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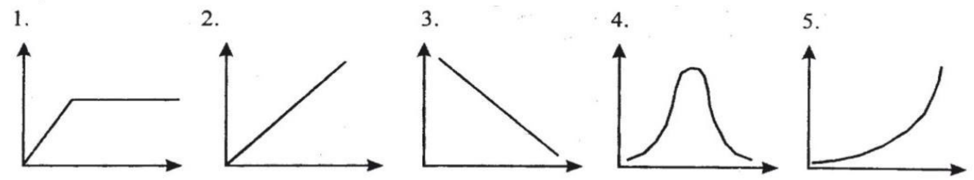
MCQ

1. Which of the following statement is incorrect regarding enzymes?
(1) One enzyme can act in presence of another. (2) Enzymes get damaged at high temperatures. (3) Enzymes are specific in function. (4) Enzyme concentration effect function. (5) Enzymes are not active outside cells.

2. Which of the following sentence is incorrect regarding enzymes
(1) All enzymes are proteins (2) enzymatic reactions are reversible (3) Enzymes interact with the substrate through active site (4) Enzyme molecule react only once with the substrate (5) Enzyme and the substrate form an unstable complex

3. Which of the following statements are incorrect of regarding enzymes
(1) They are polymer of amino acids (2) Heat stable (3) Heavy metals affect the reactivity
(4) pH affects the reactivity (5) They are required in small quantities

4. Which of the following graph show the relation between substrate concentration and rate of enzyme reaction.



X – Substrate concentration Y – Rate of enzyme reaction

5. Which of the following statement regarding enzymes is incorrect?
(1) Enzymes are made of amino acids (2) Enzymatic reactions are reversible.
(3) Enzymes do not change the nature of end products.
(4) Some of the enzyme structure consists of non protein part. (5) Enzymes molecules are smaller than substrate molecules.

6. Which of the statement regarding cofactors is/are correct?
(A) They are non– protein components.
(B) They are always needed for enzyme activity.
(C) They could be permanently bound to enzyme molecule.
(D) They could be temporarily bound to enzyme molecule.
(E) They are always organic compounds. 2019 AL/Old 41

7. ATP
(1) is a nucleoside containing pentose sugar, adenine and phosphate groups.
(2) can be produced by oxidative phosphorylation using solar energy.
(3) hydrolyses to ADP releasing 3()5 kJ/mol of energy.
(4) is formed in pyruvate oxidation through substrate level phosphorylation.
(5) contains deoxyribose. 2020 AL/6

8. Which of the following is a characteristic of enzymes?
(1) They do not alter the nature of end products.
(2) They increase the activation energy of a reaction.
(3) They are not substrate specific.
(4) A small amount of enzyme is used up during the reaction.
(5) Any part of the enzyme molecule can catalyse a reaction. 2020 AL/7

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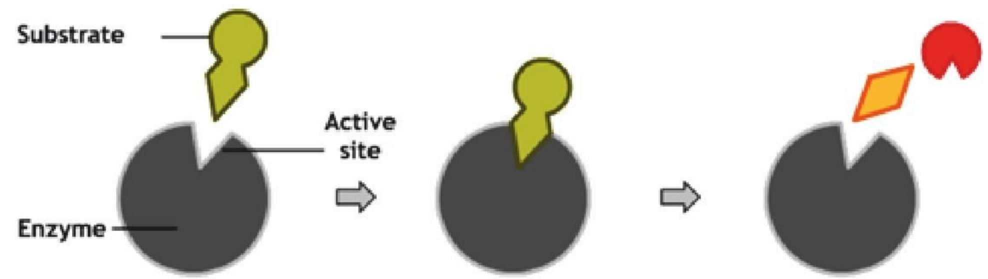
The mechanisms of enzyme action

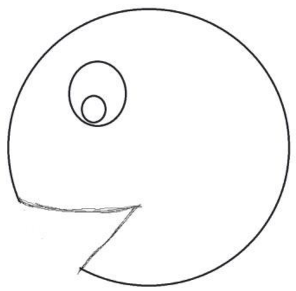
The reactant on which the enzyme acts on is referred to as the substrate. The enzyme binds to its substrate forming …………...………………….. …………………….. While enzyme and substrate form their complex, catalytic action of the enzyme converts the substrate to the product.

Enzyme + substrate ↔ Enzyme-substrate complex ↔ Enzyme + Product

The reaction catalyzed by each enzyme is very specific. The specificity of an enzyme results from its ……………... The substrate binds to a specific region of the enzyme. This region is called the …………….. site. The active site is formed by only a few ……….. ………………..

Other amino acids are needed to maintain the shape of the enzyme molecule. The shape of the active site of the enzyme is ………………………………….. to the shape of its specific substrate. The shape of the active site of an enzyme is not always fully complementary to its substrate. As enzymes are not ………………….. structures, the interactions between substrate and active site may slightly change the shape of the active site, so that the substrate and the active site become complementary to each other. This is called ……….…… …………... mechanism. The tight fit not only brings the substrate molecules and the active site close to each other, but also ensures the correct ………………..…… of the molecules to help the reaction to proceed and catalyzes the conversion of substrate to product. Thereafter, the product departs from the active site of the enzyme. The enzyme is then ………… to take ……………... ……………………….. molecule into its active site.





11. Describes the importance of co-factors for enzymatic activities

12. Explains how pH, temperature, substrate concentration, and inhibitors (competitive and non competitive) affect the rate of enzyme activity. **Essay**

13. State regulation mechanisms found in cells

14. Briefly describe a suitable experiment to demonstrate enzyme activity and how temperature affects the rate of enzyme activity using starch amylase reaction

7. Discusses the structure and the importance of ATP as a universal energy currency unit

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8. Definition 'enzymes'

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9. Explains the general characteristics of enzymes and states their role

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10. Describes a mechanism for enzyme activity using suitable diagrams

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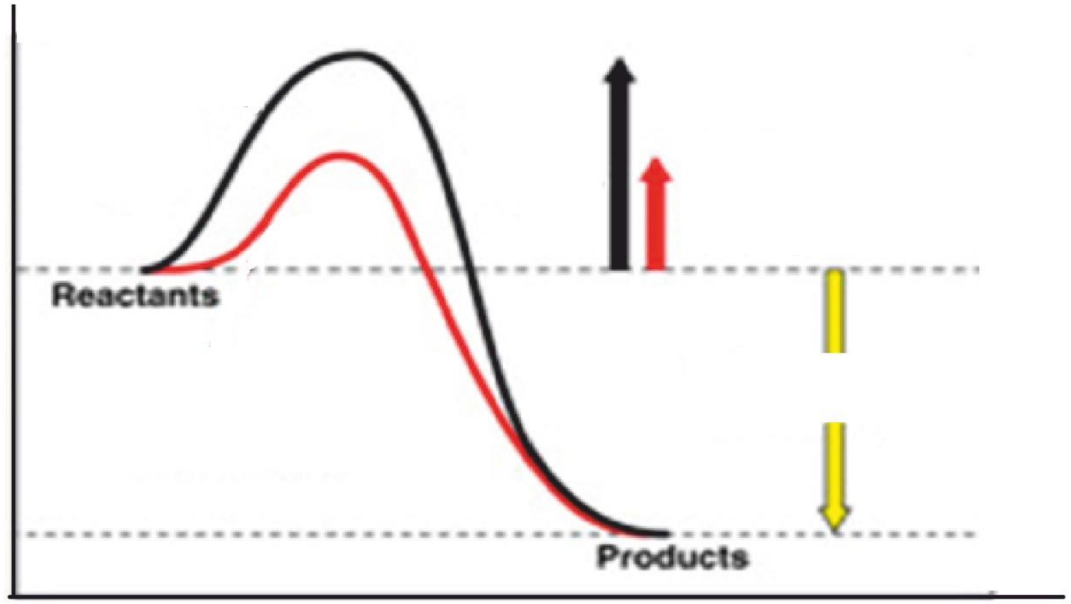

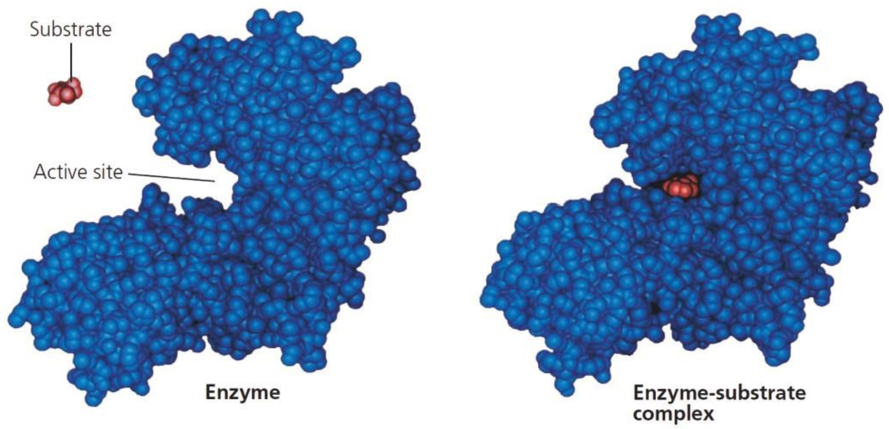

Cofactors

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These cofactors bind to the enzymes in two ways. Some ………………. bind and remain …………………….. and others ……………………... bind …………………………...

Loosely bound cofactors are ………………………... under certain circumstances. Organic cofactors are called ………………………... eg. derivatives of vitamins e.g. NAD+, FAD and biotin.

Inorganic co-factors — e.g. ………..…., ……………….., …………….

Factors affecting the rate of enzymatic reactions

1. …………………………………..
2. …………………………………..
3. …………………………………..
4. …………………………………..

Temperature

Increase in temperature increases …………………. ……………………….. Therefore the speed of the moving molecules of both …………………... as well as the ………………... will be …………………………... This will enhance the ……………………… probability for both enzyme …………………….. …………………... and substrate molecules. More collision between the enzyme active sites and substrate molecules generate ……………….. chances for the reaction to occur. This can continue up to a certain temperature, after which there is a rapid …………………….. in enzyme activity.

Learning outcome:

1. Explains what is metabolism.

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2. Explain the role of ATP in universal energy transaction.

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3. Lists the cellular processes involving energy

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4. Explain how energy requirements of organisms vary in relation to body size, activity and environment

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5. Explains what is catabolic and anabolic reactions with examples

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6. State the role of electron carriers (NAD +, NADP+ and FAD)

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- Test a drop of reaction mixture with a drop of Iodine solution on the white porcelain tile at 2 minute intervals.
- Continue the test until the dark blue colour will not appear.
- Observe the time taken.
- Tabulate the results indicating time elapsed and change of the colour.
- Repeat the above procedure for different temperatures (5^0C, room temperature, 40^0C, 60^0C- Temperature can be maintained by adding cold or hot water to the water bath).
- Assist students to plot a graph using the results obtained (1/t vs temperature).
- Guide them to interpret their findings analytically.

This temperature is referred to as ………………….. ………………………….. This may vary from organism to organism. e.g. most of the human enzymes have …………………… temperature around the body temperature (35°C- 40°C). Optimum temperature of bacteria in ……………. …………………………... is above 70°C. When the temperature increases beyond the optimum temperature, the hydrogen bonds, ionic and other weak chemical bonds of enzyme active sites may be disrupted.

This will result a change in the …………………... of the ………………… ……………. of enzyme which will alter the complementary nature of the active site of enzyme molecules. Therefore, the complementary binding of enzyme active sites and substrate molecules will be prevented. The above event is called as ………………………... of enzyme molecules.

Therefore the rate of enzyme catalyzed reaction will start to decline when the temperature increases ………………..the ………………….. …………………. and stops completely at certain temperature, although rate of collision will keep on increasing

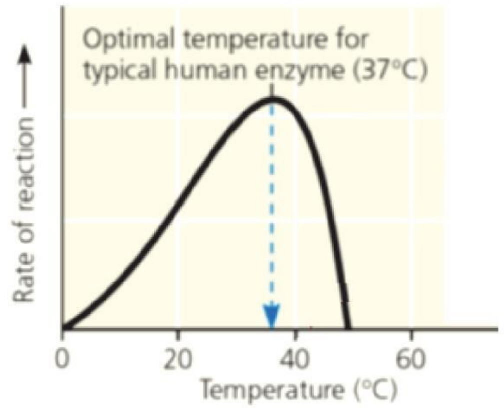
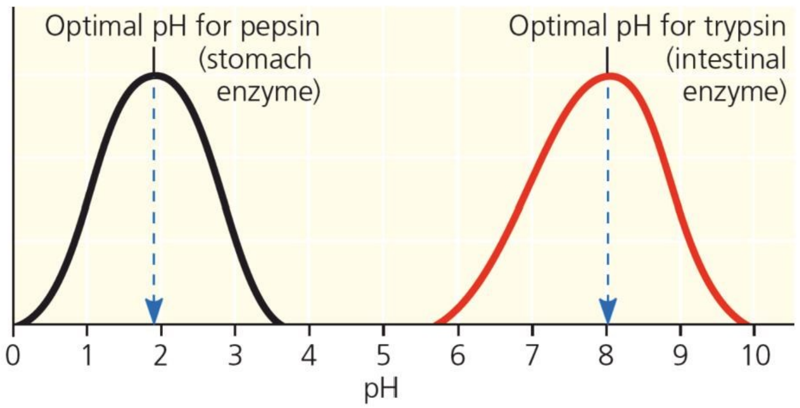


Fig -2.32 The graph of Rate of reaction (V) vs (Temperature)

pH

Enzymes function most efficiently within a certain pH range despite maintaining temperature of the environment constant.

The narrow range of pH in which a particular enzyme catalyzed reaction takes place is named as the ……………… ……... The pH at which the highest rate of reaction occurs is the …………………… ……... of the enzyme. The alteration in pH above or below the optimum pH may lead to decline in enzyme activity. This is due to the ………………… of ………………… ………. involving in formation of enzyme substrate complex. In most enzymes Optimum pH range is ………………., but there are exceptions. Pepsin works best at ……………. and optimum pH for Trypsin is ………...



Substrate concentration

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…………………………………………………………. However the enzyme molecules will be saturated after a particular concentration and therefore there will not be any further increase in the rate of reaction.

Enzyme inhibitors

Certain molecules or ions selectively bind ………………….. or ………………………. to the enzyme molecules and ………………………... them from forming enzyme-substrate complex. These substances are called inhibitors.

They are either binding …………………...with …………………… interactions or binding ……………………..…….. through ………………….. bonds.

eg. Irreversible inhibitors: ………………., …………………..

Reversible inhibitors - ……………………………………………………...

c.) Feedback inhibition

In feedback inhibition, a metabolic pathway is ……………………….. by the inhibitory binding of its …………….. product to the enzyme involved, thereby, limit the production of ……………….. end products than required. Thus, prevents the wastage of chemical resources. Feedback inhibition is an essential process regulates the end products produced in metabolism. In case ATP supply exceeds demand, catabolism slows down as ATP molecules function as allosteric inhibitor.

PRACTICAL NO.5

Laboratory experiment to demonstrate enzyme activity and to determine the effect of temperature on the rate of enzymatic reaction (starch - amylase)

Objectives

Students should be able to

- set up the starch-amylase reaction,
- record the time taken for the reaction,
- tabulate the results and observations,
- conduct the experiment set different temperatures,
- interpret the observation analytically.

Materials and equipment

- 1% (w/v) amylase solution
- 1% (w/v) starch solution
- Iodine solution (I_2 / KI)
- Stop watch
- White porcelain tile
- Thermometer
- Pipettes
- Water bath
- Boiling tubes and test tubes

Instructions

- Instruct students to set up the experiments as given below.
- Measure definite volumes (5 ml) of amylase solution and (10 ml) of starch solution into separate test tubes.
- Allow the solutions to attain the same temperature.
- Mix up the two solutions and start the stop watch (starch to amylase).

Regulation mechanism of enzymatic activity in cells

Allosteric regulation of enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave like …………………………. non-competitive …………………….. Regulatory molecules (either activators or inhibitors) bind to specific regulatory sites elsewhere (other than the active site) of the enzyme molecule via non-covalent interactions and affect the shape and function of the enzyme. It may result in either inhibition or stimulation of an enzyme activity.

a.) Allosteric activation and inhibition

Most enzymes regulated by allosteric regulation are made from ………….. or ………….. subunits. Each sub unit composed of a …………………………. chain with its own active site. The entire complex ………………………………..between two different shapes one catalytically ……………………. and other …………………………... In this two forms regulatory molecules bind to a ………………... site called ……………………. site, often located where …………………. …………….. When an ……………... ………….. with this regulatory site, ………………………... the shape with …………………. active sites. Whereas the inhibitor binds with the regulatory site, it ……………………. the inactive form of enzyme. Subunits of allosteric enzyme arranged in such a way that a shape alteration in one unit is transmitted to all other subunits. Through the interaction of subunits even a ………………..activator or inhibitor molecule that bind to ……………. …………….. site will affect the active site of all sub units. eg. ……………. function as allosteric ……………… bind to the enzyme and stimulates the production of ATP by catabolism. If the …………… of ATP ………….. demand catabolism slows down as ATP bind to the ………………... enzyme as ……………………...

b.) Cooperativity

This is another type of …………………... activation. Binding of ………. ………………... molecule can ………………………... binding or ………………….. at other active site. Thereby increase the catalytic activity.

eg, Hemoglobin (not an enzyme) is made up of four subunits each with an O_2 binding site. The binding of a molecule of O_2 to the first binding site increases the affinity in the remaining binding sites. Cooperativity work similarly in multi subunit enzymes too.

Competitive inhibitors

Most of these are ……………………….. inhibitors. These chemicals …………………. the shape and nature of the substrate. Therefore they ………………………. with the substrate ………………………... for the active site of certain enzymes. As a result of the above, the number of active sites available for the substrate may …………….………. and therefore reduces the ………………………. of enzyme catalyzed reactions. The above situation may be reversed by ………………………... the ……………………………... concentration.

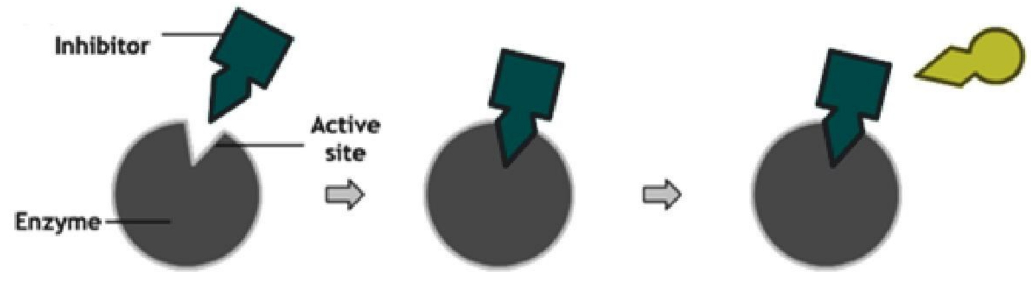

Fig 2.34: Competitive inhibitors

Non-competitive inhibitors

These chemicals do not ……………………... with ……………………. molecules. They ………………………….. enzymatic reaction by binding to a part of the enzyme other than the active site. This causes the enzyme molecule to change its shape in such a way that the active site becomes less effective for the formation of enzyme substrate complex.

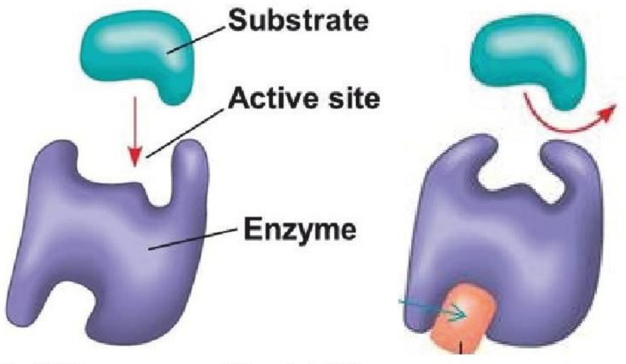

Fig 2.35: noncompetitive inhibitors