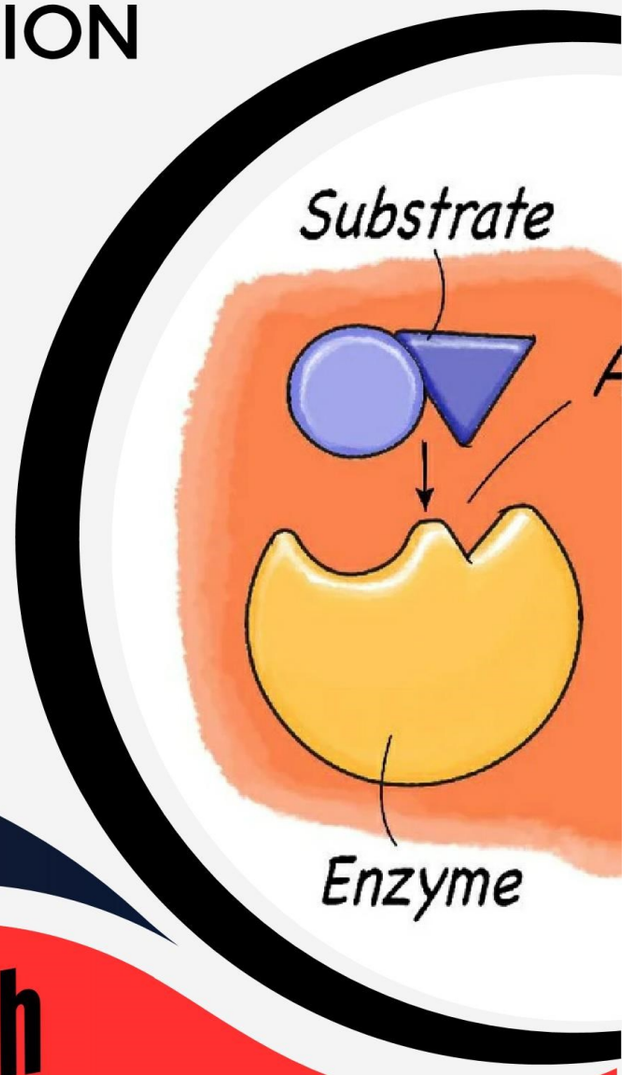
Biology

2025 REVISION

UNIT 02

ENZYMES



Sampath Lankadheera

The energy relationships in metabolic processes

Sum of all biochemical reactions of living being is known as the metabolism and it consists of all catabolic and anabolic reactions.

Catabolism is breaking down of complex molecules into simple molecules by releasing free energy. Therefore it is an exergonic reaction. Anabolism is making complex molecules from the simple molecules by absorbing free energy. Hence it is an endergonic 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;

- Synthesis of substances
- Active transport across plasma membrane
- Transmission of nerve impulses
- Muscle contraction
- Beating of cilia and flagella
- Bioluminescence
- Electrical discharges.

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.

10. Give different examples for allosteric regulations

Allosteric Activation	
Allosteric Inhibition	
Cooperativity	
Feedback Inhibition	

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1. (a) Briefly describe the general characteristics 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.

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Temperature (°C)	Rate of Reaction (arbitrary units)
0	5
10	15
20	30
30	65
40	90
50	50
60	10
70	0

7. State regulation mechanisms found in cells

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

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9. Two types of inhibition shown in the diagram. Identify them

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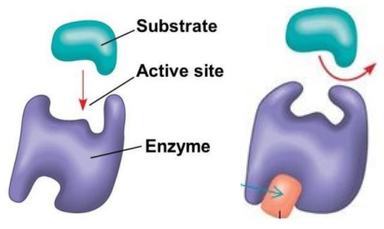
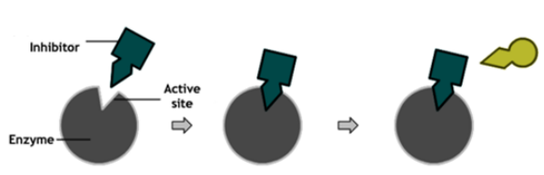
	

ATP (Adenosine Tri Phosphate)

ATP is a nucleotide, consisting of,

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

During the hydrolysis of ATP, ADP and Pi are produced due to the removal of terminal phosphate. As a result, a very high 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/mol.

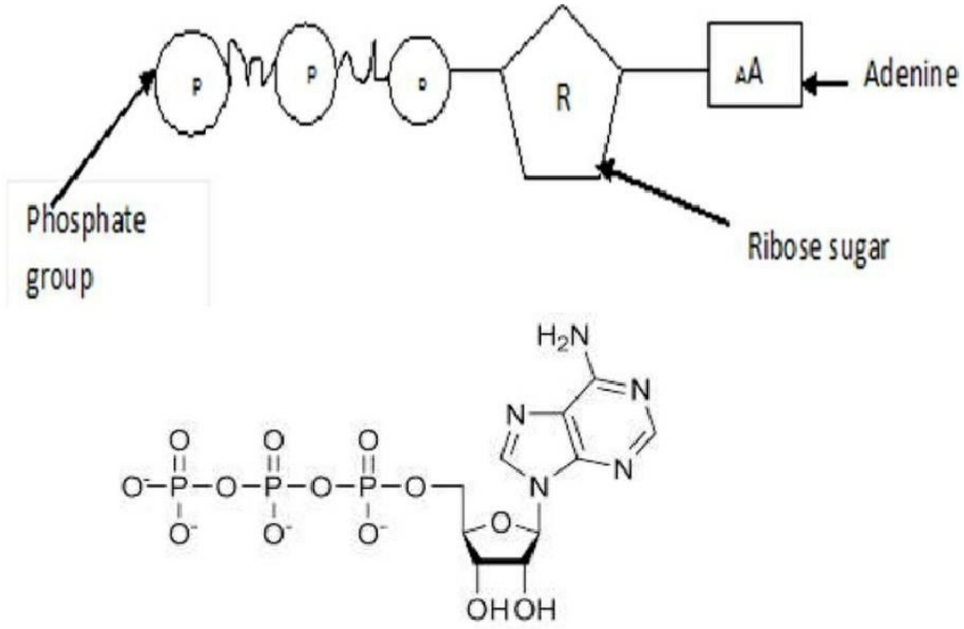


Fig 2.29: Chemical structure of ATP molecule (need not be memorized)

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;

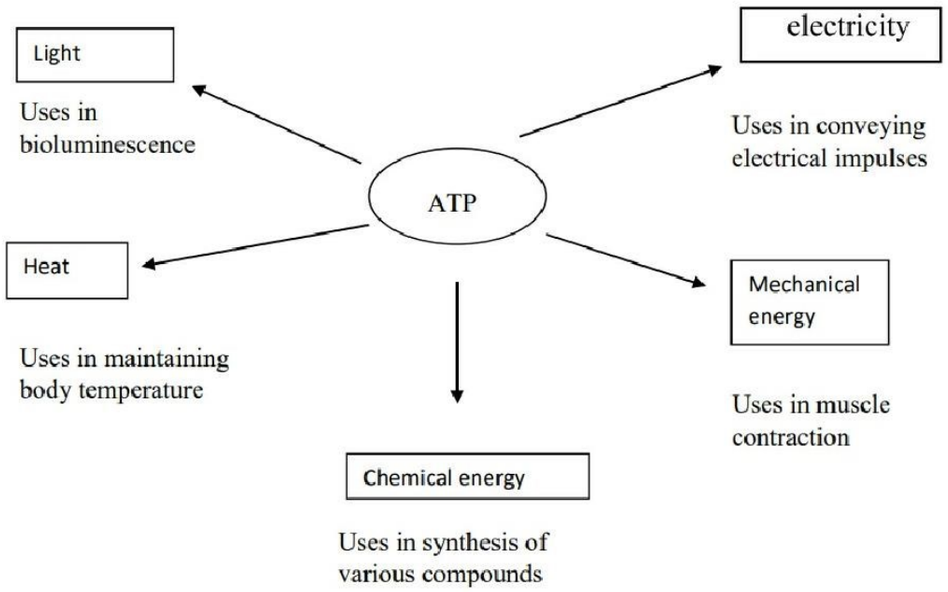
i. Photophosphorylation – synthesis of ATP using solar energy in photosynthesis

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

iii. Oxidative phosphorylation – synthesis of ATP using energy released as a result of oxidation of molecules.

} In cellular respiration

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



4. Fill in the blanks with appropriate terms to explain the mechanism of enzyme action:
The reactant on which the enzyme acts on is referred to as the _____. The enzyme binds to its substrate forming _____ complex. While enzyme and substrate form their complex, _____ action of the enzyme converts the substrate to the _____.
Enzyme + substrate ↔ _____ ↔ Enzyme + Product
The reaction catalyzed by each enzyme is very _____. The specificity of an enzyme results from its _____. The substrate binds to a specific region of the enzyme. This region is called the _____. The active site is formed by only a few _____. Other amino acids are needed to maintain the _____ 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 _____ 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 _____ from the active site of the enzyme. The enzyme is then free to take another substrate molecule into its active site.

5. Cofactors are …….... components which are essential for the catalytic activities of ……... enzymes. These cofactors bind to the enzymes in two ways. Some …… bind and remain ……. and others …….. bind temporarily. Loosely bound …….. are reversible under certain circumstances. Organic cofactors are called ……..., e.g., derivatives of vitamins e.g. NAD+, FAD+ and biotin. Inorganic co-factors include metal ions such as Zn2+, Fe2+, Cu2+.

6. Following shows the rate of enzyme action against temperature

Structured Essay

1. What is defined by followings

   (a) Sum of all biochemical reactions of living beings, consisting of all catabolic and anabolic reactions.

   ……

   (b) which acts as the energy carrier in all living organisms including the simplest bacteria.

   ……

   (c) The capacity to do work. ……..

   (d) Synthesis of ATP using solar energy in photosynthesis …….

   (e) Synthesis of ATP using energy released by the breaking down of complex molecules

   ……..

   (f) Synthesis of ATP using energy released as a result of oxidation of molecules during cellular respiration.

   ……..

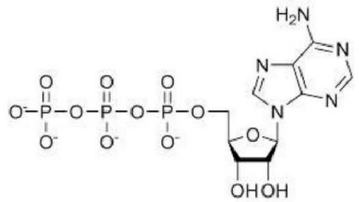
   (g) Non-proteinous components essential for the catalytic activities of certain enzymes.

   …….

   (h) Molecules that naturally regulate enzyme activity by binding to specific regulatory sites elsewhere

   …….

2. (a) Highlight high energy phosphate phosphate bond in following molecule



   (b) State a similarity between ATP, NAD+ and FAD.

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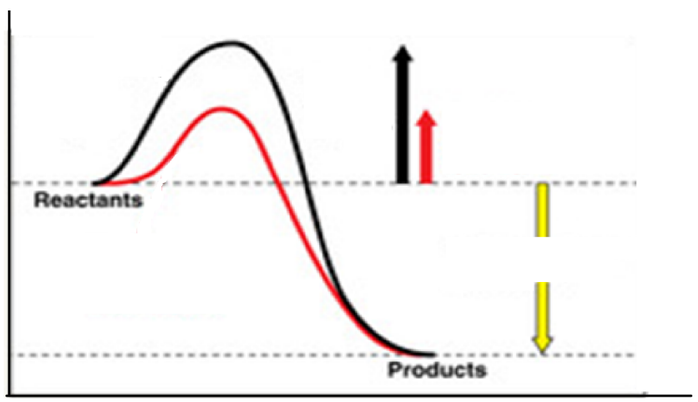
3. Explain the role of ATP in universal energy transaction.



Label E_A with enzyme, EA without enzyme and ΔG in above graph.

Enzymes

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:

1. Most of the enzymes are globular proteins.
2. Enzymes are biological catalysts. They lower the activation energy of the reaction they catalyze (increases the rate of reaction).
3. Most enzymes are heat labile/ sensitive
4. Their presence does not alter the nature or properties of the end products of any reaction.
5. Enzymes are highly specific to the substrate (substrate specific)
6. Most enzyme catalyzed reactions are reversible.
7. The rate of enzyme activity is affected by pH, temperature, substrate concentrations and inhibitors.
8. They are not being used up during the reaction.
9. Enzymes possess active sites where the reaction takes place.
10. Some enzymes need non-proteinous components to catalyse the reaction which are known as cofactors.

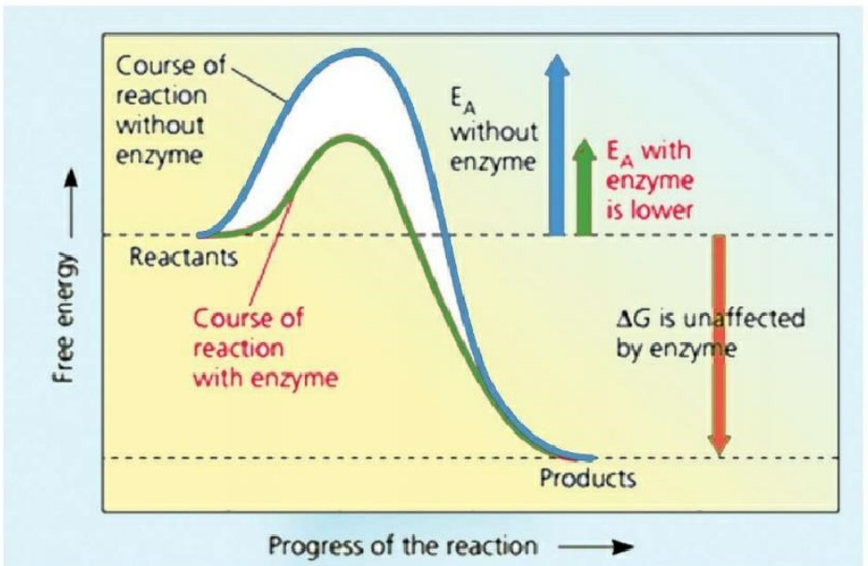


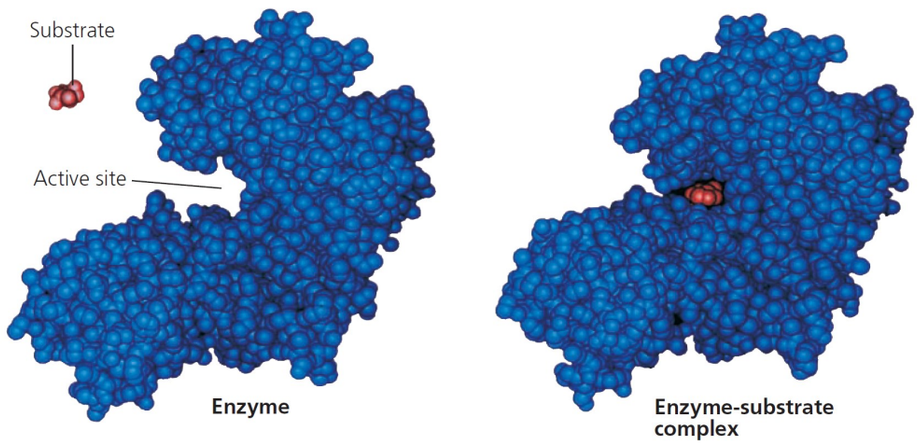
Fig 2.30 - The relationship between activation energy and the enzyme

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 enzyme-substrate complex. 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 shape. The substrate binds to a specific region of the enzyme. This region is called the active site. The active site is formed by only a few amino acids. Other amino acids are needed to maintain the shape of the enzyme molecule. The shape of the active site of the enzyme is complementary 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 rigid 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 induced fit mechanism. The tight fit not only brings the substrate molecules and the active site close to each other, but also ensures the correct orientation 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 free to take another substrate molecule into its active site.



(3) Reaction rate fluctuates (4) Reaction rate becomes zero
(5) Reaction rate plateaus as enzymes become saturated

25. Which type of inhibitor binds to a site other than the active site of the enzyme?
(1) Competitive inhibitor (2) Non-competitive inhibitor (3) Irreversible inhibitor
(4) Allosteric inhibitor (5) Reversible inhibitor

26. Competitive inhibitors function by:
(1) Permanently binding to the enzyme (2) Changing the shape of the enzyme
(3) Competing with the substrate for the active site (4) Binding to a regulatory site
(5) Denaturing the enzyme

27. 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 30.5 kJ/mol of energy.
(4) is formed in pyruvate oxidation through substrate level phosphorylation.
(5) contains deoxyribose.

28. 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.

29. The example of cooperativity mentioned:
(1) ATP production (2) Enzyme-substrate complex formation
(3) Hemoglobin binding with oxygen (4) Feedback inhibition (5) Allosteric regulation

30. 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 are proteinous components which are permanently or temporarily bound to enzymes.

13. Which of the following is NOT a general characteristic of enzymes?
(1) Most enzymes are globular proteins (2) Enzymes are biological catalysts
(3) Enzymes alter the properties of end products (4) Enzymes are heat labile/sensitive
(5) Enzymes are highly specific to the substrate

14. The activation energy (EA) in enzyme-catalyzed reactions:
(1) Increases (2) Decreases (3) Remains constant (4) First increases then decreases
(5) First decreases then increases

15. 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.

16. The specific region of the enzyme where the substrate binds is called:
(1) Allosteric site (2) Active site (3) Regulatory site (4) Cofactor site (5) Inhibitory site

17. The mechanism where substrate binding causes slight changes in the shape of the active site is known as:
(1) Lock and key model (2) Induced fit mechanism (3) Allosteric regulation
(4) Competitive inhibition (5) Feedback inhibition

18. 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.

19. Which of the following is an example of an organic cofactor?
(1) Zn^{2+} (2) Fe^{2+} (3) Cu^{2+} (4) NAD^{+} (5) H^{+}

20. The optimum temperature for most human enzymes is around:
(1) 25°C-30°C (2) 35°C-40°C (3) 45°C-50°C (4) 55°C-60°C (5) 65°C-70°C

21. What happens to enzyme activity when temperature increases beyond the optimum temperature?
(1) It continues to increase (2) It remains constant (3) It starts to decline
(4) It fluctuates randomly (5) It increases exponentially

22. The optimum pH for pepsin is approximately:
(1) 2 (2) 4 (3) 6 (4) 8 (5) 10

23. The optimum pH for trypsin is approximately:
(1) 2 (2) 4 (3) 6 (4) 8 (5) 10

24. What happens when substrate concentration increases beyond a certain point in enzyme-catalyzed reactions?
(1) Reaction rate continues to increase (2) Reaction rate declines

Cofactors

Non-proteinuos components which are essential for the catalytic activities of certain enzymes are called cofactors.

These cofactors bind to the enzymes in two ways. Some tightly bind and remain permanently and others loosely bind temporarily. Loosely bound cofactors are reversible under certain circumstances.

Organic cofactors are called co-enzymes. e.g. derivatives of vitamins e.g. NAD^{+}, FAD^{+} and biotin

Inorganic co-factors – e.g. Zn^{2+}, Fe^{2+}, Cu^{2+}

Factors affecting the rate of enzymatic reactions

1. Temperature
2. pH
3. Substrate concentration
4. Inhibitors

Temperature

Increase in temperature increases molecular motion. Therefore the speed of the moving molecules of both enzymes as well as the substrate will be accelerated. This will enhance the colliding probability for both enzyme active sites and substrate

molecules. More collision between the enzyme active sites and substrate molecules generate greater chances for the reaction to occur. This can continue up to a certain temperature, after which there is a rapid decline in enzyme activity. This temperature is referred to as optimum temperature. This may vary from organism to organism.

e.g. most of the human enzymes have optimum temperature around the body temperature (35˚C-40˚C). Optimum temperature of bacteria in hot springs 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 shape of the active site 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 denaturation of enzyme molecules.

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

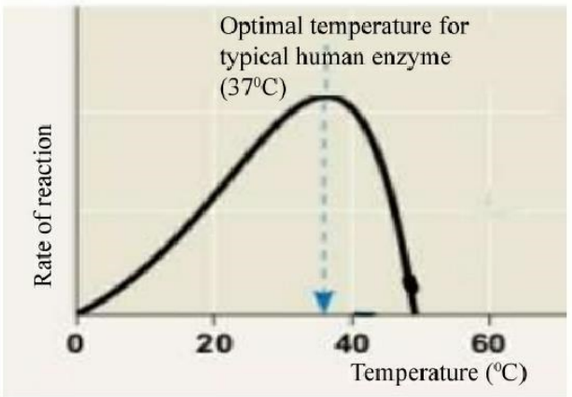


Fig -2.32 The graph of Rate of reaction (V) vs Temperature(T)

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 pH range. The pH at which the highest rate of reaction occurs is the optimum pH 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 alteration of chemical bonds involving in formation of enzyme substrate complex. In most enzymes optimum pH range is 6-8, but there are exceptions. Pepsin works best at pH 2 and optimum pH for Trypsin is 8.

MCQs

1. The sum of all biochemical reactions in a living organism is known as:
   (1) Respiration (2) Metabolism (3) Catabolism (4) Anabolism (5) Phosphorylation

2. Which of the following is an exergonic reaction?
   (1) Anabolism (2) Synthesis of proteins (3) Catabolism (4) ATP formation
   (5) Active transport

3. Which of the following is an endergonic reaction?
   (1) Breaking down of glucose (2) Hydrolysis of ATP
   (3) Making complex molecules from simple molecules (4) Cellular respiration
   (5) Glycolysis

4. ATP is often referred to as:
   (1) Energy storage molecule (2) Universal currency of energy transactions
   (3) Primary energy source (4) Coenzyme (5) Enzyme cofactor

5. Which of the following is NOT listed as a process requiring energy in living organisms?
   (1) Synthesis of substances (2) Active transport across plasma membrane
   (3) Muscle contraction (4) Allosteric regulation (5) Bioluminescence

6. The primary energy source for biological systems is:
   (1) ATP (2) Glucose (3) Solar radiation (4) Chemical bonds (5) Heat

7. ATP consists of:
   (1) Ribose sugar, adenine, and two phosphate groups
   (2) Deoxyribose sugar, adenine, and three phosphate groups
   (3) Ribose sugar, guanine, and three phosphate groups
   (4) Ribose sugar, adenine, and three phosphate groups
   (5) Deoxyribose sugar, adenine, and four phosphate groups

8. The free energy yield when ATP is hydrolyzed is approximately:
   (1) -50.5kJ/mol (2) -20.5kJ/mol (3) -30.5kJ/mol (4) -40.5kJ/mol (5) -60.5kJ/mol

9. Which of the following is NOT a form of energy ATP can be converted into in living cells?
   (1) Light (2) Heat (3) Mechanical energy (4) Chemical energy (5) Nuclear energy

10. The process of ATP production within cells using ADP, inorganic phosphate, and energy is called:
    (1) Respiration (2) Metabolism (3) Phosphorylation (4) Hydrolysis (5) Catabolism

11. The process of ATP synthesis using solar energy in photosynthesis is known as:
    (1) Oxidative phosphorylation (2) Substrate phosphorylation (3) Photophosphorylation
    (4) Chemiosmotic phosphorylation (5) Glycolysis

12. An enzyme is defined as:
    (1) A protein catalyst (2) A macromolecule acting as a biological catalyst
    (3) A non-proteinous component essential for catalytic activities
    (4) A biological substrate (5) A complex of substrate and active site

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).
- 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.

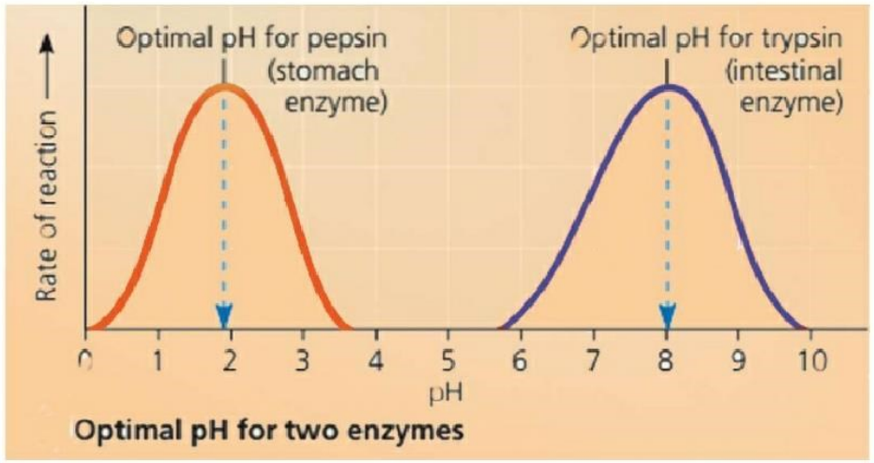


Fig -2.33 Rates of reaction of two enzymes at various pH values

Substrate concentration

Increasing substrate concentration increases the probability of collision between the enzyme and substrate molecules with correct orientation. 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 permanently or temporarily to the enzyme molecules and prevent them from forming enzyme-substrate complex. These substances are called inhibitors.

They are either binding reversibly with weak interactions or binding irreversibly through covalent bonds.

e.g. Irreversible inhibitors: toxins, poisons

Reversible inhibitors- drugs used against microbes

Competitive inhibitors

Most of these are reversible inhibitors. These chemicals resemble the shape and nature of the substrate. Therefore they compete with the substrate selectively for the active site of certain enzymes. As a result of the above, the number of active sites available for the substrate may decline and therefore reduces the rate of enzyme catalyzed reactions.

The above situation may be reversed by increasing the substrate concentration.

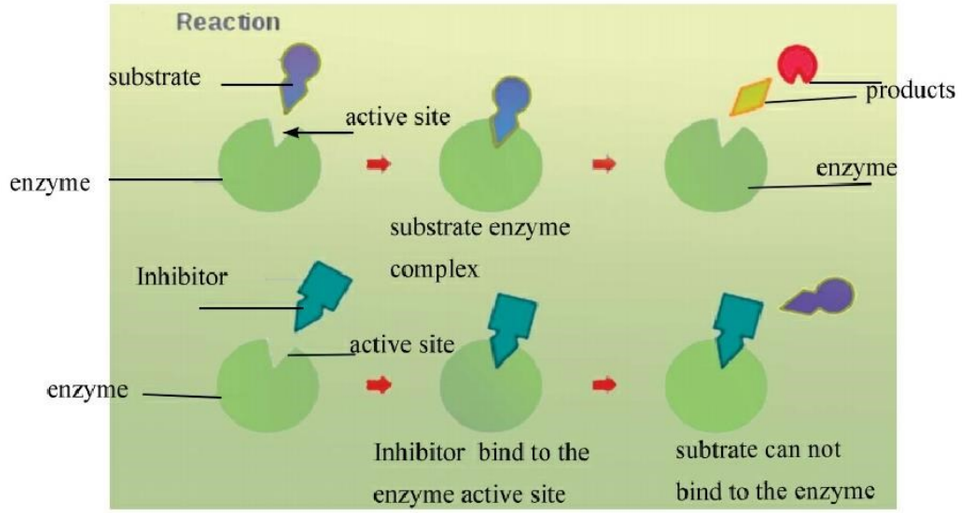


Fig 2.34: Competitive inhibitors

Non-competitive inhibitors

These chemicals do not compete with substrate molecules. They interrupt 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.

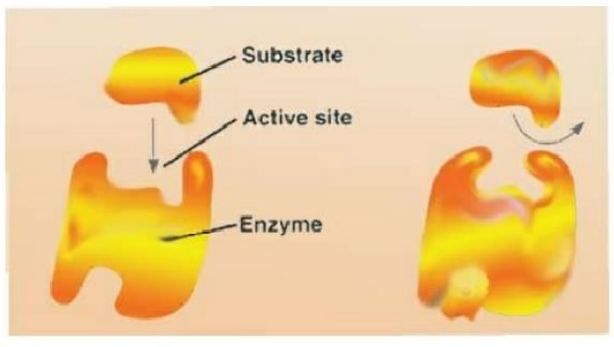


Fig 2.35: noncompetitive inhibitors

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 reversible non-competitive inhibitors. 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 two or more subunits. Each sub unit composed of a polypeptide chain with its own active site. The entire complex oscillates between two different shapes one catalytically active and other inactive. In this two forms regulatory molecules bind to a regulatory site called allosteric site, often located where subunits join.

When an activator binds with this regulatory site, stabilizes the shape with functional active sites. Whereas the inhibitor binds with the regulatory site, it stabilizes 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 single activator or inhibitor molecule that bind to one regulatory site will affect the active site of all sub units. e.g. ADP function as allosteric activator bind to the enzyme and stimulates the production of ATP by catabolism. If the supply of ATP exceed demand catabolism slows down as ATP bind to the same enzyme as inhibitor.

b.) cooperativity

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

e.g. 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.

c.) Feedback inhibition

In feedback inhibition, a metabolic pathway is stopped by the inhibitory binding of its end product to the enzyme involved, thereby, limit the production of more 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.