EXPERIMENT 12 STUDY OF ENZYME ACTION

Structure

12.1 INTRODUCTION

An enzyme is a protein which increases the rate at which a chemical reaction in the cell takes place without being used up in the rcaction it carries out. In other words, an enzyme is a biological catalyst. Enzymes arc highly specific. An enzyme usually catalyses only a single chemical rcaction or, at most, a very few closely related reactions.

Enzymes are grouped into the following major classes on the basis of chemical rcactions they catalyse (Table 12.1)

Table 12.1 The major enzyme classes and subclasses

Class end Subclass	General Reaction Type
Oxidoreductases Oxidases Reductases Dehydrogenases	Remove and add electrons or electrons and hydrogen. Oxidases transfer electrons or hydrogen to O_2 only
Transferases Kinases	Transfer chemical groups Transfer phosphate groups, especially from ATP
Hydrolases Proteinases Ribonucleases Deoxyribonuclcascs Lipascs	Break chemical bonds (e.g., amides, esters, glycosides) by adding the elements of water Hydrolyse proteins (peptidc bonds) Hydrolyse RNA (phosphate cstcrs) Hydrolysc DNA (phosphate esters) Hydrolyse fats (csters)
Lyases	Form double bonds by elimination of a chemical group
Isomerases	Rearrange atoms of a molcculc to form a structural isomer
Ligases or Synthetases	Join two molecules coupled with hydrolysis of ATP or other nucleosidc triphosphatc
Polymerases	Link subunits (monomers) into a polymer such as RNA or DNA

All kinds of enzyme cannot bc studied by simple laboratory techniques. Therefore, in this laborslory course you arc going to sludy the action of oxidoreductases and hydrolases only that arc easy Io sludy.

Objectives

After doing the experiments on enzyme aclion, you should bc able to:

- demonstrate the aclion of xanthine oxidnsc, phenolase complex, catalase, anaerobic dehydrogenase and amplasc,
- explain the chemical rcaclions involved in the enzymes studied.

12.2 OXIDOREDUCTASES

They catalyse addition of O_2 or removal of clcclrons from substrate and thus effect oxidation or reduction. Probably you arc aware that oxidation reactions arc common in catabolism and reduction rcaclions in anabolism in the cell. Oxidoreductases arc grouped according to their mode of aclion.

1) Enzymcs that directly involve oxygcn.

2) Enzymes that do not directly involve oxygcn, but transfer clcclrons through a scries of carriers likc various cytochromes in respiratory clcctron transfer chain to oxygen forming water. These arc called **anaerobic dehydrogenases.**

1. Enzymes **that directly** involve **oxygen**

There arc three types of cnzymes in this group.

i) Enzymes that transfer 2 to 4 clcclrons from substrate to oxygcn as shown below. These arc called **oxidases.**

$$2H^+ + 2e^- + O_2 \longrightarrow H_2O_2$$

$$4H^+ + 4e^- + O_2 \longrightarrow 2H_2O$$

Examples arc cytochrome oxidascs which catalyse the oxidation of cytochromes (Fe^{2+}) in respiratory clcclron transfer chain.

Some cnzymes of this category can transfer clcclrons Io dyes such as methylene blue as well.

ii) These cnzymes incorporate one atom of oxygcn in the substrate and the other oxygcn atom reacts with 2 clcclrons donated by electron donors like $NADH_2$ or NADPH, Io form water. Therefore these cnzymes arc called **mixed function oxidases.** Examples arc phenolase complex, catalase and peroxidasc.

iii) Enzymcs that transfer both the atoms of oxygcn to the substrate. These arc calicd **oxygenases.** For example lipoxidasc, tryptophanc pyrrolasc.

$$\underset{\text{substrate}}{X} + O_2 \longrightarrow XO_2$$

Reclox Indicators

Certain dyes can accept electrons from the substrate during enzymatic redox reaction. The dye changes colour when reduced and thus indicates the occurrence of reaction (redox indicator). Such artificial electron acceptors are commonly used to study the action of oxidoreductases. For example, methylene blue (MB) and dichlorophcnol indophenol (DCPIP) are blue in the oxidised state and colourless when reduced.

$$\underset{\substack{\text{Melhylene blue}\\ \text{oxidised}\\ \text{(blue)}}}{MB} + 2e^- + 2H^+ \longrightarrow \underset{\substack{\text{Methylene blue}\\ \text{reduced}\\ \text{(colourless)}}}{MB(H)_2}$$

12.2.1 Xanthine Oxidase

This enzyme is present in milk. It can transfer electrons (and H^+) from hydrated aldehyde such as formaldehyde to O_2 or to dye like methylene blue.

$$\underset{\text{Formal-dehyde}}{HCHO} + H_2O \longrightarrow \underset{\text{Hydrated formaldehyde}}{HCH(OH)_2} + \underset{\text{Methylene blue}}{MB} \xrightarrow{\text{Xanthine oxidase}} \underset{\text{Formic acid}}{HCOOH} + \underset{\text{(colourless)}}{MBH_2}$$

Materials

1. Unpasteurised milk
2. 0.02% Methylene blue solution
3. 0.4% Formaldehyde solution
4. Paraffin oil.

Procedure

Take three test tubes and number them 1 to 3. Pour in each 5 ml of unpasteurised milk. Boil tube 1 for 2 minutes and cool. Add 1 ml of 0.02% methylene blue solution in each tube. Then add 0.4% formaldehyde to tube 1 and 2 but not in tube 3. Mix the contents of the tube by gentle rotation and add 1-2 ml of paraffin oil and place at 40°C. Record observations.

Tube No.	Contents in the Tube	Change in the colour of dye
1.	Boiled (cooled) milk + melhylene blue + formaldehyde	
2.	Unboiled milk + melhylene blue + formaldehyde	
3.	Unboiled milk + melhylene blue	

SAQ 1

a) Why do wc usc unpasteurised milk?

.....

.....

b) What is thc substrate in this reaction?

.....

.....

c) Why is it ncccssary to use paraffin oil?

.....

12.2.2 Phenolase Complex

This cnzymc oxidises naturally occurring phenols to quinones which then polymerisc to give a brownblack compound—the quinones. This reaction causes browning of peelcd fruits or potatoes that we commonly observe.

Monohydric phenol + $2\bar{e}$ + O^{2-} ⟶ O-Diphenol + O^{2-}

2 O-Diphenol + O_2 ⟶ 2 O-Quinone + $2H_2O$

sum : Monophenol + O_2 ⟶ 2 O-Quinone + $2H_2O$

Materials

1. Grated potato or.apple slices
2. Cheese cloth
3. 1% catechol solution

Procedure

Take a small picce (about 5 g) of potato and grind it in a mortar and pestle with 10 ml of distilled water. Filicr through cheese cloth and use immediately. To 5 ml of potato extract add 10 drops of 1% catechol and mix wcll. Observe any change in colour. Rcpcat thc cxpcriment with a piece of potato that has been kept in boiling waicr for 5 min.

SAQ 2

a) Why is it ncccssary to use the potato extract immediately?

.....

.....

b) If lcmon juicc is poured on freshly cut apple slices, they do not turn brown. Can you offer an explanation?

.....

.....

12.2.3 Catalase

Hydrogen peroxide, a toxic chemical for protoplasm, is produced in **the** actively metabolising aerobic tissues. It is produced when electrons are ultimately donated to oxygen by dehydrogenases. The enzyme **catalase** speeds **up** the break down of H_2O_2 to O_2 and thus detoxifies the tissue.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

This is an oxidation-reduction reaction in which one molecule of hydrogen peroxide is **oxidised** to yield oxygen and the other molecule is reduced to form water.

Materials

1. Animal tissue (liver, or muscle) or
   Plant tissue (potatoes or apple)
2. 'Hydrogen peroxide
3. Buffer solution pH 6.8

Procedure

Take four test tubes and number them 1 to 4. Add in each tube 5 ml of buffer. Take 10g of liver or potatoes and cut it into small pieces, divide it in two portions and place them in tubes 2 and 3. Boil the contents of tube 2 for two minutes and cool. Macerate 5g of liver or potato in a mortar and pestle and place in tube 4. Now, add 5 ml of H_2O_2 to each test tube and record your observations as follows:

Observations	Record
No effervescence	O
Moderate effervescence	+
Good effervescence	++
High effervescence	+++

Tube No.	Contents of the Tube	Observation
1.	Buffer + H_2O_2	
2.	Buffer + liver pieces (Boiled) + H_2O_2	
3.	Buffer + liver pieces + H_2O_2	
4.	Buffer + macerated liver pieces + H_2O_2	

SAQ 3

a) Is there any difference between the reactions carried out with cut pieces of tissue and macerated tissue?

.....

.....

.....

.....

b) Why are the results of tube 2 different from that of tube 3?

.....

.....

12.2.4 Dehydrogenases

They form the most important group of enzymes in respiratory metabolism. A few examples are succinic acid dehydrogenase (Krebs cycle), alcohol dehydrogenase (fermentation) and NADH dehydrogenase (electron transfer chain). You will study dehydrogenases of yeast.

Materials

1. Four test tubes (15 ml)
2. Four clean rubber stoppers
3. Four pipettes
4. 5% solution of glucose
5. 2% solution of starch
6. Methylene blue .05% solution
7. Formaldehyde 4%

Procedure

Arrange four test tubes in a rack and number them 1 to 4.
Introduce the various material in the test tubes as listed in the table.

Tube No.	Distilled Water	Glucose	Starch	Formal-dehyde	Active yeast	Methylene blue
1.	10 ml	x	x	x	5 ml	√
2.	5 ml	5 ml	x	x	5 ml	√
3.	5 ml	x	5 ml	x	x	√
4.	x	5 ml	x	5 ml	5 ml	√

introduce yeast at last. Fill all the tubes to the brim by adding distilled water, if necessary. Now, add 2 drops of methylene blue in each tube. Mix the contents of the tubcs by placing your thumb on the tube and inverting it. Make surc the initial intensity of the colour is same in all the tubcs. Stopper each tube and place them in rack. Let the liquid overflow. Record your observations after 20, 40, 60 min and 2 hours. Grade the intensity of colour as follows. Nolc the evolution of gas also and interpret the results.

Colour	Intensity grade
Colourless	O
Pale	+
Light-blue	++
Moderate light blue	+++
Deep blue	++++

SAQ 4

a) Interpret the results (intensity grade) keeping in view the contents in each of the tube.

.....

.....

b) What gas is evolved during the reaction?

.....

12.3 HYDROLASES

These enzymes breakdown larger molecules into smaller units. The mast common enzymes in this category arc digestive cnzymcs — amylases, lipascs and proteinases that break down starch, lipids and protcins respectively.

12.3.1 Salivary Amylase

Salivary amylase is present in the saliva of most mammals. It initiates the hydrolysis of starch.

Materials

1. Starch (Cracker)
2. Benedicts solution
3. Iodine solution
4. 3 ml of saliva

Procedure

Collect 3 ml of saliva in a test tube by chewing a piece of paraffin wax. Take four test tubes and label them 1-4. Add the reagents as shown in the lable.

Test tube	Starch (cracker)	Water	Iodine	Benedicts Solution	Saliva	Obser-vation
I.	x	10 ml	10 drops	5 ml	x	
2.	√	10 ml	10 drops	x	5 ml	
3.	√	10 ml	x	5 ml	x	
4.	√	10 ml	x	5 ml	5 ml	

After adding Benedicts solution heat over a flame until it boils gently. Benedicts reagent gives positive reaction if reducing sugars are present

SAQ 5

a) Explain the results of your experiment.

.....

.....