

EXPERIMENT 12 STUDY OF ENZYME ACTION

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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 reaction it carries out. In other words, an enzyme is a biological catalyst. Enzymes are highly specific. An enzyme usually catalyses only a single chemical reaction or, at most, a very few closely related reactions.

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

Table 12.1 The major enzyme classes and subclasses

Class and 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 Deoxyribonucleases Lipases	Break chemical bonds (e.g., amides, esters, glycosides) by adding the elements of water Hydrolyse proteins (peptide bonds) Hydrolyse RNA (phosphate esters) Hydrolyse DNA (phosphate esters) Hydrolyse fats (esters)
Lyases	Form double bonds by elimination of a chemical group
Isomerases	Rearrange atoms of a molecule to form a structural isomer
Ligases or Synthetases	Join two molecules coupled with hydrolysis of ATP or other nucleoside triphosphate
Polymerases	Link subunits (monomers) into a polymer such as RNA or DNA

All kinds of enzyme cannot be studied by simple laboratory techniques. Therefore, in this laboratory course you are going to study the action of oxidoreductases and hydrolases only that are easy to study.

Objectives

After doing the experiments on enzyme action, you should be able to:

- demonstrate the action of xanthine oxidase, phenolase complex, catalase, anaerobic dehydrogenase and amylase,
- explain the chemical reactions involved in the enzymes studied.

12.2 OXIDOREDUCTASES

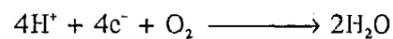
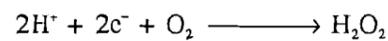
They catalyse addition of O_2 or removal of electrons from substrate and thus effect oxidation or reduction. Probably you are aware that oxidation reactions are common in catabolism and reduction reactions in anabolism in the cell. Oxidoreductases are grouped according to their mode of action.

- 1) Enzymes that directly involve oxygen.
- 2) Enzymes that do not directly involve oxygen, but transfer electrons through a series of carriers like various cytochromes in respiratory electron transfer chain to oxygen forming water. These are called **anaerobic dehydrogenases**.

1. Enzymes that directly involve oxygen

There are three types of enzymes in this group.

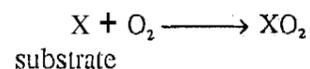
- i) Enzymes that transfer 2 to 4 electrons from substrate to oxygen as shown below. These are called **oxidases**.



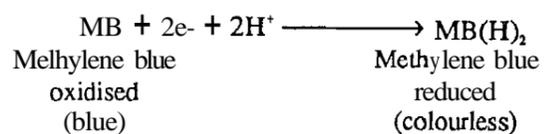
Examples are cytochrome oxidases which catalyse the oxidation of cytochromes (Fe^{2+}) in respiratory electron transfer chain.

Some enzymes of this category can transfer electrons to dyes such as methylene blue as well.

- ii) These enzymes incorporate one atom of oxygen in the substrate and the other oxygen atom reacts with 2 electrons donated by electron donors like $NADH_2$ or $NADPH$, to form water. Therefore these enzymes are called **mixed function oxidases**. Examples are phenolase complex, catalase and peroxidase.
- iii) Enzymes that transfer both the atoms of oxygen to the substrate. These are called **oxygenases**. For example lipoxidase, tryptophan pyrrolase.

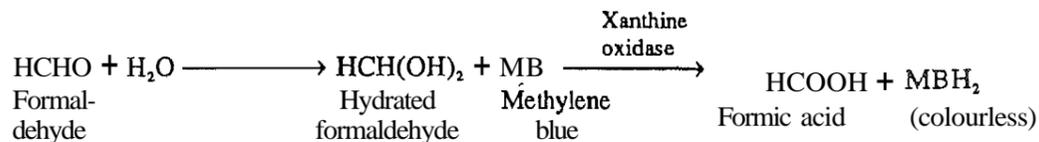


Certain dyes can accept electrons from the substrate during enzymatic **redox** reaction. The dye changes colour when **reduced** and thus indicates the occurrence of reaction (redox indicator). Such artificial electron acceptors are commonly used to study the action of oxidoreductases. For example,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



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.



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 + methylene blue + formaldehyde	
2.	Unboiled milk + methylene blue + formaldehyde	
3.	Unboiled milk + methylene blue	

SAQ 1

a) Why do we use unpasteurised milk?

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b) What is the substrate in this reaction?

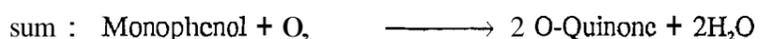
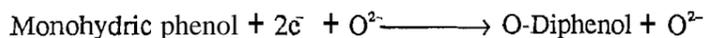
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c) Why is it necessary to use paraffin oil?

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12.2.2 Phenolase Complex

This enzyme oxidises naturally occurring phenols to quinones which then polymerise to give a brown/black compound—the quinones. This reaction causes browning of peeled fruits or potatoes that we commonly observe.



Materials

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

Procedure

Take a small piece (about 5 g) of potato and grind it in a mortar and pestle with 10 ml of distilled water. Filter through cheese cloth and use immediately. To 5 ml of potato extract add 10 drops of 1% catechol and mix well. Observe any change in colour. Repeat the experiment with a piece of potato that has been kept in boiling water for 5 min.

SAQ 2

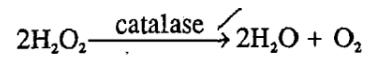
a) Why is it necessary to use the potato extract immediately?

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b) If lemon juice 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.



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	0
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?

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

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12.2.4 Dhydrogenases

They form the most important group of enzymes in respiratory metabolism. A few examples are succinic acid dhydrogenase (Kreb cycle), alcohol dhydrogenase (fermentation) and NADH dhydrogenase (electron transfer chain). You will study dhydrogenases 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	Formaldehyde	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 tubes by placing your thumb on the tube and inverting it. Make sure the initial intensity of the colour is same in all the tubes. 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. Note the evolution of gas also and interpret the results.

Colour	Intensity grade
Colourless	0
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.

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b) What gas is evolved during the reaction?

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12.3 HYDROLASES

These enzymes breakdown larger molecules into smaller units. The most common enzymes in this category are digestive enzymes — amylases, lipases and proteinases that break down starch, lipids and proteins 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. Benedict's 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 table.

Test tube	Starch (cracker)	Water	Iodine	Benedicts Solution	Saliva	Observation
1.	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.

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