EXERCISE 5

Enzymes

LEARNING OBJECTIVES

- Demonstrate enzyme activity by the hydrolysis of starch by amylase.
- Determine the effect of different temperatures on the rate of starch hydrolysis.
- Determine the effect of different pHs on starch hydrolysis.
- Demonstrate the presence of the enzyme catalase in living tissues
- Compare the relative amounts of catalase in different tissues.
- Demonstrate that oxygen is produced when hydrogen peroxide is decomposed by catalase.

Answer these questions before you come to lab:

1. Define each of the following terms:

Catalyst	
Activation energy	
Enzyme	
Substrate	
Product	
Denaturation	
Hydrolysis	
Metabolism	

2. Why do living cells need enzymes?

_____

_____

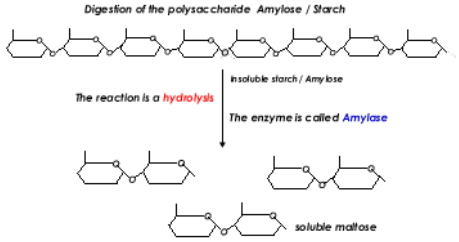
INTRODUCTION

Enzymes are proteins folded into their tertiary structure which give them a particular 3-dimensional shape. This highly specific folding creates a groove in the enzyme molecule, called the active site. The active site has a very specific shape which only one substrate (with a complementary shape) can bind. Any condition that causes the enzyme to unfold and lose its

shape will result in its inability to function.

An example of an enzyme is amylase, found in human saliva and also in germinating seeds. Its substrate is the polysaccharide starch. Amylase catalyzes hydrolysis of starch to the disaccharide, maltose.

$$H_2O + \text{amylase} + \text{starch} \longrightarrow + \text{amylase-starch complex} \longrightarrow \text{maltose} + \text{amylase}.$$



In this lab we will investigate how two conditions, temperature and pH affect the ability of amylase to hydrolyze (digest) starch.

Lugol's iodine is used to detect starch which stains dark-blue/black in its presence. If starch is digested by amylase to maltose, this color will disappear since maltose does not react in this way with iodine. The color that remains will be that of iodine.

IMPORTANT NOTE: **Wear gloves.**

Rinse spot plates of Lugol's Iodine and Amylase + Starch + Lugol's with water over waste tub.

Empty tubes with Amylase + Starch solution in waste tub.

Exercise 1: Starch hydrolysis by the enzyme amylase

Procedure

1. Obtain a spot plate that has many small depressions on its surface.

2. Place a drop of iodine into each of the depressions. Each well will be used to detect the presence of starch.

3. Obtain a test tube and fill it with 10 ml of starch. This is the reaction tube.

4. Add 1 ml of 1% amylase. Mix carefully by inversion, after covering the tubes with a small piece of Parafilm®. Immediately remove 1 drop from the reaction tube and place into one of the wells on the spot plate. A positive test for starch should be observed.

5. Remove a small sample from the reaction tube and test for starch at 2 minute intervals. Continue this for a 10-minute period, and record the time at which no starch is detected (the time at which all the starch is converted to maltose).

6. Tabulate your observations making a note of the time interval and what you observed.

Time (min)	Starch present? (+ or -)
0	
2	
4	
6	
8	
10	

If starch is still present even after 10 minutes of reaction time, can you detect a lighter color when you add the sample to the drop of iodine? If so, why?

_____

_____

Exercise2: Effect of Temperature on Starch Hydrolysis

What effect do you predict temperature will have on the reaction of amylase with starch?

_____

_____

At which temperature do you think the reaction will proceed best? Why? (Hint: Where is amylase found?)

_____

_____

Procedure

1. Get 4 test tubes and label one each: ice; RT (room temperature); 40°C; boiling.

2. Into each tube, pipette 10 ml of starch solution, and place each tube in a water bath of appropriate temperature. Incubate the tubes for 5 minutes to allow the starch to come to that temperature (to equilibrate).

3. During the 5-minute incubation, prepare your spot plate by adding a drop of iodine to each well.

4. After the 5-minute incubation, add 1 ml of the 1% amylase solution. Mix carefully by inversion, after covering the tubes with a small piece of Parafilm®. Remove the Parafilm® and return the tubes to the water baths. **(Do not mix the tube in the boiling water bath!)**

5. At 2-minute intervals, remove 1 drop from each tube and test for the presence of starch by dropping the solution into a well containing iodine.

6. Continue testing for the presence of starch at 2-minute intervals, as described above, until no starch is detected, that is, until the hydrolysis is complete, in each of the 4 reaction tubes. Use the following table to keep track of the results for each test by placing a + or - to indicate the presence or absence of starch.

Temp. (°C)	**2 min.**	**4 min.**	**6 min.**	**10 min.**	**12 min.**	**14 min.**	**16 min.**	**18 min.**
4 (ice)								
25 (RT)								
40 (warm)								
100 (boiling)								

7. From the above table, note the time needed for complete hydrolysis, and write the times for each corresponding temperature in the table below.

Temp. (°C)	Time to complete hydrolysis (min.)
4 (ice)	
25 (RT)	
40 (warm)	
100 (boiling)	

Exercise 3: Effect of pH on Starch Hydrolysis

What effect do you predict pH will have on the hydrolysis of starch by amylase?

_____

_____

At what pH do you think the reaction will proceed best? (Hint: What is the pH where amylase is found?)

_____

Procedure

1. Get 3 test tubes and label one each: pH 4, pH 7, and pH 10.

2. Pipette 5 ml of the appropriate pH buffer into each tube. Add 5 ml of the starch solution to each tube, and mix by swirling.

3. Add 0.5 ml of the amylase solution to each tube; mix by inversion. Take 1drop from each tube and test for the presence of starch, as described above.

4. Continue to test for starch every 2 minutes until the reaction is complete in each tube, that is, until the iodine solution no longer changes color to dark-blue/black. Use the following table to keep track of the results for each test by placing a + or - to indicate the presence or absence of starch.

pH of Buffer	**2 min.**	**4 min.**	**6 min.**	**10 min.**	**12 min.**	**14 min.**	**16 min.**	**18 min.**
4								
7								
10								

5. Note the time it takes for the hydrolysis to reach completion at each pH from the table above and fill in the table below.

pH of Buffer	Time to complete hydrolysis (min.)
4	
7	
10	

Were your results what you had predicted before starting the experiment? Why or why not?

_____

_____

_____

Exercise 4: To demonstrate that different cell types produce varying amounts of the enzyme CATALASE.

During cellular metabolism, cells produce a by-product, **hydrogen peroxide** (H_2O_2) which is toxic to cells. To get rid of the hydrogen peroxide, cells produce an enzyme called **catalase** which breaks down the harmful hydrogen peroxide into the harmless **water** and **oxygen**.

Hydrogen peroxide	=	water	+	oxygen
H_2O_2	=	H_2O	+	O_2

When hydrogen peroxide is added to living cells, this reaction takes place and the oxygen released can be observed as bubbles leaving the tissue.

In this experiment you will investigate the varying amounts of **catalase** in different cell types.

Procedure

1. Weigh out 1g of each of the tissues provided.

2. Cut up the tissues and place each of the chopped tissues into a test tube. Use a spatula or a glass rod to push all of the tissue to the bottom of the test tube. You need to use all identical test tubes for this experiment.

3. Place 1g of chopped potato into a test tube with about 10 ml of water and boil for five minutes. Drain off the water.

   What is the purpose of this test tube?

   _____

4. Add 5 ml of hydrogen peroxide to each tube and mark the level of the hydrogen peroxide ***immediately***. Do one tube at a time so that you can mark the level of the hydrogen peroxide as soon as you add it.

5. Allow the tubes to stand for 5-7 minutes and then mark the height to which the foam rises.

6. While you are waiting you can confirm that the gas being produced is oxygen by doing the test for oxygen gas. Place a glowing splint into the test tube. Describe what happens.

   _____

6. Measure the height of the foam in mm and record your results in the table below.

Cell type	**Height of foam (mm)**
Boiled potato	

Review Questions

1. Consider the chemical reaction:

   starch + water = maltose

   a. Specifically what type of chemical compound is starch? maltose?

      starch _____ maltose_____

   b. What type of chemical reaction is represented by the equation above?

      _____

   c. In this reaction what is the substrate? Product?

      substrate _____ product _____

2. (a) At what temperature did the reaction proceed best? Why do you think this is the ideal temperature for this enzyme?

   _____

   _____

   (b) Explain why was there no breakdown of starch in the tubes placed at

   (i) 100°C

   _____

   _____

   (ii) 4°C?

_____

_____

3. (a) At what pH did this enzyme work best?

   _____

   (b) Amylase is an enzyme produced in the human body. State 2 areas of the **human body** where amylase is found.

   _____

   _____

   (c) Name an enzyme in the human body that works best at acid pH.

   _____

4. What is the active site of an enzyme?

   _____

   _____

5. From your results, which tissue type produced the most catalase?_____

   Which produce the least? _____

   Explain why there was no bubbling in the tube with the boiled potato.

   _____

   _____

   In which organelle of the cell would you expect to find the enzyme catalase?

   _____