

Enzymes

Learning Goals: After completion of this laboratory exercise you will be able to:

1. Explain the importance of enzymes in biology.
2. Explain the basic properties of an enzyme as a catalyst.
3. Discuss the effect of temperature, pH, substrate concentration, and enzyme concentration on the rate of an enzymatic reaction.

Introduction

Enzymes are biological catalysts that constitute the largest and most highly specialized class of protein molecules. Enzymes act as catalysts, to increase the rates of chemical reactions, but they do not cause a reaction to occur that would not proceed spontaneously without the enzyme. The reactions of metabolism would occur at extremely slow rates at normal body temperature and pH in the absence of enzymes. An appreciation of the catalytic efficiency of enzymes can be gained by realizing that, under optimal conditions, most enzymatic reactions proceed 10^8 to 10^{11} times more rapidly than the corresponding non-enzymatic reactions. Without the enzymes in our digestive tract, for example, it would take us about 50 years to digest a single meal!

Enzymes participate in the reaction, which they catalyze, but they emerge unchanged at the end of the reaction, i.e., they are not altered or used up. Thus, a few enzyme molecules can go a long way. This can be represented by the following generalized equation:



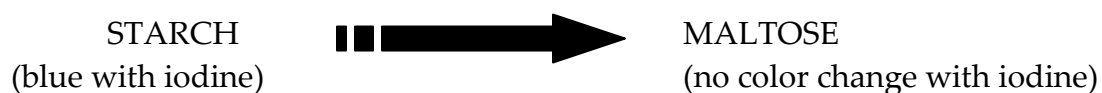
One very important property of enzymes is their specificity. Any one enzyme will only catalyze a single class of chemical reactions. In this lab you will be studying a single reaction carried out by one enzyme. Many thousands of enzymes exist, each catalyzing a different chemical reaction. Many of these enzymes have been isolated, purified and crystallized: the amino acid sequences of many have also been determined.

Because enzymes are proteins, they are affected by environmental conditions. Hence, changes in temperature, pH, and salt concentration can change the biological activity of enzymes by affecting the configuration of the enzyme molecules.

We shall demonstrate the activity of one enzyme in this lab. Saliva contains the enzyme amylase. Amylase catalyzes the hydrolysis of starch (a mixture of the polysaccharides amylopectin and amylose) to a disaccharide, maltose. Maltose can be

hydrolyzed to glucose by another enzyme, maltase (in the small intestine). In this lab we will study the action of the enzyme amylase on starch.

The action of amylase on starch can be followed readily with the iodine test. Iodine stains starch a deep blue color, but iodine does not stain maltose. When iodine is added to starch alone, the solution turns blue. But if all the starch has been hydrolyzed to maltose, the solution will not turn blue (it remains the yellowish color of the iodine solution. In between these two extremes, as hydrolysis is taking place, there are shades of reddish-brown.



Procedure Part I: Reaction rate vs. Enzyme Concentration.

0. Guide to colors: Each group should prepare one spot plate with a drop of iodine test solution in each of the first two depressions. To one of the iodine drops, add a drop of starch solution. To the other iodine drop, add a drop of water. Record the colors and make it clear in your notes what happened in each case.

1. Each group: prepare 6 test tubes, each containing 4 ml of the "stock starch solution" (This solution contains 0.5 grams starch boiled in 100 ml. water, and, after cooling, 100 ml of pH 6.8 buffer was added). Be as accurate as possible in your measurements (instructor should demonstrate the method of pipetting). **BE SURE TO SWIRL THE STOCK STARCH SOLUTION BEFORE EACH PIPETTING.** Label each tube "SS" for "Stock Starch", and place all the tubes in the 37°C water bath.

2. Prepare solutions as follows:

DILUTION		CONCENTRATION	
(ml amylase	:	OF STOCK AMYLASE	
ml H ₂ O)			
1	:	9	10%
1	:	19	5%
1	:	49	2%
1	:	99	1%

DO NOT THROW AWAY ANY OF THESE SOLUTIONS (nor the stock) UNTIL THE END OF THE LAB; you may need them for part 2 or for other dilutions later on.

3. Prepare a series of 4 test tubes, labeled 10%, 5%, 2%, and 1%. Pipet one ml. from each of the above enzyme dilutions into the appropriate labeled test tube. Place these 4 test tubes in the 37°C water bath and allow a few minutes for the samples to reach the bath temperature (equilibration).

4. Clean a spot plate and place one drop of iodine solution in each depression. The top left-hand depression will be called the first depression, and you will be testing samples starting with the iodine in the first depression and moving to the right with successive samples. You could number the depressions if you think you might lose track of which ones have been used.

5. One student in the group should record the actual start time (hour, minutes, seconds) while another student in the group pours the contents of one of the "SS" (stock starch) test tubes from the water bath into the test tube labeled 10%. This tube contained 1 ml of 10% amylase, and it will now contain in addition 4 ml of the starch solution. Swirl this tube and RETURN IT TO THE WATER BATH. We want the reaction, which is now starting, to take place at 37°C.

6. Remove ONE drop using a Pasteur pipette or plastic transfer pipette, as soon as the tube is back in the bath, and place it on your first iodine drop in the spot plate. Record the time and color of the iodine-solution mixture in the spot plate. If the color is blue, you still have starch present and should proceed with the remaining steps below. If the color does not change from the yellowish iodine color, either you forgot to add starch or your amylase concentration is too high and all starch has been digested: consult your instructor.

7. Continue as follows if the first test drop turned blue: take another drop from your 10% tube in the water bath 15 seconds after you took the first drop, and place it in the second drop of iodine in your spot plate. Record color and time. Keep taking new drops and testing them at the same time intervals with new iodine drops until the color no longer is blue, but remains yellowish or faint reddish-yellow. Keep a careful record of the colors and the times. Then work out how much time it took for your 10% solution to "reach end point". (The time for the starch to be digested.)

Usually the 10% amylase concentration will digest all of your starch quickly, within 1-2 minutes. Therefore your sampling intervals must be short, 10- 20 seconds. Consult your instructor if your solution takes a long time - you might want to make up stronger concentrations. If the amylase is too active you may want to make up weaker concentrations. Just be sure to record any modifications you make.

8. Determine the end points for your other amylase concentrations, using the same exact technique in steps 6, 7, and 8. Except that you may want to lengthen the sampling intervals (time = 30 or 60 seconds) since the more dilute amylase will not digest the starch as quickly. You made up six tubes of "SS" (stock starch) though you only need four, one for each amylase concentration. You have two extra tubes in case you require them for higher or lower amylase concentrations or if you make a mistake and need to repeat an end point determination.

9. Summarize your data as follows:

Amylase concentration	Time to reach endpoint in Minutes (transform data from seconds)	Rate of Reaction (100 units/Time to reach endpoint)
10%		
5%		
2%		
1%		

Note: We are getting the rate of the reaction (3rd column above) by assuming that we started with 100 units of starch in each tube, and then dividing 100 by the number of minutes that it took to digest all the starch. For instance, if it took 5 minutes, the rate would be $100/5 = 20$ units per minute -- that is, 20 units of starch were digested each minute.

10. Plot a graph showing the rate of the reaction (dependent variable) vs. the concentration of amylase (independent variable). Before the lab period ends, take the results from one of the groups and plot a rough graph on the board, so that you can review the rules of making graphs. In your lab report you will discuss the relationship between enzyme concentration (in this case, amylase concentration) and the rate of the reaction which the enzyme catalyzed.

Part II: Reaction Rate as a Function of Temperature.

1. Choose which dilution of amylase which gave you an end point in 2-4 minutes at 37°C. Make up at least six tubes containing 4 ml. of the stock starch (SS) solution and start them equilibrating at the various temperatures listed below. Make sure you have at least six ml. of the amylase dilution you have chosen (otherwise make up a new batch from your original amylase solution). Pipette 1 ml. portions of the amylase dilution into six new test tubes, labeled to correspond to the temperatures at which you will incubate (below). Then start these tubes also equilibrating at the various temperatures.

Temperatures to be used:

a) near freezing (use the ice bath to cool the test tubes, record the actual temperature)

b) room temperature (leave test tubes in a rack on the table top)

c) 40-50°C record the actual temperature

d) 55-65°C record the actual temperature

e) 67-77°C record the actual temperature

f) boiling water bath (100°C) (Keep tube in boiling bath.)

2. Use the same techniques as in Part I, at some recorded time, pour contents of SS tube at a given temperature into the tube containing amylase at that temperature. Keep this tube at the given temperature and start taking drops at intervals to test with iodine for the presence of starch. Groups might split up and each person can run a different temperature assay. Be sure to swap data at the end, and set up a table showing the end point and rate of reaction at each temperature.

3. Plot a graph showing the rate of the reaction (dependent variable) vs. temperature (independent variable). In your lab report, be sure to discuss the apparent optimal temperature for amylase and relate it to the body temperature of the animal from which the amylase was obtained (What is your body temperature in °C?). Also discuss in detail the effect of very low and very high temperatures on enzyme activity. If you got

no activity, spots stayed blue for over 30 minutes, at low and high temperatures do you think this means the enzymes' activity is "destroyed" by those temperatures? Try a quick experiment at the end of the lab: Take your tubes which were in the ice bath and in the boiling water bath and place them in the 37°C bath. Monitor them by taking drops at intervals to test for starch. Monitor them for a least as long as you did the original tube which was started at 37°C. Now do you know whether cold or boiling "destroys" (denatures) enzymes? Discuss in your report the mechanism of action of cold and hot temperatures on protein molecules in general and enzymes in particular.

Notebook Entries

Address the issues highlighted in the lab manual and by your instructor. Include the following :

1. Include a summary of your data and graphs.
2. Discuss the effects of temperature and enzyme concentration on the rate of an enzymatic reaction.
3. Include results as described in part I. # 9 and #10 and part II # 3.