EXPERIMENT 36

Enzymes

Performance Goals

36–1 Determine the activity of an enzyme.

36–2 Determine how the rate of an enzyme-catalyzed reaction changes under different conditions.

CHEMICAL OVERVIEW

An enzyme is a biological molecule that increases the rate of a reaction by lowering the activation energy. This allows reactions to occur approximately a million times faster and at much lower temperatures. Without increasing the rate of most biochemical reactions, life would not be possible.

Nearly all known biological catalysts are proteins that function by folding into a specific three-dimensional form called the tertiary structure. This structure is primarily due to four intermolecular forces: hydrogen bonding, nonpolar interactions, ionic bonds, and disulfide linkages. The enzyme's ability to function will be diminished or stopped if these intermolecular forces are disrupted, which, in turn, will alter the tertiary structure. This process of diminishing, or stopping the enzyme's function by altering its shape, is called denaturing. There are two major ways in which an enzyme can be denatured: alter its environment or introduce an enzyme inhibitor.

Most enzymes function in a specific biological environment. Some common causes of denaturing include heat and pH changes. In this experiment, both of these factors will be used to make the enzymes inoperative.

Silver nitrate will be used as an inhibitor. Heavy metals, like silver, often bind to the sulfhydryl groups of the amino acid cysteine to reduce or stop enzyme activity. Silver nitrate was used in newborn infants' eyes as an antibacterial agent.

This experiment will use catalase as an enzyme to work on the substrate hydrogen peroxide to form water and oxygen. Hydrogen peroxide is toxic to cells, so this enzyme's function is to decrease peroxide concentrations. Catalase is found in red blood cells and in many plants.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O(l) + O_2(g) \qquad (36.1)$$

The rate of the reaction will be measured by collecting and measuring the amount of oxygen generated in 5 minutes. Oxygen will be collected in a graduated cylinder initially full of water. The amount of water displaced

will equal the volume of oxygen gas generated from the enzyme. The time of the reaction will be measured, and the rate will then be reported as milliliters per minute.

Volume of $O_2 = V_{final} - V_{initial}$

Rate of catalase (mL/min) = Volume of O_2/5 minutes

SAFETY PRECAUTIONS AND DISPOSAL METHODS

Be sure to wear safety goggles or safety glasses while performing this experiment. Acids are corrosive and contact with the skin should be avoided. Any spilled acid should be washed off promptly.

Dispose of the waste as directed by your instructor.

PROCEDURE

NOTE: Record all volume measurements to the nearest 0.1 mL.

1. Standardization of Enzyme Activity

A. Put about 400 mL of water into a 500-mL beaker. Next, take a 250-mL beaker and fill it near the top with water and place on a hot plate.

B. Set up the apparatus according to Figure 36.1. Heat the water bath to 35–37°C. Often when the graduated cylinder is tipped over, a small amount of air will be trapped at the top. Record this initial amount of air in the 25-mL graduated cylinder.

C. Using a graduated, cylinder measure 5.0-mL of an 18% solution of H_2O_2. Place a magnetic stirring bar into a test tube and then pour in the solution. Place the stopper in the test tube.

D. Open the stopper and add 5.0 mL of the catalase solution. Immediately stopper the test tube and begin recording the time. This step must be completed quickly so no oxygen is lost. Shake the test tube to insure mixing. Turn on the magnetic stirrer.

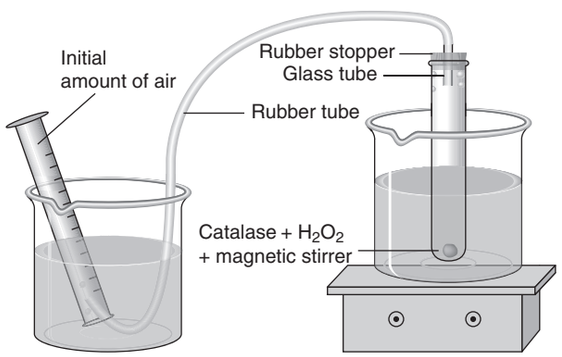

Figure 36.1
Enzyme rate measuring apparatus

E. Wait until 5 minutes have elapsed. Record the final volume of oxygen in the graduated cylinder.

F. Repeat steps B through E. Record both results and average them.

2. Double Substrate Concentration

A. Follow the directions outlined above except for step 1C. Instead, pour 10.0 mL of the catalase solution into the test tube and stopper.

3. Double Enzyme Concentration

A. Follow the directions outlined above except for step 1D. Instead, pour 10.0 mL of an 18% solution of H_2O_2 into the test tube and stopper.

4. Heat Effect

A. Place a test tube containing 5.0 mL of the catalase solution into a boiling-water bath for 5 minutes. Remove and let it cool.

B. Pour 5.0 mL of an 18% solution of H_2O_2 into another test tube and put it in the water bath at 35–37°C and stopper it. Record the initial amount of air in the graduated cylinder.

C. Open the stopper and add 5.0 mL of the catalase that had previously been heated in part 4A. Immediately stopper the test tube and begin recording the time. This step must be completed quickly so no oxygen is lost.

D. Wait until 5 minutes have elapsed. Record the final volume of oxygen in the graduated cylinder.

5. Acidic Conditions

A. Add 5 drops of 0.1 M HCl to a test tube containing 5.0 mL of the catalase solution.

B. Measure the activity of the enzyme by following the procedure above (part 1, steps B through E).

6. Inhibitor

A. Add 5 drops of 0.1 M $AgNO_3$ solution to a test tube containing 5.0 mL of the catalase solution.

B. Measure the activity of the enzyme by following the procedure above (part 1, steps B through E).

Name _____ Date _____ Section _____

Experiment 36

Advance Study Assignment

1. Calculate the rate of catalase if the final volume after 10 minutes is 34.5 mL. The initial volume was 4.2 mL.

2. Identify the substrate in the experiment.

3. What enzyme rate is expected for the boiled catalase?

4. What happens to a protein when it is denatured?

5. What is an enzyme and what is its function?

Name _____ Date _____ Section _____

Experiment 36

Work Page

Results

	Water bath Run 1	*Water bath Run 2*	*Double substrate conc.*	*Double enzyme conc.*	*Boiled catalase*	*Acidic condition*	*Inhibitor $AgNO_3$*
Final volume (mL, 5 min)							
Initial volume (mL)							
Volume of O_2 (mL)							
Rate of enzyme (mL/min)							

Average of rate for part 1 (water bath at 35–37°C). _____

Show all your calculations below:

Questions

1. Which conditions resulted in the largest enzyme rate?

2. Did boiling the enzyme and using an inhibitor decrease the rate of the enzyme to the same degree?

3. What rate was measured for the boiled catalase? Explain this result.

4. What rate was measured for the acidic catalase? Explain this result.

5. What rate was measured when the substrate concentration was doubled? Was this new rate expected?

Name *Date* *Section*

Experiment 36

Report Sheet

Results

	Water bath Run 1	*Water bath Run 2*	*Double substrate conc.*	*Double enzyme conc.*	*Boiled catalase*	*Acidic condition*	*Inhibitor $AgNO_3$*
Final volume (mL, 5 min)							
Initial volume (mL)							
Volume of O_2 (mL)							
Rate of enzyme (mL/min)							

Average of rate for part 1 (water bath at 35–37°C). _____

Show all your calculations below:

Questions

1. Which conditions resulted in the largest enzyme rate?

2. Did boiling the enzyme and using an inhibitor decrease the rate of the enzyme to the same degree?

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5. What rate was measured when the substrate concentration was doubled? Was this new rate expected?