CAROLINA

Student Guide

Name/Group # _____

Date _____

AP® Biology Laboratory 2
Enzyme Catalysis

Objectives

- Observe the action of an enzyme
- Determine the rate of an enzyme-catalyzed reaction
- Study the characteristics of an enzyme-mediated reaction
- Observe the effect of heat on enzyme activity

Background to Activity A

In this lab, you will study some characteristics of enzyme action. The specific reaction you will investigate is the decomposition of hydrogen peroxide by the enzyme, catalase. At room temperature, hydrogen peroxide very slowly decomposes into water and oxygen. The addition of catalase lowers the activation energy of the reaction until it proceeds swiftly at room temperature. At the end of the reaction, the catalase is unchanged and is available to catalyze the reaction of more hydrogen peroxide. Catalase, like most enzymes, is a protein. Its ability to form a complex with hydrogen peroxide is based on its molecular shape. Any factor that can alter the shape of a protein molecule can be expected to impact the ability of catalase to facilitate the decomposition of hydrogen peroxide.

Activity A: Observing the Reaction

Materials

H_2O_2 in labeled cup, catalase on ice, unlabeled 60-mL cup, test tube, test tube holder, test tube rack, syringe labeled H_2O_2, transfer pipets, glass rod, scalpel or other sharp instrument, piece of potato or liver, boiling water bath, *Wash Water* cup, *Waste* cup.

Caution: *You will be working with and around hot liquids. You will use a sharp scalpel or other instrument to cut the cube of potato or liver.*

Introduction

In Activity A, you will make your first observations of the interaction of catalase and hydrogen peroxide.

Procedure

Testing for enzyme activity

1. Use the labeled H_2O_2 syringe to transfer 10 mL of H_2O_2 into the unlabeled 60-mL cup. Use a transfer pipet to add 1 mL of catalase solution to the unlabeled cup. Observe for 30 to 60 seconds. Do you observe anything that indicates that a chemical change is taking place? If so, explain.

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2. On the basis of your observation of the reaction and your knowledge of chemistry, write a balanced equation for the reaction.

3. Give the following for this reaction:

   a. Substrate: _____

   b. Enzyme: _____

   c. Intermediate complex that formed (not observed): _____

   d. Product(s): _____

The effect of boiling on enzyme activity

1. Use the same transfer pipet (not the H_2O_2 syringe) to transfer 3–5 mL of catalase to a test tube. Place the test tube in a boiling water bath for five minutes.
2. While waiting, rinse your unlabeled cup. Use the H_2O_2 syringe to transfer 10 mL of H_2O_2 into the rinsed unlabeled cup. Use a clean, unused transfer pipet to add 1 mL of the boiled catalase to the unlabeled cup. Observe the results. What is the effect of boiling the catalase? Explain how boiling affects catalase activity.

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3. List at least three other factors that could affect the activity of the catalase and explain why they would have an effect.

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Testing for catalase in living tissue

1. Rinse your unlabeled 60-mL cup. Use a scalpel or other sharp instrument to cut a cube about one cm on a side from a piece of potato or liver. Place the cube in the unlabeled cup and macerate with a glass rod. **Caution:** *Do not use so much force that you break the rod.*
2. Use the H_2O_2 syringe to add 10 mL of H_2O_2 to the cup of tissue, and then observe. Does the tissue contain catalase? Explain your answer.

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Background to Activities B, C, and D

In Activity B, you will determine the amount of H_2O_2 initially present in solution. In Activity C, you will determine the rate at which H_2O_2 spontaneously decomposes at room temperature, and in Activity D, you will determine the rate at which catalase decomposes H_2O_2. In all of these activities, you will need to determine the amount (concentration) of H_2O_2 left in solution. You will make this determination by using a titration procedure. In a titration, you use a solution of known concentration to determine the concentration of an unknown. An example would be using a solution of 0.1 N (Normal) NaOH to determine the concentration of an unknown acid. To do the titration, you might put 5 mL of the acid in a beaker and add a pH indicator. The indicator is yellow in an acid but becomes green at a pH of 7 (neutral). Then you add 0.1 N NaOH drop-by-drop, stirring between drops until the solution in the beaker turns green and remains green. This is the endpoint of the titration. The NaOH has reacted with (neutralized) all of the acid in solution; therefore, the amount of NaOH used to reach the endpoint is directly proportional to the amount of acid that was in solution. Let us say that it took 25 mL of 0.1 N NaOH to titrate to the endpoint. Then the concentration of acid in solution is equivalent to 25 mL of 0.1 N NaOH.

In the following activities, you will use 2% potassium permanganate ($KMnO_4$) to titrate H_2O_2 in solution. As potassium permanganate (dark purple) is added to the H_2O_2 solution, the permanganate ion (MnO_4^-) is reduced and becomes colorless. When all the H_2O_2 in solution has reacted, the solution becomes a persistent pink or pale brown. This is the endpoint of the titration. You will use the volume of $KMnO_4$ needed to titrate to the endpoint as a measure of the amount of H_2O_2 present in the solution.

Titration Protocol

The titrations can be done with a 5- or 6-mL titration syringe (a syringe with a piece of Tygon® tubing attached) or with a buret. If you are using a buret, fill it with $KMnO_4$ before beginning. If using a titration syringe, draw in about 0.2 mL of air, then about 5 mL of $KMnO_4$. If you do not draw in the air, it will be almost impossible to read the level of $KMnO_4$ from the scale on the syringe.

1. Record the initial volume reading for the titration syringe or buret.
2. Add one drop of $KMnO_4$ to the solution in your titration cup. Gently swirl the cup until the purple color disappears.
3. Repeat Step 2 until the solution in the cup becomes a persistent pink or brown; that is, continue until the pink or brown color persists after swirling the cup. This is the endpoint of the titration.
4. Once you have reached the endpoint, record the final volume of $KMnO_4$.
5. Rinse the titration syringe by drawing up water from your *Wash Water* cup and expelling it into the *Waste* cup. Rinse your other equipment, also.

Activity B: Determining the Initial (Baseline) Amount of H_2O_2 in Solution

Materials

H_2O_2 in labeled cup, H_2SO_4 in labeled cup, $KMnO_4$ in labeled cup, cup labeled *Baseline*, cup labeled *Titration*, syringe labeled H_2O_2, syringe labeled H_2SO_4, syringe labeled *Transfer*, transfer pipet, titration syringe, *Wash Water* cup, *Waste* cup, distilled water in cup labeled dH_2O.

Caution: Use Extreme Care When Handling Acids. *H_2SO_4 can irritate or burn the eyes, skin, and mouth. Avoid all skin contact with this and other chemicals. Your teacher will instruct you about the proper safety procedures for handling hazardous materials. $KMnO_4$ can stain skin and clothes. H_2O_2 can discolor clothes.*

Introduction

In this activity, you will use the Titration Protocol to determine the initial amount of H_2O_2 present in solution. You will use this amount as a baseline for activities C and D.

Procedure

Preparation of Baseline Sample

1. Use the syringe labeled H_2O_2 to put 10 mL of H_2O_2 in the 60-mL plastic cup labeled *Baseline*.
2. Use the plastic transfer pipet to add 1 mL of distilled water from the dH_2O cup to the cup labeled *Baseline*. (Distilled water is used to replace the 1 mL of catalase that is added to the H_2O_2 in Activities A and D.)
3. Use the syringe labeled H_2SO_4 to add 10 mL of 1 M H_2SO_4 from the H_2SO_4 cup to the cup labeled *Baseline*.
4. Gently swirl the *Baseline* cup to mix the contents.
5. Use the 5- or 6-mL syringe labeled *Transfer* to remove 5 mL of solution from the cup labeled *Baseline* and put it in the cup labeled *Titration*. Rinse the *Transfer* syringe by drawing up some water from the *Wash Water* cup and expelling it into the *Waste* cup.
6. Titrate your 5-mL sample (refer to the Titration Protocol) to determine the baseline amount of H_2O_2. Record your data in Table 1 below.

Table 1: Baseline

Initial Volume	mL
Final Volume	mL
Δ Volume	mL

Δ Volume = change in volume
(Initial – Final) for titration syringes; (Final – Initial) for burets

7. You will use the value of Δ Volume from Table 1 as the baseline amount of H_2O_2 present in solution. Record the baseline below.

   Baseline = _____ mL

Activity C: The Uncatalyzed Rate of Decomposition of H_2O_2

Materials

H_2O_2 in labeled cup, H_2SO_4 in labeled cup, $KMnO_4$ in labeled cup, cup labeled *Uncatalyzed Decomposition*, cup labeled *Titration*, syringe labeled H_2O_2, syringe labeled H_2SO_4, syringe labeled *Transfer*, titration syringe, transfer pipet, *Wash Water* cup, *Waste* cup, distilled water in cup labeled dH_2O, cup labeled H_2O_2 *Overnight*.

Introduction

In this activity, you will determine the rate at which H_2O_2 spontaneously decomposes when exposed to room temperatures and ambient light for a 24-hour period.

Preparation

Use the H_2O_2 syringe to transfer 15 mL of H_2O_2 from the H_2O_2 cup to the 60-mL plastic cup labeled H_2O_2 *Overnight*. Store the cup uncovered at room temperature for approximately 24 hours.

Wait 24 hours before proceeding with Procedure Step 1, below.

Procedure

1. Use the syringe labeled H_2O_2 to transfer 10 mL of H_2O_2 from the H_2O_2 *Overnight* cup to the 60-mL plastic cup labeled *Uncatalyzed Decomposition*.
2. Use the transfer pipet to add 1 mL of distilled H_2O from the dH_2O cup to the cup labeled *Uncatalyzed Decomposition*. (Distilled water is used to replace the 1 mL of catalase that is added to the H_2O_2 in activities A and D.)
3. Use the 10- or 12-mL syringe labeled H_2SO_4 to add 10 mL of 1 M H_2SO_4 from the labeled H_2SO_4 cup to the cup labeled *Uncatalyzed Decomposition*.
4. Gently swirl the *Uncatalyzed Decomposition* cup to mix the contents.
5. Use the 5- or 6-mL syringe labeled *Transfer* to remove 5 mL of the reaction mixture and put it in the cup labeled *Titration*. Rinse the *Transfer* syringe by drawing up water from the *Wash Water* cup and expelling it into the *Waste* cup.
6. Titrate your sample (refer to the Titration Protocol) to determine the amount of H_2O_2 left in solution after 24 hours. Record your data in Table 2 below.

Table 2: Uncatalyzed Decomposition

Initial Volume	mL
Final Volume	mL
Δ Volume	mL

The amount of H_2O_2 that has decomposed is equal to the Baseline determined in Activity B minus Δ Volume from Table 2. The amount of H_2O_2 that spontaneously decomposed at room temperature was _____ mL/24 hours.

Activity D: The Catalyzed Rate of Decomposition of H_2O_2

Materials

H_2O_2 in labeled cup, H_2SO_4 in labeled cup, $KMnO_4$ in labeled cup, catalase on ice, cup labeled *Titration*, syringe labeled *H_2O_2*, syringe labeled *H_2SO_4*, syringe labeled *Transfer*, titration syringe, transfer pipet, *Wash Water* cup, *Waste* cup, and cups labeled respectively *10 sec*, *30 sec*, *60 sec*, *120 sec*, and *180 sec*.

Introduction

In this activity, you will determine the rate at which catalase decomposes H_2O_2. To do this, you will add catalase to a measured amount of H_2O_2, and after a predetermined lapse of time (10 sec, 30 sec, 60 sec, etc.) you will add H_2SO_4 to halt the reaction. Once the reaction is stopped, you will titrate to determine the amount of H_2O_2 left in solution. You will subtract this amount from the baseline that you determined in Activity B to determine the amount of H_2O_2 decomposed over the timed interval. You will graph the resulting data and calculate the rate at which catalase was decomposed by catalase.

Read the following directions thoroughly before beginning.

Procedure

Line up the 60-mL plastic cups with the labels *10 sec*, *30 sec*, *60 sec*, *120 sec*, and *180 sec*. Use the syringe labeled *H_2O_2* to add 10 mL of H_2O_2 to each cup.

Before beginning each test, premeasure 10 mL of H_2SO_4 in the *H_2SO_4* syringe, so you can stop the reaction precisely when required.

One student should add the reagents while another student keeps the time.

For the 10-second time trial:

1. Use the transfer pipet to add 1 mL of catalase extract to the *10 sec* cup.
2. Gently swirl the *10 sec* cup to mix the contents.
3. At 10 seconds, add 10 mL of H_2SO_4 to the cup.

For each time trial, repeat steps 1 through 3, as above, but allow the reactions to proceed for 30, 60, 120, or 180 seconds, as assigned, before adding the 10 mL of H_2SO_4.

Titrating the Time Trials

Use the rinsed syringe to remove 5 mL of solution from the *10 sec* reaction cup and place it in the plastic cup labeled *Titration*. Rinse the *Transfer* syringe by drawing in water from the *Wash Water* cup and expelling it into the *Waste* cup. Titrate according to the Titration Protocol. If you overshoot an endpoint, you will have enough solution for a second titration. Record your results in Table 3. Repeat for each assigned time trial.

Table 3: Catalyzed Decomposition

	10 Sec	30 Sec	60 Sec	120 Sec	180 Sec
Initial Volume					
Final Volume					
Δ Volume					
H_2O_2 Decomposed (Baseline – Δ Volume)					

Analysis of Results, Activity D: The Catalyzed Rate of Decomposition of H_2O_2

1. Why was it necessary to determine a baseline for the H_2O_2?

2. Why did the addition of H_2SO_4 stop the reaction?

3. Graph your data from Table 3 for H_2O_2 decomposed by catalase. Title the graph and supply the following information:

   a. The independent variable is _____.

   b. The dependent variable is _____.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

4. What does the line on your graph represent?

5. What does your graph tell you about the rate of the reaction over time?

6. When an enzyme is mixed with an excess of its substrate, it will react with the substrate at a constant rate. The reaction will continue at the same constant rate as long as the substrate is present in excess. As the substrate is consumed by the reaction, there will be less substrate available to react with the enzyme, and the rate of the reaction will slow. The rate of the reaction while the substrate is present in excess is called the initial rate. At a given temperature and pH, the initial rate of a given enzyme/substrate reaction will always be the same.

   Look at your graph. The initial rate of the catalase/H_2O_2 reaction is represented by the linear (straight) portion of the line on your graph. Using your graph and data, calculate the rate of the catalase/H_2O_2 reaction for the following time intervals: 0–10 sec, 10–30 sec, 30–60 sec, 60–120 sec, and 120–180 sec. This is easily done by referencing your graph. For example, suppose your graph shows that 0.1 mL of H_2O_2 had been decomposed at 10 seconds.

$$\text{Reaction Rate (mL/sec)} = \frac{y_2 - y_1}{x_2 - x_1} \quad \text{or} \quad \frac{\Delta y}{\Delta x}$$

Where $y_1 = 0.0$ mL
$y_2 = 0.1$ mL
$x_1 = 0$ sec
$x_2 = 10$ sec

then

$$\text{Reaction Rate} = \frac{0.1 \text{ mL}}{10 \text{ sec}} = 0.01 \text{ mL/sec}$$

Calculate the results for your data. Record your results in a Data Table that you construct below.

7. On the basis of your results, what is the initial rate of the catalase/H_2O_2 reaction?

Title: _____