Enzyme Catalysis

Background

In general, enzymes are proteins produced by living cells; they act as catalysts in biochemical reactions. A catalyst affects the rate of a chemical reaction. One consequence of enzyme activity is that cells can carry out complex chemical activities at relatively low temperatures.

In an enzyme-catalyzed reaction, the substance to be acted upon, the substrate (S), binds reversibly to the active site of the enzyme (E). One result of this temporary union is a reduction in the energy required to activate the reaction of the substrate molecule so that the products (P) of the reaction are formed. In summary:

$$E + S \rightarrow ES \rightarrow E + P$$

Note that the enzyme is not changed in the reaction and can be recycled to break down additional substrate molecules. Each enzyme is specific for a particular reaction because its amino acid sequence is unique and causes it to have a unique three-dimensional structure. The active site is the portion of the enzyme that interacts with the substrate, so that any substance that blocks or changes the shape of the active site affects the activity of the enzyme. A description of several ways enzyme action may be affected follows:

1. **Salt Concentration**. If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract each other. The enzyme will denature and form an inactive precipitate. If, on the other hand, the salt concentration is very high, normal interaction of charged groups will be blocked, new interactions will occur, and again the enzyme will precipitate. An intermediate salt concentration, such as that of human blood (0.9%) or cytoplasm, is the optimum for many enzymes.
2. **pH**. pH is a logarithmic scale that measures the acidity, or H+ concentration, in a solution. The scale runs from 0 to 14 with 0 being highest in acidity and 14 lowest. When the pH is less than 7, a solution is said to be acidic; if the pH is 7, the solution is neutral; and if the pH is greater than 7, the solution is basic. Amino acid side chains contain functional groups, such as -COOH and -NH2, that readily gain or lose H+ ions. As the pH is lowered an enzyme will tend to gain H+ ions, and eventually enough side chains will be affected so that the enzyme's conformation is disrupted. Likewise, as the pH is raised, the enzyme will lose H+ ions and eventually lose its active shape. Many enzymes perform optimally in the neutral pH range and are denatured at either an extremely high or low pH. Some enzymes, such as pepsin, which acts in the human stomach where the pH is very low, have a low pH optimum.
3. **Temperature**. Generally, chemical reactions speed up as the temperature is raised. As the temperature increases, more of the reacting molecules have enough kinetic energy to undergo the reaction. Since enzymes are catalysts for chemical reactions, enzyme reactions also tend to go faster with increasing temperature. However, if the temperature of an enzyme-catalyzed reaction is raised still further, a temperature optimum is reached; above this value the kinetic energy of the enzyme and water molecules is so great that the conformation of the enzyme molecules is disrupted. The positive effect of speeding up the reaction is now more than offset by the negative effect of changing the conformation of more and more enzyme molecules. Many proteins are denatured by temperatures around 40-50˚C, but some are still active at 70-80˚C, and a few even withstand boiling.
4. **Activations and Inhibitors**. Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of the reaction it is an activator, and if it decreases the reaction rate it is an inhibitor. These molecules can regulate how fast the enzyme acts. Any substance that tends to unfold the enzyme, such as an organic solvent or detergent, will act as an inhibitor. Some inhibitors act by reducing the disulfide bridges that stabilize the enzyme's structure. Many inhibitors act by reacting with side chains in or near the active site to change its shape or block it. Many well-known poisons, such as potassium cyanide and curare, are enzyme inhibitors that interfere with the active site of critical enzymes.

The enzyme used in this lab, catalase, has four polypeptide chains, each composed of more than 500 amino acids. This enzyme is ubiquitous in aerobic organisms. One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide formed as a byproduct of metabolic processes. Catalase might also take part in some of the many oxidation reactions that occur in all cells. The primary reaction catalyzed by catalase is the decomposition of H_2O_2 to form water and oxygen:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2\ (gas)$$

In the absence of catalase, this reaction occurs spontaneously but very slowly. Catalase speeds up the reaction considerably. In this experiment, a rate for this reaction will be determined.

Much can be learned about enzymes by studying the kinetics (particularly the changes in rate) of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped.

In this experiment, the disappearance of the substrate, H_2O_2, is measured using the general procedure outlined below:

1. A purified catalase extract is mixed with substrate (H_2O_2) in a container. The enzyme catalyzes the breakdown of the substrate.
2. Before all of the substrate is reacted with, sulfuric acid (H_2SO_4) is added to stop the catalytic activity. The acid lowers the pH of the solution, thereby denaturing the enzyme, disallowing it from functioning.
3. After the reaction is halted, the remaining hydrogen peroxide in the container is measured using a titration procedure. To assay (measure) this quantity, potassium permanganate is used. Potassium permanganate ($KMnO_4$) in the presence of H_2O_2 and H_2SO_4 reacts as follows:

$$5\ H_2O_2 + 2\ KMnO_4 + 3\ H_2SO_4 \rightarrow K_2SO_4 + 2\ MnSO_4 + 8\ H_2O + 5\ O_2$$

Potassium permanganate, which has a distinct dark pink/purple color, when reacted with hydrogen peroxide produces a colorless solution. Once all hydrogen peroxide has been reacted with, a persistent pink or brown color remains. The amount of $KMnO_4$ used is proportional to the amount of H_2O_2 in the solution.

Objective(s)

- ✓ to observe the function of an enzyme
- ✓ to understand the relationship between the structure and function of an enzyme
- ✓ to understand the concept of the initial reaction rates of enzymes
- ✓ to measure the amount of substrate remaining after a reaction using a titration procedure
- ✓ to explain why the rate of an enzyme-catalyzed reaction changes over time

Materials

- medicine cups (x7)
- transfer pipette
- "H_2SO_4" syringe
- "X-FER" syringe
- "$KMnO_4$" syringe
- small flask
- 80 mL H_2SO_4
- 40 mL $KMnO_4$
- 100 mL H_2O_2
- 7 mL catalase
- timer

★ *Do not rest any of the syringes in the solutions! Lay them flat on the lab bench on a paper towel!*

Pre-Lab Questions

Answer the following questions on your lab paper. For actual questions, you must either write out the questions, or include the questions in your responses. Be sure to use complete sentences and show your work for math problems.

1. Define the following terms:
   a. catalyst
   b. substrate
   c. product
2. Why might a change in temperature of pH alter an enzyme's ability to function properly?
3. What is the difference between: catalyst, catalysis, and catalase?
4. What is the purpose of the sulfuric acid in **Part C** of the activity?
5. Why must you not discard any of your solutions until you've completed the lab?

6. What color is a solution of potassium permanganate? What color is a solution of potassium permanganate and hydrogen peroxide?
7. From 30 to 60 seconds of a reaction, the amount of product created increases by 5 µmoles. Calculate the rate of the reaction for that time interval.

✓ Use a ruler to recreate the Data Tables below neatly on your lab paper, and be sure they are drawn approximately the same size

Safety

★ *WARNING – 1.0 M sulfuric acid is a skin, eye, and mucous membrane irritant*

★ *WARNING – Potassium permanganate readily stains skin and clothing*

Procedure – Part A: Base Line Assay

1. Pour 10 mL of 1.5% H_2O_2 into a clean medicine cup.
2. Use a transfer pipette to add 1 mL of water to the H_2O_2.
3. Use the "H_2SO_4" syringe to add 10 mL of sulfuric acid to the medicine cup.
4. Carefully mix the solution.
5. Use the "X-FER" syringe to remove a 5 mL sample of solution from the medicine cup and deposit it into a small flask.
6. Use the appropriate syringe, draw up a full syringe of $KMnO_4$, making sure to study and understand how to read the graduations on the syringe.
7. Over a white background, add – one drop at a time – potassium permanganate to the flask. Be sure to swirl the flask between each drop. The endpoint of the titration is met when a persistent pink or brown color is achieved.
8. Collect the base line values from all other lab groups and determine the average base line value. Remember, the amount of $KMnO_4$ is proportional to the amount of H_2O_2 in the initial solution. Record the average amount of H_2O_2 in **Data Table 1** (row a).

Procedure – Part B: Uncatalyzed Rate of H_2O_2 Decomposition

To determine the rate of spontaneous conversion of H_2O_2 to H_2O and O_2 in an uncatalyzed reaction, put a small quantity of 1.5% H_2O_2 – about 15 mL – into a small beaker. Set it in an area where it can remain undisturbed at room temperature for approximately 24 hours. After that time has elapsed, conduct a titration by repeating steps #**2 – 8** from **Part A**. Complete **Data Table 1**.

Data Table 1

Baseline H_2O_2 (from **Part A**)	mL

Amount of $KMnO_4$ titrant (from **Part B**)	mL
H_2O_2 spontaneously decomposed = Baseline – $KMnO_4$	mL
% H_2O_2 spontaneously decomposed*	%

* % H_2O_2 spontaneously decomposed = [(baseline – H_2O_2 spontaneously decomposed)/baseline] x 100

Procedure – Part C: Enzyme-Catalyzed Rate of H_2O_2 Decomposition

In this experiment, you will determine the rate at which a 1.5% H_2O_2 solution decomposes when catalyzed by the purified catalase extract. To do this, you should determine how much H_2O_2 has been consumed after 10, 30, 60, 90, 120, 180, and 360 seconds.

1. 10 seconds
   a. Pour 10 mL of 1.5% H_2O_2 into a clean medicine cup.
   b. Use a transfer pipette to add 1 mL of catalase enzyme.
   c. Swirl gently for 10 seconds.
   d. At 10 seconds, use the "H_2SO_4" to add 10 mL of sulfuric acid to the medicine cup.
   e. Follow steps #**5 – 7** in **Part A** to conduct the titration and record the results in **Data Table 2**.
2. Repeat the steps outlined above for the following time intervals: 30, 60, 90, 120, 180, and 360 seconds.

   ★ NOTE: Each time, use the "X-FER" syringe to remove a 5 mL sample and conduct a titration in a small flask to determine the amount of H_2O_2 remaining in the sample. Do not discard any of the solutions until the entire lab is completed in the event that the endpoint of the titration – a persistent pink or brown color – is achieved. Be sure to dispose of the flask solutions properly and rinse it thoroughly between titrations.

Data Table 2

$KMnO_4$ (mL)	Time (sec)						
	10	30	60	90	120	180	360
a) Base Line (from **Part A**)							
b) Amount of $KMnO_4$ Consumed							
c) Amount of H_2O_2 Used (a – b)							
d) Class Average H_2O_2 Used							

Clean Up

- ✓ chemical waste: titration solution, excess reagents
- ✓ rinse (no need to dry): beakers, flask, medicine cups, syringes (remove plunger to rinse)
- ✓ trash: transfer pipette
- ✓ everything returned to its original location

Results & Analysis

Answer the following questions on your lab paper. For actual questions, you must either write out the questions, or include the questions in your responses. Be sure to use complete sentences and show your work for math problems.

1. Create an appropriate graph to plot the class average H_2O_2 used from **Data Table 2** (row d).
2. Determine the rate of reaction for each of the following time intervals:
   a. 0 – 10 seconds
   b. 10 – 30 seconds
   c. 30 – 60 seconds
   d. 60 – 120 seconds
   e. 120 – 180 seconds
   f. 180 – 360 seconds
3. When is the rate of reaction highest? Explain.
4. When is the rate of reaction lowest? Explain.
5. Explain the inhibiting effect of sulfuric acid on the function of catalase. Relate this to enzyme structure and chemistry.
6. Predict the effect that lowering the temperature slightly would have on the rate of enzyme activity. Explain your prediction in terms of the nature of chemical reactions.