AP Biology Lab #2: Enzyme Catalysis

OVERVIEW:
In this lab you will:

1. Observe the conversion of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) to water and oxygen gas by the enzyme catalase.
2. Measure the amount of oxygen generated and calculate the rate of the enzyme-catalyzed reaction.

OBJECTIVES:
Before doing this lab you should understand:

A) The general functions and activities of enzymes.
B) The relationship between the structure and function of enzymes.
C) The concept of initial reaction rates of enzymes.
D) How the concept of free energy relates to enzyme activity.
E) That changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions.
F) Understand the terms catalyst, catalysis, and catalase.

After doing this lab you should be able to:

A) Measure the effects of changes in temperature, pH, enzyme concentration, and substrate concentration on reaction rates of an enzyme-catalyzed reaction in a controlled experiment.
B) Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

INTRODUCTION:

In this laboratory you will observe the conversion of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) to water and oxygen gas by the enzyme liver catalase. You will then measure the amount of oxygen generated and calculate the rate of the enzyme-catalyzed reaction.

Organisms use enzymes as catalysts to reduce the amount of energy required to initiate a chemical reaction, and, therefore, to allow the reaction to proceed at temperatures which will not destroy cells. The enzyme liver catalase has a molecular weight of approximately 240,000 Daltons and contains 4 polypeptide chains, each composed of more than 500 amino acid residues. Liver catalase is used by aerobic organisms to convert hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), a poisonous compound formed by many cellular reactions, into water. Liver catalase might also take part in some of the many oxidation reactions going on in all cells. In this laboratory you will use a chemical titration to measure and then calculate the rate of conversion of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) to water and oxygen gas using the enzyme liver catalase. The primary reaction catalyzed by catalase is the decomposition of H\textsubscript{2}O\textsubscript{2} to form water and oxygen.

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \text{(gas)} \]

In this experiment, a rate for this reaction will be determined. Enzymes are proteins produced by living cells; they act as catalysts in biochemical reactions. A catalyst affects the rate of a chemical reaction but is NOT CHANGED in the reaction and can recycle 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. One benefit of enzyme catalysis is that the cell can carry out complex chemical activities at a relatively low temperature that will not harm an organism. 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 HI concentration in a solution. The scale runs from 0 to 14 with 0 being highest in acidity and 14 lowest. When the pH is in the range of 0-7, a solution is said to be acidic; if the pH is around 7, the solution is neutral; and if the pH is in the range of 7-14, the solution is basic. Amino acid side chains contain groups such as -COOH and -NH\textsubscript{2} that readily gain or lose H\textsuperscript{+} ions. As the pH is lowered an enzyme will tend to gain H\textsuperscript{+} ions, and eventually enough side chains will be affected so that the enzyme’s shape is disrupted. Likewise, as the pH is raised, the enzyme will lose H\textsuperscript{+} 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 information 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 -S-S- 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.

In biochemical reactions the enzyme, $E$, combines reversibly with its specific substrate, $S$, to form a complex, $ES$. One result of this temporary union is a reduction in the energy required to activate the reaction of the substrate molecule so that $P$, the products of the reaction, are formed.

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

Much can be learned about enzymes by studying the kinetics (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. If the amount of product formed is measured at 30-sec intervals and this quantity is plotted on a graph, a curve like the one in the figure opposite is obtained.

Observe the solid line for this reaction. At time 0 there is no product. After 30 seconds, 5 µmoles have been formed; after 1 minute, 10; after 2 minutes, 20. The rate of this reaction could be given as 10 µmoles of product formed per minute for this initial period. Note, however, that by the third and fourth minutes, only about 5 additional µmoles of product have been formed. During the first 3 minutes, the rate is constant. From the third minute through the eighth minute, the rate is changing—it is slowing down. For each successive minute after the first 3 minutes, the amount of product formed in that interval is less than in the preceding minute. From the seventh minute onward, the reaction rate is very slow, as the substrate is used up.

Suppose you wanted to compare the effectiveness of catalase obtained from potato with that of catalase obtained from liver. Would you want to compare the two reactions during the first few minutes when the rate is constant or later when the rates are changing? It is best to compare the reactions when the rates are constant. In the first few minutes of an enzymatic reaction such as this, the number of substrate molecules is usually so large compared to the number of enzyme molecules that changing the substrate concentration does not (for a short period at least) affect the number of successful collisions between substrate and enzyme. During this early period, the enzyme is acting on substrate molecules at a constant rate. The slope of the graph line during this early period is called the **initial velocity** of the reaction. The initial velocity (or rate) of any enzyme-catalyzed reaction is determined by the characteristics of the enzyme molecule. It is always the same for an enzyme and its substrate as long as temperature and pH are constant and the substrate is present in excess.

The rate of the reaction, therefore, is the slope of the linear portion of the curve. To determine a rate, pick any two points on the straight-line portion of the curve. Divide the difference in the amount of product formed between these two points by the difference in time between them. The result will be the rate of the reaction, which, if properly calculated, can be expressed as µmoles product/sec.
The rate then is: $\mu$moles$_2 - \mu$moles$_1 / t_2 - t_1$ or from the graph $\Delta y / \Delta x$

Procedure:

General Procedure:
In this experiment, we will be measuring the rate of a chemical reaction through the disappearance of substrate (in this case, H$_2$O$_2$) as follows:

1. A raw liver catalase extract is mixed with substrate (H$_2$O$_2$) in a beaker. The enzyme catalyzes the conversion of H$_2$O$_2$ to H$_2$O and O$_2$.

2. Before all of the H$_2$O$_2$ is converted to H$_2$O and O$_2$, the reaction is stopped by adding sulfuric acid (H$_2$SO$_4$). The H$_2$SO$_4$ lowers the pH, denatures the enzyme, and thereby stops the enzyme's catalytic activity.

3. After the reaction is stopped, the amount of substrate (H$_2$O$_2$) remaining in the beaker is measured. To assay this quantity, potassium permanganate is used. Potassium permanganate (KMnO$_4$), in the presence of H$_2$O$_2$ and H$_2$SO$_4$, reacts as follows:

$$H_2O_2 + KMnO_4 + H_2SO_4 \rightarrow K_2SO_4 + MnSO_4 + H_2O + O_2 \text{ (unbalanced equation)}$$

H$_2$O$_2$ is essential for this reaction. Once all the H$_2$O$_2$ has reacted, any more KMnO$_4$ added will be in excess and will not be decomposed. The addition of excess KMnO$_4$ causes the solution to have a permanent pinkish brown color. Therefore, the amount of H$_2$O$_2$ remaining is determined by adding KMnO$_4$ until the KMnO$_4$ color no longer disappears and the whole mixture stays a faint pinkish brown, permanently. Add no more KMnO$_4$ after this point. The amount of KMnO$_4$ added is a proportional measure of the amount of H$_2$O$_2$ remaining (1 molecule of KMnO$_4$ decomposes 2.5 molecules of H$_2$O$_2$).

PART 1: Test of Catalase Activity

To observe the reaction to be studied, transfer 10 ml of 1.5% (0.44-M H$_2$O$_2$) into a 50 ml glass beaker and add 1 ml of the liver catalase solution. The bubbles coming from the reaction mixture is O$_2$ that results from the breakdown of H$_2$O$_2$ by catalase. How could you show that the gas evolved is O$_2$?

To demonstrate the effect of boiling on enzymatic activity, transfer 5 ml of purified catalase extract to a test tube and place it in a boiling water bath for 5 minutes. Transfer 10 ml of 1.5% H$_2$O$_2$ into a 50-ml glass beaker and add 1 ml of the boiled catalase solution. How does the reaction compare to the unboiled catalase?

PART 2: The Base Line Assay
To determine the amount of H$_2$O$_2$ initially present in a 1.5% solution, you need to perform all the steps of the procedure without adding catalase (enzyme) to the reaction mixture. This amount is known as the baseline and is an index of the initial concentration of H$_2$O$_2$ in solution. In any series of experiments, a baseline should be established first.

**Procedure for Establishing a Baseline**

1. Put 10 ml of 1.5% H$_2$O$_2$ in a beaker.
2. Add 1 ml of H$_2$O (instead of enzyme solution).
3. Add 10 ml of H$_2$SO$_4$ (1.0-M). USE EXTREME CARE IN HANDLING ACIDS.
4. Use a stirring rod and mix well.
5. Remove 5 ml and assay for the amount of H$_2$O$_2$ present. Use a burette to add KMnO$_4$ a drop at a time to the solution until a persistent pinkish brown color is obtained. Remember to gently swirl the solution after adding each drop. Check to be sure that you understand the calibrations on the burette. Record below.

   **Note:** Handle KMnO$_4$ with care. Avoid contact with skin (it stains you brown for several days) and eyes.

   Remember, the amount of KMnO$_4$ used is proportional to the amount of H$_2$O$_2$ that was in the solution. Record your results in a data table.

**PART 3: The Uncatalyzed Rate of H$_2$O$_2$ Decomposition**

1. To determine the rate of spontaneous conversion of H$_2$O$_2$ to H$_2$O and O$_2$ in an uncatalyzed reaction, transfer approximately 15 ml of 1.5% H$_2$O$_2$ to a 50-ml glass beaker.
2. Store it uncovered at room temperature for approximately 24 hours.
3. Repeat steps 1-5 from Part 2 to determine the proportional amount of H$_2$O$_2$ still present (for ease of calculation assume Table 1.

<table>
<thead>
<tr>
<th>KMnO$_4$ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final reading</td>
</tr>
<tr>
<td>Initial reading</td>
</tr>
<tr>
<td>Amount of KMnO$_4$ used</td>
</tr>
<tr>
<td>Amount of H$_2$O$_2$ spontaneously decomposed in 24 hours</td>
</tr>
</tbody>
</table>

**PART 4: An Enzyme-Catalyzed Rate of H$_2$O$_2$ T$_2$ - t$_1$ Decomposition**

In this experiment, you will determine the rate at which a 1.5% H$_2$O$_2$ solution decomposes when catalyzed by the
liver catalase extract. To do this, you should determine how much H₂O₂ has been consumed after 10, 30, 60, 120, and 180 seconds.

Procedure for a Time-Course Determination

10 seconds

1. Put 10 ml of 1.5%H₂O₂ in a 50-ml glass beaker.
2. Add 1 ml of catalase extract.
3. Swirl gently for 10 seconds.
4. At 10 seconds, add 10 ml of H₂SO₄ (10 M)

30 seconds, 60, 120, and 180 seconds

1. Each time, repeat steps 1 through 4, as above, except allow the reactions to proceed for 60, 120, and 180 seconds, respectively.

2. Each time remove 5 ml and assay for the amount of H₂O₂ remaining. Use a burette to add KMNO₄ a drop at a time, to the solution until a persistent pinkish brown color is obtained. Should the end point be overshoot, there is sufficient sample left to repeat the titration. Record your results in Table 2.

* Remember that the baseline tells how much H₂O₂ is in the initial 5 ml sample. The difference between the initial and final readings tells how much H₂O₂ is left after the enzyme-catalyzed reaction. The shorter the reaction time, the more H₂O₂ remains and therefore the more KMNO₄ will be used in titrating.

3. Graph liver catalase activity as a function of time for your data by plotting the amount of H₂O₂ used on the y-axis and time (in seconds) on the x-axis.

4. From the formula described earlier, determine the initial rate of the reaction and the rates between each of the time points.

Table 2.

<table>
<thead>
<tr>
<th>KMNO₄ (ml)</th>
<th>TIME (sec)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
</tr>
<tr>
<td>Final reading</td>
<td></td>
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<tr>
<td>Initial reading</td>
<td></td>
</tr>
<tr>
<td>Amount of KMNO₄ used</td>
<td></td>
</tr>
<tr>
<td>Amount of H₂O₂ used</td>
<td></td>
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</tbody>
</table>

DISCUSSION:

1. From Part 1, discuss how you could show that the gas evolved is O₂?
2. From Part 1, how did boiling the liver catalase affect the rate of reaction? What was occurring?
3. Based on facts related to enzyme structure and chemistry, explain the inhibiting effect of sulfuric acid on the function of liver catalase.
4. After calculating your reaction rates for both the catalyzed and spontaneously decomposed reactions, discuss the trend.
5. You need to refer again to the most prominent errors already detailed in your data analysis.
6. Be sure to make references to what was previously expected (from your introduction)