Enzyme Activity Lab

**Essential Question:** How do abiotic or biotic factors influence the rates of enzymatic reactions?

**Background**

Enzymes are the catalysts of biological systems. They speed up chemical reactions in biological systems by lowering the activation energy, the energy needed for molecules to begin reacting with each other. Enzymes do this by forming an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. The enzyme’s active site is very selective, allowing only certain substances to bind. If the shape of an enzyme is changed in any way, or the protein denatured, then the binding site also changes, thus disrupting enzymatic functions.

Enzymes are fundamental to the survival of any living system and are organized into a number of groups depending on their specific activities. Two common groups are catabolic enzymes (“*cata*” or “ket” from the Greek “to break down”) — for instance, amylase breaks complex starches into simple sugars — and anabolic enzymes (“*a*” or “*an*” from the Greek “to build up”). (You may know this second word already from stories about athletes who have been caught using anabolic steroids to build muscle.)

Catalytic enzymes, called proteases, break down proteins and are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin. Bromelain often is an ingredient in commercial meat marinades. Papain is an enzyme that comes from papaya and is used in some teeth whiteners to break down the bacterial film on teeth. People who are lactose intolerant cannot digest milk sugar (lactose); however, they can take supplements containing lactase, the enzyme they are missing. All of these enzymes hydrolyze large, complex molecules into their simpler components; bromelain and papain break proteins down to amino acids, while lactase breaks lactose down to simpler sugars.

Anabolic enzymes are equally vital to all living systems. One example is ATP synthase, the enzyme that stores cellular energy in ATP by combining ADP and phosphate. Another example is rubisco, an enzyme involved in the anabolic reactions of building sugar molecules in the Calvin cycle of photosynthesis.

To begin this investigation, you will focus on the enzyme peroxidase obtained from a turnip, one of numerous sources of this enzyme. Peroxidase is one of several enzymes that break down peroxide, a toxic metabolic waste product of aerobic respiration. Using peroxidase, you will develop essential skills to examine your own questions about enzyme function.

Later, you will have an opportunity to select an enzyme, research its properties and mode of reaction, and then design an experiment to explore its function. The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the levels of protein structure, energy transfer, abiotic and biotic influences on molecular structure, entropy and enthalpy, and the role of enzymes in maintaining homeostasis.

**Learning Objectives**

- To understand the relationship between enzyme structure and function
- To make some generalizations about enzymes by studying just one enzyme in particular
- To determine which factors can change the rate of an enzyme reaction
- To determine which factors that affect enzyme activity could be biologically important

**General Safety Precautions**

Follow general laboratory safety procedures. Wear proper footwear and safety goggles. Use proper pipetting techniques, and use pipette pumps, syringes, or rubber bulbs. Never pipette by mouth! Dispose of any broken glass in the proper container. Since the concentrations of the reactive materials in this laboratory are environmentally friendly (0.1% hydrogen peroxide and 0.3% guaiacol), they can be rinsed down a standard laboratory drain. The concentrations used here are deemed to be safe by all chemical standards, but recall that any compound has the potentiality of being detrimental to living things and the environment. When you develop your individual investigations you must always consider the toxicity of materials used.
Key Vocabulary
- **Baseline** is a universal term for most chemical reactions. In this investigation the term is used to establish a standard for a reaction. Thus, when manipulating components of a reaction (in this case, substrate or enzyme), you have a reference to help understand what occurred in the reaction. The baseline may vary with different scenarios pertinent to the design of the experiment, such as altering the environment in which the reaction occurs. In this scenario, different conditions can be compared, and the effects of changing an environmental variable (e.g., pH) can be determined.

- **Rate** can have more than one applicable definition because this lab has two major options of approach, i.e., using a color palette and/or a spectrophotometer to measure percent of light absorbance. When using a color palette to compare the change in a reaction, you can infer increase, decrease, or no change in the rate; this inference is usually called the relative rate of the reaction. When using a spectrophotometer (or other measuring devices) to measure the actual percent change in light absorbance, the rate is usually referred to as absolute rate of the reaction. In this case, a specific amount of time can be measured, such as 0.083 absorbance/minute.

The Investigations

**Experiment 1: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline**

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes.

The general reaction can be depicted as follows:

\[
\text{Enzyme} + \text{Substrate} \rightarrow \text{Enzyme-Substrate Complex} \rightarrow \text{Enzyme} + \text{Product(s)} + \Delta G
\]

For this investigation the specific reaction is as follows:

\[
\text{Peroxidase} + \text{Hydrogen Peroxide} \rightarrow \text{Complex} \rightarrow \text{Peroxidase} + \text{Water} + \text{Oxygen}
\]

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \text{ (gas)}
\]

Notice that the peroxidase is present at the start and end of the reaction. Like all catalysts, enzymes are not consumed by the reactions. To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In a decomposition reaction of peroxide by peroxidase (as noted in the above formula), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the actual volume of oxygen gas released or by using an indicator. In this experiment, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen to form tetraguaiacol, which is brownish in color. The greater the amount of oxygen gas produced, the darker brown the solution will become.

The color change that occurs in this investigation will be recorded as a change in absorbency using the Vernier Colorimeter probes. To correctly use a Colorimeter cuvette, remember:
- All cuvettes should be wiped clean and dry on the outside with a piece of lens paper.
- Handle cuvettes only by the top edge of the ribbed sides.
- All solutions should be free of bubbles.
- Always position the cuvette with its reference mark (blue pencil) facing toward the white reference mark at the right of the cuvette slot on the Colorimeter.

Materials
- Turnip peroxidase
- 0.1% hydrogen peroxide (H₂O₂)
- Guaiacol
- Distilled water
- 2 test tubes
- Test tube rack
- Wax pencil
- 3.5 mL cuvette
- Timer
- 10 mL syringes
- 0.5 mL pipets
- Vernier Colorimeter probe system
Procedure:

Step 1: Wear your goggles (over your eyes) throughout the entire experiment.

Step 2: Be sure that you have all of the materials listed above. If not, get what you need from your instructor.

Step 3: Calibrate the Colorimeter.
   a. Place the blank ("_") in the cuvette slot of the Colorimeter and close the lid.
   b. Set the dial on the Colorimeter to 0% T.
   c. Click Experiment > Calibrate > LabPro: 1 CH1: Colorimeter
      • Check the One Point Calibration box and click Calibrate Now.
      • Enter “0.0” in the box under Reading 1 and click Keep.
      • Set the dial on the Colorimeter to Blue (470 nm). Leave it on this setting for the rest of the lab.
      • Click Experiment > Calibrate > LabPro: 1 CH1: Colorimeter
      • Check the One Point Calibration box and click Calibrate Now.
      • Enter “100.0” in the box under Reading 1 and click Keep.

Step 4: Determine the baseline.
   a. Using two test tubes, label one “substrate” and the other “enzyme.”
      Substrate tube: 7 mL of distilled water, 0.5 mL of H\textsubscript{2}O\textsubscript{2}, and 0.5 mL guaiacol (total volume 8 mL)
      Enzyme tube: 6 mL of distilled water and 2 mL of peroxidase (total volume 8 mL)
   b. Combine the materials of the substrate and enzyme tubes. Mix the tubes twice and quickly pour some of the mixture into a Colorimeter cuvette. Place a lid on the cuvette.
   c. Place the cuvette into the Colorimeter and record transmittance; this is your initial or “0” time reading. Remove the tube. Repeat recording transmittance at 1, 2, 3, 4, and 5 minutes. Be sure to correctly align the cuvette in the Colorimeter each time.

Step 5: Record and graph your data in the space below. Add your % change in transmittance after 5 minutes to the class data displayed on the SMART Board.
Consider the following questions before you proceed to the next experiment:

- You measured the color change at different times. Which time will you use for your later assays? Why? (The time/color change that you select will serve as your baseline for additional investigations.)
- When you use this assay to assess factors that change enzyme activity, which components of the assay will you change? Which will you keep constant?

Experiment 2: Determining the Effect of an Environmental Factor on Enzymatic Activity

Numerous variables can be employed to observe the effects on the rate of an enzymatic reaction and possibly the specific fit of the enzyme with the substrate.

Materials
- Turnip peroxidase
- 0.1% hydrogen peroxide (H$_2$O$_2$)
- Guaiacol
- Buffers with range of pH
- Distilled water
- 12 test tubes
- Test tube rack
- Timer
- 10 mL syringes
- 0.5 mL pipets
- Vernier colorimeter probe system

Designing and Conducting Your Investigation

You now have the basic information and tools needed to explore enzymes in more depth on your own. In this part of the lab, you will do just that. You will have the chance to develop and test your own hypotheses about enzyme activity. To help you get started, read the following questions, and discuss the answers among the members of your group.

- In Experiment 1, was the limiting factor of your baseline reaction the enzyme or the substrate? How could you modify the procedure used in Experiment 1 to answer this question?
- What are factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? For instance, what do you predict will occur if the pH in the reaction changes? How do you justify your predictions? How could you modify the procedure used in Experiment 1 to answer this question?

Design an experiment to investigate the answer to one of the questions above or another question that might have been raised as you conducted Experiment 1. Remember, the primary objective of the investigation is to explore how biotic and abiotic factors influence the rate of enzymatic reactions. Describe your experiment in detail in the space below. Be sure to include the research question, hypothesis, prediction, variables (independent, dependent, controlled), materials, and procedure in your description. Also include in your description an explanation for why you expect the results to turn out the way you expect them to turn out. (Use the back of this page, if necessary)
Analyzing Results

Record the data that you collected from your independent investigation and graph the results in the space below.

Based on the graph and your observations, compare the outcome of your experiment to your hypothesis/prediction and explain any unexpected results. Be sure to identify any possible sources of error that may have affected the results of your experiment.