Investigation 13

ENZYME ACTIVITY

BACKGROUND

Enzymes speed up chemical reactions by lowering activation energy (that is, the energy needed for a reaction to begin). In every chemical reaction, the starting materials (the substrate(s) in the case of enzymes) can take many different paths to forming products. For each path, there is an intermediate or transitional product between reactants and final products. The energy needed to start a reaction is the energy required to form that transitional product. Enzymes make it easier for substrates to reach that transitional state. The easier it is to reach that state, the less energy the reaction needs.

Enzymes are biological catalysts. They are large protein molecules, folded so that they have very specifically shaped substrate binding sites. These binding sites make substrates go into the transition state. To catalyze the reaction, several regions of the binding site must be precisely positioned around the substrate molecules. Any change in the shape of the overall folded enzyme molecule can change the shape of the binding site.

The optimum reaction conditions are different for each enzyme. The correct environmental conditions, proper substrates, and, often, particular cofactors associated with an enzyme are needed. In some instances, the optimum conditions can be deduced fairly accurately based on the following:

- The organism from which the enzyme is derived
- The part of the organism in which the enzyme functions
- The environmental conditions in which that organism lives

For example, lactase, the enzyme that catabolizes the disaccharide sugar lactose into the two monosaccharides, glucose and galactose. In humans, lactase is found mostly in the small intestine, where the pH is around 7. It would be reasonable to hypothesize that human lactase is optimally active at pH 7 and at 37°C. Free-living decomposer fungi in soil also produce lactase. However, soil pH usually is between 5 and 6.5. As could be predicted, the purified enzyme from a common soil fungus has a pH optimum of 5.5. The main enzyme for this lab, peroxidase, is found in many different forms, with optimum pHs ranging from 4 to 11 depending on the source and optimum temperatures varying from 10 to 70°C.

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 "*kata*-" 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.

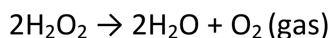
Procedure 1: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline

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:

Enzyme + Substrate --> Enzyme-Substrate Complex --> Enzyme + Product(s) + ΔG

For this investigation the specific reaction is as follows:

Peroxidase + Hydrogen Peroxide --> Complex --> Peroxidase + Water + Oxygen



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

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 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.

Qualifying color is a difficult task, but a series of dilutions can be made and then combined on a palette, which can represent the relative changes occurring during the reaction. A color palette/chart ranging from 1 to 10 (Figure 1) is sufficient to compare relative amounts of oxygen produced. Alternatively, the color change can be recorded as a change in absorbency using a variety of available meters, such as a spectrophotometer or a probe system.

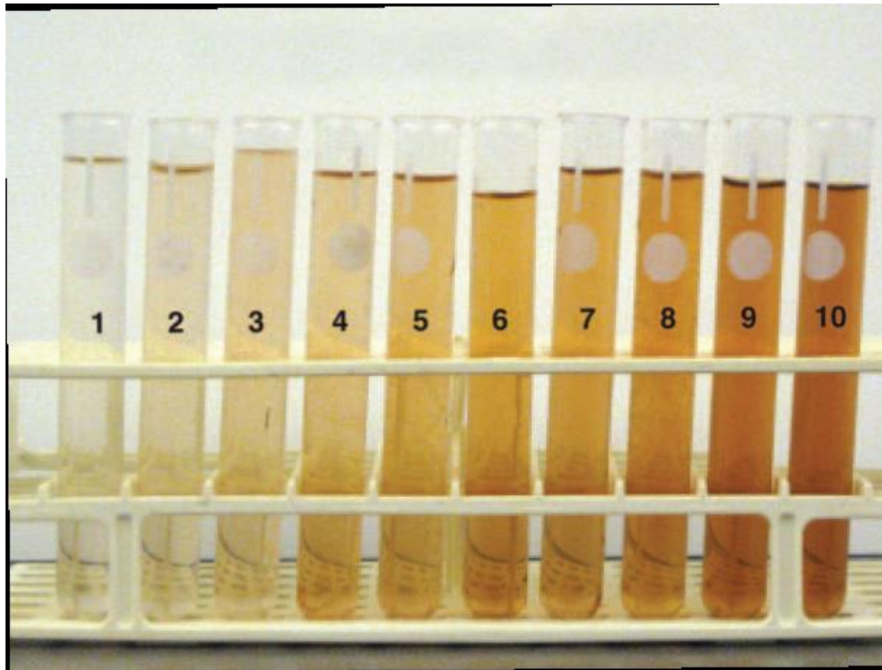


Figure 1. Turnip Peroxidase Color Chart

Materials

- Turnip peroxidase*(see appendix for preparation)
- 0.2% hydrogen peroxide (3mL of 3% H_2O_2 diluted w/distilled H_2O to final volume of 500mL)
- Guaiacol (0.2%)
- Distilled (deionized) water
- Spectrophotometer or Vernier Probeware
- Timer
- 1 mL graduated pipettes
- 13 x 100 mm cuvettes (3 per group)

Spectrophotometer Procedure

The use of measuring devices can better quantify your results. Using a spectrophotometer, you can select a specific wavelength to fit the color/pigment expected in an experiment. The change in the amount or concentration of color/pigment may be measured as absorbance (amount of the wavelength trapped by the pigment) or transmittance (amount of the wavelength that is not trapped by the pigment).

1. **Read the entire procedure before proceeding**. Pay special attention to the requirements for mixing the contents of the substrate and enzyme tubes and timing the reaction. **Precise volume** and **accurate timing** are crucial for rate studies.
2. Turn on your spectrophotometer approximately 10 to 15 minutes prior to starting the investigation so that it will warm up appropriately.
3. To measure the amount of the compound tetraguaiacol, set the wavelength to 500 nm.
4. Prepare a “blank” by combining the appropriate amounts of each material found in **Table 1** in a 13 x 100 mm cuvette.
5. Set your machine at zero absorbance using the blank containing all the appropriate materials *except* the substrate.
6. This is your baseline.

7. Using two 13 x 100 mL cuvettes, label one “substrate” and the other “enzyme.”
8. Follow **Table 1** to prepare each cuvette for the appropriate trial.
9. Combine the materials of the substrate and enzyme tubes. Mix the tubes twice and pour into the enzyme (E) cuvette. (When mixing or rotating always cover the opening of the cuvette with Parafilm and clean its surface with a scientific cleaning wipe before each reading..) **The elapsed time between mixing the tubes and recording the first absorbance measurement should be no greater than 20-40 seconds.**
10. Place the cuvette into the spectrophometer and record absorbance; this is your initial or “0” time reading. Remove the tube.
11. Repeat recording absorbance at 1, 2, 3, 4, and 5 minutes. Be sure to check zero absorbance with your blank before each reading.
12. Record and graph your data.

Vernier SpectroVis Plus Procedure

1. Connect the spectrophotometer to LabQuest and choose New from the File menu.
2. Prepare a “blank” by combining the appropriate amounts of each material found in **Table 1** in a 13 x 100 mm cuvette. Transfer 3 mL of the solution to a SpectroVis Plus cuvette.
3. Calibrate the spectrophotometer.
    a. Choose Calibrate from the Sensors menu.
    b. When the warm-up period is complete, place the Blank in the spectrometer. Make sure to align the cuvette so that the clear sides are facing the light source of the spectrometer.
    c. Tap Finish Calibration, and then select OK. The meter should be running, showing zero absorbance.
4. Using two 13 x 100 mL cuvettes, label one “substrate” and the other “enzyme.”
5. Follow **Table 1** to prepare each cuvette for the appropriate trial.
6. Combine the materials of the substrate and enzyme tubes. Mix the tubes twice and pour into the enzyme (E) cuvette. (When mixing or rotating always cover the opening of the cuvette with Parafilm.)
7. Quickly transfer 3 mL of the combined material to a SpectroVis Plus cuvette**. The elapsed time between mixing the tubes and recording the first absorbance measurement should be no greater than 20-40 seconds.**
8. Place the cuvette into the SpectroVis Plus, allow 10 seconds for the readings displayed in the meter to stabilize, and then hit the stop button. This ends run 1.

9. Keep the cuvette in the SpectroVis Plus.
10. At the one minute mark, tap the play button.
11. Store the data for run 1. (run 1 = 0 minutes)
12. Allow 10 seconds for the readings displayed in the meter to stabilize, and then hit the stop button. This ends run 2. (run 2 = 1 minute)
13. Repeat this procedure to record the absorbance of the sample for each minute, of the 5 minute time period.
14. To view your data, tap the table button at the top of your screen.
15. Remember, each run represents data from a one minute interval, starting at time zero.
16. Scroll to 500nm and record the absorbance for each time interval.

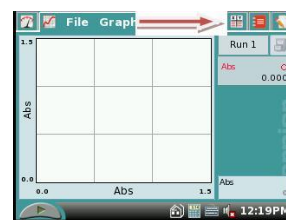


Table 1

		Trial 1		Trial 2		Trial 3	
Tube	**Blank**	**S1**	**E1**	**S2**	**E2**	**S3**	**E3**
pH 5 Buffer	4 ml	1 mL	2 mL	1 mL	2 mL	1 mL	2 mL
0.02% H_2O_2	2 mL	2 mL		2 mL		2 mL	
0.2% Guaiacol	1 mL	1 mL		1 mL		1 mL	
Enzyme Extract			0.5 mL		1 mL		.25 mL
Phosphate Buffer	2 mL		1.5 mL		1 mL		1.75 mL

Procedure 2: Developing an experiment to test how a biotic or abiotic factor may influence the rate of an enzyme-catalyzed reaction.

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 hypothesis about enzyme activity. To help you get started, consider the following while reflecting upon your knowledge:

- In Procedure 1, was the limiting factor of your reaction the enzyme or the substrate? How could you modify the procedure to answer the question?
- What are the different factors that vary in the environment in which organisms live? Which of these factors do you think could affect enzyme activity? How could you modify the procedure to answer the question?
- Do metal ions (co-factors) activate or inhibit the rate of enzyme-catalyzed decomposition of hydrogen peroxide? How could you modify the procedure to answer the question?
- Do peroxidase enzymes from different vegetables have similar activity? How could you modify the procedure to answer the question?

Plan, discuss, execute, and evaluate an experiment to test a question regarding the rate of an enzyme-catalyzed reaction and the model of enzyme activity.

- Decide upon one question that your group would like to explore.
- Develop a testable hypothesis (sign-up topic with teacher)
- Discuss and design a controlled experiment to test the hypothesis.
- List any safety concerns or precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experiment.
- Determine what and how you will collect and record the data.
- How will you analyze the data to test your hypothesis?
- Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
- Once the experiment is complete, analyze the data and evaluate whether the experimental evidence supports, refutes or provides no information concerning the hypothesis.
- Explain the results in terms of the mechanism of enzyme action, structure-function relationships involving proteins, and metabolic control of biological reactions.
- Make suggestions for additional experiments to modify or revise the hypothesis.

Appendix

Enzyme Extraction:

- Peel and cut a turnip root into small cubes, about 1 cm on each side. (no outer peel)
- Measure about 2g (about 2 pieces) in a weighing dish.
- Place 500 mL of pH 7 phosphate extraction buffer and the turnip pieces in a blender.
- Pulse the turnip root in 1-3 minute bursts three times, with two minutes rest between pulses, to homogenize and extract the enzyme.
- Filter the turnip enzyme extract through filter paper and store over ice in the refrigerator (Use within one week)