

Interactions

INVESTIGATION 13

ENZYME ACTIVITY*

How do abiotic or biotic factors influence the rates of enzymatic reactions?

■ 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, this investigation mentions 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.

* Transitioned from the *AP Biology Lab Manual* (2001)



One suggestion for extending the inquiry activities in this lab is to have students compare peroxidase extracted from different vegetables. Ask them, *What could you predict based on what you know about how each vegetable grows? What observations could you make of the vegetables before extracting enzyme?* With this prompt, it is very likely that one or more students will think about potential differences in tissue pH or normal growing temperature for root versus leaf versus fruit. A pH test strip would give a reasonable estimate of tissue pH, and there will be a large difference (which students can look up or measure themselves) in the average temperature of a turnip root in soil versus the aboveground leaves of the same plant.

Before starting this laboratory, students should understand how proteins are made and establish their final structure. The final structure of the protein (in this investigation, an enzyme) is determined by interactions between its amino acids and the surrounding environment. Primary structure is the protein's unique sequence of amino acids. The protein chain will contain hundreds to a few thousand amino acids (sometimes more) and can be identified with an amino end and a carboxyl end. Secondary structure produces β pleated sheets or α helices formed by hydrogen bonding throughout the molecule. Tertiary structure occurs because of the numerous interactions of the backbone amino acids with various side chains (R groups), such as hydrophilic or hydrophobic interactions, ionic bonds, and disulfide bridges associated with the amino acid cysteine. At this point the protein may be active or become a component of the quaternary structure when two or more subunits unite to form a larger protein. A good example of a quaternary protein is hemoglobin, which is made up of two α and two β subunits associated with four molecules of heme (a nonpolypeptide component), each of which contains an iron atom that binds oxygen.

Enzymatic proteins 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 word for “break down”; for instance, amylase breaks complex starches into simple sugars) and anabolic enzymes (“a-” or “an-” from the Greek word *anabole*, meaning to “build up”). You can remind students of stories about athletes who have been caught using anabolic steroids to build muscle.

Catalytic enzymes that break down proteins, which are called proteases, are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin and is often 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); 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, students will focus on a specific enzyme, peroxidase, which is obtained from turnips, one of numerous sources of this enzyme. (The peroxidases are a large family of catalytic enzymes that include “cousins” cytochrome c peroxidase and catalase.) Using peroxidase, students will develop essential skills to explore their own questions about enzymes, including the following:

What is the effect of using a variety of temperature ranges on the overall rate of reaction?

How will different pH buffers in the reaction affect the rate of reaction?

Which has a greater effect on the rate of reaction—changing the concentration of enzyme or changing the concentration of substrate?

What other abiotic or biotic changes could affect an enzyme’s activity?

After developing specific skills to determine enzymatic activity of peroxidase, students will have an opportunity to explore the properties of this enzyme on their own. The investigation provides an opportunity for students to apply and review concepts they have studied previously, including the levels of protein structure, energy transfer, entropy and enthalpy, abiotic and biotic influences on molecular structure, and the role of enzymes in maintaining homeostasis. The laboratory allows students to investigate more deeply the relationship between structure and function of enzymes; to develop a concise understanding of a specific enzymatic reaction; and then to apply their knowledge and newly acquired skills to answer their own question(s) about enzymatic activity.

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 point 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.

■ PREPARATION

Materials and Equipment

- Turnip peroxidase: Extracted from a turnip of choice (possibly one grown in your local area): Cut the outer 2–4 mm of the root surface (a potato peeler is recommended) and use a blender in the pulse mode to liquefy 20 grams in 500 mL of distilled water. Filter through triple layers of cheesecloth and then filter the filtrate using coarse grade filter paper or a coffee filter. Keep refrigerated. Store in brown bottles.
- Some root vegetables have a large amount of starch. Prepared extracts may contain a variable amount of fine white powder floating in suspension. This is excess starch, and it will not go into solution. If this should appear, simply place the solution in the refrigerator for a few hours so that the starch will settle. Then, decant the liquid and proceed with the experiment. Peroxidase can also be extracted from rutabaga (*Brassica napobrassica*), which is available from commercial vendors and produces no powdery starch.
- Concentrations of peroxidase vary from species to species, from different growing environments and even the time of the year. Thus, the sample you make must be sufficient to run all intended experiments so that there is consistency of the enzyme concentration in each phase. Your sample may be frozen if time does not permit you to complete your intended series and used at a later time, as long as the frozen material is not older than three months.
- Hydrogen Peroxide (0.1%): A standard solution of 3% H_2O_2 is available at most drug stores. Combine 15 mL of H_2O_2 with 435 mL of distilled water to make a 0.1% solution. Keep refrigerated, and store in brown bottles.
- Guaiacol: Available from numerous suppliers. Dilute 1.5 mL with 500 mL distilled water. Although very rarely, guaiacol has been reported as a skin irritant at high concentrations. Care should be taken when making the first dilution since the stock solution is 96–98 percent pure. Guaiacol is weakly soluble in water, so make the solution the day before and store it in a refrigerator. On the day of the experiment, place the solution in small brown bottles and keep cold. Keep out of direct light because guaiacol is light sensitive. As long as the original stock bottle is kept in the refrigerator, it will last for years.
- Buffers: You can make your own phosphate buffers to fit the needs of your experiment, but for simplicity and storage, pHDrion buffers are recommended. A good range of buffers is pH 3 - 5 - 6 - 7 - 8 - 10.

Other Supplies

- Laboratory notebook
- Distilled or deionized water
- Test tubes of approximately 16 x 150 mm and appropriate test tube rack. Each student group will need approximately 14 test tubes.
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes, probably as series of 1, 2, 5, and 10 mL.

This laboratory investigation is designed to be performed without a spectrophotometer, but a spectrophotometer or probes with computer interface can be used.

■ Timing and Length of Lab

This investigation requires approximately three to four lab periods of about 45 minutes each, depending on student interest and how far students want to take their investigation. The skills set requires approximately 40 minutes for students to work through it. Students can work in pairs or small groups to accommodate different class sizes. Time should be allotted for students to research their questions before designing their experiment. Plan additional time for students to present their results and conclusions to their peers, perhaps in the form of a mini-poster session or traditional laboratory report.

■ Safety and Housekeeping

Instructors and students should always adhere to general laboratory safety procedures and wear proper footwear, safety goggles or glasses, laboratory coats, and gloves. Use proper pipetting techniques; use pipette pumps, syringes, or rubber bulbs, and never use your 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 in the investigations are deemed to be safe by all chemical standards, but recall that any compound has the potential to harm the environment.

■ ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to cellular processes (big idea 2), including the structure and function of enzymes, or while exploring interactions at the molecular level (big idea 4). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

■ Enduring Understandings

- 2D1: All biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.
- 4A1: The subcomponents of biological molecules and their sequence determine the properties of that molecule.
- 4B1: Interactions between molecules affect their structure and function.



■ Learning Objectives

- The student is able to design a plan for collecting data to show that all biological systems are affected by complex biotic and abiotic interactions (2D1 & SP 4.2, SP 7.2).
- The student is able to use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule (4A1 & SP 6.1, SP 6.4).
- The student is able to analyze data to identify how molecular interactions affect structure and function (4B1 & SP 5.1).

■ ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

Before students investigate enzymes, they should be able to demonstrate understanding of the following concepts. The concepts may be scaffolded according to level of skills and conceptual understanding.

- Basic protein structure
- The concept of induced fit
- The role of enzymes
- That structure, function, and environment are all required for maximal function of enzymatic reactions

■ Skills Development

Students will develop/reinforce the following skills:

- Using pipettes to measure solutions
- Using pH indicators to determine environmental conditions that may influence the reaction activity of enzymes

■ Potential Challenges

One challenge for students is coordinating the timing of the enzymatic reaction(s) and collecting data. Instruct students that as soon as they combine the contents of the test tube with substrate and the test tube with enzyme, they must immediately begin timing the reaction.

The key to having excellent results is measuring the amount of each solution as accurately as possible. Check that both tubes (substrate and enzyme) have equal amounts of liquid by holding the tubes next to each other. The student must always use a clean measuring apparatus for each solution. Make sure that students label each measuring device for the specific solution used and then use it for only that material (e.g., distilled water, guaiacol, hydrogen peroxide, and enzyme).

For Procedure 2, students must replace the 6.0 mL of distilled water in their original enzyme tube with 6.0 mL of a specific pH buffer. Students then compare reaction rates.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

Have students review the importance of the structure of an organic molecule to its overall function. Make sure that students review the laboratory equipment they are to use and understand units of measure, especially since a 1.0 mL pipette is used and its divisions are 0.1 and 0.01 mL. An error in measurement may then be expressed by a power of 10.

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

Procedures 1 and 2 are designed to help students understand concepts related to the activity of enzymes before they design and conduct their own investigation.

A basic enzymatic and substrate reaction can be depicted as follows:



For this investigation the specific reaction is as follows:



Peroxidase is an enzyme that breaks down peroxides, such as hydrogen peroxide, and is produced by most cells in their peroxisomes. Peroxide is a toxic byproduct of aerobic metabolism. Various factors — abiotic and biotic — could have a major influence on the efficiency of this reaction.

To determine the rate of an enzymatic reaction, a change in the amount of at least one specific substrate or product is measured over time. In a decomposition reaction of peroxide by peroxidase (as noted in the above formula), the easiest molecule to measure is oxygen gas, a final product. This can 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 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 in the reaction. A color palette ranging from 1 to 10 (Figure 1) is sufficient to compare relative amounts of oxygen produced, or 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. Using a color palette is a relative way to compare a change and is therefore qualitative. To collect quantitative data, a spectrophotometer or probe system is required.

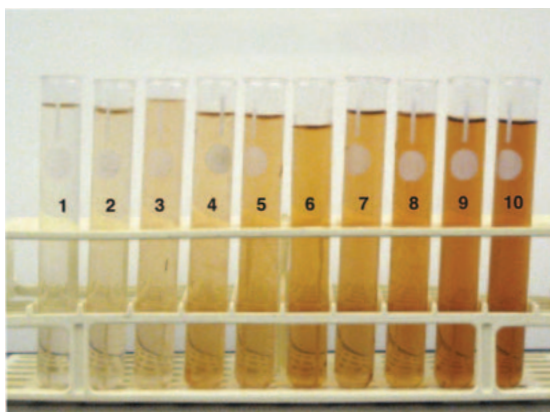


Figure 1. Turnip Peroxidase Color Chart

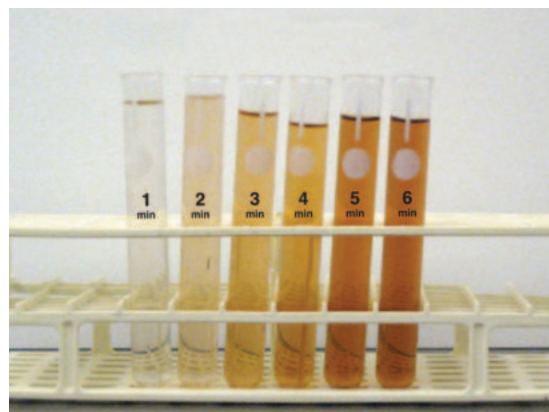


Figure 2. Turnip Peroxidase Baseline

A sample baseline palette is shown in Figure 2.

Another suggestion is to make a maximally converted solution of tetraguaiacol and then prepare serial dilutions.

1. Prepare a mixture equivalent to 10 replicate reactions containing the buffer, enzyme, and substrate.
2. Incubate the mixture overnight so that all of the guaiacol converts to product. The concentration of guaiacol in the original mixture is known, so the concentration of brown product is simply that same concentration divided by 4 (tetraguaiacol). Make a series of dilutions (10% brown product, 20% brown product, etc.) and place them in test tubes. Students now have a color comparison chart that provides a specific concentration of final product.
3. You and your students can use the amount of product formed overall to construct a graph and calculate enzyme reaction rate.

■ Procedure 2: Determining the Effect of pH 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. In Procedure 2, students examine the effects of various pH solutions via the color change and then compare their results by graphing pH versus color change (if using a spectrophotometer the pH to percent of light absorbance, as shown in the sample data table in Table 1).

Table 1. Sample Data Table: Changes in Absorbance at Different pH

pH	3	5	6	7	8	10
	-0.001	0.663	0.347	0.170	0.047	0.003

■ Designing and Conducting Independent Investigations

The following questions are presented in the student version of this investigation under Designing and Conducting Your Investigation.

- In Procedure 1, was the limiting factor of your base line reaction the enzyme or the substrate? How could you modify the procedure you learned to answer this question?

If students are not able to think this through on their own, remind them that in an enzymatic reaction a “fit” of the substrate and enzyme must be accomplished. The reaction is dynamic and requires the two to join together. Whichever material is present in smaller amount will thus determine the rate of the reaction. Therefore, students simply would perform two tests varying the substrate in one while keeping the enzyme the same and the reverse in the other. Remember, the volume must always be the same; thus, if students reduced the enzyme by 0.5 mL, they would have to increase the distilled water by 0.5 mL. In another test they would modify the hydrogen peroxide in a similar manner.

- What are three or four factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? How would you modify your basic assay to test your hypothesis?

Students already have the basic assay needed to explore enzymes in different environments. They need only to develop a hypothesis and decide how to vary the basic experimental procedures to answer the question above. Then students perform the experiment(s) and report whether their results support or contradict their hypotheses. (It is not recommended that students pursue the role of inhibitors in enzymatic reactions. Many inhibitors will also work on the human’s enzymatic networks, so you do not want students to handle inhibitors in the laboratory.)

Sample results for investigations that students might choose to explore are shown in the following figures and tables. Figure 3 shows a typical color palette for the effect of different temperatures or enzyme concentration on peroxidase activity.

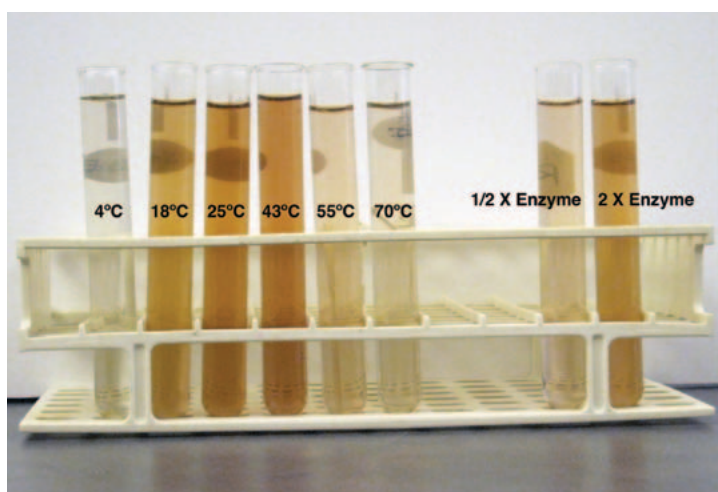


Figure 3. The Effect of Temperature and Enzyme Concentration on Enzymatic Reactions

Table 2 and Figure 4 show possible results for the effect of different temperatures on peroxidase activity (changes in light absorbance) if students use a spectrophotometer to collect data.

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Table 2. The Effect of Various Temperatures on Peroxidase Activity

Temperature	4°C	15°C	25°C	43°C	55°C	70°C	100°C
	0.106	0.177	0.251	0.312	0.289	0.164	0

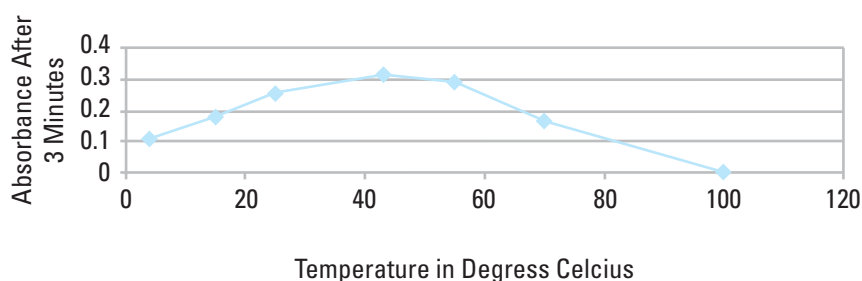


Figure 4. Effects of Temperature Change on Light Absorbance After Three Minutes

Table 3 and Figure 5 show possible results for the effect of different enzyme and/or substrate concentrations on peroxidase activity (changes in light absorbance) if students use a spectrophotometer to collect data.

Table 3. Effect of Different Enzyme and Substrate Concentrations on Peroxidase Activity

	0 min.	1 min.	2 min.	3 min.	4 min.	5 min.
Baseline	0	0.083	0.141	0.198	0.287	0.314
2X enzyme	0	0.131	0.244	0.366	0.433	0.469
1/2X enzyme	0	0.038	0.070	0.091	0.120	0.151
2X substrate	0	0.080	0.152	0.241	0.298	0.345
1/2X substrate	0	0.080	0.139	0.198	0.270	0.287

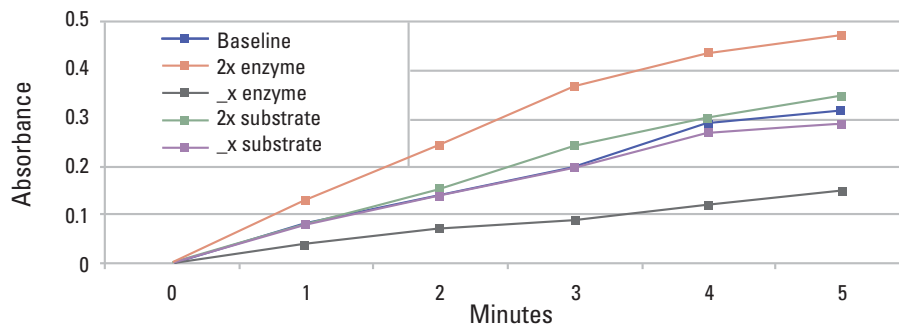


Figure 5. Effect of Different Enzyme and Substrate Concentrations on Peroxidase Activity

Investigating Other Enzymes and Reactions


This section is supplemental, as not all students will be ready for it. Similarly, not all schools will have the requisite time and resources. It requires that the student “think outside the box” to conduct outside online or library research on an enzyme of specific interest or explore a particular enzyme-catalyzed process in more detail. If included, a nonlab mini-research project requires additional time for students to present their results and conclusions to their peers, perhaps in the form of a mini-poster session or traditional laboratory report.

Before students proceed to designing and conducting their own nonlab investigations/research projects based on the following suggested topics, it is helpful to steer them in the right direction to gather some preinvestigation information.

Topic A: Ask students to investigate enzymes that appear to be conserved across all living domains (bacteria, fungi, plants, and animals). Students’ research should raise questions, including *How similar are the enzymes in function, structure, and usage? For example, a specific dehydrogenase has been found in E. coli, corn, horse liver, and sheep. Does this mean that the enzyme evolved numerous times in numerous organisms or just once early in the history of life? Does the enzyme function the same in each organism? How similar is the DNA for this enzyme? How do organisms live in extreme environments, such as hot springs?* There are many similar enzyme systems — both catabolic and anabolic — that are found across our current and historical living domains. Students can find examples on the Internet and develop presentations for the class that address concerns, ideas, and conclusions.

Considering Topic A: Students must search the Internet for a specific enzyme. Using bioinformatics, students can investigate specific DNA sequences that are common for a particular enzyme. Resources for this type of information include <http://www.ncbi.nlm.nih.gov/Class/minicourses/> and “Incorporation of Bioinformatics Exercises into the Undergraduate Biochemistry Curriculum” (see Supplemental Resources).

Topic B: To understand how organisms’ survival is linked to enzymatic reactions, the role of abiotic factors needs to be addressed. Ask the students to assume the role of a farmer growing soybeans. What would be the best soil conditions for maximum productivity? Can you find examples that would support or reject the concept that survival is a matter of a best-fit scenario for the organism and its abiotic and biotic pressures as related to enzymes? Can you suggest which abiotic factors need to be examined and then perform an experiment that might support your hypothesis? Develop a presentation for the class that helps to address your conclusions.



Considering Topic B: Soil science is a major course of study in agricultural programs. Many specialty farmers use soil science to enhance their crops. For example, Nalo Farms in Hawaii will condition the soil to be slightly acidic when growing leafy vegetables or slightly basic when growing tomatoes. Some seeds will germinate better in light (tobacco) while others germinate in darkness. The same preferences can be noted for temperature conditions and soil salinity. Numerous colleges and universities have available soil science sites on the Internet. Students can explore these websites to gather information to begin their investigative research.

Topic C: Ask students to consider evolutionary questions such as *Have plants evolved different characteristics to cope with specific abiotic conditions, such as salt marsh (salinity issues), high mountain pastures, deserts, acidified environments (acid rain), and estuaries? Are there different optimums for the same abiotic factors within different plants? Have plants evolved over time because of the influence or selectivity due to the abiotic factors associated with their environments?* Students can develop presentations for the class that help address their conclusions.

Considering Topic C: The key to understanding evolution and natural selection is grasping that variations in a population of a species aid in the selective forces of the environment. To investigate this concept, some students might simply buy a few packages of seeds of a particular leafy vegetable, root vegetable, tomato, etc., and then grow them in varying conditions (salinities, temperatures, and so forth). Other students may choose to do nonlab mini-research projects on the topic. Although the links among growth, environment, and enzymes are more difficult to connect, regardless of their approach, students are establishing a protocol to do further work on those plants that vary tremendously from each other.

■ Summative Assessment

1. Review the learning objectives and students' answers to analysis questions that you ask. Do students understand the concepts? You can use the learning objectives to generate questions.
2. Review the students' experimental evidence. Did they make the appropriate measurements and graphs to reflect their results? Can they draw conclusions from their data?
3. Have the students prepare laboratory notebooks and record their experimental designs, data, graphs, results, and conclusions. They can also present their work in the form of a mini-poster.

Homeostasis is also discussed when studying hormonal interactions (e.g., sugar balance with insulin and glucagon or water balance with diuretics and antidiuretics). Ask students to make a strong argument for the role of enzymes in maintaining homeostasis at the cellular level.

SUPPLEMENTAL RESOURCES

Assay for peroxidase and guaiacol, Faizyme Laboratories,

<http://faizyme.com/assaperg.htm>

This website contains general information about the peroxidase and guaiacol reaction.

“Incorporation of Bioinformatics Exercises into the Undergraduate Biochemistry Curriculum” provides information if students opt to investigate Topic A as a supplemental mini-research project. http://bioquest.org/bedrock/san_diego_01_07/projectfiles/fulltext_ID=113449540&PLACEBO=IE.pdf

Kubo, Akihiri, Hikan, et al., “Cloning and Sequencing of a cDNA Encoding Ascorbate Peroxidase from *Arabidopsis thaliana*,” *Molecular Biology*, Vol. 18, Number 4, 691–701. This paper supports supplemental research Topic A.

<http://www.ncbi.nlm.nih.gov/pubmed/1558944>

<http://www.ncbi.nlm.nih.gov/Class/minicourses/> This site introduces available online resources and is valuable if students choose to investigate supplemental research Topic A.

Soil Food Web. Students who would like to investigate supplemental research Topic B can perform a Google search to explore literally thousands of entries on soil, food, plant growth, and the role of the environmental in crop production.

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INVESTIGATION 13

ENZYME ACTIVITY*

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 “*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.

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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, safety goggles or glasses, a laboratory coat, and gloves. 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

Getting Started

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:



For this investigation the specific reaction is as follows:



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.

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. (Information about the use of spectrophotometers and/or probe systems is found in the Additional Information section of this investigation.)

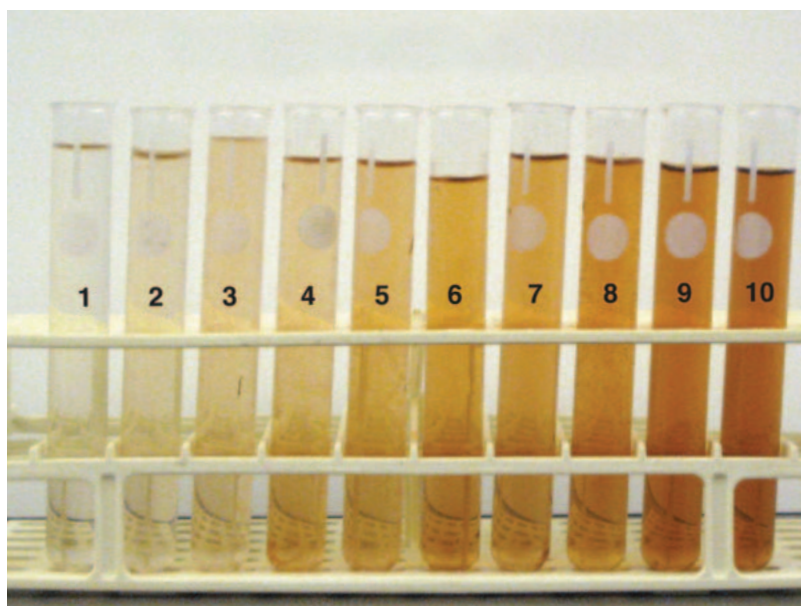


Figure 1. Turnip Peroxidase Color Chart

Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Distilled (deionized) water
- 2 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)

This investigation is designed to be performed without a spectrophotometer, but your teacher may ask you to use a spectrophotometer or probe system. If so, additional equipment may be required.

Step 1 Using two 16 x 150 mm test tubes, mark one “substrate” and the other tube “enzyme.” To the substrate tube, add 7 mL of distilled water, 0.3 mL of 0.1 percent hydrogen peroxide, and 0.2 mL guaiacol for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm® and gently mix.

Step 2 To the enzyme tube, add 6.0 mL of distilled water and 1.5 mL of peroxidase for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm and gently mix.

Step 3 Combine the contents of the two tubes (substrate and enzyme) in another 16 x 150 mL test tube, cover the tube with Parafilm, invert twice to mix, and place the tube in a test tube rack. Immediately begin timing the reaction.

Step 4 Observe the color change for the next 5 minutes. Rotate the tube before each reading. Record the observed color at 0, 1, 2, 3, 4, and 5 minutes. (A cell phone and/or camera are excellent ways to record color change.)

Step 5 Use the color palette/chart (Figure 1) to help you quantify changes in color over time. Graph your data in your laboratory notebook.

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?



■ Procedure 2: Determining the Effect of pH 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.

- What do you predict will occur if the pH in the reaction changes? How do you justify your prediction?

Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Buffers with range of pH
- Distilled (deionized) water
- 12 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)
- Spectrophotometer or probe system

Step 1 Using clean 16 x 150 mL test tubes, make six sets of pairs of original substrate and enzyme tubes for a total of 12 tubes or 6 pairs. This time you will substitute a different pH buffer for the distilled water used in the original enzyme tubes. Prepare the tubes as follows and be sure to label them.

- For each substrate tube in a pair, add 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL of guaiacol for a total volume of 7.5 mL.
- For each enzyme tube in the pair, add 6.0 mL of a specific pH solution and 1.5 mL of peroxidase for a total volume of 7.5 mL. For example, in the enzyme tube of the first pair, you can substitute 6.0 mL of buffer solution of pH 3 for the distilled water; in the enzyme tube of the second pair, you can substitute 6.0 mL of buffer solution of pH 5 for the distilled water, and so forth.
- Cover each test tube with a piece of Parafilm, and gently mix.

Step 2 Combine the substrate and enzyme tubes for all six pairs (total volume 15.0 mL per pair), cover with Parafilm, gently mix, and place the tubes back in the test tube rack. Immediately begin timing the reactions.

Step 3 Record the observed color for each tube at 0 minutes and again at the time you chose based on your results in Procedure 1. (Again, a cell phone and/or camera are excellent ways to record color change.)

Step 4 Use the palette/color chart (Figure 1) to help you quantify the changes you observe. Graph your data as color intensity versus pH. What conclusions can you draw from your results?

■ 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 write your answers in your laboratory notebook.

- In Procedure 1, was the limiting factor of your baseline reaction the enzyme or the substrate? How could you modify the procedure you learned to answer this question?
- What are three or four factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? How would you modify your basic assay to test your hypothesis?

Design and conduct an experiment to investigate an answer(s) to one of the questions above or another question that might have been raised as you conducted Procedures 1 and 2. Remember, the primary objective of the investigation is to explore how biotic and abiotic factors influence the rate of enzymatic reactions.

■ Analyzing Results

From the data that you collected from your independent investigation, graph the results. Based on the graph and your observations, compare the effects of biotic and abiotic environmental factors on the rate(s) of enzymatic reactions and explain any differences.


■ Additional Information

If a spectrophotometer is available, the following information is useful.

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).

For Procedure 1:

1. Turn on your spectrophotometer approximately 10 to 15 minutes prior to starting the investigation so that it will warm up appropriately.
2. To measure the amount of the compound tetraguaiacol, set the wavelength to 470 nm.

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- 3.** Set your machine at zero absorbance using a blank containing all the appropriate materials *except* the substrate (i.e., 13.3 mL of distilled water, 0.2 mL of guaiacol, and 1.5 mL of enzyme extract = 15 mL total).
 - 4.** Determine the baseline.
 - A.** Using two 16 x 150 mm test tubes, label one “substrate” and the other “enzyme.”
Substrate tube: 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL guaiacol (total volume 7.5 mL) Enzyme tube: 6 mL of distilled water and 1.5 mL of peroxidase (total volume 7.5 mL)
 - B.** Combine the materials of the substrate and enzyme tubes. Mix the tubes twice and pour into a cuvette. (When mixing or rotating always cover the opening of the cuvette with Parafilm.)
 - C.** Place the cuvette into the spectrophotometer and record absorbance; this is your initial or “0” time reading. Remove the tube. Repeat recording absorbance at 1, 2, 3, 4, and 5 minutes. Be sure to rotate (use Parafilm to cover) the tube and also clean its surface with a scientific cleaning wipe before each reading.
 - 5.** Record and graph your data.

For Procedure 2:

Follow steps 1, 2, and 3 above. In step 4, set up as outlined above. Make an initial reading at time “0” and a second reading at the time you chose as optimal based on results obtained in Procedure 1. Record and graph your data.