Enzyme Activity Guided Inquiry Lab Turnip Peroxidase

Introduction

Peroxidase enzymes are widely distributed in plants and animals, including bacteria, to protect cells against the effects of oxidative stress and cell damage due to hydrogen peroxide. Peroxidases are easily extracted from turnips and other root vegetables and provide a model enzyme for studying enzyme activity—how the rate of an enzyme-catalyzed reaction depends on biotic and abiotic factors. Enzyme activity studies reflect enzyme structure and function and provide the foundation for understanding the mechanism or theory of enzyme action.

Background

The term *peroxidase* refers to both a class of oxidoreductase enzymes and to specific enzymes within that class. As a general class of enzymes, peroxidases catalyze the oxidation–reduction decomposition reaction of hydrogen peroxide. There are two general types of peroxidases—catalase and peroxidase. Catalase catalyzes the disproportionation reaction of hydrogen peroxide to water and oxygen gas (Equation 1). In reactions mediated by catalase, hydrogen peroxide substrate molecules act as both oxidizing agent (electron acceptor) and reducing agent (electron donor). In contrast, peroxidase acts in the presence of other naturally occurring organic reducing agents, such as ascorbic acid and glutathione, to catalyze the decomposition of hydrogen peroxide. Organic reducing agents, abbreviated AH_2, transfer hydrogen atoms and electrons to hydrogen peroxide, resulting in the formation of water and oxidized organic substrates such as A_2 in Equation 2. Oxygen gas is not produced in this latter reaction.

Catalase-catalyzed reaction $2H_2O_2 \rightarrow 2H_2O + O_2$ *Equation 1*

Peroxidase-catalyzed reaction $2H_2O_2 + 2AH_2 \rightarrow 4H_2O + A_2$ *Equation 2*

The differences in the two equations shown above provide a basis for studying the enzyme activity of turnip peroxidase in this guided-inquiry laboratory investigation. Many endogenous organic compounds may be used as reducing agents in Equation 2. One of the most common and convenient reducing agents for this purpose is guaiacol, a colorless compound having the formula $C_7H_8O_2$. Oxidation of guaiacol according to Equation 2 converts it to a dark orange compound called tetraguaiacol. The rate of the reaction may be followed by measuring the absorbance or color intensity of the orange product as a function of time.

Materials

Buffer capsules, pH 3–8, 100 mL each
Distilled or deionized water
Guaiacol solution, $C_7H_8O_2$, 0.2%, 1 mL
Hydrogen peroxide, H_20_2, 3%, 3 mL
Isopropyl alcohol, $(CH_3)_2CHOH$ 70%, 100 mL
Phosphate buffer, pH 7, 500 mL, NaH_2PO_4 and Na_2 HPO_4
Turnip (root/tuber)
Ice and water baths
Blender
Erlenmeyer flask, 500-mL
Filter paper and funnel
Hot plate
Knife, paring
pH paper, narrow range
Pipets, serological, 2- and 5-mL
Spectrophotometer
Test tubes, 13 × 100 mm, 6, and rack
Thermometer
Timer

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Safety Precautions

The guaiacol solution contains isopropyl rubbing alcohol (70%) and is a flammable liquid. Keep away from heat, flames, and other sources of ignition. Dilute hydrogen peroxide solution (3%) may be irritating to the eyes and skin. Exercise care when using a knife to peel and cut the turnip. Avoid contact of all chemicals with eyes and skin. Pure guaiacol is toxic by ingestion. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

1. Extraction Buffer: Prepare 500 mL pH 7 phosphate buffer by mixing equal volumes, 250 mL each, of 0.1 M sodium phosphate monobasic and sodium phosphate dibasic solutions.
2. Reaction Buffers: Dissolve one each pH 3–8 buffer capsules in 100 mL distilled or deionized water according to instructions.
3. Hydrogen Peroxide: Dilute 3 mL of 3% hydrogen peroxide to a final volume of 500 mL using distilled or deionized water. Store in a dark bottle protected from heat and light.

Enzyme Extraction

Peel and cut a turnip root into small cubes, about 1 cm on each side. Measure approximately 2 g (about 2 pieces) in a weighing dish and add to 300 mL of pH 7 phosphate extraction buffer in a blender. Pulse the turnip root in 1–3 minute bursts three times, with 2-minutes rest between pulses, to homogenize and extract the enzymes. Filter the *enzyme extract* through filter paper and store the extract over ice or in the refrigerator.

Baseline Activity—Peroxidase-Catalyzed Oxidation Decomposition of Hydrogen Peroxide

Read the entire procedure before beginning. Pay special attention to the requirements for mixing the tubes and timing the reaction. Accurate timing is crucial for rate studies.

1. Turn on the spectrophotomer, adjust the wavelength setting to 500 nm, and allow the instrument to warm up for 15–20 minutes.
2. Prepare separate 13 × 100 mm test tubes containing substrates (tube S) and enzyme (tube E) in pH 5 buffer as shown below. The presence of pH 7 extraction buffer in tube E makes it possible to vary the enzyme concentration while maintaining the overall buffer composition constant. The concentration of the diluted hydrogen peroxide is described in the *Preparation* section.

Tube S:	2 mL pH 5 buffer	*Tube E:*	2 mL pH 5 buffer
	2 mL dilute H_2O_2		1.5 mL pH 7 phosphate extraction buffer
	1 mL 0.2% guaiacol		0.5 mL enzyme extract

3. Prepare a "blank" by combining 4 mL pH 5 buffer, 2 mL dilute H_2O_2, 1 mL guaiacol solution, and 2 mL pH 7 phosphate buffer in a 13 × 100 mm test tube.
4. Zero the spectrophotometer (zero absorbance, 100% transmittance) at 500 nm using the blank solution.
5. When ready to begin a kinetics run, carefully pour the contents of tube S into tube E and ***immediately start timing.*** Carefully pour the combined contents back into tube S, wipe the outside of the tube with lab tissue, and place the test tube in the spectrophotometer cell holder.
6. Measure and record the absorbance as a function of time every 20 seconds. Ideally, the elapsed time between mixing the tubes and recording the first absorbance measurement should be no more than 20–40 seconds!
7. Graph absorbance versus time to obtain the reaction rate from the slope of the line.

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Enzyme Activity—Opportunities for Inquiry

Vary the enzyme and substrate concentrations and investigate the effects of pH, temperature, and possible inhibitors on the rate of the peroxidase-catalyzed decomposition of hydrogen peroxide. Analyze and graph the results and explain in terms of the mechanism of enzyme action and structure–function relationships involving biological protein molecules. Precise volume transfers and accurate "time zero" measurements are essential for meaningful results and analysis.

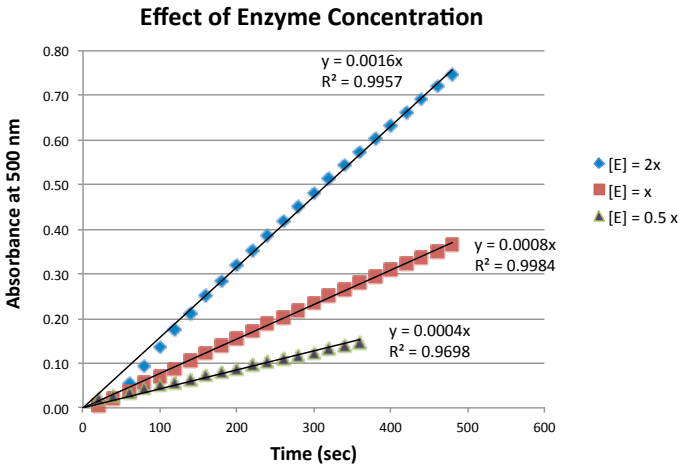
AP Biology Curriculum Framework (2012)

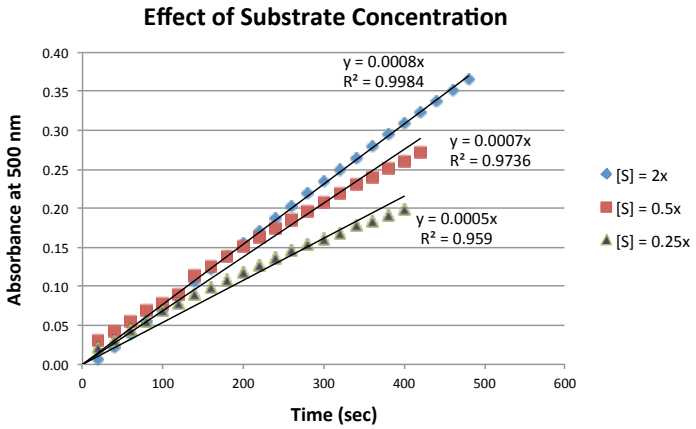
Essential Knowledge 2.D.1. All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

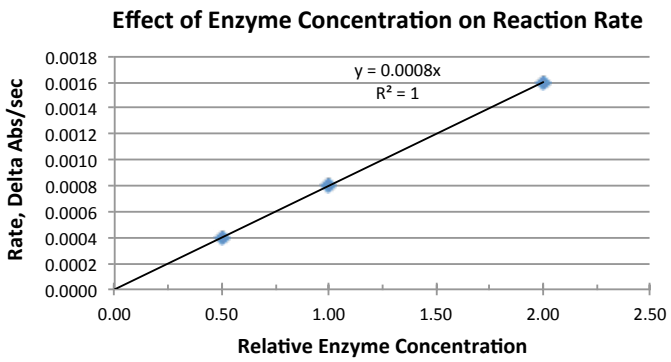
Essential Knowledge 4.A.1. The subcomponents of biological molecules and their sequence determine the properties of that molecule.

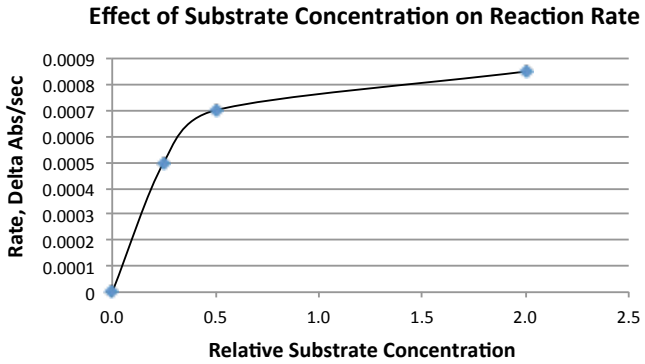
Essential Knowledge 4.B.1. Interactions between molecules affect their structure and function.

Sample Results









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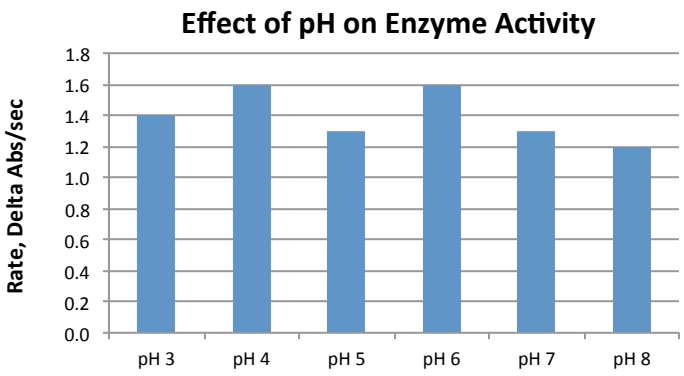

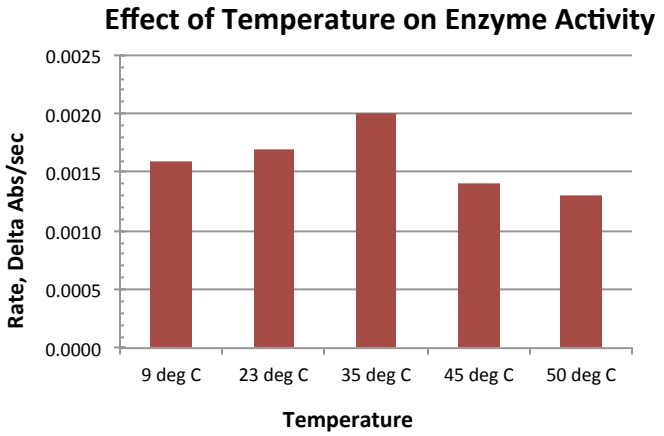

Materials for *Enzyme Activity Guided Inquiry Lab* are available from Flinn Scientific, Inc.

Catalog No.	Description
B0227	Buffer Capsules, pH 2–12
B0099	Buffer Solution Concentrated, pH 7, 500 mL
G0054	Guaiacol Solution, 0.2%, 100 mL
H0009	Hydrogen Peroxide, 3%, 473 mL
I0021	Isopropyl Alcohol, 70%, 500 mL
AP4369	Blender, Single-Speed
FB2039	Peroxidase Enzyme Activity—Advanced Inquiry Lab

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

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