



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.

Catalase-catalyzed reaction



Equation 1

Peroxidase-catalyzed reaction



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, $C_7H_8O_2$, 1 mL

Hydrogen peroxide, H_2O_2 , 3%, 3 mL

Isopropyl alcohol, $(CH_3)_2CHOH$ 70%, 100 mL

Phosphate buffer, pH 7, 300 mL, NaH_2PO_4 and Na_2HPO_4 , 0.05 M

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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Javier Perez-Villasenor



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