

LABORATORY 2: ENZYME CATALYSIS

OVERVIEW

In this laboratory you will observe the conversion of hydrogen peroxide (H_2O_2) to water and oxygen gas by the enzyme catalase. You will then measure the amount of oxygen generated and calculate the rate of the enzyme-catalyzed reaction.

OBJECTIVES

Before doing this laboratory you should understand:

- the general functions and activities of enzymes;
- the relationship between the structure and function of enzymes;
- the concept of initial reaction rates of enzymes;
- how the concept of free energy relates to enzyme activity; and
- that changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions.

After doing this laboratory you should be able to:

- measure the effects of changes in temperature, pH, enzyme concentration, and substrate concentration on reaction rates of an enzyme-catalyzed reaction in a controlled experiment; and
- explain how environmental factors affect the rate of enzyme-catalyzed reactions.

INTRODUCTION

In general, **enzymes** are proteins produced by living cells; they act as catalysts in biochemical reactions. A **catalyst** affects the rate of a chemical reaction. One consequence of enzyme activity is that cells can carry out complex chemical activities at relatively low temperatures.

In an enzyme-catalyzed reaction, the substance to be acted upon (the **substrate** = **S**) binds reversibly to the **active site** of the enzyme (**E**). One result of this temporary union is a reduction in the energy required to activate the reaction of the substrate molecule so that the **products** (**P**) of the reaction are formed.

In summary:



Note that the enzyme is not changed in the reaction and can be recycled to break down additional substrate molecules. Each enzyme is specific for a particular reaction because its amino acid sequence is unique and causes it to have a unique three-dimensional structure. The **active site** is the portion of the enzyme that interacts with the substrate, so that any substance that blocks or changes the shape of the active site affects the activity of the enzyme. A description of several ways enzyme action may be affected follows.

1. **Salt concentration.** If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract each other. The enzyme will denature and form an inactive precipitate. If, on the other hand, the salt concentration is very high, normal interaction of charged groups will be blocked, new interactions will occur, and again the enzyme will precipitate. An intermediate salt concentration such as that of human blood (0.9%) or cytoplasm is the optimum for many enzymes.
2. **pH.** pH is a logarithmic scale that measures the acidity of H^+ concentration in a solution. The scale runs from 0 to 14 with 0 being highest in acidity and 14 lowest. When the pH is in the range of 0-7, a solution is said to be acidic; if the pH is around 7, the solution is neutral; and if the pH is in the range of 7-14, the solution is basic. Amino acid side chains contain groups such as $-\text{COOH}$ and $-\text{NH}_2$ that readily gain or lose H^+ ions. As the pH is lowered an enzyme will tend to gain H^+ ions, and eventually enough side chains will be affected so that the enzyme's shape is disrupted. Likewise, as the pH is raised, the enzyme will lose H^+ ions and eventually lose its active shape. Many enzymes perform

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