

# Enzyme Catalysis

## 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 relative 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.

The enzyme is not changed in the reaction and can even 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:

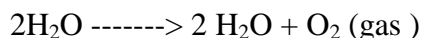
1. Salt Concentration. If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract to each other. The enzyme will denature and form an inactive precipitate. If, on the other hand, the salt concentration is too high, normal interaction of charged 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. Amino acid side chains contain groups such as - COOH and  $\text{NH}_2$  that readily gain or lose  $\text{H}^+$  ions. As the pH is lowered an enzyme will tend to gain  $\text{H}^+$  ions, and eventually enough side chains will be affected so the enzyme's shape is disrupted. Likewise, as the pH is raised, the enzymes will lose  $\text{H}^+$  ions and eventually lose its active shape. Many of the enzymes function properly in the neutral pH range and are denatured at either an extremely high or low pH. Some enzymes, such as pepsin, which acts in the human stomach where the pH is very low, have a low pH optimum.

3. Temperature. Generally, chemical reactions speed up as the temperature is raised. As the temperature increases, more of the reacting molecules have enough kinetic energy to undergo the reaction. Since enzymes are catalysts for chemical reactions, enzyme reactions also tend to go faster with increase temperature. However, if the temperature of an enzyme-catalyzed reaction is raised still further, a temperature optimum is reached; above this value the kinetic energy of the enzyme and water molecules is so great that the conformation of the enzyme molecules is disrupted. The positive effect of speeding up the reaction is now more than offset by the negative effect of changing the conformation of more and more enzyme molecules. Many proteins are denatured by temperatures around 40-50 degrees C, but some are still active at 70-80 degrees C, and a few even withstand boiling.

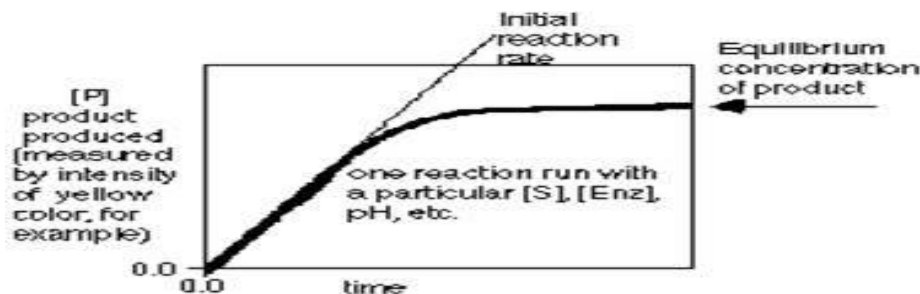
4. Activation's and Inhibitors. Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of the reaction it is an activator, or if it decreases the reaction rate it is an inhibitor. These molecules can regulate how fast the enzymes acts. Any substance that tends to unfold the enzyme, such as an organic solvent or detergent, will act as an inhibitor. Some inhibitors act by reducing the -S-S- bridges that stabilize the enzyme's structure. Many inhibitors act by reacting with the side chains in or near the active site to change its shape or block it. Many well known poisons such as potassium-cyanide and curare are enzyme inhibitors that interfere with the active site of critical enzymes.

The enzyme used in this lab, catalase, has four polypeptide chains, each composed of more than 500 amino acids. This enzyme is ubiquitous in aerobic organisms. One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide formed as a by-product of metabolic processes. Catalase might also take part in some of the many oxidation reactions that occur in the cell.



In the absence of catalase, this reaction occurs spontaneously, but very slowly. Catalase speeds up the reaction considerably. In this experiment, a rate for this reaction will be determined. Much can be learned about enzymes by studying the kinetics of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped. If the amount of product formed is measured at regular intervals and this quantity is plotted on a graph, a curve like the one that follows is obtained.

Study the solid line of the graph of this reaction. At time 0 there is no product. As time progresses the production of product increases at a steady rate. After a period of time this rate slows down and at a certain point the reaction rate is very slow.



#### Part One: Test of Catalase Activity

1. To observe the reaction to be studied, transfer 10 ml of 1.5% (0.44M)  $\text{H}_2\text{O}_2$  into a small beaker and add six chunks of potato approximately 1 cm<sup>3</sup> in size to the beaker. The bubbles coming from the reaction mixture are oxygen, which results from the breakdown of  $\text{H}_2\text{O}_2$  by catalase contained in peroxisomes in the potato.

- a. what is the enzyme in this reaction? \_\_\_\_\_
- b. What is the substrate in this reaction? \_\_\_\_\_
- c. What is the product in this reaction? \_\_\_\_\_
- d. How could you show that the gas evolved is oxygen ? \_\_\_\_\_

2. To demonstrate the effect of boiling on enzymatic activity, transfer six different chunks of potato approximately 1 cm<sup>3</sup> in size to a test tube and place this test tube in a boiling water bath for five minutes. Transfer 10 mL of 1.5% H<sub>2</sub>O<sub>2</sub> into a 50 mL glass beaker and add boiled potato solution from your test tube. Record your observations.

How does the reaction compare to the one using the unboiled catalase? Explain the reason for this observation.

3. To demonstrate the effect of acid on an enzyme in living tissue, take a small beaker and add six chunks of potato approximately 1 cm<sup>3</sup> in size to the beaker. Then cover the chunks of potato with acid provided by your teacher and wait one minute. Then add 10 ml of 1.5% H<sub>2</sub>O<sub>2</sub> to this solution. Record your observations.

4. Mix a teaspoon of table salt into the catalase extract. Wait for several minutes and observe its activity. Record your observations.

#### [Analysis Questions]

1. Explain the effect you observed with boiling the catalase in the potato with its function. Relate this to enzyme structure and chemistry.
2. Explain the inhibiting effect of acid on the function of catalase in your potatoes.. Relate this to enzyme structure and chemistry.
3. Explain why adding an ionic solution like sodium chloride may influence the function of an enzyme. Relate your explanation to your knowledge of enzyme structure and chemistry.
4. Predict the effect lowering the temperature would have on the rate of enzyme activity. Explain your prediction.
5. Why is there peroxide inside the cells of our body or the cells of a potato?
6. What is the biological role of the enzyme catalase?
7. State the overall chemical equation for cellular respiration and compare the product water in this reaction to the formula for hydrogen peroxide.