LAB TOPIC 4

Enzymes

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Define *enzyme* and describe the activity of enzymes in cells.
2. Differentiate competitive and noncompetitive inhibition.
3. Discuss the effects of varying environmental conditions such as pH and temperature on the rate of enzyme activity.
4. Discuss the effects of varying enzyme and substrate concentrations on the rate of enzyme activity.
5. Discuss the scientific process, propose questions and hypotheses, and make predictions based on hypotheses and experimental design.
6. Practice scientific thinking and communication by constructing and interpreting graphs of enzyme activity.

Introduction

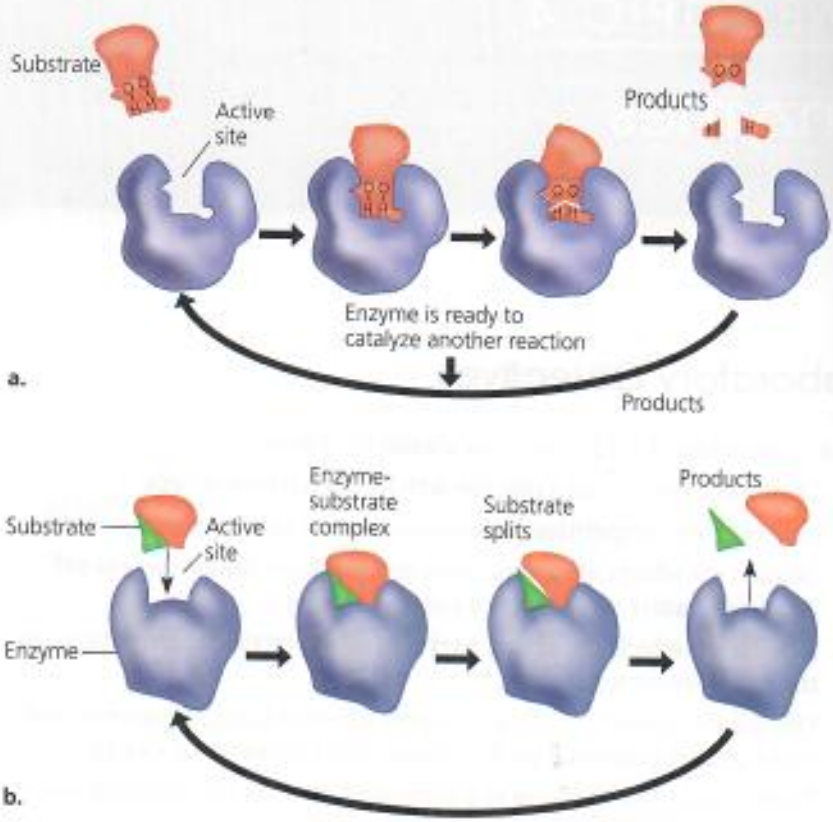
Living cells perform a multitude of chemical reactions very rapidly because of the participation of enzymes. **Enzymes** are biological **catalysts,** compounds that speed up a chemical reaction without being used up or altered in the reaction. The material with which the catalyst reacts, called the **substrate,** is modified during the reaction to form a new product (see Figure 4.1). But because the enzyme itself emerges from the reaction unchanged and ready to bind with another substrate molecule, a small amount of enzyme can alter a relatively enormous amount of substrate.

The **active site** of an enzyme will bind with the substrate, forming the **enzyme-substrate complex.** It is here that catalysis takes place, and when it is complete, the complex dissociates into enzyme and product or products.

Enzymes are, in part or in whole, proteins and are highly specific in function. Because enzymes lower the energy of activation needed for reactions to take place, they accelerate the rate of reactions. They do not, however, determine the direction in which a reaction will go or its final equilibrium.

Enzyme activity is influenced by many factors. Varying environmental conditions, such as pH or temperature, may change the three-dimensional shape of an enzyme and alter its rate of activity. Specific chemicals may also bind to an enzyme and modify its shape. Chemicals that must bind for the enzyme to be active are called **activators. Cofactors** are nonprotein substances that usually bind to the active site on the enzyme and are essential for the enzyme to work. Organic cofactors are called **coenzymes,** but other cofactors may simply be metal ions. Chemicals that shut off enzyme activity

FIGURE 4.1
Enzyme activity. A substrate or substrates bind to the active site of the enzyme, forming the enzyme-substrate complex, which then dissociates into enzyme and product(s). The enzyme may catalyze the addition or removal of a molecule or a portion of a molecule from the substrate to produce the product (a), or the enzyme may catalyze the splitting of a substrate into its component subunits (b).



are called **inhibitors,** and their action can be classified as **competitive** or **noncompetitive inhibition.**

Review Figure 4.1, illustrating enzyme activity. There are two ways to measure enzyme activity: (1) Determine the rate of disappearance of the substrate, and (2) determine the rate of appearance of the product.

In this laboratory, you will use both methods to investigate the activity of two enzymes, **catechol oxidase** and **amylase.** You will use an inhibitor to influence the activity of catechol oxidase and determine if it is a competitive or noncompetitive inhibitor. Additionally, you will investigate the effect of changing environmental conditions on the rate of amylase activity.

EXERCISE 4.1
Experimental Method and the Action of Catechol Oxidase

Materials

test-tube rack
3 small test tubes
small Parafilm™ squares
calibrated 5-mL pipette
3 calibrated 1-mL pipettes
disposable pasteur pipettes
pipette filler
pipette bulb
distilled or deionized (DI) water
Irish potato extract
catechol
disposable gloves (optional)

Introduction

This exercise will investigate the result of catechol oxidase activity. In the presence of oxygen, catechol oxidase catalyzes the removal of electrons and hydrogens from **catechol,** a phenolic compound found in plant cells. Catechol is converted to benzoquinone. Molecules of benzoquinone subsequently polymerize (combine) to form a dark pigment called catechol melanin to distinguish it from the melanin found in animals. The hydrogens combine with oxygen, forming water (Figure 4.2). The melanin is responsible for the darkening of fruits and vegetables, such as apples and potatoes, after exposure to air.

In this exercise you will use an extract of potato tuber to test for the presence of catechol oxidase and to establish the appearance of the products when the reaction takes place.

FIGURE 4.2
The oxidation of catechol. In the presence of catechol oxidase, catechol is converted to benzoquinone. Hydrogens removed from catechol combine with oxygen to form water.

Question

Remember that every experiment begins with a question. Review the information given earlier about the activity of catechol oxidase. You will be performing an experiment using potato extract.

Formulate a question about catechol oxidase and potato extract. The question may be broad, but it must propose an idea that has measurable and controllable elements.

Hypothesis

Construct a hypothesis for the presence or absence of catechol oxidase in potato extract. Remember, the hypothesis must be testable. It is possible for you to propose one or more hypotheses, but all must be testable.

Prediction

Predict the result of the experiment based on your hypothesis. To test for the presence or absence of catechol oxidase in potato extract, your prediction would be what you expect to observe as the result of this experiment (if/then).

Catechol is a poison! Avoid contact with all solutions. Do not pipette any solutions by mouth. Wash hands thoroughly after each experiment. If a spill occurs, notify the instructor. If the instructor is unavailable, wear disposable gloves and use dry paper towels to wipe up the spill. Follow dry towels with towels soaked in soap and water. Dispose of all towels in the trash.

Procedure

1. Using Table 4.1, prepare the three experimental tubes. Note that all tubes should contain the same total amount of solution. Do not cross-contaminate pipettes! After each tube is prepared, use your finger to hold a Parafilm™ square securely over the tube mouth and then rotate the tube to mix the contents thoroughly. Use a fresh square for each tube.

TABLE 4.1 Contents of the Three Experimental Tubes

Tube	Distilled Water	Catechol	Distilled Water	Potato Extract
1	5 mL	0.5 mL (10 drops)	0.5 mL (10 drops)	—
2	5 mL	0.5 mL (10 drops)	—	0.5 mL (10 drops)
3	5 mL	—	0.5 mL (10 drops)	0.5 mL (10 drops)

2. Explain the experimental design: What is the purpose of each of the three test tubes? Which is the control tube? Is more than one control tube necessary? Explain. Which is the experimental tube? Why is an additional 0.5 mL of distilled water added to tubes 1 and 3, but not tube 2?

3. Observe the reactions in the tubes, and record your observations in the Results section below. Explain your conclusions in the Discussion section.
4. After recording your results, dispose of the solutions in the test tubes in the waste container indicated by your instructor. Do not pour down the drain.

Results

Design a simple table to record results (Table 4.2).

TABLE 4.2 Results of Catechol Oxidation Experiment

Discussion

1. Explain your results in terms of your hypothesis.

2. In this experiment, the enzyme catechol oxidase was extracted from potato. However, this was not a purified preparation; it contained hundreds of enzymes. What evidence supports the assumption that catechol oxidase was the enzyme studied?

EXERCISE 4.2
Inhibiting the Action of Catechol Oxidase

Materials

test-tube rack
3 small test tubes
small Parafilm™ squares
calibrated 5-mL pipette
4 calibrated 1-mL pipettes
disposable pasteur pipettes
pipette bulb
distilled water
potato extract
catechol
phenylthiourea (PTU)
disposable gloves (optional)

Introduction

This exercise will investigate the inhibition of enzyme activity by specific chemicals called **inhibitors.** The specific inhibitor used is **phenylthiourea (PTU).** To be active, catechol oxidase requires copper as a cofactor. PTU is known to combine with the copper in catechol oxidase and inhibit its enzymatic activity.

An inhibitor molecule affects an enzyme in one of two ways. **Competitive inhibition** takes place when a molecule that is structurally similar to the substrate for a particular reaction competes for a position at the active site on the enzyme. This ties up the enzyme so that it is not available to the substrate. Competitive inhibition can be reversed if the concentration of the substrate is raised to sufficiently high levels while the concentration of the inhibitor is held constant (Figure 4.3a and b).

In **noncompetitive inhibition,** the inhibitor is not structurally similar to the substrate and does not compete for position in the active site. It may

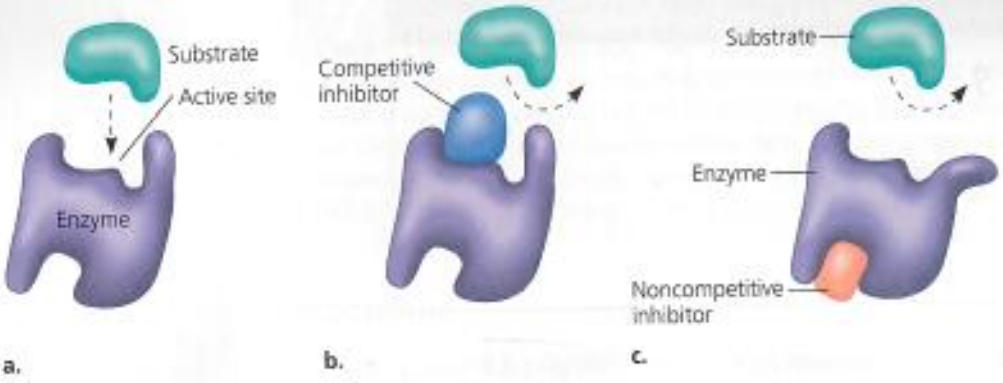


FIGURE 4.3
Action of enzyme inhibitors—competitive and noncompetitive. (a) Without inhibition, the substrate binds to the active site of an enzyme. (b) A **competitive inhibitor** mimics the substrate and competes for the position at the active site on the enzyme. (c) In some examples of noncompetitive inhibition, the inhibitor binds to the enzyme at a location away from the active site, changing the conformation of the enzyme, rendering it inactive.

physically block the access to the active site or it may bind to a part of the enzyme that is not the active site, causing the conformation of the protein to change (Figure 4.3c). A noncompetitive inhibitor may also inhibit the active site by interfering with a cofactor. In noncompetitive inhibition the inhibitor can become unbound, reversing the inhibition. However, unlike in competitive inhibition, *adding additional substrate* will not reverse the inhibition.

In the following experiment, you will determine if PTU is a competitive or noncompetitive inhibitor.

Question

Pose a question about the activity of PTU.

Hypothesis

Hypothesize about the nature of inhibition by PTU.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

PTU and catechol are poisons! Avoid contact with solutions. Do not pipette any solutions by mouth. Wash hands thoroughly after the experiment. If a spill occurs, notify the instructor. If the instructor is unavailable, wear disposable gloves and use dry paper towels to wipe up the spill. Follow dry towels with towels soaked in soap and water. Dispose of all towels in the trash.

TABLE 4.3 Contents of the Three Experimental Tubes

Tube	Distilled Water	Potato Extract	PTU	Distilled Water	Catechol
1	5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL
2	5 mL	0.5 mL	0.5 mL	—	1 mL
3	5 mL	0.5 mL	—	1 mL	0.5 mL

1. Using Table 4.3, prepare three experimental tubes. Be sure to add solutions in the sequence given in the table (water first, potato extract next, PTU next, etc.). Cover each tube with a fresh Parafilm™ square and mix.
2. Which test tube is the control?

3. Why was the concentration of catechol increased in test tube 2?

4. Why should the catechol be added to the test tubes last?

5. Record your observations in the Results section, and explain your results in the Discussion section.
6. After recording your results in Table 4.4, dispose of the solutions in the test tubes in the waste container indicated by your instructor. Do not pour down the drain.

Results

Design a table to record your results (Table 4.4) in the margin.

TABLE 4.4 Results of Inhibition Experiment

Discussion

1. Explain your results in terms of your hypothesis.

2. One member of your team is not convinced that you have adequately tested your hypothesis. How could you expand this experiment to provide additional evidence to strengthen your conclusion?