Enzymes

Introduction

Biological processes depend on molecular catalysts called **enzymes** to speed up the chemical reactions that are necessary for cells to function. It takes energy to initiate some chemical reactions. Enzymes work by lowering that amount of energy so it's easier for the reaction to get started. Without enzymes the reactions would take place far too slowly to support life. The basic components of an enzyme-catalyzed reaction are the substrate(s), the product(s), and the enzyme itself.

The **substrate** is the reactant molecule that is changed by the enzyme. Each enzyme has a specific substrate or type of substrate. For example, the substrate sucrose is acted upon by the enzyme sucrase. In the reaction shown in Figure 5.1, a disaccharide, sucrose, is broken down into its component monosaccharides glucose and fructose, which are the **products** of this reaction. The enzyme itself is neither changed nor destroyed during the reaction.

Enzymes speed up the synthesis of biological molecules as well as the breakdown, but synthesis is usually more complex than breakdown and requires a series of reactions. When sucrose is synthesized from glucose and fructose, for example, there are six steps to the process. Each step is catalyzed by a different enzyme.

There are many chemical reactions that are common to most organisms, so the enzymes that catalyze these reactions are also common. For example, the process by which energy is harvested from glucose molecules is nearly universal, so almost all organisms have common enzymes that are used in this common pathway However, the specific properties and behaviors of enzymes may be different in different organisms. Even within the same organism, the version of an enzyme found in one organ may be slightly different from the version found in a different organ.

In addition to differences in the enzyme molecules themselves, the environment in which an enzyme works is an important influence on the reaction rate.

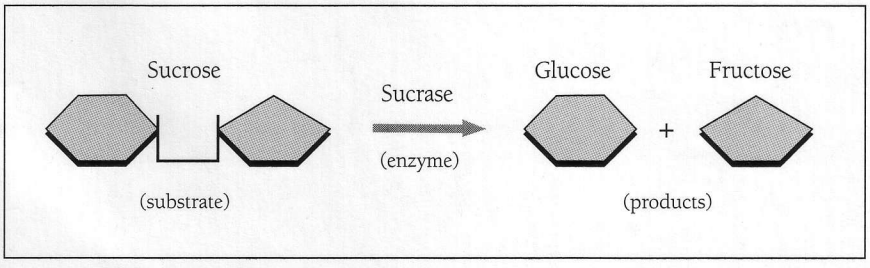

Figure 5.1.
Breakdown of sucrose by sucrase into glucose + fructose.

In this lab topic you will learn a method for investigating catecholase, an enzyme that is found in many plants. Your lab team will then design and perform your own experiment using this method.

Outline

EXERCISE 5.1

Factors Affecting Reaction Rate

Objectives

After completing this exercise, you should be able to

1. Make predictions regarding the effects that the amounts of enzyme and substrate present will have on the rate of an enzyme-catalyzed reaction.
2. Make predictions regarding the effects that physical factors such as pH, salt concentration, and temperature will have on the rate of an enzyme-catalyzed reaction.

Activity A: Quantity of the Reactants

Enzyme molecules do not undergo permanent changes during a reaction. After "turning over" one substrate molecule into product, the enzyme is free to engage with another substrate molecule and repeat the process. Enzymes work at a steady pace, turning over substrate into product for as long as substrate is available.

If substrate is abundant, what should happen to the reaction rate (amount of product formed/unit time) when **more enzyme** molecules are added to the reaction mixture? Sketch your prediction on the axes in Figure 5.2. Remember to graph the independent variable on the horizontal axis and the dependent variable on the vertical axis. Label both axes.

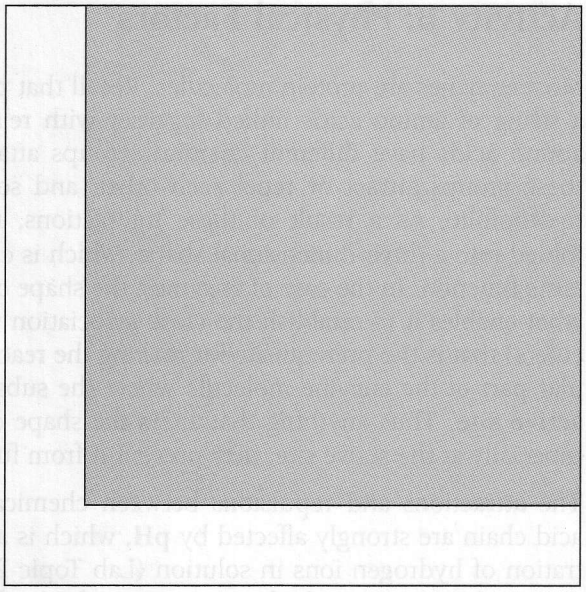

Figure 5.2.
Effect of enzyme concentration on reaction rate.

If the number of enzyme molecules is constant and the number of substrate molecules is low, what do you expect to happen to the reaction rate when **more substrate** is added to the reaction mixture? Sketch your prediction on the axes in Figure 5.3 and label both axes.

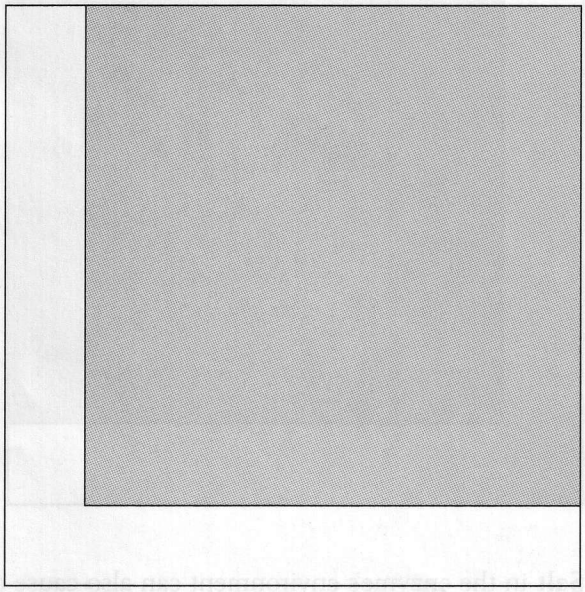

Figure 5.3.
Effect of substrate concentration on reaction rate.

Activity B: Physical Factors

Most enzymes are protein molecules. Recall that proteins are composed of a string of amino acids linked together with relatively tight bonds. The amino acids have different chemical groups attached to them. Some of these groups attract or repel each other, and some are hydrophobic or hydrophilic. As a result of these interactions, the protein molecule is folded into a three-dimensional shape, which is closely related to the protein's function. In the case of enzymes, the shape of the protein molecule is what enables it to establish the close association with the substrate molecule(s) that is the prerequisite for making the reaction happen. The particular part of the enzyme molecule where the substrate fits in is called the **active site.** Thus anything that alters the shape of the enzyme molecule, especially at the active site, may prevent it from functioning correctly.

The attractions and repulsions between chemical groups on the amino acid chain are strongly affected by **pH,** which is a measure of the concentration of hydrogen ions in solution (Lab Topic 2, pH and Buffers). Each enzyme has a pH at which it is most perfectly shaped; it therefore functions best at that pH. If the pH is too low (acidic) or too high (basic), the enzyme molecule loses its shape and thus its ability to catalyze reactions.

How do you think reaction rate will change in solutions of different pH? Sketch your idea on the axes shown in Figure 5.4 and label both axes.

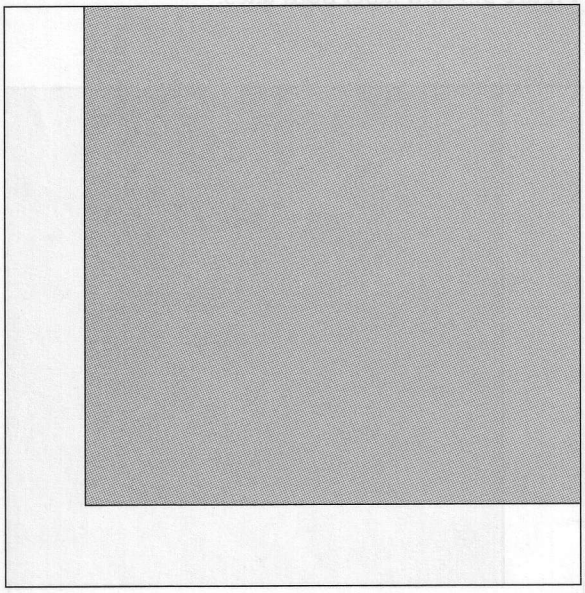

Figure 5.4.
Effect of pH on reaction rate.

Salt in the enzyme's environment can also cause distortion of its shape by changing the interactions of the chemical groups on the amino acids. Just as with pH, there is an optimum salt concentration at which the enzyme is shaped perfectly to engage the substrate.

Temperature also has a profound effect on enzyme reactions. Since the enzyme molecules must be in actual contact with substrate molecules for the enzyme to catalyze the reaction, anything that increases the number of

collisions between enzyme and substrate is expected to increase the reaction rate. Sketch this relationship on the axes in Figure 5.5 and label both axes.

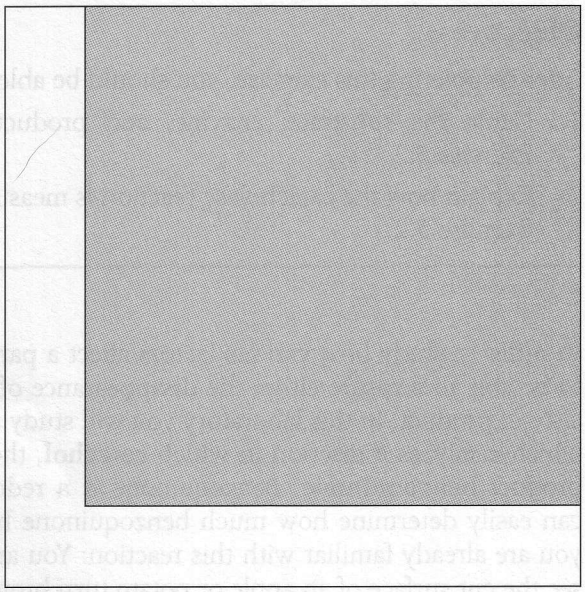

Figure 5.5.
Effect of temperature on reaction rate.

But heat energy has another effect in addition to speeding up the movement of molecules. It can also disrupt the delicate attractions between the chemical side groups of amino acids, causing the enzyme to change its shape and perhaps even be denatured. So when the temperature is too high, the enzyme can't catalyze the reaction. Therefore, like pH and salt concentration, enzymes work best in an optimum temperature range. Adding this information to the graph you just drew, sketch how temperature is related to reaction rate on the axes in Figure 5.6.

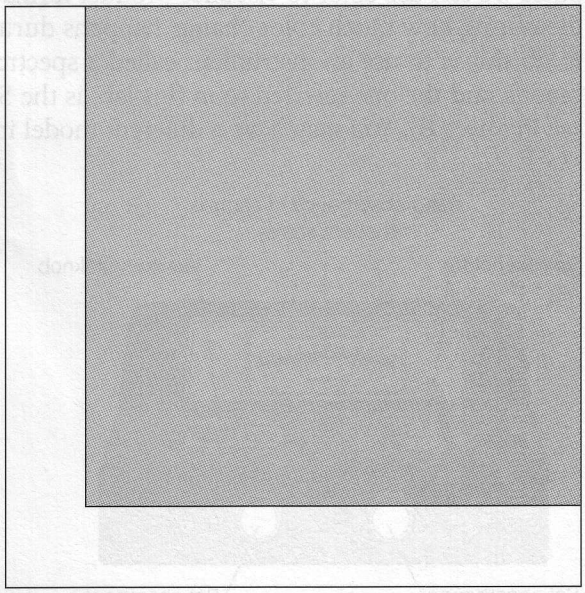

Figure 5.6.
Effect of temperature on reaction rate.

EXERCISE 5.2

The Catecholase-Catalyzed Reaction

Objectives

After completing this exercise, you should be able to

1. Name the substrate, enzyme, and product in the experiment in Exercise 5.2.
2. Explain how the catecholase reaction is measured in the experiment in Exercise 5.2.

In order to study how various factors affect a particular enzyme, we need to be able to measure either the disappearance of substrate or the appearance of product. In this laboratory you will study the enzyme **catecholase,** which catalyzes a reaction in which **catechol,** the substrate, becomes the product **benzoquinone.** Benzoquinone is a reddish-brown color, so we can easily determine how much benzoquinone has been formed. In fact, you are already familiar with this reaction: You are observing it when you see the cut surface of an apple or potato turn brown. A good source of catecholase is an extract made from potatoes.

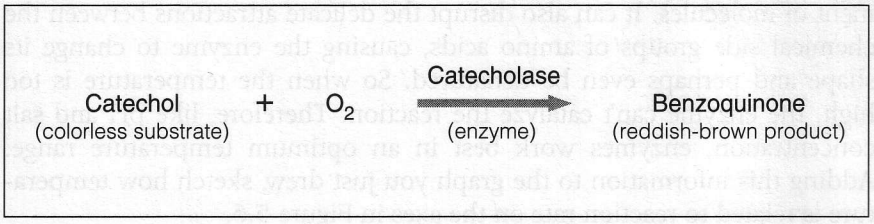

Figure 5.7.
Catecholase reaction. The colorless substrate is converted to a reddish-brown product.

Since we can use color to visualize product formation, we need a means of measuring how much color change happens during the reaction. One way to do this is to use an instrument called a spectrophotometer. A common model, and the one referred to in this lab, is the Spectronic 20 (Spec 20—see Figure 5.8). You may have a different model in your laboratory.

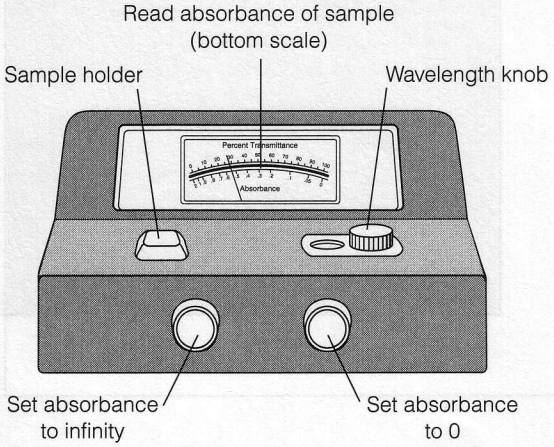

Figure 5.8.
Spec 20.

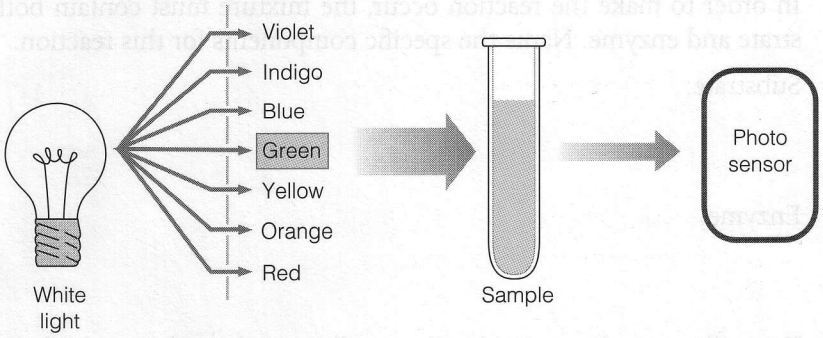

Figure 5.9.
Diagram of how a Spec 20 works. Like a prism, a spectrophotometer divides white light into its component wavelengths. In this diagram, green light has been selected to shine through the sample.

The Spec 20 measures catecholase activity by measuring the color change in reaction mixtures. As shown in Figure 5.9, this instrument shines light through a sample of reactants in a test tube and measures the amount of light that penetrates through the tube. This tells us how much light was absorbed by the sample, which in turn is a measure of how much product has been formed. The more of the reddish-brown product that has been made, the more light will be absorbed.

If spectrophotometers are not available, you may use a color chart to determine how much reaction has occurred. Compare your samples with Plate 1 and record the number for color intensity that matches the sample most closely. The more intense the color, the more benzoquinone has been formed.

On the axes in Figure 5.10, sketch how absorbance or color intensity (y-axis) varies in relation to product formation (x-axis) for the catecholase reaction. Label both axes.

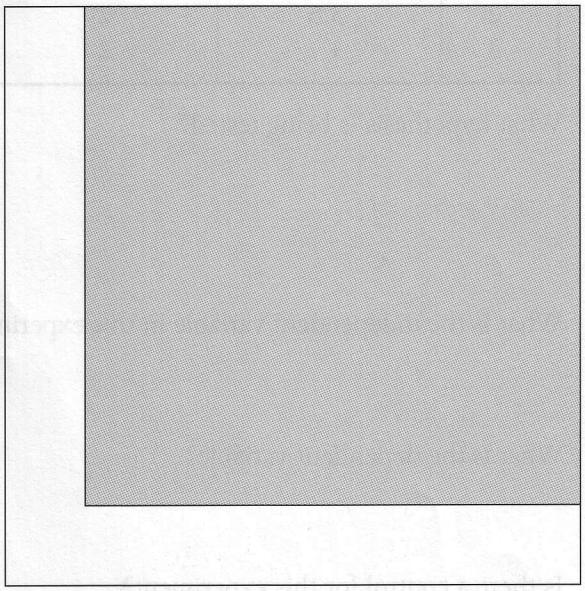

Figure 5.10.
Relationship of product formation and absorbance.

In order to make the reaction occur, the mixture must contain both substrate and enzyme. Name the specific components for this reaction.

Substrate:

Enzyme:

You will set up three sample tubes to illustrate how this method works. If you are using a Spec 20 or other spectrophotometer, you must first zero the instrument using a blank. (If you are using the color chart method, you may skip the rest of this paragraph.) The blank is a type of control. You will notice that the potato extract itself has color; it already absorbs some light. That amount of light must be subtracted from the amount of light absorbed by the product of the reaction. This procedure is analogous to taring a balance. The blank contains enzyme (potato extract) but no substrate, so very little reaction will take place. (There will be some reaction, though, because the potato extract itself contains substrate.)

Look at Table 5.1 to see how the experiment has been designed and answer the following questions.

Table 5.1
Reaction Mixtures for Catecholase Experiment

Tube	Water (mL)	Catechol (mL)	Extract (mL)
1	3	2	1
2	3.5	2	0.5
3	4	2	0

What hypothesis is being tested?

What is the independent variable in this experiment?

What is the dependent variable?

Is there a control for this experiment?

Predict the outcome of your experiment in terms of your hypothesis. Explain what results would support your hypothesis and what results would prove your hypothesis false.

Procedure

Zeroing the Spec 20 (skip steps 1–9 if you are using the color chart)

1. Get a clean test tube (either a small test tube or a special Spec tube) and use a wax pencil to label it B (for blank). If the tube does not already have a short vertical mark at the top, draw one with wax pencil. When you put the tube into the sample holder, the mark should always face front.
2. Measure 1 mL potato extract into Tube B.
3. Add 5 mL distilled water to Tube B.
4. Cover Tube B tightly with Parafilm and invert it to mix the contents.
5. Set the wavelength knob on the Spec 20 to 540 nm (see Figure 5.8).
6. Using the knob on the left (Figure 5.8), set the absorbance reading (bottom scale) to infinity.
7. Wipe Tube B with a cleaning tissue and insert it into the sample holder of the Spec 20 with the vertical mark facing front.
8. Using the knob on the right, set the absorbance reading to 0.
9. Set Tube B aside. You will need to rezero the Spec 20 later.

The Spec 20 is now zeroed and ready to measure the tubes in which the catecholase reaction has occurred.

Measuring Color Change in Samples

10. Use a pipet to measure the water into Tubes 1, 2, and 3 (see Table 5.1).
11. Use a different pipet to add 2 mL catechol to each tube.
12. Use a third pipet to add 1 mL potato extract to Tube 1. Add 0.5 mL potato extract to Tube 2. Do not add any potato extract to Tube 3.
13. Place Parafilm tightly over the top of each tube and invert the tubes to mix their contents.
14. Shake each tube gently at 1 minute intervals to keep the reactants well mixed.

Spec 20 Method

15. After 3 minutes, use the blank to rezero the Spec 20 (follow steps 6–8).
16. Insert each tube in turn into the sample holder and read the absorbance (bottom scale). Record the absorbance reading for each tube in Table 5.2.

Color Chart Method

15. After 5 minutes, compare each tube to the catecholase chart on Color Plate 1. Observe the intensity of the colors (the intensity is more important than the actual color). In Table 5.2 record the number of the color intensity that most closely matches the color intensity of each tube.

Table 5.2
Results of Catecholase Experiment (absorbance or color intensity is recorded after 3 minutes)

	Tube		
	1	2	3
Absorbance/color intensity			

Was your hypothesis proven false or supported by the results? Use data to support your answer.

Predict the color change of a tube containing 3.75 mL water, 2 mL catechol, and 0.25 mL of potato extract. Explain how you derived this prediction from your data.

Why is it necessary to add the potato extract to each tube last, after the water and catechol are already measured?

Why was a different amount of water added to each tube?

EXERCISE 5.3

Designing an Experiment

Objective

After completing this exercise, you should be able to

1. Design an original experiment to investigate some aspect of enzyme activity.

In Exercise 5.2 you learned a method of measuring the reaction as the enzyme catecholase converts catechol to benzoquinone. In Exercises 5.3 and 5.4 your lab team will design an experiment using this method, perform your experiment, and present and interpret your results. You may want to review Exercise 5.1 to help you decide on an independent variable for your investigation.

The following materials will be supplied for your group.

50 mL potato extract	30 Parafilm squares
2 1-mL pipets	30 test tubes
3 5-mL pipets	test tube rack
pi-pump for 1-mL pipet	50 mL of 0.05% catechol
pi-pump for 5-mL pipet	bottle of distilled water
wax marker	

Your instructor will be able to tell you what additional materials will be available.

Describe your experiment below.

Question or Hypothesis

Dependent Variable

Independent Variable

Explain why you think this independent variable will affect catecholase activity.

Control Treatment(s)

Replication

Brief Explanation of Experiment

Predictions

What results would support your hypothesis? What results would prove it false?

Method

Include the levels of treatment you plan to use. It might be helpful to make a table like Table 5.1, showing the contents of each reaction tube.

Design a Table to Collect Your Data

List Any Additional Materials You Will Require

EXERCISE 5.4

Performing the Experiment and Interpreting the Results

Objectives

After completing this exercise, your should be able to

1. Perform the experiment your lab team designed.
2. Present and interpret the results of your experiment.

Before you do the experiment, be sure that everyone on your lab team understands the techniques that will be used. You may want to divide up the tasks before you begin work. Since it is important to measure the volumes of reactants accurately, you may want to ask your instructor to review pipet use with you.

Be thorough in collecting data. Don't just write down numbers; record what they mean as well. Don't rely on your memory for information that you will need when reporting on your experiment later! If you have any questions, doubts, or problems during the experiment, be sure to write them down, too.

Results

Before you begin to prepare your results for presentation, decide on the best format to use. Remember, you want to give the reader a clear, concise picture of what your experiment showed. Refer to the data presentation exercise in Appendix A (Tools for Scientific Inquiry) for help. If you are drawing graphs, use graph paper. Complete your tables and/or graphs before attempting to interpret your results.

Write a few sentences *describing* the results (don't explain why you got these results or draw conclusions yet).

Discussion

Look back at the hypothesis or question you posed in this experiment. Look at the graphs or tables of your data. Do your results support your hypothesis or prove it to be false? Use your data to support your answer.

Did your results correspond to the prediction you made? If not, explain how your results are different from your expectations and why this might have occurred.

Describe how your data are supported by information from other sources (for example, textbooks or other lab teams working on a similar problem).

If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.

If you had an opportunity to repeat and extend this experiment to make your results more convincing, what would you do?

Summarize the conclusion you have drawn from your results.

Questions for Review

1. A freshly cut potato turns brown when left standing. Why do mashed potatoes stay white?

2. The three graphs in Figure 5.11 represent three different enzymes that are found in a unicellular pond organism. Assuming that these enzymes must function in order for the organism to grow, what water pH is best for this organism? Explain your answer.

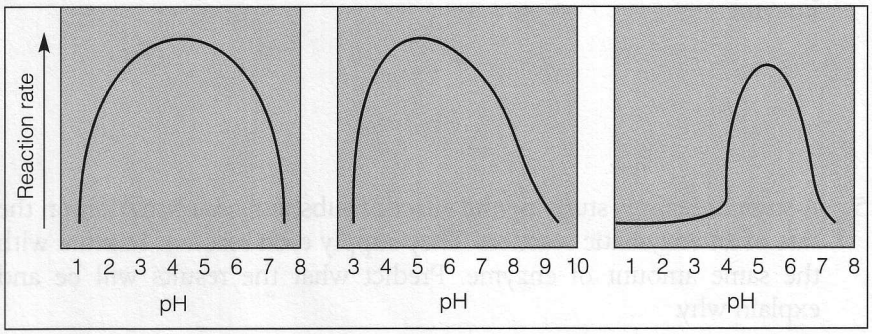

Figure 5.11.
Reaction rate versus pH of water for three enzymes.

3. Lemon juice, which has a pH of about 3, can be sprinkled on freshly cut fruit to keep it from turning brown. Propose a hypothesis to explain this observation.

   How could you test your hypothesis?

   Propose an alternative hypothesis. (Hint: Lemon juice is a complex substance.)

4. You hypothesize that reaction rate is proportional to enzyme concentration and design an experiment to test your hypothesis. Fill in the blanks below to complete the design.

	Water	**Substrate**	**Enzyme**
Tube A	20 drops	_____	_____
Tube B	15 drops	10 drops	5 drops
Tube C	_____	10 drops	_____
Tube D	_____	_____	20 drops

   Explain your answers:

   Water:

   Substrate:

Enzyme:

5. A student team is studying the effect of substrate concentration on the rate of an enzymatic reaction. They supply each reaction mixture with the same amount of enzyme. Predict what the results will be and explain why.