LAB TOPIC 4: ENZYMES

Objectives

- Define enzyme and describe the activity of enzymes in cells.
- Discuss the effects of varying enzyme concentrations on the rate of enzyme activity.
- Discuss the effects of varying environmental conditions such as pH and temperature on the rate of enzyme activity.

Introduction

The chemical reactions in cells would not occur fast enough to support life without the action of enzymes. **Enzymes** are organic catalysts that greatly accelerate the rate of chemical reactions in cells by reducing the required activation energy. All chemical reactions require a certain amount of activation energy to start. By lowering the required activation energy, an enzyme greatly increases the rate of a chemical reaction by up to a million times a second in some cases (Figure 4.1)

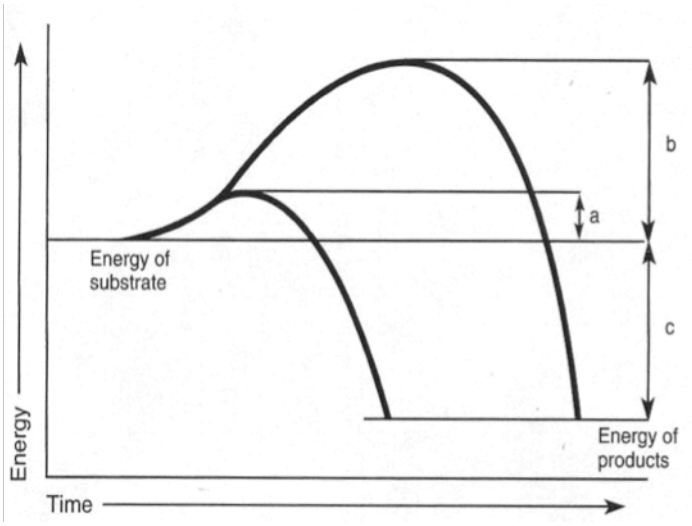

Figure 4.1 Activation energy and enzymes. (a) Activation energy required with enzyme. (b) Activation energy required without enzyme. (c) Net energy released by the reaction.

Enzymes are proteins; therefore, each enzyme consists of a specific sequence of amino acids. Weak hydrogen bonds that form between some of the amino acids help to determine the three dimensional shape of the enzyme, and it is this shape that allows the enzyme to fit on to a specific **substrate molecule** (the substance the enzyme acts upon). The enzyme and the substrate molecule must fit together like a lock

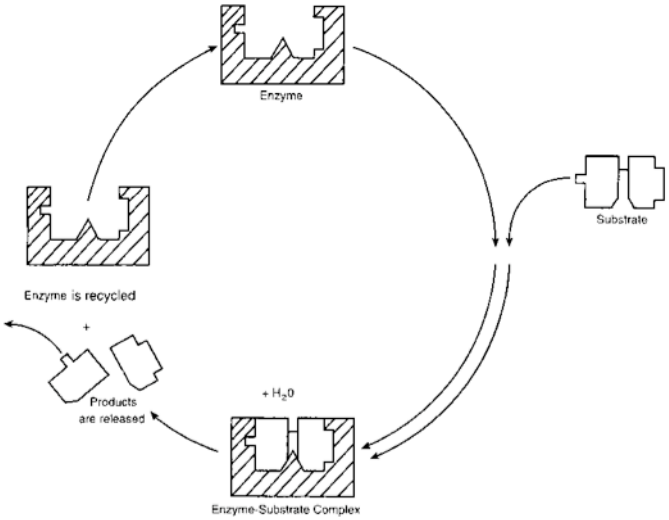

Figure 4.2. The mechanism of enzyme action.

Enzyme catalyzed reactions can be expressed in the following way:

$$E + S \Rightarrow ES \Rightarrow E + P$$

The enzyme (E) combines with the substrate molecules (S) to form a temporary **enzyme substrate complex** (ES), in which the specific reaction occurs. Then the product molecules (P) separate from the enzyme, and the unchanged enzyme is recycled to combine with another substrate molecule. Note that the enzyme is not altered in the reaction, which means that a few enzyme molecules can catalyze a great number of reactions.

An enzyme is inactivated by a change in shape (i.e. **denatured**), and its shape is altered by anything that disrupts the enzyme's pattern of hydrogen bonding. For example, many enzymes function best within rather narrow temperature and pH ranges, because substantial changes in temperature or pH

disrupt their hydrogen bonds and alter their shape. However, some enzymes function well over rather broad temperature and pH ranges because their hydrogen bonds are not easily disrupted. It is the unique hydrogen-bonding pattern of each enzyme that determines the sensitivity of the enzyme to changes in temperature and pH.

Exercise 4.1
The Action of Peroxidase: Effect of Enzyme Concentration

All organisms using molecular oxygen produce hydrogen peroxide (H_2O_2) as a harmful byproduct of some cellular reactions. **Hydrogen peroxide** is a strong oxidizing agent that can cause serious damage to cells. Fortunately, cells have an enzyme, **peroxidase** that quickly breaks down hydrogen peroxide into water and oxygen, preventing cellular damage.

$$\text{R (reduced) } H_2 + H_2O_2 \xrightarrow{\text{(peroxidase)}} \text{R (oxidized) } + 2\ H_2O$$

In this exercise you will use an extract of horseradish as your source of peroxidase. You will mix the horseradish extract with a solution of hydrogen peroxide to examine the activity of horseradish peroxidase. This reaction will be coupled to a reaction with the dye **guaiacol**, which turns from colorless to brown as it is oxidized by the enzyme peroxidase in the presence of H_2O_2. We can monitor this reaction by using a spectrophotometer to measure changes in light absorption.

$$\text{Guaiacol-reduced (colorless)} \Rightarrow \text{Guaiacol-oxidized (brown)}$$

In the first exercise, you will use varying amounts of horseradish extract to test for the presence of peroxidase and to establish the appearance of the products when the reaction takes place. 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?

Hypothesis

Construct null and alternative hypotheses for the effect of peroxidase concentration on the reaction rate. Remember, your hypotheses must be testable.

Prediction

Predict the result of the experiment based on your hypotheses. Your prediction would be what you expect to observe as a result of this experiment (if/then).

Procedure
You will set up four sample tubes to illustrate how this reaction works and to examine the effects of enzyme concentration on the rate of activity. Remember when using a spectrophotometer, you must first zero the instrument using a blank (see Lab Topic 2). The blank consists of everything in the reaction mixture, **except the horseradish extract** (Tube 1).

What are the specific components for this reaction?

Substrate:

Enzyme:

Product:

Using Table 4.1, prepare the blank and three experimental tubes. **HINT:** Fill the tubes in the order presented in the table (i.e., buffer first, then the guaiacol, then the extract, and finally hydrogen peroxide). Use labeling tape and a marker to label each of the tubes near the top. After adding the solutions to the tubes cap them and invert to mix the contents.

After three minutes you will measure absorption. Use 470 nm as your wavelength. (see Lab Topic 2 for review of spectrophotometer use). Record the absorbance of each sample tube in Table 4.1. Use your results to construct a graph of absorbance (enzyme activity) vs. enzyme concentration.

What is your dependent variable? Independent variable? Remember to label the axes appropriately.

Do you accept or reject your null hypothesis based on the results? Use your data to support your answer.

Predict the color change of a tube containing 5.0 mL of buffer, 0.2 mL of hydrogen peroxide, 0.1 mL of guaiacol, and 0.7 mL of horseradish extract. Explain how you derived this prediction from your data.

Table 4.1 Reaction mixtures for the peroxidase enzyme concentration experiment

Tube	Buffer (mL)	Guaiacol (mL)	Enzyme (mL)	Hydrogen Peroxide (mL)	Absorbance (470 nm)
1 (blank)	5.7	0.1	0	0.2	
2	5.5	0.1	0.2	0.2	
3	5.2	0.1	0.5	0.2	
4	4.7	0.1	1.0	0.2	

Exercise 4.2
Designing an Experiment: Effects of Environmental Condition

In Exercise 4.1 you learned a method of measuring the reaction as peroxidase converts hydrogen peroxide to oxygen and water. In exercise 4.1 we used a pH 7 buffer and ran the experiment at room temperature or approximately 20°C.

In Exercise 4.2, **your lab group will design an experiment** using the method described above to examine the effect of **either** pH or temperature on peroxidase activity.

There is a set of buffers of pH 4, 7, and 10 and temperature treatments of 4, 20, and 80°C.

The first step in this exercise is for your group to decide which variable (pH or temperature) you will test. Remember you should only vary one factor all others should remain constant.

Hypothesis
Construct null and alternative hypotheses for the effect of pH or temperature on the reaction rate. Remember, your hypotheses must be testable.

Prediction
Predict the result of the experiment based on your hypotheses. Your prediction would be what you expect to observe as a result of this experiment (if/then).

Procedure
Follow the same procedure used for Exercise 4.1, EXCEPT, you will use either different pH buffers for each treatment tube OR place each treatment tube at a different temperature to observe the effect of these factors on peroxidase activity. Add all ingredients except substrate. After 10 minutes; add substrate to the pH tubes at each table or AT THE TEMPERATURE STATION (do not remove tubes from the treatment) and continue exposure for 3 minutes. Remember to mix the tubes after adding substrate.

Measure absorbance at 470 nm. Record your treatments (pH OR temperature) and results in table 4.2 below.

Use your results to construct a graph of absorbance (enzyme activity) vs. environmental condition.

What is your dependent variable? Independent variable? Remember to label the axes appropriately.

Do you accept or reject your null hypothesis based on the results? Use your data to support your answer.

NOW SHARE YOUR DATA WITH THE CLASS

Table 4.2 Reaction mixtures for the peroxidase enzyme environmental condition experiment

Tube	pH	Temp	Buffer (mL)	Guaiacol (mL)	Enzyme (mL)	Hydrogen Peroxide (mL)	Absorbance (470 nm)
1 (blank)	7	20°C	5.7	0.1	0.0	0.2	
2			4.7	0.1	1.0	0.2	
3			4.7	0.1	1.0	0.2	
4			4.7	0.1	1.0	0.2	

Questions for Review

Explain your answers:

1. Do you think that there is a correlation between the widespread presence of peroxidase among organisms and the ranges of temperature and pH within which this enzyme is active (Explain)?

2. We extracted enzyme X from an **arctic** cod. Our results suggest that this enzyme has a temperature optimum of 50°C. Does this result make sense or do we need to repeat the experiment, explain?

Vocabulary list
Enzyme
Substrate
Product
Active site
Enzyme-substrate complex
Peroxidase
Guaiacol
Hydrogen peroxide
pH
Denature

3. You are designing an experiment to examine the effect of substrate concentration on enzyme activity. State null and alternative hypotheses for this experiment:

   Null:

   Alternative:

Fill in the blanks below to complete the design.

Tube	Buffer (mL)	Guaiacol (mL)	Enzyme (mL)	Hydrogen peroxide (mL)
1 (blank)	4.7	0.1	1.0	0.2
		0.1		0.4
3	4.3	0.1	1.0	