

## Biology 3A Laboratory: Enzyme Function

### Objectives

- To be able to list the general characteristics of enzymes.
- To study the effects of enzymes on the rate of chemical reactions.
- To demonstrate the effect of some environmental conditions on enzymatic reactions such as temperature, pH, substrate concentration, enzyme concentration and inhibitors.

### Introduction

All living organisms require energy in order to sustain the many processes involved in life. The energy for these processes is provided by **cellular respiration**, a catabolic process that releases energy (exergonic), most often as ATP. It is essential that the chemical reactions involved in cellular respiration occur at a rapid rate and within optimum conditions. Enzymes are critical in this process.

Enzymes are biological **catalysts** that accelerate the multitude of anabolic and catabolic chemical reactions (movement, cellular respiration, digestion, growth, etc.), which occur in living organisms. Many of these reactions are not only accelerated by enzymes, but would not occur to any appreciable extent at body temperature without them.

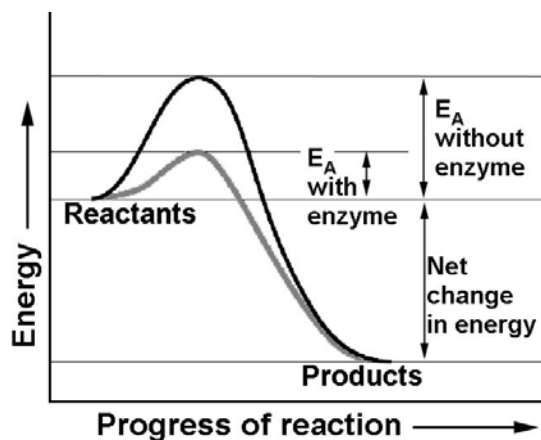


Figure 1. Energy of activation with and without an enzyme.

Before any chemical reaction can occur, molecules must obtain enough energy (**energy of activation -  $E_A$** ). This energy may be provided when one molecule collides with another or from an external source of energy such as heat. The amount of energy required by chemical reactions varies. The greater the energy barrier, the more energy required to drive the reaction. Enzymes increase reaction rates by lowering the energy of activation (Figure 1).

Each enzyme catalyzes a specific chemical reaction. The molecules that enzymes work on are called **substrates**. Each enzyme has one or a few substrates. Whenever a group of substrates are susceptible to

catalysis by a particular enzyme, those substrates are closely related compounds, demonstrating the **specificity** of enzymes. This specificity is dependent on the three dimensional shape of the enzyme.

The catalytic cycle (Figure 2) for enzymatic reactions begins as the **reactants** (enzyme and substrate) collide and the substrate fits into the **active site** (Figure 3) of the enzyme. The substrate and enzyme forms the **enzyme-substrate complex** held together by temporary bonding (hydrophobic interactions, hydrogen and ionic bonds). It is during the complex formation that the chemical reaction(s) takes place resulting in the **product(s)**. Notice that the enzyme also appears with the product(s) in the equation below (Figure 2). Enzymes emerge essentially unchanged upon completion of the chemical reaction and are capable of further catalysis (**reusable**).

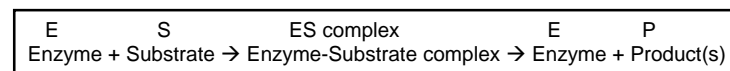


Figure 2. Catalytic cycle for enzymatic reactions

### A: TYROSINASE ACTIVITY

The enzyme **tyrosinase** is naturally found in potatoes. The substrate, **pyrochatechol** is a clear colorless liquid. The product formed from these **hydroxyquinone**, which has a yellowish color. The appearance of this color indicates that a reaction has occurred between the enzyme and the substrate as shown in equation 1. The degree of color from light yellow to dark yellow corresponds to the amount of end product produced. To quantify the reaction, we will use a spectrophotometer to measure the absorbance of light at 400 nm (the maximum absorbance for the product). Since absorbance is directly related to concentration, a higher value at an absorbance of 400 nm ( $A_{400}$ ) indicates more end products are produced. In other words, there was more enzymatic activity.

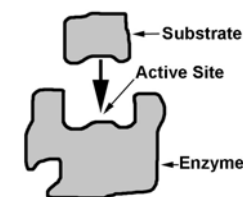
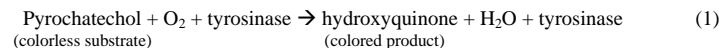


Figure 3. Binding of the substrate at the active site of the enzyme



### A1 Procedure: Characteristics of the Enzyme Reaction

1. Turn on the spectrophotometers and allow them to warm-up for 10 minutes.
2. Make sure the correct filter is inserted, set the spectrophotometer at 475 nm ( $A_{475}$ ) and calibrate with distilled (DI) water. Remember to recalibrate each time if you change the wavelength.  
NOTE: When placing tube in the spectrophotometer:
  - Try not to get fingerprints and smudges on the tubes – wipe off any liquids or prints
  - Mark numbers of on top of the tube, not where the light is passing
  - Remove parafilm before placing tube into the spectrophotometer
3. Obtain four tubes and label them on the top 1 – 3 and B.
4. Fill tubes 1 – 3 according to Table 1. Fill the fourth tube, “B” with DI water. This will be the tube to zero or blank the spectrophotometer for the remainder of the lab exercises.
5. Using a small piece of parafilm, cover the top of each tube and invert several times to mix.
6. Note the color and record it on the worksheet.
7. Place the tubes upright in the test tube rack. After 5 minutes determine the  $A_{475}$  and record.



**CAUTION: Pyrocatechol 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.

**Table 1.** Experimental Conditions to Test the Trypsinase Activity

Tube	DI water	Enzyme	Substrate	Sucrose
1	5 ml	1 ml	1 ml	---
2	6 ml	---	1 ml	---
3	5 ml	1 ml	---	1 ml

**A2 Procedure: The Effects of Enzyme Concentration**

1. Label three test tubes 1 – 3.
2. Fill each tube according to the Table 2 (add the *SUBSTATE last* to all tubes).
3. Cover with parafilm and mix by inverting.
4. Note and record the color of each tube.
5. Determine the at  $A_{475}$  of each tube after **3 minutes**. Please be consistent with the timing.

**Table 2.** Experimental Conditions to Test the Effects of Enzyme Concentration

Tube	DI water	Enzyme	Substrate
1	5.5 ml	0.5 ml	1 ml
2	5 ml	1 ml	1 ml
3	4 ml	2 ml	1 ml

**A3 Procedure: The Effects of Substrate Concentration**

1. Label three test tubes 1 – 3.
2. Fill each tube according to the Table 3 (add the *ENZYME last* to all tubes).
3. Cover with parafilm and mix by inverting.
4. Note and record the color of each tube.
5. Determine the  $A_{475}$  of each tube after **5 minutes**. Please be consistent with the timing.

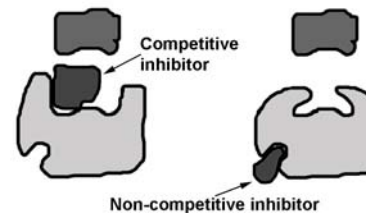
**Table 3.** Experimental Conditions to Test the Effects of Substrate Concentration

Tube	DI water	Enzyme	Substrate
1	5.5 ml	1 ml	0.5 ml
2	5 ml	1 ml	1 ml
3	4 ml	1 ml	2 ml

**B. THE EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE ACTIVITY OF ENZYMES**

Each enzymatic reaction has an optimum set of conditions which produces the most efficient enzymatic activity (fastest reaction rate). Optimum conditions may vary with different enzymes and with the location of the reaction in the body. Many enzymes also require the presence of inorganic or organic

enzyme helpers (cofactors and coenzymes) in order to function properly. Other factors that affect enzyme activity include: temperature, pH, the concentration of substrates, and the concentration of enzymes. Enzyme inhibitors (Figure 4) can also affect the binding of substrates by causing the active site to undergo a conformational change preventing substrate binding. When the three dimensional shape of the enzyme is disrupted, the protein is said to be **denatured** and the enzyme becomes inactivated.



**Figure 4.** Interference of substrate binding by two types of inhibitors

**B1 Procedure: The Effect of Temperature on Enzyme Activity**

1. Label five tubes 1 – 5.
2. Place 4 ml DI water and 1 ml enzyme into each tube. Then place each tube into the respective environmental condition listed in Table 4.
3. **After 10 minutes**, do not bring the enzyme tube back to your desk, but rather add the 1 ml of substrate to each tube at the environmental location, and hold at temperature for 5 more minutes.
4. Remove the tubes, dry them, and determine and record the  $A_{475}$  for each. *Make sure you wipe off any condensation or liquids before you place the tubes into the spectrophotometer. Note: If there are solids floating in the high temperature tube, you will need to set up a funnel with qualitative filter paper to remove the solids before you read the absorbance. Your instructor will demonstrate this.*

**Table 4.** Experimental Set-up to test the Effects of Temperature on Enzyme Activity

Tube	DI water	Enzyme	Substrate	Temperature	
1	4 ml	1 ml	1 ml	0 C	Ice bath
2	4 ml	1 ml	1 ml	22 C	Water bath
3	4 ml	1 ml	1 ml	40 C	Water bath
4	4 ml	1 ml	1 ml	60 C	Water bath
5	4 ml	1 ml	1 ml	100 C	Boiling water

**B2 Procedure: The Effect of pH on Enzyme Activity**

1. Label five test tubes 1 – 5.
2. Fill each test tube with 5 ml with **buffer** solutions of pH levels: 3, 5, 7, 9, 11 (**CAUTION – Do not get any of the buffer solutions on your body or clothing, especially pH 3 & 11!**)
3. Add 1 ml of enzyme (tyrosinase) to each tube.
4. Add 1 ml of substrate (pyrocatechol) to each tube.
5. Mix thoroughly and place the tubes upright in the test tube rack. After 5 minutes, determine the  $A_{475}$  for each tube and record. Please be consistent with the timing.

### **B3 Procedure: The Effect of Inhibitors on Enzyme Activity**

1. Label seven test tubes 1 – 7.
2. Fill each tube according to Table 5. **Pay close attention to what you're using!**
3. Add the substrate (pyrochatechol) last to all of the tube and mix thoroughly.
4. Determine the  $A_{475}$  after 5 minutes for each tube. Please be consistent with the timing.



**CAUTION: Phenylthiourea 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.

**Table 5.** Experimental Set-up to test the Effects of Enzyme Inhibitors

<b>Tube</b>	<b>DI water</b>	<b>Enzyme</b>	<b>Phenylthiourea</b>	<b>Tyrosine</b>	<b>Substrate</b>
1	4.9 ml	1 ml	0.1	---	1 ml
2	4.5 ml	1 ml	0.5	---	1 ml
3	3.5 ml	1 ml	1.5 ml	---	1 ml
4	4.9 ml	1 ml	---	0.1	1 ml
5	4.5 ml	1 ml	---	0.5	1 ml
6	3.5 ml	1 ml	---	1.5 ml	1 ml
7	6 ml	1 ml	---	---	1 ml

Enzyme → tyrosinase

Substrate → pyrochatechol