

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.

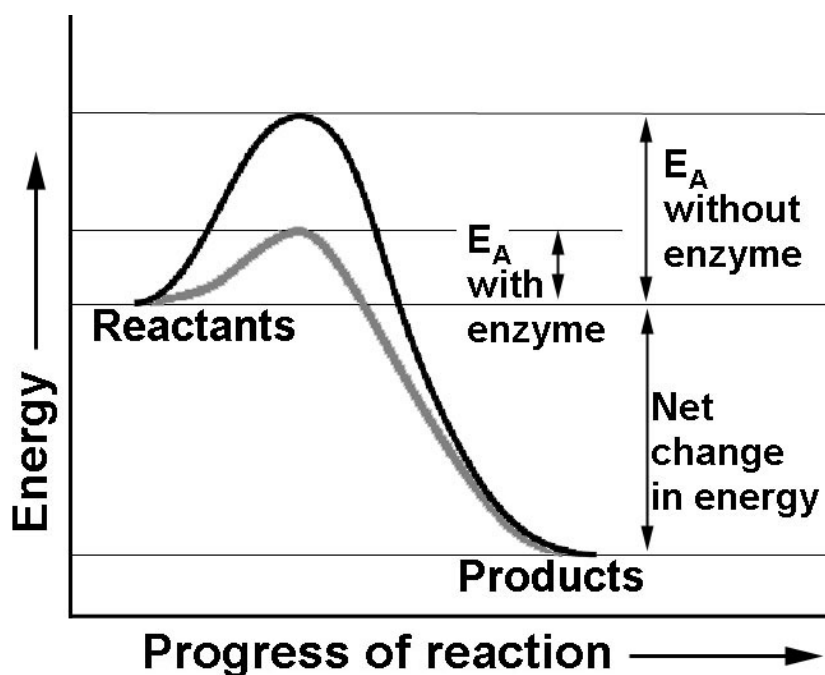


Figure 1. Energy of activation with and without an enzyme.

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



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 the Table 2 (add the *substrate 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 **3 minutes**.

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 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**.

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.

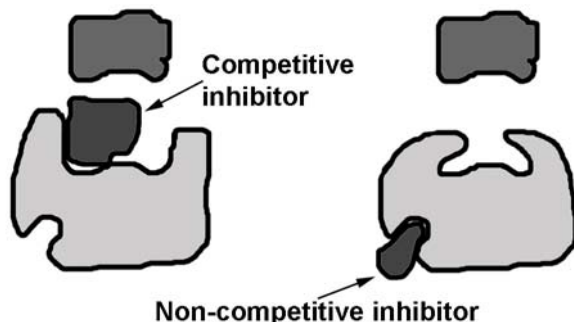


Figure 4. Interference of substrate binding by two types of inhibitors

B1 Procedure: The Effect of Temperature on Enzyme Activity

1. Label five tubes 1 – 5.
2. Fill each tube according to Table 4 and immediately place each test tube into the respective environmental condition. (**Add the enzyme just as you place the tube into the proper temperature – DO NOT MIX**).
3. Place the tubes upright in the test tube rack. After 10 minutes, mix the tubes; determine the A_{400} for each tube and record.
4. Make sure you wipe off any condensation or liquids before you place the tubes into the spectrophotometer.

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 (pyrochatechol) 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.

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.



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

Tube	DI water	Enzyme	Phenylthiourea	Tyrosine	Substrate
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

****Record the color at the beginning for all data tables below.****

A1 Procedure: Characteristics of the Enzyme Reaction

1. Data Table 1: Tyrosinase Enzymatic Activity

Tube	Color	A ₄₇₅ after 5 minutes
1		
2		
3		

2. What were the enzyme, substrate and product of the enzymatic reaction?
3. Which tube was the control?
4. Why is it important to have controls?
5. What do the results of tube 3 demonstrate?
6. Explain if tube 3 really proves “substrate specificity”?

A2 Procedure: The Effects of Enzyme Concentration

7. Data Table 2: The Effects of Enzyme Concentration

Tube	Enzyme Concentration	Color	A ₄₇₅ after 3 minutes
1			
2			
3			

8. How does changing the concentration of the enzyme affect the rate of the reaction?
9. If you start with 1 ml of substrate and add no more, but you continue to add amounts of enzyme (say 1 ml every 5 minutes), what would happen to enzymatic activity? Graphically illustrate what would occur below:

A3 Procedure: The Effects of Substrate Concentration

10. Data Table 3: The Effects of Substrate Concentration

Tube	Substrate Concentration	Color	A₄₇₅ after 5 minutes
1			
2			
3			

11. Why does increasing the concentration of the substrate promote enzyme activity?
12. If you allowed the reaction to continue until all the substrate was converted to product and then you added more substrate but did not add any more enzyme; *explain* if a reaction would occur.

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

B1 Procedure: The Effect of Temperature on Enzyme Activity

13. Data Table 4: The Effects of Temperature on Enzyme Activity

Tube	Temperature	Color	A₄₇₅
1	0 C		
2	22 C		
3	40 C		
4	60 C		
5	100 C		

14. Construct a graph using Excel for the temperature data and indicate the optimal temperature for this reaction to occur.
15. If the tube incubated at 100 C was placed back in the optimum temperature, would a reaction occur? **Explain.**

B2 Procedure: The Effect of pH on Enzyme Activity

16. Data Table 5: The Effect of pH on Enzymatic Activity

Tube	pH	Color	A ₄₇₅
1	3		
2	5		
3	7		
4	9		
5	11		

17. Graph the results of this experiment to show the optimum pH for this reaction (Use Excel).

18. Why does the enzyme reaction fail to occur at very low and very high pH?

19. Explain what “optimum” means. Do all enzymes have the same optimum pH?

20. What would be the adaptive advantage of maintaining a constant blood pH?

B3 Procedure: The Effect of Inhibitors on Enzyme Activity

21. Data Table 6: The Effects of Enzyme Inhibitors

Tube	Contents	Color	A ₄₇₅
1			
2			
3			
4			
5			
6			
7			

22. Which of the substances used (phenylthiourea or tyrosine) was an inhibitor for the reaction?

23. Tyrosine is a competitive substrate. Do your results support this? Explain.

24. Based on the results of all experiments, type a paragraph summarizing the optimum conditions for this enzymatic reaction and whether or not these conditions are the same for every enzyme. Put the two graphs (using Excel) and the paragraph on the same page.