Biology 20 Laboratory

ENZYMES & CELLULAR RESPIRATION

OBJECTIVE

- 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.
- To study anaerobic and aerobic respiration.
- To study enzymes involved in the Krebs cycle of aerobic respiration.
- To study and differentiate between oxidation and reduction.

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 a 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.

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 5).

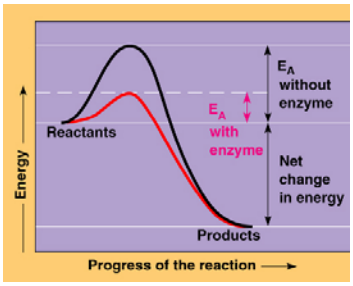


Figure 5. Energy of activation with and without an enzyme.

Each enzyme catalyzes a specific chemical reaction. The molecules in which enzymes catalyze 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 5.1) for enzymatic reactions begins as the **reactants** (enzyme and substrate) collide and the substrate fits into the **active site** (Figure 5.2) 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 5.1). Enzymes emerge essentially unchanged upon completion of the chemical reaction and are capable of further catalysis (**reusable**).

E	S		ES complex		E	P
Enzyme	+ Substrate	→	Enzyme-Substrate complex	→	Enzyme	+ Product(s)

Figure 5.1. Catalytic cycle for enzymatic reactions.

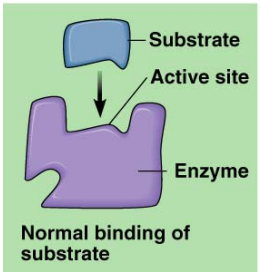


Figure 5.2. Binding of the substrate in the active site of the enzyme.

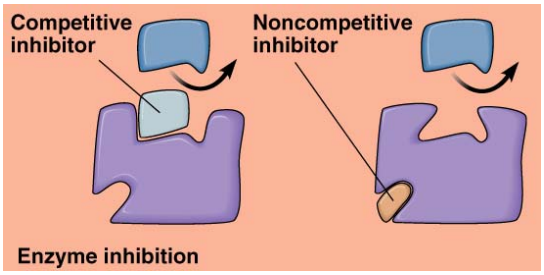


Figure 5.3. Interference of substrate binding by two types of inhibitors.

Each enzymatic reaction has an optimum set of conditions, which produce 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 5.3) 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.

Let's take a look at one example of an enzymatic reaction. Our cells utilize glucose for energy. To obtain energy from glucose, a number of reactions occur involving the **oxidation/reduction** of substrates, rearrangement of molecules, or removal of carbon dioxide. The initial use of glucose occurs in the cytoplasm of the cell. It involves oxidation/reduction reactions and molecular rearrangements, resulting in two molecules of *pyruvic acid (pyruvate*), two molecules of *ATP* and two molecules of reduced *coenzyme, NADH*. This series of reactions is known as **glycolysis**, an since the presence of oxygen has not been required, the process is considered **anaerobic**.

The fate of the two pyruvic acids is dependent on the presence of oxygen. If oxygen is not present, the two pyruvic acids will remain in the cytosol and undergo the anaerobic process called **fermentation**. There is no "extra" energy yield from fermentation. If oxygen is present,

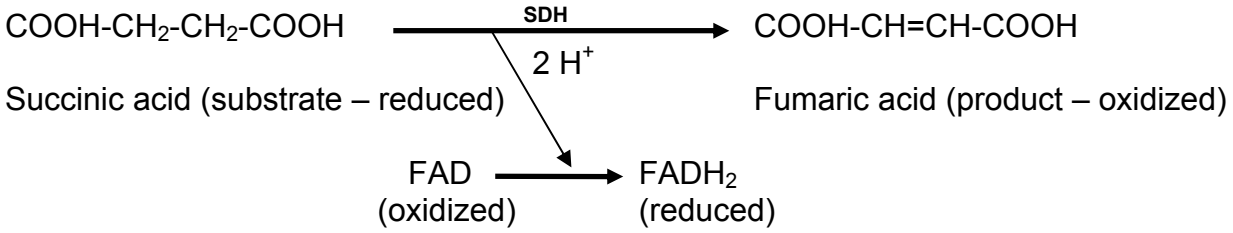
the two pyruvic acids will be shuttled to mitochondria, altered and enter into a series of reactions involving the **Krebs Cycle** and the **Electron Transport Chain (ETC)**. Both are dependent on oxygen and are aerobic in nature. The Krebs cycle only produces 1 ATP molecule directly per cycle. However, it is indirectly responsible for the greatest ATP production by generating coenzymes, both NADH and $FADH_2$. When these coenzymes are reoxidized in the Electron Transport Chain, many molecules of ATP are generated (a theoretical 36 ATP per 1 glucose).

A. AEROBIC RESPIRATION
SUCCINIC ACID DEHYDROGENASE ACTIVITY IN THE KREBS CYCLE

Succinic acid dehydrogenase (SDH) is an oxidative enzyme that catalyzes the removal of hydrogen atoms from succinic acid, the substrate, according to Figure 5. This reaction is a vital step in the Krebs Cycle, which is a sequence of biochemical reactions occurring in the mitochondria of cells.

Succinic acid loses two hydrogen atoms and is transformed into *fumaric acid*, which has a double bond between the middle two carbon atoms (Figure 5.4). Since two hydrogen atoms are given up in the reaction, there must be some molecule, which accepts them. **Flavin adenine dinucleotide (FAD)** is a molecule called a "coenzyme" which works along with SDH and accepts the hydrogen atoms released during the oxidation of the substrate, succinic acid, and passes them to an acceptor molecule.

Figure 5.4: The conversion of succinic acid to fumaric acid.



The FAD molecule with its hydrogen atoms, $FADH_2$, passes its hydrogens on through a series of biochemical steps located in the inner mitochondrial membrane involving cytochrome enzymes. The hydrogens and their electrons (one electron/hydrogen atom) are eventually accepted by oxygen to form water (Figure 5.5). This stepwise sequence of events, involving electron transfer and cytochrome enzymes, is known as the **electron transport chain (ETC)** or the respiratory chain. Because the final reaction involves oxygen, this process is known as **oxidation**, the loss/removal of electrons from a molecule. SDH facilitates the oxidation of succinic acid to fumaric acid, removing hydrogen atoms and their electrons. Meanwhile FAD becomes reduced as it picks up the two hydrogens. **Reduction** is the gain of electrons (follow the hydrogens in Figure 5.5).

Figure 5.5: The oxidation of $FADH_2$ and the reduction of O_2.

$$FADH_2 + ½\ O_2 \rightarrow FAD + H_2O$$

The activity of enzymes can be inhibited in many ways. Some inhibitors "poison" the system by irreversibly binding to the enzymes **active site** or altering their structure. Other inhibitors, disguised as the normal substrate, "compete" for the active site on enzymes (Figure 5.2). An enzyme molecule is very large compared to its substrate and may contain several active sites. When **inhibitors** or *"false" substrates* bind to the active site, the substrate cannot undergo the desired reaction at the rate ordinarily observed.

Malonic acid has the formula: $COOH\text{-}CH_2\text{-}COOH$. Its structure is very similar to that of succinic acid ($COOH\text{-}CH_2\text{-}CH_2\text{-}COOH$), lacking only a second CH_2 group. Since it strongly resembles the normal substrate of SDH, it can *"trick"* the enzyme into binding with it. Hydrogens cannot be removed from the malonic acid, however, and the enzyme is temporarily inactive. When succinic acid and malonic acid are mixed together, succinic acid must compete with malonic acid for an active site on the enzymes therefore hydrogens are not passed on to methylene blue (Figure 5.6) as readily. If malonic acid is removed, the active site can be cleared and the SDH enzyme will return to its original active form. Figure 5.4 shows the relationship of the structure of fumaric acid to succinic acid.

Experiment:

In this experiment, a hydrogen acceptor that does not occur in nature, the dye methylene blue, is used. When oxidized it is blue, but when it accepts hydrogen and is thus reduced, it becomes colorless (Figure 5.6). Therefore, it can be used as an indicator to show when the reaction mixture has undergone some oxidative activity.

Figure 5.6: The reduction of methylene blue.

$$FADH_2 + \text{methylene blue} \rightarrow FAD + \text{methylene blue-}H_2.$$
(blue colored) (colorless)

Since muscle is an excellent source of mitochondria, hamburger meat will serve as the source of the succinic acid dehydrogenase (SDH) enzyme of the Krebs Cycle. Succinic acid and malonic acid will both be available in dropper bottles.

Materials:
Homogenized hamburger, succinic acid, malonic acid, methylene blue, test tubes, stir rods, incubator (37C), DI water.

Procedure:

1. Obtain 4 test tubes and set them up as listed below.
2. Test tube #1:
    a. Place approximately 2 cm (depth) of homogenized hamburger in the test tube.
    b. Add 10 drops of succinic acid.
    c. Add 3 drops of methylene blue.
    d. Mix thoroughly with a clean stir rod.
    e. NOTE the time the tube was setup and how long it takes for any color change to occur. This will indicate reaction rate.
3. Test Tube #2:
    a. Place approximately 2 cm of homogenized hamburger in the test tube.
    b. Add 10 drops of **DI water** acid.

c. Add 3 drops of methylene blue.
d. Mix thoroughly with a clean stir rod.
e. NOTE the time the tube was setup and how long it takes for any color change to occur. This will indicate reaction rate.

4. Test Tube #3:
    a. Place approximately 2 cm of homogenized hamburger in the test tube.
    b. Add 10 drops of **malonic** acid.
    c. Add 3 drops of methylene blue.
    d. Mix thoroughly with a clean stir rod.
    e. NOTE the time the tube was setup and how long it takes for any color change to occur. This will indicate reaction rate.
5. Test Tube #4:
    a. Place 5 ml of DI water in the test tube – NO meat.
    b. Add 10 drops of succinic acid.
    c. Add 3 drops of methylene blue.
    d. Mix thoroughly with a clean stir rod.
    e. NOTE the time the tube was setup and how long it takes for any color change to occur. This will indicate reaction rate.
6. DO NOT SHAKE OR STIR THE TUBES, it will change the results.
7. Make some predications regarding this experiment as the experiment is proceeding. Do not forget to check on your tubes.
8. Do not allow the experiment to exceed 45 minutes.
9. Carefully remove the test tubes and note any change on your worksheet.
10. Have your instructor confirm your results and then answer question #11 on page 7.

B. ANAEROBIC FERMENTATION

Fermentation involves the oxidation of NADH by the removal of electrons (or hydrogens) from the NADH + H^+ and their acceptance by pyruvic acid, forming either **lactic acid** or **ethyl alcohol**. The products, which result from the reduction of pyruvic acid, depend upon the presence of the specific enzymes of the organisms involved. Many cells are capable of fermentation, but animal cells can produce only lactic acid. Bacterial cells can produce not only lactic acid, but also many other products, including ethyl alcohol. Yeast and certain other fungi are known for their fermentation abilities, producing ethyl alcohol and CO_2 in the process.

Materials:
3 fermentation tubes, water, molasses solution, yeast, metric ruler, cork

Procedure:

1. Three fermentation tubes will be used by the entire lab table and labeled #1 - #3.
2. Test tube #1: fill the entire tube with DI water.
3. Test tube #2: fill with the molasses solution.
4. Test tube #3: fill with the molasses and activated yeast (that has been heated slightly).
5. Develop a hypothesis.
6. Make some predications regarding this experiment on your worksheet.
7. After 20 – 30 minutes, measure any gas production with a millimeter ruler and note the appearance of the tubes.

Biology 20 **Name:** _____
Cellular Respiration & Enzymes Worksheet **Lecture Day & Time:** _____

A. SUCCINIC ACID DEHYDROGENASE ACTIVITY IN THE KREBS CYCLE

Data Table 1: Tube contents and time required for color change.

Tube #	**Tube Contents**	Time Required for Color Change (minutes)
1		
2		
3		
4		

1. What is the hypothesis that is being tested?

2. Make 2 – 3 predictions regarding this experiment:

3. Was your hypothesis validated by your experiment? **Explain** why or why not.

4. What is the substrate in this experimental setup? _____

5. What enzyme is changing the substrate? _____

6. Where, in the hamburger, are the enzymes-substrate complexes located?

7. What is the role of methylene blue in this experiment?

8. Although the process that is occurring is called “oxidation,” no oxygen is used. **Explain**.

9. Did a color change occur in Tube #2? Since no succinic acid was added, should there have been a color change? Is there a difference in what you observed and what you expected? If so, explain.

10. Why did the methylene blue become **colorless** at the bottom of the tube, yet remain colored (blue) at the surface of the hamburger?

11. Would shaking or stirring the tube affect the color of the tube? Answer this question after your instructor has confirmed your results. If a change occurred, what was the cause?

12. Graph the reaction rate results for this experiment. Do not forget to include all the components of a graph.

B. ANAEROBIC RESPIRATION

Data Table 2: Anaerobic respiration.

Tube #	**Gas Amount (mm)**	Appearance
1		
2		
3		

13. Develop a hypothesis:

14. Make 2 – 3 predictions regarding this experiment:

15. Was your hypothesis validated by your experiment? **Explain** why or why not.

16. What is the purpose of Tubes #1 and #2?

17. What is occurring in Tube #3 to make it look differently?

18. Do similar reactions occur in your body? If so, where?

19. What was the role of the yeast in the experimental setup?