LAB 6 - Enzymes

Objectives

1. Carry out several enzyme catalyzed reactions and analyze the products.
2. Examine the effect of pH on enzyme activity.

INTRODUCTION

Chemical Reactions

The cells of organisms, from bacteria to plants to animals, carry out hundreds to thousands of chemical reactions that must be properly coordinated and controlled. We call the molecules at the start of a chemical reaction the **reactants**, and the resulting molecules are called the **products**. Chemical reactions can be represented as shown below:

	Reactants				Products		
1)	A	+	B	→	C		
2)			X	→	Y	+	Z

In the first example, molecules **A** and **B** undergo a chemical reaction to form a larger product **C**. In the second example molecule **X** undergoes a chemical reaction to form two smaller molecules **Y** and **Z**. This is exactly what occurs in two common biological reactions:

3)	Glucose	+	Fructose	→	Sucrose		
4)			Sucrose	→	Glucose	+	Fructose

In many plants, the monosaccharides glucose and fructose are combined in a chemical reaction to form the disaccharide sucrose as shown for reaction 3. Organisms that consume sucrose from plants (such as you!) carry out reaction 4 to "digest" the disaccharide sucrose to the monosaccharides glucose and fructose, which can then be effectively absorbed into the bloodstream and used by cells. Biochemical reactions that build larger molecules from smaller ones (such as reactions 1 and 3) are generally referred to as **anabolic**. Reactions that break down larger molecules into smaller ones (such as reactions 2 and 4) are referred to as **catabolic**. The sum of all biochemical reactions in a living organism, both anabolic and catabolic, is referred to as **metabolism**.

Energy

Another way of looking at chemical reactions is in terms of ***energy***. All chemical reactions involve changes in the energy state of the reactants relative to the products. If the stored or **potential** energy of the products of a reaction are greater than that of the reactants, then the reaction requires a net *input* of energy. Such reactions are called **endergonic** (endo- "into" and -ergonic "energy") and absorb energy. Other chemical reactions have a net *release* of energy since the products contain less potential energy than the reactants. Such reactions are called **exergonic** (exo- "off or out" and -ergonic "energy") and release energy.

ENDERGONIC: amino acid-1 + amino acid-2… + ***ENERGY*** → polypeptide

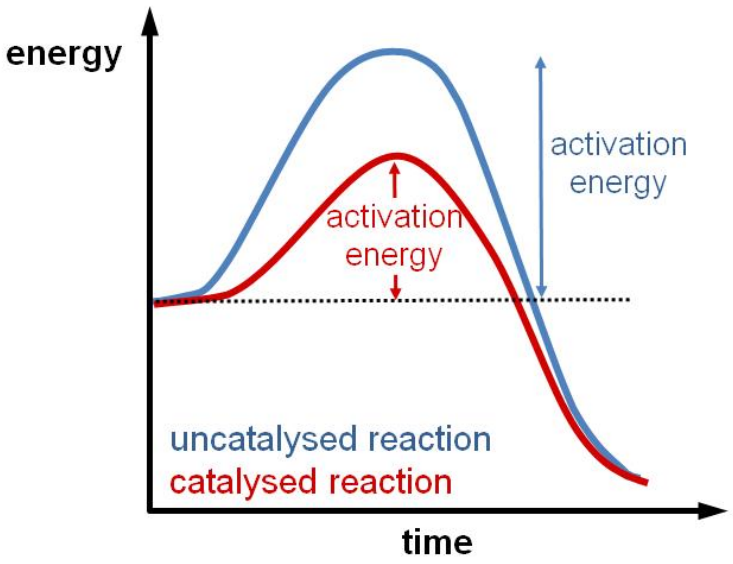
EXERGONIC: gasoline + O_2 → CO_2 + H_2O + ***ENERGY***

As indicated above, the synthesis of a polypeptide from free amino acids is a complex series of chemical reactions that requires energy and thus is **endergonic**. There is more stored energy in a polypeptide than in the free amino acids from which it is made. The burning of gasoline, on the other hand, is a chemical reaction that releases energy and thus is **exergonic**. There is less stored energy in the CO_2 and water products than in the reactants gasoline and oxygen. As a general rule, **anabolic** reactions such as polypeptide synthesis are **endergonic** and **catabolic** reactions are **exergonic**, with exergonic reactions providing the needed energy for endergonic ones.

Activation Energy and Catalysts

Another important aspect of chemical reactions and energy is the concept of **activation energy** (**E_a**). Regardless of whether a chemical reaction is endergonic or exergonic, every chemical reaction requires a certain amount of energy to get the reaction started. It is clear that the burning of gasoline is an exergonic reaction, however gasoline doesn't just burn spontaneously, it requires some sort of energy input to "spark" the reaction. This is why car engines have spark plugs, to supply the necessary activation energy to burn gasoline in a very controlled manner. When you strike a match on a rough surface and then light a candle you are doing the same thing, providing the necessary activation energy to get each of these exergonic reactions started.

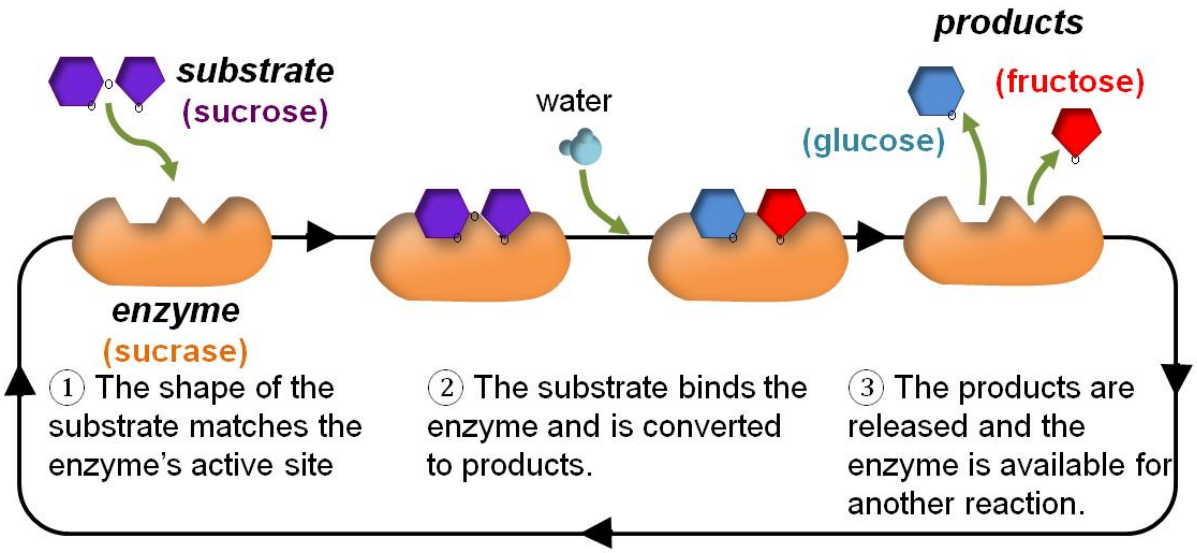
Chemical reactions in biological systems generally occur at a negligible rate by themselves. Without changing the amount of reactants, the only ways to increase the rate of a chemical reaction are to 1) increase the temperature (which is what you do when you light a match, burn a candle, or burn gasoline), or 2) introduce a **catalyst**. Catalysts are substances that interact directly with chemical reactants and position them so that they react more easily. No increase in temperature is necessary. Catalysts actually lower the activation energy requirement for a reaction, allowing it to occur much more readily as illustrated in this graph:



Enzymes

Since it would be impossible for living cells to control and coordinate their many biochemical reactions by adjusting the temperature, cells rely on *biological* **catalysts**. The biological catalysts in cells are proteins called enzymes, and just about every biochemical reaction in a cell has its own *specifically shaped* enzyme catalyst. By controlling the production and activity of enzymes (which are encoded by genes), cells control and coordinate their biochemical activity, i.e., their metabolism.

As with any catalyst, an enzyme works by binding and positioning the reactant(s) for a specific reaction in a way that lowers the activation energy. The biochemical reactant(s) that a given enzyme binds to is referred to as its **substrate**, and the part of the enzyme that binds the substrate is called the **active site**. The diagram below illustrates this for the enzyme **sucrase** and its substrate **sucrose** (*the suffix* ***–ase*** *denotes an enzyme, whereas* ***–ose*** *denotes a carbohydrate*):



As you can see, the enzyme sucrase, a protein, binds directly to its substrate sucrose and positions it so the covalent bond between the monosaccharides glucose and fructose is strained in a way that lowers the activation energy enough to break the bond. This yields the products glucose and fructose, which are then released. The enzyme is free to repeat this process, catalyzing the reaction over and over again until it is no longer active.

Like any protein, the action of an enzyme is dependent upon its unique three-dimensional shape. Anything that causes an enzyme to adopt a non-functional shape is said to **denature** the enzyme. Factors that can denature an enzyme and cause it to become non-functional include changes in temperature, pH and salt concentration. For example, most human enzymes have evolved to function best at normal cellular conditions: 37^{o} C, pH 7.4 and 0.9% NaCl. If the temperature, pH or salt concentration deviates significantly from the "normal" state, enzymes and other proteins will begin to denature and lose their function. This is largely why high fevers and deviations in pH (acidosis, alkalosis), for example, can be so dangerous.

In today's lab you will examine the functions of three digestive enzymes and test the effect of denaturing conditions on one of these enzymes…

DIGESTIVE ENZYME FUNCTION

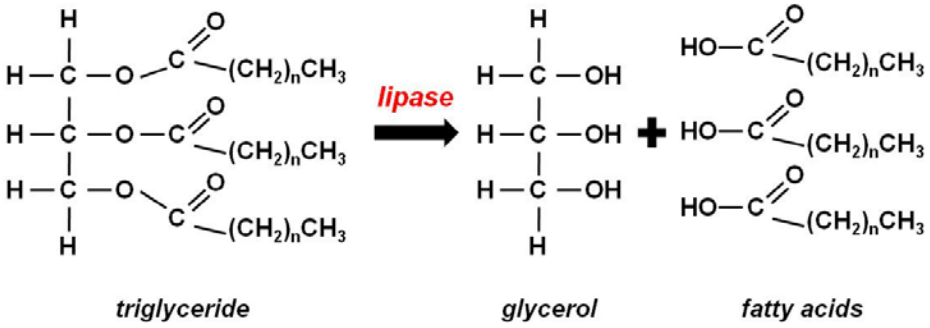
In the first three exercises you will observe how three different digestive enzymes catalyze biochemical reactions that break down their substrates into smaller molecules. Below is a list of each enzyme with its substrate and resulting product(s):

Enzyme	Substrate	Product
lipase	triglycerides	fatty acids, glycerol
trypsin	proteins	smaller polypeptides
amylase	starch	glucose

Each of these digestive enzymes is produced in the pancreas as part of a cocktail of digestive enzymes in what we call "pancreatic juice". Pancreatic juice is released into the first section of the small intestine, the duodenum, when it receives partially digested food called **chyme** from the stomach. The enzymes contained in pancreatic juice will complete the chemical digestion of a meal so that the *monomeric* nutrients it contains (e.g., amino acids, monosaccharide sugars, fatty acids) can be absorbed.

Digestion of Triglycerides by the Enzyme Lipase

The first enzyme you will examine is **pancreatic lipase**. Lipase is produced by the pancreas to catalyze the breakdown of lipids such as triglycerides into free fatty acids and glycerol:



Triglycerides are the main form of lipid found in animal fats (such as milk cream) and vegetable oils, however they must be digested to fatty acids and glycerol via pancreatic lipase to be absorbed. Since lipids are not soluble in water they will form large droplets to minimize contact with the surrounding aqueous environment. This limits their interaction with lipase thus making their digestion very slow and inefficient. To avoid this problem, your liver produces bile, a greenish fluid with properties similar to soap that helps to emulsify lipids, i.e., break them up into smaller droplets. Bile is stored in the gall bladder until your partially digested meal reaches the duodenum. This triggers the release of bile into the duodenum to help emulsify the lipids.

In the laboratory, digestion of triglycerides to fatty acids and glycerol can be detected by a decrease in pH. As their name implies, fatty acids are acidic (they release H^+ into solution) due to their carboxyl groups. The release of fatty acids from neutral triglycerides will thus result in an increase in the H^+ concentration (i.e., *lowering* of the pH value). In the following exercise, you will detect such changes in pH by observing changes in color of the pH indicator **litmus**.

Exercise 1 – Digestion of cream by lipase

*In this exercise you will test the ability of pancreatic lipase to digest triglycerides in milk cream, both with and without bile powder. The pH indicator **litmus** has been added to the cream. Litmus is red under acidic conditions, blue under basic conditions, and is purple at neutral pH. The "litmus cream" you will use is a light purple color (neutral pH) and will gradually turn reddish-pink if free fatty acids are released due to the digestion of triglycerides in the cream.*

1. Label four test tubes **1**, **2**, **3** & **4** and add the indicated components below to each tube in the order listed (i.e., first add bile powder to tubes 2 and 4, next add 2 ml of litmus cream to each tube, etc).

	Tube 1	Tube 2	Tube 3	Tube 4
bile powder	*none*	1 spatula	*none*	1 spatula
litmus cream	2 ml	2 ml	2 ml	2 ml
water	0.5 ml	0.5 ml	*none*	*none*
1% lipase	*none*	*none*	0.5 ml	0.5 ml
TOTAL	2.5 ml	2.5 ml	2.5 ml	2.5 ml

2. Mix each tube well and incubate them in a 37° C water bath for 1 hour.
3. On your worksheet, state your hypothesis regarding any changes in pH for each tube. When the 1 hour incubation is complete record the results and answer the associated questions.

NOTE: For exercises 1 & 2, begin the next exercise while the current one is incubating.

Digestion of Proteins by the Enzyme Trypsin

The next enzyme you will examine is **trypsin**, one of many enzymes your body produces to digest or break down proteins. Trypsin will catalyze the breakage of peptide bonds in proteins at lysine and arginine amino acid residues. This results in larger polypeptides being broken down into smaller polypeptides (commonly referred to as "peptides").

The protein source you will subject to trypsin digestion is gelatin. Gelatin consists primarily of the protein collagen extracted from animal bones and other connective tissues. At room temperature and below, gelatin is a semisolid gel due to interactions between the collagen fibers that form a fishnet-like structure. Trypsin will partially digest the collagen fibers, disrupting their interaction and causing the gelatin to liquefy and remain liquid, even at cool temperatures.

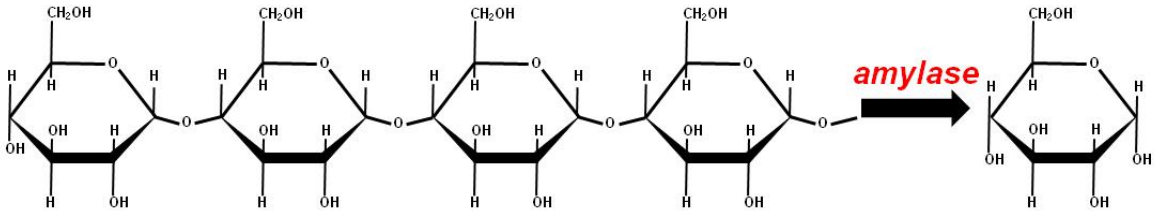
Exercise 2 – Digestion of gelatin by trypsin

To demonstrate the ability of trypsin to catalyze the partial digestion of gelatin, you will carry out two reactions as described below. If digestion of the gelatin has occurred, you will see that the gelatin remains liquid even on ice:

1. Obtain two tubes of molten gelatin from the 37° C water bath and label them **1** & **2**.
2. Add 0.5 ml of water to tube **1** and 0.5 ml of 1% trypsin solution to tube **2**.
3. Mix well and place both tubes in the 37° C water bath for 30 minutes.
4. Place both tubes on ice for 15 minutes and then let them gradually warm up at room temperature.
5. Invert each tube periodically to see which tube liquefies first and record on your worksheet.

Digestion of Starch by the Enzyme <u>Amylase</u>

Starch is a large polymer of the monosaccharide glucose. In order for your body to obtain glucose from the starch you eat, it must be digested by the enzyme **amylase**:



starch ***free glucose***

Amylase is present in human saliva as well as pancreatic juice. The amylase you will be using is produced by a fungus. As you learned in the previous lab, starch can be detected by the addition of a small amount of iodine solution. If starch is present the sample will turn dark blue or black when iodine solution is added, if there is no starch then the sample should be a clear light brown color. **The *complete* digestion of starch to free glucose should thus result in a clear light brown color when iodine solution is added. Partial digestion of starch will result in a color that is somewhat dark but not as dark as an undigested starch sample.** In the next exercise, you will use iodine solution to determine if starch is digested by amylase.

<u>Exercise 3</u> – Digestion of starch by amylase

In this exercise you will set up three reaction tubes. Two reactions will serve as controls, one lacking the enzyme and the other lacking starch. The third reaction will contain both the enzyme amylase and its substrate, starch. Your three reactions should be performed as follows:

1. Label three test tubes **1**, **2** & **3** and add the indicated components in the order listed.

	Tube 1	**Tube 2**	**Tube 3**
starch solution	2.5 ml	*none*	2.5 ml
water	0.5 ml	2.5 ml	*none*
1% amylase	*none*	0.5 ml	0.5 ml
TOTAL	3.0 ml	3.0 ml	3.0 ml

2. Mix well and incubate each tube at room temperature for 10 minutes.
3. Add 3 drops of iodine to each tube, mix and record the results on your worksheet.

(Record the color <u>immediately</u> after mixing since it may fade)

NOTE: Save your control tubes (tubes 1 & 2) from Exercise 3 for use in Exercise 4.

Effect of pH on Enzyme Function

As you learned earlier, enzymes are proteins and proteins only function properly in their native **conformation** or shape. If anything causes an enzyme to lose is shape, i.e., to become **denatured**, it will no longer function as a catalyst since it can no longer bind to its substrate. Changes in temperature, pH and salt concentration all can denature an enzyme and destroy its activity. To illustrate this, you will design an experiment to test the effects of changes in pH on the activity of the enzyme you used in the previous exercise – amylase.

Human pancreatic enzymes such as lipase, trypsin and amylase normally carry out their catalytic activities at relatively neutral pH values of 7 to 8 in the small intestine. Fungi digest food sources containing starch externally in slightly acidic environments close to pH 5. **You will be using <u>fungal</u> amylase for following experiments**, so keep this in mind when you come up with your hypothesis**.**

<u>Exercise 4</u> – Effect of pH on amylase activity

In this exercise you will design an experiment to carry out five amylase reactions much like you did for Tube 3 in Exercise 3, however each reaction will occur at a different pH. <u>The control tubes from Exercise 3 can be used as controls for this experiment</u>.

1. Buffered solutions of pH **2**, **4**, **7**, **10** & **12** are available at the front of the lab.
2. Write your hypothesis on your worksheet regarding the pH value(s) at which you predict fungal amylase will have the highest activity.
3. Design an experiment to test fungal amylase activity at all five pH values being sure that pH is the only independent variable.
4. Record your plan on your worksheet and carry out the experiment.
5. Record the results of your experiment on your worksheet.

NOTE: Be sure to <u>immediately</u> record the color of each reaction on your worksheet (the color may fade), and score each reaction for amylase activity as indicated below:

0 – no amylase activity (completely dark blue/black – no starch hydrolysis)
1 – a little amylase activity (dark but not completely dark blue/black)
2 – significant amylase activity (slightly darkened)
3 – high amylase activity (clear light brown – complete starch hydrolysis)

7. Graph your results (pH vs amylase activity) and conclude whether or not your hypothesis is supported.

PLEASE be sure to <u>dispose of all tube contents in the chemical disposal jug</u> in the flow hood and <u>wash all of your tubes with soap and hot water</u> leaving them upside in your test tube rack in the sink.

We don't have the staff to wash so many tubes and your cooperation will be greatly appreciated. THANK YOU!

Before you leave, please make sure your table is clean, organized, and contains all supplies listed below so that the next lab will be ready to begin. Thank you!

SUPPLY LIST

- 12 clean test tubes in test tube rack
- Marker pen or China marker
- Container of deionized water

Also PLEASE be sure to do the following before you leave:

- *Melt the gelatin in the water bath, pour it down the sink with running hot water, wash the tubes with soap and water, and leave in the pan next to the water bath.*

LABORATORY 6 WORKSHEET

Name _____

Section_____

Exercise 1 – Digestion of triglycerides by lipase

Background questions:

1. When triglycerides in cream are digested, what are the products? _____
2. Which of these products will a) cause a change in pH, and b) what will this change be?

   a) _____ b) _____

3. What pH indicator will you be using to detect changes in pH in this experiment? _____
4. What is the biological role of bile? _____
5. What is the biological role of lipase? _____
6. Bile is produced in the _____ and stored in the _____.
7. Lipase is produced in the _____.

HYPOTHESIS –*Hypothesize which tube will show the most triglyceride digestion*:

RESULTS – *Predict the color of each tube at the end of the experiment based on your hypothesis, and once the experiment is complete record the actual color for each tube and how the color relates to pH:*

tube #	original color	predicted color	actual color	pH change (↑, ↓ or none)	Cause of pH change (if applicable)
1	purple				
2	purple				
3	purple				
4	purple				

CONCLUSION

State whether or not the results support your hypothesis and be sure to explain <u>why</u>.

Exercise 2 – Digestion of gelatin by trypsin

Background questions:

1. What is gelatin? _____
2. What is the biological role of trypsin? _____

HYPOTHESIS – ***Review the experiment & hypothesize which tube will show the most gelatin digestion:***

RESULTS

After cooling, which of your tubes liquefied first? _____

CONCLUSION

State whether or not the results support your hypothesis and be sure to explain why.

Exercise 3 – Digestion of starch by amylase

Background questions:

1. When starch is digested, what *product* is produced? _____
2. Is the product of starch digestion (see above) detected by iodine? _____
3. What is the biological role of amylase? _____
4. What two organs in the body produce amylase? _____

HYPOTHESIS – ***Review the experiment & hypothesize which tube will show the most starch digestion*:**

RESULTS – ***Predict the color of each tube after adding iodine based on your hypothesis, then perform the experiment and record the actual color and whether or not starch was digested:***

tube #	predicted color	actual color	Was starch digested?
1			
2			
3			

Which tube is a control for the *presence* of starch? _____ for the *absence* of starch? _____

Did the results for your control tubes turn out as expected? If not, why?

CONCLUSION

State whether or not the results support your hypothesis and be sure to explain why.

Exercise 4 – Effect of pH on amylase activity

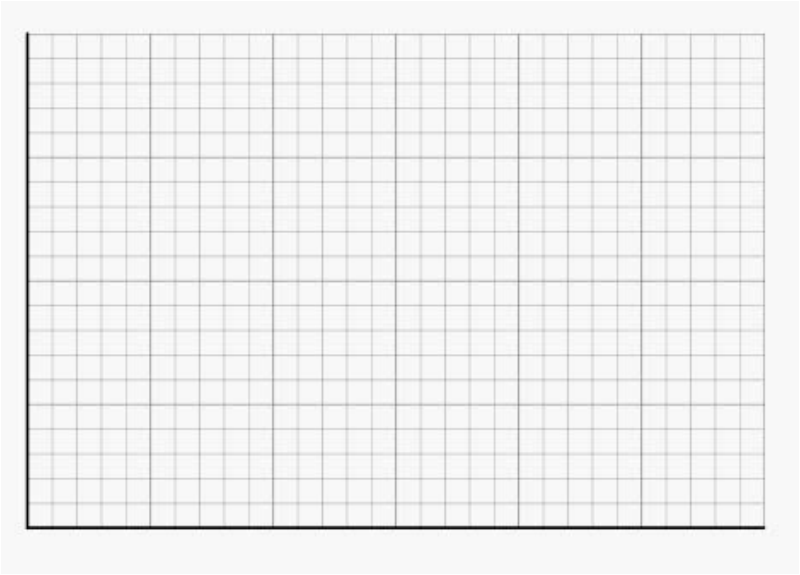
HYPOTHESIS – ***Hypothesize the pH at which fungal amylase will have the greatest activity*** *(see pg 93)*:

What is the independent variable for this experiment? _____

What is the dependent variable for this experiment? _____

RESULTS – ***Record the color of each tube immediately after adding iodine in the table below and score each tube for amylase activity. Graph pH vs amylase activity in the grid below.***

pH	color	amylase activity
2		
4		
7		
10		
12		



At what pH value(s) does amylase have the highest activity? _____

At what pH value(s) does amylase have the lowest activity? _____

CONCLUSION

State whether or not the results support your hypothesis and *explain* your conclusion with regard to your results.

Were there any pH values at which the enzyme amylase was non-functional? _____

If so, explain what happened to the enzyme to make it non-functional.