Amylase: a sample enzyme

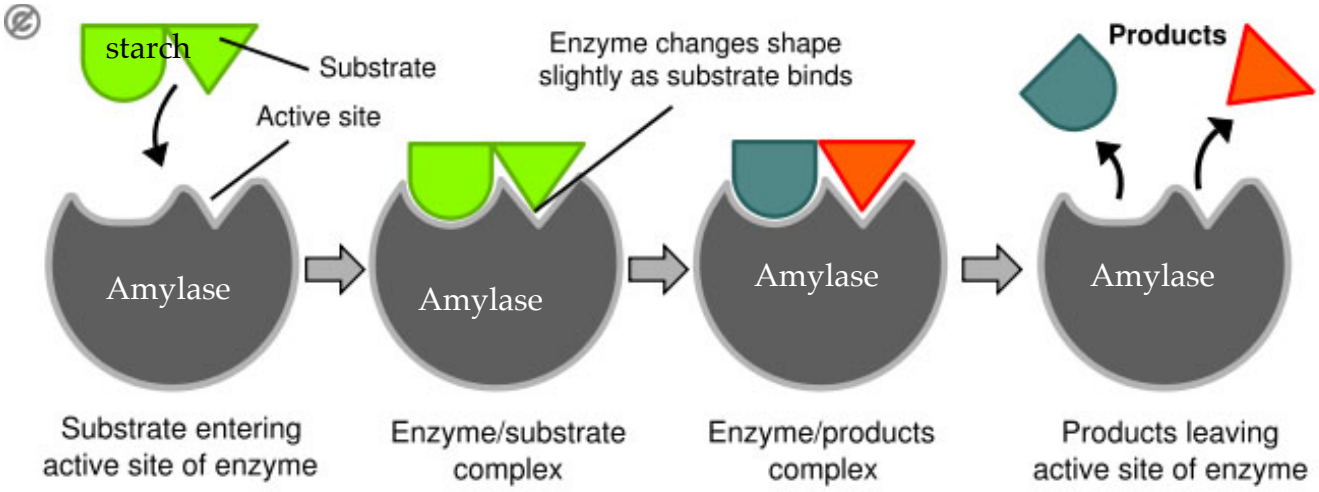
Objectives: After completion of this laboratory exercise you will be able to:

1. Explain the importance of enzymes in biology.
2. Explain the basic properties of an enzyme as a catalyst.
3. Discuss the effect of enzyme concentration and various inhibitors on the rate of an enzymatic reaction.
4. Evaluate the efficacy of nutritional supplements called "starch blockers"

Introduction:

Enzymes are biological catalysts that constitute the largest and most highly specialized class of protein molecules. Enzymes act as catalysts to increase the rates of chemical reactions, but they do not cause a reaction to occur that would not proceed spontaneously without the enzyme; that is, the reaction must have $\Delta G < 0$. The reactions of metabolism would occur at extremely slow rates at normal body temperature and pH in the absence of enzymes. An appreciation of the catalytic efficiency of enzymes can be gained by realizing that under optimal conditions, most enzymatic reactions proceed 10^8 to 10^{11} times more rapidly than the corresponding reactions without enzymes. Without the enzymes in our digestive tract for example, it would take us about 50 years to digest a single meal!

One very important property of enzymes is their specificity. Any one enzyme will only catalyze a single class of chemical reactions. Some enzymes act on one substrate only; other enzymes act on a family of related molecules. Enzymes participate in the reaction that they catalyze, but they emerge unchanged at the end of the reaction, i.e., they are not used up. Thus, a few enzyme molecules can go a long way. This reaction can be represented by the following cartoon:



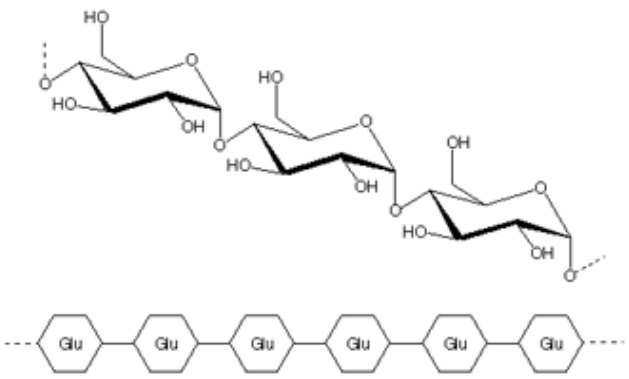
http://www.phoenixhealth.me

Most enzymes are proteins with specific three dimensional shapes necessary for the proper function of the enzyme (some RNA molecules also have catalytic activity). As with any protein, the shape of an enzyme can be affected by different environmental conditions. An increase in temperature can speed up the rate of an enzymatic reaction but it can also change the shape of the enzyme causing it to become inactive because the substrate can no longer bind to it. The pH of the environment is also important to the proper functioning of an enzyme. Enzymes work best at a

specific pH and a change in this value can also cause the enzyme to denature (change its shape) and become inactive.

In this lab we will demonstrate the hydrolysis of starch to glucose using the enzyme amylase which is found in saliva and in secretions from the pancreas. Starch (also known as amylose) is a polymer made from many glucose molecules connected together in a long chain via covalent bonds. It is produced by dehydration synthesis and is used by many organisms as a way to store glucose for later use. Its structure is shown in figure 1 (right).

Figure 1–Structure of amylose



Adapted from BeMiller, J. N., and Whistler, R. L. (1996). Carbohydrates. In *Food Chemistry* (3rd ed., pp. 157-223.) New York, New York: Marcel Deker.

For organisms to be able to use the energy in starch the polymer must be broken down into its simpler glucose units. In animals, this hydrolysis reaction is catalyzed by the enzyme amylase. In fact, amylase converts starch to glucose dimers (called maltose) and trimmers (called maltotriose); these are then converted by other enzymes to glucose which can then be used for cellular respiration, etc. We will study the effect of enzyme concentration on the rate of the conversion of starch to maltose.

There are a variety of nutritional supplements that call themselves "starch blockers". Their manufacturers claim that they contain extracts from the common bean (*Phaseolus vulgaris*) that inhibit amylase. Beans are known to contain amylase inhibitors – these are proteins that bind to the active site of amylase and prevent it from catalyzing the breakdown of starch. However, the supplement industry is very lightly regulated, so manufacturers are able to make claims of efficacy without having to provide evidence to support these claims. As a result, not all supplements claiming to be "starch blockers" will necessarily contain amylase inhibitors. In this lab, we will measure one or more "starch blockers" to see if in fact they do inhibit amylase.

The action of amylase on starch can be readily followed with the IKI (a mixture of iodine and potassium iodide) test. IKI stains starch a blue-black color, but it does not stain maltose or maltotriose. When all of the starch has been hydrolyzed to these sugars, the solution will remain the yellowish-brown color of IKI. As hydrolysis is taking place and some of the starch has been hydrolyzed and some has not, the solution will show intermediate shades of brownish-black. The diagram below shows these changes. Please note that a positive test for starch means that the enzyme has not broken down all of the starch in the sample yet and a negative test for starch means that the enzyme has broken down all of the starch in the sample to sugars.

Time →

1. START: **only un-hydrolyzed starch** (BLUE-BLACK)
2. {some starch hydrolyzed to maltose}
3. MIDDLE: **Mixture of starch and glucose** (BROWN-BLACK)
4. {finally, all the starch has been hydrolyzed to maltose}
5. END: **No starch; only maltose** (YELLOW-BROWN)

First: Notebook Review

To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- What do the terms 'substrate', 'enzyme', and 'product' mean and how do they apply to today's lab?

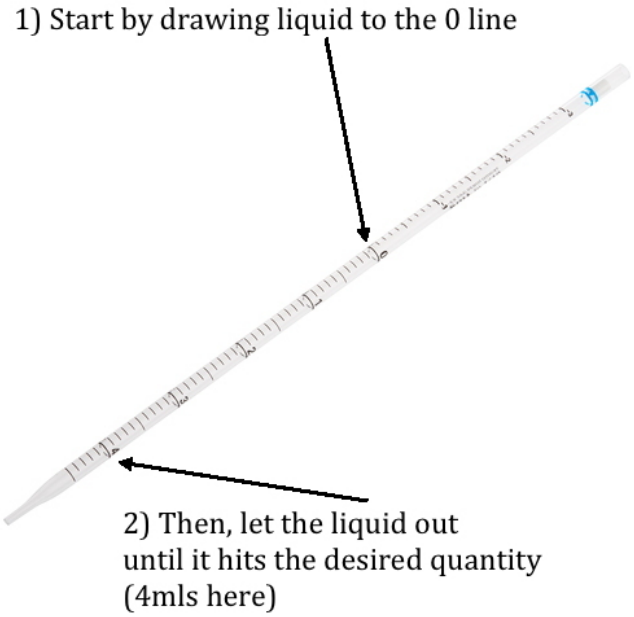
You will then discuss these with your TA as a class to clarify any issues that remain.

Procedure

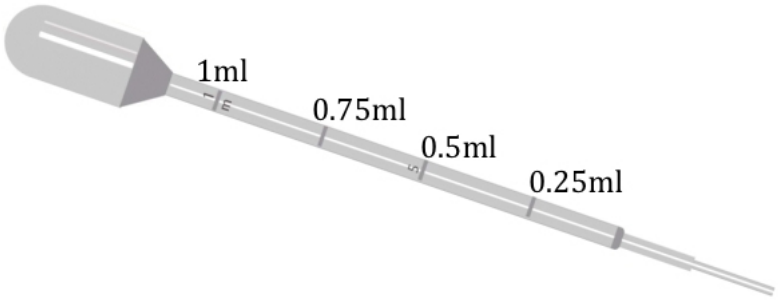
PART 0: PIPETTE PRACTICE

You should practice using pipettes to measure precise volumes of water into a test tube until you're sure you can do it quickly and repeatably. The best practice is to show that you can add and subtract volumes accurately – for example, put two samples of 2.5ml each into a test tube and be sure that you can pipette out exactly 5ml.

The figure below shows how to measure 4ml using a 5ml pipette as an example:



You will also use transfer pipettes like this one (check the markings carefully as they may be different):



PART I: REACTION RATE

0. Guide to colors: Each group should prepare one spot plate with a drop of IKI test solution in each of two of the depressions. To one of the IKI drops, add a drop of starch solution. Record the colors and make it clear in your notes what happened in each case. The depression with the IKI only is the standard or reference that shows the color of the solution when no starch is present and the depression with the drop of starch is the standard that shows the color of the solution when high amounts of starch are present.

1. Label one test tube "SS" for "Stock Starch" and your group name. Pipette 4 ml of the stock starch solution into this test tube. Be as accurate as possible in your measurements. BE SURE TO SWIRL THE STARCH SOLUTION BEFORE EACH PIPETTING TO GET THE PROPER CONCENTRATION OF STARCH.

2. Take a different test tube, label it "10%" and pipette 1 ml from the 10% amylase solutions into it. Swirl the amylase flask before pipetting your sample. Place this test tube in a rack while you get other things ready.

You should now have 2 tubes:

- 1 tube with 4ml starch stock solution; labeled "SS"
- 1 tube with 1ml 10% amylase; labeled "10%"

3. Obtain a spot plate and place one drop of IKI solution in each depression. The top left-hand depression will be called the first depression, and you will be testing samples starting with the first depression and moving to the right with successive samples. Number the depressions if you think you might lose track of which ones have been used.

4. One student in the group should record the time while another student in the group pours the contents of one of the "SS" test tubes into the test tube labeled 10%. Swirl this to mix the solution.

5. Remove ONE drop immediately using a plastic pipette and place it on the first IKI drop in your spot plate. If there is any blue-black color in this depression, put a check mark next to the "time 0" line in the 10% amylase column showing that there is still starch in the solution.

6. Continue taking one drop of the solution and putting it in successive depressions of the spot plate every 30 seconds until you have a negative starch reaction which will show you the original color of the IKI solution (your negative standard). Put a check mark next to each line corresponding to the time that continues to show any blue-black color. Stop testing at 10 minutes.

7. When your drop tests negative for starch, put an "x" in the line next to the time when it happened. Take two more drops at 30-second intervals to be sure that the test is correct. Then determine how much time it took for your 10% solution to "reach end point" – that is, how long did it take for all the starch to be digested?

Data Table
Put a check (√) in the box when any blue-black color is visible. Put an "x" when there is a no color change (the color looks like the "no starch standard" from (0) above). Show 3 Xs in a row to be sure the reaction is complete.

Time (min:sec)	10%
0:00	
0:30	
1:00	
1:30	
2:00	
2:30	
3:00	
3:30	
4:00	
4:30	
5:00	
5:30	
6:00	
6:30	
7:00	
7:30	
8:00	
8:30	
9:00	
9:30	
10:00	

8. Calculate the rate of the reaction by assuming that we started with 100 units of starch in each tube, and then dividing 100 by the number of minutes that it took to digest all the starch. For instance, if it took 5 minutes, the rate would be 100/5 = 20 units per minute – that is, 20 units of starch were digested each minute. Note it below:

_____ Units/minute

9. Compare your end point with that of your fellow students. You will likely note significant discrepancies.

Special Assignment for Bio 111 Fall 2023

We've been doing this lab in Bio 111 for more than 10 years and, each year, we try to be more quantitative. By "quantitative" we mean "getting results that are reproducible enough to really measure the effects of temperature and various inhibitors on amylase activity". In practice, this means two things:

- *Level 1*: if you measure the activity of identical samples under identical conditions, you should get pretty close to the same activity.
- *Level 2*: if you measure the activity of samples that should give different activities, you should see clearly different activities.

It has become increasingly clear that the lab as described on the previous pages is not sufficiently quantitative – typically, we don't even get to Level 1.

So the special assignment for Bio 111 this fall is to figure out what we need to fix to make the lab quantitative. This is likely to require a combination of tools, techniques, and attention to detail. The only way to find out what is required is to have Bio 111 students test drive different versions of the lab and see which gives the best results.

As a first step, you will all follow the directions on the previous pages and report your results. It is likely that there will be significant disagreement – this is the problem we've been having. It is likely the result of small group-to-group variations in what you are doing.

You will then decide what aspects of the lab to try to be more precise about and repeat the lab making these changes and see if they have an effect. The first goal is to get to Level 1; if you get there, then go for Level 2. We will then pool these results to revise the lab manual so future generations of Bio 111 students will benefit.

Although this does not "seem like science", it turns out that a large part of research involves getting your techniques working well so you can get good data. This sort of "debugging" is a crucial, unpublicized, and under-appreciated part of science.

To give you some ideas of what the problem might be, here are some suggestions from past students and TAs:

1. *Not being careful about measurements*. There are many measurements of liquid volume in this lab. Some measurements are probably more critical to reproducible results. You could try having people use the pipettes more carefully or use pipettors to measure some of the volumes (see your TA for details on pipettors).
2. *The color of the IKI solution*. Sometimes, the IKI is more brown; other times, it's more orange. Different people think that one color works better than the other. You could try using only one color of IKI for all reactions.
3. *Swirling the amylase solution*. The amylase solution is cloudy and it tends to settle out. Some wonder if the activity of the settled out stuff is higher than the clear solution. You could try having everyone use a well-mixed solution or let it settle and pick from the top or bottom.
4. *Swirling the starch solution*. Same as with (3).
5. *Timing*. A big part of each reaction is timing it properly. You could set up a procedure to increase the accuracy of the timing and have everyone follow it.

6. *Something else that seems likely to be an issue*. There are lots of tiny details to this lab that are not spelled out in the lab manual – maybe some of them matter. For example:
    - Does it matter if you take a new sample for the reaction for each drop into the IKI solution or is it OK to leave the solution in the pipette in between samples?
    - Does it matter how big the drop of reaction you add to the IKI is?
    - Contamination – are people being careless and getting amylase in the starch and/or vice-versa?

You should discuss this as a class, decide what you want to try, and devise instructions for the whole class to follow. You will then see if your changes make a difference.

Here's a blank data table to record your results as you try different approaches

Time (min:sec)			
0:00			
0:30			
1:00			
1:30			
2:00			
2:30			
3:00			
3:30			
4:00			
4:30			
5:00			
5:30			
6:00			
6:30			
7:00			
7:30			
8:00			
8:30			
9:00			
9:30			
10:00			
10:30			
11:00			
11:30			

Preparing for the Take Home Exam

Take Home Exam 4 will be, in part, based on this lab. You should look at the exam on Blackboard before you leave lab today. You may want to use some of the remaining time in lab to prepare for the exam.