

Name _____

Pre-lab 1–Enzyme Activity: Part 1

1. When a potato slice, which contains the enzyme peroxidase, is added to its substrate (peroxide):
 - A. nothing happens (no product is formed)
 - B. gas will be produced but no water will be produced
 - C. water will be produced but no gas will be produced
 - D. gas and water will both be produced

2. Matching: use each letter only once

___enzyme

___ substrate

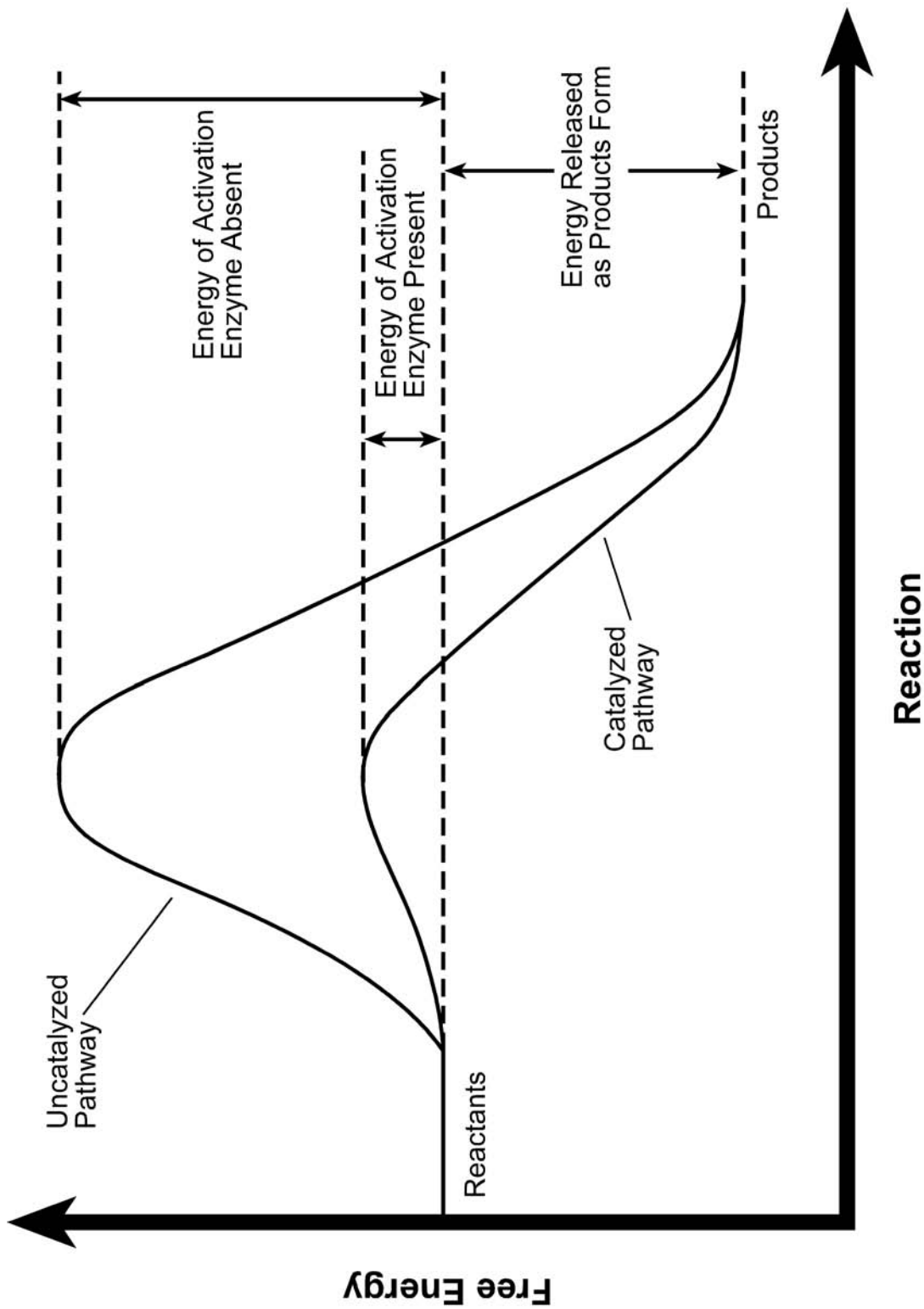
___pH

___product

___transition state

___optimal conditions

- a. the point in a reaction when the substrate's chemical bonds are stressed and the substrate is more reactive
- b. refers to the concentration of hydrogen ions present in solution or more commonly thought of as how acidic or basic something is
- c. the molecule which the enzyme binds to and forms product from
- d. refers to a type of protein molecule that catalyzes reactions faster than if the reaction were to occur alone
- e. pH, temperature, salinity, concentration, etc. which make each reaction occur at the fastest rate
- f. the molecule or molecules that are formed by the enzyme substrate complex; (the enzyme is left unchanged)



LABORATORY

1

Enzyme Activity: Part 1 Design Your Own Experiment

BACKGROUND

Most chemical reactions within cells do not occur spontaneously. Molecules within cells are relatively stable, and many metabolic reactions must be **catalyzed** (accelerated) by protein molecules called **enzymes**. An enzyme is a protein that catalyzes specific reactions. Since they are not altered or used up in the reaction, enzymes can keep catalyzing reactions over and over again. Enzymes act by binding to the substrate (the molecule being changed) forming an enzyme-substrate complex that may stress certain chemical bonds of the substrate. When the substrate's chemical bonds are stressed, the substrate is more reactive and is said to be in its transition state. The energy needed to get the substrate to its transition state is called the **energy of activation**. The reaction is complete when the reactants (either the substrate alone or the substrate and another molecule) form a new product and the enzyme releases the product(s) and returns to its original condition. The enzyme molecule can then repeat the process with more substrate molecules. A reaction where the enzyme breaks down the substrate is represented in Figure 1.1. Remember that enzymes can also be used to catalyze reactions where new chemical bonds are formed.

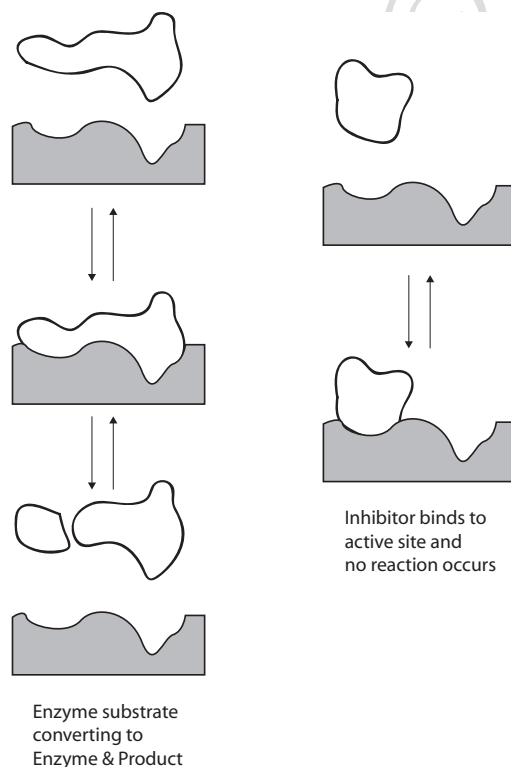


Figure 1.1 Schematic of Enzyme Action and Enzyme Inhibitor.

The rate of enzymatic catalysis depends on factors in the immediate environment that affect the shape of the enzyme and modify the interactions between the enzyme and its substrate. Remember that an enzyme is a protein and therefore has a very complex 3-dimensional structure that is very sensitive to environmental conditions. Cells contain many kinds of enzymes and each enzyme will catalyze only a specific reaction using a specific substrate. Such specificity results from the precise 3-dimensional structure of the enzyme. The substrate-binding site on the surface of the enzyme is unique and usually couples with only one substrate. Any structural change in the enzyme may alter the substrate binding site and slow down the reaction rate. An example of this structural specificity occurs during the production of stomach acid; the shape of the enzyme that produces stomach acid is inhibited by products such as pepcid and axid and consequently cannot bind its substrate (that's why these compounds are used in treatment for people suffering from over-production of acid). Sometimes a molecule, called a cofactor, may be required by the enzyme for proper folding.

There is a set of environmental conditions at which the enzyme works at its highest rate, known as the **optimal conditions** for the enzyme's activity. Seemingly small changes in temperature can have a great effect on the rate at which an enzyme will act. Other factors, such as the amount of substrate versus the amount of enzyme present, can also affect the reaction rate. During this laboratory you will determine some of the optimal ranges for temperature, enzyme concentration, and substrate concentration as well as the effects of environmental factors such as temperature, pH, and heavy metals on reaction rates.

Since enzymes act on specific substrate molecules and produce specific products, it is possible to determine enzyme activity by measuring the disappearance of the substrate or the appearance of the product, or in some cases both. This analysis is facilitated by the availability of purified enzyme preparations, which can be mixed with the appropriate purified substrate.

Using the materials available, you will design and conduct an experiment illustrating the effect of environmental factors on enzyme activity. Upon completion of the experiment each student will either write up the results in scientific format, or be prepared to present results (show graphs) at the beginning of the next lab session. Review the guidelines for writing lab reports in Lab 4.

DESIGNING AN EXPERIMENT

Asking a Question about Enzymes

Consider the information you have about enzymes and what types of factors may influence the rate at which they catalyze reactions. We have 3 different types of enzymes (each needs its own specific substrate) and 3 different types of conditions/variables that could be used in your experiment (see template on next page). Use this information to ask a question that you have about what may affect the rate of enzyme activity.

Your question may follow one of the following formats (which help lead into designing an experiment), or you can ask the question in your own way.

What is the effect of _____ on the rate of _____ activity?

What is the effect of the concentration of _____ on the rate of _____ activity?

your question:

Writing Hypotheses

Based upon your question above, write simple hypotheses about your experiment.

Experimental Design (using the choices in the template below, choose one item in each category by underlining your choice). Be sure to show your design to your instructor before you perform the experiment.

ENZYME	SUBSTRATE
Amylase: found in the mouth	Starch
Peroxidase: found in potato cells	Hydrogen Peroxide Solution
Proteases	Proteins

VARIABLES:

pH—Buffered solutions are available with pH of 4, 6, 7, and 9.

Temperature—Some temperatures to consider are 0°C, 27°C, 37°C, and 70°C.

Salinity—A 10% NaCl stock solution is available; you can make various dilutions from this as required.

DETECTION:

Methods to monitor substrate disappearance or product formation:

KI—Will detect the presence of starch. (colorimeter, 605 nm)

Collect oxygen gas—This is produced by the breakdown of hydrogen peroxide into oxygen and water, and can be quantified by measuring the collection of gas in a graduated centrifuge tube.

Biuret's solution—Detects the presence of proteins. Can be used to quantify protein levels by measuring how the samples absorb light (colorimeter, 530 nm).

Materials

0.5% starch solution	potato
hydrogen peroxide solution	Colorimeter (or spectrophotometer)
1% albumin solution	Fresh Biuret's solution
distilled water	potassium iodide
amylase solution	test tubes
0.12 gm/200 mL)	triple beam balances
peroxidase (in potato form)	beakers
protease solution	10% sodium chloride solution
graduated centrifuge tubes	graduated cylinders
buffered pH solutions (pH 4, 7, 10)	water baths (at least 3 different temperatures)

Use your choices above to design your experiment. **Remember to include controls.**

DESIGN:**Suggested Reaction Components**

If you are testing pH, run reactions with at least 3 different buffers. If you are testing salt, run reactions with 3 different salt concentrations. If you are testing temperature, test 3 different temperatures. If you are testing inhibitors, test 3 different concentrations of inhibitor.

1. Peroxidase.

- *If you are testing the effect of salt*, pipet 10 mL of 3% hydrogen peroxide and 1 mL of pH7 buffer into three tubes. Add 4 mL water to 1 tube. Add 2 mL water and 2 mL salt to the 2nd tube. Add 4 mL salt to the 3rd tube.
- *If you are testing the effect of pH*, add 10 mL of hydrogen peroxide and 4 mL water to three 15 mL tubes. Next, add 1 mL of low pH buffer to 1 tube, 1 mL medium pH buffer to the 2nd tube, and 1 mL high pH buffer to the 3rd tube.

When you are ready to start the reaction, add 2 cubes of potato (0.5 cm on each side), place 50 mL beaker on top of tube and invert, IMMEDIATELY noting the volume of air space in the graduated tube. You can quantitate the amount of peroxidase activity in 2 ways: 1) if bubbles are rising slowly (this may happen if the potato has been stored a long time), then count the number of bubbles rising for 30 seconds, wait 2-5 minutes and count again for 15 seconds, etc. until you have made 5-6 measurements; 2) if bubbles are rising rapidly, record the volume of the air space at 5-6 time intervals.

2. Amylase.

- *If you are testing the effect of salt*, pipet 8 mL of 0.5% starch solution and 1 mL of pH7 buffer into three 16 x 125 tubes. Add 6 mL water to 1 tube. Add 3 mL water and 4 mL salt to the 2nd tube. Add 6 mL salt to the 3rd tube.
- *If you are testing the effect of pH*, add 8 mL of 0.5% starch solution and 6 mL water to three 16 x 125 tubes. Next, add 1 mL of low pH buffer to 1 tube, 1 mL medium pH buffer to the 2nd tube, and 1 mL high pH buffer to the 3rd tube.
- *If you are testing the effect of temperature*, add 8 mL starch solution, 1 mL pH 7 buffer, and 6 mL water to each tube. Next, expose each tube to different temperatures.

Remove 1 mL of this solution and add to the 2 mL of H₂O and 3 drops KI and label this sample “time zero.” To the rest of the starch/buffer solution, add 1 mL of amylase, mix. At 1 minute intervals, remove 1 mL of reaction and add to 2 mL of H₂O and 3 drops KI (mix!) until you have 5 time measured samples. Using a colorimeter (or spectrophotometer), measure the absorbance of the samples using a 605 nm filter. To make a “reagent blank” for the colorimeter, make a test tube containing 3 mL of water and 3 drops of KI solution. Use this for “zeroing” the colorimeter.

3. Protease.

- *If you are testing the effect of salt*, pipet 8 mL of 1% albumin and 1 mL of pH7 buffer into three 16 x 125 tubes. Add 6 mL water to 1 tube. Add 3 mL water and 3 mL salt to the 2nd tube. Add 6 mL salt to the 3rd tube.
- *If you are testing the effect of pH*, add 8 mL of 1% albumin and 6 mL water to three 16 x 125 tubes. Next, add 1 mL of low pH buffer to 1 tube, 1 mL medium pH buffer to the 2nd tube, and 1 mL high pH buffer to the 3rd tube.
- *If you are testing the effect of temperature*, add 8 mL albumin, 1 mL pH 7 buffer, and 6 mL water to each tube. Next, expose each tube to different temperatures.

Remove 1 mL of this solution and add to 2 mL H₂O and 10 drops Bituret, mix, and label this sample “time zero”. To the rest of the albumin/buffer solution, add 1 mL “protease,” mix. At 5 minute intervals, remove 1 mL of the reaction and add 2 mL H₂O and 10 drops Bituret (mix!) until you have 4-5 time-measured samples. Using a colorimeter (or spectrophotometer), measure the absorbance of the samples using a 530 nm filter. To make a “reagent blank” for the colorimeter, make a test tube containing 3 mL of water and 10 drops of Biuret solution. Use this for “zeroing” the colorimeter.

Once you have outlined your procedure and discuss it with your instructor you may begin. Remember to take measurements at the start of your experiment and at appropriate intervals.

Record your results in the data tables and graphs provided or in a format you design. Prior to the next lab session, a Lab Report may be written following the outline below, **or** you may be asked to prepare graphs from your results to present to the class at the beginning of the next lab session.

Lab report

- A. Introduction—Write a brief introductory paragraph on the topic of enzyme activity. Include your question, your hypotheses statements (ideas the experiment tests) and the information you used to derive your hypotheses.
- B. Materials and Methods—What you used to test your hypotheses and how you went about testing them. Clearly explain the experimental design.

- C. Results—Describe the experimental results. What happened during and after your experiment? Present results both written (in a table) and graphically (but do not discuss them here).
- D. Discussion—Provide an interpretation and assessment of results in relation to the introductory paragraph. Was each hypothesis supported or rejected? What do your results mean in terms of how the enzyme you studied may work in cells?

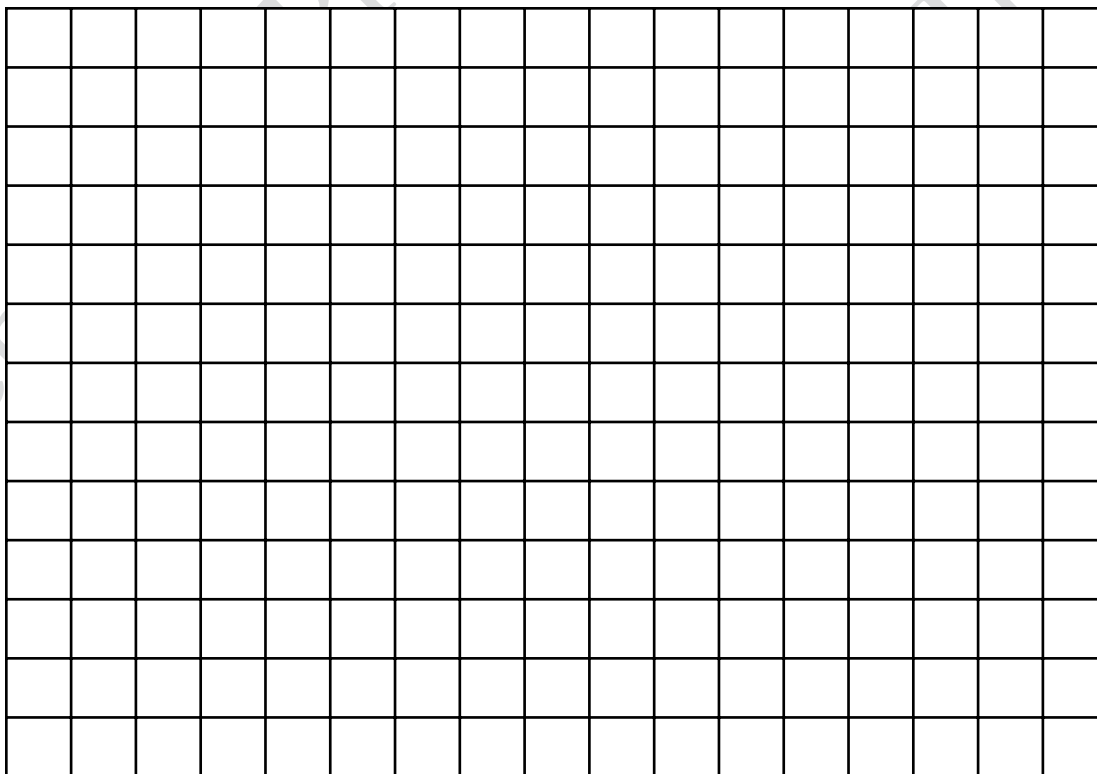
Results

Table 1.1 Timed detection results (presence or absence of substrate or product, or volume of gas produced).

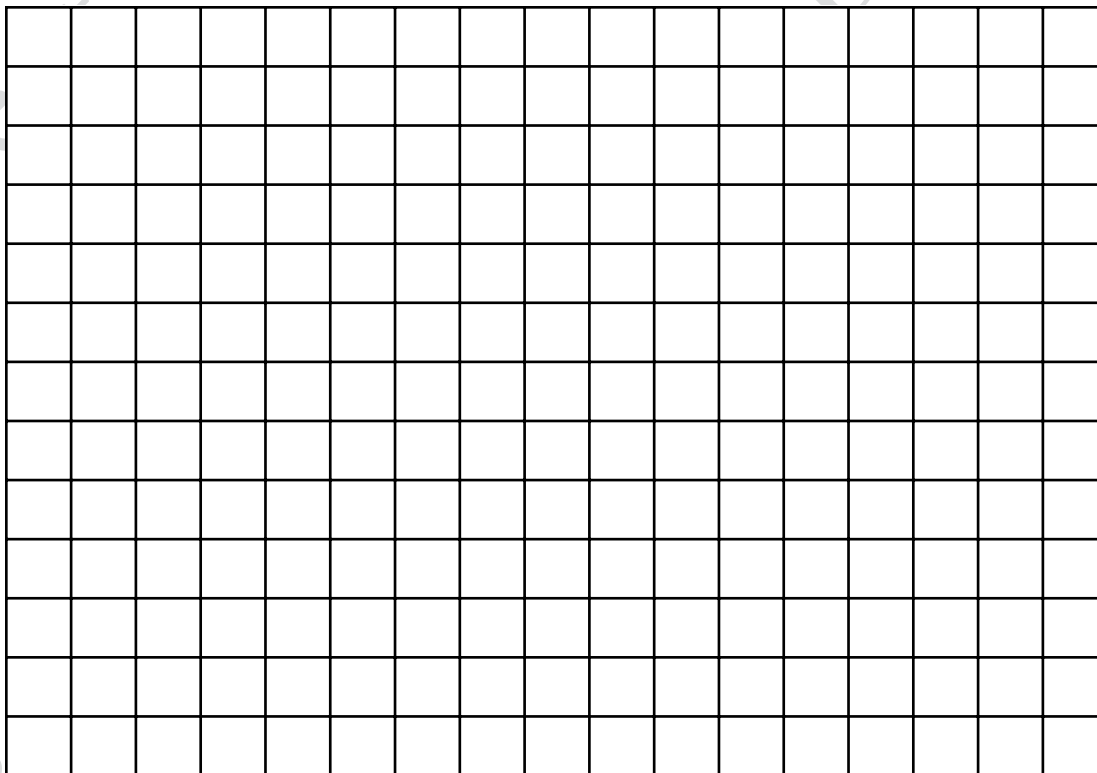
TEST CASES				
TIME OF MEASUREMENT	reaction 1	reaction 2	reaction 3	reaction 4
time 0 (starting)				
time 1				

If you do not have use of a colorimeter, it may be necessary for your group to grade your color change test based on the range of results obtained during your experiment. This grade scale should include a negative result (0) and include as many numbers as you have distinguishable color variants. Shake tubes before grading and consider amount of precipitate present. Use this scale for presenting and explaining your results in your report.

Graph 1.1 Growth of gas bubbles over time: peroxidase.



Graph 1.2 Disappearance of substrate (starch or protein) over time.



INTERPRETING RESULTS

Answer these questions to assist you in interpreting your results.

For the enzyme/substrate pair in your experiment answer the following questions.

What was the environmental variable used in your experiment? _____

What were the specific values of the environmental variable tested in your experiment?

_____, _____, _____, _____

What conditions were held constant during your experiment?

What was used as a control?

In which cases did the enzyme work fastest? _____ slowest? _____

How long did the enzyme require to consume the substrate in each case?

reaction 1 _____ reaction 2 _____ reaction 3 _____ reaction 4 _____

END OF CLASS DISCUSSION

What is the function of each of the three enzymes used in the lab?

What factors affect enzyme activity?

What are the properties that make a protein an enzyme?

DISCUSSION FOR LAB REPORT OR FOR BEGINNING OF THE NEXT LAB SESSION

What were the results of your experiment?

Were there any results of your experiment that surprised you?

Can you explain these results?

Do the results of your experiment tell you anything about conditions found in living cells in which enzymes function?