Name: Date: AP Biology

LAB : FACTORS INFLUENCING ENZYME ACTIVITY

Background

Enzymes are biological catalysts capable of speeding up chemical reactions by lowering activation energy. One benefit of enzyme catalysts is that the cell can carry out complex chemical activities at a relatively low temperature.

Most enzymes are proteins and their 3-dimensional shape is important to their catalytic activity.
Two specific regions on the enzyme structure play an important role in catalytic activity: the **active site** and the **allosteric site**. The **active site** is the area of the enzyme which binds to the substance(s) (substrate) and aids in the chemical reaction. The **allosteric site** is involved in forming the proper 3-dimensional shape when linked with specific cofactors. As a result of the unique characteristics of these sites, enzymes are highly specific in terms of the reactions they will catalyze and the condition under which they work best.

In biochemical reactions the **enzyme**, combines reversibly with its specific **substrate**, to form an enzyme-substrate complex. One result of this temporary union is a reduction in the energy required to activate the reaction of the substrate molecule so that the **products of the reaction**, are formed. This can be summarized in the equation:

Enzyme + Substrate –> Enzyme-Substrate Complex –> Enzyme + Product

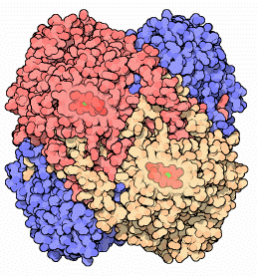
Note that the enzyme is not consumed in the reaction and can recycle to work with additional substrate molecules. Each enzyme is specific for a particular reaction because its amino acid sequence is unique which causes it to have a unique 3-dimensional structure. The **active site** is the portion of the enzyme that interacts with the substrate, so that any substance that blocks or changes the shape of the active site affects the activity of the enzyme.

A description of several ways enzyme action may be affected follows:

1. **Salt Concentration:** If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract each other. The enzyme will denature and form an inactive precipitate. If, on the other hand, the salt concentration is very high, normal interaction of charged groups will be blocked, new interactions will occur, and again the enzyme will precipitate. An intermediate salt concentration such as that of human blood (0.9%) or cytoplasm is optimum for many enzymes.
2. **pH:** Amino acid side chains contain groups such as –COOH and $–NH_2$ that readily gain or lose H^+ ions. As the pH is lowered an enzyme will tend to gain H^+ ions, and eventually enough side chains will be affected so that the enzyme's shape is disrupted. Likewise, as the pH is raised, the enzyme will lose H^+ ions and eventually lose its active shape. Many enzymes perform optimally in the neutral pH range and are denatured at either an extremely high or low pH. Some enzymes, such as pepsin, which acts in the human stomach where the pH is very low, work best at a low pH.
3. **Temperature:** Generally, chemical reactions speed up as the temperature is raised. As the temperature increases, more of the reacting molecules have enough kinetic energy to undergo the reaction. Since enzymes are catalysts for chemical reactions, enzyme reactions also tent to go faster with increasing temperature. However, if the temperature of an enzyme – catalyzed reaction is raised still further, a temperature optimum is reached. Above this value, the kinetic energy of the enzyme and water molecules is so great that the conformation of the enzyme molecules is disrupted. The positive effect of speeding up the reaction is now more than off-set by the negative effect of changing the conformation of more and more enzyme molecules. Temperatures around 40-50 degrees Celsius denature many proteins, but some are still active at 70-80 degrees Celsius, and a few even withstand boiling.
4. **Activators and Inhibitors:** Many molecules other than the substrate may interact with an enzyme. If such a molecule increases the rate of the reaction it is an **activator**, and if it decreases the reaction rate it is an **inhibitor**. These molecules can regulate how fast the enzyme acts. Any substance that tends to unfold the enzyme, such as an organic solvent or detergent will act as an inhibitor. Some inhibitors act by reducing the S-S bridges that stabilize the enzyme's structure. Many inhibitors act by reacting with side chains in or near the active site to change its shape or block it. Many well-known

poisons such as potassium cyanide and curare are enzyme inhibitors that interfere with the active site of critical enzymes.

We will be working in this lab with a representative enzyme - **catalase**. Catalase has a molecular weight of approximately 240,000 daltons and contains 4 polypeptide chains, each composed of more than 500 amino acid monomers. This enzyme occurs universally in aerobic organisms. One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide (H_2O_2) formed as a by-product of metabolic processes. Catalase might also take part in some of the many oxidation reactions going on in all cells. The primary reaction catalyzed by catalase is the decomposition of H_2O_2 to form water and oxygen.



The catalase that works in liver

$$\mathbf{2\ H_2O_2} \rightarrow \mathbf{2\ H_2O + O_2}\ (\text{gas})$$

In the absence of catalase, this reaction occurs spontaneously, but very slowly. Catalase speeds up the reaction considerably. Much can be learned about enzymes by studying the kinetics (changes in rate) of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped.

In this experiment, a rate for this reaction will be determined indirectly. The assay system used in this lab consists of a filter paper disk that is coated with the enzyme and then dropped into a cup of substrate (hydrogen peroxide). As the catalyst breaks down the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen collect underneath the filter paper disk and make it rise to the surface of the hydrogen perioxide. The time it takes for the filter paper disk to rise (from the bottom of the cup) is an indication of the rate of enzyme activity.

$$\mathbf{RATE_{ENZYME\ ACTIVITY} = DISTANCE_{DEPTH\ OF\ HYDROGEN\ PEROXIDE\ IN\ MM} / TIME_{IN\ SEC}}$$

We will assume that each filter disk is coated with the same amount of catalase (except in the investigation of the effect of enzyme concentration of enzyme activity).

LAB PROCEDURE PART A:

(**Safety:** You will be using glass, hot water, acids, and bases. Use caution and wear goggles.)

1. Prepare a beaker or clear cup with H_2O_2 that is 4 cm deep.
2. Using a single hole punch, cut individual single layer disks of coffee filter paper.
3. Pour a small amount of 100% catalase in a second cup. **Shake the bottle of solution BEFORE pouring** to make sure it is well mixed.
4. Pick up a single disk with forceps (tweezers) and dip the disk in your catalase enzyme solution in the cup.
5. Still using the forceps, toss the disk into the H_2O_2. Watch the disk carefully and start timing when the disk hits the bottom of the cup. Stop timing when the disk reaches the surface.
6. Repeat steps 4-5 for a total of five trials to perfect your technique.
7. When you are cleaning up, make sure the filter disks do NOT go down the drain.

Practice - Number of Seconds Required For a Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H_2O_2

Trial	Seconds to Rise	Reaction Rate (Distance/Time)
1		
2		
3		
4		
5		

8. Now get a small amount of the boiled catalase and run 2 timed trials.

Practice - Number of Seconds Required For a Boiled Catalase-coated Filter Paper Disk to Rise to the Top of 4 cm of H_2O_2

Trial	Seconds to Rise	Reaction Rate (Distance/Time)
1		
2		

a. What happened? Why do you think this occurred?

LAB PROCEDURE PART B:

For this part of the lab you will work in groups of two people. Each group will test and report data on one of the following questions (two groups will do each question and their data will be pooled for the class use). EVERY STUDENT will be responsible for getting the class data for all four questions, answering the analysis questions, and evaluating all four situations in his/her lab report.

Question #1: What is the effect of enzyme concentration on enzyme activity?

1. Set up 3 fresh cups of 1% H_2O_2 that are 4 cm deep.
2. Begin with the enzyme solution. Make a dilution of the enzyme so that you have 3 strengths of enzyme: one at 200% enzyme strength (~4 mL solution), one at 100% enzyme strength (~4 mL), and one at 50% enzyme strength (2 mL 100% solution + 2 mL water).
3. Run 5 trials using each of the 3 catalase solutions (200%, 100%, and 50%)
4. Record your data in the table below.

Question #2: What is the effect of substrate concentration on enzyme activity?

1. Take a fresh amount of 1% H_2O_2 and dilute it to 0.5% (1/2 H_2O_2 and and 1/2 water). Swirl so the solution mixes well then set up a cup that is 4 cm deep with this 0.5% solution.
2. Set up a second cup with 4 cm of fresh 1% H_2O_2 and a third with 4 cm of the 2% H_2O_2.
3. Run 5 trials using each of the 3 substrate solutions (1%, 0.5%, and 2% H_2O_2). Again, make sure you mix the catalase solution before you pour it into your cup for dipping the disks.
4. Record your data in the table below.

Question #3: What is the effect of acid pH on enzyme activity?

1. Set up 3 fresh cups of 1% H_2O_2 that is 4 cm deep.
2. Label 3 additional cups pH 3, pH 5, and pH 7.
    a. In the pH 3 cup, put 5 mL double strength (200%) catalase (after shaking the bottle to mix it well) with 5 mL pH 3 buffer. Swirl the cup to mix well.
    b. In the pH 5 cup, put 5 mL double strength catalase with 5 mL pH 5 buffer. Swirl.
    c. In the pH 7 cup, put 5 mL double strength catalase with 5 mL pH 7 buffer. Swirl.
3. Run 5 trials using each of the 3 catalase solutions (pH 3, pH 5, pH 7). Again, make sure you mix the catalase solution before dipping the disks.
4. Record your data in the table below.

Question #4: What is the effect of base pH on enzyme activity?

1. Set up 3 fresh cups of 1% H_2O_2 that is 4 cm deep.
2. Label 3 additional cups pH 7, pH 9, and pH 11.
    a. In the pH 7 cup, put 5 mL double strength (200%) catalase (after shaking the bottle to mix it well) with 5 mL pH 7 buffer. Swirl the cup to mix well.
    b. In the pH 9 cup, put 5 mL double strength catalase with 5 mL pH 9 buffer. Swirl.
    c. In the pH 11 cup, put 5 mL double strength catalase with 5 mL pH 11 buffer. Swirl.
3. Run 5 trials using each of the 3 catalase solutions (pH 7, pH 9, pH 11). Again, make sure you mix the catalase solution before dipping the disks.
4. Record your data in the table below.

	Group 1			Group 2		
Question #1	200% catalase	100% catalas	50% catalase	200% catalase	100% catalas	50% catalase
Trial 1						
Trial 2						
Trial 3						
Trial 4						
Trial 5						
Avg Reaction Rate						
Question #2	2% substrate	1% substrate	0.5% substrate	2% substrate	1% substrate	0.5% substrate
Trial 1						
Trial 2						
Trial 3						
Trial 4						
Trial 5						
Avg Reaction Rate						
Question #3	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7
Trial 1						
Trial 2						
Trial 3						
Trial 4						
Trial 5						

Avg Reaction Rate						
Question #4	pH 7	pH 9	pH 11	pH 7	pH 9	pH 11
Trial 1						
Trial 2						
Trial 3						
Trial 4						
Trial 5						
Avg Reaction Rate						

Make a graph of enzyme concentration vs reaction rate. Write 1-2 sentences summarizing the effect of enzyme concentration on the rate of reaction. Which enzyme percentage was the "control" group?

Make a graph of substrate concentration vs. reaction rate. Write 1-2 sentences summarizing the effect of substrate concentration on the rate of reaction. Which substrate percentage was the "control" group? Why?

Make a graph of pH vs. reaction rate. Write 1-2 sentences summarizing the effect of pH on the rate of reaction. Which pH was the "control" group? Why?

Write-up:

You will be writing a lab report

1. **Make a title that reflects our independent and dependent variables.**
2. **List the materials and methods for this experiment.**
3. **Results: Include the data table, all graphs listed above, and 1-2 sentence summaries of graphs.**
4. **ANALYSIS:**
   Write an analysis for the experiments. Be sure to answer the following in your analysis:
   What is the enzyme, substrate, and products in this reaction?
   What is the gas you see bubbling up?
   How did each treatment affect the reaction rate? Predict and explain why the treatments affected the reactions the way they did.
5. **CONCLUSION:**
   Write a conclusion for this lab. Be sure to answer the following in your conclusion:
   To which "big idea" did this experiment relate?
   What did you learn from this lab?
   Why is a high fever (108 degrees F) so harmful to the human body?
   Why must pH remain constant (be homeostatic) if life is to flourish?
   This would be a good time to include some research about the enzymes and their work. Cite all references used in your research.

This will be due_____

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Teacher Notes:

Option 1 – Use dried yeast, simply dissolve a 7 g package of yeast in 1000 ml warm (not hot) water and wait, at room temperature, for an hour or so. Strain off the liquid using cheesecloth and discard the solids. Keep the liquid on ice (or refrigerated) until needed.
Option 2 – Use fresh potatoes. For each gram of potato add 1 ml of water and mix in a blender briefly. Strain off the liquid using cheesecloth and discard the solids. Keep the liquid on ice until needed.
Option 3 – Use fresh or frozen liver. For each gram of liver use 10 ml water and mix in blender briefly. Use the same technique as in option 1.
Whichever option is used for preparing the catalase, the same general technique should be applied to make a double strength solution.
Have students mark all data tables other than theirs with the following: "**See Attached Class Data**". I prefer to create the class data tables in Excel and provide these data tables to the students on my website.