Name_____ Date_____ Period_____ Bio I, Ms. D _

What Affects Enzyme Activity? Lab

Introduction

Enzymes are **biological catalysts** that help to carry out the thousands of chemical reactions that occur in living cells. They are generally large proteins made up of several hundred amino acids.
In an enzyme-catalyzed reaction, the substance to be reacted, the **substrate**, binds to the **active site** of the enzyme. The enzyme then converts the substrate to products. Finally, the products are released into solution and the enzyme is ready to help with another reaction. As is true of any catalyst, the enzyme is not used up as it carries out the reaction but is **recycled again and again**. One enzyme molecule can carry out *thousands* of reaction cycles every minute!!

Each enzyme is **specific** for a certain reaction because its amino acid sequence is unique and causes it to have a unique three-dimensional **structure**. The "business" end of the enzyme molecule, the **active site**, also has a specific shape. Because the active site is so specific it will only bind with one kind of molecule.
An enzyme can be **denatured** (unfolded) by extreme **heat**, **pH, or ionic concentration**. If this happens, the enzyme will no longer be functional because the shape of the active site will be destroyed.

In this exercise you will study the enzyme **catalase**, which speeds up the breakdown of hydrogen peroxide, H_2O_2, (a common waste product of cellular metabolism) into water and oxygen.

$$2H_2O_{2\,(l)} \xrightarrow{\text{catalase}} 2H_2O_{\,(l)} + O_{2\,(g)}$$

It's important for cells to be able to break down hydrogen peroxide as soon as it's generated in the cell because hydrogen peroxide is quite toxic for cells. The products of the reaction, water and oxygen, are not toxic for the cell. So catalase helps to protect the cell from damage that could be caused by its own metabolic waste. Catalase is a very important enzyme! Because it's so important, catalase is found in many animal and plant tissues.

Objectives:

1. Determine whether potato plants and/or carrot plants contain the enzyme catalase.
2. Analyze the effect of high heat, low pH, and high ionic concentration (saltiness) on the function of the catalase enzyme.

Pre Lab Questions:

1. What is the reactant (substrate) in the reaction above?_____
2. What are the products in the reaction above?_____
3. Would this reaction happen by itself without the help of catalase? Explain._____
   _____
4. What do the subscripts after each formula mean? (the little *l* and g)_____

Materials:

For each group:
Safety spectacles or goggles
Test tubes
Potato puree carrot puree
Test tube racks
Dropper pipet
Test tube clamp
Acid solution
Salt solution
Scoopula
Spatula
Graduated cylinder
Paper cups
Labeling tape
Markers

For the class lab:
Hot plates
Beaker

Part I: Organisms that have catalase

In this section, you will determine whether or not two different organisms have catalase in their tissues. We will examine potato plants and carrot plants to see if they have catalase. If they do have catalase in the tissue, it would be located inside the cells.

Procedure:

1. At your station, label one paper cup "potato puree" and another "carrot puree."
2. Go to the demo bench and use a scoopula to obtain a scoop of potato puree (blended up potato) and a scoop of carrot puree (blended up carrot) and put them in their cups.
3. Back at your station, USE TAPE to label one test tube "P" for "potato puree" and another "C" for "carrot puree."
4. Use a spatula to transfer a small scoop (about 25 chunks) of each type of puree into the respective test tubes. Push the puree down to the bottom of the test tube.
5. Use a graduated cylinder and funnel to measure out 10 ml of hydrogen peroxide. Pour the hydrogen peroxide into the potato puree test tube.
6. Record your observations on the data sheet provided.
7. Repeat steps 4-5 this time using carrot puree.

Part II: The Effect of Extreme Heat, pH & Salt on Catalase Function

In this section, you will use the potato puree to determine how heat, low pH, and high ionic concentration affect the activity of catalase.

Heat Procedure:

1. USE TAPE to label a test tube "BP" for "boiled potato puree." Use a spatula to take some of your potato puree (about 5 chunks) and transfer it into the "BP" test tube. Push it down to the bottom of the test tube.
2. Bring the "BP" test tube over to the hot water bath and hold it in the hot water (with a test tube clamp!!!!) for 3 minutes. **Your hand will get too hot if you keep holding it with one hand! Alternate hands!!! Allow to cool for 3 minutes before proceeding.**
3. Back at your station, use a graduated cylinder and funnel to measure out 10 ml of hydrogen peroxide. Pour the hydrogen peroxide into the "BP" test tube.
4. Record your observations on the data sheet provided.

pH Procedure

1. USE TAPE to label a test tube "AP" for "acid & potato puree." Use a spatula to take A SMALL AMOUNT (about 5 chunks) of your potato puree and transfer it into the "AP" test tube. Push it down to the bottom of the test tube.
2. Use a the dropper in the acid bottle to add 50 drops of acid to the AP test tube.
3. Use your spatula to mix the puree in with the acid. Rinse the spatula off in the sink.
4. Use a graduated cylinder and funnel to measure out 10 ml of hydrogen peroxide. Pour the hydrogen peroxide into the "AP" test tube.
5. Record your observations on the data sheet provided.

High Ionic Concentration (High Salt) Procedure

1. USE TAPE to label a test tube "SP" for "Salty potato puree." Use a spatula to take A SMALL AMOUNT (about 5 chunks) of your potato puree and transfer it into the "SP" test tube. Push it down to the bottom of the test tube.
2. Use a dropper pipet to add 10 drops of salt solution to the SP test tube.
3. Use your spatula to mix the puree in with the salt solution. Rinse the spatula off in the sink.
4. Use a graduated cylinder and funnel to measure out 10 ml of hydrogen peroxide. Pour the hydrogen peroxide into the "SP" test tube.
5. Record your observations on the data sheet provided.

CLEAN UP INSTRUCTIONS:

You CANNOT remove your goggles until AFTER you have cleaned up AND Ms. D has OKed your station!!!!

1. Make sure you have emptied the contents of your test tubes into the regular trash BEFORE you wash them in the sink. Otherwise, the sink will clog!!
2. Remove the labels from your test tubes and throw in the regular trash.
3. Use ALCONOX (in the squeeze bottles on your lab bench) and test tube brushes to clean the test tubes. Make sure to get all of the "gunk" out of them or Ms. D will not OK your station clean up!!
4. Rinse the graduated cylinders with water. (No alconox necessary)
5. Clean any spatulas and scoopulas on your bench with alconox.
6. Wipe down your station with a damp paper towel. Don't leave any puddles of water on the station!
7. ORGANIZE all the supplies neatly on the table.
8. PUSH YOUR STOOLS IN!!
9. Get Ms. D to OK your station. AFTER your station has been OKed by Ms. D, you can return to the classroom and remove your goggles.

DATA TABLES

Part I: Organisms that have catalase

Type of Plant Tissue	Observations when H_2O_2 was added	Evidence of a reaction? (Y/N)	Is catalase present? (Y/N)
Potato			
Carrot			

Part II: The Effect of Extreme Heat, pH & Salt on Catalase Function

Type of Puree	Observations when H_2O_2 was added	Evidence of a reaction? (Y/N)	Is catalase functional? (Y/N)
Boiled Potato Puree			
Low pH Potato Puree			
Salty (High Ionic Concentration) Potato puree			

Data Analysis and Conclusions: Questions:

1. What are you looking for in your observations when you are looking for evidence of a reaction?
   You are looking for bubbles as evidence of a chemical reaction. According to this equation,

$$2H_2O_{2\,(l)} \xrightarrow{\text{catalase}} 2H_2O_{(l)} + O_{2\,(g)}$$

   a gas is formed (oxygen gas) only if the reaction happens and the products are formed. When a gas is formed inside of a liquid, we see the gas as bubbles.

2. Do the two plant tissues that we tested contain catalase? *Yes* How do you know (i.e. explain how your data supports your answer to this question.
   You should have gotten the following data for Part I.

Part I: Organisms that have catalase

Type of Plant Tissue	Observations when H_2O_2 was added	Evidence of a reaction? (Y/N)	Is catalase present? (Y/N)
Potato	*Lots of bubbles are present, creating foam that rises near the top of the test tube.*	*Yes*	*Yes*
Carrot	*Bubbles are present (not as many as were created with the potato)*	*Yes*	*Yes*

According to this equation,

$$2H_2O_{2\,(l)} \xrightarrow{\text{catalase}} 2H_2O_{(l)} + O_{2\,(g)}$$

a gas is formed (oxygen gas) only if the reaction happens and the products are formed. When a gas is formed inside of a liquid, we see the gas as bubbles. Because we see bubbles being formed in the test tube with potato as well as the test tube with carrot, it indicates that the reaction took place. The only way that the reaction could take place at a fast enough rate for us to see the bubbles is if the catalase enzyme is there to speed up the reaction. Therefore, catalase must be present in both potato and carrot.

Questions 3, 4, and 5 relate to the data from part II. You should have gotten the following data for Part II. It's ok if your data doesn't match this data perfectly. It's science! You just have to be able to explain what might have gone wrong with your data.

Part II: The Effect of Extreme Heat, pH & Salt on Catalase Function

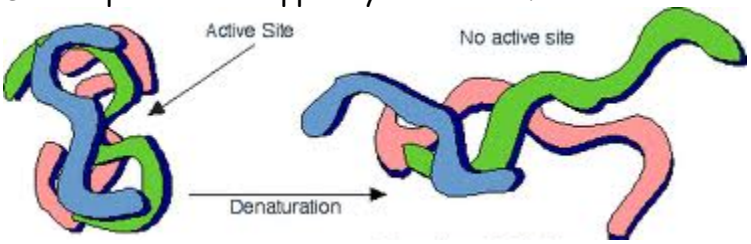
Type of Puree	Observations when H_2O_2 was added	Evidence of a reaction? (Y/N)	Is catalase functional? (Y/N)
Boiled Potato Puree	*No bubbles*	*No*	*No*
Low pH Potato Puree	*No bubbles*	*No*	*No*

Salty (High Ionic Concentration) Potato puree	*No bubbles*	*No*	*No*

What effect did high temperature have on the catalase activity? *High temperature caused the catalase to have NO ACTIVITY whatsoever. (The catalase is not functional) You know this because you did NOT see bubbles . Therefore, the reaction was not sped up by catalase and products were not formed in the lab time frame.*

3. Explain what happened to the catalase at the molecular level. *At the molecular level, the catalase was **denatured** by the high heat. Heat causes the enzyme molecule to have more kinetic energy. This means that the enzyme molecule will begin to move more rapidly (a.k.a. shake and shimmy). When the enzyme "shimmies" it unfolds. When an enzyme unfolds, it has **denatured** and no longer has shape or function. Therefore it cannot bind to the substrate anymore so it cannot speed up the reaction anymore.*

   Draw a picture to support your answer.



4. What effect did low pH have on the catalase activity? *Low pH caused the catalase to have NO ACTIVITY whatsoever. (The catalase is not functional) You know this because you did NOT see bubbles . Therefore, the reaction was not sped up by catalase and products were not formed in the lab time frame.*

   Explain what happened to the catalase at the molecular level. *At the molecular level, the catalase was **denatured** by the low pH. In low pH (or acid) the R groups of the amino acids in the enzyme begin to get H+ ions attached. This, in essence, caused the parts of the molecule that were originally hydrophobic to become hydrophilic and vice versa. The effect on the enzyme is that it gets "flipped inside out." When an enzyme gets flipped inside out, that constitutes unfolding. Therefore, it has **denatured** and no longer has shape or function. Therefore it cannot bind to the substrate anymore so it cannot speed up the reaction anymore.*

5. What effect did salt (high ionic concentration) have on the catalase activity? *Salt (high ionic concentration) caused the catalase to have NO ACTIVITY whatsoever. (The catalase is not functional) You know this because you did NOT see bubbles . Therefore, the reaction was not sped up by catalase and products were not formed in the lab time frame.*

   Explain what happened to the catalase at the molecular level.
   *At the molecular level, the catalase was **denatured** by the saltiness (high ionic concentration). In high ionic concentration the R groups of the amino acids in*

the enzyme begin to get ions attached. This, in essence, caused the parts of the molecule that were originally hydrophobic to become hydrophilic and vice versa. The effect on the enzyme is that it gets "flipped inside out." When an enzyme gets flipped inside out, that constitutes unfolding. Therefore, it has ***denatured*** *and no longer has shape or function. Therefore it cannot bind to the substrate anymore so it cannot speed up the reaction anymore.*

6. Part I of this lab did not have a control!! What could you have done as a control?
   Positive control: A test tube with purified active catalase enzyme + 10 ml hydrogen peroxide. This is a POSITIVE control because we expect to see bubbles. That is we expect the reaction to occur. It would give us a sense of what the bubbles formed by this reaction are supposed to look like, and it would also let us know that the hydrogen peroxide is fresh (not degraded).
   Negative control: A test tube with water + 10 ml hydrogen peroxide. This is a NEGATIVE control because we DON'T expect to see bubbles. That is, we DON'T expect the reaction to occur (because there is no catalase). It would also let us be sure that bubbles are not forming in the test tubes for other reasons unrelated to the experiment(like leftover soap in the test tube, for example.)

7. Why was it necessary to puree the plant tissue in order to test for catalase activity?

   The catalase enzyme is located inside of the cells. Since we wanted to work with the catalase enzyme, we had to break open the cells to release the catalase from inside the cells. Pureeing the plant tissue helps to break open the cells.