

Laboratory - Digestive Enzymes

Purpose/Objectives

- Observe and describe the function of bile and lipase in fat digestion
- Observe and describe the effects of lactase on lactose
- Test the effects of temperature and pH on enzyme activity
- Describe how each compartment of the digestive tract must have a particular environment for optimal enzyme function

Background

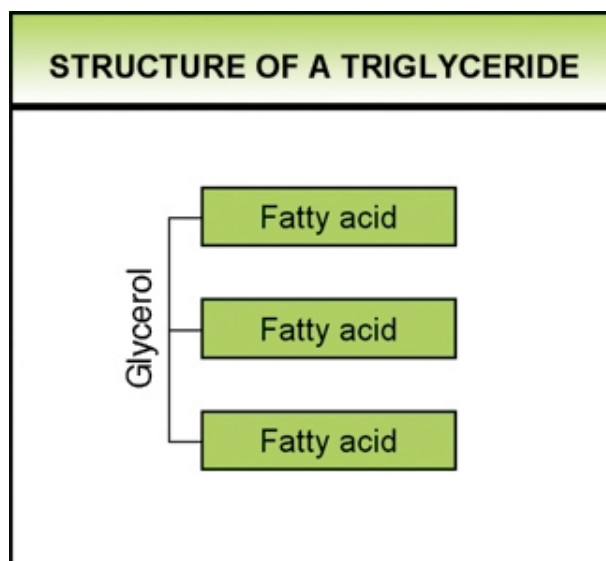
Enzymes are a type of protein a biological macro-molecule composed of amino acids, Enzymes make chemical reactions occur much easier and faster. They usually break or make bonds, subsequently breaking apart larger molecules into smaller molecules OR assembling the pieces of larger molecules. In the digestive system, enzymes break the large macromolecules found in food down to their monomer form or smaller pieces making them more easily absorbed into the blood or lymph.

The enzymes of the digestive system are generally names for the substance they break down. For example the main categories of enzymes include proteases to break down proteins, lipases to break down lipids, and amylases to break down starches known as amylose. Within these general categories, there are enzymes with more specific jobs (i.e. lactase breaks down lactose).

In this experiment, you will be testing the performance of two enzymes: pancreatic lipase and lactase. You will be preparing eight test tubes: 5 for experimenting with pancreatic lipase and 4 for experimenting with lactase. Think about which test tube(s) is/are serving as the control(s) as you set up the experiment. Keep in mind that the different parts of your body (and the different compartment of the digestive tract) have specific environments and enzymes are designed to work best in one type of environment.

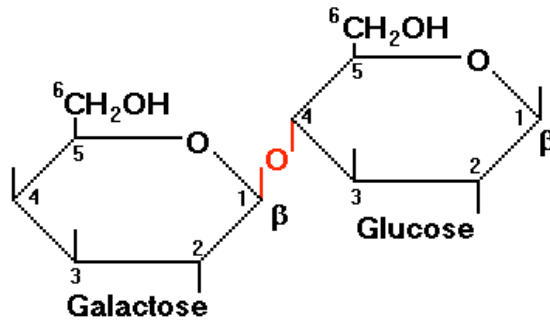
Pancreatic Lipase

Pancreatic lipase is made by the pancreas and breaks down triglycerides into glycerol and free fatty acids. To observe the effects of pancreatic lipase, you will be testing the performance of the enzyme under a variety of environmental conditions. To visualize the changes caused by digestion of triglycerides, the “pink” cream has a pH indicator added to it. When the color is pink, the pH is basic. As the free fatty acids are released through digestion and the activity of pancreatic lipase, the color will become orange then yellow as the pH becomes more acidic.



Lactase

Lactase is the enzyme that breaks lactose (a disaccharide) into glucose and galactose. To observe the action of lactase, we will test for the presence of glucose in milk after treating it with lactase enzyme. For one reaction, the lactase enzyme was boiled BEFORE it is added to the milk. This demonstrates denaturing of an enzyme.



Materials

Materials shared with the class:

37°C water bath
70°C water bath
ice bucket

Materials for each group (or shared with one other group):

China marker , Green pipette pump
3 X 10mL glass pipettes
Pink Cream (with NaOH added) in flask
White Cream in flask
sucrose solution
8 test tubes in holder
Lactase enzyme
Boiled lactase enzyme
1% paeceatin
1% bile salts
Glucose test strips (4)

Name _____

Procedure

1. Label the tubes 1-9.
2. Add a mark to the tubes so that you can remember which tubes are yours when mixed in with the rest of the class.
3. The pink cream should be pink because phenol red (a pH indicator) was added to it. Phenol red turns a pinkish color at high/basic pH and an orange color at lower/acidic pH. Your tubes should all be the same color – a fuchsia or magenta color representing a basic pH at the start.
4. As you fill the tubes, use the table below to help keep track of everything.
5. Fill each of test tubes 1-5 with 2mL of “pink” cream using the green pipette pump and a 10mL glass pipette.
6. Fill each of test tubes 6-8 with 2mL “white” cream using the green pipette pump and a NEW 10mL glass pipette.
7. Fill test tube 9 with 2mL sucrose solution.
8. Add nothing else to test tube 1 and 6.
9. Add ~8 drops of pancreatin solution (a mixture of pancreatic enzymes including pancreatic lipase) to test tubes 2-5.
10. Add ~8 drops of bile to test tube 3.
11. Add ~8 drops of lactase enzyme to test tube 7 and 9.
12. Add ~8 drops of boiled lactase enzyme to test tube 8.
13. Swirl all the test tubes gently to mix.
14. Place test tubes 1, 2, 3 and 6, 7, 8, 9 in the 37°C water bath.
15. Place test tube 4 in the 70°C water bath.
16. Place test tube 5 in the ice at 0°C.
17. After about 40 minutes (not too much more), collect all your test tubes.
18. Observe the tubes 1-5 for color changes (use test tube 1 for comparison). Record your results in the table on your assignment.
19. Test tubes 6, 7, 8, and 9 for glucose by dipping the glucose test strips in (pink means no glucose, purple means glucose is present). Record your results in the table on your assignment.
20. Clean all test tubes with test tube cleaners and acetone, return all materials to storage, and clean desktop.
21. Complete the remaining questions on the assignment.

Table of Tube Contents

Place a mark in the white boxes as you fill the appropriate tube with that component. A black box means you DO NOT add that component.

Tube	Pink Cream	White Cream	Sucrose solution	Pancreatin	Bile	Lactase	Boiled lactase	Temperature
1								37°C
2								37°C
3								37°C
4								70°C
5								0°C
6								37°C
7								37°C
8								37°C
9								37°C

Name _____

Lab Assignment – Digestive Enzymes

Data:

Tube	Color Before	Color After	Glucose present after?
1			N/A
2			N/A
3			N/A
4			N/A
5			N/A
6	N/A	N/A	
7	N/A	N/A	
8	N/A	N/A	
9	N/A	N/A	

1. Which test tube(s) is/are the controls?
2. WHEN YOU FIRST placed test tubes 1-5 into the different temperatures (at time = 0), which test tubes best corresponded to a basic pH (remember the pink color represent something) **and body temperature** (as found in the small intestine where pancreatic lipase works)?
3. What do the changes in colors in test tubes #1-5 represent chemically?
4. Which test tube of #1-5 demonstrated the strongest reaction? Why?
5. What is the scientific reason for either adding bile salts or omitting them as in test tubes #2 and #3?
6. The temperature in test tubes 4 and 5 should have been too extreme for the enzyme to work. Propose a reason why it might be possible to get a slight color change in these test tubes anyway. Hint: review your procedure for this activity for potential sources of experimental error.

Name _____

7. Which of tubes #6-9 showed the strongest reaction? Why?

8. Why **might** there be some reaction in test tube #6? Even if you didn't get a reaction in test tube #6, think about why there could be a reaction in test tube #6.

9. What happened to the tube that had boiled enzyme added (#8)? Why?

10. What happened to the tube that had sucrose solution (#9)? Why?

11. What would happen if we added lactase to a solution of maltose?

11. Describe how a peanut butter and jelly sandwich on whole wheat bread is processed by your digestive system beginning with sight of the sandwich and ending with elimination. Be **organized and thorough** in your explanation. Note: a peanut butter & jelly sandwich has carbohydrates, lipids, proteins, vitamins, minerals, and water - that means you need to discuss how all those nutrients are handled through the digestive tract!