Digestive Enzymes

Background
All living organisms must break down foods into its most simple forms in order to absorb it from the digestive tract (small intestine). From there, most nutrients are used to produce ATP. This is accomplished in humans via various organs and digestive enzymes throughout the digestive tract. In this lab, we will examine some of the major digestive enzymes and their effects under various conditions that simulate the internal environment of the digestive system.

Digestion begins in the mouth. Here, the salivary glands secrete a liquid substance that contains various elements including water, mucin (a protein) and the enzyme amylase. Amylase works to begin the breakdown of polysaccharides to disaccharides.

Protein digestion begins in the stomach where the enzyme pepsin breaks down polypeptide chains into shorter chains of amino acids. Pepsinogen is released by the chief cells in the gastric pits (mini-exocrine glands) and is converted to its active form, pepsin, in the presence of HCl.

Bile salts are not enzymes, but aid in the digestion of lipids. They work as an emulsifying agent which will make fat droplets smaller (greater surface area) so that the enzyme lipase can be more efficient. Lipase will not work well in an acidic pH like the stomach environment. To create the optimal environment for lipase, the pancreas and liver will also secrete sodium bicarbonate to create an environment in the small intestine with a pH around 7.8.

Exercise 1: Salivary Digestion of Carbohydrates
Aim: To test the action of salivary amylase on starch (a polysaccharide).

Procedure
1 Collect 10 ml of saliva in a test tube (how else did you think we were going to get amylase 😊).
2 Prepare 5 labeled test tubes with the following contents:
   a. Tube 1: 3ml starch + 3ml distilled H₂O @ 37°C
   b. Tube 2: 3ml starch + 3ml saliva @ 37°C
   c. Tube 3: 3ml pre-cooled starch + 3 ml pre-cooled saliva @ 4°C (ice bath)
   d. Tube 4: 3ml starch + 3ml saliva + 5 drops conc. HCL @ 37°C
   e. Tube 5: 3ml starch + 3ml distilled H₂O + 5 drops conc. HCL @ 37°C
3 Incubate the tubes for 1 hour at the designated temperature.
4 While these tubes are incubating, conduct this simple test:
   Place a small drop of saliva on a spot plate and add a few drops of 1% acetic acid. If enough mucin (a protein) is present, a precipitate will form.
5 After the incubation is over, pour ½ of each tube into a new labeled test tube. You will test ½ for maltose and the other ½ for glucose and starch (see directions below).

Thought Questions
1. In which test tube did amylase work best to break down starch? Is this what you would expect? Why or why not?
2. What action does amylase have on carbohydrates?

Exercise 2: Digestion of Proteins
Aim: To illustrate the environmental conditions necessary for the optimal activity of pepsin.

Procedure
1 Your instructor will have cut uniform pieces of egg whites. The egg whites are high in a protein called albumin. Note the appearance of each piece of egg as you place one piece in 5 numbered test tubes.
2 Add the following to the test tubes and take the pH of each of the initial solutions:
   a. Tube 1: 5ml pepsin (5% solution) + 5 ml HCL (0.5% solution)
   b. Tube 2: 5ml pepsin + 5 ml distilled H₂O
   c. Tube 3: 5ml HCL + 5ml distilled H₂O
   d. Tube 4: 5ml pepsin + 5ml NaOH (0.25% solution)
   e. Tube 5: 5ml distilled H₂O + 5ml NaOH
3 Incubate all test tubes in a 37°C water bath for 1 hour.
4 Test the final pH of the solutions after incubation.
5 Estimate the about of egg that was broken down using the scale (+++) completely gone, (++) moderately, (+) slightly digested, (-) no change in color.
Thought Questions
1. In which test tube did pepsin work best to break down albumin? Is this what you would expect? Why or why not?

2. What action does pepsin have on proteins?

Exercise 3: Digestion of Fat with Pancreatic Lipase and Bile Salts
Aim: To illustrate how environmental conditions affect pancreatin (a form of lipase) activity.

Procedure
1. In the 37°C water bath in the back of the room, there is a beaker with blue colored litmus cream and a beaker with 1% pancreatin solution. Use these to prepare the following 4 test tubes:
   a. Tube 1: 3ml cream + 3 ml pancreatin
   b. Tube 2: 3ml cream + 3ml distilled H₂O
   c. Tube 3: 3ml cream + 3ml pancreatin + pinch bile salts
   d. Tube 4: 3ml cream + 3ml distilled H₂O + pinch bile salts

2. Put these tubes in the 37°C water bath, and check them every 2-3 minutes for the first 15 minutes, and then every 5 minutes for a total of 60 minutes. Note color and odor (blue = basic, red = acidic).

3. While these 4 tubes are incubating, label 2 more test tubes A and B. Place 3ml of distilled water + 3ml of vegetable oil in each test tube. To test tube B, add a small pinch of bile salts. Then, shake each tube vigorously (with thumb over the top) for 30 seconds. Set the test tubes in a rack, and make observations for several minutes.

Thought Questions
1. In which test tube did pancreatin work best to break down fat? Is this what you would expect? Why or why not?

2. What action do bile salts have on fats?