Digestive Enzymes Demo

Introduction

People must eat to live but how does the body transform food into the essential nutrients (peptides, amino acids, fatty acids, and glucose) needed to carry out cell processes and cell growth? This demonstration introduces the biochemistry of digestion.

Concepts

- Catalysts
- Digestion
- Enzymes
- Gastrointestinal tract

Background

The human body is composed of millions of cells that need oxygen, water, and nutrients to survive. The amazing transformation of food into simpler molecules that can be absorbed for use by the cells is called *digestion*. Digestion occurs in the *gastrointestinal (GI) tract*. The GI tract is a mucous membrane-lined tube that extends from the mouth to the anus. While in the GI tract, food is first mechanically broken down and then chemically treated with acids, bases, and enzymes within the organs of the digestive system. Enzymes are biochemical catalysts. A catalyst is any substance that causes a chemical reaction to occur without being permanently altered in the process. A single molecule of a catalyst can perform the same reaction thousands of times in a single second. Enzymes are globular, three-dimensional proteins with characteristic shapes that allow only a few specific substances to temporarily bond with the enzyme. Because of the exclusive nature of enzyme/substrate binding, the human body contains thousands of different enzymes that are needed to catalyze all the different biochemical reactions that must occur.

Digestion begins in the mouth. The food mixes with saliva while the teeth grind the food. Saliva provides the first chemical treatment of the food. Saliva is composed of a neutral pH mixture of water, mucus, proteins, mineral salts, and the enzyme amylase. *Amylase* begins the breakdown of starch, a carbohydrate, into glucose (see Figure 1). Glucose is the sugar used during cellular respiration as a source of cellular energy.

In the stomach gastric juices containing mucus, hydrochloric acid, pepsinogen, and small amounts of other enzymes continue the process of digestion. Hydrochloric acid acts to denature (uncoil) the proteins in food and activates pepsinogen, the inactive precursor of the enzyme *pepsin*. Glucose, alcohol, fat-soluble drugs, some salts, and small amounts of water are absorbed through the walls of the stomach directly into the bloodstream for transport to the liver, where they are metabolized or sent on to other cells in the body.

Once in the small intestine, the remaining food combines with enzymes from the pancreas and epithelial cells of the small intestine and with bile salts from the liver. The digestion of carbohydrates into glucose and other simple sugars is completed in the small intestine by the enzymes sucrase, maltase, lactase, and pancreatic amylase. The resulting sugars are absorbed through the mucous lining of the small intestine into the bloodstream for transport to the liver.

The partially digested proteins from the stomach are still too large to be absorbed through the small intestine. Pancreatic juice contains three peptidases that complete the digestion of protein into amino acids for absorption into the bloodstream. Each peptidase in the pancreatic juice is very specific and splits the bonds only between particular combinations of amino acids. Nucleases found in pancreatic juice convert the nucleic acids found in the food into nucleotides, which are also

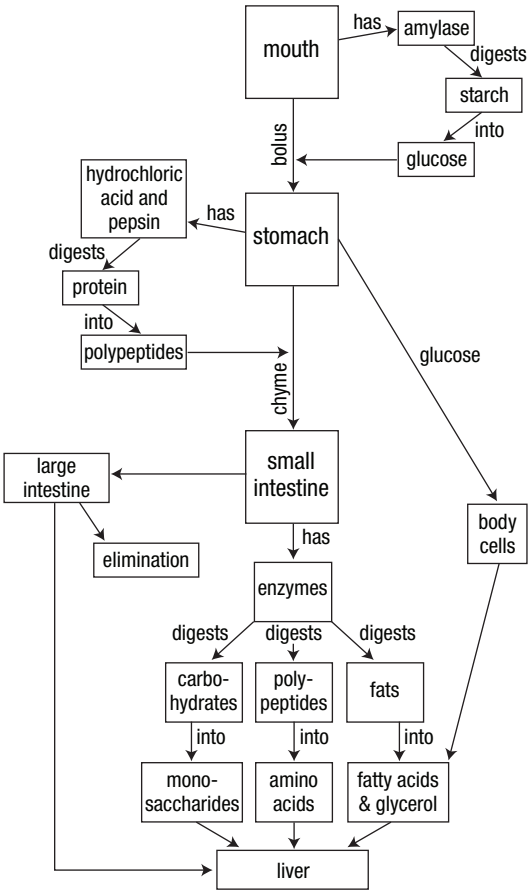

Figure 1.

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absorbed and transported to the liver.

Fats (lipids) are hydrolyzed into fatty acids and glycerol by intestinal and pancreatic lipase with help from bile salts secreted by the liver. Hepatic cells of the liver produce bile, which is stored in the gall bladder before being excreted into the small intestine. Bile salts help with the digestion of fat globules by acting like soap. The globules of fat are small clumps of lipids that stick together during digestion. Bile salts break the globules into smaller drops, creating greater surface area for pancreatic lipase to break the lipids into fatty acids and glycerol which are also transported to the liver. The material remaining in the small intestine travels to the large intestine where more mucous is added and where water and electrolytes are absorbed before the "waste" is expelled from the body.

Materials (for each demonstration)

Albumin, 1 g
Amylase, 1 g
Biuret test solution, 20 mL
Hydrochloric acid solution, 0.01 M, 50 mL
Iodine solution, I_2/KI, 1 mL
Pepsin, 0.5 g
Starch solution, 0.5%, 100 mL
Water, deionized or distilled
Graduated cylinders, 100-mL, 3
Marker
Plastic cups, clear, 16-oz, 7
Stirring rods

Safety Precautions

Biuret test solution contains copper(II) sulfate and sodium hydroxide and is a corrosive liquid. It is moderately toxic by ingestion and is dangerous to skin and eyes. Hydrochloric acid solution is an eye and skin irritant. Iodine solution contains iodine and potassium iodide and is an eye and skin irritant; it will stain skin and clothing. Avoid contact of all chemicals with eyes and skin. Wear chemical splash goggles and chemical-resistant gloves and apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Prepare the following solutions up to five days in advance of the lab.

- Use 100 mL of DI water to prepare a 1% albumin (protein) solution. Add 100 mL of the DI water to 1 g of albumin. Gently mix and refrigerate. *Note:* Either egg or bovine albumin will provide accurate results however, bovine albumin is easier to dissolve in water.
- The starch solution may either be purchased (Flinn Catalog No. S0151) or prepared. To prepare, boil 100 mL of DI water. Add a small amount of the boiling DI water to 0.5 g of starch. Mix well, forming a paste. Continue to add 10 mL of boiling water to the bottle until the entire 100 mL of boiling water has been added. Allow the solution to slowly cool to room temperature or refrigerate.

Prepare the following solution the day of the lab.

- Prepare a 1% pepsin solution by adding the 50 mL of 0.01 M hydrochloric acid to 0.5 g of pepsin. Mix well. The solution should have a pH of 1.5 to 2.5.
- Use 100 mL of DI water to prepare 1% amylase solution. Add 100 mL DI water to 1 g of amylase. Mix well.

Procedure

Part A. Protein Digestion

1. Add 50 mL of the 1% albumin solution to each of two clear plastic cups.
2. Add 50 mL of DI water to one of the two plastic cups. Mix well.
3. Add 50 mL of the 1% pepsin solution to the second plastic cup. Mix well.
4. Wait 2 minutes before adding 10 mL of biuret test solution to each cup. Mix well.

5. Observe the color and appearance of the resulting solution in each cup and record the observations in the data table on the Digestive Enzyme Demo Worksheet. *Note:* Biuret test solution is bluish-purple in the presence of polypeptides and lavender pink in the presence of amino acids.
6. Answer questions 1 and 2 on the Digestive Enzyme Worksheet.

Part B. Carbohydrate Digestion

7. Add 50 mL of the 1% starch solution to each of two clear plastic cups.
8. Add about 5 drops of iodine to each of the two clear plastic cups.
9. Add 50 mL of DI water to one of the two plastic cups. Mix well.
10. Add 50 mL of the 1% amylase solution to the second plastic cup. Mix well.
11. Observe the color and appearance of the resulting solution in each cup and record the observations in the data table on the Digestive Enzyme Demo Worksheet. *Note:* Dark black color indicating a positive starch test will fade in the cup containing the amylase solution as the enzyme digests the starch into sugars.
12. Answer questions 3 and 4 on the Digestive Enzyme Demo Worksheet.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Excess biuret test solution may be neutralized with acid according to Flinn Suggested Disposal Method #10. Excess hydrochloric acid may be neutralized with base according to Flinn Suggested Disposal Method #24b. Excess iodine solution may be reduced with sodium thiosulfate solution according to Flinn Suggested Disposal Method #12a.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evolution and equilibrium
Form and function
Content Standards: Grades 5–8
Content Standard A: Science as Inquiry
Content Standard C: Life Science, structure and function in living
Content Standard F: Science in Personal and Social Perspectives, personal health
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard C: Life Science, matter, energy, and organization in living systems
Content Standard F: Science in Personal and Social Perspectives, personal and community health

Tips

- Biuret test solution does not contain the compound biuret. Biuret is the simplest compound that gives a positive test result with biuret test solution.
- Catalysts cause slow reactions to occur more quickly by lowering the activation energy necessary for the reaction to occur. A ski lift is an analogy for a catalyst. If the reaction is "skiing," then the skier must first get to the top of the ski hill. One option is for skiers to climb to the top and once they reach the top, enjoy the potential energy they earned as they ski back down the hill. The ski lift allows many more skiers to reach the top of the hill very quickly without the skiers expending much energy. Once at the top, they still enjoy the same energy release as they ski down the ski hill. The reaction can occur many, many more times and the ski lift (the catalyst) is not changed during the process.
- For an advanced class you may want to use this activity to introduce the terms substrate, active site, cofactors, coenzymes, and the induced fit theory.

- In order to help students understand that amylase is found in saliva, have each student chew on an unsalted, unsweetened saltine type cracker until the cracker tastes sweet. The sweetness is due to the amylase hydrolyzing the starch into glucose and other mono- and disaccharides. Complete this activity in a food-appropriate area.

Sample Data Table *(Student data will vary.)*

Cup Contents	Observations
Protein, water, and biuret	*Bluish-purple, cloudy*
Protein, pepsin, and biuret	*Pinkish-purple, clear*
Starch, water, and iodine	*Dark blue-black*
Starch, amylase, and iodine	*Brown*

Answers to Questions *(Student answers will vary.)*

1. Compare and contrast the observations of the biuret test results. Describe the evidence, if any, for the digestion of protein using pepsin.

   The cup containing protein solution, water and biuret test solution is the negative control. The solution is a cloudy, blue-purple color indicating this cup is positive for polypeptides. The cup containing protein solution, pepsin and biuret is a clear, pink-purple color. Pepsin digests the albumin protein leaving peptides which gives a pink-purple biuret test.

2. The pepsin solution was prepared using 0.01 M hydrochloric acid in order to optimize the pepsin enzyme. Why was this necessary?

   Pepsin is active in the acidic environment of the stomach. A basic or neutral pH would inactivate the enzyme.

3. Compare and contrast the iodine test results for starch and starch/amylase. Explain the test results based on the activity of amylase.

   The cup containing the starch, water, and iodine solution is the control sample yielding a positive iodine test for starch. The remaining cup contains starch, amylase, and iodine. It has a negative iodine test because the amylase has hydrolyzed the starch to glucose.

4. Summarize the digestion of a steak and baked potato dinner. Indicate the enzymes responsible for digestion in the mouth, stomach, and small intestine for the protein and starch components of the meal.

   Amylase in the mouth starts digesting the starch of the baked potato. Pepsin and acid begin digesting the steak proteins in the stomach. The carbohydrates (starch) leave the stomach first and are digested into simple sugars in the small intestine by sucrase, maltase, lactase, and pancreatic amylase. The protein digestion of the steak continues in the small intestine when the three peptidases finish splitting the protein into amino acids for absorption into the bloodstream. Nucleases in the small intestine convert nucleic acids into nucleotides. Finally, fats are hydrolyzed into fatty acids and glycerol by lipases in the small intestine.

Materials for *Digestive Enzymes Demo* are available from Flinn Scientific, Inc.

Catalog No.	Description
FB1862	Digestive Enzymes at Work—Student Laboratory Kit
A0300	Bovine Serum Albumin, 10 g
A0283	Amylase, 10 g
B0051	Biuret Test Solution, 500 mL
H0014	Hydrochloric Acid Solution, 0.1 M, 500 mL
I0038	Iodine–Potassium Iodide Solution, 100 mL
P0006	Pepsin, 25 g
S0151	Starch Solution, 0.5%, 500 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Name: _____

Digestive Enzyme Demo Worksheet

Data Table

Cup Contents	Observations
Protein, water, and biuret	
Protein, pepsin, and biuret	
Starch, water, and iodine	
Starch, amylase, and iodine	

Questions

1. Compare and contrast the observations of the biuret test results. Describe the evidence, if any, for the digestion of protein using pepsin.

2. The pepsin solution was prepared using 0.01 M hydrochloric acid in order to optimize the pepsin enzyme. Why was this necessary?

3. Compare and contrast the iodine test results for starch and starch/amylase. Explain the test results based on the activity of amylase.

4. Summarize the digestion of a steak and baked potato dinner. Indicate the enzymes responsible for digestion in the mouth, stomach, and small intestine for the protein and starch components of the meal.