Teacher Activity
Yams, Soybeans, and Pharmaceuticals: Starch Digestion by Amylase
Companion Web page:
http://www.chemheritage.org/classroom/chemach/pharmaceuticals/index.html

Lesson Concepts

Analysis in chemistry involves many tools for identifying the results of a chemical reaction. The methods for identifying particular chemicals not involved in a chemical reaction can be the same. The group of chemicals of interest in this lab is representative of some important organic chemicals associated with living systems. In addition, the study of the chemical reaction in this lab involves catalysis and the dimensions of such a system where the catalyst is another organic compound, an enzyme. The rate of the reaction being studied is influenced by temperature and concentration of reactants, among other parameters.

Pre-Lab Procedures

1. Students need to practice generating saliva; chewing on a clean rubber band is the best stimulus for producing the saliva to be collected in the individual tubes. If there is a negative reaction by students, amylase powder can be substituted. In this case prepare a fresh solution of amylase according to the supplier's instructions.
2. Depending on the background of the students, some basics on molecular structure of starch and the breakdown products of maltose and glucose would be helpful. Provide visuals of the hydrolysis of starch.
3. To save time students could prepare their reaction test tubes by marking off the different levels for fluids: 1 cm, 2 cm, and numerical sequence per tube.

Materials required per group are

- Lugol's solution in dropper bottles (dissolve 10.0 g KI and 5.0 g I_2 in 100 mL of distilled water; this is a stock solution; dilute in a 1:10 ratio, stock to water)
- Benedict's solution (commercial)
- 5 test tubes (18 x 150 mm) per group
- Marking pencil or pen
- Metric ruler
- Distilled water
- 2% starch solution (either from powder or add spray starch to water)
- Saliva (from students); an alternate is commercial amylase powder from science-education supply companies
- Hot-water bath arrangement (preferable to use hot plates with large beakers of hot water at various lab bench stations rather than individual setups)

Procedure

1. Label five test tubes #1 through #5, and mark each tube at 1 cm and 2 cm from the base.

2. Fill test tubes #1 through #4 with your saliva to the 1-cm mark. Fill #5 to the 1-cm mark with distilled water.

Classroom activities are provided through the generous support of the Biogen Idec Foundation.

3. Add starch solution to #1 to the 2-cm mark and conduct the iodine test IMMEDIATELY.

4. Add starch solution to #2 to the 2-cm mark and conduct the Benedict's test IMMEDIATELY.

5. Add starch solution to #3 through #5 to the 2-cm mark and allow the tubes to sit for 30 minutes.

6. After 30 minutes conduct the iodine test on #3 and the Benedict's test on #4 and #5.

Data:

Tube	Test Conducted	Timing of Test	Results of Test
1.			
2.			
3.			
4.			
5.			

Post-Lab Procedures

1. Have students post their data to compare results with other teams.

2. Implications from the results of doing the Benedict's test at the beginning and later: What if there is a positive test for sugar in test tube #2 at the beginning of the experiment? Would you expect this? (***No***) If it does happen, how would you explain a positive result? (***Partial hydrolysis of the starch solution before adding to the tube or some hydrolysis in the tube, depending on how long students waited after combining starch and saliva)***. Implications of doing the iodine test at the beginning and later? What would students expect to happen? ***Since the iodine test is done to test for the presence of starch, doing the test at the beginning should show a positive test. Doing the test later may show less starch present or none at all and should complement the Benedict's test for sugars that are produced from the digestion of the starch by the amylase enzyme in the saliva.***

Use the post-lab questions in the student version of this lab for discussion before their lab write-up.

3. Ask students what they predict would happen if they repeated the experiment but changed the temperature (higher and lower than lab temperature) and tested for reaction products every minute for up to 5 minutes.

Student Skills Required

Before carrying out the activity, students should

- be able to accurately measure reactants used in the activity and skillfully manipulate basic lab equipment;

Classroom activities are provided through the generous support of the Biogen Idec Foundation.

- have a conceptual understanding of basic molecular structure and bonding in organic molecules;
- be familiar with metric units of measurement; and
- have a conceptual and mathematical understanding of Dalton's law of partial pressures.

Student Misconceptions

1. "The term *organic* means *natural*." Historically, organic substances were thought to be those that could only be produced by living substances. The synthesis of urea by Friedrich Wöhler in 1818 forced a change in the definition to mean any compound that contains carbon-carbon or carbon-hydrogen bonds. Many organic compounds are synthesized outside living systems and in reaction vessels.
2. "An enzyme is part of the reaction and disappears into the formation of product." An enzyme is a catalyst, which means it accelerates the rate of a reaction but is the same before and after a reaction. However, it can undergo changes during the reaction that involve breaking and forming bonds in the enzyme as well as in the substrate. As with other catalysts, an enzyme forms an enzyme-substrate complex, equivalent to the activated complex formed by most chemical catalysts.
3. "High temperatures (higher than room temperature) are needed for chemical reactions to occur. Chemical mixtures must always be heated to cause the components to react." For reactions to occur, a minimum activation energy must be present. Depending on the physical state of one or more reactants, the reaction can occur at temperatures below room temperature as well as above room temperature. Increasing the temperature of a chemical system will increase the rate of the reaction.
4. "A polymer is a plastic substance—'not natural.'" A polymer is any molecule with a large molecular weight. Many polymers occur in nature and are therefore "natural." Polymers include such things as human hair, protein of various types, spider "silk," cotton, cellulose, and starch. Some polymers are synthesized without biological systems, producing such things as nylon, rubber, and rayon.

Answers to Pre-Lab Questions

1. Starch is a food substance. What are some of its sources? Starch is produced by plants as a storage form of glucose produced in photosynthesis. Starch can be found in the leaves and roots of plants. Tuberous plants have large quantities in their tubers: for example, potatoes (stem tuber), yams, sweet potatoes, and cassava (root tubers) all contain starch.
2. The reaction mechanism to be used in your lab exercise is called *hydrolysis*. What does the word mean in chemical terms? *Hydrolysis* means to "cut" (lysis) with water. Water molecules chemically join where individual monomers of a polymer like starch are bonded to each other. Where the bond is broken, a hydrogen from a water molecule is added to one of the products that split off, and an –OH is added to the other product of the lysis.
3. How are the terms *enzyme* and *catalyst* related? The term *catalyst* refers to any substance that accelerates the rate of a chemical reaction without undergoing a permanent chemical change itself. Enzymes are proteins that catalyze reactions in biological systems. Enzymes are organic compounds. There are also inorganic catalysts, but they are not enzymes.

Classroom activities are provided through the generous support of the Biogen Idec Foundation.

4. Starch and sugar are organic molecules. What is common to all organic molecules? Organic molecules contain carbon-carbon bonds and/or carbon-hydrogen bonds.
5. Starch is considered to be a polymer, and a sugar such as glucose is a monomer. What are the meanings of these two terms, *polymer* and *monomer*? *Polymer* means many “mers” or parts. A polymer is a compound of very high molecular weight, built up from a large number of simple molecules or monomers. *Monomer* means one part or unit.

Answers to Post-Lab Questions

1. “What was the purpose of using two tests, iodine and Benedict’s. What is each test used for?” The starting substance, starch (the substrate), is detected with the iodine test. If the starch were hydrolyzed into monomers of glucose, its presence would be detected with the Benedict’s test.
2. “Why were the tests performed immediately and after 30 minutes?” The immediate test was to ensure that the original starch was not converted to glucose at the start, when adding the enzyme amylase in the saliva. “How did they serve as indicators of successful digestion by the enzyme?” If the starch were digested (hydrolyzed), then the reaction product of glucose would be detected through a positive Benedict’s test and a negative test for starch using iodine.
3. “From what structure in your mouth did you obtain the salivary amylase?” Amylase is produced in the saliva glands scattered in your mouth. “Where else in your body could you find amylase?” Amylase is also produced in the pancreas and secreted into the digestive tract at the small intestine.
4. “Why do you think that you need two sources of amylase for successful digestion of starch?” It is possible that not all the starch in food is converted to glucose and maltose in the mouth before it moves on to the stomach and intestinal area. Therefore, amylase produced by the pancreas will be added to the food as it passes into the small intestine for “digestion” of remaining starch to glucose.
5. “What specific disaccharide is formed when amylase works on starch?” Maltose. What additional step in digestion needs to occur to fully digest starch into glucose monomers? Another enzymatic hydrolysis must occur to convert the disaccharide maltose into glucose units.
6. “What is the fate of the glucose monomers digested from the starch?” Glucose molecules are now small enough to pass through the cell membrane. Then they are oxidized in the mitochondria in a chemical process known as respiration, by which the potential energy associated with the bonds in glucose can be incorporated into the potential energy bonds of a molecule called adenosine triphosphate, or ATP.

Assessment

1. Redo the experiment but substitute a different enzyme—catalase. This enzyme is available in powder form. A reaction is carried out in which hydrogen peroxide, the substrate, is catalyzed to produce oxygen gas and water. The rate of the reaction is measured based on the amount of oxygen gas produced per unit of time (see references under “Resources” below for lab setup). Students would investigate the effect of temperature, pH, and concentration of both the substrate and the enzyme itself. A good reference for doing this lab exercise is found at http://www.accessexcellence.org/AE/ATG/data/released/0074-GenNelson/index.php.

Classroom activities are provided through the generous support of the Biogen Idec Foundation.

2. Many animals are incapable of digesting cellulose (i.e., converting the polysaccharide to glucose) unless they possess certain bacteria and protozoans that are able to produce an enzyme, *cellulase*, which catalyzes the breakdown of the cellulose. Students could do a study on the implications of providing humans with these bacteria or the enzyme in order to expand their diet to include cellulose-containing foods. They could also research the present interest in using cellulotic sources for producing ethanol and methanol as fuel alternatives to gasoline.

Extension Activities

1. Students could research the role of vitamins and minerals as coenzymes in biochemical reactions.
2. Students could research the role of enzyme inhibitors that include heavy metals, bacterial toxins, and certain poisons (organophosphates in insecticides, cyanide, arsenic).
3. Students could do research on the lives of the three most prominent chemists involved in the development of the steroidal compounds synthesized from extracts of the Mexican yam tubers. These chemists are Percy Julian, Carl Djerassi, and Russell Marker (a starting point would be the Chemical Heritage Foundation publication, *Chemical Achievers*, found online at http://www.chemheritage.org/classroom/chemach/pharmaceuticals/julian-djerassi.html; other biographical references on Djerassi are found at http://www.stanfordalumni.org/news/magazine/1998/novdec/articles/djerassi.html and http://www.djerassi.com/

Additional Teacher Notes

1. Polysaccharides are composed of many monosaccharide units connected by what are known as *glycosidic* linkages. The polymer starch consists entirely of D-glucose units. Through enzymatically influenced hydrolysis with amylase, these glycosidic bonds are broken, producing various fragments, including glucose and maltose. The enzyme maltase hydrolyzes the maltose to glucose. One of the tests used in this lab for detecting the presence of a sugar—in this case, glucose—uses Benedict's solution. This test is specific for only one general type of sugar, that of a reducing sugar, of which glucose is an example (along with fructose). Sucrose (common table sugar, a disaccharide made up of glucose and fructose) is a non-reducing sugar and will not give a positive test when using Benedict's solution.

2. Although this lab has focused on experimenting with the chemical action of an enzyme, the substrate starch is a plant material, a polysaccharide. One category of plant, Dioscoreaceae, or the yam family, possesses tubers in which starch and other chemical products are stored. The following provides some botanical, biochemical, and historical information about the importance of the nonstarch chemicals in the tubers that have been used to synthesize a number of very important corticosteroids, substances that are the same in structure and function to animal corticosteroids and are produced in the adrenal gland, among other locations, in humans. The ability to synthesize these important pharmaceutical substances means availability, assuming that an inexpensive synthesis route can be found.

References

1. This reference provides interesting botanical and biochemical background to the yam family. It also discusses the role of the Mexican yam in providing starter chemicals to synthesize steroidal chemicals, particularly the sex hormones and cortisone (see summary

Classroom activities are provided through the generous support of the Biogen Idec Foundation.

under “Teacher Notes”) (http://www.botgard.ucla.edu/html/botanytextbooks/economicbotany/Dioscoreamed/).

2. The basics of enzyme catalysis and an alternate quantitative lab exercise that uses the enzyme catalase (found in both plants and animals) can be found in *ChemSource* (http://intro.chem.okstate.edu/ChemSource/Enzymes/enzymetableofcont.html).
3. At this teacher’s site you can access a catalase lab that is highly quantitative and easy to manage by students with good results (http://www.jdenuno.com/APBiology/APBIO.htm).
4. Another source of ideas for an enzyme lab is found at http://www.accessexcellence.org/AE/ATG/data/released/0167-JudyBrown/index.php

Classroom activities are provided through the generous support of the Biogen Idec Foundation.