Santa Cruz-Watsonville Inquiry-Based Learning in Environmental Sciences
An NSF GK-12 Project

Spit Lab

Macromolecules & environmental effects on enzymes

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Field-tested with: 10th grade students in multiple Biology Courses, ESNR & BATA Academy
Watsonville High School, Watsonville, CA (Fall, 2010)

Concepts: Macromolecules and their components, enzyme action, anabolic and catabolic activity, environmental effects on enzymes

Skills: Performing an experiment; displaying information graphically; making graphs on a computer

Module Type: Two block periods (4 hours total)

Duration: 2 days

Key materials:

- Glucose test strips
- Iodine
- Pipettes
- Rice crackers
- Toothpicks
- Small scoops
- Mortar and pestles
- Hot bath (boiling capacity)
- Ice bath
- Deionized water
- 7 – 1.5 ml or 2 ml micro centrifuge tubes
- Tube rack to hold the tubes
- Permanent marker
- Cut-out worksheets (attached)

Science Education Standards:

National: A. Science As Inquiry; B. Physical Science; C. Life Science
California: Biology-Life Sciences: **1. b.** & **9. f.***

Overview: In this module students will do an activity and a lab. A cut-out activity illustrates the anabolic and catabolic functions of enzymes, enzyme specificity, and the individual components of macromolecules. Students then test the effects of temperature on enzymatic activity in spit.

This project is an opportunity for students to:

- Understand the individual components of macromolecules.
- Understand anabolic and catabolic enzyme activity.
- Understand enzyme specificity and how environmental variables like temperature affect enzyme activity.

Background for Teachers

Enzymatic activity is important in all biological reactions. Students should recognize the four types of macromolecules important in biology (proteins, lipids, carbohydrates, and nucleic acids) and the kinds of monomers that they are built from. Understanding that enzymes are proteins that catalyze all reactions in the body is critical to understand how the body works.

Science Education Standards Addressed

This module focuses on experimentation, chemical reactions, macromolecule identity, and environmental effects on enzyme function. The module addresses the following NSES & SCSCPS content standards:

NSES: A. Science As Inquiry; B. Physical Science; C. Life Science

SCSCPS

Biology-Life Sciences

1. The fundamental life processes of plants and animals depend on a variety of chemical reactions that occur in specialized areas of the organism's cells. As a basis for understanding this concept:

> 1. b. Students know enzymes are proteins that catalyze biochemical reactions without altering the reaction equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings.
>
> 9. f.* Students know the individual functions and sites of secretion of digestive enzymes (amylases, proteases, nucleases, lipases), stomach acid, and bile salts.

NSES (http://www.nap.edu/catalog/4962.html)

SCSCPS (http://www.cde.ca.gov/be/st/ss/documents/sciencestnd.pdf)

Common Student Misconceptions/Notes to Teachers

Students are often unclear about differences among lipids, carbohydrates, nucleic acids, and protein, because they have not had a chemistry class before taking 10th grade biology. Even quality lessons on the monomer components of these macromolecules leave students with lots of unfamiliar words with definitions that lack personal meaning. For example, words like polysaccharide, monosaccharide, glucose, fatty acid, glycerol, amino acid, and nucleic acid are difficult for the students to grasp and can present an obstacle to real understanding.

Project Description

In this module students are assumed to have already had instruction about macromolecules. This module picks up right after a macromolecule lesson to reinforce macromolecule vocabulary but then expands into the role of enzymes. After an introductory lecture on enzyme activity (included in this module), students do a cut-out activity and a laboratory experiment. The cut-out activity requires that students cut out individual monomers and build them into macromolecules using specific enzymes. Some cut-out enzymes are designated as catabolic enzymes, while others are anabolic enzymes. Students must build macromolecules using the correct anabolic enzyme and then destroy them using the correct catabolic enzyme. The laboratory exercise uses amylase in spit to convert the starch in a rice cracker into sugar. Students observe the reaction by testing for the presence of starch and sugar before and after spit contact with the cracker. To test for the effect of temperature on enzyme activity, students boil or cool their spit on ice before mixing it with the cracker. The results from the lab can be hand-written or typed up in a separate computer activity. The results can be graphed on the computer to add skill-building activities to the module.

Materials

For the cut-out activity (can be done in partners):

- Cut-out worksheets (attached)
- Glue and scissors

For the laboratory exercise (can be done in groups of 4):

Each group will require:

- 5 URS 1G urine glucose test kits with 100 strips each can be ordered at http://www.testyourselfathome.com/Urinalysis_Test_Strips.htm (URS 1 G Urine Glusose Test Kit-GlucoseCHECK – Urine reagent diagnostic test strips, urinalysis test strip for detection and screening of Glucose in urine. (FDA 510K#:K020175). These should cost $6.95 for a bottle of 100 strips.)
- Mortar and pestle (size and material doesn't matter)
- 2 crackers (crackers cannot have any sugar in them.) I use rice crackers. Sesmark brand brown rice thins works well:

- Small scoop (you can use your fingers)
- Seven toothpicks
- One 50-ml beaker with deionized or distilled water
- One 1-ml plastic squeeze pipette
- Seven 1.5-ml or 2-ml micro centrifuge tubes (ex. Fisher brand 05408129)
- Tube rack to hold the tubes
- Permanent marker
- Iodine dropper bottle

Preparation

The teacher should have already covered macromolecules and their individual components (monomers). Prior to conducting this module it is important to order the glucose test strips in advance and find the right crackers. You cannot use a cracker with sugar in it. Even crackers with no added sugar may still test positive for glucose. It is best to use the rice crackers that are specified above or just be sure to test a different type of cracker before hand. The introductory lecture on enzyme function requires at least 25 minutes of class time. The cut-out activity will take 30-40 minutes. Both of these activities could be done on the same day as the teacher's macromolecule lesson. The spit lab activity requires a full 2-hour block period.

Timeline

1. Lecture on enzyme function – 25 minutes

2. Macromolecule cut out activity – 30-40 minutes
   Instructions and recap of macromolecules (10 minutes)
   Students cut out shapes and glue them together using the anabolic enzyme cut outs – 25 min
   Students use the catabolic enzymes to break apart the macromolecules that they have just built – 5 min
   Recap – 5 min
   Students fill out the worksheet for homework

3. Spit Lab – full 2-hour block period
   Teacher sets up all the materials at each group station prior to class
   Group reading of the lab & instructions using power point – 25 min
   Demonstration of the "spit tube"
   Discussion of indicators, controls, and expectations
   Students get in groups and begin the lab – 75 min
   Discussion of the results- 15 min
   Optional computer lab/ homework – overnight or 30 min in computer lab

Procedure

1. Enzyme lecture - 20 minutes

   Please see attached Introductory Enzyme Lecture

   Introductory enzyme lecture
   Instructions and recap of macromolecules: Review the 4 major macromolecules. This leads to a discussion of what is responsible for the construction of these molecules.
   Introduce enzymes. Enzymes are proteins that build other molecules. Introduce the words **anabolic** and **catabolic**. Anabolic enzymes build new molecules and catabolic enzymes break macromolecules apart. Each enzyme acts on specific **substrates** or **monomers.** These **substrates** enter the **active site** of the enzyme where they fit like a **key in a lock**. The enzyme will **catalyze** a reaction between the substrates. The conversation can be framed in the context of eating, digestion, and macromolecule synthesis. For example, you consume formed macromolecules in your food, the body breaks them down during digestion using catabolic enzymes, and you use the resulting monomers to rebuild new macromolecules using anabolic enzymes.

2. Macromolecule cut out activity – 30-40 minutes

   See Attached: Cut-out activity worksheets (with instructions for students), Cut-outs

   Cut-out Activity: Students cut out shapes and glue them together using the anabolic enzyme cut-outs. It's important that the students use the anabolic enzymes by putting the individual monomers into the active site. Once in the active site, they glue the monomers together. Students do this for all three macromolecules (we have left out nucleic acids for simplicity and since most

biology classes cover this separately).

Students use the catabolic enzymes to break apart the macromolecules that they have just built.

Recap – 5 min: Clarify what you would like them to turn in (the worksheet). Students fill out the worksheet for homework

3. Spit Lab – full 2 hour block period

See Attached: Spit Lab (with instructions for students), Spit Lab introductory lecture

Teacher sets up all the materials at each group's station prior to class. Each station should have all of the supplies listed under "supplies" in the Spit Lab document. See the supply list at the beginning of this module for specific instructions for teachers. Glucose test strips and crackers must be purchased beforehand. One package of crackers per class.

Group reading of the lab & instructions – 25 min:
Have the class read over the lab in whichever method you prefer (popcorn style etc.). You can use the **Spit Lab introduction lecture** during this discussion.

Discuss the indicators used in this lab (iodine and glucose test strips). When drops of iodine are placed in the tube it will change color (turns purple) in the presence of starch. When the glucose test strips are dipped into the tube they will change color depending on the amount of glucose present. There is a color chart on the test strip bottle indicating the glucose concentration. Discuss the importance of controls (why do we measure starch and glucose both before and after contact with spit), and discuss the expected results. A critical point is that the students understand that the enzyme **amylase** in their spit converts starch to sugar. There was no glucose (sugar) in the spit control, and no glucose to begin with in the cracker water slurry. When the cracker mixes with amylase in spit, glucose is produced. **The teacher should emphasize that the amount of glucose produced is equivalent to enzyme activity.**
See **Spit Lab introductory lecture** for good visuals.

Demonstration of the "spit tube": Making a good spit tube is important for the lab to work successfully. The teacher needs to demonstrate how to make a spit tube properly. The teacher chews a cracker for at least 30 seconds. It's helpful to explain what is happening, "the cracker is currently dry, I'm still chewing, don't

swallow, you have to keep it in your mouth and get it really wet with spit, resist the urge to swallow, ok now it's a fine wet slurry with no chunks." Slowly spit the cracker goo into a small tube to fill it half way up. Each group will need to create a tube like this. They will be grossed out. Tell them only to handle their own spit tubes. Do not touch other people's spit.

Students get in their groups and begin the lab – 75 min: Circulate around the room and make sure they are doing the lab. See the **Spit Lab** and **Spit Lab introduction lecture** for details.

Discussion of the results- 15 min: Talk about the results. What effect did temperature have on enzyme activity? They should see that the boiled enzyme stopped converting starch to sugar and that the chilled enzyme barely worked at all. The room temperature enzyme worked best. They should also have established that the cracker control was positive for starch and negative for sugar. The spit control was negative for both starch and sugar.

Computer lab/ homework – overnight or 30 min in computer lab

Starting Point For Further Inquiry: The end of the lab asks students to speculate on the optimal temperature for enzymes in a hot water vent bacteria. They have to use some background about evolution and their experience in the lab to guess that enzymes are going to be optimized for the conditions they occur in. The lab we did today tests for the activity of amylase at three different temperatures (room temp, iced, and boiled). Ask the class what they think the optimal temperature for amylase is. (It is likely that amylase works faster at the temperature inside your mouth.)

Analyzing and Presenting Final Results:

The lab activity asks the students to graph their final results using the computer. Some teachers ask the students to type the entire lab, other prefer the whole lab to be hand written. We have prepared the materials to that a hand written lab can be turned it.

Assessment Methods

You will know how well the students understand the concepts by their answers to the macromolecule activity and the Spit Lab.

Appendices

1) Enzyme introductory lecture
2) Cut-out activity worksheet (with instructions for students)
3) Cut-outs

5) Spit Lab introductory lecture
6) Spit Lab (with instructions for students)