Stressed Membranes

Introduction

Cell membranes help hold cell contents within the confines of the cell. If a cell membrane is damaged physically or chemically, the contents of the cell can "leak" out. In this activity, students can observe the red pigment (betacyanin) after it has left beet cells that have been damaged to varying degrees.

Concepts

- Hydrophilic/hydrophobic
- Bipolar membrane

Materials

Acetone, 10 mL
Methanol, 10 mL
Fresh beets
Beaker, 250-mL
Cork borer
Corks, #4, 6
Forceps
Graduated cylinder, 10-mL
Metric ruler
Razor blade
Refrigerator with freezer
Test tube rack
Test tubes, 13 × 100 mm, 6
Thermometer

Safety Precautions

This activity requires the use of hazardous components and/or has the potential for hazardous reactions. The solvents in Part II of the experiment should be handled carefully. Both are highly flammable and should be kept away from any open flame. Both are dangerous by ingestion or inhalation. Care should be used in handling these chemicals. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Secure enough fresh beets for the number of student groups who will conduct the laboratory.

Prepare the 6 treatment solutions for Part II—1%, 25%, and 50% (v/v) solutions of acetone to water and 1% and 25%, and 50% (v/v) solutions of methanol to water.

Procedure

Part I. Temperature Stress

1. Use a cork borer and razor blade to cut six sections of beet tissue into cylinders 15 mm long and 5 mm in diameter.
2. When beet cores are cut some cells rupture and the red pigment (betacyanin) leaks out. Rinse the beet sections to remove the pigment from the damaged cells.
3. Look at Data Table 1. Use the six beet sections for each of the six temperature treatments outlined in the Data Table and described below:

#1: Place beet section in test tube and then place in freezer (–5 °C) for 30 minutes. After 30 minutes, add 10 mL of water and place it in the test tube rack at room temperature.

#2: Place beet section in test tube and then place in refrigerator (5 °C) for 30 minutes. After 30 minutes, add 10 mL of water to the beet section and place it in the test tube rack at room temperature.

#3: Add tap water with a temperature of 20 °C to a beaker. Submerge a beet section in the 20° water for one minute. Remove the beet and place it in a test tube with 10 mL of room temperature water. Place the test tube in the test tube rack at room temperature.

Publication No. 10159
061616

#4: Heat water to 40 °C in a beaker. Submerge a beet section in the 40° water for one minute. Remove the beet and place it in a test tube with 10 mL of water and add it to the test tube rack.

#5: Repeat procedure #4 using hot water at 55 °C.

#6: Repeat procedure #4 using hot water at 70 °C.

4. After the six beet sections have been treated, allow all the test tubes to stand for 30 minutes. Shake the tubes occasionally and equally during the 30 minutes. Note that the water surrounding the stressed beet sections starts to contain various amounts of the red pigment.
5. After 30 minutes, remove the beet sections from the tubes.
6. Compare the intensity of color in each test tube. Use judgment values of 0–10 to rate the color intensity in each test tube. Give the lightest colored tube a value of 0 and the darkest a value of 10. Rate all the other tubes in between. Enter your ratings in Data Table 1.
7. If you have access to a spectrophotometer or a colorimeter, take samples from your test tubes and measure the absorbance of 460 nm light by betacyanin. Record your results in the absorbance column of Data Table 1.
8. Answer the following questions:

   A. Which temperature(s) stressed and damaged the beet cell membranes the most? What is your evidence?

   B. After reading about membrane structure, offer an explanation for how temperature extremes might damage cell membranes.

   C. Graph your absorbance data if available (temperature on *x*-axis, absorbance on *y*-axis). Compare your visual observations with your absorbance data. Explain any differences and/or similarities.

Data Table 1. Temperature Stress

Tube #	Treatment (°C)	Color Intensity (0–10)	Absorbance (460 nm)
1	–5		
2	5		
3	20		
4	40		
5	55		
6	70		

Part II. Solvent Stress

Caution: The solutions used in this part of the lab are flammable and volatile. Handle them carefully, avoid breathing fumes, skin contact, and inform your instructor of any spills or accidents.

1. Cut and rinse six beet sections using the same procedure as step 1 of Part I.
2. Place one beet section in each of six labeled test tubes. Add 10 mL of each of the treatment solvents outlined in Data Table 2.
3. Seal the test tubes with corks to prevent fumes from escaping.
4. After 30 minutes, remove the beet sections. Compare the betacyanin solutions left in the test tubes as you did in Part I. Use the 0–10 rating scale again trying to be as objective as possible about your ratings. Record your color ratings in Data Table 2.
5. If you have access to a spectrophotometer or colorimeter, use samples from each of the test tubes and measure the absorbance of 460 nm light by betacyanin. Record your results in Data Table 2.
6. Answer the following questions:

A. Which solvent caused the most stress?

B. Is there any relationship between concentration of solvent and stress damage? Use your results to explain any trends.

C. After reading about membrane structure, offer an explanation of how solvents might damage cell membranes.

Data Table 2. Solvent Stress

Tube #	Treatment (Solvent)	Color Intensity (0–10)	Absorbance (460 nm)
1	1% Acetone		
2	25% Acetone		
3	50% Acetone		
4	1% Methanol		
5	25% Methanol		
6	50% Methanol		

Discussion

Membranes are important to the cell because they separate and organize molecules within the cell by allowing selective passage of materials across their boundaries. Cell membranes are composed of a double layer of phospholipid molecules interspersed with protein molecules. A phospholipid molecule is a combination of a phosphate group and two fatty acids bonded to a three-carbon glycerol chain (see Figure 1). The polar (charged) phosphate group is *hydrophilic* (water-loving) and the non-polar fatty-acid groups are *hydrophobic* (water-fearing).

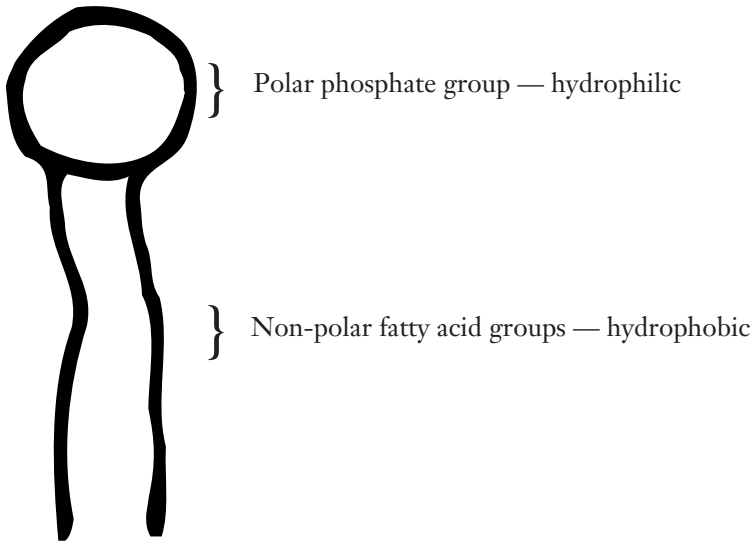

Figure 1. Schematic of phospholipid molecule — a membrane building block

Polarized phospholipids will self-assemble into a double-layered sheet of molecules forming a membrane. The hydrophobic tails of the lipids form the core of the membrane and the hydrophilic phosphate groups line both the inside and outside surfaces of the cell (see Figure 2).

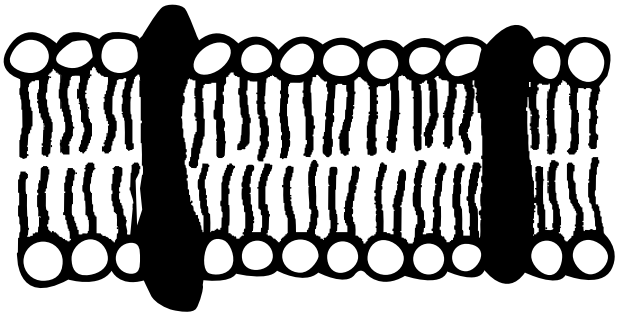

Figure 2 Schematic of bilayer membrane

Beet cells contain large amounts of a red pigment called *betacyanin*. The pigment is stored in the large central vacuole of the cells. The vacuole is surrounded by a vacuolar membrane and the entire beet cell is surrounded by a cell membrane. As long as the beet cells are whole and their membranes are undamaged, the betacyanin will remain in the vacuoles. However, if the membranes are damaged or stressed, betacyanin will diffuse through the vacuolar and cell membranes and produce the red color in the water surrounding the stressed beet. The color intensity in the surrounding water will be proportional to the level of stress on the membranes.

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. Used solvents from this experiment should be collected and disposed of by the instructor following Flinn Suggested Disposal Procedure #18a.

Materials for *Stressed Membranes* Available from Flinn Scientific, Inc.

Catalog No.	Description
GP1020	Beaker, 250 mL
GP6010	Test Tubes, 13 × 100 mm
AP8304	Corks, #4
AP1452	Thermometer
AP1872	Metric Ruler
AP4417	Test Tube Rack
AP8326	Cork Borer
AP4287	Graduated Cylinder, 10 mL
M0054	Methanol
A0009	Acetone
AP8685	Flinn Scientific Spectophotometer

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