

Diffusion

It is important to remember that cell membranes are differentially permeable NOT semi-permeable. That is, while the membrane is permeable to water and impermeable to mannitol, other solutes must be permeant (able to cross the membrane). Were this not so, cells could not obtain minerals, photosynthate, or amino acids needed for metabolism and growth. Membrane proteins must assist in acquiring and retaining essential solutes.

Unfortunately most metabolic solutes are invisible and difficult to detect with the simple means available to us. We will turn to the betacyanin pigment of beet roots for assistance. This is normally sequestered in the vacuole and, by means of the properties of the tonoplast and cell membrane, does not leak into the cytosol or the extra-cellular sap of the beet root. Of course when we cut the beet root, we slice cells open and the pigment spills out, but can we alter the membrane (phospholipid bilayer + proteins) more subtly to induce leakage (diffusion) of betacyanin?

1. The instructor will use a mandoline to cut uniform sticks of tissue from the beet root. Use a razor blade to slice the ends from the sticks and cut the sticks to equal length.
2. Place the sticks into a plastic cup of distilled water. Stir gently to rinse the sticks. Pour off the now-red distilled water. Repeat this step until the rinse water comes off colorless. The goal is to wash all betacyanin released from injured cells.
3. Prepare large test tubes with one stick of rinsed beet root and 10 ml of a test solution. Your tests should answer the following questions (or others you may think of). Can a nasty ion (NaCl) "salt out" the betacyanin? Can a membrane-stabilizing ion (CaCl_2) prevent salting out? Can a less-polar solvent (acetone) interfere with the phospholipid bilayer? Can the stabilizing ion prevent that? Does temperature influence diffusion? How about a freezing treatment or a boiling treatment? Distilled water, 4% (W/V) NaCl, 4% (W/V) KCl, 0.2% (W/V) CaCl_2 , 0.2% (W/V) MgCl_2 , 80% (V/V) acetone/water, 80% (V/V) ethanol/water, an ice bath, an ice-salt bath, warm water, and boiling water baths are available for your use.
4. Any pre-treatments should probably last about 20 min.
5. Incubation periods for final treatments should be 45 minutes for all of the tubes.
6. After the 45-minute incubation period, decant the fluid from each tube into a spectrophotometer cuvette. Be sure to keep track of the treatments with respect to your spectrophotometer cuvette. Observe the intensity of the color in each fluid.
7. Measure the absorbance of each fluid at 475 nm in the spectrophotometer. Use distilled water as your blank. Why would you use 475 nm (=blue) light to measure absorbance of a red pigment?
8. At home write an abstract of this beet-root project. Be sure to explain what you think each of the treatments did to alter the permeability of the tonoplast and cell membrane.