Methods

Detailed experiment notes and procedures are available online at:

http://www.nonanone.com/invertase/

Preparation of invertase reagent. A 1.00 mg/mL solution of invertase is prepared by dissolving 0.0100 g of yeast (*Saccharomyces cerevisiae*) invertase (Sigma-Aldrich I9253: pH 4.5, 55 °C, 30 units/mg solid) in 10.00 mL of cold pH 4.5 acetate buffer solution. Transfer 1 mL aliquots into fresh Eppendorf tubes, and store on ice until ready to use. Just before use, vortex the tube thoroughly to redissolve any solid that may have precipitated out of solution.

Activity of invertase with β-fructofuranosides. Prepare fresh 0.3390 M solutions of raffinose, melezitose, and sucrose. For each sugar create a set of tubes with varied amounts of sugar solution, pH 4.5 acetate buffer totaling 590 µL. For each tube, add 10 µL of invertase solution. Let reaction proceed at room temperature for exactly five minutes, and then immediately add 400 µL of 3,5-DNS in 0.6 M NaOH solution to each tube which stops the reaction. Bathe in a boiling water bath for 5 minutes to develop the color. Add 3 mL of deionized water to each tube. Create a blank, as above, only without sugar and enzyme, and zero a spectrophotometer at 540 nm. Measure absorbance of all tubes. Determine

reducing sugars with a standard curve made with standards containing glucose+fructose, buffer to 600 μL, 400 μL of 3,5-DNS, incubation at 100 °C, and 3 mL of water.

Verification of hydrolysis of a reducing sugar whose products are also reducing sugars. Prepare a 1.00 mg/mL solution of lactase (Sigma-Aldrich G5160: pH 4.5, 30 °C, 8 units/mg solid) similar to above for invertase. Prepare a lactose blank with 120 μL of 0.0100 M lactose, 480 μL buffer, 400 μL 3,5-DNS, bathed in boiling water bath for 5 minutes, 3 mL of water. Prepare the analytical sample with 120 μL of 0.0100 M lactose, 280 μL of buffer, and 200 μL of lactase solution. Let reaction proceed for 5 minutes, add 400 μL 3,5-DNS, bathe at 100 °C, add 3 mL water. Measure absorbance at 540 nm. The analytical sample should be at least 0.050 absorbance units higher than the blank.

Activity of invertase with α-glucosides. Use the method above with invertase in place of lactase, and maltose, cellobiose in place of lactose. Also use the method with sucrose and invertase to verify that the invertase is active.

Inhibition of invertase. Proceed as stated for the activity of invertase with β-fructofuranosides, with the following changes. New standards should be prepared with the addition of 60 μL of 30 mM fructose. Run samples of sucrose with 60 μL of 30 mM fructose added. Repeat with sucralose (including new standards). Repeat with just sucrose for a set of uninhibited activities for comparison with the inhibited runs.