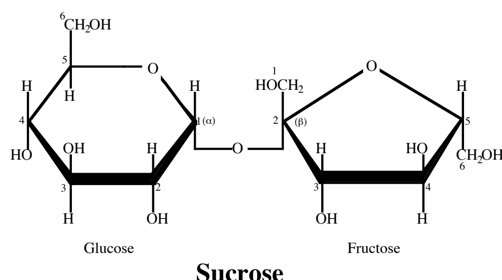
Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 1: Activity of Invertase with β-fructofuranosides

Experiment 1 Introduction

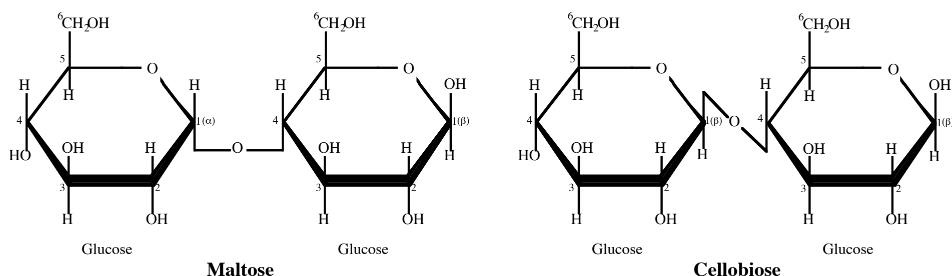
It is known that invertase hydrolyzes *sucrose* (glucose α(1 → 2) fructose; non-reducing sugar) to form *glucose* (a α-glucoside monosaccharide unit) and *fructose* (a β-fructofuranoside).



Sucrose

The primary goal of the activity experiments is to determine which type of monosaccharide units, if any, the enzyme looks for when it cleaves the disaccharide.

Other disaccharides and trisaccharides will be tested with invertase to see if any reaction occurs. The α-glucosides to test are *maltose* (glucose α(1 → 4) glucose; reducing sugar) and *cellobiose* (glucose β(1 → 4) glucose; reducing sugar). If the reaction succeeds, we can characterize invertase as an α-glucosidase.



Maltose **Cellobiose**

The β-fructofuranosides to test are *raffinose* (galactose α(1,6) glucose β(1,2) fructose; non-reducing sugar) and *melezitose* (glucose α(1 → 3) fructose unit of sucrose; non-reducing sugar). If the reaction succeeds, we can characterize invertase as a β-fructofuranosidase.

Raffinose is referred to as an *unsubstituted* β-fructofuranoside, because the fructose unit does not have any other groups attached to it. Melezitose is a *substituted* β-fructofuranoside, because the fructose unit that is attached to one glucose unit also has another glucose unit attached to it.

The journal articles indicate that invertase is a β-fructofuranosidase, so we expect it to hydrolyze raffinose and possibly melezitose, while we expect it to have no effect on maltose and cellobiose.

If raffinose is hydrolyzed by the enzyme at a greater rate than melezitose, then the enzyme prefers that the fructose unit not have anything attached to it. That could mean that the enzyme needs to bind such that steric hindrance from a substituted group would cause the binding to be less efficient, or not work at all.

Experiment 1 Outline

Show that raffinose and melezitose are cleaved by invertase. Do *Michaelis-Menton* kinetic study on raffinose and melezitose (as done for *A Manual for Biochemistry I Laboratory: Experiment 7 Part D*).

Experiment 1 Materials

Stockroom:

- 10.00 mL volumetric flask
- 5.000 mL volumetric flask
- 3.000 mL volumetric flask
- β-Fructose
- α-Glucose
- Raffinose • $5H_2O$
- Melezitose • $1H_2O$
- A hot water bath spec tube holder
- Small magnetic stirring bar

In the lab:

- 3,5-dinitrosalicylate
- pH 4.5 or 4.7 buffer
- Invertase
- Sucrose
- Boileezers®
- Spectrophotometer
- Hot plate
- Magnetic stirrer
- Deionized water
- Oakton® pH Meter
- Eppendorf tubes

In the lab drawer:

- (43) Clean spec tubes
- Beakers for water baths
- (8) 50-mL beakers for:
  - Invertase solution
  - 3,5-dinitrosalicylate
  - Buffer
  - 10 mM solution of glucose+fructose
  - 1.0 M sucrose solution
  - 0.3390 M raffinose solution
  - 0.3390 M melezitose solution
  - Deionized water

Experiment 1 Procedure

Check the pH and temperature requirements for the invertase (see the bottle, or the entry on sigmaaldrich.com). For now, assuming that invertase from *Saccharomyces cerevisiae* is used (pH 4.5, 55 °C, 30 units/mg solid).

If needed, prepare a stock of 0.0500 M acetate buffer solution, pH 4.5:
Add 25.00 mL of 0.5000 M acetic acid to a clean 250.00 mL volumetric flask.
Add ≈ 200 mL of water.
0.9567 g of 100% sodium acetate trihydrate (136.08 g/mol).
Adjust to pH 4.5 dropwise with HCl or NaOH (if needed).
Dilute (add remaining ≈ 25 mL) to 250.0 mL.

Prepare the enzyme solution, 1 mg/mL solution of invertase:
Add 0.0100 g invertase to a clean 10.00 mL volumetric flask.
Dilute with *cool* buffer solution (pH 4.5) to 10.00 mL.
Transfer to a small clean beaker and use a magnetic stirrer to facilitate dissolution of the enzyme.
Upon dissolution, transfer 1000 μL aliquots to 2 or 3 fresh Eppendorf tubes.
Keep the tubes on ice at 4 °C until ready to use.
Vortex Eppendorf tubes thoroughly to redissolve solid just before using.

Prepare a 1.0 M solution of sucrose:
3.4402 g of 99.5% sucrose (342.30 g/mol)
Dilute with deionized water to 10.00 mL

Prepare a 10 mM solution of glucose+fructose:
0.0900 g fructose (180.16 g/mol)
0.0900 g anhydrous α-glucose (180.16 g/mol)
Dilute with deionized water to 100.0 mL

Prepare a 0.3390 M solution of raffinose:
1.0283 g of 98.0% raffinose pentahydrate (594.51 g/mol)
Dilute with deionized water to 5.00 mL

Prepare a 0.3390 M solution of melezitose:
0.5182 g of 99.0% melezitose monohydrate (504.44 g/mol)
Dilute with deionized water to 3.00 mL

Follow the procedure from *A Manual for Biochemistry I Laboratory: Experiment 7 Part A* (preparation of standards) and *D* (uninhibited enzyme with substrate) for each sugar (sucrose, raffinose, melezitose).