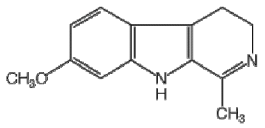
Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 3: Inhibition of Invertase

Experiment 3 Introduction

It is known that *harmaline* is a non-competitive inhibitor of invertase as we have seen from *A Manual for Biochemistry I Laboratory: Experiment 7 Part E.*

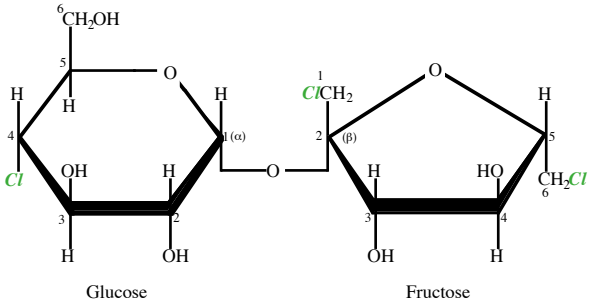


Harmaline

In this experiment, we repeat the procedure with two additional candidates, *fructose* and *sucralose*. Fructose is a product of invertase's primary enzymatic function, the hydrolysis of sucrose into glucose and fructose. It is hypothesized that fructose may act as a feedback inhibitor of invertase, so that sucrose is not hydrolyzed when fructose is already in plentiful supply.

Sucralose is an artificial sweetener marketed commercially as Spenda®. Its structure is very similar to that of sucrose, except some hydroxyl groups have been replaced with chlorine atoms.

Since the point of an artificial sweetener is to taste the same as sugar but not be metabolized as much, perhaps sucralose may be able to fit into the active site of invertase but is not able to be hydrolyzed. If this were true, then sucralose should be a competitive inhibitor of invertase.



Sucralose

Experiment 3 Outline

Repeat *A Manual for Biochemistry I Laboratory: Experiment 7 Part E* with fructose and sucralose in place of harmaline. New sets of standards will need to be made with fructose and sucralose, and their standard curves should be used to obtain product concentrations from the absorbance readings obtained. Do *Michaelis-Menton* kinetic study, and produce a Lineweaver-Burk plot with uninhibited sucrose, sucrose with fructose, and sucralose.

Experiment 3 Materials

Stockroom:

10.00 mL volumetric flask
20 mL beaker for invertase solution
β-Fructose
α-Glucose
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

In the lab drawer:

(53) Clean spec tubes
Beakers for water baths
(7) 50-mL beakers for:
- 3,5-dinitrosalicylate
- Buffer
- 1.0 M sucrose solution
- 10 mM solution of glucose+fructose
- 30 mM solution of fructose
- 30 mM solution of Splenda® (sucralose)
- Deionized water

Bring from home:

Splenda® (sucralose)

Experiment 3 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 anhydrous 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 30 mM solution of fructose:
- 0.0540 g fructose (180.16 g/mol)
- Dilute with deionized water to 10.00 mL

Prepare a 30 mM solution of Splenda® (sucralose):
- 0.1463 g sucralose pentahydrate (487.72 g/mol)
  - or 0.1193 g sucralose (397.64 g/mol)
- Dilute with deionized water to 10.00 mL

Follow the procedure from *A Manual for Biochemistry I Laboratory: Experiment 7 Part A* (to prepare the standards), *D* (uninhibited invertase with sucrose) and *E* (inhibited invertase; fructose and sucralose are used instead of harmaline).