Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 4: Stability of Various Invertase Preparations

Experiment 4 Introduction

Enzymes are sensitive to pH and temperature, and evolution has optimized their performance to be most efficient at the optimal environment for the organism. We have seen first-hand how finicky a preparation of enzyme can be. In this experiment, we try to determine which factors in the preparation are most important, so that subsequent experiments do not fail as a result of a "dead" enzyme.

Five different preparations of invertase are examined. Each uses a combination of these three variables: pH, storage temperature, and initial temperature of the solvent used during dilution.

These preparations will be denoted by the following. Potential mistakes are underlined. It is hypothesized that the first preparation is the optimal method, and that deviations from the optimal method are mistakes that may lead to reduced enzymatic performance.

ID	Preparation Description	Mistakes
CI4	*C*hilled buffer, stored on *I*ce, pH *4.5*.	0
CR4	*C*hilled buffer, stored at *R*oom temperature, pH *4.5*.	1
CI7	*C*hilled water, stored on *I*ce, pH *7*.	1
RI4	*R*oom temperature buffer, stored on *I*ce, pH *4.5*.	1
RR7	*R*oom temperature water, stored at *R*oom temperature, pH *7*.	3

These invertase preparations will be made, and each preparation will be tested with an identical sample of sucrose. They will be tested several times over the course of a few hours, so that enzyme performance over time can be monitored. It is hypothesized that the enzyme will degrade over time, so this data should be useful to determine the stability of the enzyme over time.

Experiment 4 Materials

Stockroom:

(nothing required)

In the lab:

3,5-Dinitrosalicylate
Invertase
Sucrose
Boileezers®
Spectrophotometer
Hot plate
Deionized water
Kimwipes®

In the lab drawer:

(30) Clean spec tubes
Beakers for water baths
(4) 50-mL Beakers for:
- Chilled water
- Water
- Chilled pH 4.5 buffer
- pH 4.5 buffer
- 3,5-Dinitrosalicylate

(2) Resealable 10-mL tubes for:
- 0.1 M Sucrose
- pH 4.5 buffer

(5) Eppendorf tubes for:
- "CI4" Invertase
- "RI4" Invertase
- "CI7" Invertase
- "CR4" Invertase
- "RR7" Invertase

Experiment 4 Procedure

Start a 55 °C warm water bath.
Get a hot plate and two 150 mL beakers filled about 2/3rds full of tap water.
Put the beakers on the hot plate and set it to level "2" or so, and add a thermometer. Keep an eye on this and adjust the temperature so it stays at about 55 °C.

Start a vigorously boiling hot water bath.
Set up a hot plate with a few beakers of water.
Add Boileezers to the beakers so the water doesn't boil over.

Get the clean spec tubes. Make sure they are dry, or start drying them now.

If needed, prepare a stock of 0.0500 M acetate buffer solution, pH 4.5:
25.00 mL of 0.5000 M acetic acid
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 a fresh 0.1000 M solution of sucrose in water:
0.3440 g of 99.5% sucrose (342.30 g/mol)
Dilute with deionized water to 10.00 mL.

Prepare beakers of water, pH 4.5 buffer, chilled water, and chilled pH 4.5 buffer.

Allow sealed tubes of pH 4.5 buffer solution and sucrose solution to incubate in the warm water bath.

Preparing the tubes.
Prepare one tube as a water blank (and label it):
Add 600 µL of deionized water to the tube.
Add 400 µL of 3,5-DNS to the tube.
Add 3000 µL of deionized water to the tube.
Prepare one tube as a sucrose blank (and label it):
Add 200 µL of 0.1 M sucrose solution.
Add 400 µL of deionized water to the tube.
Add 400 µL of 3,5-DNS to the tube.
Add 3000 µL of deionized water to the tube.
Label the tubes with unique identifiers.

Prepare the first batch of (***C***hilled dilution, Stored on ***I***ce, pH ***4***.5 = ***CI4***) tubes.
Need (4) initial CI4/CR4 tubes, (2) RI4 tubes, (2) CI7 tubes, (2) RR7 tubes.
390 µL of pH 4.5 buffer solution warmed to 55 °C.
Put these tubes back in the warm water bath until ready for use.

Prepare the CI4 invertase solution:
Add 0.0100 g invertase to a clean 10.00 mL volumetric flask.
Note exact mass used, and try to be consistent so batches are comparable.
Dilute with *cool* buffer solution (pH 4.5) to 10.00 mL.
Immediately note the time water was added.
Transfer to a clean 25-mL Erlenmeyer flask.
Use a magnetic stirrer to facilitate dissolution of the enzyme.
As soon as it dissolves, prepare two Eppendorf tubes.
Label one "CI4" and the other "CR4."
Keep the "CI4" tube on ice at 4 °C until ready to use.
Do not let the ice melt too much!
Keep the "CR4" tube at room temperature.

Immediately run the initial CI4 tubes.
Add 200 µL of 0.1 M sucrose solution warmed to 55 °C.
Add 10 µL of CI4 invertase.
Note the time of addition.
Move the tubes back to the warm water bath.
Note: Don't forget to move the CI4 tube back to the ice bath.
Exactly 5 minutes later, add 400 µL of 3,5-DNS.
Move the tubes to a boiling water bath to develop the color.

Prepare the first batch of (***R***oom temperature dilution, Stored on ***I***ce, pH ***4.5*** = ***RI4***) tubes.
Need (2) initial RI4 tubes.
390 µL of pH 4.5 buffer solution warmed to 55 °C.

Prepare the RI4 invertase solution:
Clean the volumetric and Erlenmeyer flask now if you reuse them.
Add 0.0100 g invertase to a clean 10.00 mL volumetric flask.
Note exact mass used, and try to be consistent so batches are comparable.
Dilute with deionized water (pH ≈7.0) at *room temperature* to 10.00 mL.
Immediately note the time water was added.
Transfer to a clean 25-mL Erlenmeyer flask.
Use a magnetic stirrer to facilitate dissolution of the enzyme.
As soon as it dissolves, prepare an Eppendorf tube.
Label it "RI4," and keep it on ice until ready to use.

Immediately run the initial RI4 tubes.
Add 200 µL of 0.1 M sucrose solution warmed to 55 °C.
Add 10 µL of RI4 invertase.
Note the time of addition.
Move the tubes back to the warm water bath.
Note: Don't forget to move the RI4 tube back to the ice bath.
Exactly 5 minutes later, add 400 µL of 3,5-DNS.
Move the tubes to a boiling water bath to develop the color.

Prepare the first batch of (***C***hilled dilution, Stored on ***I***ce, pH ***7*** = ***CI7***) tubes.
Need (2) initial CI7 tubes.
390 µL of pH 4.5 buffer solution warmed to 55 °C.

Prepare the CI7 invertase solution:
Clean the volumetric and Erlenmeyer flask now if you reuse them.
Add 0.0100 g invertase to a clean 10.00 mL volumetric flask.
Note exact mass used, and try to be consistent so batches are comparable.
Dilute with *cool* deionized water (pH ≈7.0) to 10.00 mL.
Immediately note the time water was added.
Transfer to a clean 25-mL Erlenmeyer flask.
Use a magnetic stirrer to facilitate dissolution of the enzyme.
As soon as it dissolves, prepare an Eppendorf tube.
Label it "CI7."
Keep it on ice at 4 °C until ready to use.

Immediately run the initial CI7 tubes.
Add 200 µL of 0.1 M sucrose solution warmed to 55 °C.
Add 10 µL of CI7 invertase.
Note the time of addition.
Move the tubes back to the warm water bath.
Note: Don't forget to move the CI7 tube back to the ice bath.
Exactly 5 minutes later, add 400 µL of 3,5-DNS.
Move the tubes to a boiling water bath to develop the color.

Prepare the first batch of (***R***oom temperature dilution, Stored at ***R***oom temperature, pH ***7*** = ***RR7***) tubes.
Need (2) initial RR7 tubes.
390 µL of pH 4.5 buffer solution warmed to 55 °C.

Prepare the RR7 invertase solution:
Clean the volumetric and Erlenmeyer flask now if you reuse them.

Add 0.0100 g invertase to a clean 10.00 mL volumetric flask.
Note exact mass used, and try to be consistent so batches are comparable.
Dilute with deionized water (pH ≈7.0) at *room temperature* to 10.00 mL.
Immediately note the time water was added.
Transfer to a clean 25-mL Erlenmeyer flask.
Use a magnetic stirrer to facilitate dissolution of the enzyme.
As soon as it dissolves, prepare an Eppendorf tube.
Label it "RR7."
Keep it at *room temperature* until ready to use.

Immediately run the initial RR7 tubes.
Add 200 µL of 0.1 M sucrose solution warmed to 55 °C.
Add 10 µL of RR7 invertase.
Note the time of addition.
Move the tubes back to the warm water bath.
Exactly 5 minutes later, add 400 µL of 3,5-DNS.
Move the tubes to a boiling water bath to develop the color.

Run the additional time intervals with sets of tubes (2 per set) for each of the types of invertase made (CI4, RI4, CI7, CR4, RR7). Be sure to vortex the Eppendorf tube thoroughly (at least 15 seconds) before each use. Keep the temperature of both water baths constant. Try to keep the amount of time the tubes spend in the boiling water bath constant for all tubes.

Each tube should be made fresh, with warm solution:
Add 390 µL of pH 4.5 buffer solution warmed to 55 °C.
Add 200 µL of 0.1 M sucrose solution warmed to 55 °C.
Add 10 µL of RR7 invertase.
Note the time of addition.
Move the tube back to the warm water bath.
Exactly 5 minutes later, add 400 µL of 3,5-DNS.
Move the tube to a boiling water bath to develop the color.

Measure the absorbance of the tubes.
Turn on a spectrophotometer.
Set it to absorbance mode. (MODE button)
Zero it at 540 nm with the tube you made as the blank.
Add 3000 µL of deionized water to each tube that has finished bathing in the hot water bath.
Take readings for all tubes. Be sure to use Kimwipes first.