Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment 5: Activity of Invertase with Varied pH

Experiment 5 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. In this experiment, we wish to narrow down the optimal pH for invertase from this species of yeast.

The method we used in *A Manual for Biochemistry I Laboratory: Experiment 7 Part C* worked well, and it was determined that the best pH was 4.4 ± 1.0 unit. For this experiment, we try to narrow this optimal pH range to ± 0.1 unit. The same basic procedure is used, except we will be preparing the buffer solutions for each pH.

Experiment 5 Materials

Stockroom:

(nothing required)

In the lab:

3,5-Dinitrosalicylate
Invertase
Sucrose
Boileezers®
Spectrophotometer
Hot plate
Deionized water
Oakton® pH Meter
Kimwipes®
Sodium Acetate
0.5 M Acetic Acid
6 M HCl
6 M NaOH

In the lab drawer:

(30) Clean spec tubes
Beakers for water baths
100-mL Graduated cylinder
400-mL Beaker
150-mL Beaker
Eyedroppers
(4) 50-mL Beakers for:
- pH calibration buffer, pH 4
- pH calibration buffer, pH 7
- pH calibration buffer, pH 10
- 3,5-Dinitrosalicylate

Experiment 5 Procedure

Preparation of spec tubes.

Get 30 spectrophotometer tubes, and put them in a small plastic bucket.

Add a drop or two of dish soap. Fill the bucket with warm water.

Make sure all the tubes are fully submerged so they are not floating.

For each tube, rinse it several times with tap water to get the bulk of the soap out, and then finally rinse it twice with distilled deionized water from your water bottle. The experiment is pH sensitive, so it will be important to use deionized water to thoroughly clean the tubes. There must not be any soap suds or any other contaminants left over in the tubes which could change the pH.

Put the clean tubes in a large beaker, and let them dry in the oven.

Start a 55 °C 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 "3" or so, and add a thermometer. Keep an eye on this and adjust the temperature so it stays at about 55 °C.

Calibration of pH meter.

Get 3 small (50 mL) beakers. Label them "pH 4," "pH 7," and "pH 10."

Get the 3 types of calibration solution. They should be pink, yellow, and blue solutions for pH 4, 7, and 10, respectively. Pour about 30 mL of each into their respective beaker.

Turn on the pH meter. Press the "CAL/MEAS" button to start the calibration. The first number you should see should be "4.01."

Rinse the pH meter's electrode with distilled water, then dip it into the beaker with pH 4 calibration buffer. Be careful not to touch the sides or bottom of the beaker with the electrode. Let the pH meter stabilize (until it says "READY"). Then press the "CON" button to confirm the calibration for pH 4.

The next number you should see is "7.00." Re-rinse the electrode with water. Repeat the process with the pH 7 buffer solution and press "CON."

The last number you should see is "10.01." Re-rinse the electrode with water. Repeat the process with the pH 10 buffer solution and press "CON."

Press the "CAL/MEAS" button again to end the calibration mode. The pH meter is now ready for measurements. Keep the electrode in water or the pH 7 buffer solution until you are ready to take readings.

Preparation of 0.0500 M acetate buffer solution, pH 4.70.

Add 25.00 mL of 0.5000 M acetic acid to a clean, large (400-mL) beaker.

Fill a 100 mL graduate cylinder with 100 mL of deionized water from the water jug. Pour the water into the beaker.

Fill the graduate cylinder again with 100 mL of deionized water from the water jug. Pour the water into the beaker. There should be about 225 mL of solution in the beaker at this point.

Add 1.701 g of 100% sodium acetate trihydrate (136.08 g/mol) to the beaker, and make sure it is thoroughly dissolved. There should be some fizzing to indicate that the base is reacting with the acid you added before.

Adjust to pH 4.70 dropwise with HCl or NaOH, using the pH meter.

For example, if the pH meter read 4.61, you would need to add NaOH to get to 4.70. If it read 4.97, you would need to add HCl to get back down to pH 4.70.

Mix the beaker thoroughly while you are adding acid or base, being careful not to touch the sides or bottom of the beaker with the pH meter's electrode.

Make sure the pH reading is exactly 4.70 before continuing.

Pour about 100 mL of the newly made buffer solution into a clean 150-mL beaker ("the small beaker"). Leave the pH electrode in the big beaker for now.

If not already done, 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 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.

Preparing the tubes.

Get the tubes out of the oven. Make sure they are dry before continuing.

Set aside 3 of the tubes for spares.

Prepare one tube as a blank (and label it):

Add 60 µL of 1.0 M sucrose solution to each tube.

Add 540 µL of pH 4.7 buffer (pipette it directly from the big beaker).

Add 400 µL of 3,5-DNS to the tube.

Add 3000 µL of deionized water to the tube.

Label the rest of the tubes A1, A2, B1, B2, C1, C2, …, M1, M2.

Adding individual pHs to the tubes.

Start with the big beaker.

Into tubes G1 and G2, add 530 μL of buffer (pH 4.7).

Use the pH meter and add HCl dropwise until you reach pH 4.60.

Write down the exact pH in the notebook. Hopefully that will be 4.60, but 4.59 or 4.58 would be OK.

Into tubes F1 and F2, add 530 μL of buffer (pH 4.6).

Use the pH meter and add HCl dropwise until you reach pH 4.50.

Write down the exact pH in the notebook.

Into tubes E1 and E2, add 530 μL of buffer (pH 4.5).

Continue decreasing the pH by 0.1 units and adding to tubes D, C, B, A.

Go back to the small beaker.

Rinse the electrode thoroughly with water.

The pH of the buffer in this beaker should already be 4.70.

Use the pH meter and add NaOH dropwise until you reach pH 4.80.

Write down the exact pH in the notebook.

Into tubes H1 and H2, add 530 μL of buffer (pH 4.8).

Use the pH meter and add NaOH dropwise until you reach pH 4.90.

Write down the exact pH in the notebook.

Into tubes I1 and I2, add 530 μL of buffer (pH 4.9).

Continue increasing the pH by 0.1 units and adding to tubes J, K, L, M.

Start a 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.

Turn it up on high and continue on to the next step when the water is boiling.

As the experiment progresses, you'll need to make sure there is enough water in the beakers so they can bathe the tubes.

Enzymatic reaction with invertase.

This step is time sensitive. The invertase must be stopped exactly 5 minutes after it is added to the tubes. It is stopped by the addition of 3,5-DNS, so have your pipette set to 400 µL and ready to go.

Get a small beaker with 3,5-DNS.

Set a P1000 to 400 µL.

Get the Eppendorf tube with invertase.

Inspect the invertase. There should be no solid on the bottom.

If there is solid, vortex (about a minute) to redissolve the invertase.

Run the tubes in sets of 3, for example, the first set would be A, B, and C (tubes A1, A2, B1, B2, C1, C2).

For each tube in the set:

Add 60 µL of 0.1 M sucrose solution, then immediately add 10 µL of invertase.

Mix briefly with the vortex. Do not use the pipette to mix the tubes or else you won't be finished with the last tube in time.

Immediately move the tube into the 55 °C water bath.

Write down the time, in *hours:minutes:seconds* in the notebook.

Let the tubes simmer until it is time to add the 3,5-DNS.

After the last tube in the set is finished, you should have about a minute and a half to go before the first tube is ready to be stopped. Put the invertase back on ice!

Watch the clock until it is time to add the 3,5-DNS to the first tube (5 minutes since the start time). Then add 400 µL of 3,5-DNS to the tube at the five minute mark. Write down the stop time in the notebook. Add the tube to a boiling water bath, and let it bathe for about 5 minutes.

Add DNS to the other tubes as their 5 minute times elapse.

Make sure the tubes bathe in the hot water bath after adding the DNS.

Move the tubes out of the hot water bath after at least 5 minutes.

Add 3000 µL of deionized water to each tube that has finished bathing in the hot water bath.

Repeat this entire step for the other sets of tubes.

Measuring 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.

Take readings for all tubes. Be sure to use Kimwipes first.