Release of O-Glycans by β-Elimination

MATERIALS

- 30% Acetic Acid
- $NaBH_4$ (Sigma, 213462)
- 100 mM NaOH
- Dowex 50Wx8 (H^+ Form) (BioRad, 142-1451**)**
- Methanol (Sigma, 322415)
- 9:1 Methanol:Acetic Acid
- Polyprep Column (BioRad, 731-1550)
- Sep Pak C18 Column (100mg, CAT# 023590 from Waters)

2M $NaBH_4$ in 100mM NaOH:
(Make fresh right before use)

- 75.66 mg of $NaBH_4$

Weight the $NaBH_4$ in a centrifuge tube and then add 1 mL of 100 mM NaOH and mix.

9:1 Methanol:Acetic Acid:

- 450 mL Methanol
- 50 mL Acetic Acid

Mix the two solutions in a graduated cylinder and then transfer to a 500 mL glass bottle.

PROCEDURE:

NOTE: In order to obtain pure O-glycans, first perform a release of the N-glycans as these can also be removed during β-elimination.

1. Dissolve the glycoprotein sample in Milli-Q water, sonicate if necessary.

2. Transfer the solution to a glass reaction tube and add an equal volume of 2 M $NaBH_4$ in 100 mM NaOH. Add a stirbar, cap the tube, and incubate at 45 ^oC with stirring overnight.

3. After incubation centrifuge the tube briefly to remove condensation from the cap. Place the tube on ice.

4. Neutralize the reaction mixture by adding cold 30% acetic acid drop-wise. When the mixture is neutralized the effervescence will stop and bubbles will dissipate. Check the pH to make sure your sample is neutral before moving to the next step.

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CAUTION: Heat is generated upon the addition of acid to the samples. De-sialylation of glycans can take place under conditions of extreme heat so add the acid slowly and keep the samples cooled on ice during acid addition.

5. Taking a polyprep column, add 1-2 mL of the cleaned Dowex (H^+ Form). Wash the resin with several milliliters of Milli-Q water.

6. Add the neutralized sample to the column and collect the flow-thru. Wash the resin with 5 mL of Milli-Q water and collect.

Note: The O-glycans will pass through the column so make sure to collect as soon as you load your sample.

7. Freeze the collected fraction and lyophilize.

8. To remove the borate add 200 µL of methanol to the sample, vortex, and evaporate by dry nitrogen flush. Repeat two more times.

9. Next add 200 µL of 9:1 Methanol:Acetic Acid, vortex, and evaporate by dry nitrogen flush. Repeat two more times. (At this point the sample should start to look translucent)

10. Dissolve the dried sample in Milli-Q water and load on a pre-cleaned SepPak C18 column and wash the column with 3 mL of Milli-Q water, collecting the flow thru.

Note: The O-glycans will pass through the column so make sure to collect as soon as you load your sample.

11. Freeze the collected flow-thru and lyophilize.

12. Perform a monosaccharide analysis on a portion of the sample to confirm the presence of O-Glycans.

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COLUMN PREPARATION:

Sep Pak C-18:

- wash with 3 mL of 10% acetic acid
- wash with 3 mL of 50% Methanol
- wash with 3 mL of 100% Methanol
- wash with 3 mL of 72% Isopropanol/28% Methanol with 0.1% formic acid
- wash with 2 mL of anhydrous ethyl acetate
- wash with 3 mL of Chloroform
- wash with 3 mL of 100% Methanol
- wash with 3 mL of 50% Methanol
- wash with 3 mL of Milli-Q water

Preparing Dowex resin:

- Regenerate Dowex AG50W-X8 resin (cation exchange) with 1 M HCl, use at least one bed volume.
- Wash with Milli-Q water until pH returns to neutral (check with pH paper).

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