Permethylation of N- and O-Glycans

MATERIALS

- Mortar and Pestle
- Dimethyl Sulfoxide (Sigma, 276855)
- NaOH Pellets
- Iodomethane (Sigma, 289566)
- Chloroform (Sigma, 288306)

PROCEDURE:

NOTE: In order to perform permethylation the sample needs to be free of water. It is best to place the sample in a glass reaction tube and lyophilize it to thoroughly dry the sample.

1. Dissolve the dried glycans sample in 500 µL of DMSO, add a stirbar, and stir for 15-30 min, while the sample tube is capped.

2. Place 2 pellets of NaOH per sample (10-20 µg) in a mortar and crush it into a powder using the pestal. Add DMSO to the freshly ground NaOH, making a slurry.

3. While stirring with the pipette tip take up 500 µL of the slurry and add it to the sample tube and recap.

4. Using a gas-tight syringe add 200 µL of methyliodide to the sample and recap. Allow the sample to stir for 30 min. The reaction is complete when the mixture is milky white in appearance. Add 50 µL of methyliodide to the reaction mixture and allow it to react for another 15-20 mins. (This addition should be performed in a chemical hood.)

   NOTE: Methyliodide should be kept in a container with desiccant at 4 ^{o}C. Monitor the temperature of the reaction and place the sample tubes on ice for 1 min when the sample becomes warm and then return it to stirring.

5. Place the sample tubes on ice and add 1 mL of cold Milli-Q water to stop the reaction. The contents of the tube will bubble as water is added.

6. Extract the permethylated glycans by adding 1 mL of chloroform to the sample and vortexing. Centrifuge the sample at 2000 rpm for 2 min.

7. After centrifugation remove the water layer and discard. Again add water and vortex the sample and then allow it to sit for 2 min for the phases to clearly separate. Remove the water layer and discard. Repeat this step 3-4 times.

8. Remove the chloroform using a stream of dry nitrogen.

9. Once dried the sample is ready for MALDI-TOF analysis or conversion to PMAA for linkage analysis.