Monosaccharide and Glycan Analysis by Liquid Chromatography Mass Spectrometry

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Glycosylation is a common posttranslational modification of proteins. Many important biological functions have been attributed to glycosylation, including cell-cell interactions, cell adhesion, and pathogen-host interactions. Mass spectrometry is considered the most common analytical approach employed to characterize the enzymatically released N-glycans. However, this enzymatic release is time consuming. Faster methods employing PNGase enzyme-reactor for the rapid release of N-glycans have been reported. However, none of these methods were efficient for the simultaneous detection of both neutral and acidic glycans. Here, an approach for the rapid release of N-glycans, employing a monolithic PNGase F reactor, and the online LC-MS/MS detection is described. The approach allows the simultaneous profiling of neutral and acidic N-glycans as well as the determination of glycosylation sites.

Monolithic enzyme reactor (MER) with immobilized PNGase was developed. For profiling experiments, glycoproteins were injected onto the MER. A C-8 trap was employed to capture the proteins while a porous graphitized carbon (PGC) trap was employed to capture the glycans. Glycans were transferred to the PGC column (10.0x0.075 mm) and detected by LTQ-Orbitrap. For determining the sites of glycosylation, a tryptic digest was injected into the MER. The peptides were captured on a C-18 trap while the glycans were washed-out. The peptides were resolved on C-18 column and detected on LTQ-Orbitrap. The tryptic digest was re-injected into the system bypassing the MER. By comparing the MS spectrums of the two run the sites of glycosylation was determined.

First, the chromatographic conditions for optimum the separation and the PGC LC-MS/MS detection were investigated. A binary gradient was used to perform the separation. For optimum retention, component A of the mobile phase was 7.5 mM

Ammonium acetate; pH 8.4 while component B was acetonitrile. A linear gradient from 3% B to 35.0 %B in 35 minutes was applied and the ions were detected in the positive ion-mode. N-glycans were eluted from the column in less than 30 minutes. It was found that high pH is required to elute the highly sialylated N-glycans from PGC. Other mobile phase systems were employed and the effect of pH and the ionic strength of the mobile phase on the N-glycans retention will be shown. For example, if a low pH mobile phase is used in Component A of the mobile phase, a high ionic strength is required to elute the highly sialylated structures. The different parameters allowing efficient and rapid release using the enzyme reactor were optimized using glycoproteins standard. These parameters include: the reaction time, reaction temperature and the release buffer pH and ionic strength. Results indicate that the optimum loading flow rate falls between 2 µL/min to 5 µL/min. The enzyme reactor performance at room temperature was shown to be comparable to that at 37oC with no significant decrease in performance. Ammonium Bicarbonate can be used as loading mobile phase at different concentrations (i.e. 10 mM, 20 mM and 25 mM) without the need for pH control. Neutral and acidic N-glycans released as well as the location of the glycosylation site from 10 ng fetuin were detected using the above-described approaches.