ASMS 2005

FRACTIONATION OF NEUTRAL AND SIALYLATED N-LINKED GLYCANS USING MICRO SCALE HILIC SPE FOR MALDI-TOF MS ANALYSIS

Ying Qing Yu, Jennifer Kaska, Martin Gilar and John C. Gebler
Waters Corporation, Milford, MA, 01757

OVERVIEW

A micro scale hydrophilic interaction chromatography (HILIC) SPE cartridge is applied to enrich and fractionate N-linked glycans released from glycoproteins. The fractionation of glycans from HILIC SPE is achieved by using low and high pH stepped elutions. Fractionation enables isolation and enrichment of sialylated glycans, therefore, simplifying the identification and structural characterization of these glycans using MALDI MS analysis.

INTRODUCTION

Characterization of oligosaccharides from glycoproteins is challenging due to their structural heterogeneity and low abundance. Glycans released from glycoproteins contain both neutral and acidic glycans. Extensive fragmentation of sialylated glycans (loss of sialic acids) occurs with MALDI and multiple peaks are generally observed as a result of the fragmentation. Since the desialylated fragment ions can have the same mass as the corresponding native neutral glycans, this complicates glycan assignment.

HILIC SPE was used for the removal of salts and detergents from hydrophilic analytes such as oligosaccharides. In addition, we developed a method for SPE fractionation of glycans based on their acidity. The fractionation is performed using two elution steps (Scheme 1). The first elution uses an acidic solution and neutral glycans are released from the SPE exclusively; the second elution is a pH neutral solution which elutes the remaining glycans that contain acidic glycans only.

The fractionated glycans are analyzed separately, which reduces the complexity of the sample, allows an easier assignment of sialylated glycans.

METHODS

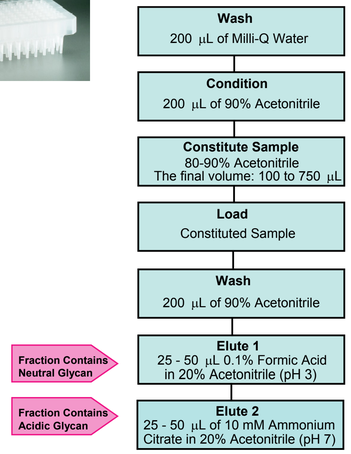
Deglycosylation

Glycoproteins, RNase B, and α1-acid glycoprotein (Sigma) were solubilized in 0.1% surfactant, RapiGest™ SF. DTT was added to the solution to a final concentration of 10mM before heating the sample at 100ºC for 5 minutes. The protein sample was buffered using equal volume of 100 mM NH_4HCO_3. PNGase F was used to remove N-linked glycans.

The released glycans were desalted using Waters 96 well MassPREP™ HILIC µElution plate.

Sialic acids from AGP glycans were removed by using neuraminidase (sigma).

Scheme 1. Micro scale HILIC SPE Protocol



A 96 well micro scale HILIC SPE plate operated via a vacuum manifold was used to extract and fractionate neutral and acidic glycans.

MALDI MS

- Waters MALDI micro MX™ , MALDI QTof Ultima™
- MALDI Matrix: MassPREP™ DHB (20 mg/ml in 100% ETOH)
- HILIC SPE extracted sample was mixed with MALDI matrix in 1:1 ratio directly on the target and dried at ambient temperature. After crystallization, 0.8 µl of EtOH was spotted on the target to recrystallize the sample. The glycans spotted on the MALDI target are released from 10 to 100 pmol of proteins
- MALDI-TOF positive and negative ion mode was used to acquire spectrum
- MS/MS fragmentation was performed on the AGP glycans after neuraminidase treatment

RESULTS

1. High pH elution buffer elutes acidic Glycan (A2F)

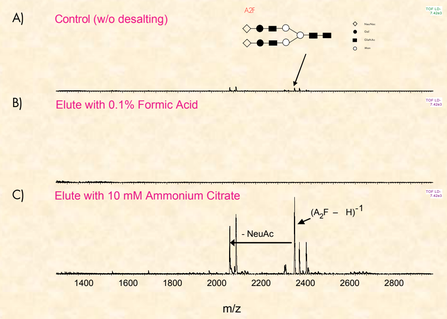


Figure 1. MALDI-TOF MS negative ion analysis of 25 ng of sialylated glycan A2F. A) Control sample, A2F was analyzed directed without desalting. B) HILIC µElution SPE was used to desalt A2F (see method for protocol). No A2F ions were observed from the first elution fraction which contains 0.1% formic acid. C) A2F was eluted from the HILIC SPE using 10 mM ammonium citrate (pH 7).

2. Fractionation of neutral and acidic glycan

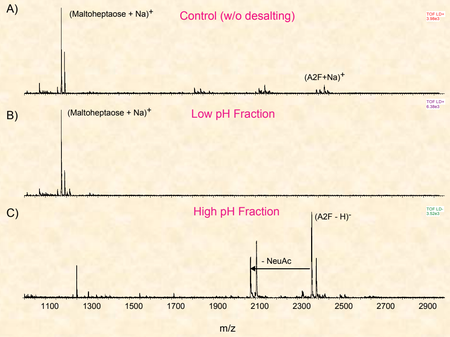


Figure 2. MALDI-TOF MS of mixed glycans. A) MALDI TOF MS positive ion mode of control sample of mixed neutral glycan (Maltoheptaose) and acidic glycan A2F, extensive fragmentation of A2F ions were observed. B) MALDI-TOF positive ion mode showed that Maltoheptaose was fractionated in the low pH elution. C) MALDI-TOF negative ion showed that the A2F was eluted using the high pH elution.

3. Mixed glycans released from glycoprotein, RNase B and α1-Acid Glycoprotein (AGP)

RNase B contains neutral glycans while AGP contains only sialylated glycans

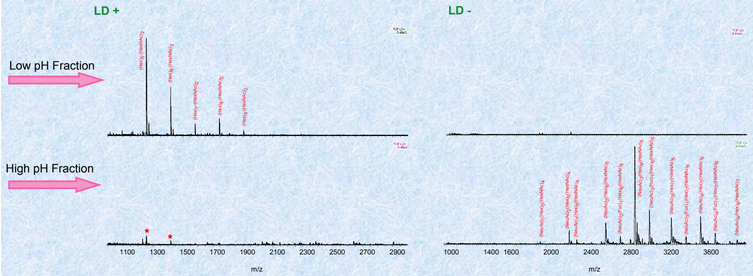


Figure 3. Glycans released from RNase B and AGP were mixed. The mixed glycans were desalted and fractionated using micro scale HILIC SPE. The fractions collected from low and high pH elutions were analyzed individually using MALDI-TOF in both positive and negative ion modes. The glycans identified in the low pH elution were all RNase B glycans released from 1.0 µg protein, no AGP glycans were observed in the low pH fraction. The glycans identified from the high pH elution were all sialylated AGP glycans released from 2.0 µg proteins, only trace amount of RNase B glycans (labeled with *) were observed. The sialic acids were removed using neuraminidase, and the structure of the desialylated glycans were further characterized using MALDI-QTof MS/MS (Figure 4).

CONCLUSIONS

- This micro scale HILIC SPE device removes salts and detergents from glycans released from glycoproteins enzymatically prior to mass spectrometry analysis
- The low and high pH stepped elution can effectively fractionate neutral and acidic glycans (sialylated), since
  1. Neutral glycans retain on HILIC SPE due to hydrophilic interaction
  2. Acidic glycans retain on HILIC SPE due to both hydrophilic interaction and weak anion exchange mechanism
- The fractionation of glycans simplifies glycan samples and the sialylated glycan assignment

4. MS/MS structure characterization of AGP glycans after the neuraminidase treatment

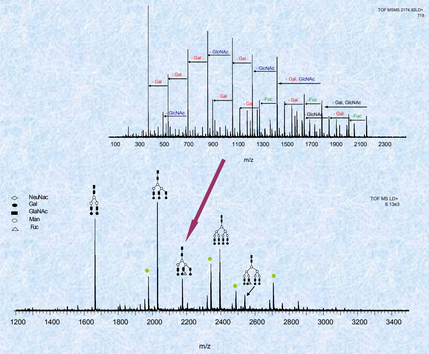


Figure 4. MALDI-QTof MS/MS fragmentation was performed on the desialylated AGP glycans. The fragment ion peak list and their intensity was submitted to Glycosuite database (ProteomeSystems) for structural identification. The structure with the highest score was proposed for the likely structure and showed in the bottom spectrum. Ions labeled with a solid green circle were partially desialylated ions. The top MS/MS spectrum is shown as an example. Ions generated from glycosidic bond cleavages are labeled.

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS

Lit Code Number 720001213EN