Analysis of Derivatized Glycans using Differential Mobility Spectrometry

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INTRODUCTION

Several factors can impact an ion's observed behavior during differential mobility spectrometry (DMS) analyses, including steric shielding of the charge site (Campbell et al., 2014; Liu et al., 2015), electronic delocalization of charge (Campbell et al., 2012), and the presence of intramolecular hydrogen bonds (Lintonen et al., 2014) or other forms of charge shielding (Maccarone et al., 2014).

However, we have only observed these properties and DMS behaviors in molecules as they exist in nature. In this study, we introduced these different DMS behaviors in analytes by derivatization. Here, we have taken three identical mixtures of glycans and derivatized each with one of the three isomers of aminobenzoic acid. The DMS separation of these isomeric species is due primarily to the differing degrees of intramolecular hydrogen bonding within each isomer.

In short, this study represents the first purposeful isomerization of analyte populations to exploit anticipated differences in DMS behavior based upon differences in ionic structure.

MATERIALS AND METHODS

Sample Preparation. Glycans (Figure 1), the aminobenzoic acid isomers, and sodium cyanoborohydride solution (0.1 M in THF) were purchased from Sigma-Aldrich (Oakville ,ON). GalNAc and GlcNAc were purchased from Dextra Laboratories (Reading, UK), and diluted to 1 mg/mL in methanol.

Glycan analytes were subjected to derivatization using a standard reductive amination protocol (Maxwell et al., 2011). Each individual glycan standards was reacted with one of three isomeric aminobenzoic acids (2-, 3-, or 4-aminobenzoic acid) as reagents (Figure 3).

DMS-MS Conditions. A differential mobility spectrometer (Figure 2) was mounted in the atmospheric region between a quadrupole time-of-flight mass spectrometer's sampling orifice and its electrospray ionization (ESI) source (-4500V) (Figure 2). The temperature of the DMS cell was maintained at 150 °C, and the nitrogen curtain gas was operated at 10 psi. Chemical modifiers (water, methanol, 2-propanol, or (±)-2-butanol) were added into the nitrogen curtain flow at 1.5% (v/v). The fundamentals of the DMS device have been described elsewhere. (Schneider et al., 2010) In this study, the separation voltage (SV) was held at 4000 V while the compensation voltage (CV) was scanned from -40 V to +20 V in 0.25 V increments.

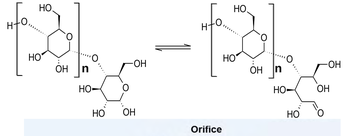


Figure 1. Generic structures of the pyranose and linear isomers of linear glycans (n ≥ 0).

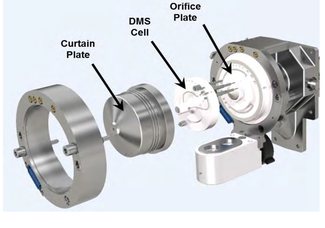


Figure 2. Exploded view of the DMS mounted on the quadrupole time-of-flight mass spectrometer.

RESULTS

DMS Behavior of Derivatized Linear Glycans

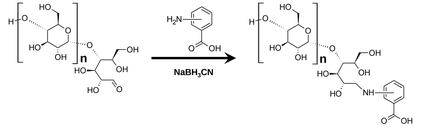


Figure 3. Reaction of generic glycans with aminobenzoic acid isomer (either 2-, 3-, or 4-aminobenzoic acid) via reductive amination.

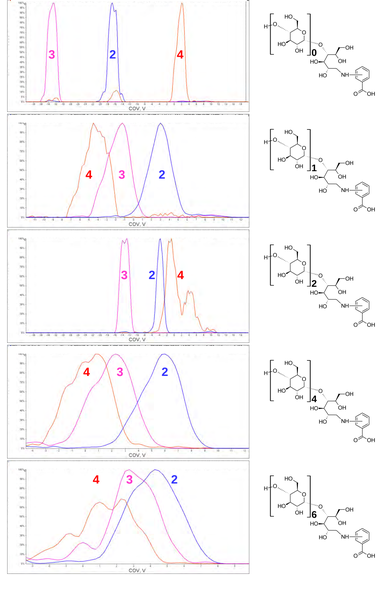


Figure 4. Ionograms displaying the separation for the derivatized linear glycans by DMS using 2-propanol (1.5%) as the chemical modifier.

We observed separation of the derivatized glycan species as a function of the aminobenzoic acid isomer derivatives employed.

The degree of separation of the isomers decreases with increasing MW of the linear glycans (i.e., increasing m/z of the deprotonated derivatized glycans), which was expected.

Interesting trends in relative CV shifts are observed, which are presently being examined using computational modeling.

RESULTS

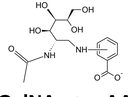
GalNAc and GlcNAc: Interesting Separations using Aminobenzoic Acid

We also used DMS to separate isomeric glycans: N-acetylgalactosamine (GalNAc) and N-acetylglucosamine (GlcNAc) after derivatization with isomers of aminobenzoic acid (AA).

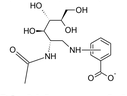
Figure 5. Structures of GalNAc and GlcNAc derivatized with either 2-, 3-, or 4-aminobenzoic acid via reductive amination.

The 2-aminobenzoic acid derivatized isomers of GalNAc (CV = -23V) and GlcNAc (CV = -13V) are easily separated, while interestingly, the 3- and 4-aminobenzoic acid derivatized isomer pairs are separated to much lesser degrees. This is also under further investigation using computational modeling.

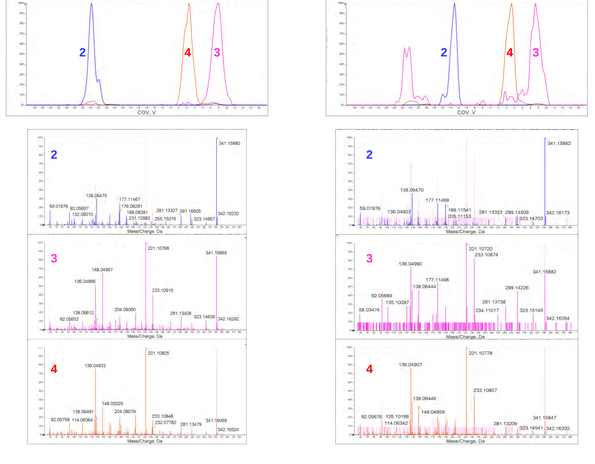
Each isomer's structure was verified by performing MS/MS on each DMS-separated species (see below).



GalNAc + x-AA



GlcNAc + x-AA



CONCLUSIONS

In this study, differential mobility spectrometry was used to separate isomeric derivatized glycans; these derivatives, prepared using isomeric reagent tags, deliberately induced DMS separation as a function of the expected differences in CV shifts resulting from different levels of intramolecular hydrogen bonding (and charge solvation).

Besides the separation of linear glycans, we also observed the separation of isomeric GalNAc and GlcNAc after derivatization with 2-aminobenzoic acid, which did not occur for the simpler protonated underivatized forms of these glycans.

The separation of the isomeric glycan species studied here was accomplished because of differences in the DMS behavior between each isomer pair. The ions bear subtle structural differences that allow the DMS to separate them based on their differing mobilities during the high- and low-field portions of the asymmetric waveform applied across the DMS cell. As such, each isomer required different DC compensation voltages (CVs) to bring their trajectories on-axis for successful sampling by the MS. Besides the differences between the isomers' structures, differences in how these species bind to added volatile chemical modifiers in the DMS cell made their DMS behavior differences more prominent, yielding increased selectivity and peak capacity for the DMS experiments.

In choosing the use of different isomeric derivatives, these species each maintain the charge differently by virtue of the location of the charge group (e.g., the carboxylate group for the aminobenzoic acids). Other types of derivatizing reagents are also under investigation in our labs, as well as other forms of targeted analytes.

The degree of competition between the **intra**molecular solvation of the charge and the **inter**molecular bonding between the ions and the DMS chemical modifier determines the relative binding energy of the derivatized glycans and therefore the observed DMS CV shift (i.e., stronger binding energy with chemical modifier molecules leads to more negative CV shifts).

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