Separation and Characterization of Isomeric Glycans by Gated TIMS MS/MS

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Abstract

The vital roles glycans play in living systems derive from their structural complexity and diversity. Unlike linear biopolymers such as proteins, glycans can assume branched topologies with different stereochemical and linkage configurations. In nature, glycans often exist as mixtures of related structures, including many isomers. Characterization of glycans thus demands analytical methods that can effectively separate and identify isomeric structures. In this study, we utilized gated-trapped ion mobility spectrometry (TIMS) for isomer separation and electronic excitation dissociation (EED) tandem mass spectrometry for structure determination. We show that isomeric tetrasaccharides, LNT and LNnT, differing by a single linkage variation, can be resolved by gated-TIMS based on their conformational difference, and discerned by EED based on the relative abundance of linkage-informative secondary fragments: Z−CH3OH and Z−OH. Gated-TIMS-EED-MS/MS appears to be a promising new tool for glycan mixture analysis, and should find ample applications in structural glycomics.

Introduction

The study of glycan structure-function relationship is essential to understanding their diverse and crucial roles in a wide range of physiological and pathological processes, including immune response, tumorigenesis, and embryonic development. In many cases, variation in even a single linkage can profoundly affect the glycan function. For example, the difference in the terminal sialic acid-galactose linkage between the α2−6 and α2−3 forms is a key determinant for direct avian to human influenza. The rapid growth of glycomics is largely fueled by the recent development of new instrumentation and analytical methods for glycan structure elucidation. In particular, tandem mass spectrometry (MS/MS) has been the enabling technique for structural glycomics, where the glycan sequence and linkage information are deduced from its glycosidic and cross-ring fragments (Figure 1). Here, we report a novel strategy that combines the power of ion mobility separation with EED tandem MS analysis for characterization of isomeric glycan mixtures. The key components, including glycan derivatization, separation, tandem MS analysis, and spectral interpretation, will be described in detail. A pair of glycan linkage isomers, lacto-N-tetraose and lacto-N-neotetraose (LNT and LNnT, structures shown in Figure 2), were used as the model system to investigate the potential of this approach for isomeric glycan mixture analysis.

Methods

LNT and LNnT were derivatized with the proton reagent for acid-catalyzed glycan sequencing (PRAGS), followed by methylation of its pyridinium methyl. Me-PRAGS labeling led to a 120.0687-Da mass shift (Figure 4). The Me-PRAGS label is a fixed-charge tag that promotes detection of reducing-end fragments while minimizing proton-mediated gas-phase structural rearrangement.

Structural isomers may be separated by IMS based on their gas-phase collisional cross sections (CCS). Here, Me-PRAGS-labeled LNT and LNnT were separated by gated-TIMS (Figure 5). Inside the TIMS device, ions are pushed downstream by a carrier gas flow with a velocity of \( v_1 \), while an axially varying electric field (E) is applied to stop the ion motion. When these opposing forces balanced out, the ion becomes stationary, or trapped, in a region where its velocity, \( v_1 = KE \) (where \( KE \) equals zero. An ion with a lower mobility requires a stronger electric field to be trapped. During the TIMS analysis, the axial electric field is slowly reduced, allowing sequential elution of ions from the TIMS analyzer, in ascending mobility order. A downstream ion gate (split lens) can be programmed to allow passage of only ions with mobility of interest. Mobility-selected ions can then be analyzed by EED (Figure 6).

Results

The EED spectra of LNT and LNnT are very similar as they share the same sequence and linkage configurations except for the linkages between the non-reducing-end galactose (Gal1) and N-acetylgalcosamine (GlcNAc2). This subtle difference gave rise to different fragmentation patterns at the GlcNAc site. As illustrated in Figure 11, for LNnT, loss of the C5 substituent from Z2, is energetically more favorable than loss of the C3 substituent due to the resonant stabilization in the Z−CH3OH product ion, thus LNnT produces more Z−CH3OH ions than Z−CH2 ions. For LNT, loss of CH3OH at C6 must be preceded by 1,2-hydrogen migration with a substantial energy barrier, thus Z−CH2 for the favorable product in the LNT spectrum. This difference allows structural elucidation on each mobility-separated species by IMS.

Discussion

Linkage isomers LNT and LNnT were successfully separated by gated TIMS. Each isomer may exist either in a mixed form (with the anomeric carbon in a or β configuration) or as an open-ring structure (with the C−N bond in either cis- or trans- form, Figure 10). Reductive amination may be performed to reduce conformational heterogeneity for easier analysis.

Conclusion

Isomeric glycans can be separated by gated-TIMS.

Fixed charge-derivationization leads to simplified glycan tandem mass spectra by promoting detection of reducing-end fragments.

EED of Me-PRAGS-labeled glycans produces unique fragmentation patterns to aid differentiation of linkage isomers.

Gated-TIMS-EED-MS/MS analysis is a powerful tool for characterization of glycan mixtures.

References


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All experiments were carried out on a 12-T solidX™ hybrid Qb-Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics) equipped with a modified funnel for TIMS separation and a cathode for EED analysis (Figure 3).