Frequency Encoding the Mobility of Isomeric Glycans: Separation Using Drift Tube Ion Mobility and Tandem Mass Spectrometry

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INTRODUCTION

- Collision induced dissociation is routinely used for carbohydrate composition assignment, but the branching and linkage combinations in glycans can be problematic for isomer distinction.
- High resolution IMS has been shown to provide separation of isomeric carbohydrates, but IM-MS data alone still lends uncertainty to isomer identification.
  - This challenge is exacerbated using low duty cycle instruments which often precludes concurrent fragmentation.
- By combining CID with Fourier transform ion mobility spectrometry and metal adduction, glycan isomer distinction in a mixture is realized.

Atmospheric Pressure, Dual-Gate Ion Mobility Linear Ion Trap Mass Spectrometer

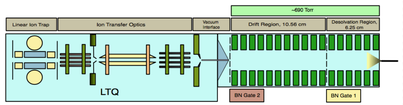


Figure 1. ExcelliMS MA3100 atmospheric pressure, dual-gate drift tube ion mobility spectrometer coupled to Thermo LTQ linear ion trap mass spectrometer. The drift tube can be coupled to an LTQ mass spectrometer without any modifications to either system.

AIMS

- Apply Co^{2+} adduction to a system of NMR-identified isomeric glycans to enhance drift time separation.
- Utilize Fourier transform ion mobility-mass spectrometry combined with concurrent CID to provide another dimension for isomer determination.

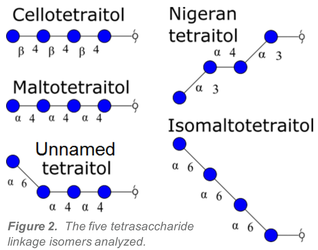


Figure 2. The five tetrasaccharide linkage isomers analyzed.

METHODS

- Drift time and mass spectra were obtained using a dual gate drift tube coupled to a linear ion trap mass spectrometer (Figure 1).
- Applying a frequency chirp of 5-10,005 Hz to both gates, the ion current was frequency modulated such that drift times were encoded in the resulting ion current decay (Figure 3).
- Solutions of each individual isomeric glycan (Figure 2) were prepared in MeOH with 0.1% formic acid at a concentration of 3 uM each, with $CoCl_2$ added to a final concentration of 50 uM; mixtures of glycans prepared with 3 uM of each isomer.

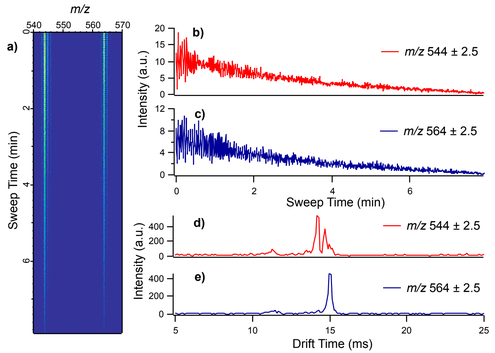


Figure 3. A 2D plot of the scan number vs m/z is shown for the range 540-570 (a). The extracted ion chromatograms for 544 (b) and 564 (c), corresponding to CID fragments of the cobalt-glycan adduct mixture, show the characteristic ion current decay produced by Fourier multiplexing of the ion current. With Fourier transformation of the extracted ion chromatograms, the resultant arrival time distributions for the precursor of the fragments m/z 544 (d) and 564 (e) can be found.

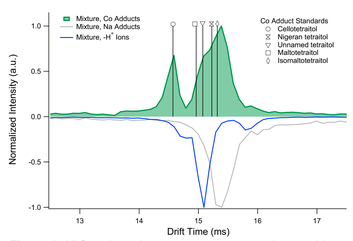


Figure 4. All five glycan isomers were run as a mixture adducted with Co^{2+} cations and analyzed by FT-IM-MS. Each individual glycan isomer adduct with Co^{2+} is shown with an outline, each with a differing line pattern. The common Na^+ adducts and deprotonated glycans of the same mixture are shown for comparison.

Table 1. Primary fragments produced by each respective cobalt-isomeric glycan adduct.

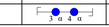
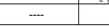
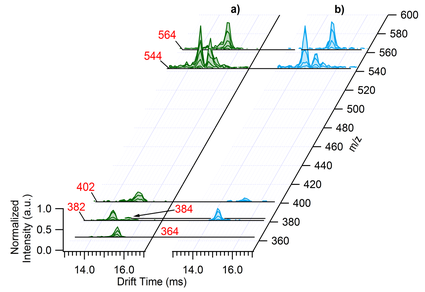
Co-Glycan Adduct Fragment *m/z*	Cellotetraitol	Nigeran tetraitol	Maltotetraitol	Isomaltotetraitol	Unnamed tetraitol
564.11	----		----		
544.08				----	
402.06	----		----		----
384.05	----		----	----	----
382.03		----	----	----	
364.02	----		----	----	----

Figure 5. a) Three dimensional waterfall plot of the full mixture of cobalt-isomeric glycan adducts obtained with FT-IM-CID-MS. b) Three dimensional waterfall plot of the three cobalt-glycan adducts that can be fully separated, which are cellotetraitol, maltotetraitol, and isomaltotetraitol.

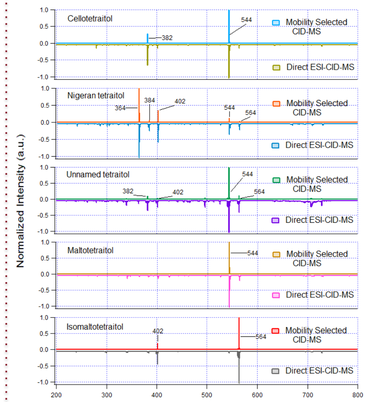
RESULTS

- Co^{2+} adduction improved separation of the five isomers to partial separation (Figures 4, 5a), and nearly complete separation of three isomers (Figure 5b).
- Primary fragments produced by CID of cobalt-glycan adducts resulted from the cleavage of glycosidic bonds (Table 1).



- When the CID spectra for cobalt-glycan adducts are obtained by drift time selection, resultant spectra are simplified compared to direct ESI-CID-MS spectra (Figure 6).

Figure 6. The Co^{2+}-glycan adducts were subject to CID both direct electrospray and FT mobility separation beforehand; the mobility selected spectra were obtained by selection of the CID mass spectra for an individual Co^{2+}-glycan adduct.



CONCLUSIONS

- Adduction of Co^{2+} with tetrasaccharide glycan isomers provides improved drift time separation relative to the sodium adducts and deprotonated glycans for select isomeric systems.
- High duty cycle Fourier transform-IMMS generates enough ion current to permit CID immediately following drift time separation, allowing identification of four of five isomers in a mixture according to both precursor drift time and characteristic fragments.
- Drift time selected CID spectra provide more accurate representation of relative peak intensities for desired species than from direct ESI-CID-MS.

FUTURE DIRECTIONS

- Investigate the use of other divalent cations for enhancing glycan drift time separation.
- Apply other fragmentation techniques to Fourier multiplexed drift time separations.

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