Evaluation of Ion Mobility Mass Spectrometry (IMMS) for Determining the Isomeric Heterogeneity of Bovine Submaxillary Mucin (BSM)

Hongli Li[1], Brad Bendiak[2], Kimberly Kaplan[1],William Siems[1], and Herbert H. Hill Jr.[1]

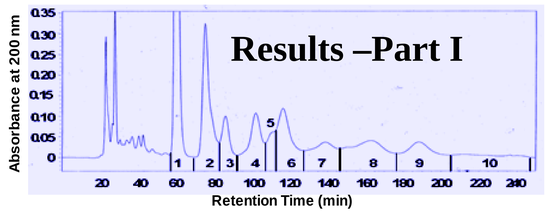
1. Department of Chemistry, Washington State University, Pullman, Washington, USA

2. Cell and Developmental Biology Program in Structural Biology and Biophysics, University of Colorado, Health Sciences Center, Anschutz Medical Campus, Aurora, Colorado, USA

Abstract

- Rapid separation and analysis of isomeric species are essential steps in the analytical protocol of carbohydrate structure analysis for glycomics research.
- This study evaluated the use of ion mobility spectrometry (IMS) prior to MS as an additional rapid isomer separation step prior to mass spectral analysis.
- Neutral oligosaccharide alditols of BSM were investigated in positive mode.
- Multiple isomers were detected for specific *m/z* on millisecond time scale, which would be difficult or impossible using MS alone or single LC column.
- Tandem MS provide valuable information for elucidating glycan structures and understanding the isomeric nature of glycans.

Results –Part I



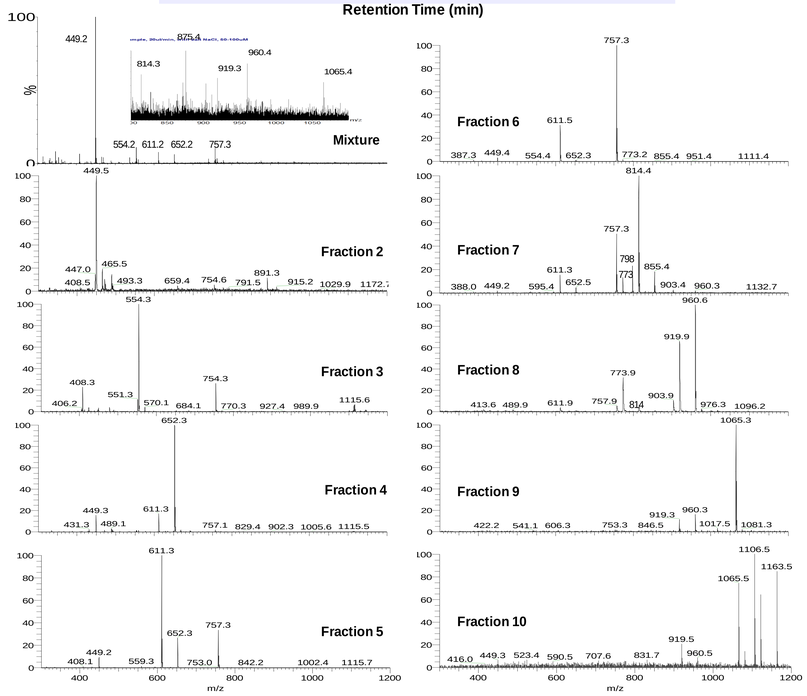


Figure 1: HPLC elution profile of neutral oligosaccharide-alditols isolated from BSM and mass spectra of BSM mixture and individual HPLC fractions.

Experimental

ESI voltage (V)	14.5 KV
First ring V	11 KV
Gate voltage	9007 V
Last ring V	773 V
Gate width	100 µs
Electric field	412V/cm
Drift gas	Nitrogen

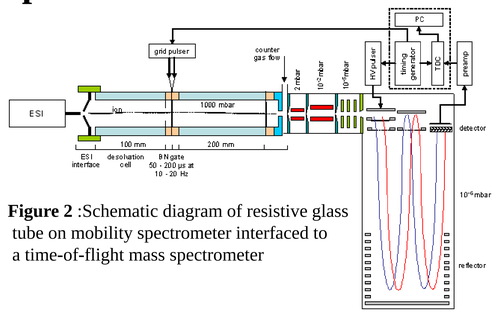


Figure 2 :Schematic diagram of resistive glass tube on mobility spectrometer interfaced to a time-of-flight mass spectrometer

Ion mobility quadrupole ion trap mass spectrometer was also employed in this study.

Results –Part II

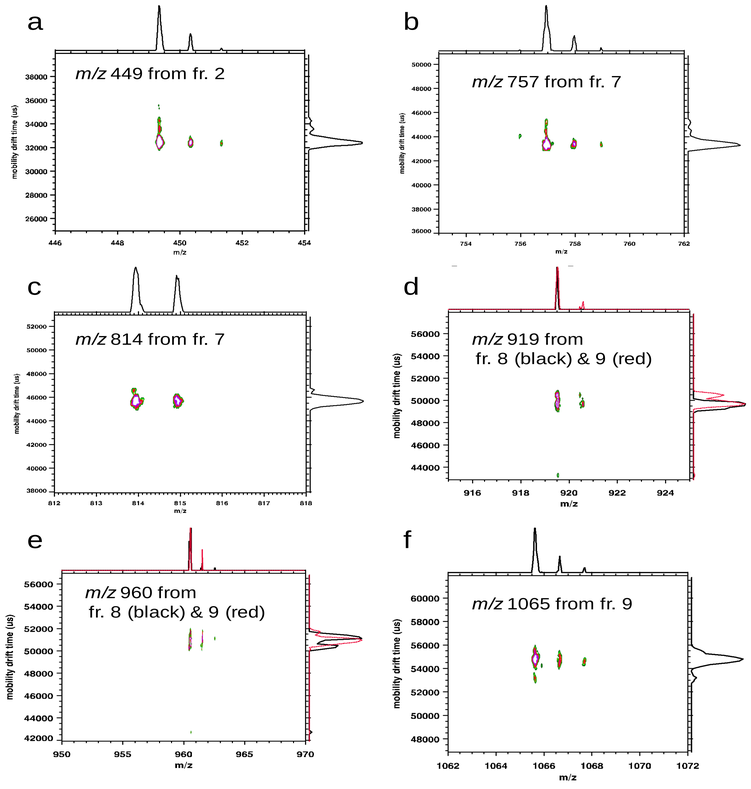
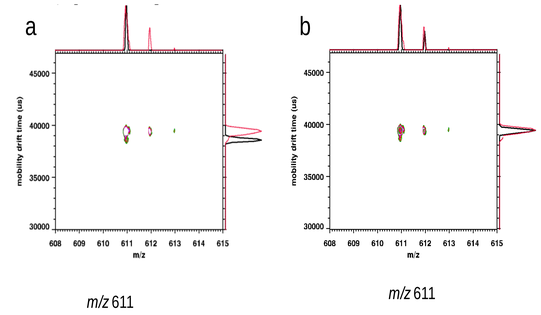


Figure 3: Two-Dimensional IM-MS plots of different compounds in BSM fractions: (a): m/z 449 in BSM Fr. 2; (b): m/z 757 in BSM Fr. 7; (c): m/z 814 in BSM Fr. 7; (d): m/z 919 in BSM Fr. 8 & 9; (e): m/z 960 in BSM Fr. 8&9; (f): m/z 1065 in BSM Fr. 9.

Results –Part III



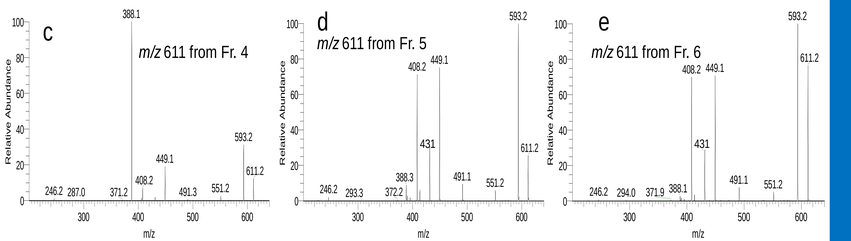


Figure 4: Ion mobility and MS/MS analysis of *m/z* 611 from HPLC fraction 4, 5 and 6. (a): Overlaid 2D IMMS spectra of *m/z* 611 from BSM Fr. 4 (black trace) and Fr. 5 (red trace); (b): Overlaid 2D IMMS spectra of m/z 611 from BSM Fr. 5 (red trace) and Fr. 6 (black trace); (c): The MS/MS Spectrum of *m/z* 611 eluted from HPLC fraction 4; (d): The MS/MS spectrum of *m/z* 611 eluted from HPLC fraction 5; (e): The MS/MS spectrum of *m/z* 611 eluted from HPLC fraction 6.

Conclusions

- It is demonstrated that IMS has the capability to rapidly differentiate glycan isomers, ranging from disaccharides (*m/z* 449) to hexasaccharides (*m/z* 1065).
- IMS resolved isomeric peaks for the *m/z* species that appeared as single peak in a HPLC fraction.
- The number of isomers of a given *m/z* species able to be identified by LC/MS proved to be very limited compared to IMMS.
- Ion mobility coupled to mass spectrometry represents a significant advancement in biological glycan analysis.

Acknowledgement

This work is supported by the National Institutes of Health.
Grant #: 5R33RR020046-05