Enhancing Carbohydrate Isomer Separation with Ion Mobility Spectrometry-Mass Spectrometry

Xueyun Zheng[1], Xing Zhang[2], Nate Schocker[3], Roger A. Ashmus[3], Igor C. Almeida[4], Keqi Tang[1], Catherine E. Costello[5], Richard D. Smith[1], Katja Michael[3], Erin S. Baker[1]

[1] Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA
[2] Skaggs School of Pharmacy and Pharmaceutical Sciences, Anschutz Medical Campus, University of Colorado, Denver, CO
[3] Department of Chemistry, [4] Department of Biological Sciences, University of Texas, El Paso, TX
[5] Center for Biomedical Mass Spectrometry, Boston University School of Medicine, Boston, MA

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Overview

- An ion mobility spectrometry-mass spectrometry (IMS-MS) platform was applied to characterize/separate standard glycan isomers in both positive and negative ion modes.
- Metal ions were used to enhance isomer separations.
- This platform was applied to study biologically active αGal-containing O-glycans that are important during Chagas disease infection.

Introduction

- Glycomics has become an important field of research since glycans play a critical role in many biology processes such as molecular recognition, signaling and cell communication [1, 2].
- Glycans are difficult to characterize due to the variety and complexity of their structures, e.g. the configuration of their glycosidic linkages and their connectivities.
- Here we use an ion mobility spectrometry-mass spectrometry (IMS-MS) platform to characterize standard glycan isomers and biologically relevant αGal-containing O-glycans that are important targets for protective anti-α-Gal antibodies against *T. cruzi*, which causes Chagas disease (Fig. 1) [3-6].

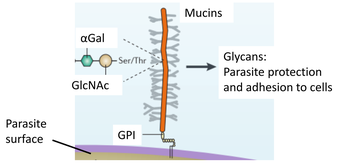
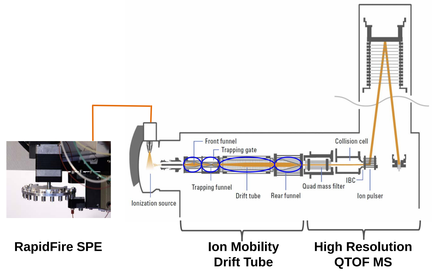


Figure 1 αGal-O-glycans on mucins of *T. cruzi* surface [4]

Methods



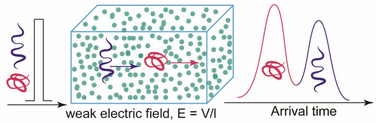


Figure 2 Schematic of IMS-MS platform and IM separation of conformation

IMS-QTOF-MS method:

- Glycan standards and synthetic O-glycans were analyzed with both a home-built IMS-QTOF MS [7] and an Agilent 6560 IMS-QTOF MS platform (Fig. 2) [8].
- Ions generated by ESI were transmitted through the inlet glass capillary, focused by a high pressure ion funnel, and accumulated in a lower pressure ion funnel trap. The ions were then pulsed into an 78 cm long IMS drift tube filled with ~ 4 torr of nitrogen gas for the drift field separation. Ions exiting the drift tube were refocused by a rear ion funnel prior to QTOF MS detection.

Results

1. IMS profiling of standard glycans with different ion polarities

- The observed IMS profile was different between positive ion mode and negative ion mode (Fig. 3A and B).
- Trend lines of arrival time vs. m/z ratios for glycan standards (monosaccharides to hexasaccharides) in both positive and negative ion modes (Fig. 3C and D) were plotted and revealed different separation capabiilities in each mode.

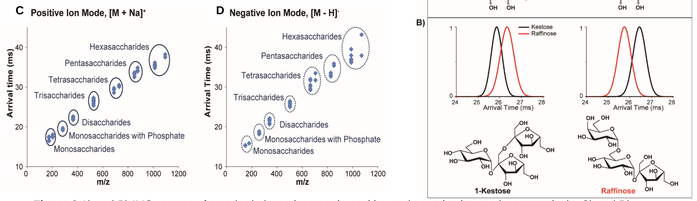
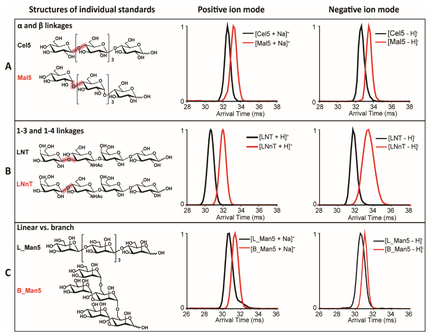


Figure 3 A) and B) IMS spectra of standard glycan isomers in positive and negative ion mode, respectively; C) and D) Arrival times and evident trend lines for IMS profiling of 41 standard glycans (Monosaccharides to hexasaccharides)

2. Separation of glycans with subtle structural differences

- Standard glycan isomers with subtle structural differences, such as α- versus β-linkages, 1→3 vs. 1→4 linkages, and linear vs. branched configurations, are separated with using IMS-MS platform (Fig. 4A-C).



- Metal ions[9] were used to enhance isomer separations for LNT and LNnT isomers (Fig. 4D)

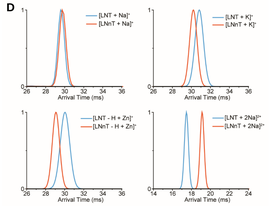


Figure 4 A), B) and C), IMS spectra of standard glycan isomers with subtle structural differences in different polarities; D) IMS spectra of LNT/LNnT with addition of different metal ions

3. Understanding biologically relevant αGal-O-glycans

- O-glycan isomers KM1, KM11 and KM23 could be partially separated with IMS in positive or negative mode (Fig. 5A).
- KM20 and KM24 have similar IMS profiles in positive mode but were well separated in negative mode (Fig. 5B).

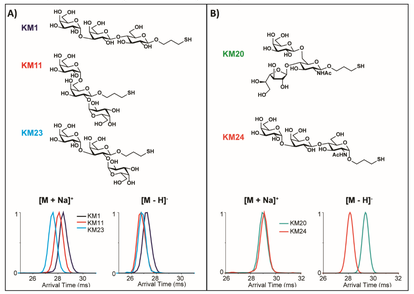


Figure 5 A) IMS spectra of O-glycan isomers KM1, αGal-(1, 3)-βGal-(1, 4)-βGlc-$(CH_2)_3$SH; KM11, αGal-(1, 6)-[αGal-(1, 2)]-βGal-$(CH_2)_3$SH; and KM23, αGal-(1, 3)-[αGal-(1, 2)]-βGal-$(CH_2)_3$SH; B) IMS spectra of O-glycan isomers KM20, βGalf-(1, 4)-[βGal-(1, 6)]-αGlcNAc-$(CH_2)_3$SH, and KM24, αGal-(1, 3)-βGal-(1, 4)-αGlcNAc-$(CH_2)_3$SH

- Using metal ions to enhance separation of KM6 and KM10 that were not separable in either positive or negative mode (Fig. 6)

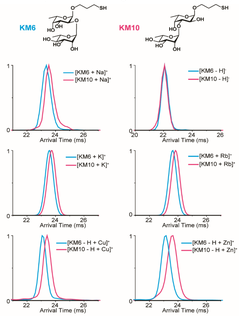


Figure 6 Metal ions enable better separations of KM6 (αRha-(1, 2)-αFuc-$(CH_2)_3$SH) and KM10 (αRha-(1, 3)-αFuc-$(CH_2)_3$SH)

Conclusions

- IMS separations can distinguish standard glycans with the use of both ionization modes
- Application of IMS-MS can separate glycan isomers with subtle structural differences
- Metal ion adduction can enable better separation of glycan isomers
- IMS-MS can improve the understanding of biologically active αGal-containing O-glycans

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CONTACT: Xueyun Zheng, Ph.D.
Biological Sciences Division
Pacific Northwest National Laboratory
E-mail: xueyun.zheng@pnnl.gov

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