Glycan Characterization using Trapped Ion Mobility Mass Spectrometry

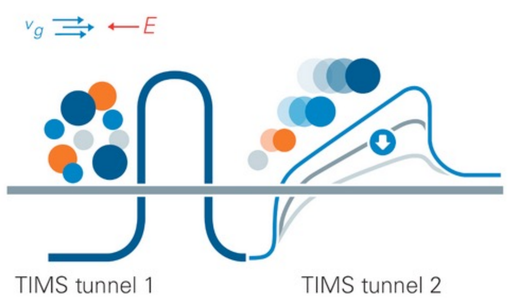
David Harris, W. Keith Ray, Richard F. Helm
Department of Biochemistry, Virginia Tech, Blacksburg, VA

Introduction

The structural analysis of glycans is difficult. Multiple monomers and the stereochemistry of the glycosidic bond lead to complex isobaric structures that limit full characterization. Current methods rely on fragmentation-based mass spectrometry to differentiate between two similar glycans, which requires further manual interpretation. **Trapped Ion Mobility mass spectrometry** (TIMS) takes advantage of differences in electric densities between molecules, allowing for their separation based upon their differential ion mobilities in the gas phase.

What is Trapped Ion Mobility?

TIMS uses a gas flow opposing an electric field to both trap and separate ions (see below). The first "tunnel" traps the molecules, which are then released to a ramped electric field, allowing for their separation, with larger molecules eluting first. The results of the separation are reported as a **mobiligram**.



Research Question

Can TIMS-based mass spectrometry techniques be used to distinguish between two similar isobaric glycans (**Fig. 1**)?

Methods

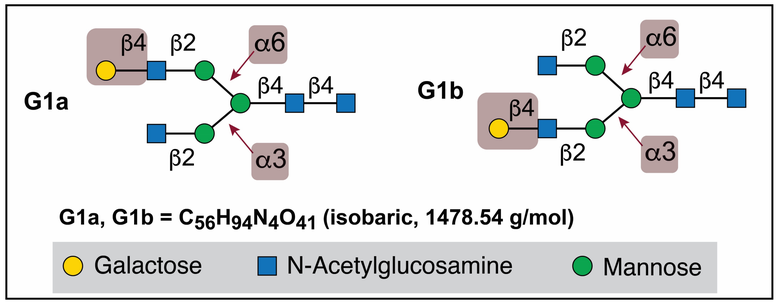


Figure 1. *Glycans G1a and G1b differ at the location of the terminal galactose.*

Glycans G1a and G1b were analyzed using matrix-assisted laser desorption ionization (MALDI) with TIMS on a Bruker timsTOF™ fleX MALDI-2 system. Glycans were ionized with dihydroxybenzoic acid (DHB) as the matrix.

Results

G1a and G1b were analyzed through MALDI-TIMS at a $1/K_0$ window of 1.300-1.800. The $1/K_0$ values were found to be 1.525 and 1.575 for G1a and G1b, respectively. $[M+Na]^+$ values were found to be 1501.5260 for G1a (**Fig. 2**) and 1501.5256 for G1b (Not shown).

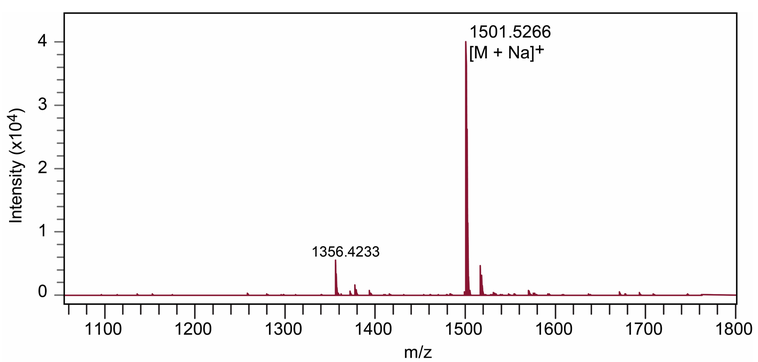


Figure 2. *MALDI-based Mass spectrum of G1a.*

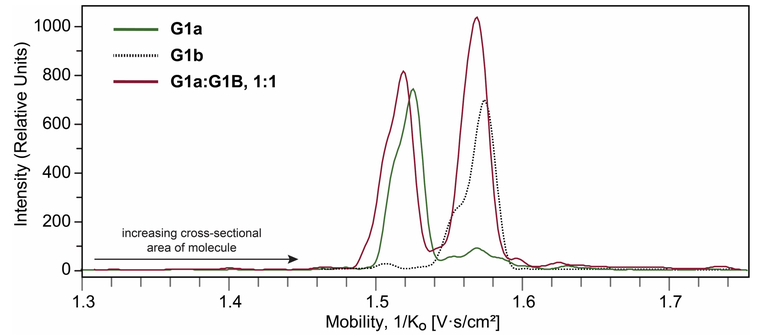


Figure 3. *TIMS mobilogram of G1a and G1b.*

A 1:1 mixture (red) showed two distinct peaks centered at $1/K_0$ = 1.520 and 1.570. The mixture result is shown overlayed with the individual peaks for G1a (green) and G1b (dashed) and shows a strong similarity between the two isolated samples, indicating separation and differentiation.

Conclusion/Future Work

TIMS can be used differentiate two isobaric glycans. Additional structures will be investigated to assess TIMS as a general too for glycan work. Additionally, TIMS will be combined with liquid chromatography to give higher resolution mobilograms with more complex samples.

Acknowledgement

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References

Mechref, Y.; Kang, P.; Novotny, M. V., Differentiating structural isomers of sialylated glycans by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry. *Rapid Commun Mass Spectrom* **2006,** *20* (8), 1381-9.

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