Ganglioside Isomer Differentiation by Trapped Ion Mobility Spectrometry Combined with Anion-Electron Reactions

Emily E. Hughes[1,2], Steven A. DeFiglia[1,2], Christopher A. Wootton[3], Kristina Hakansson[1,2]

[1] Florida State University; [2] National High Magnetic Field Laboratory; [3] Bruker Daltonics GmbH & Co.

OVERVIEW

- Trapped ion mobility spectrometry (TIMS) enables rapid separation of ganglioside isomers and species with varying ceramide tail lengths; however, conventional tandem mass spectrometry (CID) methods often lack sufficient structural resolution, particularly for isomers such as GD1a / GD1b and GT1a / GT1b.
- We examined ganglioside species from a porcine brain extract using TIMS coupled with electron detachment dissociation (EDD), building on previous studies of disialylated gangliosides.[1]
- We found that EDD generates a high number of structurally informative fragments, including cross-ring and glycosidic cleavages, enabling improved differentiation of isomeric species and more confident structural characterization.

INTRODUCTION

- Gangliosides are involved in various biochemical processes including essential roles in immune recognition and cellular communication.[1]
- GD1a/b have been studied using negative-ion liquid chromatography-collision induced dissociation (LC-CID) MS/MS, but LC separations are slow, while TIMS enables ganglioside isomer separation in milliseconds.[1,2]
- CID MS/MS of sialylated ganglioside anions shows poor differentiation between GD1a and GD1b.[1,2]
- EDD can provide rich structural information, including cross-ring fragments, from anionic gangliosides that allow for further characterization of species by giving unique fragmentation.[3]
- This work couples TIMS with EDD for rapid and improved ganglioside isomer characterization.

METHODS

- Total ganglioside mix standard (Avanti) was prepared at 5 μM in 50:50 isopropanol:H_2O with 0.1% triethylamine.
- EDD and CID experiments were performed on doubly deprotonated precursor ions.
- Experiments were conducted on a prototype timsMRMS 9.4 T mass spectrometer (Bruker). Ions were accumulated before TIMS for 100 ms and separated at ~2.65 mbar with a sweeping ramp time of 500 ms. TIMS voltage ramp performed for voltages 15 - 190V with 50 bins.

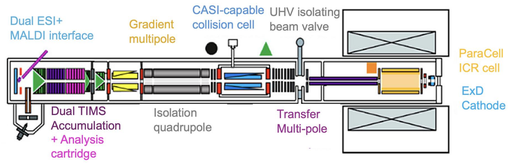

Fig. 1. Schematic of timsMRMS FT-ICR mass spectrometer.[4]

RESULTS

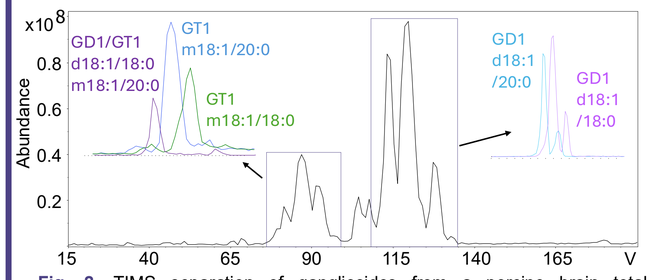

Fig. 2. TIMS separation of gangliosides from a porcine brain total ganglioside mixture.

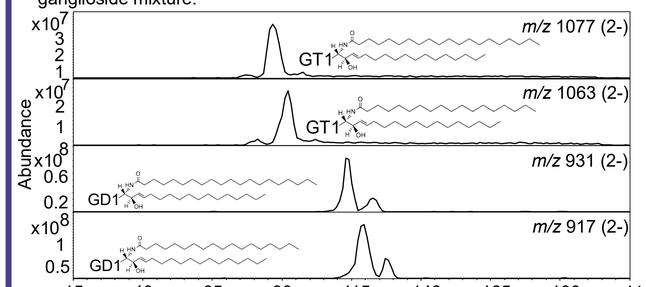

Fig. 3. Extracted ion TIMS voltage elution profiles of GT1 (m/z 1077 and m/z 1063) and GD1 (m/z 931 and m/z 917) from the total ganglioside mixture shown in Fig. 2.

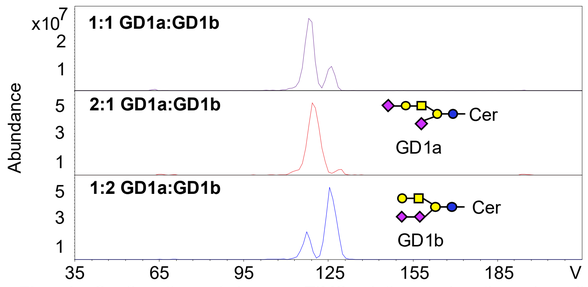

Fig. 4. Confirmation of isomer TIMS elution order through spike-in experiments of GD1a and GD1b into a total ganglioside mixture.

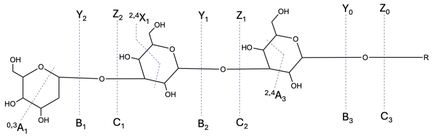

Fig. 5. Nomenclature for possible ganglioside fragmentation pathway.

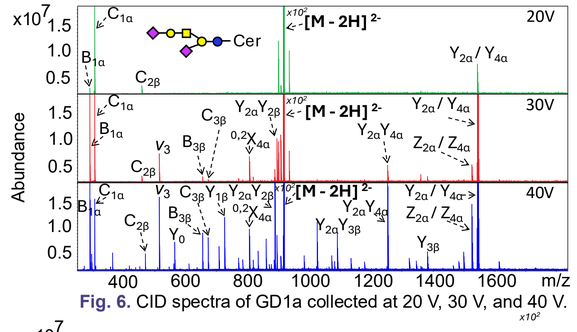

Fig. 6. CID spectra of GD1a collected at 20 V, 30 V, and 40 V.

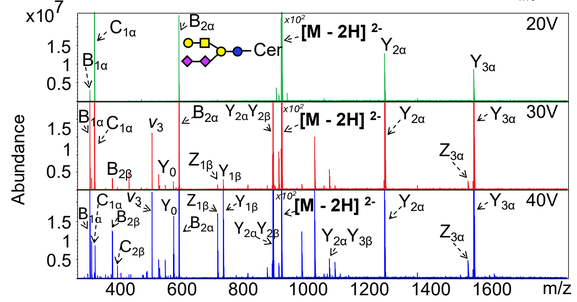

Fig. 7. CID spectra of GD1b collected at 20 V, 30 V, and 40 V.

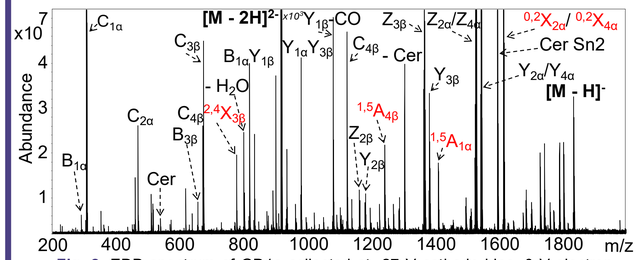

Fig. 8. EDD spectrum of GD1a collected at -27 V cathode bias, 0 V electron extraction anode, 500 ms irradiation. Cross ring fragments are labeled in red.

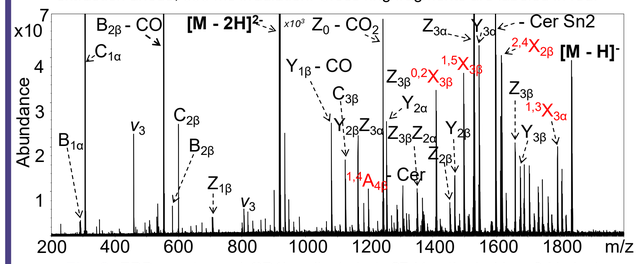

Fig. 9. EDD spectrum of GD1b collected at -27 V cathode bias, 0 V electron extraction anode, 500 ms irradiation. Cross ring fragments are labelled in red.

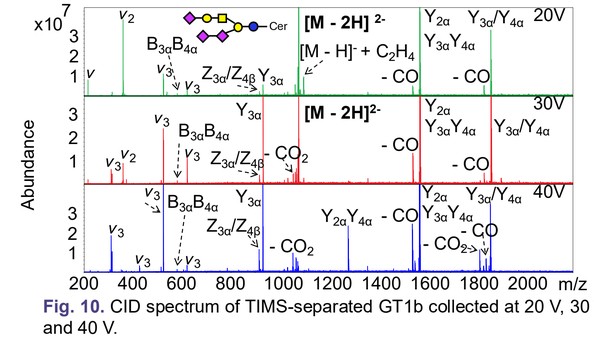

Fig. 10. CID spectrum of TIMS-separated GT1b collected at 20 V, 30 V, and 40 V.

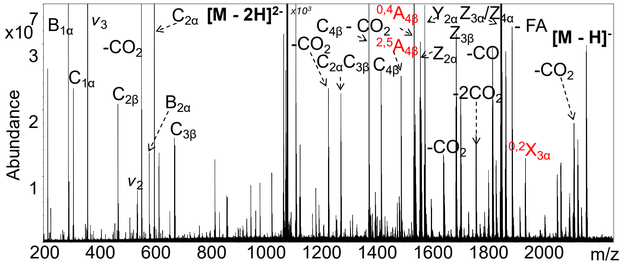

Fig. 11. EDD spectrum of GT1b collected at -30 V cathode bias, -20 V electron extraction anode, 500 ms irradiation. Cross ring fragments are labeled in red.

CONCLUSIONS

- Ganglioside species analyzed using TIMS-EDD showed rapid separation and produced abundant, structurally informative fragments, including cross-ring and glycosidic cleavages.
- These results demonstrate that coupling TIMS with EDD improves both the speed and confidence of ganglioside isomer characterization, providing a powerful alternative to traditional LC-CID MS/MS approaches.

ACKNOWLEDGMENTS

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[1] Djambazova, et al.. *Anal. Chem.* **2022,** *95,* 1176. [2] Li, et al. *Anal. Bioanal. Chem.* **2021**;413:3269. [3] McFarland, et al. *J. Am. Soc. Mass Spectrom.* **2005**;16:752. [4] Wootton, et al. *Anal. Chem.* **2024**, 96, 11343. [5] Seymour, et al;. *J. Am. Soc. Mass Spectrom.* **2006**;17:844.