UNRAVELLING THE COMPLEXITY OF HUMAN BRAIN GLYCOSAMINOGLYCANS: A STUDY OF CHONDROITIN/ DERMATAN SULFATES BY ION MOBILITY MASS SPECTROMETRY

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Abstract
Glycosaminoglycans (GAGs), particularly chondroitin sulfate (CS) and dermatan sulfate (DS), regulate brain processes through specific sulfation motifs. We report the first application of ion mobility spectrometry (IMS) mass spectrometry (MS) to neural GAG analysis. CS/DS octamers from mouse brain were profiled, revealing separation by sulfation content and glucuronic acid type. Eighteen distinct DS-rich domains were identified, including rare under- and oversulfated motifs. IMS MS/MS further resolved isomers and uncovered unusual trisulfated and pentasulfated structures. This approach advances brain glycosaminoglycomics and highlights novel, potentially bioactive CS/DS domains.

Introduction
Glycosaminoglycans are sulfated, linear *O*-glycan chains abundantly present in the extracellular matrix (ECM). Among these, CS and DS play key roles in the brain, where the specific pattern and positioning of sulfate groups along the CS/DS chains influence a wide range of biological processes [1]. The structural complexity and diversity of neural hybrid CS/DS in the brain have driven significant efforts to develop analytical techniques capable of identifying both regular and irregular sulfation patterns. In this context, we present here the first application of IMS MS to the study of brain glycosaminoglycans.

Experimental
Brains from 14-week-old C57BL/6 wild-type mice were incubated overnight in 4 M guanidinium chloride. Following the centrifugation, the supernatant was dissolved in Tris/HCl (pH 7.4) containing 150 mM NaCl. The solution was applied on a DEAE-anion exchange column. GAG chains were released by β-elimination. Depolymerization of CS/DS was carried out by digestion with 1 mU chondroitin AC lyase, which cleaved the linkage between GalNAc and D-GlcA. The size fractionation was performed on a Superdex Peptide HR10/30 column. The pooled octamer fraction was dissolved in methanol to a concentration of 10 pmol/μL and submitted to IMS MS. The IMS MS experiments were performed on a Synapt G2S (Waters, Manchester, UK) with nanoESI source, tuned in the negative ion mode, running MassLynx (version V4.1, SCN 855) and Driftscope (version V2.7).

Results and discussion
IMS MS profiling revealed that the species were not only separated according to the charge state, but, most importantly, to the sulfation content and the saturated (GlcA) or unsaturated (4,5-Δ-GlcA) type of glucuronic acid. The high separation efficiency of the IMS MS allowed

the discrimination of 18 distinct DS-rich CS/DS domains, of which nine undersulfated, five oversulfated and four regularly sulfated. Except for the most common motif, the tetrasulfated-[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$], several unusual under- and oversulfated, as well as saturated structures were discovered. Among these, of major importance is the pentasulfated-[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$], for which the IMS MS/MS disclosed the incidence of two isomers. Moreover, the generated fragment ions revealed an uncommon trisulfated and an uncommon pentasulfated motif.

Conclusion

By IMS MS and MS/MS, introduced here for the first time in brain glycosaminoglycomics, neural CS/DS domains of atypical sulfation patterns were discovered and structurally characterized in detail. This workflow discriminated isomers, defined sulfation signatures, and revealed novel motifs, pointing to potentially bioactive brain GAG species.

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References

[1] O. Habuchi, Glycobiology (2022) 664-678.