CHARACTERIZATION OF HYBRID CHONDROITIN/DERMATAN SULFATE OCTASACCHARIDE DOMAINS IN HUMAN BRAIN BY ION MOBILITY TANDEM MASS SPECTROMETRY

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Abstract
We report here on the introduction of a rapid, highly sensitive and reliable approach in a single run, based on ion mobility separation (IMS), high resolution and tandem MS (MS/MS) by collision-induced dissociation (CID) for compositional and structural elucidation of neural chondroitin sulfate (CS) and dermatan sulfate (DS) domains, which implies the determination of the epimerization and the sulfation code of regular and irregular structures. By IMS MS and CID MS/MS, we were able to characterize in details CS/DS octasaccharides from brain obtained after CS/DS chain depolymerization by chondroitin B lyase and to detect sequences that were never found before in the octasaccharide domains of the investigated CS/DS brain fraction.

Introduction
Proteoglycans represent heavily glycosylated proteins, covalently linked to glycosaminoglycans (GAGs), which are sulfated linear polysaccharides. Among GAGs, CS and DS frequently arise as hybrid CS/DS motifs predominantly expressed in mammalian tissue. In brain, CS/DS modulate glial cell maturation, cellular behavior and are implicated in several neurological events. CS/DS are sulfated at either GalNAc and/or IdoA/GlcA in a variety of combinations to yield characteristic patterns and a large structural diversity of domains in human brain, some of which being associated to the diseases of the central nervous system. Therefore, the analysis of CS/DS oligosaccharides in brain and the identification of regularly, over-, and undersulfated alternating motifs became the focus of the research in the field.

Experimental
The CS/DS chain extracted from brain was deploymerized by using chondroitin B lyase, which specifically cleaves the 3GalNAcβ1–4IdoAα1 bond irrespective of the sulfation status of the molecule. Following the size exclusion chromatography, the separated octasaccharide fraction was collected. For the IMS MS analysis, the octamer pool was dissolved in pure methanol to the concentration of 5 pmol/μL and infused into a Synapt G2S mass spectrometer by nanoESI. The signal was acquired in the negative ion mode at 0.9 kV ESI and 30 V cone voltage respectively. IMS wave velocity was set at 650 m/s and IMS wave height at 40 V. Fragmentation analyses for detailed structural investigation was performed by MS/MS using CID at low collision energies within 30-50 eV range.

Results and discussion

The driftscope display (drift time *vs. m/z*) of the total distribution of CS/DS octamer fraction ions has shown that the brain-derived octasaccharide species were separated by IMS MS into mobility families based on the charge state and the sulfation content. Following the digestion with chondroitin B lyase and the IMS MS separation and screening, several distinct unsaturated and saturated GlcA-rich species were detected and identified in the investigated octasaccharide fraction. Apart from the regularly tetrasulfated-[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$] octasaccharide bearing one sulfate group per disaccharide repeat, detected as a tetradeprotonated molecule, a number of unusual species, highly interesting from the structural and biological point of view, were for the first time discovered in brain tissue due to their separation by IMS and the high resolution and mass accuracy provided by the employed MS instrument. These motifs are GlcA-rich chains characterized by either undersulfation such as the monosulfated-[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$] and the trisulfated -[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$] species or oversulfation such as the pentasulfated-[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$], the hexasulfated-[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$] and the octasulfated -[4,5-Δ-IdoAGalNAc(GlcAGalNAc)$_3$] octasaccharides.

In the last stage of research, the rare CS/DS domains, exhibiting the atypical sulfation pattern *i.e.* the under and oversulfated CS/DS chains discriminated by IMS MS were submitted to CID MS/MS, which was carried out after mobility separation in the transfer cell. CID MS/MS data revealed one mobility feature for every isolated ion and a set of product ions, which are of diagnostic value for the determination of the sulfate sites and complete characterization of the sulfation code.

Conclusion

The optimized IMS MS has revealed different species in the CS/DS octasaccharide pool from human brain obtained after chain depolymerization using chondroitin B lyase. Since, to prevent the sample loss, rechromatography was avoided, the octamer fraction contained traces of hexasaccharides, which could be also discriminated by the present approach. Except for the regularly sulfated hexa- and octasaccharide domains, containing one sulfate group per disaccharide repeat, we have discovered five oversulfated structures and one undersulfated motif. Among these, the saturated tetrasulfated- IdoAGalNAc[GlcAGalNAc]$_2$ and pentasulfated- IdoAGalNAc[GlcAGalNAc]$_2$ document the oversulfation of the GAG chain reducing end. CID MS/MS of the $[M\text{-}3H^+]^{3-}$ detected at *m/z* 484.64, corresponding according to mass calculation to the tetrasulfated 4,5-Δ-IdoAGalNAc[GlcAGalNAc]$_2$ generated fragment ions consistent with a hexasaccharide motif having all GalNAc moieties and the first GlcA from the non-reducing end sulfated.

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