IDENTIFICATION AND STRUCTURAL CHARACTERIZATION OF CHONDROITIN SULFATE DISACCHARIDES IN HUMAN BIGLYCAN BY NANOELECTROSPRAY IONIZATION-ION MOBILITY TANDEM MASS SPECTROMETRY

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Abstract
We have implemented a superior glycomics method based on ion mobility separation (IMS) mass spectrometry (MS) and tandem MS (MS/MS) to characterize the chondoritin sulfate (CS) disaccharide domains in human biglycan (BGN). The high separation efficiency and sensitivity of IMS MS technique allowed the discrimination of five distinct CS motifs, of which four irregulated in their sulfation pattern. The structural investigation by IMS MS/MS disclosed that in one or both of the CS/DS chains, the non-reducing end is 3-*O*-sulfated GlcA in a rare bisulfated motif having the structure 3-*O*-sulfated -GlcA-4-*O*-sulfated GalNAc. Considering the role played by BGN in cancer cell spreading, the influence on this process of the newly identified sequences is to be investigated in the future.

Introduction
Biglycan (BGN) is a small leucine-rich repeat proteoglycan involved in a variety of pathological processes including malignant transformation, for which the upregulation of BGN was found related to cancer cell invasiveness. Since the functions and interactions of BGN are mediated by its chondroitin/dermatan sulfate (CS/DS) chains through the sulfates, the determination of CS/DS sulfation pattern is of major biological importance [1]. In the past years, ion mobility separation (IMS) combined with mass spectrometry (MS) has emerged as a reliable analytical platform able to separate and identify isomers, isobars, and conformers, thus decoding the structural information of functional components in biological mixtures [2]. In this context, in the present study we have implemented IMS MS and tandem MS (MS/MS) for the characterization of CS disaccharide domains in BGN, with a particular emphasis on the determination of the sulfation code *i.e.* the number of the sulfate groups and their exact location.

Experimental
CS/DS chains were released from HEK293 cells BGN by β-elimination and submitted to partial depolymerization with AC I lyase. The mixture was fractionated on a Superdex Peptide HR10/30 column. Following the purification, the pooled disaccharide fraction was dissolved in pure methanol to a concentration of 10 pmol/μL and infused by negative ion nanoelectrospray (nanoESI) into a Synapt G2S (Waters, Manchester, UK) mass spectrometer at a nanoESI potential of 1.4 kV, a cone voltage of 15 V, IMS gas flow 90 mL/min, IMS wave velocity 650

m/s and IMS wave height 40 V. MS/MS experiments were performed by collision induced dissociation (CID) at collision energies ramped from 25 to 35 eV.

Results and discussion

The CS disaccharide pool infused by (-) nanoESI was subjected to a two dimensional separation of the ions: the initial separation took place in the IMS sector according to the mobilities of the ions under the electric field, while the second separation occurred in the TOF analyzer according to *m/z* values of the ions. The generated ion mobilograms revealed that BGN: a) contains heterogeneous CS disaccharide domains and b) the species were separated not only according to their charge state but also to the number of the sulfate groups and the saturated (GlcA) or unsaturated (4,5-Δ-GlcA) type of glucuronic acid. Ten molecular ions corresponding to five distinct disaccharides differing in the number of sulfate groups and the saturated/unsaturated type of GlcA were discovered. As expected, the most abundant ions correspond to [4,5-Δ-GlcAGalNAc], the regularly sulfated unsaturated disaccharide, containing one SO_3 group. Except for the regularly sulfated species a non-sulfated [4,5-Δ-GlcAGalNAc] and a series of three oversulfated structures bearing two or three sulfate groups were also identified. According to mass calculation, the monodeprotonated species at *m/z* 538.021 corresponds to the unsaturated bisulfated-[4,5-Δ-GlcAGalNAc] whereas the $[M-H]^-$ detected as an ion of fair abundance at *m/z* 617.970 is attributable to the unsaturated trisulfated-[4,5-Δ-GlcAGalNAc]. The latter domain is among the CS disaccharides containing the highest number of sulfates ever detected by MS. Of a particular importance is the discovery of the saturated bisulfated-[GlcAGalNAc], which might originate from the terminus of the original chain. The detailed structural analysis carried out by IMS CID MS/MS disclosed that one or both of the non-reducing ends of BGN consist of an atypical oversulfated motif having the structure 3-*O*-sulfated GlcA-4-*O*- sulfated GalNAc.

Conclusion

We have reported here on the discovery of CS disaccharide domains in BGN by one of the most advanced glycomics methods based on IMS MS and CID MS/MS. Considering the role of BGN in the biological processes related to malignant transformation, the novel structural CS domains of irregular sulfation, discovered here, are to be further investigated from this perspective. Such domains may influence, by triggering or obstructing, the functional interactions of BGN involved in tumour growth.

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