Structural analysis of human decorin dermatan sulfate by nanoelectrospray ionization quadrupole time-of-flight tandem mass spectrometry

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Introduction

Decorin (DCN, Figure 1) is an extracellular matrix proteoglycan on average 90 -140 kDa in size. DCN belongs to the small leucine-rich proteoglycan (SLRP) family and consists of a protein core containing leucine repeats with a glycosaminoglycan (GAG) chain that can be either chondroitin sulfate (CS) or dermatan sulfate (DS).

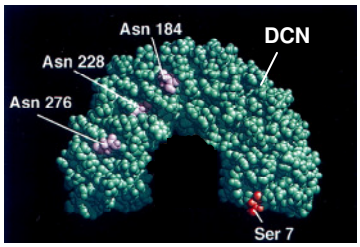


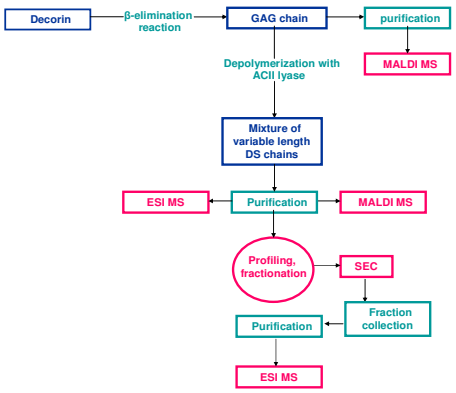
Figure 1. Model of the DCN. The glycosaminoglycan attachment site on Ser7 is in *red*, and the *N*-linked oligosaccharide attachment sites are in *purple*.

GAG primary structure is organized macroscopically by segregation into disaccharide repeats of specific sulfation patterns. CS is composed of glucuronic acid-(β-1, 4)-N-acetyl-galactosamine-(α-1, 4). Depending on tissue, the glucuronic acid (GlcA) can be epimerized to L-iduronic acid (L-IdoA) forming DS. For structure investigation of DCN CS/DS glycoforms, development of high performance specific methods was lately required, among which, electrospray ionization (ESI) mass spectrometry (MS) contributed essential progress to the field [1-4].

In this study the GAG chain from DCN secreted by human skin fibroblasts was released and depolymerized using chondroitin ACII lyase. After size exclusion separation, DS hexa-, octa and decasaccharide were pooled in one fraction, purified and analyzed by nanoESI quadrupole time-of-flight (QTOF) MS and tandem MS for gathering accurate data upon molecule epimerization, the sulfate content and its distribution.

Experimental

1. Strategy for DCN DS chain extraction and analysis



2. Mass Spectrometry

•NanoESI MS was performed on a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (QTOF Micro Quattro Ultima, Micromass Waters, Manchester, UK).
•All mass spectra were recorded in the negative ion mode.
•For sulfation assessment and delineating the real oversulfated species from non- and regularly sulfated ones, nanoESI conditions were optimized to enhance the formation and transfer into MS of intact molecular ions and prevent the in-source fragmentation of labile sulfate groups.
•MS/MS was performed by collision-induced dissociation (CID) at low ion acceleration energies using argon as a collision gas.
•Collision energy and gas pressure were readjusted during an ongoing experiment to provide the full set of fragment ions fingerprint for the localization of sulfate groups along the chain.

References
[1] Zaia, J. *Mass Spectrom. Rev.* 23, 161-227, 2004.
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[3] Hitchcock, A.M., Costello, C.E. and Zaia, J. *Biochemistry* 45, 2350-2361, 2006.
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Results and Discussion

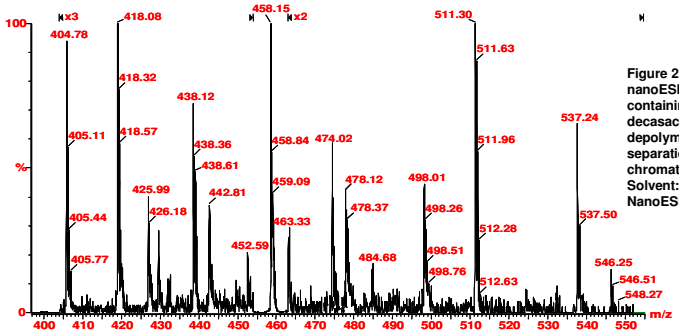

Figure 2. Negative ion mode nanoESI QTOF MS of the DS fraction containing hexa-, octa- and decasaccharides obtained after depolymerization with ACII lyase and separation by size-exclusion chromatography (SEC). Solvent: MeOH/H_2O/NH_4OAc; NanoESI: 650-780 V; Cone: 15-25V.

m/z	Type of ion	Composition
404.78	$[M-3H]^{3-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_2$](1S)
418.08	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_2$](2S)
425.99	$[M-5H]^{5-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_4$](3S)
438.12	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$](3S)
458.15	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$](4S)
474.02	$[M-5H]^{5-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_4$](6S)
478.12	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$](5S)
484.68	$[M-3H]^{3-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_2$](4S)
498.01	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$](6S)
511.30	$[M-3H]^{3-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_2$](5S)
537.24	$[M-4H]^{4-}$	[4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_4$](3S)
546.25	$[M-4H]^{4-}$	n.a

Table 1. Assignment of the molecular ions corresponding to DS species detected by nanoESI QTOF MS. The assignment indicates DS species of repeating (IdoAGalNAc) unit with terminal CS disaccharide (GlcAGalNAc). The CS disaccharide is bearing a double bond (4,5-Δ) induced by the specific eliminative action of chondroitin AC lyase.

blue = undersulfated species; purple = regularly sulfated species (1SO_3/disaccharide unit); red = oversulfated species; n.a = not assigned; nS = nSO_3

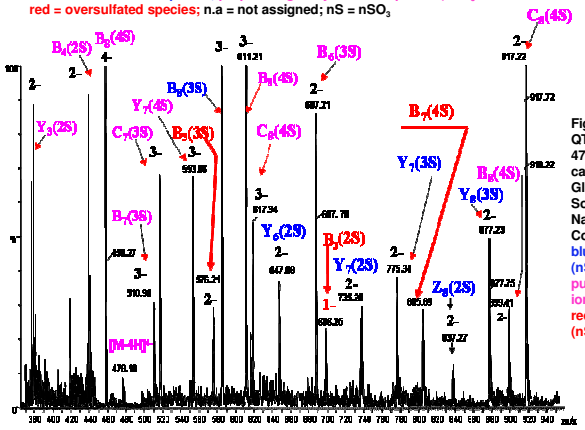

Figure 3. Negative ion mode nanoESI QTOF CID MS/MS of the $[M-4H]^{4-}$ ion at 478.12 assigned according to mass calculation to [4,5-Δ-GlcAGalNAc(IdoAGalNAc)$_3$](5S). Solvent: MeOH/H_2O/NH_4OAc; NanoESI: 650 V; Cone: 20 V. Collision energy (lab system): 4-40 eV
blue = undersulfated fragment ions (nS < $n_{disaccharides}$);
purple = regularly sulfated fragment ions (nS =$n_{disaccharides}$);
red = oversulfated fragment ions (nS>$n_{disaccharides}$).

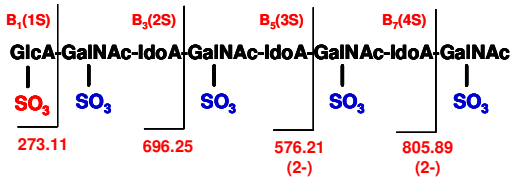

Figure 4. Structure of the pentasulfated octasaccharide as deduced from the spectrum in Figure 3. In red are highlighted the fragment ions diagnostic for SO_3 localization at GlcA.

Conclusions

•By nanoESI QTOF MS we were able to identify under-, regular and oversulfated hexa ,octa- and decasaccharide regions within the DS chain released from human skin fibroblast DCN.
•By tandem MS using collision-induced dissociation at low ion acceleration energies a fragmentation pattern indicating the localization of the additional sulfate group at the GlcA moiety in a pentasulfated octasaccharide was found.
•The corroborated data are consistent with the presence within the human decorin of DS motifs containing sulfated glucuronic acid.
•The obtained results show that the presence of irregular DS domains, playing important biological role, can be directly recognized by mass spectrometric screening and sequencing.

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