Negative Ion Electron Capture Dissociation of Synthetic Heparan Sulfate Oligosaccharides

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OVERVIEW

- Heparan sulfate (HS) oligosaccharides are structurally complex carbohydrates implicated in a myriad of biological activities [1].
- The inherent structural complexity has been attributed to variations in oligomer length, sulfo modifications and the C-5 uronic acid stereochemistry.
- Whereas most tandem mass spectrometric methods have been developed to assign sites of sulfo modifications, very few including electron detachment dissociation (EDD) have been successful at assigning stereochemical differences [2].
- Recent niECD of chondroitin and dermatan sulfate tetrasaccharides by the Håkansson's group demonstrated the ability to differentiate epimers in a single stage MS/MS [3].
- This work extends niECD to Heparan sulfate tetrasaccharides

METHODS

niECD MS/MS experiments were performed on a Bruker 12T SolariX FT-ICR mass spectrometer fitted with a hollow cathode as an electron source. Synthetic HS tetrasaccharides were directly infused at concentrations of 0.02 mg / mL using static nano spray and ionized in negative mode. Abundant singly deprotonated molecular ions of each HS oligomer were isolated in the quadrupole, accumulated in the hexapole for 1.0 to 4.0 s and transmitted to the ICR cell for niECD activation. Two isomeric HS oligomers were considered for this work

RESULTS AND DISCUSSIONS

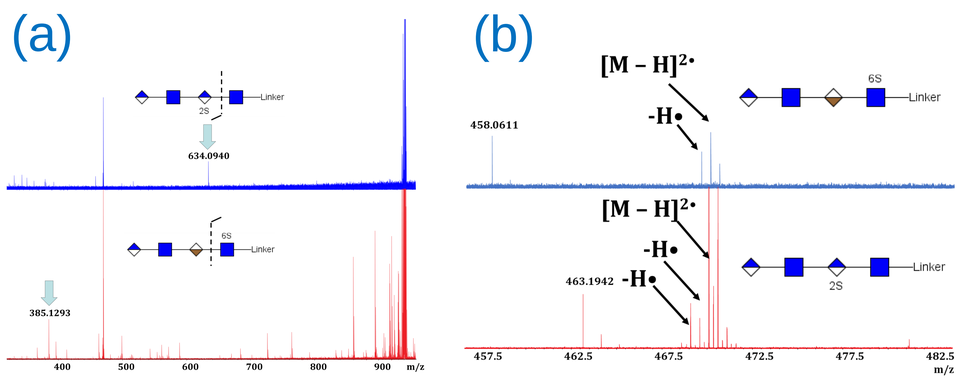

Figure 1. (a) niECD of HS tetrasaccharides GlcA-GlcNAc-GlcA2S-GlcNAc-$(CH_2)_5NH_2$) and (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$) (b) Expanded electron caption product ion region for the two HS tetrasaccharides.

ESI-MS of HS tetrasaccharides GlcA-GlcNAc-GlcA2S-GlcNAc-$(CH_2)_5NH_2$ and (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$) produced abundant singly deprotonated ions for the niECD experiment. Evidence of electron capture on the HS anionic precursor ion is confirmed by loss of H• and 2H• from the charged increase product ion [M – H]2-• (Fig 1b).

The two isomeric HS tetramers were differentiated using glycosidic bond cleavages. The niECD results of (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$) are discussed below.

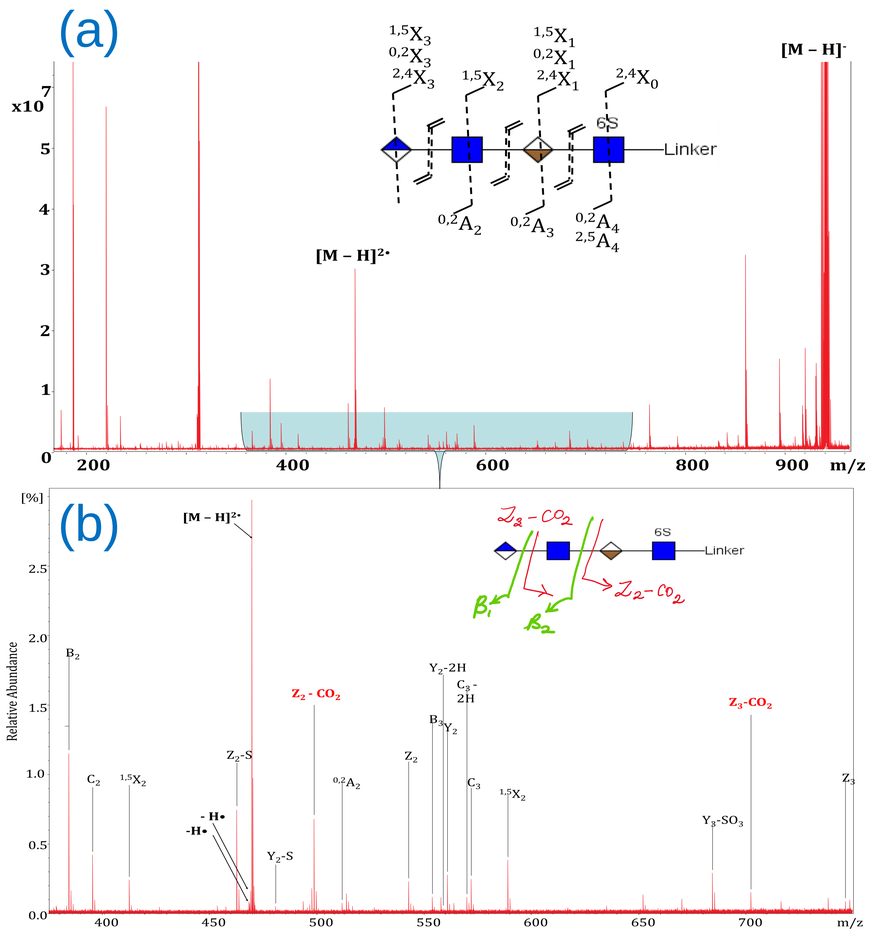

Figure 2. (a) Annotated niECD product ion structure for (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$) (b) Expanded annotated niECD spectrum for and (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$) (m/z 380 – 750).

The MS/MS generated abundant cross-ring fragments on both uronic acid residues including the non-reducing end N-acetylglucosamine unit (Fig 2). A full set of glycosidic product ions were observed, which afforded the unambiguous assignment of the sulfo group on the reducing end N-acetylglucosamine unit.
Unambiguous assignment of the 6-O sulfo group was achieved via a combination of cross-ring product ions.

Product ions associated with loss of low molecular weight species example (B_3'- CO_2) have proven to be diagnostic for glucuronic acid (GlcA) in differentiating heparan sulfate epimers via EDD activation.

Careful examination of the niECD MS/MS spectra confirmed earlier EDD reports showing the absence of (B_3'- CO_2) for the IdoA residue in the HS oligomer (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH_2)_5NH_2$)). Interestingly, we do observe CO_2 loss from the reducing end fragments Z_3 and Z_2 both containing the IdoA residue (Fig 2b). The position of the GlcA residue necessitates the use of non-reducing end (NRE) fragments for assigning possible CO_2 losses. None of the B and C type fragment ions containing the GlcA residue had CO_2 loss associated with them.

CONCLUSIONS

niECD of two synthetic HS tetrasaccharides has been achieved. Extensive niECD cross-ring and glycosidic product ions from (GlcA-GlcNAc-IdoA-GlcNAc6S-$(CH2)_5NH_2$) allowed the assignment of the 6-*O* sulfo position. Subsequent work will to explore the use of radical generated product ions for the assignment of the C-5 uronic acid stereochemistry.

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