Identification of Synthetic Heparan Sulfate Glycosaminoglycan Hexasaccharides Epimers by Capillary Zone Electrophoresis Negative Electron Transfer Dissociation Tandem Mass Spectrometry

UNIVERSITY OF GEORGIA

Marshall Liss[1]; Lauren Pepi[1]; Pradeep Chopra[2]; Geert-Jan Boons[2]; I. Jonathan Amster[1]

[1]University of Georgia, Chemistry Department, Athens, GA; [2]University of Georgia, Complex Carbohydrate Research Center, Athens, GA

Introduction

- Electron detachment dissociation (EDD) and negative electron transfer dissociation (NETD) have previously been demonstrated for analysis of highly sulfated glycosaminoglycans (GAGs)
- NETD rivals EDD fragmentation patterns but can easily be paired with separation techniques.
- Past work has addressed the ability of EDD to distinguish synthetic heparan sulfate (HS) hexamers differing in glucuronic acid (GlcA) vs iduronic acid (IdoA).
- MS/MS of HS hexamers in EDD and NETD produced abundant glycosidic products and cross-ring fragmentation.

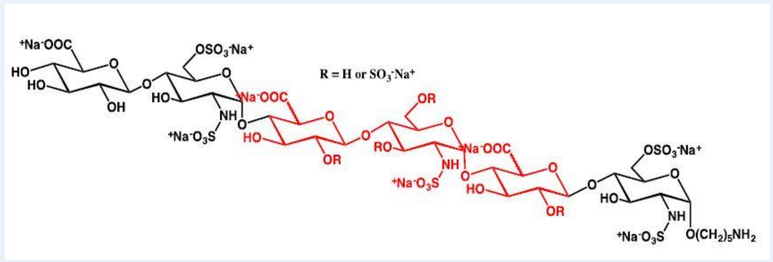

Figure 1. Backbone structure for synthetic HS epimers. Red highlights regions which sulfation and stereochemistry vary. Eighteen unique HS hexamers were synthesized using a modular approach and investigated for this work.

Experimental

- NETD and EDD experiments were performed on a 12T Bruker solariX XR FT-ICR MS. (Figure 2).
- Each sample was injected at a rate of 10 uL/h in 50:50 MeOH: H_2O and ionized by nanoESI at a concentration of 0.2mg/mL.
- Capillary-Zone Electrophoresis (CZE) -MS was performed on an Agilent G1600AX 3D CE coupled to a Thermo Scientific Velos Orbitrap Elite by an EMASS-II interface.

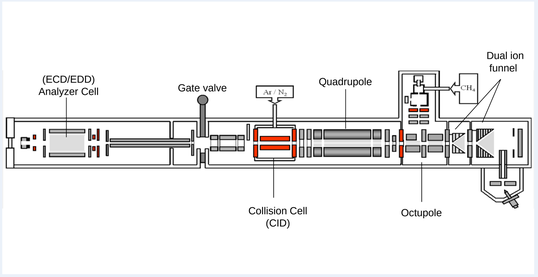

Figure 2. Diagram of the 12T Bruker solariX XR FT-ICR MS

Results and Discussion

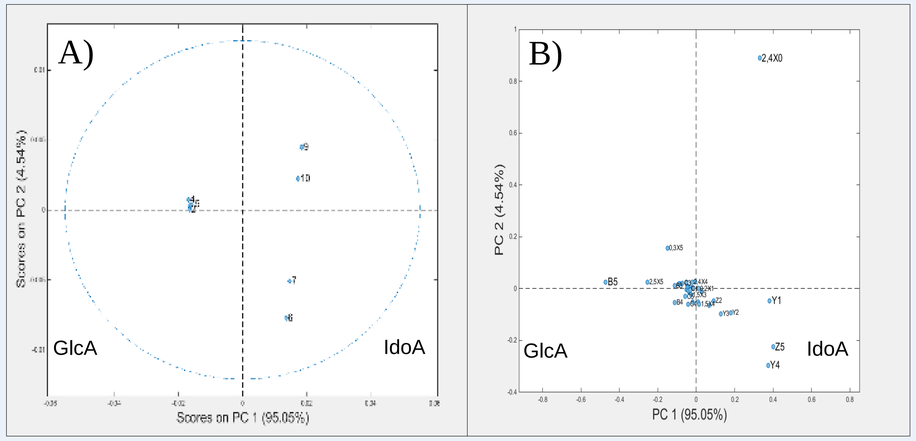

Figure 4. GlcA-GlcNS6S-IdoA-GlcNS6S-GlcA/IdoA-GlcNS6S-O$(CH_2)_5NH_2$ (A) scores plot and (B) loadings plot from a PCA analysis. Left cluster contains GlcA at reducing end, right cluster contains IdoA at reducing end. B_5 ions were found to be diagnostic for GlcA, Y_4, Z_5, Y_1 and $^{2,4}X_0$ ions to be diagnostic for IdoA

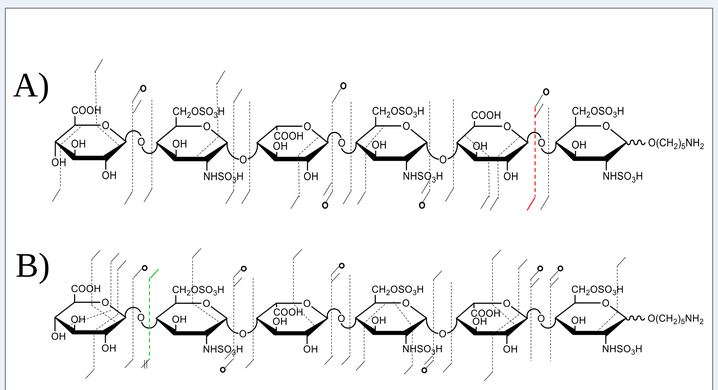

Figure 5. (A) GlcA sample fragment map using EDD fragmentation of precursor $[M-6H+Na]^{5-}$. B_5 fragment ion determined to be diagnostic for GlcA as the reducing end uronic. (B) IdoA sample fragment map using EDD fragmentation of precursor $[M-6H+Na]^{5-}$. The Z_5 fragment ion is determined by PCA to be diagnostic for IdoA.

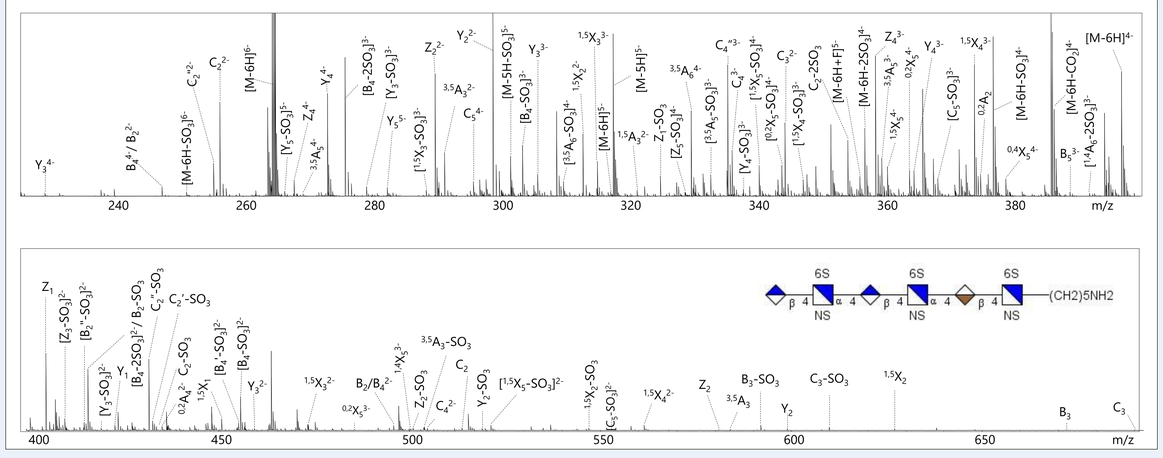

Figure 6. NETD of GlcA-GlcNS6S-GlcA-GlcNS6S-IdoA-GlcNS6S. Fragments found in the PCA of EDD spectra were also produced in this NETD spectrum such as Y_4, and B_5 ions.

EDD and NETD Analysis

- PCA analysis of EDD spectra shows the effectiveness of electron-based ion activation methods. NETD techniques produce similar fragmentation patterns with respect to cross-ring and glycosidic bond cleavages.
- Diversity of ions (shown as in Figure 4) are diagnostic for GlcA/IdoA.
- Due to scarce sample quantities and impure samples, further analysis is not effective without CZE separations.
- Radial repulsion between a negatively charged ion cloud and electron beam make EDD ineffective on short time scales

Capillary-Zone Electrophoresis

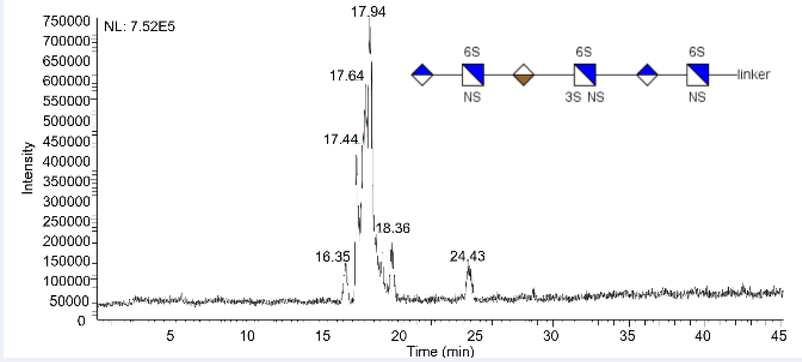

Figure 3. Electropherogram of a HS hexamer. Peak at 17.64 depicts the expected structure, with the 17.94 peak depicting one less sulfate from the expected structure.

- CZE separates analytes based on their differences in effective electrophoretic mobilities. Since glycosaminoglycans can be highly charged substances, CZE becomes an effective separation technique.
- HS Hexamer samples can contain other unwanted GAGs or organic compounds.
- CZE sample introduction requires minimal sample for tandem MS, (~1uL).

Conclusions

- EDD displays restricted capabilities when coupled with electrophoretic separation techniques such as CZE.
- NETD is an effective technique when used with shorter analyte time scales.
- CZE elucidates a method to purify and conserve samples.
- Future work will pair CZE sample introduction with NETD methods in FT-ICR MS utilizing PCA analysis.

References

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- Smith, R. D., Olivares, J. A., Nguyen, N. T., & Udseth, H. R. (1988). Capillary zone electrophoresis-mass spectrometry using an electrospray ionization interface. Analytical Chemistry, 60(5), 436–441. doi: 10.1021/ac00156a013

Acknowledgements

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