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Negative Electron Transfer Dissociation of Glycosaminoglycans

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Structural characterization of glycosaminoglycans (GAGs) has been a challenge in the field of mass spectrometry, and the application of electron detachment dissociation (EDD) Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) has shown great promise to GAG oligosaccharide characterization in a single tandem mass spectrometry experiment. In this work, we apply the technique of negative electron transfer dissociation (NETD) to GAGs on a commercial ion trap mass spectrometer. NETD of GAGs, using fluoranthene or xenon as the reagent gas, produces fragmentation very similar to previously observed EDD fragmentation. Using fluoranthene or xenon, both glycosidic and cross-ring cleavages are observed, as well as even- and odd-electron products. The loss of SO_3 can be minimized and an increase in cross-ring cleavages is observed if a negatively charged carboxylate is present during NETD, which can be controlled by the charge state or the addition of sodium. NETD effectively dissociates GAGs up to eight saccharides in length, but the low resolution of the ion trap makes assigning product ions difficult. Similar to EDD, NETD is also able to distinguish the epimers iduronic acid from glucuronic acid in heparan sulfate tetrasaccharides and suggests that a radical intermediate plays an important role in distinguishing these epimers. These results demonstrate that NETD is effective at characterizing GAG oligosaccharides in a single tandem mass spectrometry experiment on a widely available mass spectrometry platform.

Glycosaminoglycans (GAGs) are sulfated carbohydrates present in organisms ranging from bacteria to humans[1] that participate in many important biological processes including the regulation of biochemical pathways and disease progression.[2−9] GAGs are linear biomolecules, but complexity arises on the basis of a series of nontemplate-based enzymatic modifications including sulfation, N-modification of the amino sugar, and stereochemical modification of the hexuronic acid.[10] 1D and 2D NMR can be used to determine the type and location of GAG modification as well as hexuronic acid stereochemistry,[11] but the technique requires milligram quantities of a high purity sample. Mass spectrometry and tandem mass spectrometry (MS/MS) are viable alternatives to NMR-based GAG identification, as microgram quantities or less of a mixture can be used for analysis. A number of different mass spectrometry[12−18] and tandem mass spectrometry[19−21] techniques, including electron-based ion activation methods, have been developed for the analysis of sulfated GAGs. The oligomer length and extent of sulfation can be determined based on an accurate mass measurement, but MS/MS is necessary to determine the location of a modification within a saccharide ring. Tandem mass spectrometry of sulfated GAGs also presents an analytical challenge because the labile sulfate group is readily lost from the GAG

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during low-energy or threshold-type dissociation techniques, hampering efforts to locate this modification.

Electron detachment dissociation[22] (EDD), the negative ion complement of electron capture dissociation[23] (ECD), has recently been applied to the activation of acidic biomolecules for tandem mass spectrometry.[22,24−28] For EDD, multiply charged anions are irradiated with moderate energy (~19 eV) electrons, detaching an electron and producing a radical species. We have recently demonstrated the utility of EDD for the analysis of GAG oligosaccharides.[27] EDD produces more abundant glycosidic and cross-ring fragmentation than low energy or threshold-type dissociation methods and has been shown to effectively dissociate GAGs up to 10 saccharides in length,[29] suggesting GAG sequencing can be performed with a single MS/MS experiment. EDD has also been shown to distinguish glucuronic acid (GlcA) from iduronic acid (IdoA) in heparan sulfate tetrasaccharides.[30]

In their original inception, ECD and EDD could only be performed in the analyzer cell of a Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS), and the ability to fragment biomolecules and determine sites of modification on peptides and proteins made ECD a very desirable technique to adapt to less expensive mass spectrometers. Both ECD and EDD have been demonstrated on 3D ion traps modified with magnets to assist in electron trapping[31,32] and in a digital ion trap in the absence of any magnetic fields.[33] To overcome the difficulties of performing electron−ion reactions in the high pressure, RF region of the ion trap, separate traps that are external to the mass analyzer have been developed. For example, ECD has been demonstrated in linear ion traps to which both RF and magnetic fields were applied[34,35] and in a linear radio frequency-free cell using static electric and magnetic fields.[36]

To facilitate electron−ion reactions in quadrupole ion trap mass spectrometers, ion−ion reactions were developed to induce electron transfer. Electron transfer dissociation (ETD) utilizes radical anions produced in a chemical ionization (CI) source external to the trap.[37] Multiply charged precursor ions are reacted with the radical anions in a linear ion trap, and an electron is transferred from the radical anion to the precursor ion that generates an odd-electron species that undergoes further fragmentation. Compared to ECD, ETD produces similar fragmentation on a shorter time scale and, therefore, is ideally suited for applications such as HPLC, where time is limited.

Negative electron transfer dissociation (NETD) is the negative ion complement of ETD. The gas phase electron transfer from a multiply charged oligonucleotide anion to a rare gas cation was initially demonstrated by McLuckey and co-workers[38] and later applied to peptide anions.[39] In this later work, the utility of xenon as well as fluoranthene was examined as an electron acceptor. Abundant EDD-like fragmentation of a phosphopeptide was observed using xenon radical cations ($Xe^{+\bullet}$), while NETD using fluoranthene produced only loss of CO_2 from the precursor ion. The absence of EDD-like fragmentation was attributed to using protonated fluoranthene instead of the fluoranthene radical cation. Recently, Polfer and co-workers have examined the energetics of NETD between phosphopeptide anions and the radical cations of either fluoranthene or xenon.[40] NETD with fluoranthene retains the labile phosphorylation site whereas the reaction with xenon results in the neutral losses of CO_2 and H_3PO_4 and adds complexity to the spectrum. This result was rationalized as due to the difference in recombination energy between the two radical cations with xenon ~4 eV more energetic.

In addition to rare gas cations and fluoranthene, metal ions have also been employed to promote electron transfer. Negative electron transfer has been reported between the $[M - 5H]^{5-}$ charge state of insulin and Fe^{+}, resulting in disulfide bond cleavage and formation of the A-chain and B-chain anions.[41] Metal ion complexes with phenanthroline have also shown utility in accepting an electron from peptide anions, but this reaction can also result in proton transfer depending upon the reagent metal.[42]

In this paper, we describe the application of NETD to the dissociation of GAGs. This technique has wide application to the field of GAG sequencing and analysis because ion traps are significantly more prevalent than FTICR-MS mass spectrometers. We examine a number of factors that influence GAG dissociation (radical cation species, degree of sodiation, and oligomer length) and demonstrate the ability of NETD to determine the C5 stereochemistry of the hexuronic acid residues.

EXPERIMENTAL SECTION

Preparation of GAG Oligosaccharides. Dermatan sulfate (DS) tetrasaccharide and octasaccharide, Structures **1** and **2**, respectively (Chart 1), were prepared by partial enzymatic depolymerization of porcine intestinal mucosa dermatan sulfate (Celsus

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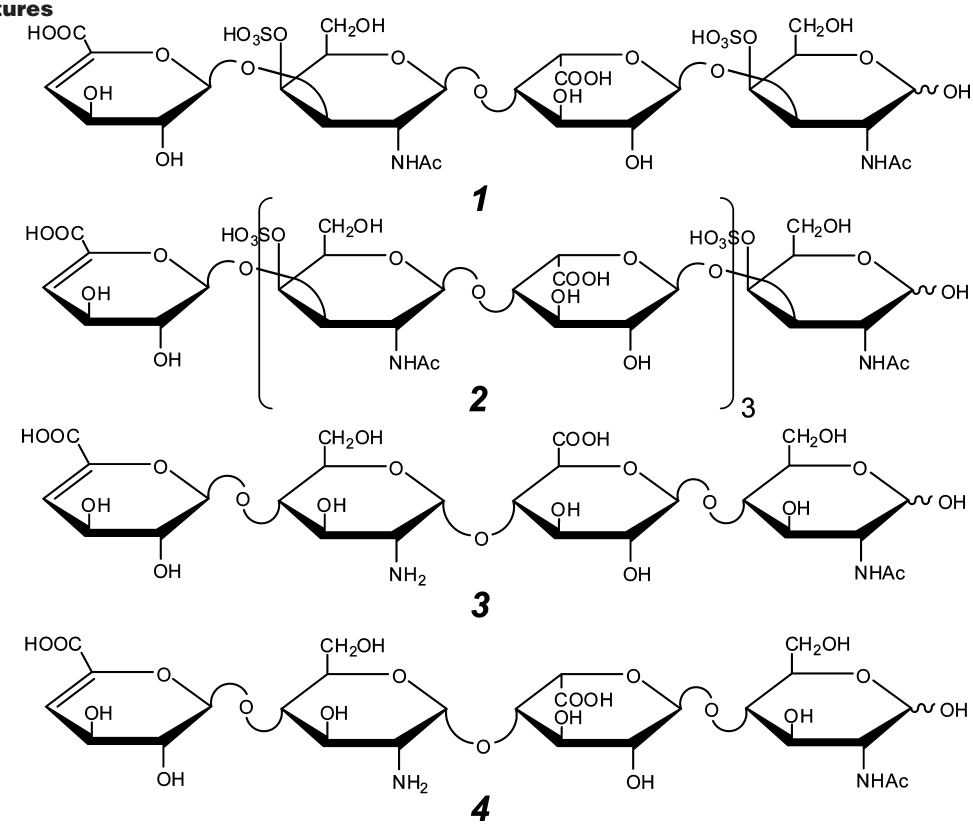
Chart 1. Structures



Laboratories, Cincinnati, OH). Heparan sulfate tetrasaccharide **3** was prepared by partial enzymatic depolymerization of heparan sulfate sodium salt (Celsus Laboratories, Cincinnati, OH). A 20 mg/mL dermatan sulfate or heparan sulfate solution in 50 mM Tris−HCl/60 mM sodium acetate buffer, pH 8, was incubated at 37 °C with chondroitin ABC lyase from *Proteus vulgaris*, EC 4.2.2.4. (Seikagaku, Japan) or heparinase II (Sigma, St. Louis, MO). After the absorbance at 232 nm indicated the digestion was 50% completed, the digestion mixture was heated at 100 °C for 3 min. High-molecular-weight oligosaccharides and the enzyme were removed by ultrafiltration using a 5000 MWCO membrane. The resulting oligosaccharide mixture was concentrated by rotary evaporation and fractionated by low pressure GPC on a Bio-Gel P10 (Bio-Rad, Richmond, CA) column. Fractions containing oligosaccharides **1**, **2**, and **3** were desalted by GPC on a Bio-Gel P2 column and freeze-dried.[43] Further purification of Structures **1**−**3** was carried out using strong anion exchange high-pressure liquid chromatography (SAX-HPLC) on a semipreparative SAX S5 Spherisorb column (Waters Corp, Milford, MA). The SAX-HPLC fractions containing >90% of Structures **1**−**3** were collected, desalted by GPC, and freeze-dried. The solid was reconstituted in water and purified a second time by SAX-HPLC. Only the top 30% of the chromatographic peak was collected, desalted, and freeze-dried. Concentration of the oligosaccharide solutions was determined by measuring the absorbance at 232 nm ($\varepsilon = 3800$ $M^{-1}cm^{-1}$). The resulting fractions containing individual Structures **1**, **2**, and **3** were characterized by polyacrylamide gel electrophoresis (PAGE), electrospray ionization (ESI)-MS, and high-field nuclear magnetic resonance (NMR) spectroscopy.[44] Structure **4** was prepared from ΔUA-GlcNSO$_3$-IdoA-GlcNAc (isolated from the preceding protocol) using the following protocol: ΔUA-GlcNSO$_3$-IdoA-GlcNAc sodium salt was converted to a pyridinium salt using a Dowex 50W cation exchange column (Sigma, St. Louis, MO). The pyridinium salt of ΔUA-GlcNSO$_3$-IdoA-GlcNAc (200 μg) was dissolved in 10 μL of dimethyl sulfoxide (Acros, Geel, Belgium) containing 5% methanol and incubated for 1.5 h at 50 °C.[45] The desulfated product, Structure **4**, was then purified on a Bio-Rad P-2 spin column and freeze-dried.[45] The product of the desulfation reaction differs in mass from the starting material and can be easily isolated from each other for the EDD experiment. The desulfation reaction does not affect the stereochemistry of the hexuronic acid residue, and so, the chirality of the product is the same as that of the reactant as established by NMR analysis.

Mass Spectrometry Analysis. Experiments were performed on a HCT PTM Discovery ion trap (Bruker Daltonik, Bremen Germany). This ion trap is a 3-D high capacity ion trap fitted with the ETD module. The GAGs were introduced at a concentration of 5−10 μM in 50:50:0.1 methanol/H_2O/formic acid (Sigma, St. Louis, MO) by nano ESI in negative ion mode. Formic acid was added to reduce Na/H heterogeneity during the ESI of each GAG analyte.[48] Samples were infused at a flow

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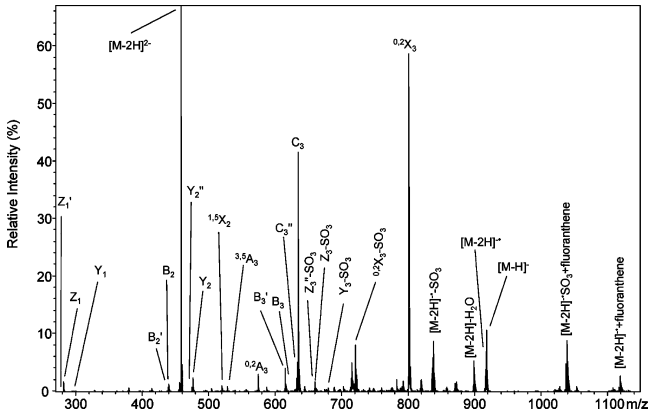

Figure 1. NETD of the $[M - 2H]^{2-}$ precursor ion of Structure **1** using fluoranthene as the radical cation. The NETD spectrum demonstrates abundant glycosidic and cross-ring bond cleavage, similar to the published EDD for the same precursor ion.

rate of 300 nL/min using a pulled fused silica tip (FS360−75−15, New Objective, Woburn, MA). For experiments requiring the addition of sodium, the solution was modified to contain 10 mM sodium acetate. For ETD experiments, precursor anions were accumulated in the trap for 10−500 ms and were then isolated with a 4 m/z window. The reagent cation, either fluoranthene or xenon produced external to the ion trap in the CI source, was admitted to the trap for 50−500 ms and allowed to react with the precursor anions. The ion trap was scanned from m/z 150−1500 for GAG tetrasaccharides (**1**, **3**, and **4**), and m/z 150−2000 for the dermatan sulfate octasaccharide (**2**). Spectra were acquired for 5−10 min and then averaged using Bruker DataAnalysis 4.0 (Billerica, MA) to produce a single mass spectrum. For background spectra, all NETD conditions were maintained, but the voltages on the CI source were set to block transmission of the fluoranthene or xenon radical cations. For the work presented here, fragmentation of the oligosaccharides is presented using a modification of the Domon and Costello annotation[46] that presents GAG fragmentation with SO_3 loss and hydrogen rearrangement that is observed in EDD of GAGs.[29]

RESULTS AND DISCUSSION

NETD of Dermatan Sulfate Tetrasaccharide with Fluoranthene. NETD of the $[M - 2H]^{2-}$ precursor ion of dermatan sulfate dp4, Structure **1**, is shown in Figure 1. The product ions assigned from the NETD fragmentation are shown in Figure 2A. The charge reduced species, $[M - 2H]^{-\bullet}$, is observed, as well as $[M - H]^-$, presumably from a hydrogen transfer reaction between the reactive odd-electron charged reduced species and water present as a background neutral in the ion-trap instrument, although it is possible that it could arise from a proton transfer reaction from the fluoranthene radical cation and the dp4 dianion. The observation of both such products when xenon radical cation is used for NETD strongly suggests that this product results from the former channel rather than the latter. The EDD mass spectrum of the $[M - 2H]^{2-}$ precursor ion of Structure **1** has been previously published,[29] and products observed from EDD from the $[M - 2H]^{2-}$ precursor ion of Structure **1** are shown in Figure 2B for comparison purposes. When compared to EDD of Structure **1**, NETD of Structure **1** produces remarkably similar dissociation. Both glycosidic and cross-ring fragmentation is observed using both NETD and EDD, and many glycosidic bond cleavages are accompanied by the loss of 1 or 2 hydrogen atoms. For example, for both NETD and EDD of Structure **1**, the B_2 is accompanied by the loss of 1 hydrogen atom, labeled B_2' in Figure 1, and the C_3 glycosidic cleavage is accompanied by the loss of 2 hydrogen atoms, labeled C_3''. Odd electron products, such as the $^{0,2}X_3$ cross-ring cleavage, are also observed with NETD. Product ions accompanied by SO_3 loss, indicated by the hatch mark with the open circle at the end, are observed in both the NETD and EDD mass spectrum of Structure **1** and occur primarily near the nonreducing end. Similar to EDD, no fragmentation of the reducing end of Structure **1** is observed.

NETD of Structure **1** also produces a few different products than are found in the EDD spectrum, in particular the ions at m/z 1116.1 and m/z 1036.2, which are assigned as fluoranthene adducts of the charge reduced species without and with SO_3 loss, respectively. No other product ions accompanied by fluoranthene addition are observed. Fewer product ions that can be assigned as glycosidic cleavages are observed in the NETD mass spectrum when compared to the EDD mass spectrum. For example, the B_1 and C_1 glycosidic cleavages are not observed even though the mass spectrometer is set to detect down to m/z 150. No doubly charged product ions are observed, and less cross-ring fragmentation of the IdoA residue next to the reducing end is observed.

We have previously proposed a mechanism for GAG dissociation by EDD based on the observed product ions.[27,30] In EDD, multiply charged GAG anions are excited by 19 eV electrons, producing an excited precursor ion. The excited precursor ion can undergo electron detachment to produce a radical species that may undergo further fragmentation. Alternatively, the excited anion can undergo dissociation through the process of EID. Our previous work with EID of GAG tetrasaccharides demonstrated that both even- and odd-electron product ions can result from electronic excitation of the precursor ion.[47] For NETD, electron transfer from the precursor ion to the radical cation produces a radical GAG anion, and therefore, products can only arise from dissociation of the odd-electron precursor ion. The products formed via EID and NETD offer insight into the mechanism of EDD. Many of the product ions are observed in both the EID and NETD mass spectra, indicating that these products can form via either mechanism. For example, the odd-electron product $^{0,2}X_3$ and all the glycosidic bond cleavages are observed in both the NETD and EID mass spectra and are, therefore, proposed to form via either pathway. However, some of the product ions are observed exclusively by NETD or EID, providing a method to distinguish products of electron detachment from electron excitation. Such products include the $^{0,2}X_1$ and $^{1,5}X_1$ product ions that are observed only in the EID and EDD mass spectra and are, thus, proposed to arise only through electronic excitation. Conversely, the $^{0,2}A_3$ product ion is only observed in the EDD and NETD mass spectra and is proposed to arise through electron detachment fragmentation.

We have previously investigated the influence of precursor ion charge state and sodium adduction on the EDD products of dermatan sulfate glycosaminoglycans.[48] Briefly, we demonstrated

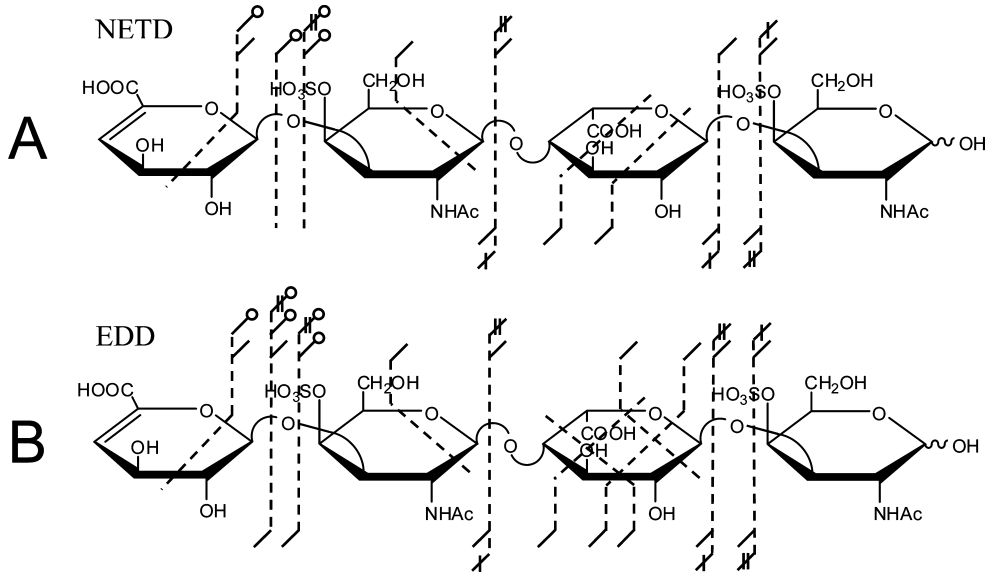

Figure 2. Product ions assigned from the (A) NETD and (B) EDD of the $[M - 2H]^{2-}$ precursor ion of Structure **1**. The EDD spectrum of Structure **1** has been previously published. Similar fragmentation is observed for both activation methods, although two cross-ring cleavages are absent in the NETD spectrum. The C_2 and Z_2 cleavages are not assigned because they are isobaric with the precursor ion.

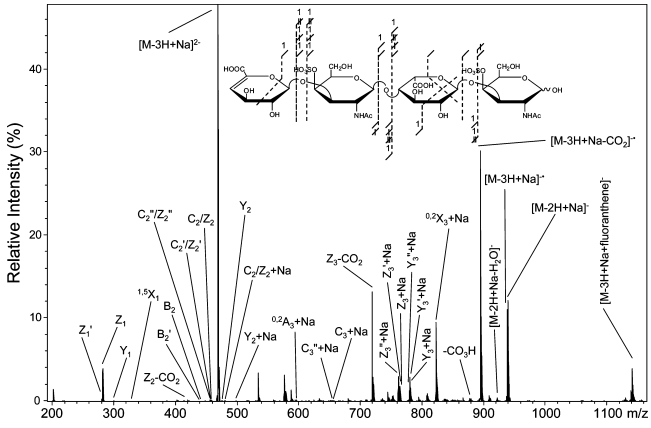

Figure 3. NETD of the $[M - 3H + Na]^{2-}$ precursor ion of Structure **1** using fluoranthene as the radical cation. Products assigned to the observed fragmentation are shown in the inset. Selection of a precursor ion in which all sulfates are ionized or paired with a Na minimizes the loss of SO_3.

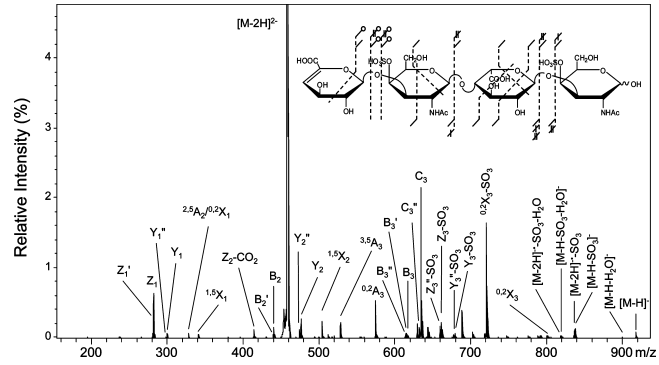

Figure 4. NETD of the $[M - 2H]^{2-}$ precursor ion of Structure **1** using xenon as the radical cation. Products assigned to the observed fragmentation are shown in the inset. Although the use of xenon as a reagent gas increases the number of cross-ring cleavages observed, SO_3 loss is also increased.

that SO_3-loss was minimized and cross-ring products maximized when the number of sodium atoms plus the charge state is one greater than the number of sulfate groups. This was proposed to occur due to preferential electron detachment from a carboxylate versus a sulfate anion. NETD of the $[M - 3H + Na]^{2-}$ precursor ion of Structure **1** was performed to determine if similar behavior is observed. The product ion mass spectrum from NETD of the $[M - 3H + Na]^{2-}$ precursor of Structure **1** is shown in Figure 3, and product ions assigned to the NETD fragmentation are shown in Figure 3, inset. When compared to the product ions observed from NETD of the $[M - 2H]^{2-}$ precursor ion of Structure **1** shown in Figure 1, NETD of the $[M - 3H + Na]^{2-}$ precursor ion produces similar glycosidic and cross-ring cleavages, and no product ions accompanied by the loss of SO_3 are observed. The suppression of SO_3 loss is similar to the observations from EDD of dermatan sulfate octasaccharides.[48]

NETD of Dermatan Sulfate Tetrasaccharide with Xenon. NETD has been previously reported using xenon radical cations as the electron acceptor for NETD experiments.[39,40] NETD of the $[M - 2H]^{2-}$ precursor ion of Structure **1** using $Xe^{+\bullet}$ as the electron acceptor is shown in Figure 4, and the product ions assigned to the fragments are shown in Figure 4, inset. When compared to NETD of Structure **1** with fluoranthene (Figure 1), xenon NETD produces similar glycosidic bond cleavages. There are a number of differences in the product ions using xenon as the reagent gas. For example, no reagent ion adduct species (e.g., $[M - 2H + Xe]^{-\bullet}$) is observed. While more cross-ring cleavages are observed with the xenon reagent gas, more product ions accompanied by the loss of SO_3 are observed. Also, the product ions accompanied by SO_3 loss are typically greater in intensity than product ions unaccompanied by SO_3 loss. For example, the cross-ring cleavage $^{0,2}X_3$-SO_3 is significantly more abundant than the product ion $^{0,2}X_3$ when Xe is used as the electron acceptor, as shown in Figure 4. In contrast, the $^{0,2}X_3$ product is significantly more abundant than the $^{0,2}X_3$-SO_3 product ion when fluoranthene is used as the electron acceptor, as shown in

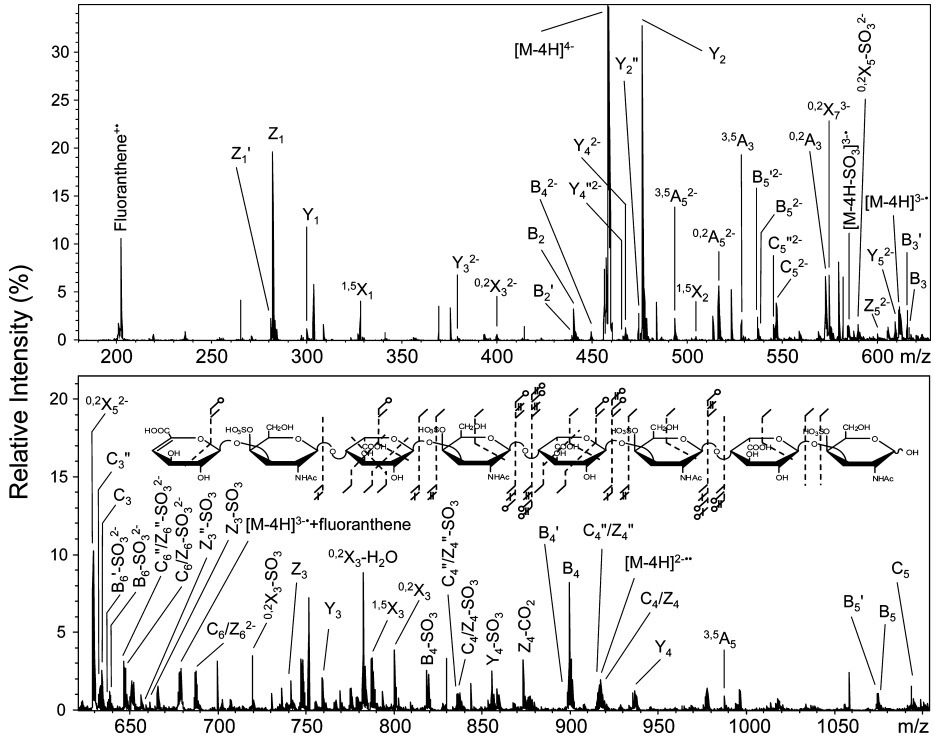

Figure 5. NETD of the $[M - 4H]^{4-}$ precursor ion of Structure **2** using fluoranthene as the radical cation. The *m*/*z* axis is divided into two sections for clarity, *m*/*z* 180−630 (top) and *m*/*z* 620−2000 (bottom). Inset: observed products from the NETD data of Structure **2**. NETD is shown to produce glycosidic and cross-ring fragmentation in an octasaccharide, but the number of confident assignments is limited due to the limited mass resolution in an ion trap.

Figure 1. These results can be rationalized by the thermodynamics of the ion−ion reaction and are consistent with the observations of Polfer and co-workers.[40] The ionization energy of xenon is 12.1 eV, while the ionization energy of a fluoranthene cation is 7.9 eV.[49] Therefore, recombination of an electron with $Xe^{+\bullet}$ will produce approximately 4 eV more energy than recombination of an electron with a fluoranthene radical cation. The additional 4 eV of energy produces greater fragmentation of the most labile bond, namely the sulfate half ester, and dissociation of this bond will lead to loss of SO_3, observed when xenon is used as the radical cation.

NETD of Dermatan Sulfate Octasaccharide. EDD has been shown to effectively fragment GAGs up to 10 saccharides in length.[29] In this prior work, we demonstrated that EDD produced more abundant fragmentation with far less SO_3 loss when compared to infrared multiphoton dissociation (IRMPD) of the same precursor ions, giving EDD the potential to characterize a GAG with a single tandem mass spectrometry experiment. The mass spectrum from NETD of the $[M - 4H]^{4-}$ precursor ion of Structure **2** with fluoranthene is shown in Figure 5. Products identified from NETD are shown in the inset of Figure 5; product ions observed from EDD of the same precursor ion have been previously published.[29] While abundant fragmentation is observed from NETD of Structure **2**, the low resolution of the ion trap makes identifying isobaric species difficult, and therefore, many product ions cannot be assigned. For example, the *m*/*z* 775−800 region of NETD mass spectrum of Structure **2** contains at least six product ions. Only three of these product ions can be confidently assigned, specifically the ones with high abundance and low charge state, and a number of the remaining product ions cannot be assigned due to their overlapping isotope patterns. For larger oligosaccharides, the high resolving power and mass accuracy of FTMS is necessary to resolve and identify the product ions produced by NETD and EDD fragmentation.

NETD of Heparan Sulfate Epimers. We have previously reported the ability to distinguish IdoA from GlcA in heparan sulfate tetrasaccharides using EDD.[30] The presence of $^{0,2}A_3$, B_3', and B_3'-CO_2 product ions in the EDD mass spectrum indicate the presence of the GlcA saccharide, while the absence of these product ions indicates the presence of IdoA. The ability to distinguish IdoA from GlcA is proposed to occur through a radical intermediate that permits transfer of a local hydrogen atom to the carboxy radical, with further fragmentation producing the differences in the observed product ions. These diagnostic products were proposed to form through a radical intermediate, and our previous work with EID of GAG epimers suggested that the radical intermediate plays a significant role in producing these product ions.[47] The radical species produced by NETD can be used to probe the importance of the proposed radical intermediate in distinguishing IdoA from GlcA.

NETD of ΔUA-GlcN-GlcA-GlcNAc, Structure **3**, with fluoranthene is shown in Figure 6A, while NETD of its epimer ΔUA-GlcN-IdoA-GlcNAc, Structure **4**, with fluoranthene is shown in Figure 6B. A zoom of the region of interest is shown in Figure 6, and the product ions assigned from NETD of Structures **3** and **4** are shown in the insets of Figure 6A,B, respectively. The NETD spectra of Structures **3** and **4** are similar to the previously reported EDD spectra. Similar to the EDD mass spectra, the $^{0,2}A_3$, B_3', and B_3'-CO_2 product ions are observed in the NETD mass spectrum

(49) Bartmess, J. E. In *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; Linstrom, P. J., Mallard, W. G., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, 2009.

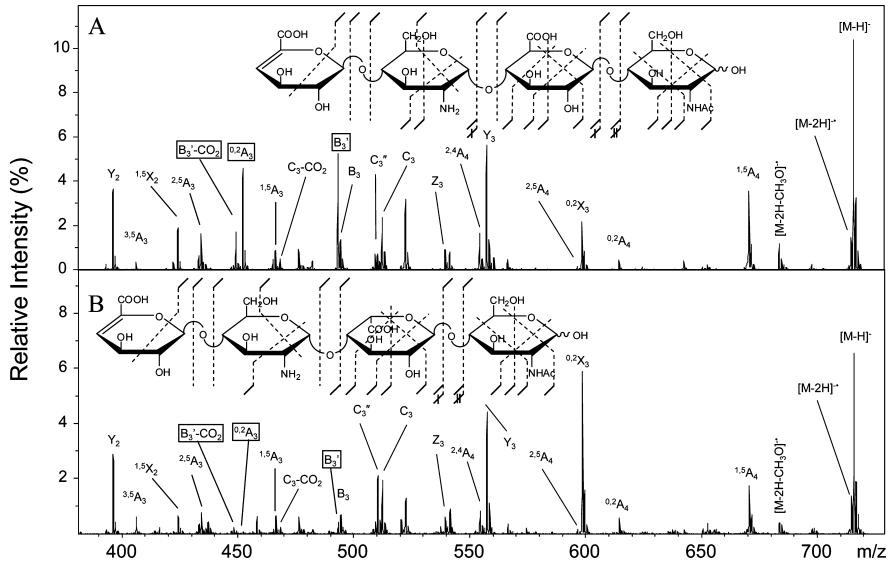

Figure 6. NETD of the $[M - 2H]^{2-}$ precursor ion of the epimer pair Structures (A) **3** and (B) **4** using fluoranthene. The *m*/*z* scale is zoomed to the region of interest where product ions used to distinguish GlcA from IdoA are observed, which are enclosed in a box. Insets: structures indicating observed product ions from NETD of Structures (A) **3** and (B) **4**. Previously observed cleavages indicative of hexuronic stereochemistry during EDD are also present in the NETD spectrum.

of Structure **3**, the tetrasaccharide containing GlcA. If the B_3' is observed in the NETD spectrum of Structure **4**, it is less abundant than the B_3 product ion, where the B_3' product ion is significantly more abundant than the B_3 product ion in NETD of the GlcA containing tetrasaccharide, Structure **3**. B_3'-CO_2 and $^{0,2}A_3$ are also observed in the NETD spectrum of Structure **4** but occur as minor products when compared to their respective intensities in Structure **3**. EID of Structures **3** and **4** were unable to distinguish IdoA from GlcA,[47] while NETD is able to distinguish these epimers. This data suggest that it is the radical intermediate, and not electronic excitation, which produces the diagnostic product ions. Alternatively, dissociation through the radical intermediate may occur much faster than electronic excitation to produce the diagnostic product ions. The similarity of the NETD spectra to the EDD spectra demonstrates the importance of the radical intermediate in generating the products that distinguish IdoA from GlcA.

CONCLUSIONS

NETD has been shown to effectively dissociate GAG oligosaccharides, producing abundant and useful fragmentation very similar to EDD. Glycosidic and cross-ring fragmentation is observed, in the form of both even- and odd-electron ions. While SO_3 loss is observed using NETD, SO_3 loss can be minimized through the addition of sodium atoms, or increasing the charge state, to produce a negatively charged carboxylic acid. Both xenon and fluoranthene work well in NETD as the reagent gas, but loss of SO_3 is less pronounced when fluoranthene is used as the reagent gas, while xenon produces a higher yield of product ions, as expected from its higher recombination energy. Similar to EDD, NETD is able to distinguish the epimers GlcA from IdoA in heparan sulfate tetrasaccharides. Production of the diagnostic product ions (B_3', B_3'-CO_2, and $^{0,2}A_3$ only in GlcA) suggests the importance of the radical intermediate in producing these ions.

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