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Electron capture dissociation, electron detachment dissociation and infrared multiphoton dissociation of sucrose octasulfate

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The structural analysis of sulfated carbohydrates such as glycosaminoglycans (GAGs) has been a long-standing challenge for the field of mass spectrometry. The dissociation of sulfated carbohydrates by collisionally-activated dissociation (CAD) or infrared multiphoton dissociation (IRMPD), which activate ions via vibrational excitation, typically result in few cleavages and abundant SO_3 loss for highly sulfated GAGs such as heparin and heparan sulfate, hampering efforts to determine sites of modification. The recent application of electron activation techniques, specifically electron capture dissociation (ECD) and electron detachment dissociation (EDD), provides a marked improvement for the mass spectrometry characterization of GAGs. In this work, we compare ECD, EDD and IRMPD for the dissociation of the highly sulfated carbohydrate sucrose octasulfate (SOS). Both positive and negative multiply-charged ions are investigated. ECD, EDD and IRMPD of SOS produce abundant and reproducible fragmentation. The product ions produced by ECD are quite different than those produced by IRMPD of SOS positive ions, suggesting different dissociation mechanisms as a result of electronic versus vibrational excitation. The product ions produced by EDD and IRMPD of SOS negative ions also differ from each other. Evidence for SO_3 rearrangement exists in the negative ion IRMPD data, complicating the assignment of product ions.

Keywords: carbohydrate, sulfation, electron capture dissociation, electron detachment dissociation, infrared multiphoton dissociation, Fourier transform mass spectrometry

Introduction

The structural characterization of sulfated carbohydrates such as glycosaminoglycans (GAGs) has been a long-standing problem for mass spectrometry. Sulfate half-ester modifications in carbohydrates are labile and difficult to characterize by tandem mass spectrometry (MS/MS). A number of mass spectrometry and MS/MS spectrometry techniques have been developed for the analysis of this class of molecules.[1–23] The challenge of determining sites of modification in GAGs by MS/MS spectrometry is to produce abundant glycosidic and cross-ring fragmentation without loss of the labile sulfate group, a challenge similar to determining sites of post translational modification on proteins. The development of electron capture dissociation (ECD)[24] and its negative ion complement electron detachment dissociation (EDD),[25] has greatly increased the

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analytical utility of MS/MS for characterizing labile modifications in biomolecules.

Recently, we have reported the utility and application of EDD for the structural analysis of GAGs.[26] EDD has been shown to be a powerful tool for determining the sites of modification of GAGs ranging in size from tetrasaccharides to decasaccharides.[27] EDD can also distinguish iduronic acid from glucuronic acid in GAG tetrasaccharides based on the presence of key product ions.[28] ECD has been shown to be useful for determining sites of post-translational modification in peptides and proteins, including sulfation.[29–34] However, ECD requires multiply-charged positive precursor ions, which are often difficult to produce from highly acidic molecules such as GAGs. In order to form multiply-charged positive ions suitable for analysis by ECD, carbohydrates have been complexed with divalent metal ions.[35] However, multiply-charged positive ions can also be produced with monovalent cations such as sodium. In this work we make a comparison of ECD and EDD for the model sulfated disaccharide, sucrose octasulfate (SOS), which can form both positive and negative multiply-charged ions. In addition, we compare these data with those obtained by infrared multiphoton dissociation (IRMPD) of the same precursors.

Materials and methods

Pharmaceutical purity sucrose octasulfate (sodium salt) was a gift from Bukh Meditec (Farum, Denmark). Experiments were performed with a 9.4 T Bruker Apex IV QhFTMS (Billerica, MA, USA) fitted with an Apollo II dual source, a 25 W CO_2 laser (Synrad model J48-2, Mukilteo, WA, USA) for IRMPD and an indirectly heated hollow cathode for generating electrons for ECD and EDD. For positive ion analyses, SOS was diluted to a concentration of 1 mg mL^{-1} in 50 : 50 methanol : H_2O (Sigma, St Louis, MO, USA). For negative ion analyses, SOS was diluted to a concentration of 0.025 mg mL^{-1} in 50 : 50 methanol : H_2O (Sigma, St Louis, MO, USA). All samples were analyzed using ESI at an infusion rate of 2 µL min^{-1}. For ECD, EDD and IRMPD experiments, multiply-charged precursor ions were selected in the external quadrupole and stored in the external hexapole for 1–6 s before injection into the FT-MS analyzer. For ECD, the multiply-charged precursor ions were irradiated for 0.05 s with the cathode set to –1.5 V, the ECD lens was set to 15 V and the heater current was set to 1.5 A. For EDD, the multiply-charged precursor ions were irradiated for 1 s with the cathode set to –19 V, the ECD Lens set to –19 ± 0.5 V and the heater current set to 1.5 A. For IRMPD experiments, conditions were similar to the ECD/EDD experiments but the electron pulse was replaced with the laser pulse. For IRMPD, ions were irradiated for 0.01–0.2 s with beam attenuation set to pass from 40–60% of full power. Ions were excited with an RF frequency chip that covered the range *m/z* 100–2000. Twenty four acquisitions were co-added for each mass spectrum. 512 K points were acquired at a 2.4 MHz digitization rate, padded with one zero fill and apodized using a sinebell window. For the work presented here, fragmentation of the tetrasaccharides is presented using the Domon and Costello annotation.[36]

Results and discussion

SOS contains eight acidic sulfate half-esters. While this highly acidic molecule readily forms multiply-charged negative ions,[7] the formation of positive ions requires cationization, for example, by using sodium as a counter ion for the sulfate groups. With all eight sulfate groups ion cationized by sodium ions, the doubly-charged species of SOS, $[M-8H+10Na]^{2+}$, is observed as shown in Figure 1(a). Using a more dilute sample, SOS forms the multiply-charged negative ion $[M-8H+6Na]^{2-}$, as shown in Figure 1(b). The use of sodiated SOS allows the production of both multiply-charged positive and negative ions for tandem mass spectrometry experiments.

IRMPD and ECD of SOS positive ions

IRMPD of the $[M-8H+10Na]^{2+}$ precursor ion of SOS produces the mass spectrum shown in Figure 2(a) (peak list and intensities available in supplemental data). IRMPD produces only singly-charged even-electron product ions, as shown in Figure 2(a). The most abundant product is the ion at *m/z* 1180.626, resulting from loss of Na^+ from the precursor to yield $[M-8H+9Na]^+$. The peaks at *m/z* 1100.659, *m/z* 1020.701 and *m/z* 940.749 differ from the $[M-8H+9Na]^+$ product ion by multiples of the exact mass of SO_3, 79.956 u, indicating loss of up to three equivalents of SO_3.

Other peaks in the IRMPD mass spectrum are difficult to assign and do not seem to correspond to common glycosidic or cross-ring cleavage products. The IRMPD mass spectrum was internally calibrated from the assignable peaks mentioned above to provide mass accuracy better than 2 ppm. Many products in the IRMPD mass spectrum cannot be assigned even with accurate mass data. In an attempt to identify the observed products, the *m/z* value were calculated for cross-ring cleavages that have been observed in MS/MS spectra of glycosaminoglycan oligosaccharides and the values were compared to the data from the IRMPD mass spectrum, but none of these calculated products were observed. For example, the peak at *m/z* 796.704 [indicated by the asterisk over the peak in Figure 2(a)] cannot be assigned, as the $^{0,3}X_1$–SO_3 cleavage (theoretical *m/z* = 796.731) due to the large mass error of 35 ppm, well outside the mass accuracy of 2 ppm expected for an internal calibration. Similarly, the products at *m/z* 590.716, *m/z* 568.734 and *m/z* 546.751 were tentatively identified as B_1/Y_1, B_1/Y_1–Na and B_1/Y_1–2Na, respectively, prior to internal calibration. However, attempts to use any of these products for internal calibration resulted in large mass calibration errors indicating that these are not arising from the expected glycosidic cleavages. Despite the small number of identified products, a number of patterns exist in the IRMPD data. Many of the product ions in the IRMPD

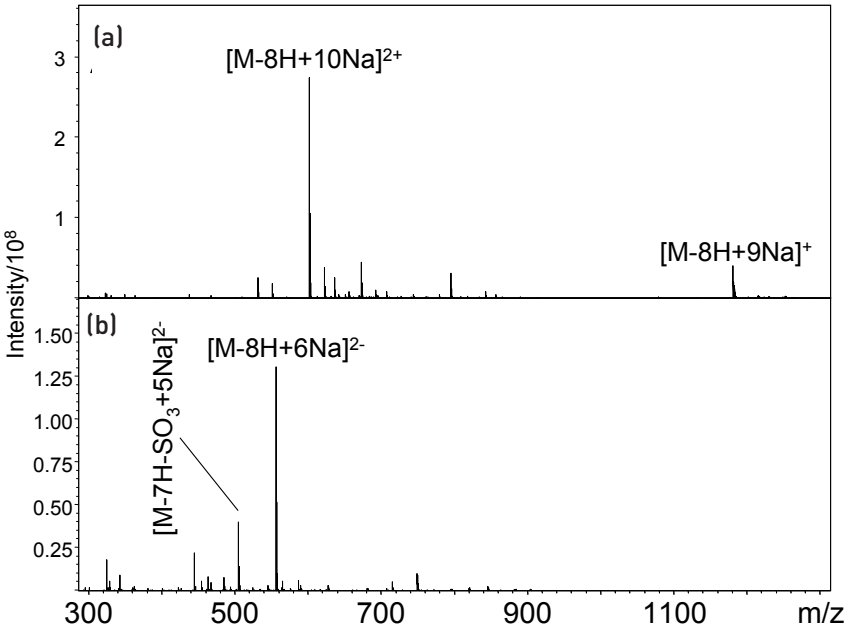


Figure 1. Electrospray ionization Fourier transform ion cyclotron resoanance mass spectra of SOS in (a) positive ion mode and (b) negative ion mode. Sodium allows abundant multiply-charged precursor ions to be produced by both positive and negative ionization.

mass spectrum differ by the exact mass of SO_3, resulting from the sequential loss of this labile group. Also, a number of peaks in the IRMPD mass spectrum differ by 21.982 u, implying Na/H heterogeneity in the product ions. These products are unusual because all ionizable hydrogen atoms have been replaced by sodium atoms in solution. Therefore, these peaks indicate that Na/H exchange occurs at carbon–hydrogen bonds during fragmentation.

The ECD mass spectrum of the $[M-8H+10Na]^{2+}$ precursor ion of SOS is shown in Figure 2(b) (peak list and intensities available in supplemental data). Predominantly singly-charged even-electron product ions are observed, but three odd-electron singly-charged products are also observed. Similar to IRMPD of the same precursor ion, the $[M-8H+9Na]^{+}$ ion is the most abundant product and is accompanied by peaks resulting from the loss of up to two SO_3 moieties. Interestingly,

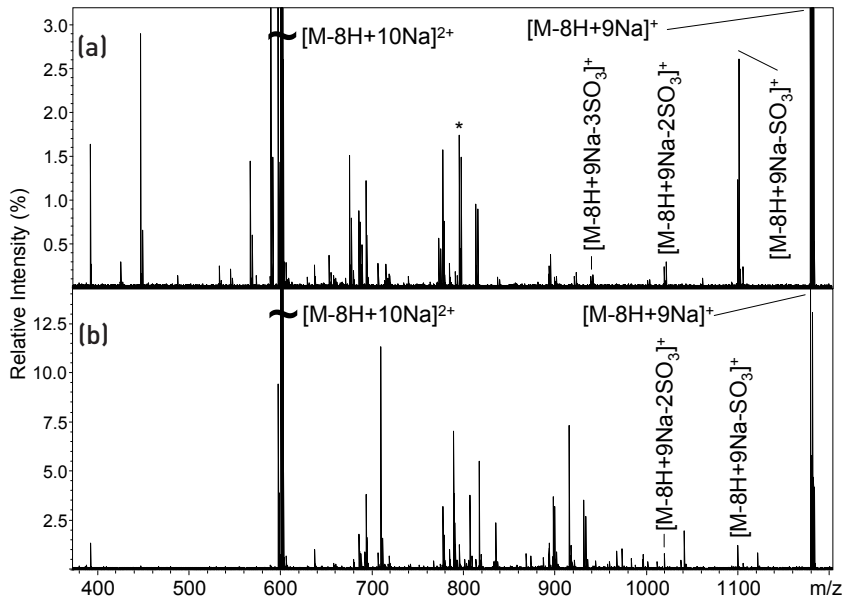


Figure 2. MS/MS spectra of the $[M-8H+10Na]^{2+}$ precursor ion of SOS produced by (a) IRMPD and (b) ECD.

the charge-reduced precursor ion ($[M-8H+10Na]^{+\bullet}$) is not observed. The ECD mass spectrum was internally calibrated on the precursor and confidently assigned product ions (for example, the precursor ion, charge reduced species and the charge reduced species minus SO_3) and resulted in mass accuracies ⩽ 0.5 ppm. However, aside from the product ions used for internal calibration, no other products in the ECD mass spectrum could be assigned using accurate mass measurement. For example, the abundant product ion at *m/z* 710.665 falls close in mass to a $^{0,2}X_1$ product, but can be discounted as this product is due to the large difference between measured and calculated values (calculated *m/z* = 710.793), which differ by ~180 ppm.

Many of the peaks in the ECD mass spectrum are not observed in the IRMPD mass spectrum. For example, aside from the $[M-8H+9Na]^+$ and its satellites resulting from SO_3 loss, only three other product ions are common to both mass spectra. Differences are also observed in product ions accompanied by SO_3 loss and Na/H heterogeneity. For example, abundant SO_3 loss accompanies many peaks in the IRMPD mass spectrum, but only two products in the ECD mass spectrum have accompanying peaks from SO_3 loss. Also, while abundant Na/H heterogeneity is observed in the IRMPD mass spectrum, in the form of peaks with mass differences of 21.982 u, this mass spacing between product ions is not observed in the ECD mass spectrum. It is important to note that while many of the ECD product ions cannot be assigned to common glycosidic or cross-ring cleavages, the ECD mass spectra are very reproducible and produce identical MS/MS spectra for data acquired months apart. This suggests that the ECD fragmentation of SOS is not random, but rather occurs by some specific mechanisms that are as yet unreported for saccharides.

IRMPD and EDD of SOS negative ions

IRMPD of the $[M-8H+6Na]^{2-}$ precursor ion of SOS produces the mass spectrum shown in Figure 3(a) (peak list and intensities available in supplemental data). Predominantly singly-charged product ions are observed and no odd-electron products are observed. The only doubly-charged product ion that is observed is $[M-8H+6Na-SO_3]^{2-}$, which occurs with low abundance. Aside from loss of SO_3 from the precursor ion, the only peak that can be assigned to expected cleavages is the isobaric C_1/Y_1 glycosidic cleavage product at *m/z* 586.811. This product ion is accompanied by product ions that differ by the addition or loss of sodium (*m/z* 608.794 and *m/z* 564.829, respectively) and a product that differs by the addition of SO_3 at *m/z* 666.769. This latter peak is unusual in that it indicates the possibility of SO_3 rearrangement occurring as a result of ion activation and subsequent fragmentation. Such SO_3 migrations have been reported before for singly-charged chondroitin sulfate anions,[19] and may be a cause of the difficulty in assigning products.

Neither the isobaric B_1/Y_1 glycosidic cleavage, nor any products ions differing by Na/H heterogeneity or SO_3 loss accompanying the B_1/Y_1 glycosidic cleavage, are observed in the IRMPD spectrum of the dianion. In contrast to IRMPD of the $[M-8H+10Na]^{2+}$ precursor ion of SOS, IRMPD of the $[M-8H+6Na]^{2-}$ precursor ion produces many low abundance product ions. Very few product ions differ by 79.956 u, suggesting that SO_3 loss is not a significant process for negative ions of SOS. This latter observation is consistent with findings for GAG anions in which ionizable protons have been replaced by sodium.[37] Similar to the IRMPD of the $[M-8H+10Na]^{2+}$ precursor ion of SOS, IRMPD of the $[M-8H+6Na]^{2-}$ precursor ion produces a number of product ions separated by 21.982 u. For example, the products at

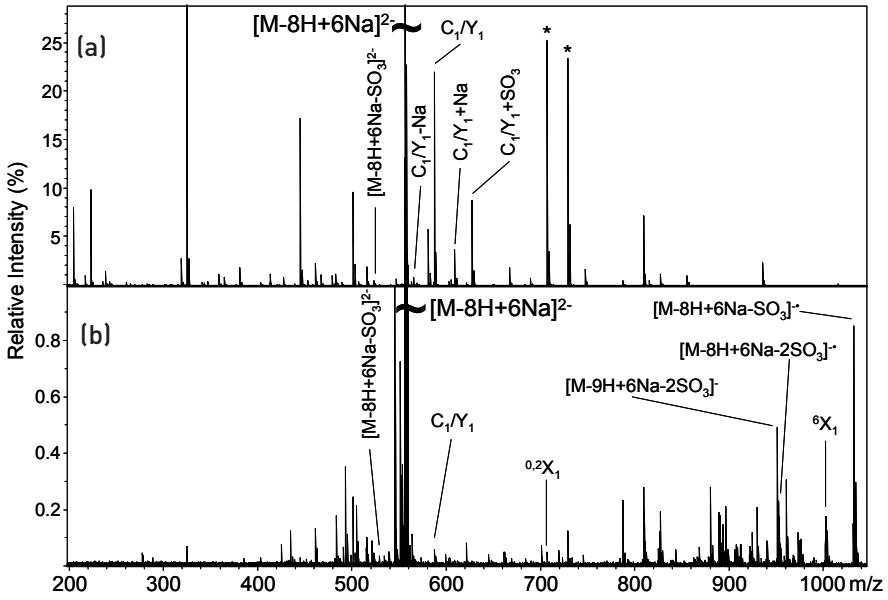
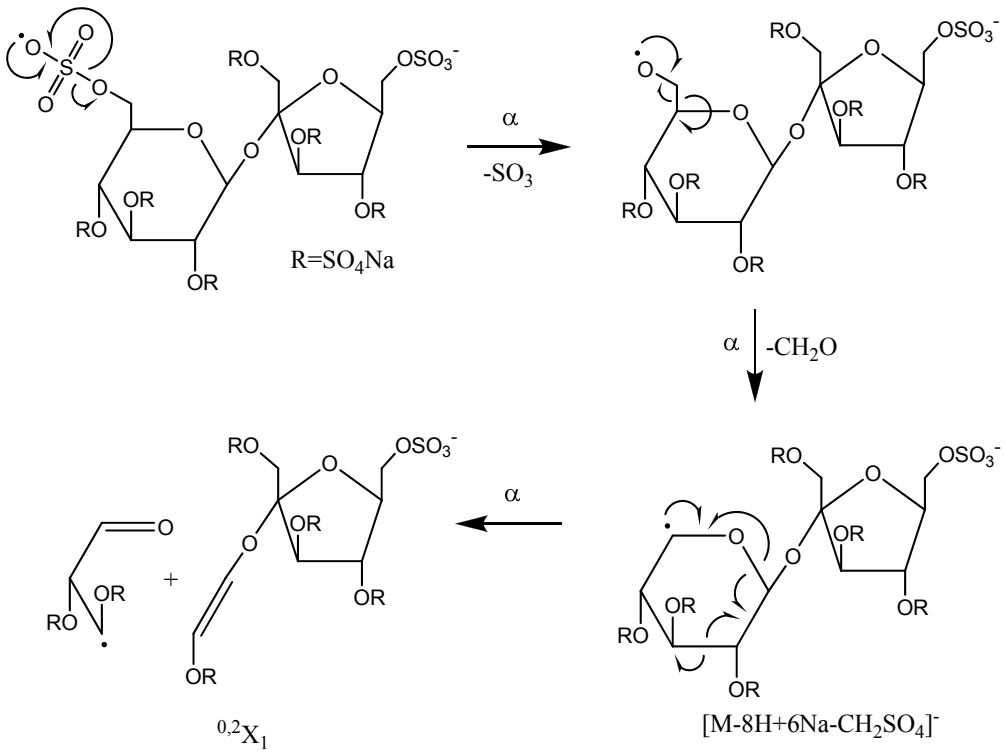


Figure 3. MS/MS spectra of the $[M-8H+6Na]^{2-}$ precursor ion of SOS obtained by (a) IRMPD and (b) EDD.



Scheme 1.

m/z 706.761 and *m/z* 728.744, indicated by the asterisk over the peaks in Figure 3(a), are a result of Na/H heterogeneity in the IRMPD mass spectrum. The Na/H heterogeneity in the negative ion IRMPD mass spectrum indicates sodium replacing hydrogen in a carbon–hydrogen bond, similar to the trend observed in the positive ion IRMPD mass spectrum of SOS. The reduced number of product ions and product ions accompanied by the loss of SO_3 or sodium suggests that the negative ion form of SOS is more stable than the positive ion and, therefore, is less likely to undergo fragmentation by vibrational excitation.

EDD of the $[M-8H+6Na]^{2-}$ product ion of SOS is shown in Figure 3(b) (peak list and intensities available in supplemental data). Abundant fragmentation is observed in the form of even- and odd-electron ions. No charge-reduced species is observed, but the charge-reduced species minus one or two SO_3 molecules are observed at *m/z* 1031.692 and *m/z* 951.732, respectively. Unlike IRMPD of the $[M-8H+6Na]^{2-}$ precursor ion of SOS, a number of product ions can be assigned in the EDD mass spectrum other than the charge-reduced species. For example, the peak at *m/z* 586.810 can be the isobaric C_1/Y_1 glycosidic cleavage. However, unlike the negative ion IRMPD spectrum, the C_1/Y_1 glycosidic cleavage is not accompanied by product ions that differ by the mass of sodium or SO_3. We have previously proposed that the initial formation of the radical site during EDD occurs at a site of negative charge.[26] For SOS, the radical site will form at one of the eight sulfate groups. The peak at *m/z* 1001.682 corresponds to the loss of CH_2SO_4 from the precursor ion, a product that may form due to the loss of the C6 carbon and sulfate group from radical rearrangement, as proposed in Scheme 1. Further fragmentation of this product will yield the $^{0,2}X_1$ cleavage at *m/z* 706.762, also shown in Scheme 1. Comparison of product ions observed in the EDD and negative ion IRMPD mass spectra indicate that very few product ions are common to both mass spectra, similar to the observation for the positive ion tandem mass spectra.

Conclusions

ECD, EDD and IRMPD have been used to dissociate the highly sulfated molecule SOS. Despite the abundant and reproducible fragmentation resulting from these dissociation methods, very few product ions can be assigned to expected glycosidic or cross-ring cleavages. However, a number of interesting features are produced by these dissociation methods. For example, Na/H heterogeneity with nonionizable hydrogen atoms is observed in both positive and negative ion IRMPD and ECD tandem mass spectra, but not in the EDD mass spectra. There is also evidence of SO_3 migration in the IRMPD mass spectrum of the doubly-charged anion of SOS. Because of the abundance of unidentified product ions differing by the exact mass of SO_3 for all dissociation methods, it is possible that significant SO_3 rearrangement is occurring during dissociation.

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