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Electron Transfer Dissociation and Collision-Induced Dissociation of Underivatized Metallated Oligosaccharides

Ranelle M. Schaller-Duke, Mallikharjuna R. Bogala, and Carolyn J. Cassady*

Department of Chemistry, The University of Alabama, Tuscaloosa, AL 35487

Abstract

Electron transfer dissociation (ETD) and collision-induced dissociation (CID) were used to investigate underivatized, metal-cationized oligosaccharides formed via electrospray ionization (ESI). Reducing and non-reducing sugars were studied including the tetrasaccharides maltotetraose, 3 α ,4 β ,3 α -galactotetraose, stachyose, nystose and a heptasaccharide, maltoheptaose. Univalent alkali, divalent alkaline earth, divalent and trivalent transition metal ions, and a boron group trivalent metal ion were adducted to the non-permethylated oligosaccharides. ESI generated $[M + \text{Met}]^+$, $[M + 2\text{Met}]^{2+}$, $[M + \text{Met}]^{2+}$, $[M + \text{Met} - \text{H}]^+$, and $[M + \text{Met} - 2\text{H}]^+$ most intensely along with low intensity nitrate adducts, depending on the metal and sugar ionized. The ability of these metal ions to produce oligosaccharide adduct ions by ESI had the general trend: Ca(II) > Mg(II) > Ni(II) > Co(II) > Zn(II) > Cu(II) > Na(I) > K(I) > Al(III) $\approx$ Fe(III) $\approx$ Cr(III). Although trivalent metals were utilized, no triply charged ions were formed. Metal cations allowed for high ESI signal intensity without permethylation. ETD and CID on $[M + \text{Met}]^{2+}$ produced various glycosidic and cross-ring cleavages, with ETD producing more cross-ring and internal ions, which are useful for structural analysis. Product ion intensities varied based on glycosidic-bond linkage and identity of monosaccharide sub-unit, and metal-adducts. ETD and CID showed high fragmentation efficiency, often with complete precursor dissociation, depending on the identity of the adducted metal ion. Loss of water was occasionally observed, but elimination of small neutral molecules was not prevalent. For both ETD and CID, $[M + \text{Co}]^{2+}$ produced the most uniform structurally informative dissociation with all oligosaccharides studied. The ETD and CID spectra were complementary.

Graphical abstract

* Address reprint requests to: Carolyn J. Cassady, Department of Chemistry, The University of Alabama, 250 Hackberry Lane, Tuscaloosa, AL 35401 ccassady@ua.edu phone: (205) 348-8443.

Introduction

Oligosaccharides are an important sub-class of carbohydrates containing a small number of monosaccharides including hexoses, pentoses, hexosamines, uronic acids, dexoyhexoses, and sialic acids. Each unit is bonded to the next at different positions of the pyranose or furanose ring through an α or β glycosidic bond. The variability in the bonding positions for the monosaccharide units allows a variety of highly branched, linear, or stacked structures. Oligosaccharides are found naturally with O-linked or N-linked oligomers, sulfated as glycosaminoglycans (GAGs), or as sialylated glycoconjugates [1]. These modifications are also common for glycopeptides and glycolipids. This configurational diversity allows oligosaccharides to participate in many cellular and biological processes in the body, including protein folding and stabilization, molecular recognition within cells, energy storage and extraction, protein-ligand targeting, and protein function modulation [1, 2]. The biological function of oligosaccharides can depend greatly on structure. Characterization of oligosaccharide structure requires knowledge of linkage, position, branching, sequence, anomeric configuration, and covalent modifications [3].

Mass spectrometry (MS) is an important technique for structural analysis of oligosaccharides, offering advantages such as high speed, sensitivity, and minimal sample requirements. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) have been used to produce quasi-molecular ions for high mass species in the gas phase in both positive and negative ion modes [4–6]. Many monosaccharides and polymers of monosaccharide units have the same chemical formula and mass-to-charge ratios (m/z), which limits the utility of high resolution MS. Fragmentation of the molecule via tandem mass spectrometry (MS/MS or MSⁿ) is key to distinguishing between various oligosaccharides. Glycans can undergo two major types of fragmentation in MS/MS: glycosidic cleavage and cross-ring cleavage [7]. Glycosidic cleavages are able to provide information on composition, branching, and sequence within the molecule, while cross-ring cleavages can provide information on linkage types.

Several MS/MS techniques have been utilized for carbohydrate studies, including collision-induced dissociation (CID) [8–14], post-source decay (PSD) and in-source decay (ISD) [15, 16], infrared multiphoton dissociation (IRMPD) [17–21], ultraviolet photodissociation (UVPD) [22–25], and electron-based ion activation methods (ExD) [8, 17, 18, 21, 26–38]. However, neutral underivatized oligosaccharides are difficult to protonate [7, 9, 39–43]. Duffin et al. [39] reported that protonated ions of 11 oligosaccharides were only produced via ESI after CID of ammoniated or sodiated precursor ions. Bahr et al. [44] discussed that underivatized oligosaccharides are unable to be ionized at the same sensitivity as peptides and proteins due to hydrophility and found that derivatization and nano-ESI increases surface activity and enhances protonation. When protonation is successful, fragmentation varies based on the MS/MS technique employed and often involves gas-phase rearrangements of the ions. Several studies [9, 42, 45] revealed that low-energy CID of protonated oligosaccharides produces mainly glycosidic cleavages. Use of high-energy CID [45, 46] or metallated precursor ion with low-energy CID [9, 10, 47] enhances cross-ring cleavages. Electron-based ion activation methods have been utilized to reduce the loss of

labile modifications that is observed in CID and IRMPD, and to improve sequence coverage of oligosaccharides [21, 28, 29, 32, 34, 48, 49].

Studies using a variety of metal ions have shown that metallated oligosaccharides can provide structurally informative fragmentation in both positive and negative ion modes [13, 16, 38, 46, 47, 50–60]. Adamson and Håkansson [38] complexed alkali, alkaline earth, and transition metal ions to several underivatized oligosaccharides and found that with electron capture dissociation (ECD) and IRMPD metallation greatly improved the structurally informative fragmentation, although no one metal ion stood out as optimal. Costello and co-workers [28] studied five permethylated metallated oligosaccharides, finding that magnesium(II) provided the most fragmentation by ETD and that complementary fragmentation occurs by CID and ETD. Permethylation involves conversion of the free hydroxyl groups on the sugar into methoxy groups; this can simplify MS analysis by improving ionization, and facilitating characterization of glycosidic bond positions and branching [40, 61–66]. However, even with permethylation it is common to study sodiated precursor ions [7, 10, 45] due to the difficulty of achieving protonation.

The goal of this project is to explore the ability of ETD and CID to provide structural information on several underivatized oligosaccharides with a variety of metal adducts. Alkali, alkaline earth, first row transition metals, and a boron group metal were used to investigate the effects of charge, ionic radius, ionization/recombination energy, and valency of the metal adduct on oligosaccharide dissociation.

Experimental

All experiments were performed using a Bruker HCTultra PTM Discovery System high capacity quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Sample solutions were made with a 10:1 metal ion:oligosaccharide molar ratio and infused into an ESI source at a flow rate of 180 $\mu\text{L}/\text{h}$ using a KD Scientific (Holliston, MA, USA) syringe pump. Final oligosaccharide concentrations were 10 μM in methanol:water at a 50:50 volume ratio. Source conditions for positive ion mode were capillary voltage: -3500 V ; end plate offset: -500 V ; capillary exit voltage: 120 V ; nebulizer: 10 psi ; drying gas: $10\text{ L}/\text{min}$; drying gas temperature: 250°C . The amplitude of the voltage for CID experiments was 0.8 to 1.2 V and the collision gas was helium with a 30% to 200% collision energy sweep.

Isotopic ^{18}O -labeling of the reducing end oxygen in the reducing sugars maltotetraose, galactotetraose, and maltoheptaose involved dissolving enough oligosaccharide in $100\text{ }\mu\text{L}$ of H_2^{18}O to create a 0.1 M sample. A second identical sample was prepared with a 10:1 ratio of $\text{Co}(\text{NO}_3)_2$ to oligosaccharide. Both samples were stored in sealed vials in a desiccator at room temperature for three weeks. The extent of isotopic labeling was periodically monitored by MS. The solution with the cobalt(II) exchanged most quickly. Before MS analysis, the samples were diluted to $10\text{ }\mu\text{M}$ of oligosaccharide.

The ETD experiments used fluoranthene, generated in a negative chemical ionization (nCI) source, as the reagent anion and methane as the nCI reagent gas. Reagent ions were

accumulated for 20.0 ms, the reaction time was optimized at 400 ms, and the low m/z cutoff was 120 m/z . The ETD ion charge control (ICC) target was 250,000 to 400,000. The “smart decomposition” function was employed to further dissociate any charge reduced product ions by using resonant excitation to overcome attractive forces that may hold oligosaccharide fragments together following ETD. Precursor ion isolation widths for CID and ETD were varied from 2.0 m/z to 4.0 m/z to achieve maximum product ion intensity. Standard enhanced resolution mode was employed for MS and MS/MS experiments with a scan speed of 8,100 m/z per second. Spectra shown are averages of 200 scans. Compass DataAnalysis was used for data processing.

Maltotetraose (α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 4)- α -D-Glc-(1 $\rightarrow$ 4)-Glc) was purchased from MP Biomedicals (Santa Ana, CA, USA); 3 α ,4 β ,3 α -galactotetraose (α -D-Gal-(1 $\rightarrow$ 3)- β -D-Gal-(1 $\rightarrow$ 4)- α -D-Gal-(1 $\rightarrow$ 3)-D-Gal) from Santa Cruz Biotechnology (Dallas, TX, USA); maltoheptaose (α -D-glc-(1 $\rightarrow$ 4)- α -D-glc-(1 $\rightarrow$ 4)- α -D-glc-(1 $\rightarrow$ 4)- α -D-glc-(1 $\rightarrow$ 4)- α -D-glc-(1 $\rightarrow$ 4)- α -D-glc-(1 $\rightarrow$ 4)-Glc) and nystose (β -D-Fru-(2 $\rightarrow$ 1)- β -D-Fru-(2 $\rightarrow$ 1)- β -D-Fru-(2 $\leftrightarrow$ 1)- α -D-Glc) from Sigma-Aldrich (St. Louis, MO, USA); and stachyose (α -D-Gal-(1 $\rightarrow$ 6)- α -D-Gal-(1 $\rightarrow$ 6)- α -D-Glc(1 $\leftrightarrow$ 2)- β -D-Fru) from Acros Organics (Morris Plains, NJ, USA). The metal salts were nitrates of sodium (Na(I)), potassium (K(I)), magnesium (Mg(II)), calcium (Ca(II)), chromium (Cr(III)), iron (Fe(III)), cobalt (Co(II)), nickel (Ni(II)), copper (Cu(II)), zinc (Zn(II)), and aluminum (Al(III)). H₂¹⁸O was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure Milli-Q 18 M Ω water was produced with a Barnstead (Dubuque, IA, USA) E-pure system.

Results and Discussion

Relevant metal properties can be seen in Table 1, while structures of the oligosaccharides in this study are in Figure 1. Maltotetraose consists of four glucose units with identical linkages between the rings, whereas 3 α ,4 β ,3 α -galactotetraose has galactose units with α and β linkages on different ring positions. These are both linear, reducing sugars. Stachyose and nystose are stacking, non-reducing sugars with a variety of monosaccharide units and glycosidic bond linkages. Maltoheptaose was included to examine size effects as it contains seven glucose units.

Metallated Oligosaccharide Ion Formation by ESI

The predominant ion in the ESI spectra for all oligosaccharide solutions with no added metal salt is $[M + Na]^+$, where M is the oligosaccharide. Na(I) is a common MS contaminant, most likely from the glassware or solvent system. As previously mentioned sodiated oligosaccharide ions are much more common than protonated ions [7, 9, 39–43]. Singly-protonated oligosaccharide ions, $[M + H]^+$, were generated by the addition of a small amount of acid, such as 1% acetic acid (by volume); however, degradation of the sugar occurred. In the absence of metal salt, no multiply charged ions formed, even upon the addition of up to 5% acid.

To generate metal-adducted oligosaccharide ions, each of the five oligosaccharides were complexed with all surveyed metals individually. Table 2 indicates the ions observed after additions of the various nitrate salts to the oligosaccharides. To achieve the highest intensity

of metallated ions, the molar ratio of metal ion (Met) to oligosaccharide was optimized at 10:1. Ratios of 1:1, 5:1, 20:1 and 50:1 were also studied. Our finding that one molar ratio worked well for all metal ions contrasts with the results of Leary and coworkers [54], who found that both ionic radii and counter ions affect metal ion coordination to oligosaccharides and concluded that concentration optimization of each metal and sugar may be necessary.

The monovalent group I ions Na(I) and K(I) yielded intense $[M + \text{Met}]^+$ for all tetrasaccharides, as well as $[M + 2\text{Met}]^{2+}$ for the larger maltoheptaose. Divalent metal ions from group II or the transition series (Mg(II), Ca(II), Co(II), Ni(II), Cu(II), and Zn(II)) formed predominately $[M + \text{Met}]^{2+}$ with all sugars.

Electrospray on solutions of the oligosaccharides with the trivalent metals Al(III), Cr(III), and Fe(III) did not consistently produce multiply charged ions or adduct ions; many times, adducts were not observed or occurred at low intensity. No $[M + \text{Met}]^{3+}$ was seen with $[M + \text{Met} - 2\text{H}]^+$ forming instead. (The exception is maltoheptaose, as shown in Table 2.) This lack of $[M + \text{Met}]^{3+}$ is surprising because analogous ions often form with peptides [67–71]. Fe(III), Cr(III), and Al(III) have not been included in other oligosaccharide studies. As seen in Table 1, the ionic radii of the trivalent metal ions are similar to the radii of the other metal ions that coordinate well to the oligosaccharides. In addition, Angyal [72] reported that metals with ionic radii between 0.100 nm and 0.110 nm bind most strongly to monosaccharides in solution. None of the metal ions that we studied have ionic radii in this specific range, although most are within ± 0.02 nm. Any ionic radii effect may be offset by charge density; higher charge with near equivalent ionic radii causes the trivalent metal ions to have a higher charge density than the mono- and divalent metal ions studied. The charge densities range from 284 Q/R³ for K(I) to 9750 Q/R³ for Al(III). Metal cations accept electrons from weakly coordinated oxygen atoms on monosaccharides; however, water molecules can displace the coordinated cation, allowing only the strongest metal coordination to remain [72]. Due to their high charge density, trivalent metals should more strongly coordinate to an oxyanion than to a hydroxyl group, thereby favoring the loss of protons during ESI to produce $[M + \text{Met} - 2\text{H}]^+$.

The arrangement of the hydroxyl groups on a monosaccharide affects its ability to complex to metal ions [73–75]. A series of experiments were conducted to compare the ability of the metal ions to adduct and ionize the oligosaccharides. Working with each oligosaccharide separately, a solution was first prepared that contained 1 μM of oligosaccharide and all 11 metal ions at 10 μM each; the intensities of the ions produced by ESI were examined. Then, the metal that produced the base peak in the spectrum was removed and the experiment was repeated with the remaining metals. This process was continued until only two metal ions remained. The trend in the ability of the metal ions to produce adduct ions was the same for all five oligosaccharides: Ca(II) > Mg(II) > Ni(II) > Co(II) > Zn(II) > Cu(II) > Na(I) > K(I) > Al(III) $\approx$ Fe(III) $\approx$ Cr(III). Similar results have been seen in the literature where Harvey [47] found that Ca(II) > Mn(II) > Mg(II) > Co(II) > Cu(II) for adduction to maltoheptaose and Leary and coworkers [54] reported that Ca(II) produced the most intense ions in their study of two sugars with Ca(II), Mn(II), Co(II), Zn(II), and Cu(II). Considering $[M + \text{Ca}]^{2+}$ as the most easily produced ion, the greatest ion intensity was found for stachyose and maltotetraose (absolute intensities in the low 10⁵ range), with ions from galactotetraose of

intermediate abundance (high 10^4), and nystose and maltoheptaose providing the lowest ion yields (low 10^4). This result has no obvious correlation to whether the oligosaccharides are linear or stacked.

Effect of Metal Ion on Dissociation

Domon and Costello nomenclature [76] will be used to label the ionic oligosaccharide fragments. The Domon-Costello nomenclature does not consider non-reducing sugars. In the case of the non-reducing sugars stachyose and nystose, the parent monosaccharide was considered to be the reducing end. For stachyose this is the fructose and for nystose the glucose unit. In Figure 1, all oligosaccharides are drawn with the reducing end, or parent monosaccharide on the right, except for nystose where the parent monosaccharide is on the top of the molecule. Many of the product ions are isobaric with the same chemical formula, making them indistinguishable in the spectrum. Isobaric ions are noted in the spectrum with a comma between two ion labels. Colors used to illustrate the product ions in Figures 2–8 are red for cross-ring cleavages, blue for glycosidic bond cleavages, and green for internal cleavages. The labels for internal ions indicate the glycosidic bond cleavage involved and additional mass lost from cross-ring cleavage. For example, $[C_4, Y_4 + Co - 60]$ indicates the cleavage is either C_4 or Y_4 with loss of 60 Da through a cross-ring cleavage and retention of Co(II). The various possible isobaric cross-ring cleavages, sorted by mass lost, are shown in Table 3. All product ions in Table 3 are observed with both CID and ETD, except for the loss of 120 Da, which was only observed with CID. Undissociated precursor ion is labeled in light blue with a large diamond arrow head and ions involving neutral losses are labeled in black. Han and Costello [28] found that fragmentation of the oligosaccharide is more likely on the non-reducing end than the reducing end of reducing sugars, which the ^{18}O isotopic labeling of the reducing sugars in this current study agreed with.

The identity of the metal ion affects fragmentation patterns with both ETD and CID. This can be seen in Figures 2 and 3, respectively, for maltotetraose. Maltotetraose will be discussed as representative for all oligosaccharides in this study. All ETD and CID product ions from maltotetraose with every metal studied were metallated except for products containing only one glucose unit. Protonated glucose product ions were observed with loss of one, two, three, and four water molecules in all CID spectra. These singly charged protonated monosaccharide ions occur in our CID spectra and are common in other CID studies [47]; however, they are not found by ETD.

With the singly charged cations Na(I) and K(I), little to no dissociation occurred during ETD or CID. This is illustrated by the CID spectrum of $[M + K]^+$ from maltotetraose in Supplementary Figure S1. In Figure 2(a), ETD on $[M + 2Na]^{2+}$ results in loss of an adducted sodium ion, as well as formation of the charge reduced species $[M + 2Na]^+$ and very few cleavage ions. In Figure 3(a), CID on $[M + 2Na]^{2+}$ results in loss of one adducted metal ion and few product ions. These spectra are not structurally informative. This is also true for the MS/MS spectra of sodiated and potassiated ions from the other sugars studied. This predominant pathway of metal ion loss for doubly sodiated or potassiated ions was also noted in an ECD study by Adamson and Håkansson [38].

For the alkaline earth metals, ETD and CID with Mg(II) showed significantly more product ions than with Ca(II). The spectra of $[M + Ca]^{2+}$ from maltotetraose can be seen in Supplementary Figures S2 and S3, while the ETD spectrum of $[M + Mg]^{2+}$ from maltotetraose is in Figure 2(b). The majority of the product ions are related to the terminal glucose units; the middle of the molecule undergoes limited fragmentation. The only glycosidic bond cleavage is loss of one terminal glucose unit with product ions having both loss and retention of the oxygen in the glycosidic bond. All internal ions also contain these glycosidic bond cleavages, along with either another glycosidic bond cleavage or a cross-ring cleavage. Product ions that correspond to cross ring cleavage loss of 30 Da ($C_2H_4O_2$), 60 Da ($C_3H_6O_3$), and 120 Da ($C_4H_8O_4$) are very intense and there are multiple isobaric possibilities. $^{1,5}X_2$ is the only product that solely corresponds to cleavage of one of the middle glucose rings. CID of $[M + Mg]^{2+}$ from maltotetraose results in both doubly and singly charged product ions, as can be seen in Figure 3(b). Glycosidic bond cleavage occurred between every monosaccharide sub-unit. Due to the symmetry of the oligosaccharides, there are many indistinguishable isobaric product ions. The ion at m/z 315 is the only product ion that corresponds to cross-ring cleavage alone and could be $^{0,2}A_4$, $^{0,4}X_n$, $^{1,3}X_n$, or $^{2,4}X_3$. Isotopic labeling with ^{18}O on the reducing end of the reducing sugars showed that most isobaric cross-ring cleavages are A-ions, so this product ion is more likely $^{0,2}A_4$. All other cross-ring cleavage products are a part of an internal ion and are accompanied by a glycosidic bond cleavage. The intensities of the cross-ring cleavage product ions and internal cleavages are low.

The divalent transition metals Cu(II), Ni(II), and Zn(II) produce few product ions from ETD on $[M + Met]^{2+}$, while Co(II) produces abundant product ions and is structurally informative (as discussed below). CID on $[M + Met]^{2+}$ with Ni(II), Co(II), and Zn(II) show very similar fragmentation; however, with Cu(II) there is very little product ion formation. ETD and CID spectra of $[M + Cu]^{2+}$ from maltotetraose can be seen in Figures 2(c) and 3(c), respectively. Although both ETD and CID on $[M + Cu]^{2+}$ from maltotetraose produce glycosidic bond cleavage between every monosaccharide residue, these spectra have few product ions. The ETD spectrum contains a few cross-ring and internal product ions at low intensity, while CID yields only two cross-ring product ions and no internal cleavages. The relative lack of dissociation with Cu(II) is consistent with CID studies by Leary and coworkers [14, 54, 77], Zhu et al. [57], and Harvey [47]. ETD and CID spectra of $[M + Met]^{2+}$ from maltotetraose with Ni(II) and Zn(II) can be seen in Supplementary Figures S4, S5, S6, and S7.

From our data, Co(II) appears to be the most promising metal for obtaining informative oligosaccharide fragmentation. This is consistent with work by Leary and coworkers [53, 54], where Co(II) coordinated easily to oligosaccharides and CID provided extensive structural information, including linkage positions. Our results suggest that this is also true with electron-based techniques such as ETD. Figures 2(d) and 3(d) show ETD and CID spectra for $[M + Co]^{2+}$ from maltotetraose. In addition, Figure 4 shows ETD and CID spectra from $[M + Co]^{2+}$ from isotopically labeled maltotetraose. Both dissociation methods yield complete glycosidic bond fragmentation and many other product ions. ETD produced many cross-ring cleavages on the terminal glucose units and $^{2,5}X$ cross-ring product ions that were not present in the spectra of other metal adducts. CID on $[M + Co]^{2+}$ displayed 100% fragmentation efficiency with no precursor ion remaining. Fragmentation efficiency

describes the amount of precursor ion that is dissociated into product ions and is calculated as $\sum I(\text{fragment ion})/I(\text{precursor before dissociation})$, where I is ion intensity. The CID spectrum in Figure 4 has more water loss products than the maltotetraose spectra of other metals; for example, many of the glycosidic bond product ions are accompanied by water loss. The product ions of the cobalt-adducted maltotetraose from both dissociation methods are of higher intensity than for the other metal adducts. Isotopic labeling of the oxygen on the reducing end of maltotetraose allows differentiation of the fragment ions from the reducing and non-reducing ends of the oligosaccharide. Although there is glycosidic bond cleavage between every glucose unit, all ETD and CID product ions from maltotetraose are comprised of at least two glucose units. The only product ions that do not contain at least two glucose units are internal cleavages, probably due to the fact that bond cleavage is occurring twice. Another key difference in the ETD and CID spectra is that with CID the only ions that contain the reducing end are Y ions from the non-reducing side of the glycosidic oxygen; however, in ETD, both Y and Z ions from both sides of the glycosidic oxygen are observed. The majority of the cross-ring cleavages include the non-reducing side of the molecule.

The trivalent metal cations induced more cross-ring cleavage and internal cleavage than the divalent cations with CID. However, they did not produce multiply charged precursor ions, making ETD with these metals impossible. CID of $[M + Al - 2H]^+$ and $[M + Cr - 2H]^+$ from maltotetraose produced extensive fragmentation throughout the molecule, as seen in Supplementary Figures S8 and S9. The fragmentation, with extensive cross-ring and internal cleavages, more closely resembled ETD than CID from the divalent metal adducts of Co(II), Ni(II), and Zn(II). The product ions corresponding to cross-ring cleavage of the terminal rings or cross-ring cleavages with loss of mass less than 120 Da are only observed with ETD of the divalent metal cations, although they occur with CID for the trivalent metal ions. CID of $[M + Cr - 2H]^+$ from maltotetraose produced $[B_1, Z_1 + Cr - 2H + H_2O]^+$, which was not found in any other spectra. Also, CID of these ions did not form the protonated glucose product ions with loss of water that occur in all other CID spectra. CID of $[M + Fe - H]^+$ can be seen in Supplemental Figure S10 and showed similar fragmentation to that with the other two trivalent metal cations, although not as extensive. This could be due to the fact that some Fe(III) can be reduced to Fe(II) during ionization when using our ESI source design [78, 79].

The number of structurally informative product ions generated with each metal ion varied for the other tetrasaccharides included in the study. For maltotetraose and maltoheptaose, (that have linear chains of glucose residues with 1–4 glycosidic bonds) the metals that produce the most fragmentation are Co(II) and Zn(II). For galactotetraose, a linear sugar with varying monomers and linkages, adduction with Co(II), Zn(II), and Ni(II) produced similar structural information. ETD and CID spectra of $[M + Co]^{2+}$ from isotopically labeled galactotetraose are shown in Figures 5(a) and 5(b), respectively. The non-isotopically labeled spectra are in Supplemental Figure S11. Co(II), Zn(II), and Ni(II) produced the most fragmentation for stachyose, a non-reducing stacked oligosaccharide. CID and ETD on $[M + Co]^{2+}$ from stachyose can be seen in Figures 6(a) and 6(b), respectively. Minimal structural information was obtained for nystose, a non-reducing stacked sugar, but similar patterns of fragmentation occur with Co(II), Ni(II), Cu(II), and Zn(II). Figures 7(a) and 7(b) show CID

and ETD spectra for $[M + Co]^{2+}$ from nystose. Although many fragment ions are seen in the spectra, they mainly consist of indistinguishable isobaric internal cleavages.

There are multiple metal ion properties that can influence coordination to and dissociation of the oligosaccharides. The size of the metal affects both methods of fragmentation [14, 16, 38, 54]. In the present study, the metals that produced the most consistent and informative fragmentation had ionic radii between 0.083 and 0.089 nm. Metal ions tend to coordinate to multiple oxygens on an oligosaccharide simultaneously [13, 14, 16, 55, 56, 80]. Therefore, these smaller ionic radii may allow the metals to have a choice of numerous locations, possibly behaving as a mobile cation and increasing fragmentation. The larger ions, such as Na(I), K(I), and Ca(II), with an ionic radius of greater than 0.1 nm may be restricted in where they bind or they may hold together dissociated product ions through simultaneous coordination at multiple sites. Several studies have shown that larger metal cations yield decreased fragmentation by PSD and CID [13, 14, 16].

Electron-based dissociation mechanisms have been studied more thoroughly for peptides than for glycans. Liu and Håkansson [81] proposed that the electron is first captured at the metal ion and then transferred to the peptide backbone; for divalent metal ions, a lower second ionization energy (IE) may facilitate electron movement and enhance fragmentation. Chen et al. [82], however, suggested that electronic configuration of the metal dictates ECD fragmentation to a greater degree than the second IE. This is based on their observation that metal ions with half or fully filled d orbitals show different fragmentation patterns than metals with partially filled d shells. Supporting this theory, Kleinnijenhuis et al. [83] proposed that Cu(II) captures an electron during ECD, reducing Cu(II) to the more stable d^{10} Cu(I), which explains the limited fragmentation they observed from $[Peptide + Cu]^{2+}$. These concepts should hold true for ETD and either could explain why Cu(II) adducts show less extensive dissociation for our oligosaccharides. Cu(II) has the highest second IE of the divalent metals included in the study at 1957.9 kJ/mol, as well as a $3d^9$ electronic configuration that favors metal ion reduction. This contrasts with Co(II), which has a much lower second IE of 1648.0 kJ/mol and an electron configuration of $3d^7$, where extensive fragmentation is observed for $[M + Co]^{2+}$ with oligosaccharides. The enhanced dissociation seen with Co(II) versus Ni(II) (where both metals have similar solution-phase characteristics and are part of the iron triad) may be explained by the fact that Co(II) has a slightly larger ionic radius, significantly reducing the charge density on the metal ion (2890 Q/R³ for Co(II) versus 3500 Q/R³ for Ni(II)).

In a study of glycans, Adamson and Håkansson [35] reported that coordination number of a metal ion and the second IE of the metal can affect ECD fragmentation. Metal second IE may be important but cannot be the overriding factor because in our work Co(II), Ni(II), and Zn(II) produce more fragmentation for oligosaccharides than the lower second IE metals Mg(II) and Ca(II). This may relate to the fact that Mg(II) and Ca(II) already have stable electron configurations of [Ne] and [Ar], respectively. Another factor that could explain our results was proposed by Costello and coworkers [26], who used theoretical calculations to suggest that the electron is captured at Mg(II) to form Mg(I). This reduced Mg(I) then abstracts a hydroxyl group ($\bullet OH$) from the sugar, forming a carbon radical that induces

fragmentation. In this mechanism, an electron does not need to be transferred to the sugar to induce fragmentation.

Effect of Oligosaccharide Structure on Dissociation

The identity of the oligosaccharide affects fragmentation, as can be seen in Figures 2–8. ETD fragmentation efficiency of the tetrasaccharides decreased in the order maltotetraose > stachyose > 3 α ,4 β ,3 α -galactotetraose > nystose with most of the metal ions. ETD on metallated maltotetraose and 3 α ,4 β ,3 α -galactotetraose generally produce a greater variety of product ions, while maltotetraose and stachyose generate more cross-ring cleavage ions. This could be caused by the types of glycosidic bonds or the identity of the monosaccharide units in the molecule. The reducing sugars show more extensive cross-ring and internal fragmentation near the reducing end than the non-reducing end. ^{18}O isotopic labeling of the reducing sugars indicates that majority of the cross-ring product ions contain the non-reducing end. Linkage determination is possible by examination of cross-ring cleavage product ions and secondary fragment ions. One example is in comparing ETD on galactotetraose and maltotetraose. Maltotetraose contains $[\text{B}_2, \text{Z}_2 + \text{Co} - 90]$, $[\text{C}_2, \text{Y}_2 + \text{Co} - 60]$ and $[\text{C}_2, \text{Y}_2 + \text{Co} - 90]$, which can be indicative of 1,4 glycosidic bond. These internal ions along with $[\text{B}_3, \text{Z}_3 + \text{Co} - 60]$ are not observed in the galactotetraose spectra. The ETD spectrum of nystose as seen in Figure 7 lacks the neutral loss product ions seen above m/z 600 with the other tetrasaccharides. In order to accurately assign structural information, it is important that a variety of structurally informative product ions accompany high ETD efficiency.

The low product ion formation for stachyose and nystose may lie in the fact that they are non-reducing sugars that cannot be hydrolyzed into the aldehyde structure which exists in the “open” form in solution [84]. Stachyose and nystose are also stacked sugars, where some or all the monosaccharide units in the chain are flexible due to their glycosidic bond linkage and can stack above each other. Nystose has three flexible furanose rings [85, 86], while stachyose has rotational flexibility in the terminal monosaccharides and flexibility between the two middle monosaccharides [87]. Nystose produced more electron transfer no dissociation (ETnoD) ions than the other oligosaccharides. With supplemental collisional activation on the charge reduced ETnoD ion, the fragmentation efficiency of nystose was improved and resulted in products that are a mixture of ions formed by ETD and CID. This suggests that metal cations likely coordinate more strongly to multiple oxygens between stacked monosaccharides than between linear chains. The metal ions may be able to bind at different sites on the oligosaccharide depending on these structural differences, which can influence fragmentation pathways [28]. If the metal ion is coordinated to oxygens on multiple rings along with a glycosidic oxygen, the dissociation yield may be decreased. However, as previously mentioned, the ability of metals to ionize the oligosaccharide is independent of the oligosaccharide’s structural characteristics.

The effects of structural differences can be seen by comparing the spectra from maltotetraose (Figures 2 and 3) to those from the non-reducing sugar nystose (Figure 7 for $[\text{M} + \text{Co}]^{2+}$). The ETD spectrum of nystose is dominated by product ions from internal cleavages with one glycosidic bond cleavage and one cross-ring cleavage, while CID on $[\text{M} +$

$\text{Co}]^{2+}$ yields cross-ring cleavage between every carbon on the monosaccharide unit (the exact monosaccharide unit can not be distinguished). This is indicative of each monosaccharide unit being linked together via only one carbon that is a part of the ring such as the 2-1 glycosidic bonds present in nystose. Very little cross-ring cleavage is seen by ETD of nystose; however, a product ion unique to this sugar is $^{1,2}\text{X}_2$, which corresponds to loss of a terminal monosaccharide unit and CH_2OH . Even with little dissociation, ETD and CID still yield complementary fragmentation.

Effect of Chain Length on Dissociation

Maltoheptaose was compared to maltotetraose to study the effects of chain length on fragmentation of metal-adducted oligosaccharides. ETD and CID spectra of $[\text{M} + \text{Co}]^{2+}$ from ^{18}O labelled maltoheptaose can be seen in Figure 8, while the maltotetraose spectra are in Figure 4. (Spectra from non-labeled maltoheptaose are in Supplemental Figure S12.) ETD on maltoheptaose produced the same type of fragment ions as on maltotetraose, and all 7 glucose units underwent dissociation. There was glycosidic bond cleavage between every glucose unit with both dissociation techniques. Maltoheptaose generally produced larger product ions; ETD products from maltoheptaose contain at least four glucose units, while CID products have at least three glucose units from doubly charged precursor ions and at least two units from singly charged precursor ions. In contrast, maltotetraose yields products with a minimum of two glucose units by both ETD and CID. As with maltotetraose, for CID on maltoheptaose the only ions that contain the reducing end are Y ions that occurred on the non-reducing side of the glycosidic oxygen; however, in the ETD spectra, both Y and Z ions are present, on both sides of the glycosidic oxygen. The types of product ions and efficiency of dissociation did not vary between maltotetraose and maltoheptaose. This implies that when the monosaccharide unit remains the same a longer chain does not significantly affect fragmentation.

Conclusions

The current study illustrates the effects of metal-adduction on dissociation of underivatized oligosaccharides. ESI produced intense metallated precursor ions and easily interpretable spectra. Both ETD and CID methods showed high fragmentation efficiency. Cross-ring cleavages and internal cleavages were more prevalent with ETD, while CID yields more glycosidic bond cleavage. The extent and efficiency of dissociation with both ETD and CID is dependent on the metal cation. The differences in fragmentation are likely due to ionic radius, metal coordination, and IE of the metal ions. The trivalent metals used in the study did not readily adduct to the oligosaccharides and produced inconsistent dissociation patterns, possibly due to their high charge density. The most structurally informative fragmentation by both ETD and CID is obtained from $[\text{M} + \text{Co}]^{2+}$ for all oligosaccharides in the study. Increasing the chain length while monosaccharide and glycosidic bond linkage stay the same does not affect fragmentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Varki A. Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology*. 1993; 3:97–130. [PubMed: 8490246]
2. Parodi AJ. Protein glucosylation and its role in protein folding. *Annu. Rev. Biochem.* 2000; 69:69–93. [PubMed: 10966453]
3. Ashline D, Singh S, Hanneman A, Reinhold V. Congruent strategies for carbohydrate sequencing. 1. Mining structural details by MSⁿ. *Anal. Chem.* 2005; 77:6250–6262. [PubMed: 16194086]
4. Fenn JB, Mann M, Meng CK, Wong SF. Electrospray ionization--principles and practice. *Mass Spectrom. Rev.* 1990; 9:37–70.
5. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. *Science*. 1989; 246:64–71. [PubMed: 2675315]
6. Hop CECA, Bakhtiar R. An introduction to electrospray ionization and matrix-assisted laser desorption/ionization mass spectrometry: Essential tools in a modern biotechnology environment. *Biospectroscopy*. 1997; 3530:259–280.
7. Zaia J. Mass spectrometry of oligosaccharides. *Mass Spectrom. Rev.* 2004; 23:161–227. [PubMed: 14966796]
8. Zhao C, Xie B, Chan S, Costello CE, O'Connor PB. Collisionally activated dissociation and electron capture dissociation provide complementary structural information for branched permethylated oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2008; 19:138–150. [PubMed: 18063385]
9. Harvey DJ. Collision-induced fragmentation of underivatized N-linked carbohydrates ionized by electrospray. *J. Mass Spectrom.* 2000; 35:1178–1190. [PubMed: 11110090]
10. Lemoine J, Strecker G, Leroy Y, Fournet B, Ricart G. Collisional-activation tandem mass spectrometry of sodium adduct ions of methylated oligosaccharides: Sequence analysis and discrimination between α -NeuAc-(2→3) and α -NeuAc-(2→6) linkages. *Carbohydr. Res.* 1991; 221:209–217. [PubMed: 1816919]
11. Zaia J, Miller MJC, Seymour JL, Costello CE. The role of mobile protons in negative ion CID of oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2007; 18:952–960. [PubMed: 17383193]
12. Dallinga JW, Heerma W. Positive ion fast atom bombardment mass spectrometry of some small oligosaccharides. *Biol. Mass Spectrom.* 1991; 20:99–108. [PubMed: 2069986]
13. Bythell BJ, Abutokaikah MT, Wagoner AR, Guan S, Rabus JM. Cationized carbohydrate gas-phase fragmentation chemistry. *J. Am. Soc. Mass Spectrom.* 2017; 28:688–703. [PubMed: 27896699]
14. Fura A, Leary JA. Differentiation of Ca²⁺-coordinated and Mg²⁺-coordinated branched trisaccharide isomers: An electrospray ionization and tandem mass spectrometry study. *Anal. Chem.* 1993; 65:2805–2811. [PubMed: 8250263]
15. Harvey DJ, Naven TJP, Küster B, Bateman RH, Green MR, Critchley G. Comparison of fragmentation modes for the structural determination of complex oligosaccharides ionized by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 1995; 9:1556–1561. [PubMed: 8652879]
16. Cancilla MT, Penn SG, Carroll JA, Lebrilla CB. Coordination of alkali metals to oligosaccharides dictates fragmentation behavior in matrix assisted laser desorption ionization/Fourier transform mass spectrometry. *J. Am. Chem. Soc.* 1996; 118:6736–6745.
17. Wolff JJ, Laremore TN, Leach FE III, Linhardt RJ, Amster IJ. Electron capture dissociation, electron detachment dissociation and infrared multiphoton dissociation of sucrose octasulfate. *Eur. J. Mass Spectrom.* 2009; 15:275–281.
18. Leach FE III, Xiao Z, Laremore TN, Linhardt RJ, Amster IJ. Electron detachment dissociation and infrared multiphoton dissociation of heparin tetrasaccharides. *Int. J. Mass Spectrom.* 2011; 308:253–259. [PubMed: 22247649]

19. Xie YM, Lebrilla CB. Infrared multiphoton dissociation of alkali metal-coordinated oligosaccharides. *Anal. Chem.* 2003; 75:1590–1598. [PubMed: 12705590]
20. Zhang JH, Schubotho K, Li BS, Russell S, Lebrilla CB. Infrared multiphoton dissociation of O-linked mucin-type oligosaccharides. *Anal. Chem.* 2005; 77:208–214. [PubMed: 15623298]
21. Adamson JT, Håkansson K. Infrared multiphoton dissociation and electron capture dissociation of high-mannose type glycopeptides. *J. Proteome Res.* 2006; 5:493–501. [PubMed: 16512663]
22. Ko BJ, Brodbelt JS. 193 nm ultraviolet photodissociation of deprotonated sialylated oligosaccharides. *Anal. Chem.* 2011; 83:8192–8200. [PubMed: 21913695]
23. O'Brien JP, Brodbelt JS. Structural characterization of gangliosides and glycolipids via ultraviolet photodissociation mass spectrometry. *Anal. Chem.* 2013; 85:10399–10407. [PubMed: 24083420]
24. Klein DR, Holden DD, Brodbelt JS. Shotgun analysis of rough-type lipopolysaccharides using ultraviolet photodissociation mass spectrometry. *Anal. Chem.* 2016; 88:1044–1051. [PubMed: 26616388]
25. Ko BJ, Brodbelt JS. Ultraviolet photodissociation of chromophore-labeled oligosaccharides via reductive amination and hydrazide conjugation. *J. Mass Spectrom.* 2011; 46:359–366. [PubMed: 21438085]
26. Huang Y, Pu Y, Yu X, Costello CE, Lin C. Mechanistic study on electron capture dissociation of the oligosaccharide-Mg²⁺ complex. *J. Am. Soc. Mass Spectrom.* 2014; 25:1451–1460. [PubMed: 24845360]
27. Yu X, Jiang Y, Chen Y, Huang Y, Costello CE, Lin C. Detailed glycan structural characterization by electronic excitation dissociation. *Anal. Chem.* 2013; 85:10017–10021. [PubMed: 24080071]
28. Han L, Costello CE. Electron transfer dissociation of milk oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2011; 22:997–1013. [PubMed: 21953041]
29. Yu X, Huang Y, Lin C, Costello CE. Energy-dependent electron activated dissociation of metal-adducted permethylated oligosaccharides. *Anal. Chem.* 2012; 84:7487–7494. [PubMed: 22881449]
30. Darula Z, Medzihradsky KF. Glycan side reaction may compromise ETD-based glycopeptide identification. *J. Am. Soc. Mass Spectrom.* 2014; 25:977–987. [PubMed: 24664807]
31. Yang J, Mo JJ, Adamson JT, Håkansson K. Characterization of oligodeoxynucleotides by electron detachment dissociation Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* 2005; 77:1876–1882. [PubMed: 15762599]
32. Budnik BA, Haselmann KF, Elkin YN, Gorbach VI, Zubarev RA. Applications of electron-ion dissociation reactions for analysis of polycationic chitooligosaccharides in Fourier transform mass spectrometry. *Anal. Chem.* 2003; 75:5994–6001. [PubMed: 14588042]
33. Hersberger KE, Håkansson K. Characterization of *O*-sulfopeptides by negative ion mode tandem mass spectrometry: Superior performance of negative ion electron capture dissociation. *Anal. Chem.* 2012; 84:6370–6377. [PubMed: 22770115]
34. Wolff JJ, Laremore TN, Busch AM, Linhardt RJ, Amster IJ. Electron detachment dissociation of dermatan sulfate oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2008; 19:294–304. [PubMed: 18055211]
35. Wolff JJ, Laremore TN, Aslam H, Linhardt RJ, Amster IJ. Electron-induced dissociation of glycosaminoglycan tetrasaccharides. *J. Am. Soc. Mass Spectrom.* 2008; 19:1449–1458. [PubMed: 18657442]
36. Wolff JJ, Laremore TN, Busch AM, Linhardt RJ, Amster IJ. Influence of charge state and sodium cationization on the electron detachment dissociation and infrared multiphoton dissociation of glycosaminoglycan oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2008; 19:790–798. [PubMed: 18499037]
37. Leach FE III, Wolff JJ, Xiao Z, Ly M, Laremore TN, Arungundram S, Al-Mafraji K, Venot A, Boons G, Linhardt RJ, Amster IJ. Negative electron transfer dissociation Fourier transform mass spectrometry of glycosaminoglycan carbohydrates. *Eur. J. Mass Spectrom.* 2011; 17:167–176.
38. Adamson JT, Håkansson K. Electron capture dissociation of oligosaccharides ionized with alkali, alkaline earth, and transition metals. *Anal. Chem.* 2007; 79:2901–2910. [PubMed: 17328529]

39. Duffin KL, Welply JK, Huang E, Henion JD. Characterization of N-linked oligosaccharides by electrospray and tandem mass spectrometry. *Anal. Chem.* 1992; 64:1440–1448. [PubMed: 1503219]
40. Kailemia MJ, Ruhaak LR, Lebrilla CB, Amster IJ. Oligosaccharide analysis by mass spectrometry: A review of recent developments. *Anal. Chem.* 2014; 86:196–212. [PubMed: 24313268]
41. Park Y, Lebrilla CB. Application of Fourier transform ion cyclotron resonance mass spectrometry to oligosaccharides. *Mass Spectrom. Rev.* 2005; 24:232–264. [PubMed: 15389860]
42. Reinhold VN, Reinhold BB, Costello CE. Carbohydrate molecular weight profiling, sequence, linkage, and branching data: ES-MS and CID. *Anal. Chem.* 1995; 67:1772–1784. [PubMed: 9306731]
43. Burlingame AL, Boyd RK, Gaskell SJ. Mass spectrometry. *Anal. Chem.* 1994; 66:R634–R683.
44. Bahr U, Pfenninger A, Karas M, Stahl B. High sensitivity analysis of neutral underivatized oligosaccharides by nanoelectrospray mass spectrometry. *Anal. Chem.* 1997; 69:4530–4535. [PubMed: 9375514]
45. Lemoine J, Fournet B, Despeyroux D, Jennings KR, Rosenberg R, De Hoffman E. Collision-induced dissociation of alkali metal cationized and permethylated oligosaccharides: Influence of the collision energy and of the collision gas for the assignment of linkage position. *J. Am. Soc. Mass Spectrom.* 1993; 4:197–203. [PubMed: 24234847]
46. Harvey DJ, Bateman RH, Green MR. High-energy collision-induced fragmentation of complex oligosaccharides ionized by matrix-assisted laser desorption/ionization mass spectrometry. *J. Mass Spectrom.* 1997; 32:167–187. [PubMed: 9102200]
47. Harvey DJ. Ionization and collision-induced fragmentation of N-linked and related carbohydrates using divalent cations. *J. Am. Soc. Mass Spectrom.* 2001; 12:926–937. [PubMed: 11506225]
48. Adamson JT, Håkansson K. Electron detachment dissociation of neutral and sialylated oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2007; 18:2162–2172. [PubMed: 17962039]
49. Liu H, Håkansson K. Electron capture dissociation of divalent metal-adducted sulfated oligosaccharides. *Int. J. Mass Spectrom.* 2011; 305:170–177.
50. Domann P, Spencer DIR, Harvey DJ. Production and fragmentation of negative ions from neutral N-linked carbohydrates ionized by matrix-assisted laser desorption/ionization. *Rapid Commun. Mass Spectrom.* 2012; 26:469–479. [PubMed: 22279023]
51. Asam MR, Glish GL. Tandem mass spectrometry of alkali cationized polysaccharides in a quadrupole ion trap. *J. Am. Soc. Mass Spectrom.* 1997; 8:987–995.
52. El Firdoussi A, Lafitte M, Tortajada J, Kone O, Salpin J. Characterization of the glycosidic linkage of underivatized disaccharides by interaction with Pb^{2+} ions. *J. Mass Spectrom.* 2007; 42:999–1011. [PubMed: 17567836]
53. König S, Leary JA. Evidence for linkage position determination in cobalt coordinated pentasaccharides using ion trap mass spectrometry. *J. Am. Soc. Mass Spectrom.* 1998; 9:1125–1134. [PubMed: 9794083]
54. Sible EM, Brimmer SP, Leary JA. Interaction of first row transition metals with alpha 1–3, alpha 1–6 mannotriose and conserved trimannosyl core oligosaccharides: A comparative electrospray ionization study of doubly and singly charged complexes. *J. Am. Soc. Mass Spectrom.* 1997; 8:32–42.
55. Hofmeister GE, Zhou Z, Leary JA. Linkage position determination in lithium-cationized disaccharides: Tandem mass spectrometry and semiempirical calculations. *J. Am. Chem. Soc.* 1991; 113:5964–5970.
56. Zhou ZR, Ogden S, Leary JA. Linkage position determination in oligosaccharides: MS/MS study of lithium-cationized carbohydrates. *J. Org. Chem.* 1990; 55:5444–5446.
57. Zhu F, Glover MS, Shi H, Trinidad JC, Clemmer DE. Populations of metal-glycan structures influence MS fragmentation patterns. *J. Am. Soc. Mass Spectrom.* 2015; 26:25–35. [PubMed: 25315458]
58. Penn SG, Cancilla MT, Lebrilla CB. Fragmentation behavior of multiple-metal-coordinated acidic oligosaccharides studied by matrix-assisted laser desorption ionization Fourier transform mass spectrometry. *Int. J. Mass Spectrom.* 2000; 195:259–269.

59. Huang Y, Dodds ED. Discrimination of isomeric carbohydrates as the electron transfer products of group II cation adducts by ion mobility spectrometry and tandem mass spectrometry. *Anal. Chem.* 2015; 87:5664–5668. [PubMed: 25955237]
60. Kornacki JR, Adamson JT, Hakansson K. Electron detachment dissociation of underivatized chloride-adducted oligosaccharides. *J. Am. Soc. Mass Spectrom.* 2012; 23:2031–2042. [PubMed: 22911097]
61. Reinhold VN, Sheeley DM. Detailed characterization of carbohydrate linkage and sequence in an ion trap mass spectrometer: Glycosphingolipids. *Anal. Biochem.* 1998; 259:28–33. [PubMed: 9606139]
62. Viseux N, de Hoffmann E, Domon B. Structural analysis of permethylated oligosaccharides by electrospray tandem mass spectrometry. *Anal. Chem.* 1997; 69:3193–3198. [PubMed: 9271064]
63. Ciucanu I. Per-O-methylation reaction for structural analysis of carbohydrates by mass spectrometry. *Anal. Chim. Acta.* 2006; 576:147–155. [PubMed: 17723627]
64. Heiss C, Wang Z, Azadi P. Sodium hydroxide permethylation of heparin disaccharides. *Rapid Commun. Mass Spectrom.* 2011; 25:774–778. [PubMed: 21337639]
65. Price NPJ. Permethylation linkage analysis techniques for residual carbohydrates. *Appl. Biochem. Biotechnol.* 2008; 148:271–276. [PubMed: 18418759]
66. Viseux N, de Hoffmann E, Domon B. Structural assignment of permethylated oligosaccharide subunits using sequential tandem mass spectrometry. *Anal. Chem.* 1998; 70:4951–4959. [PubMed: 9852781]
67. Chen X, Liu G, Wong YLE, Deng L, Wang Z, Li W, Chan TWD. Dissociation of trivalent metal ion (Al^{3+} , Ga^{3+} , In^{3+} and Rh^{3+})–peptide complexes under electron capture dissociation conditions. *Rapid Commun. Mass Spectrom.* 2016; 30:705–710. [PubMed: 26864523]
68. Prell JS, Flick TG, Oomens J, Berden G, Williams ER. Coordination of trivalent metal cations to peptides: Results from IRMPD spectroscopy and theory. *J. Phys. Chem. A.* 2009; 114:854–860.
69. Commodore JJ, Cassady CJ. Effects of acidic peptide size and sequence on trivalent praseodymium adduction and electron transfer dissociation mass spectrometry. *J. Mass Spectrom.* 2017; 52:218–229. [PubMed: 28170125]
70. Flick TG, Donald WA, Williams ER. Electron capture dissociation of trivalent metal ion-peptide complexes. *J. Am. Soc. Mass Spectrom.* 2013; 24:193–201. [PubMed: 23283726]
71. Commodore JJ, Cassady CJ. The effects of trivalent lanthanide cationization on the electron transfer dissociation of acidic fibrinopeptide B and its analogs. *J. Am. Soc. Mass Spectrom.* 2016; 27:1499–1509. [PubMed: 27294379]
72. Angyal SJ. Complexes of metal-cations with carbohydrates in solution. *Adv. Carbohydr. Chem. Biochem.* 1989; 47:1–43.
73. Angyal SJ. Complexes of carbohydrates with metal cations. I. determination of extent of complexing by NMR-spectroscopy. *Aust. J. Chem.* 1972; 25:1957–1966.
74. Franks F, Hall JR, Irish DE, Norris K. The effect of cations on the anomeric equilibrium of D-glucose in aqueous-solutions - a Raman-spectral study. *Carbohydr. Res.* 1986; 157:53–64.
75. Petruš L, Bílik V, Kuniak , Stanković . Chromatographic-separation of alditols on a cation-exchange resin in the lanthanum form. *Chemicke Zvesti.* 1980; 34:530–536.
76. Domon B, Costello CE. A systematic nomenclature for carbohydrate fragmentations in FAB-MS MS spectra of glycoconjugates. *Glycoconj. J.* 1988; 5:397–409.
77. Kohler M, Leary JA. LC/MS/MS of carbohydrates with postcolumn addition of metal chlorides using a triaxial electrospray probe. *Anal. Chem.* 1995; 67:3501–3508. [PubMed: 8686898]
78. Feng C, Commodore JJ, Cassady CJ. The use of chromium(III) to supercharge peptides by protonation at low basicity sites. *J. Am. Soc. Mass Spectrom.* 2015; 26:347–358. [PubMed: 25395012]
79. Lavanant H, Virelizier H, Hoppilliard Y. Reduction of copper(II) complexes by electron capture in an electrospray ionization source. *J. Am. Soc. Mass Spectrom.* 1998; 9:1217–1221.
80. Zheng YJ, Ornstein RL, Leary JA. A density functional theory investigation of metal ion binding sites in monosaccharides. *Theochem-J. Mol. Struct.* 1997; 389:233–240.

81. Liu H, Håkansson K. Divalent metal ion-peptide interactions probed by electron capture dissociation of trications. *J. Am. Soc. Mass Spectrom.* 2006; 17:1731–1741. [PubMed: 16952459]
82. Chen X, Fung YME, Chan WYK, Wong PS, Yeung HS, Chan TWD. Transition metal ions: Charge carriers that mediate the electron capture dissociation pathways of peptides. *J. Am. Soc. Mass Spectrom.* 2011; 22:2232–2245. [PubMed: 21952786]
83. Kleinnijenhuis AJ, Mihalca R, Heeren RMA, Heck AJR. Atypical behavior in the electron capture induced dissociation of biologically relevant transition metal ion complexes of the peptide hormone oxytocin. *Int. J. Mass Spectrom.* 2006; 253:217–224.
84. Voet, D., Voet, JG., Pratt, CW. *Fundamentals of Biochemistry: Life at the Molecular Level.* 4. John Wiley & Sons; 2013. p. 1200
85. French AD, Mouhous-Riou N, Pérez S. Computer modeling of the tetrasaccharide nystose. *Carbohydr. Res.* 1993; 247:51–62. [PubMed: 8221731]
86. Jeffrey GA, Huang DB. The tetrasaccharide nystose trihydrate: Crystal structure analysis and hydrogen bonding. *Carbohydr. Res.* 1993; 247:37–50. [PubMed: 8221730]
87. Jeffrey GA, Huang DB. Hydrogen bonding in the crystal structure of the tetrasaccharide stachyose hydrate: A 1:1 complex of two conformers. *Carbohydr. Res.* 1991; 210:89–104. [PubMed: 1878888]
88. Shannon RD. Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. *Acta Crystallogr. Sect. A.* 1976; A32:751–767.

Figure 1. Oligosaccharides included in this study: (a) stachyose, (b) nystose, (c) 3 α ,4 β ,3 α -galactotetraose, (d) maltotetraose, and (e) maltoheptaose.

Figure 2.

ETD mass spectra of maltotetraose for (a) $[M + 2Na]^{2+}$, (b) $[M + Mg]^{2+}$, (c) $[M + Cu]^{2+}$, and (d) $[M + Co]^{2+}$. Colors used to illustrate the product ions are red for cross-ring cleavages, blue for glycosidic bond cleavages, and green for internal cleavages. Refer to Table 2 for identities of internal cleavages indicated by mass lost. Undissociated precursor ion is labeled in light blue with a large light blue diamond arrow head. Product ions involving solely neutral losses are labeled in black.

Figure 3.

CID mass spectra of maltotetraose for (a) $[M + 2Na]^{2+}$, (b) $[M + Mg]^{2+}$, (c) $[M + Cu]^{2+}$, and (d) $[M + Co]^{2+}$. Refer to the Figure 2 caption for an explanation of the color codes.

Figure 4. Mass spectra from (a) ETD and (b) CID of $[M + Co]^{2+}$ from maltotetraose with ^{18}O isotopic labeling at the reducing end. Refer to the Figure 2 caption for an explanation of the color codes.

Figure 5.

Mass spectra from (a) ETD and (b) CID of $[M + Co]^{2+}$ from galactotetraose with ^{18}O isotopic labeling at the reducing end. Refer to the Figure 2 caption for an explanation of the color codes.

Figure 8.

Mass spectra from (a) ETD and (b) CID of $[M + Co]^{2+}$ from maltoheptaose with ^{18}O isotopic labeling at the reducing end. Refer to the Figure 2 caption for an explanation of the color codes.

Table 1

Comparison of physical properties of metal ions included in study.

Metal	Charge (Q) ^a	Ionic Radius (R) ^b	Charge Density (Q/R ³) ^c	First Ionization Energy (kJ/Mol)	Second Ionization Energy (kJ/Mol)	Third Ionization Energy (kJ/Mol)	Electron Configuration
Na	1	0.116	640	495.8	4562	6910.3	[Ne]
Mg	2	0.086	3140	737.7	1450.7	7732.7	[Ne]
Al	3	0.0675	9750	577.5	1816.7	2744.8	[Ne]
K	1	0.152	284	418.8	3052	4420	[Ar]
Ca	2	0.114	1350	589.8	1145.4	4912.4	[Ar]
Cr	3	0.0755	6970	652.9	1590.6	2987	[Ar]3d ³
Fe	3	0.0785	6200	762.5	1561.9	2957	[Ar]3d ⁵
Co	2	0.0885	2890	760.4	1648	3232	[Ar]3d ⁷
Ni	2	0.083	3500	737.1	1753	3395	[Ar]3d ⁸
Cu	2	0.087	3040	745.5	1957.9	3555	[Ar]3d ⁹
Zn	2	0.088	2940	906.4	1733.3	3833	[Ar]3d ¹⁰

^a Q in 1.6×10^{-19} C.

^b R in nm [88].

^c Q/R³ in 1.6×10^{-19} C/nm³.

saccharide
heptaose
Met] ⁺
+ Met] ⁺
2Met] ²⁺
+ 2Met + NO ₃] ²⁺
Met] ²⁺
+ Met] ²⁺
+ Met - H] ⁺
+ Met + NO ₃] ⁺
+ 2Met + NO ₃] ³⁺
+ 2Met + NO ₃ - H] ²⁺
Met] ²⁺
+ Met] ²⁺
+ Met - H] ⁺
+ 2Met - 2H] ²⁺
+ Met + NO ₃] ⁺
+ 2Met + NO ₃] ³⁺
+ 2Met + NO ₃ - H] ²⁺
+ Met - H ₂ O] ⁺
+ Al - H] ²⁺
+ Al + NO ₃ - H] ⁺
+ Cr - H] ²⁺
+ Cr + NO ₃ - H] ⁺
+ Fe] ²⁺
+ Fe + NO ₃ - H] ⁺

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Metal Adducted	Tetrasaccharides			Heptasaccharide
	Maltotetraose	3 α ,4 β ,3 α -galactotetraose	Nystose	Stachyose

S [M + Fe – H₂O]²⁺

^aB represents the base peak in the spectrum (100% intensity). S represents a strong ion intensity 70–100% of base peak, M represents a medium ion intensity 30–70% of base peak, and W represents a weak ion less than 30% of base peak ion intensity.

^bMet denotes the metal adducted to the oligosaccharide.

Table 3

Possible isobaric cross-ring cleavage assignments based on mass lost.

Mass Loss	Maltotetraose	3α,4β,3α-galactotetraose	Nystose	Stachyose	Maltoheptaose
60 Da (C ₂ H ₄ O ₂)	1,3X _n 0,4X _n 2,4X _n 0,2A _n ^a	1,3X ₁ 1,3X ₃ 0,4X ₃ 2,4X _n 0,2A _n ^a	0,4X _n 2,4X _n 1,3X ₀	1,3X _n 0,2A _n ^a 2,4X _n (not on fructose)	1,3X _n 0,4X _n 2,4X _n 0,2A _n ^a
90 Da (C ₃ H ₆ O ₃)	1,4X _n 0,3X _n 1,4A _n ^a 0,3A _n ^a	0,3X ₀ 0,3X ₂ 1,4X ₀	1,4X ₀ 0,3X ₁ 0,3X ₂ 0,3X ₃	0,3X _n 1,4X _n (not on fructose)	1,4X _n 0,3X _n 1,4A _n ^a 0,3A _n ^a
120 Da (C ₄ H ₈ O ₄)	2,4A _n 0,2X _n ^a	Not observed	Not observed	Not observed	2,4A _n 0,2X _n ^a

^a n represents the position of the monosaccharide ring that is undergoing cross-ring cleavage and can be any monosaccharide unit remaining in the sugar after the glycosidic bond cleavage, while n^{*} must be the same subscript as on the corresponding glycosidic bond cleavage.

^b Loss of 60 Da and 90 Da were observed with both CID and ETD, whereas loss of 120 Da was only observed with CID.