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Fragmentation behavior of multiple-metal-coordinated acidic oligosaccharides studied by matrix-assisted laser desorption ionization Fourier transform mass spectrometry

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Abstract

Using matrix-assisted laser desorption ionization we have been able to form gas-phase mixed metal complexes by doping acidic oligosaccharides simultaneously with two different alkali metals. Multiple metal coordination brings up several interesting points such as whether the alkali metals act like point charges by repelling one another, and whether the alkali metals bind to specific sites within the saccharide In this work we carried out collision induced dissociation (CID) on multiply coordinated oligosaccharides to gain insight into these questions. By using CID of the mixed metal complexes we find that different alkali metals show differing specificity for whether they bind to the sialic acid containing fragment, or the remaining oligosaccharide fragment. We also find that the sialic acid is not capable of solvating two small alkali metals simultaneously, but for larger alkali metals such as cesium this is not the case. Molecular modeling of acidic sugars show that there is not a "defined" binding site to which the different alkali metals bind, but many local minima exist. (Int J Mass Spectrom 195/196 (2000) 259–269) © 2000 Elsevier Science B.V.

Keywords: Oligosaccharide; Sialic acid; Metals

1. Introduction

In recent years we have shown that matrix-assisted laser desorption/ionization coupled with Fourier transform mass spectrometry (MALDI-FTMS) is a powerful tool for the analysis of neutral oligosaccharides [1–3]. More recently, however, we have become interested in understanding the behavior of acidic oligosaccharides during the MALDI-FTMS process. The acidity of oligosaccharides are often due to sialic acid residues. Sialic acid is a general term for a family of nine-carbon carboxylated sugars involved in a variety of biological functions from cell adhesion and cell recognition to cell death. By studying the acidic glycolipids or gangliosides [4], we noticed it is possible to form multiple metal coordinated species. For example, when the trisialoganglioside GT1b is doped with CsCl, an $[M + 2Cs - 3H]^-$ species was observed. Likewise for the tetrasialoganglioside, GQ1b, both the $[M + 3Cs - 4H]^-$ and $[M + 2Cs + Na - 4H]^-$ ions were observed. Multiple metal coordination brings up several interesting

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Dedicated to the memory of Robert Squires, a good friend and an excellent scientist. We will miss his many contributions.

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points. (1) Do the alkali metals act like point charges by repelling one another? (2) Do the alkali metals bind to specific sites within the saccharide? (3) How effectively does the alkali metal neutralize the acidic charge?

Another noteworthy point is that these species are not commonly seen in MALDI time-of-flight (TOF) or MALDI magnetic sector instruments. In TOF experiments, for example Harvey and co-workers [5] found that for acidic oligosaccharides the species most often observed is $[M + Na]^+$, and only in conditions of excess salt would a multiple metal species be observed. Likewise, for the MALDI-TOF of multiacidic oligosaccharides Papac and co-workers [6] observed only the $[M - H]^-$ ion in the negative mode, even when the sugar contained three sialic acid residues. The reason for the observation of these species in TOF and sector mass spectrometers could simply be due to different sample preparation or possibly the differences in lifetimes of the ions. These species may be more stable than other, nonalkali metal coordinated species that dissociate in the long time scale of MALDI-FTMS—the same reason that leads to more fragmentation being observed in MALDI-FTMS compared with MALDI-TOF.

In this article we report the use of sustained off resonance irradiation collision-induced dissociation (SORI-CID) [7,8] on multiple metal coordinated oligosaccharides to gain insight into the binding site of the alkali metals, in the hope that this understanding will lead to ways to control the very prompt sialic acid fragmentation observed when analyzing acidic oligosaccharides. Molecular modeling data is also provided to determine whether specific binding sites exist.

2. Experimental

Oligosaccharides were obtained from Oxford Glycosystems (Oxford, UK), gangliosides were obtained from Sigma Chemical Company (St. Louis, MO), and all were used without any further purification. 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid were obtained from Aldrich (Milwaukee, WI).

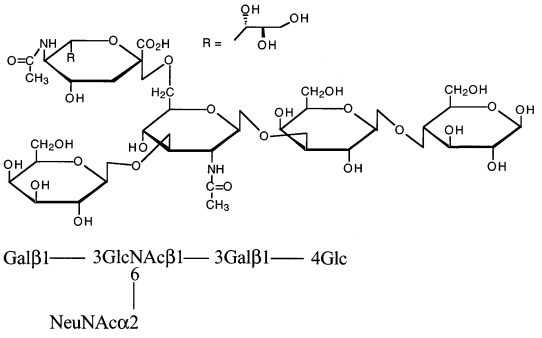
The MALDI spectra were obtained on an external source Fourier transform mass spectrometer (HiRes-MALDI, IonSpec Corporation, Irvine) instrument, equipped with a 4.7 tesla magnet, and a nitrogen laser at 337 nm. Operation of this instrument and the analysis of oligosaccharides have been described in greater detail in earlier publications [2–4,9]. All spectra were obtained by using DHB as the matrix. The DHB was used at a concentration of 0.4 M, dissolved in ethanol. The oligosaccharides were dissolved in water at a concentration of 1 mg/mL unless otherwise stated. Sample preparation involved depositing 1 μL of sample on the probe tip followed by 1 μL of each metal to be doped at a concentration of 0.01 M as the chloride salt in methanol, and finally by 1 μL of DHB. The mixture was then dried in a cold air stream until crystals started to form. The area of the probe–tip was 8 mm^2 whereas the laser spot only samples from a 0.5 mm^2 area.

Unless stated, all CID experiments were performed under sustained off-resonance collision-induced dissociation (SORI-CID) conditions [8]. The MALDI produced ions are initially trapped in the ion cyclotron resonance (ICR) cell (at time zero). After 3 s all ions except the *m/z* of interest are ejected from the cell. These ejections are carried out by using a combination of on-resonance bursts and the use of an arbitrary waveform generator. At 5 s the ions are excited at 1000 Hz below their cyclotron frequency for 1 s. Although the ions are being excited the pulse valve is fired 4 times, at 250 ms intervals, to simulate the constant pressure required for SORI-CID. During this time the pressure reaches approximately 9×10^{-6} Torr and remains approximately constant ($\pm$10%) during the CID event. After excitation, we wait a further 5 s to allow the chamber to pump down to a low pressure (10^{-10} Torr) before excitation and detection. The total length of the experiment is therefore 11 s.

3. Results

3.1. MALDI-FTMS of mixed metal complexes of the acidic oligosaccharide, LS-tetrasaccharide-b

Scheme 1 shows the structure of LS-tetrasaccharide b (LSTb). It has one sialic acid (NeuAc) and



Scheme 1.

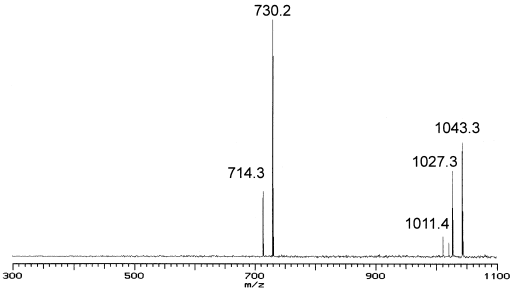


Fig. 2. MALDI-FTMS spectrum of LSTb doped with 0.01 M NaCl and 0.01 M LiCl. The quasimolecular ions are *m/z* 1011, *m/z* 1027, and *m/z* 1043. For additional details see text.

therefore can coordinate two alkali metals in the positive ion mode. LSTb was therefore chosen as a model compound, and was doped with different combinations of metals.

Fig. 1 shows a MALDI-FTMS mass spectrum of LSTb that has been doped with 0.01 M NaCl and 0.01 M CsCl. Because it can coordinate two metals, there are therefore three peaks corresponding to the quasimolecular ions: *m/z* 1263.1 $[LSTb + 2Cs - H]^+$, *m/z* 1153.2 $[LSTb + Cs + Na - H]^+$, *m/z* 1043.3 $[LSTb + 2Na - H]^+$. It should be noted that relative intensities of the three quasimolecular ions can vary considerably each time the sample is prepared, because it is very sensitive to the amount of dopant. For this reason we have not assigned special significance to the relative peak heights. The other dominant peaks in Fig. 1 are at *m/z* 730.2 and *m/z* 840.2. The *m/z* 730.2 corresponds to the loss of sialic acid, where the remaining fragment carries a Na^+ ion adduct, to give $[LSTb - NeuAc + Na]^+$. The *m/z* 730.2 ion may come from either the *m/z* 1043.3 ($[LSTb + 2Na - H]^+$) or *m/z* 1153.2 ($[LSTb + Cs + Na - H]^+$) ions. The *m/z* 840.1 corresponds to the loss of sialic acid, where the remaining fragment carried a Cs^+ ion adduct, to give $[LSTb - NeuAc + Cs]^+$. Again the *m/z* 840.1 ion can be formed from either the *m/z* 1153.1 $[LSTb + Cs + Na - H]^+$ or the *m/z* 1263.1 $[LSTb + 2Cs - H]^+$.

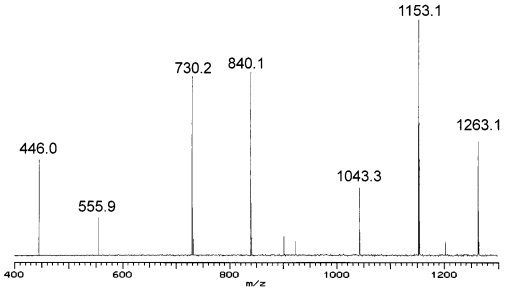


Fig. 1. MALDI-FTMS spectrum of LSTb doped with 0.01 M NaCl and 0.01 M CsCl. The quasimolecular ions are *m/z* 1043, *m/z* 1153, *m/z* 1263 corresponding to $2Na^+$, $Cs^+ + Na^+$, and $2Cs^+$. A proton is removed to yield a net charge of +1. For additional details see text.

Fragments corresponding to the sialic acid coordinated to two metals are observed. In Fig. 1 the peaks at *m/z* 446.0 corresponds to $[NeuAc + Na + Cs - H]^+$, and the peak at *m/z* 555.9 to $[NeuAc + 2Cs - H]^+$. However, we do not observe a peak at *m/z* 336 which would be $[NeuAc + 2Na - H]^+$. These species are interesting in that two large metal ions such as Cs^+ can coordinate to essentially a monosaccharide.

Fig. 2 shows the MALDI-FTMS mass spectrum of LSTb doped with 0.01 M LiCl and 0.01 M NaCl. As in the previous example we observe three peaks corresponding to the parent ion. These are *m/z* 1043.3 $[LSTb + 2Na - H]^+$, *m/z* 1027.3 $[LSTb + Na + Li - H]^+$, and *m/z* 1011.4 $[LSTb + 2Li - H]^+$. It is interesting to note that the *m/z* 1011.4, the dilithium species, is the least abundant quasimolecular ion. The lithium ion has the highest charge density and causes fragmentation leading to an attenuated quasimolecular ion [3,10]. It should be noted that it is difficult to obtain a molecular ion peak ($[LSTb + 2Li - H]^+$) even when LSTb is doped with only LiCl (example

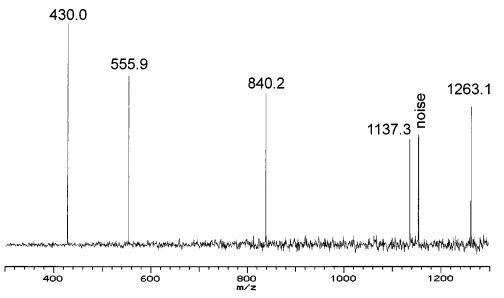


Fig. 3. MALDI-FTMS spectrum of LSTb doped with 0.01 M LiCl and 0.01 M CsCl. The quasimolecular ions are *m/z* 1137 (Cs^+ + Li^+) and *m/z* 1263 (2Cs).

not shown). The only fragments in Fig. 2 are at *m/z* 730.2 $[LSTb - NeuAc + Na]^+$ and *m/z* 714.3 $[LSTb - NeuAc + Li]^+$, which could have come from the respective *m/z* 1011.4, 1027.3, and 1043.3 ions. The *m/z* 714.3 ion is also in low abundance, for the same reasons discussed above. Note also, that there is no sialic acid fragment present either as $[NeuAc + 2Li - H]^+$ or $[NeuAc + Li + Na - H]^+$, unlike the previous example (Fig. 1).

For completeness, the sialylated oligosaccharide was doped with LiCl and CsCl. Fig. 3 shows the MALDI-FTMS spectrum of LSTb doped with 0.01 M LiCl and 0.01 M CsCl. Only two of the quasimolecular ions are observed: *m/z* 1263.1 $[LSTb + 2Cs - H]^+$, *m/z* 1137.3 $[LSTb + Cs + Li - H]^+$. The corresponding dilithiated species, *m/z* 1011 $[LSTb + 2Li - H]^+$ is not observed. There is one peak corresponding to the loss of sialic acid *m/z* 840.2 $[LSTb - NeuAc + Cs]^+$. There are two peaks corresponding to the sialic acid at *m/z* 555.9 $[NeuAc + 2Cs - H]^+$ and 430.0 $[NeuAc + Li + Cs - H]^+$, but no $[NeuAC + 2Li - H]^+$.

We may conclude from the data so far that it is difficult for the sialic acid to solvate two charges of the smaller alkali metals such as sodium and lithium. However if one or both of these ions is a larger alkali metal such as cesium, a species $[NeuAc + M1 + M2 - H]^+$ where M1 and M2 are alkali metal ions is observed in the MALDI-FTMS spectrum. This is particularly true when LSTb is doped with LiCl only. The dilithiated sialic acid fragment is not observed. When the sample is doped with NaCl, the corresponding fragment $[NeuAc + 2Na]^+$ is observed with only a very weak abundance (less than 5% of the base peak, data not shown). In the quasimolecular ion of LSTb, both Li^+, e.g. $[LSTb + 2Li - H]^+$, and Na^+ are observed, although the Li^+ species is considerably attenuated presumably because lithium causes extensive fragmentation during MALDI.

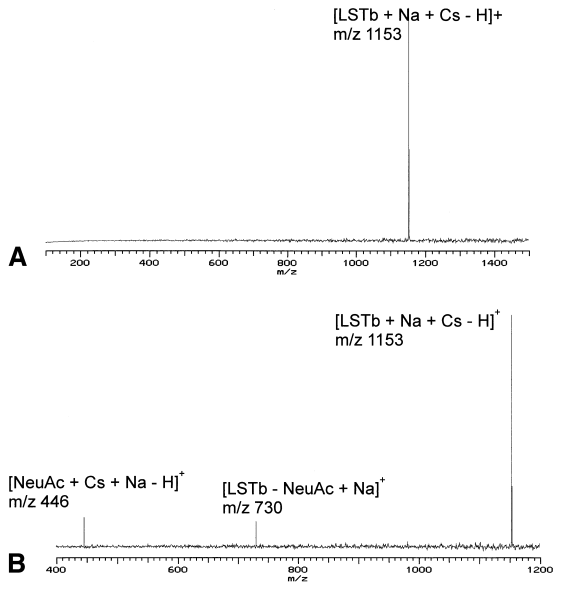


Fig. 4. (a) Isolated peak of $[LSTb + Na + Cs - H]^+$, formed by MALDI-FTMS. (b) CID spectrum of $[LSTb + Na + Cs - H]^+$ 2.25 V_{b-p} 1000 Hz off-resonance for 1 s which, by using standard equations, equates to $\langle E_{translational} \rangle$ of 3.1 eV and $\langle E_{com} \rangle$ of 0.12 eV with the ion undergoing 1000 oscillations.

3.2. CID of LS-tetrasaccharide-b

It was originally thought that the different sized metal ions may have varying affinities for different functional groups on LSTb, and therefore through the use of collision induced dissociation experiments on the mixed metal species it may be possible to identify these sites. Fig. 4(a) shows the mass spectrum of the isolation of the $[LSTb + Cs + Na - H]^+$ species, and Fig. 4(b) shows the CID spectrum at 2.25 V_{b-p}. Using standard equations [7], the ion gains 3.1 eV of translational energy, and 0.1 eV of center of mass frame energy. The CID of $[LSTb + Cs + Na - H]^+$ results in two fragments, at *m/z* 730 corresponding to

the loss of the sialic acid residue and the cesium ion, to give the fragment $[LSTb - NeuAc + Na]^+$. Also, there is a fragment at *m/z* 446 which corresponds to the sialic acid monomer with both the cesium and lithium attached, $[NeuAc + Na + Cs - H]^+$. This shows that the most dominant mode of fragmentation in this pentasaccharide is the loss of the NeuAc with either one or two metals attached to the acidic monosaccharide. This corresponds well with the fragmentation seen in the MALDI-FTMS spectra, and of other acidic oligosaccharides we have studied [4]. At higher CID energies (2.5 V_{b-p}, $\langle E_{tr}\rangle = 3.54$ eV, $\langle E_{cm}\rangle = 0.12$ eV) an additional fragment appears, corresponding to the loss of the reducing end glucose $[LSTb - Glc + Cs + Na - H]^+$ (spectrum not shown).

An analog of LSTb was also studied, namely LSTa in which the sialic acid is attached to the nonreducing end galactose through a 2–3 linkage. The CID of the quasimolecular ion, $[LSTa + Cs + Na - H]^+$, resulted in the loss of 138 mass units, which is equivalent to the loss of water and a $^{0,2}A_5$ (according to the Domon and Costello nomenclature [11], #294]) cross ring cleavage (data not shown). The same fragment is observed in the $[LSTa + 2Na - H]^+$ ion (data not shown). This would indicate that in LSTa the sialic acid is far enough from the reducing end to allow cross ring cleavages to occur, whereas in LSTb the sialic acid is more central. Therefore, the metal ions associated with the acidic group interact with many of the oxygens causing the only fragment to be loss of the sialic acid itself. This is therefore good evidence that the metals are closely associated with the sialic acid residue in the LST analogs.

Fig. 5(a) shows the mass spectrum of the isolated $[M + Li + Na - H]^+$ species, and Fig. 5(b) shows the SORI CID spectrum at 2.0 V_{b-p} ($\langle E_{tr}\rangle = 2.9$ eV, $\langle E_{cm}\rangle = 0.12$ eV). Only one fragment resulted from the CID that corresponds to the loss of the sialic acid and the lithium, the resulting fragment being at *m/z* 730. In this case we do not observe the sialic acid with the lithium and sodium attached, i.e. $[NeuAc + Li + Na - H]^+$. This is perfectly consistent with the MALDI-FTMS spectra, where we concluded that it is not possible for the sialic acid to solvate two charges

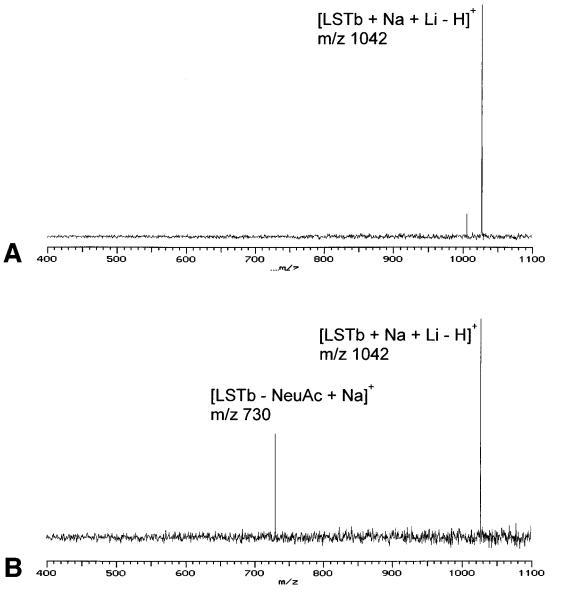


Fig. 5. (a) Isolated peak of $[LSTb + Li + Na - H]^+$, formed by MALDI-FTMS. (b) SORI CID spectrum of $[LSTb + Li + Na - H]^+$ 2.2 V_{b-p} 1000 Hz off-resonance for 1 s, which by using standard equations equates to $\langle E_{translational}\rangle$ of 2.9 eV and $\langle E_{com}\rangle$ of 0.1 eV with the ion undergoing 1000 oscillations.

from the smaller alkali metals such as sodium and lithium. This behavior is due possibly to the small size of the two metal ions that force them to be closer together.

Figs. 4 and 5 suggest a possible trend. That is, when the sialic acid is lost, as in loss of $[NeuAc + M1]^0$, it is the sodium that is left to impart charge to the remaining oligosaccharides. We infer that the sodium ion is somewhat preferentially bound in the "neutral" portion, i.e. away from the sialic acid residue, of the tetrasaccharide. To investigate this possibility further, a number of different combinations of multiple metal species were formed and subjected to CID. Table 1 shows the fragments obtained during CID for different metal doping.

Table 1 shows the CID products of LSTb coordinated to two metals, Na^+ and M^+ where M is some other alkali metal. Both off-resonance (or SORI) and on-resonance CID experiments were separately performed. However, the products of the two CID methods were identical. The major fragment is always the loss of the metal M^+ coordinated to the sialic acid

Table 1
Product ions produced by collision-induced dissociation of mixed metal complexes of $[LSTb + M_1 + M_2 - H]^+$ in both on- and off-resonance excitation modes; the same product ions are observed in both on- and off-resonance modes

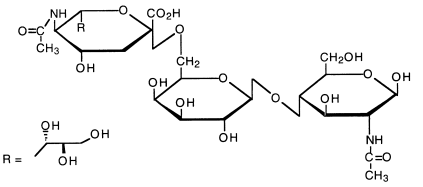
Metal	Fragments
Li, Na	*m/z* 730: $[LSTb - NeuAc - Li]^+$
Li, K	*m/z* 746: $[LSTb - NeuAc - Li]^+$ *m/z* 336: $[NeuAc + Li + K - H]^+$
Li, Cs	*m/z* 801: $[LSTb - NeuAc - Li]^+$ *m/z* 430: $[NeuAc + Li + Cs - H]^+$
Na, K	*m/z* 730: $[LSTb - NeuAc - K]^+$ *m/z* 352: $[NeuAc + K + Na - H]^+$ −60 cross ring cleavage −162 glycosidic bond cleavage
Na, Cs	*m/z* 730: $[LSTb - NeuAc - Cs]^+$ *m/z* 446: $[NeuAc + Na + Cs - H]^+$ −162 glycosidic bond cleavage
K, Cs	*m/z* 462: $[NeuAc + K + Cs - H]^+$ *m/z* 1007: −162 glycosidic bond cleavage

(NeuNAc) anion. The Na^+ always stays bound to the remaining tetrasaccharide. This indicates that there is either a selectivity for sodium by the remaining fragment Galβ1–3GlcNAcβ1–3Galβ1–4Glc, or that it is unfavorable for the Na^+ to bind to the sialic acid.

In contrast to the results in Table 1, previous work on neutral (nonsialic acid containing) oligosaccharides [12] showed that the two CID processes give very different products. With neutral oligosaccharides on-resonance CID results in primarily the loss of the metal, whereas off-resonance CID gives sugar fragmentation. In the SORI CID of $[M + Cs + Na - H]^+$, we do observe some Cs^+ at *m/z* 133, but this is not the primary fragment. We believe that in these acidic systems the metal is very tightly bound making it difficult to lose just the metal ions.

3.3. MALDI-FTMS of sialyllactose and sialy-N-acetyllactosamine

To further investigate the multiple metal adducts, two smaller systems were also studied, these being 6′sialyllactose (6′SL) and 6′ sialy-N-acetyllactosamine (6′SLN), whose structures are shown in Schemes 2 and 3. In previous work by our group, we

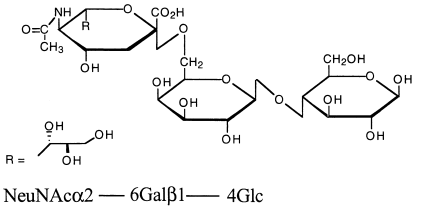


Scheme 2.

noted that for polymers of hexose sugars (such as glucose and galactose) there were a minimum number of units needed to observe a metal adducted species [3]. For example, for an $[M + Na]^+$ ion at least two subunits were needed, whereas to observe $[M + Cs]^+$ at least four subunits were needed. Therefore, by investigating small acidic saccharides this trend could be further investigated.

Harvey *et al*. [5] has also previously looked at 6′SLN using MALDI on a magnetic sector instrument. He primarily observed the species $[M + Na]^+$ (100%), with a small amount of $[M + K]^+$ (20%). Some $[M + 2Na - H]^+$ was present (65%), but only a small amount of the mixed metal species $[M + Na + K + H]^+$ (12%), although the workers were not intentionally trying to form this species. Fragments resulting from the MALDI process were $[M + Na - 18]^+$ and $[M + Na - 28]^+$, both at less than 5% intensity and presumably corresponding to loss of water and loss of methanol, respectively. However the dominant fragment is the loss of 44 mass units, corresponding to decarboxylation with approximately 45% intensity.

Fig. 6 shows the MALDI-FTMS of 6′SLN doped with 0.01 M NaCl and 0.01 M CsCl, as in the experiments discussed above for LSTb. The three



Scheme 3.

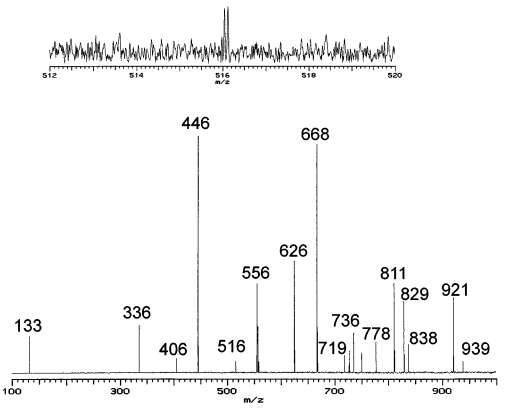


Fig. 6. MALDI-FTMS spectrum of 6′SLN doped with 0.01 M NaCl and 0.01 M CsCl. For more details see text.

parent peaks are found at *m/z* 939.0 [6′SLN + 2Cs − H]$^+$, *m/z* 829.1 [6′SLN + Cs + Na − H]$^+$, and *m/z* 719.2 [6′SLN + 2Na − H]$^+$, however there are numerous fragments also present in Fig. 6. That the di-cesium species of such a small oligosaccharide can be formed is particularly interesting and indicates that Cs^+ ions do not act like point charges by repelling one another.

The parent ion at *m/z* 939.0 [6′SLN + 2Cs − H]$^+$ loses water to give a fragment at *m/z* 921.0, an $^{0,2}A_3$ cross-ring cleavage to give a fragment at *m/z* 838.0, a $^{2,4}A_3$ fragment at *m/z* 778.0 and a C_2 fragment at *m/z* 736.0. The parent ion at *m/z* 829.1 [6′SLN + Cs + Na − H]$^+$ fragments to give loss of water at *m/z* 811.1, $^{0,2}A_3$ at *m/z* 728.1, $^{2,4}A_3$ at *m/z* 668.0 and a C_2 fragment at *m/z* 626.0. The parent ion at *m/z* 719.2 [6′SLN + 2Na − H]$^+$ gives fragments at *m/z* 558.1 $^{2,4}A_4$, and a C_2 fragment at *m/z* 516.1. The apparent lack of fragments of the *m/z* 719.2 ion [6′SLN + 2Na − H]$^+$ is possibly due to the low abundance of this species in the mass spectrum.

There are fragments due to the loss of the sialic acid residue, just as were present in the spectra of LSTb, and these are found at *m/z* 516.0 for [6′SLN − NeuAc + Cs]$^+$ and *m/z* 406.1 for [6′SLN − NeuAc + Na]$^+$. The benefit of using Fourier transform mass spectrometry is very evident when looking at the fragments. It should be noted that the mass of the [6′SLN − NeuAc + Cs]$^+$ is *m/z* 516.048 and the C_2 fragment from the parent ion [6′SLN + 2Na − H]$^+$ is *m/z* 516.131. The inset to Fig. 6 shows that these are distinguishable from one another.

The free sialic acid groups are also present at *m/z* 555.9 [NeuAc + 2Cs − H]$^+$, *m/z* 446.0 [NeuAc + Cs + Na − H]$^+$, and there is a small amount of the species [NeuAc + 2Na − H]$^+$ at *m/z* 336.1 even though we previously noted that this was a very "unstable" fragment due to the high charge density.

3.4. CID of sialyllactose and sialy-N-acetyllactosamine

Due to the complicated nature of Fig. 6, CID experiments were carried out on the isolated ions. In this set of experiments the saccharide concentrations were increased to enhance the signal sufficiently to get good signal to noise on which to carry out the isolation. As in the experiments with LSTb, mixed multiple metal species were formed and subjected to fragmentation by SORI. In the SORI of the mixed metal species [6′SLN + Na + Cs − H]$^+$ ion the fragments observed are −18 u (H_2O), −101 u ($^{0,2}A_3$, cross ring cleavage of terminal GlcNAc), −203 u (loss of GlcNAc residue), −161 u ($^{2,4}A_3$, cross ring cleavage to GlcNAc), and a fragment at *m/z* 446.0 corresponding to [NeuAc + Na + Cs − H]$^+$. Therefore in the CID of 6′SLN we see only loss of fragments of the GlcNAc residue, with both metals once again attached to the "acidic" portion of the sugar. Table 2 shows all the fragments obtained after CID of the three combinations of sodiated and cesiated species of 6′SLN.

6′Sialyllactose (6′SL) is an analog of 6′SLN which contains only a sialic acid and two hexose sugars, thus other than on the sialic acid there are no N-acetylamine groups present. In the MALDI-FTMS (not shown) it is again possible to form the [6′SL + Cs + Na − H]$^+$, [6′SL + 2Na − H]$^+$ and the [6′SL + 2Cs − H]$^+$ ion. The CID of the mixed metal species produces fragments similar to those seen in the CID of the mixed metal 6′SLN species; fragments of the reducing end sugar. For the [6′SL + Na + Cs − H]$^+$ ion the fragments observed are −60 ($^{0,2}A_3$), −120 ($^{4,2}A_3$), −162 (loss of glucose), and

Table 2
Fragments from the SORI-CID of 6′SLN

Parent ion	Fragments after SORI
$[M + 2Na - H]^+$ *m/z* 719	$-H_2O$ *m/z* 701 $^{2,4}A$ from GlcNAc − 161 (*m/z* 558) $[NeuAc + 2Na - H]^+$ −60 *m/z* 641
$[M + 2Cs - H]^+$ *m/z* 921	$-83\ ^{0,2}A$ from GlcNAc + H_2O (*m/z* 838) $[NeuAc + 2Cs - H]^+$ *m/z* 556 $-H_2O$ *m/z* 921 $-101\ ^{0,2}A$ from GlcNAc *m/z* 811 −185 (*m/z* 736) − GlcNAc + H_2O
$[M + Na + Cs - H]^+$ *m/z* 829	$-H_2O$ *m/z* 811 −101 from GlcNAc *m/z* 728 −203 − GlcNAc *m/z* 626 $[NeuAc + Na + Cs - H]^+$ *m/z* 446 $-161\ ^{2,4}A$ of GlcNAc *m/z* 668

$[NeuAc + Na + Cs - H]^+$. Table 3 shows all the fragments obtained after CID of the three combinations of the sodiated and cesiated species of 6′SL.

The species $[6'SL + Na]^+$ was also observed, albeit in low abundance, when 10 μg of sugar was used on the probe tip, and doped with NaCl only. This is the species more common in MALDI-TOF and MALDI magnetic sector as previously discussed. To form this species, the carboxylic acid is neutral and thus the metal must be on the remaining disaccharide. That a similar $[6'SL + Cs]^+$ species is not seen is therefore unsurprising. These species will behave like

Table 3
Fragments from SORI-CID of 6′SL

Parent ion	Fragments after SORI
$[M + 2Na - H]^+$ *m/z* 678	−60 + H_2O (*m/z* 516) 120 + H_2O (*m/z* 456) $[NeuNAc + 2Na - H]^+$ (*m/z* 336)
$[M + Na]^+$ *m/z* 656	−291/NeuNAc (*m/z* 365)
$[M + Na + Cs - H]^+$ *m/z* 788	−60 (*m/z* 728) −120 (*m/z* 668) −162 (*m/z* 626) $[NeuNAc + Na + Cs - H]^+$ (*m/z* 446)
$[M + 2Cs - H]^+$ *m/z* 898	$-H_2O$ (*m/z* 880) −60 (*m/z* 838) 162 (*m/z* 736) $[NeuAc + 2Cs - H]^+$ (*m/z* 556)

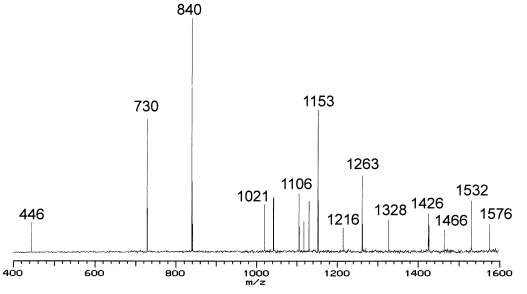
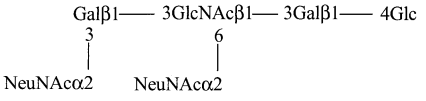


Fig. 7. MALDI-FTMS of DSLNT, a disialylated oligosaccharide, doped with 0.01 M NaCl and 0.01 M CsCl. The majority of the peaks are assigned in Table 4.

the neutral hexose polymers, requiring a minimum number of subunits to form complexes with the large alkali metals such as cesium [3]. CID of $[6'SL + Na]^+$ resulted in a single fragment, the loss of 291 u. This corresponds to the loss of the sialic acid, but with the Na^+ remaining with the disaccharide.

3.5. MALDI-FTMS of disialylated saccharides

Fig. 7 shows the MALDI-FTMS of disialyl-lacto-N-tetraose (DSLNT). This is an analog of LSTb, in which there is an additional sialic acid. The structure is shown in Scheme 4. This compound has two acidic groups and therefore in the positive ion mode it is possible to coordinate three alkali metals. In Fig. 7, DSLNT has been doped with both NaCl and CsCl. Now there are four possible combinations of quasi-molecular ion, although only two are observed in the spectrum. They are $[DSLNT + 2Cs + Na - 2H]^+$ at *m/z* 1576, and $[DSLNT + 2Na + Cs - 2H]^+$ at *m/z* 1466. We do not observe the $[DSLNT + 3Cs - 2H]^+$ nor the $[DSLNT + 3Na - H]^+$ species. Part of the reason may be that the sugar has a statistically less chance of coming into contact with only, for example, Na^+ ions (12.5%) than two Na^+ ions and a Cs^+ ion (37.5%), or two Cs^+ ions and a Na^+ ion (37.5%). The



Scheme 4.

Table 4
Peak assignments for [DSLNT + Cs + Na − H]$^+$; see mass spectrum in Fig. 7

m/z	Assignment
446	[NeuAc + Cs + Na − H]$^+$
730	[DSLNT − 2NeuAc + Na]$^+$
840	[DSLNT − 2NeuAc + Cs]$^+$
1021	[DSLNT − NeuAc + Na]$^+$
1043	[DSLNT − NeuAc + 2Na − H]$^+$
1131	[DSLNT − NeuAc + Cs]$^+$
1153	[DSLNT − NeuAc + Cs + Na − H]$^+$
1263	[DSLNT − NeuAc + 2Cs − H]$^+$
1466	[DSLNT + 2Na + Cs − 2H]$^+$
1532	−44
1576	[DSLNT + 2Na + Na − 2H]$^+$

same is true for all the Cs^+ complexes (12.5%). The parent ions that are present are however at low intensities (<20%), and fragmentation is abundant. Table 4 lists the fragments observed in Fig. 7. Previous work has shown it is more beneficial to analyze these multiacidic oligosaccharides in the negative ion mode [4]. In negative mode MALDI however, for sugars containing three or more acidic groups, multiple metal coordination would still occur.

3.6. Molecular modeling and molecular dynamics calculations of sialy-N-acetyllactosamine

In previous work on alkali metal adducts to neutral oligosaccharides, the use of molecular modeling calculations gave much insight into the fragmentation patterns seen in MALDI-FTMS [3]. We use the same method included in the DISCOVER program (Molecular Simulations, San Diego, CA) employing the AMBER forcefield to study acidic oligosaccharides. This forcefield has been shown to be particularly applicable to oligosaccharides [13]. It should be noted that within the structure a number of atom potentials were assigned; sodium ions were set to QN indicating the Na^+ state, cesium ions were set to QC indicating a Cs^+ ion and the carboxylic acid was constructed as the delocalized acid with the potentials set to O2 indicating a carboxyl nonbonded oxygen. The structures were generated by subjecting the ion first to dynamics in which the system was heated to 800 K for 75 fs, and then 300 K for 300 fs. The minimization followed the route of steepest descent for 500 iterations, followed by a conjugate descent until the derivative was within 0.0001 kcal/A. This cycle was then repeated to generate the required number of structures.

Molecular modeling was carried out on [6′SLN + Cs + Na − H]$^+$ as described above. So as not to influence final global minimum structures found by the DISCOVER program, 5 initial geometries were created for [6′SLN + Cs + Na − H]$^+$ where the metals were placed on different parts of the sugar. From these 5 initial geometries, 145 structures were generated. Within the 145 structures generated, three different dominant confirmations were found all having energies within 1 kcal/mol of each other. Fig. 8 shows these three structures. Minima A has both the Na^+ and Cs^+ ions close together (4.7 Å) in a "pocket" created by the reducing end N-acetylglucosamine (GlcNAc) and the galactose (Gal). The Na^+ ion is 4.2 and 5.1 Å from away from the carboxyl oxygens, respectively, whereas the Cs^+ ion is 4.9 and 7.0 Å away from the carboxyl oxygens, respectively. Minima B has Cs^+ interacting with the carboxylic acid, with Cs^+ to O distances of 5.6 and 4.8 Å, respectively. However, the Na^+ is far from the Cs^+ (8.8 Å) and is interacting with the carbonyl on the *n*-acetyl side chain of the sialic acid (Na^+ to O of 3.4 Å) and also with the alcohol at the C4 position (Na^+ to O of 3.3 Å). Minimum C has the metal ions interacting with one another (Na^+ to Cs^+ of 4.8 Å) but are on the opposite side of 6′SLN to the acidic function (Na^+ to carboxylic acid oxygens = 7.9 Å and 7.7 Å, and Cs^+ to carboxylic acid oxygens = 12.3 and 11.5 Å).

The same effect, where a number of minima are found, was also true when 6′SLN was modeled as [6′SLN + 2Na − H]$^+$ and [6′SLN + 2Cs − H]$^+$ (data not shown). Therefore, it would seem from molecular modeling that there is no "defined" binding site of either the sodium ion or the cesium ion in 6′SLN. There are a number of local minima with similar energies where the metal ions are found exhibiting varying coordination.

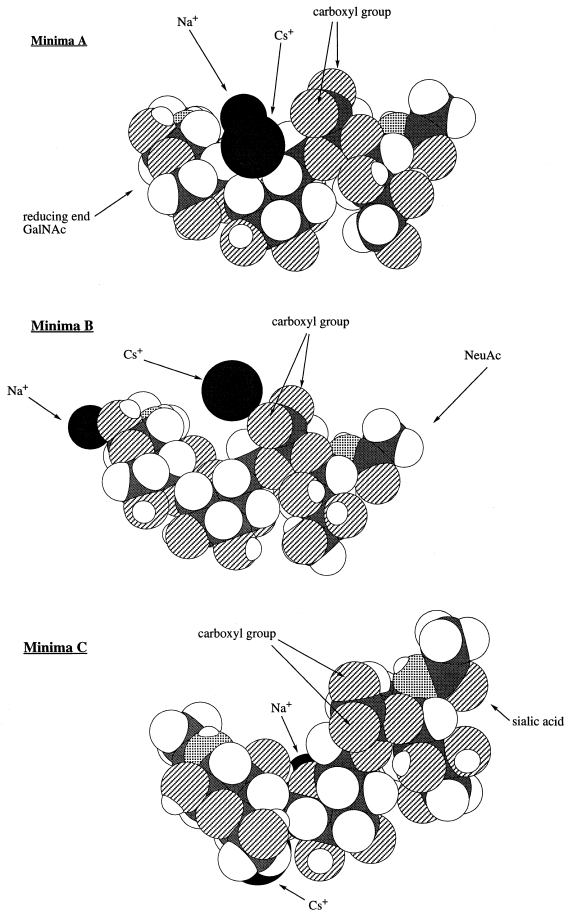


Fig. 8. The lowest energy structures generated by the DISCOVER program for $[6'SLN + Cs + Na - H]^+$. The energy of each structure vary by less than 1 kcal/mol. The three structures are representative of all the low energy structures and illustrate the mobility of the metal on the oligosaccharide surface.

4. Conclusions

In conclusion we have found that, unlike in MALDI-TOF and MALDI magnetic sector instruments, multiple metal coordination is readily observed with sugars containing acidic residues. However at high concentrations of sugar it is possible to form small quantities of single metal coordinated species when acidic groups are present. When such species are formed they act like "neutral" sugars in that a minimum number of residues are required to form a complex, thus in the case of 6′SL we observe a $[6'SL + Na]^+$ but not a $[6'SL + Cs]^+$. It appears the sialic acid residue can itself form complexes with two alkali metals simultaneously, but not if both metals have a high charge density such as lithium and sodium. These ions will act like point charges and repel one another, but two large alkali metals such as cesium can be accommodated by a single sialic acid residue.

As a final point, in LSTb the first mode of fragmentation in SORI CID was loss of the sialic acid residue either as a single or double metal coordinated species. However, in LSTa the first mode of fragmentation was loss of fragments from the reducing end. This is extremely helpful in the identification of an unknown saccharide, since the fragments from CID can give some structural indication of where the sialic acid residue is placed demonstrating that multiple metal coordination can help in structural elucidation.

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