Liquid Secondary Ion Mass Spectrometry/Fourier Transform Mass Spectrometry of Oligosaccharide Anions

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The liquid secondary ionization mass spectrometry of oligosaccharide anions is investigated with an external source quadrupole Fourier transform mass spectrometer (FTMS). Selected linear and cyclic oligosaccharides are analyzed to determine the performance of the external source FTMS in the anion mode. Fragmentation behavior of disaccharides is characterized and related to the fragmentation behavior of larger oligomers. General correlation is found between the linkages and the fragment ions observed.

INTRODUCTION

The importance of oligosaccharides in many cell/cell processes has focused many investigations toward developing rapid techniques for the structural elucidation of these important compounds. Although newer methods for ionization of nonvolatile compounds have recently become available, fast atom bombardment[1,2] (FAB) remains a reliable ionization technique in many laboratories. This is generally true except for oligosaccharides. The FAB-MS of oligosaccharides in the positive ion mode (i.e., protonated) does not produce ring fragmentation of underivatized oligosaccharides; hence sequence information is not obtained.[3] However, as early as 1982, when FAB-MS was first introduced, FAB ionization in the anion mode was known to produce fragmentation in the glycosidic ring.[2] Similar observations have followed particularly by Dell[3] and Domon and Costello[4] on sphingolipids which illustrate that ring fragmentation can be observed in the anion mode to provide linkage information. The latest studies by Garozzo et al.,[5] Ballistreri et al.,[6] and Dallinga and Heerma[7] have also shown that anion FAB-MS/MS of even underivatized oligosaccharides produces ring fragmentation and thus provides linkage information.

Recently, we have placed a major effort on understanding the formation and gas-phase chemistry of oligosaccharide ions.[8,9] With the use of an external source Fourier transform mass spectrometry[10,11] (FTMS) instrument, we have performed the cation liquid secondary ionization mass spectrometry (LSIMS)/FTMS[12-14] of oligosaccharides. Work in our group on oligosaccharides shows that the LSIMS/FTMS of these compounds in the cation mode produces a larger degree of fragmentation than with sector MS.[8,9] The detection limit is comparable in both techniques. In addition, matrix clusters are not as readily observed and in many cases are nearly absent from the LSIMS/FTMS spectra. However, it is not immediately obvious that LSIMS/FTMS can be useful for the negative ion analysis of oligosaccharides. For example, the large abundance of Cs^+ ions present in the source, from the gun, could affect the transmission of ions from the source to the analyzer cell. In fact, the anion LSIMS of biomolecules has not been well studied in the FTMS. There are, however, advantages to the use of FTMS. Simultaneous detection can be performed throughout the entire mass range. This feature has been recently exploited by Lam et al.[15-17] and Coates and Wilkins[18-20] using laser desorption FTMS on oligosaccharides. They show increased sensitivities and greater fragmentation than that obtained with FAB/MS using sector instruments. The investigation of anion LSIMS with FTMS is of further interest due to the longer time scales associated with this technique compared to other MS techniques. We have recently reported that slow fragmentation reactions are observed in the LSIMS/FTMS of large oligosaccharides.[21] These slow metastable decay reactions are the reasons for the enhanced fragmentation and the lack of matrix interference observed in cation LSIMS/FTMS spectra. Greater fragmentation and the lack of matrix interference is especially advantageous in the anion mode and would mean that collisional activated dissociation (CAD) may not be necessary to obtain linkage information. Furthermore, the low voltages with which FTMS experiments are performed can allow rapid polarity changes in turn allowing near simultaneous cation/anion MS determination. We present in this report, the first anion LSIMS/FTMS of oligosaccharides.

EXPERIMENTAL SECTION

Experiments are performed in an external source Fourier transform mass spectrometer built in our laboratory. Descriptions of the instrument have been provided in earlier publications.[8,14] Briefly, a single-stage rf-only quadrupole guides the ions from the SIMS source into the analyzer cell. The quadrupole allows differential pumping between the source and the analyzer

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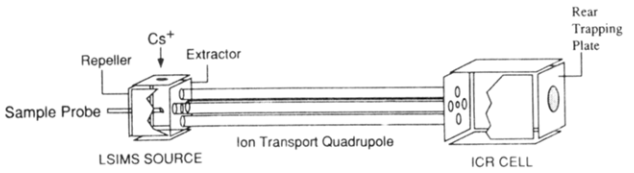
Chart I. Schematics of the Ion Source, Quadrupole Rods, and ICR Cell of External Source FTMS Instrument Built in Our Laboratory[a]



[a] The sample probe is shown in place penetrating the repeller plate. The extractor plate is directly across the repeller plate. The Cs^+ source (not shown) is mounted in the source on one of the side plates. Ions enter the ICR cell through the front trapping plate. Shown on the rear trapping plate is a large aperture covered by a high-transmittance gold mesh for performing photodissociation. The magnetic field lines are parallel to the quadrupole rods and have maximum homogeneity within the analyzer cell.

cell so that 10^{-10} Torr is maintained in the analyzer chamber even when a sample is placed in the source.

Samples are prepared in the same manner as in the cation mode. This involves preparing a 10^{-2} M solution of the oligosaccharide dissolved in deionized water. A 1–5-μL portion of the solution is placed on a copper probe tip containing 2 μL of matrix material. Matrices including glycerol, thioglycerol, and triethanolamine were all investigated. However, glycerol and thioglycerol still provided the best results. With the sample in place, the probe is degassed in a rough vacuum for about 3 min. After this period, the sample probe is positioned in the ionization source and the sample analyzed.

Samples are bombarded with a Cs^+ gun (from Antek Co.) operated at 10 kV. An Omega data system (Ionspec Co.) controls the pulse sequence as well as the voltages on the source and analyzer plates. A home-built controller powers the quadrupole rods and consists of a function generator (Wavetek) coupled to a power amplifier (ENI Co.). Typically between 10 and 50 scans are accumulated. Each spectrum including the accumulation of scans and the data workup (Fourier transform) takes usually less than 30 s. A routine analysis, including sample introduction, lasts less than 5 min.

Neither background subtraction of matrix ions nor collisionally activated dissociation has been performed to produce any of the spectra presented. The fragmentation reactions are due only to LSIMS ionization.

RESULTS

Procedural differences in obtaining spectra in the positive and negative ion modes involve primarily the inversion of dc voltages applied to the extractor plate, the quadrupole rods, and the trapping plates of the analyzer cell. The schematic of the ion source, transport region, and the analyzer cell of the instrument built in our laboratory is shown on Chart I. Cesium ions are pulsed from the cesium gun to bombard the target with an incident angle of 45° to the surface normal. A repeller plate with −200 to −400 V is used to repel the negative ions and attract much of the positive ions including the Cs^+ which is scattered from the probe tip. For the cation mode, the repeller voltages are set at nearly zero. Ions are extracted via an extractor plate which is placed between the sample probe and the quadrupole ion guide. In the anion mode, ions are extracted with voltages of typically +150 V on the extractor plate. A positive voltage (+100 to +150 V) is used to move the ions through the ion guide. Ions are then trapped in the analyzer cell with voltages of −2 to −3 V applied to the trapping plates. Voltages applied to the trapping plate are not pulsed and remain constant throughout. The values are nearly the same magnitude (opposite sign) as voltages used in the cation mode. Ion trapping is facilitated by placing typically 0 V on one transmitter plate and −0.7 to −1.0 V on the other. The voltage on the SIMS source (10 kV) and the amplitude and frequency of the rf rods remain constant. The rf frequencies

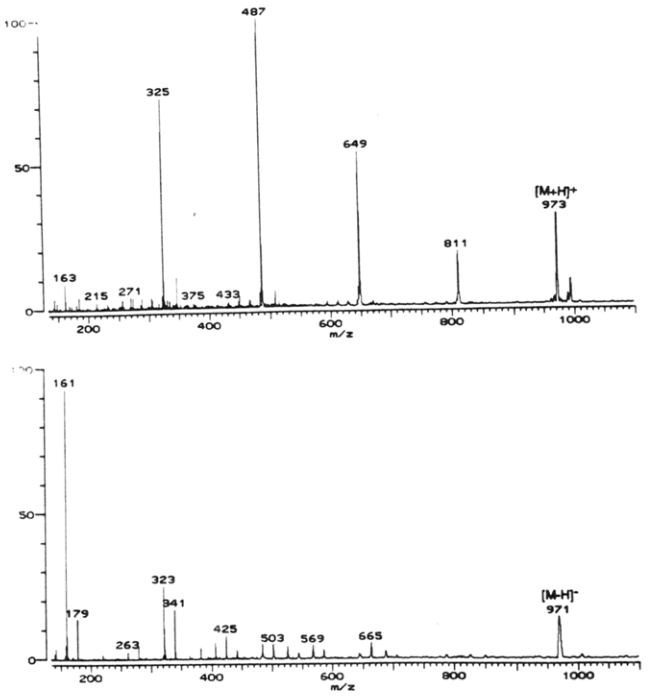
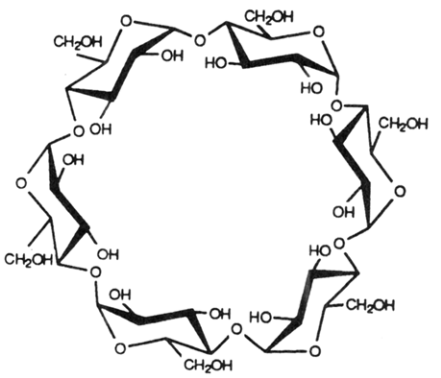


Figure 1. Positive ion (a, top) and negative ion (b, bottom) LSIMS/FTMS spectra of α-cyclodextrin in a glycerol matrix obtained with an external source FTMS instrument. The spectra were obtained by placing the sample on the probe followed by acquisition in the positive ion mode, mode change with minor tuning of source plate voltages, and acquisition in the negative ion mode. After the sample was in place, the total time of accumulation in the positive ion mode, mode change, and accumulation in the negative ion mode took less than 2.5 min. A cesium gun operating at 10 kV was used for both spectra.

applied to the quadrupole rods are typically between 0.7 and 1.4 Hz.

Secondary ion currents can be monitored along each plate. Typical currents are 200 nA on the extractor plate, 12.0 nA on the quadrupole rods, 1.4 nA on the front trapping plate, and 1.1 nA on the rear trapping plate. From the values of the current on the quadrupole rods and the trapping plates, we estimate a transmission efficiency of about 21%, similar to values obtained in the cation mode.[8]

"Simultaneous" Anion/Cation Analysis. The spectra corresponding to both charge modes for the 1–4-linked, cyclic hexasaccharide, α-cyclodextrin (I), are shown (Figure 1). We



I

emphasize that both spectra are obtained from a single sample applied to the copper probe tip. Each spectrum took less than 30 s to accumulate and transform. The switch between the anion and cation modes (with manual reversal of the polarity on various plates and minimal tuning) is accomplished in 1.5 min. The total time for obtaining both spectra including the mode change with some tuning is only about 2.5 min.

Automation of the voltages applied to the various electrodes, already available on some commercial instruments, will eventually allow near simultaneous (less than 1 min) analysis in both charge modes. This represents a great advantage and is made possible by the low voltages (with exception to the SIMS gun) associated with the FTMS technique. It is important to emphasize that the fragment ions observed are due solely to LSIMS ionization without precautions for eliminating matrix peaks. The cation spectrum is similar to those presented in an earlier paper showing primarily glycosidic bond cleavages.[8,9] The clarity of the spectrum is also noted, and the fragment ions produced by the cleavage of each glycosidic bond are represented.

The parent ion is relatively prominent in the anion spectrum, although by far the most abundant peak corresponds to m/z 161, a dehydrated monosaccharide fragment. Fragment ions in the anion mode correspond to both cleavage of glycosidic bonds and fragmentation of the glycosidic rings. Cleavage along the glycosidic bonds are represented by two series of peaks 18 mass units (mu) apart, m/z 161 (179), 323 (342), 485 (503), etc. They correspond to cleavages on both sides of the linking oxygen. Fragment ions with masses between the doublets correspond to fragmentation of the glycosidic rings. The intense fragmentation, along with abundant molecular ions, illustrates the advantages of the FTMS method even in the negative ion mode. Fragmentation of the glycosidic rings, which is never observed in the cation (protonated) mode of underivatized oligosaccharides, corresponds to losses of 60 and, in some cases, 120 mu from the glycosidic bond cleavage fragments, e.g., m/z 263 from the fragment m/z 323 and m/z 281 from the fragment m/z 341, respectively. These ring fragments are characteristic of 1–4-linked oligosaccharides and correspond to losses of $C_2H_4O_2$ units as will be discussed in greater detail below.

Disaccharides. The lack of matrix interference makes the FTMS technique highly suited for obtaining structural information of even small oligosaccharides. Spectra a-f of Figure 2 show the respective spectra of a selected group of disaccharides with linkages corresponding to 1–1 [trehalose, (Glc[α1→α1]Glc) (II)], 1–2 [sophorose (Glc[β1→α2]Glc (III)], 1–3 [laminaribiose (Glc[β1→3]Glc) (IV)], 1–4 [lactose (Gal[β1→4]Glc) (V)], [$^{13}C_1$]lactose, and 1–6 [gentiobiose (Glc-[β1→6]Glc) (VI)]. Listed (Table I) are all the disaccharides investigated for this report. From the analysis of these compounds several generalizations, with respect to the relationship between fragmentation behavior and linkage combinations, can be made about disaccharides. A lack of ring fragmentation is observed for the 1–1-linked trehalose. Ring opening via cleavage of the ring oxygen–carbon 1 bond (O–C_1) is believed to be the first step toward ring fragmentation. Ring opening is facilitated by deprotonation of the anomeric oxygen. If the anomeric oxygen is blocked by the presence of an alkyl group, then ring opening cannot occur and the subsequent ring fragmentation is not observed. Thus the lack of ring fragmentation in a 1–1-linked disaccharide is consistent with this fragmentation mechanism. A similar situation occurs in sucrose which, although 1–2 linked, also has the anomeric position blocked.

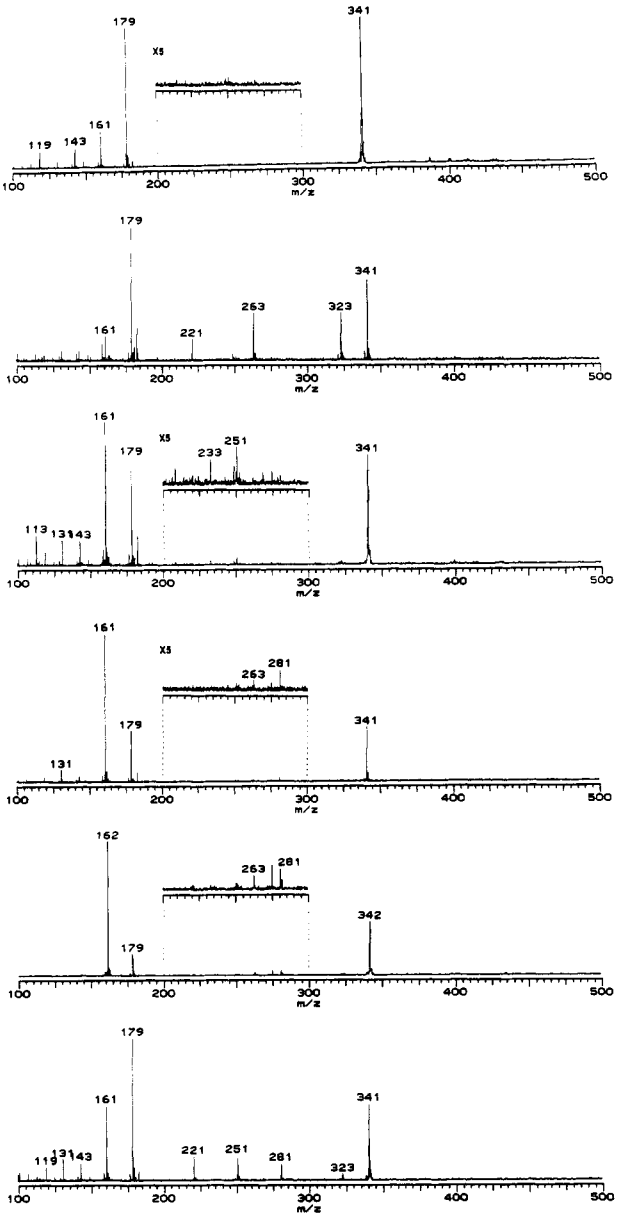


Figure 2. Negative ion LSIMS/FTMS spectra of selected disaccharides (a–f, top to bottom, respectively) with various linkage combinations including trehalose (Glc[α1→α1]Glc) (a), sophorose (Glc[β1→α2]-Glc) (b), laminaribiose (Glc[β1→3]Glc) (c), lactose (Gal[β1→4]Glc) (d), [$^{13}C_1$]lactose (e), and gentiobiose (Glc[β1→6]Glc) (f). The peak between m/z 263 and 281 in the [$^{13}C_1$]lactose spectrum (e) is a matrix peak. Relative abundances of ring and glycosidic bond fragmentation as well as the specific fragment ions produced during LSIMS/FTMS depend on the linkage combination.

Conversely, the 1–6-linked disaccharides, e.g., gentiobiose, and the 1–2-linked sophorose provide the largest abundance of ring fragments (Figure 2f and b, respectively). The 1–2-

Table I. Neutral Fragments Lost from the Deprotonated Parent of Variably Linked Disaccharides Obtained with the External Source LSIMS/FTMS Instrument[a]

		proposed ring cleavage for fragments obsd (mass loss)			
disaccharide	structure	$^{0,2}A_2$ (−60 mu)	$^{5,2}A_2$ (−78 mu)	$^{0,3}A_2$ (−90 mu)	$^{0,4}A_2$ (−120 mu)
trehalose	Glc[α1→α1]Glc				
sucrose	Glc[α1→β2]Fru				
sophorose	Glc[β1→α2]Glc		X		X
nigerose	Glc[α1→3]Glc			X	
laminaribiose	Glc[β1→3]Glc			X	
turanose	Glc[α1→3]Fru			X	
cellobiose	Glc[β1→4]Glc	X	X		
lactose	Gal[β1→4]Glc	X	X		
isomaltose	Glc[α1→6]Glc	X		X	X
gentiobiose	Glc[β1→6]Glc	X		X	X
melibiose	Gal[α1→6]Glc	X		X	X
	Gal[β1→6]Gal	X		X	X
palatinose	Glc[α1→6]Fru	X		X	X

[a] Samples are contained in a glycerol matrix and bombarded with a 10-kV Cs^+ beam: Glc, glucose; Gal, galactose; Fru, fructose.

linked disaccharide produces two major ring fragment ions corresponding to losses of 78 and 120 mu. Fragmentation of the 1–6-linked disaccharide yields neutral losses corresponding to 60, 90, and 120 mu. These fragmentations are common to all 1–6-linked disaccharides investigated in this study. That ring fragmentation should be so abundant, at least for all the 1–6-linked disaccharides, is most likely a function of the linkage. Upon opening, the reducing ring can better facilitate several configurations allowing retroaldol reactions, which have been proposed as likely mechanisms for the neutral loss reactions in lithium-coordinated species[22,23] and in laser desorption time-of-flight mass spectrometry.[24]

Disaccharides having 1–3 and 1–4 linkages have moderate ring fragmentation (Figure 2c,d). Ring fragmentation of 1–3-linked disaccharides corresponds primarily to loss of 90 mu, consistent with that reported earlier by Garozzo et al.[5] and Dallinga and Heerma[7] using high-energy CAD. Loss of 60 mu from the deprotonated parent is not observed in the LSIMS/FTMS spectra but has been reported by the same groups for 1–3-linked disaccharides.[5,7]

Neutral losses of 60 and 78 mu are characteristic of 1–4-linked disaccharides. For example, the ring fragmentation product m/z 281 ($[M-H-60]^-$) and 263 ($[M-H-78]^-$) from lactose, though relative weak, are consistently observed (Figure 2d and e, respectively). This mass corresponds to the empirical formula $C_2H_4O_2$ and $\{C_2H_4O_2 + H_2O\}$, respectively. Earlier reports have suggested that the formation of this ion involves the loss of the anomeric carbon atom (carbon 1) and the adjacent carbon atom (carbon 2).[5] We have confirmed these observations with the ^{13}C-labeled lactose (on C_1 anomeric position) and observe the loss of primarily $^{13}CCH_4O_2$ (ratio of m/z 281 to 282 is 70:30) from the deprotonated parent.

The relative abundances of fragment ions formed by cleavage along the glycosidic bonds also appear to depend on the linkage. Inspection of the spectra presented shows that the product m/z 179 is the most intense fragment ion for 1–1-, 1–2-, and 1–6-linked disaccharides. The 1–4-linked disaccharide produces primarily m/z 161, while the 1–3-linked disaccharide produces both glycosidic bond cleavage products in nearly equal abundances. Results with the ^{13}C-labeled lactose indicate that, at least for the 1–4-linked disaccharide, m/z 161 (observed predominantly as m/z 162 in labeled lactose) originates from the reducing ring exclusively, while m/z 179 (observed predominantly as m/z 179 in labeled lactose) originates from the nonreducing ring. These results are consistent with a single glycosidic bond cleavage.

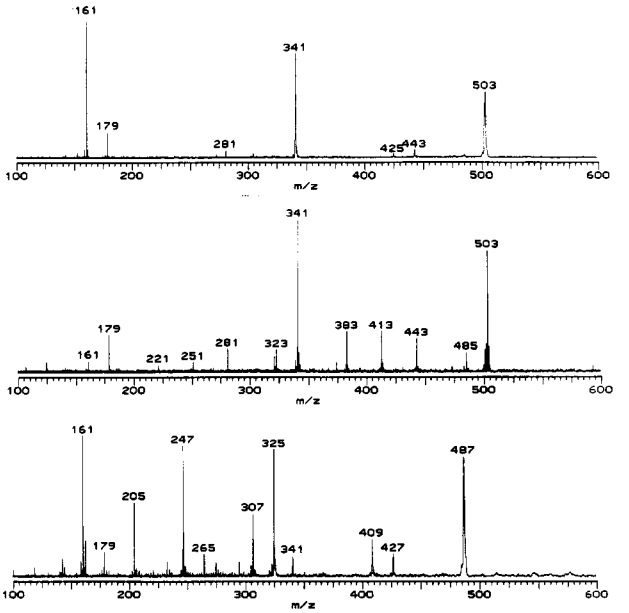


Figure 3. Negative ion LSIMS/FTMS spectra of selected trisaccharides including maltotriose (Glc[α1→4]Glc[α1→4]Glc) (a, top), isomaltotriose (Glc[α1→6]Glc[α1→6]Glc) (b, middle), and 2′-fucosyllactose (L-Fuc-[α1→2]Gal[β1→4]Glc), (c, bottom) obtained by bombarding the sample in a glycerol matrix with Cs^+ (10 kV).

Trisaccharides. A very useful feature of the spectra of small oligosaccharides is that all useful fragment ions are observed in a single analysis. Shown in Figure 3 are the spectra of 1–4-linked maltotriose (Glc[α1→4]Glc[α1→4]Glc) (a), 1–6-linked isomaltotriose (Glc[α1→6]Glc[α1→6]Glc) (b), and a mixed linked trisaccharide 2′-fucosyllactose (L-Fuc[α1→2]Gal[β1→4]Glc) (c). The spectrum of maltotriose contains the losses of 60 (to produce m/z 443) and 78 mu (m/z 425) from the deprotonated parent. Similar behavior is observed during further fragmentation of the disaccharide fragment (m/z 341) to produce m/z 281 and 263. These fragmentation reactions are consistent with the 1–4-linked disaccharides where the losses of both 60 and 78 mu are also observed.

The spectrum of the 1–6-linked isomaltotriose shows more intense ring fragmentation than the other trisaccharides, consistent with the behavior of this linkage combination in the disaccharide compound. Fragmentation of the glycosidic ring similarly produces losses of 60 (m/z 443), 90 (m/z 413), and 120 mu (m/z 383) from the trisaccharide parent. Similar

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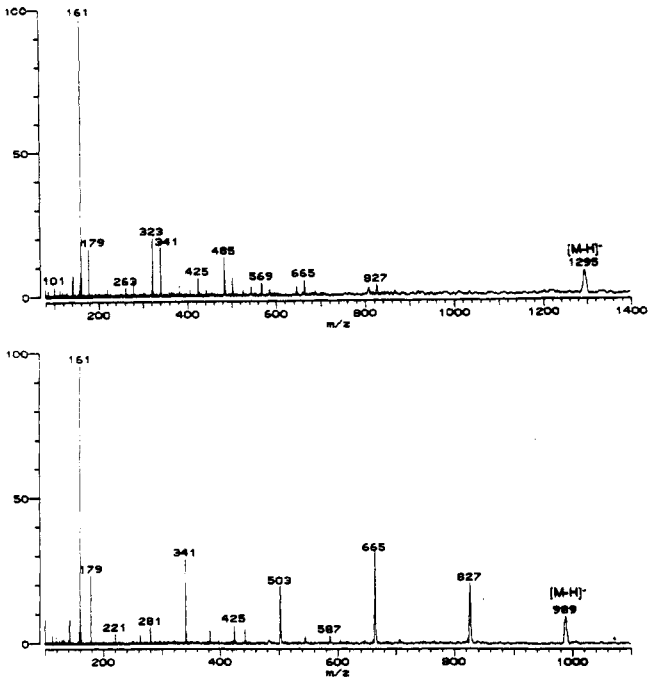
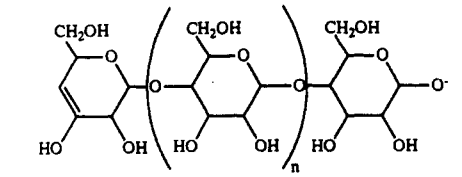


Figure 4. Negative ion LSIMS/FTMS spectra of maltohexaose (a, top) and γ-cyclodextrin (b, bottom) obtained by bombarding the sample in a glycerol/thioglycerol matrix (1:1) with Cs^+ (10 kV).

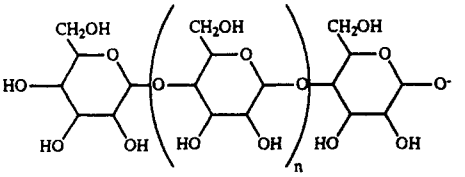
losses to produce m/z 281, 251, and 221 are observed from the disaccharide fragment.

In trisaccharides in which a mixture of linkages is present, fragment ions correspond to combinations of analogous fragment ions from similarly linked disaccharides. An example is the 2′-fucosyllactose, which is a 1–2-, 1–4-linked trisaccharide. The losses of 60 (m/z 427) and 78 mu (m/z 409) are characteristic of a 1–4-linked disaccharide. The fact that the loss occurs from the deprotonated parent tells us that the reducing monosaccharide unit is connected to another glycosidic ring via a 1–4 linkage. Fragments corresponding to m/z 307, 295, 265, 247, and 205 correspond to losses of 18, 30, 60, 78, and 120 mu from the disaccharide fragment (m/z 325). Of these fragments, the most abundant are m/z 307, 247, and 205 corresponding to losses of 18, 78, and 120 mu, respectively, consistent with fragmentation reactions of the deprotonated 1–2-linked disaccharide, sophorose. Thus from the fragmentation pattern and the mass of the parent ion, it is relatively simple to determine the linkage arrangements of this trisaccharide.

Larger Oligosaccharides. The spectra of the linear hexasaccharide, maltohexaose, and the cyclic octasaccharide, γ-cyclodextrin, provide good illustrations of the reasonably high mass capability of the FTMS in the anion mode. The deprotonated parents of γ-cyclodextrin, m/z 1295, and maltohexaose, m/z 989, are both prominent and readily observed. γ-Cyclodextrin is the largest oligosaccharide we have studied to date. The LSIMS/FTMS spectrum of this compound reveals most of the glycosidic bond cleavages, with the exception of neutral losses of one- and two-monosaccharide fragments accounting for the lack of fragment ions between m/z 1000 and 1295. Fragmentation reactions which produce ions having less than m/z 900 are, however, abundant and correspond to both glycosidic bond and ring fragmentation. The glycosidic bond cleavages are again represented by doublets 18 mu apart. By contrast, the linear oligosaccharide, maltohexaose, yields a single ion series corresponding to the higher mass ion series, i.e., m/z 179, 341, 503, 665, 827, and 989. Ring fragmentations in maltohexaose correspond to neutral losses of 60, 78, and 120 mu. Thus, for example, the fragments between 341 and 503 mu are derived from losses of three neutral products from the fragment m/z 503, which are m/z 443, 425, and 383, respectively. It appears that more glycosidic ring fragmentation products are observed for the cyclic oligosaccharides (α- and γ-cyclodextrins), although both have similar 1–4 linkages as the linear compound. In actuality, similar neutral loss products are observed but instead originate from two precursor ions rather than one as observed with linear oligosaccharides. Ring opening of the cyclic oligosaccharide and a loss of a neutral polymeric fragment most likely produce ions having structures analogous to either VII or VIII. The fragmentation products of both ion species are



VII



VIII

consistent with the presence of the charge on the reducing ring, thereby allowing ring opening reactions to occur. For example, the trisaccharide fragment ions having m/z 485 and 503 may be analogous to VII and VIII (n = 1), respectively. Neutral losses of 60 and 120 mu from m/z 485 produce ions having m/z 425 and 365, respectively. The corresponding losses from m/z 503 produce m/z 443 and 383, respectively. Interestingly, a loss of 78 mu (60 + 18) is observed from m/z 485 to produce the ion having m/z 407. A similar fragmentation is not observed from m/z 503.

DISCUSSION

The detection limit is lowest and the amount of structural information greatest in the anion mode, as compared to the cation mode, of LSIMS/FTMS. With 2 μg (3.8 mmol) of maltotriose (m/z 341), the deprotonated parent is observed with a signal to noise ratio of 3. This contrasts to analogous experiments in the cation mode where at least 6 μg is needed. In general, we find that parent ions are more readily observed in the anion mode. We have suggested that the diminished intensity of the protonated parent in the cation mode is due to slow metastable decay.[21] Although metastable decay of LSIMS-produced ions is present in all methods of mass spectrometry, its effects on larger ions are more readily observed in the FTMS due to the long time scale of the technique. Indeed, we have shown, in an earlier publication,[8] by the direct comparison of FAB sector MS and LSIMS/FTMS spectra, that identical fragment ions are observed with differences only in the relative abundances of the individual ions and that the degree of fragmentation is generally greater in the LSIMS/FTMS spectra than in FAB sector MS spectra. In the anion mode, we suspect that metastable decay rates are slower than in the cation mode. The difference derives from the ability of the proton, in the cation mode, to actually catalyze glycosidic bond cleavage. It is also for this reason that ring fragmentation is never observed in protonated underivatized oligosaccharides.

The comparison between FAB sector MS and LSIMS/FTMS is appropriate for determining whether the spectra obtained in the anion mode of the latter are unique or simply a consequence of the long time scale of the instrument.

Table II. Neutral Fragments Lost from the Deprotonated and Lithiated Parents of Variably Linked Disaccharides

	mass loss (mu)				
linkage	30	60	78	90	120
1–1					
1–2	Y	Y	XYZ		AXYZ
1–3		YZ		AXYZ	
1–4		AXYZ	XYZ		Z
1–6	YZ	AXYZ		AXYZ	AXYZ

[a] Compiled from this work with LSIMS/FTMS (X), from Garozzo et al.[5] with FAB sector MS using matrix subtraction and CAD (Y), from Dallinga et al.[7] with FAB sector MS/MS and CAD (Z), and from Hofmeister et al.[22] with FAB (BEqQ) MS/MS of lithiated cations and low-energy CAD (A).

However, a direct comparison is difficult since the fragmentation of small oligosaccharides in FAB-MS, using sector instruments, is not easily obtained. The study reported by Garozzo et al. on the anion FAB-MS spectra of several underivatized oligosaccharides, including variably linked di- and trisaccharides, was accomplished by using a combination of metastable decay and CAD, simultaneously, to obtain fragmentation.[5] Furthermore, matrix subtraction was performed to produce the reported spectra. The more recent study by Dallinga and Heerma was similarly performed in a sector instrument under separate metastable and high-energy CAD conditions, although only the CAD spectra are shown.[7] A summary comparing the neutral losses, resulting from ring cleavages, of the deprotonated parents ($[M-H]^-$) as a function of linkages under the three experimental conditions is tabulated (Table II). The results from the study of Dallinga and Heerma are not easily categorized since small abundance peaks of nearly every possible product ions are observed in most of the spectra. For example, the analysis of the 1–1-linked disaccharide shows losses of 60 and 120 mu, which would be inconsistent with the ring-opening mechanisms supported in all the three reports. These signals, produced most likely by the high-energy-collision conditions, could make differentiation between various linkages slightly more difficult. Similar, small-intensity signals are further not observed in the study by Garozzo et al., possibly because these authors use the method of background subtraction. To construct Table II, only the most abundant ions of the spectra were used to summarize the results from the work of Dallinga and Heerma. For further comparison, the study published by Hofmeister et al. on lithiated (cationized) disaccharides using a BEqQ and low-energy CAD is included since these species are also found to undergo glycosidc ring cleavages.[22]

We find general similarities in the types of neutral fragments lost in all the studies. The losses of combinations of 60, 90, and 120 mu are commonly observed under all four conditions. In the anion mode of LSIMS/FTMS, the 1–2- and 1–6-linked disaccharides produce the greatest number of different fragment ions. As expected, both the Garozzo[5] and the Dallinga[7] studies produce similar, although not identical, results. The losses of 30 mu from the 1–6 linked and 60 mu from the 1–2 and 1–3 linked are all unique of the two studies in which high-energy CAD was used. The differences between the two sector MS studies involve the presence of ions derived from losses of 30 and 60 mu in the 1–2-linked disaccharide, observed by Garazzo et al., and the loss of 120 mu from 1–4-linked disaccharides, observed by Dallinga and Heerma. The loss of both 60 and 120 mu in the 1–4-linked disaccharide is consistent with our results for the larger 1–4-linked oligomer and with studies earlier reported by Domon and Costello in the anion spectra of some glycosphingolipids and gangliosides performed under high-energy CAD.[4]

Surprisingly, we find good agreement between our results (anion LSIMS/FTMS) and those of Hofmeister et al., although the latter were obtained in the cation mode (FAB/MS/MS of lithiated disaccharides).[22] The neutral losses from our study and those of Hofmeister et al. agree for nearly all linkage combinations with the exception of the 1–2- and 1–4-linked disaccharides, where we observe the additional loss of 78 mu. The absence of some neutral losses in our experiments, compared to the sector instruments, is possibly related to the high-energy CAD conditions used in the latter. In any case, there are sufficient diagnostic fragment ions and variation between different linkage combinations to characterize linkage. The similarities between all the studies, however, may indicate analogous mechanisms and/or steric requirements for the fragmentation of the glycosidic rings even under different conditions.

In general, the fragmentation behavior of disaccharides is represented in similarly linked, larger oligomers in the FTMS. We have found only one exception, which belongs to the 1–4-linked oligosaccharides where the additional loss of 120 mu is observed only for the larger oligomers. The loss of this additional fragment for the larger oligomers makes our result even more consistent with the results obtained under high-energy CAD. We often find that both ring and glycosidic bond fragmentations are generally more abundant with the larger oligosaccharides than with the disaccharides. However, the combination of these three fragmentation products is not shared by other linkages and is still be representative of 1–4 linkages. The reason for the differences in glycosidic fragmentation between larger 1–4-linked oligosaccharides and similarly linked disaccharides is not yet clear and requires further investigation.

Finally, the rapid conversion from one charge mode to the other offers the possibility of near simultaneous analysis in both modes. In switching between charge modes, we find that the tuning parameters in the individual modes do not change greatly even over a period of several months for similar classes of compounds. This feature is important for oligosaccharides where both charge modes offer complimentary information, as illustrated by the cation and anion spectra of α-cyclodextrin where the cation mode provides all the sequence information and the anion mode provides only partial sequence information. Furthermore, anion LSIMS/FTMS similarly differentiates between different linkage combinations as FAB-MS/MS in sector instruments. The advantage of FTMS in these analysis is the possibility of obtaining high-resolution spectra of fragment ions. Furthermore, because fragmentation of the glycosidic ring occurs even without CAD, the coupling of LC/LSIMS/FTMS would be ideal for analyzing solutions of oligosaccharide mixtures.

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