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Anion Dopant for Oligosaccharides in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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A new anion dopant for oligosaccharides is developed for use in matrix-assisted laser desorption/ionization mass spectrometry. Two types of sulfate-attached quasimolecular ions are formed in the negative ion mode when neutral oligosaccharides are doped with dilute H_2SO_4 solutions. Under mild conditions, i.e., low H_2SO_4 concentration ($\sim 10^{-3}$ M) and threshold laser fluence, a sulfate adduct $[M + HSO_4]^-$ is formed. With more concentrated H_2SO_4 solutions ($\sim 10^{-2}$ M) and higher laser fluence, in situ derivatization of the oligosaccharides occurs to produce an ion whose m/z corresponds to a sulfate derivative $[M + HSO_4 - H_2O]^-$. Hydrogen sulfate appears to be a general anion dopant because it forms complexes with a wide variety of neutral oligosaccharides. Conversely, anionic oligosaccharides form neither the adduct nor the derivative. The combination of complex formation (with neutral oligosaccharides) and the deprotonation of acidic oligosaccharides allows simultaneous detection of the respective mixture.

The need to understand the roles of oligosaccharides in biological systems has necessitated the development of an efficient and sensitive analytical technique. Mass spectrometry is emerging as a critical tool for structural analysis of oligosaccharides.[1−8] However, one major limitation is its inability to simultaneously analyze neutral and acidic oligosaccharides. For the matrix-assisted laser desorption/ionization (MALDI) analysis of carbohydrates, the positive ion mode is more frequently used than the negative ion mode because neutral oligosaccharides readily complex to alkali metal ions such as Li^+, Na^+, and K^+. The corresponding complexes are the major ions normally observed during MALDI. However, the lack of an analogous anionic complexing agent (or dopant) makes the analysis of neutral oligosaccharides in the negative ion mode difficult. In this paper, a new anion dopant for oligosaccharides is presented for use with MALDI-MS.

A number of good cation dopants are already known including group I,[9−13] group II,[14] and some transition metal ions such as Co^{2+} and Mn^{2+}.[15] Group II and transition metal ion dopants are commonly used in electrospray ionization because multiply charged ions are more readily formed by this technique.[14,15] The alkali metal ion dopants are most suitable for MALDI because singly charged ions are mainly produced by this ionization method. The intrinsic interactions between alkali metal ions and oligosaccharides have been the subject of several investigations.[9−13,16−22] Although alkali metal ions complex with most oligosaccharides, they also often induce nonspecific cleavages. For example, fucosylated oligosaccharides readily lose anhydrofucose when coordinated with alkali metal ions.[12,23] Similarly, sialylated oligosaccharides also lose sialic acid readily during MALDI in the positive ion mode.[24,25] Because the fragmentation reactions are likely to be charge-induced, these ions are generally more stable in the anion mode.

There have been few studies of negative ion adducts in mass spectrometry.[26−28] The most relevant involves the chloride attach-

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ment of oligosaccharides studied by atmospheric pressure chemical ionization mass spectrometry (APCI-MS).[29]

There is no general anion dopant for oligosaccharides with MALDI. In this paper, we report the use of hydrogen sulfate as a dopant for oligosaccharides. A simple and direct method for the preparation of sulfate-attached quasimolecular ions is provided. Hydrogen sulfate anion is coordinated by the oligosaccharides to form a noncovalent complex $[M + HSO_4]^-$. In addition, under the conditions outlined below, a covalently bonded sulfate derivative $[M + HSO_4 - H_2O]^-$ is also formed. The formation of the sulfate adduct $[M + HSO_4]^-$ and the sulfate derivative $[M + HSO_4 - H_2O]^-$ depends on several parameters including the concentration of sulfuric acid and the laser fluence.

EXPERIMENTAL SECTION

Nearly all oligosaccharides were obtained from Oxford Glycosystems (Rosedale, NY). Maltohexaose and asialoganglioside were obtained from Sigma (St. Louis, MO). β-Cyclodextrin and all matrixes were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sulfuric acid was obtained from Fisher Scientific (Fair Lawn, NJ). All chemicals were used in the highest purity and without further purification.

The oligosaccharide solutions were prepared at concentrations of 1 mg/mL in either water or 50:50 water/methanol. To prepare samples for MALDI, a 1-μL aliquot of oligosaccharide solution was applied to the probe tip, followed by 1 μL of H_2SO_4 solutions and 1 μL of matrix solution.

All experiments were performed on a HiResMALDI external source FTMS instrument (IonSpec Corp., Irvine, CA) equipped with a 4.7-T magnet. Descriptions of the instrument are provided in earlier publications.[24,30]

Collision-induced dissociation (CID) experiments were performed using the method of sustained off-resonance irradiation (SORI).[31] The quasimolecular ion was isolated by a series of both rf sweeps and bursts generated by an arbitrary wave form generator (IonSpec). The quasimolecular ion was excited by a radio frequency pulse for 1000 ms at 1000 Hz below the cyclotron frequency. Four argon pulses in 250-ms intervals were applied to sustain a constant pressure of $\sim 5 \times 10^{-6}$ Torr during the CID event.

RESULTS AND DISCUSSION

Optimization of Ionization Conditions. Two sulfate-attached quasimolecular ions are produced when MALDI is performed on oligosaccharides doped with dilute H_2SO_4 solutions. The species correspond to $[M + HSO_4]^-$ and $[M + HSO_4 - H_2O]^-$, where M is the oligosaccharide. The former will be termed "adduct", as this involves a noncovalently bound complex. The latter will be termed "derivative", as this involves the formation of a covalently bound complex. We find that several factors affect the relative abundances of the two species. The most important factors include the matrix types, H_2SO_4 concentrations, and laser fluence. For analysis, the two quasimolecular ions provide complementary information. To develop the optimal conditions in producing the specific type of quasimolecular ions, these factors were examined. For these investigations, maltohexaose, a linear, six-glucose α-1,4-linked oligosaccharide, was used. The behavior of maltohexaose serves as a typical example for the optimization studies.

Four matrixes that have been shown to be effective in the anion mode were chosen for the study. They are 3-aminoquinoline (3-AQ),[30,32−34] 2,5-dihydroxyacetophenone (DHAP),[35] 2,4,6-trihydroxyacetophenone (THAP),[36] and 1-methyl-9*H*-pyrido[3,4-b]indole (harmane).[37] These matrixes were examined for their ability to produce quasimolecular ions with sulfates. The sample preparations were optimized for each matrix. With 3-AQ and THAP, a concentration of 50 mg/mL in ethanol was used, while a 20 mg/mL solution was prepared for harmane. Crystallization was performed under ambient conditions for these matrixes. With DHAP, a saturated solution of ethanol was used and crystallization was performed by exposing the sample solution to vacuum.

Of the four matrixes, harmane produces the strongest quasimolecular ion signals and the cleanest spectrum. Harmane is an ideal matrix for the sulfate adducts as it works best when the preparative solution is acidic. Under similar conditions, DHAP and THAP produced both adduct and derivative together. However, the signals were accompanied by a number of peaks due to complexes of sulfates and matrix molecules whose intensities were nearly as strong as the quasimolecular ion (spectra not shown). 3-AQ produced the sulfate adduct $[M + HSO_4]^-$ with a dilute H_2SO_4 solution (0.001 M, [acid]:[sugar] = 1:1). However, the signals are severely degraded at the higher H_2SO_4 concentrations necessary to produce the derivative (see below). There is a great deal of pH dependency in the performance of the matrix. Harmane works best at low pH, the conditions obtained with the addition of dopant. Further investigations show that adduct formation can be obtained with the other three matrixes when a more neutral form of the sulfate is used. For example, ammonium sulfate produces intense quasimolecular ions with oligosaccharides in the presence of matrixes DHAP, THAP, and 3-AQ, providing spectra of qualities similar to that of harmane. For simplicity and consistency, we used H_2SO_4 as dopant and harmane as matrix in the remainder of the study.

To determine the effects of H_2SO_4 concentration on the formation of the adduct and the derivative, a solution of maltohexaose (0.001 M) was mixed with an equivolume of sulfuric acid solutions with varying concentrations (0.1, 0.01, 0.005, and 0.001 M) on the MALDI probe tip. These experiments also provide an indication of whether the sulfuric acid degrades the oligosaccharide via acid hydrolysis. With concentrations of 0.1 M H_2SO_4, no signal was observed. With the other three concentrations, both adduct and the derivative species are observed with the higher concentrations favoring the formation of the derivative. The ratio of adduct to derivative decreases with increasing concentration (7:1 with 0.001 M, 4:1 with 0.005 M, and 2:1 with 0.01 M). In the

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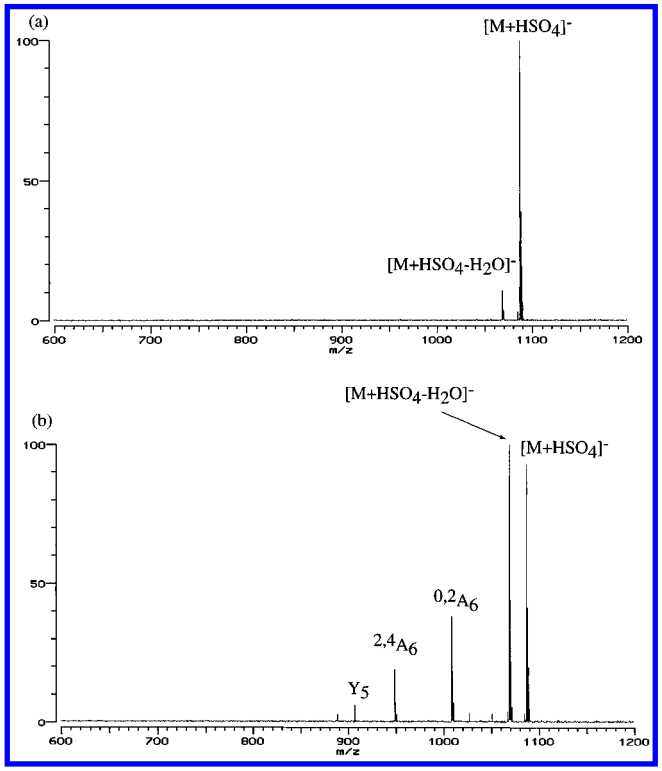

Figure 1. MALDI-FTMS spectrum of (a) maltohexaose with 0.001 M H_2SO_4 at threshold laser fluence (b) and at higher laser fluence. The base peak in (a) is the adduct ion while in (b) both derivative and adduct are in nearly equal abundances. See text for further details.

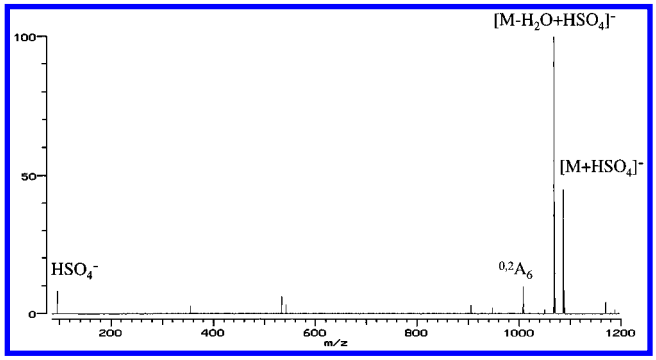

Figure 2. MALDI-FTMS spectrum of maltohexaose with higher H_2SO_4 concentration (0.01 M) and high laser fluence. The base peak is the sulfate derivative $[M + HSO_4 - H_2O]^-$.

Table 1. Adduct and Derivative Formation of Different Neutral and Acidic Oligosaccharides Doped with Hydrogen Sulfate

oligosaccharides	no. of residues	neutral (N)/ acidic (A)	adduct (m/z)	derivative (m/z)
fucose	1	N	no[a]	243.02
maltose	2	N	no	421.07
maltotriose	3	N	no	583.12
2-fucosyllactose	3	N	no	567.13
3-fucosyllactose	3	N	no	567.13
maltotetraose	4	N	763.18	745.17
G_{M1} (asialoganglioside)	4	N	1379.80	1361.80
maltopentaose	5	N	925.23	907.22
LNFP-I	5	N	950.26	932.25
maltohexoase	6	N	1087.29	1069.28
LNDFH-I	6	N	1096.33	1078.32
LNDFH-II	6	N	1096.33	1078.32
Man-5	7	N	1331.42	1313.41
β-cyclodextrin	7	N	1231.34	1213.33
NA4	13	N	2468.82	2450.80
LSTa	5	A	no	no
A1	10	A	no	no

[a] no, not observed.

remainder of the experiments, 0.01 and 0.001 M H_2SO_4 were used to produce predominately the derivative and the adduct, respectively. In addition, experiments to determine the limits of detection were crudely performed. With 10 pmol of maltohexaose, a signal-to-noise ratio of 10 was obtained, suggesting limits of detection that are comparable to the cation dopants for MALDI-MS.[9,10,12,15,22,23]

To determine whether the derivatization reaction occurs in solution or during the ionization process, the exposure time of the oligosaccharide to the acid was studied. Aliquots 10 μL of 0.01 M sulfuric acid and 0.001 M maltohexaose were mixed in Eppendorf tubes for up to 2 h both at ambient temperature and at 50 °C. The mixture was monitored every 15 min with MALDI. During the entire period, the relative intensities of the adduct and the derivative remained constant. These results demonstrate that the derivative is not formed through a solution-phase reaction but rather through the laser desorption process. It should also be noted that no acid hydrolysis of the oligosaccharide was observed under these conditions.

To investigate further the effect of the ionization process on the relative abundances of adduct and derivative, the laser fluence was varied. Maltohexaose forms the sulfate adduct $[M + HSO_4]^-$ (m/z 1087.29) with the addition of a 0.001 M H_2SO_4 solution and harmane as matrix at threshold laser fluence (Figure 1a). Under these conditions, the intensity of the adduct is optimized so that only a weak intensity of the derivative peak (m/z 1069.28, ~10% relative abundance) is observed. Note also the lack of fragmentation in the spectrum. When the laser fluence is doubled (Figure 1b**)**, the derivative-to-adduct ratio becomes nearly 1:1. In addition, cross-ring cleavages of the reducing ring are also observed ($^{0,2}A_6$, $^{2,4}A_6$, using Domon and Costello nomenclature[38]) along with minor glycosidic bond cleavage (Y_5). The sample preparation and the ionization condition can be optimized to provide primarily the derivative ion. For example, with 0.01 M H_2SO_4 and high laser fluence, the sulfate derivative becomes the base peak and is twice as large as the adduct peak (m/z 1087.29, Figure 2). Note the small amount of the hydrogen sulfate anion (HSO_4^-, ~10% relative abundance) observed at m/z 96.96. The formation of $[M + HSO_4 - H_2O]^-$ appears to be a unique in situ derivatization of the oligosaccharides.

Analysis of Neutral and Acidic Oligosaccharides. A variety of oligosaccharides is examined to study how hydrogen sulfate behaves as a general anion dopant. A total of 15 neutral and 2 anionic oligosaccharides are examined with the sulfate dopant and the results are summarized in Table 1. All neutral oligosaccharides examined readily form complexes (both adduct and derivative) with sulfates. Several notable trends are evident: (1) The derivative forms with all sizes of neutral oligosaccharides from the monosaccharide fucose to the 13-residue oligosaccharide NA4. (2) There is a minimum size needed to form the adduct. (3) Acidic oligosaccharides form neither the adduct nor the derivative.

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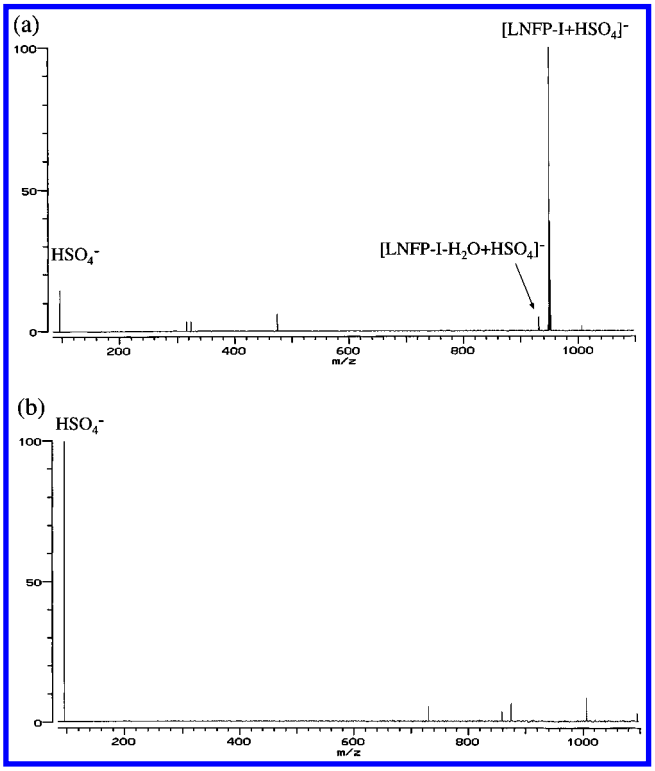


Figure 3. (a) MALDI-FTMS spectrum of LNFP-I under conditions to optimize adduct formation. See text for additional details. The base peak is the sulfate adduct $[LNFP\text{-}I + HSO_4]^-$. (b) CID of isolated sulfate adduct of LNFP-I under SORI conditions. The only product is HSO_4^-. The low abundance signals are due to noise. See text for further details.

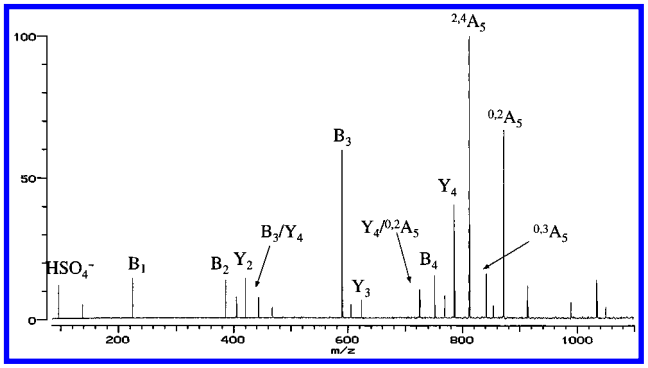


Figure 4. CID of sulfate derivative $[LNFP\text{-}I + HSO_4 - H_2O]^-$ under SORI conditions. Fragment ions are generally abundant when CID of the derivative is performed. See text for further details.

Table 2. Fragmentation Assignments of the Peaks from SORI CID of Lacto-*N*-fucopentaose I (LNFP-I)[a]

m/*z*	fragment	*m*/*z*	fragment
914.25	$[M - 2H_2O + HSO_4]^-$	590.14	B_3
872.24	$^{0,2}A_5$	444.09	B_3/Y_4
842.23	$^{0,3}A_5$	421.07	Y_2
812.22	$^{2,4}A_5$	405.07	C_2
786.20	Y_4	387.06	B_2
770.21	C_4	225.01	B_1
752.20	B_4	138.97	$^{0,2}X_0$
726.28	$Y_4/^{0,2}A_5$	96.96	HSO_4^-
624.15	Y_3		

[a] See also Figure 4 and Chart 1.

The sulfate adducts are formed only with oligosaccharides that contain at least four residues. Mono-, di-, and trisaccharides such as glucose, maltose, and maltotriose, respectively, do not form the sulfate adducts in appreciable abundances even under the most optimal conditions. Apparently, at least four monomers are necessary to stabilize the sulfate adduct to survive the ionization process. Although small oligosaccharides do not readily form adducts, they do form derivatives. For example, 2- and 3-fucosyllactose (2- and 3-FL), a pair of isomeric trisaccharides, produce the sulfate derivative $[FL + HSO_4 - H_2O]^-$ (*m*/*z* 567.13) as the base peak during MALDI-FTMS. The size dependency of the adduct is analogous to the alkali metal ions in the cation mode.[9] That a tetrasaccharide is necessary to produce an adduct ion makes the adduct effectively the same "size" as Rb^+. Rb^+ adduct is observed with linear tetrasaccharides and larger, while Cs^+ adduct is observed with pentasaccharides and K^+ with trisaccharides.

To understand the nature of the sulfate adduct, SORI CID was performed on lacto-*N*-fucopentaose I (LNFP-I). This fucosylated pentasaccharide was selected for the CID study because it contains a labile fucose group that normally dissociates readily in the cation mode. LNFP-I forms the sulfate adduct $[LNFP\text{-}I + HSO_4]^-$ under the same conditions as maltohexaose (*m*/*z* 950.26, Figure 3a). Note that the sulfate derivative $[LNFP\text{-}I + HSO_4 - H_2O]^-$ is a minor component in the spectrum (*m*/*z* 932.25, ~5%). CID of the adduct species produces a single product corresponding to HSO_4^- (Figure 3b). The loss of fucose, the predominant product in the cation mode, is not observed. The weakly abundant peaks observed in the CID spectrum are due primarily to noise. The result is consistent with the noncovalently bound nature of the complex. The interaction between the sulfate and the oligosaccharide involves mainly ion−dipole, so that the dominance of HSO_4^- as the CID product is expected. Several other adduct species were similarly examined and in each case the same product was observed.

The derivative species is likely due to the formation of a direct covalent bond. The lack of size specificity suggests multiple interactions are not important in the formation of the derivative. The mass spectra offer no evidence for site specificity in the bond formation. CID was also performed on the LNFP-I derivative for comparison. A sulfate derivative of LNFP-I is expected to produce fragments corresponding to cleavage of the oligosaccharide in contrast to the adduct that only produces HSO_4^-. The CID spectrum of the isolated derivative (*m*/*z* 932.25 Figure 4) produces several cleavage reactions including cross-ring cleavages ($^{0,2}A_5$, $^{0,3}A_5$, $^{2,4}A_5$, $^{0,2}X_0$) and glycosidic bond cleavages (B, C, and Y types). Table 2 lists the fragment ions according to the cleavages designated in Chart 1. All fragments contain the sulfate group presumably as the charge carrier. Cross-ring cleavages occur on the reducing ring, suggesting that derivatization does not occur on the anomeric position. The presence of both B- and Y-type ions means the retention of both reducing and nonreducing ends. They suggest that derivatization is random and occurs throughout the oligosaccharide chain. The large amount of fragmentation is further consistent with the formation of a true derivative. The absence of nonspecific cleavages, such as the loss of fucose in the cation mode, allows sequencing to be readily performed. In

Chart 1

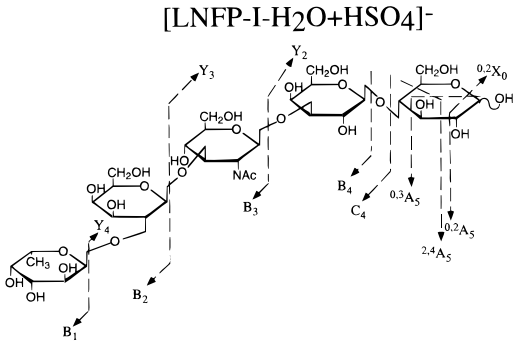


Chart 2

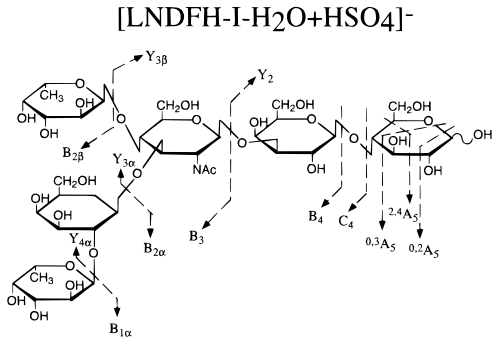
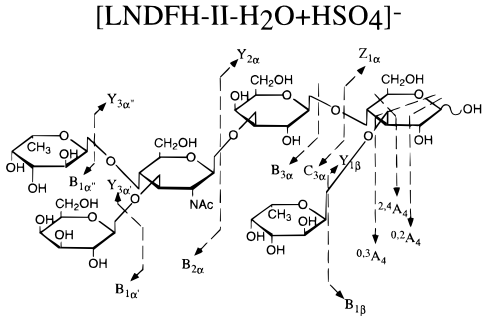


Chart 3



other words, there is no ambiguity in the CID spectrum regarding the position of the fucose residue.

The CID spectrum of the derivative in the anion mode contrasts to the CID spectrum of the underivatized parent in the cation mode. Loss of fucose typically dominates the spectrum of LNFP-I and other similarly fucosylated oligosaccharides in the cation mode making sequence determination difficult.[9,12,23] Formation of the sulfate derivative in the anion mode followed by CID provides a possible method for sequencing neutral fucosylated oligosaccharides.

To continue this theme, lacto-*N*-difucohexaose I and II (LNDFH-I and -II, Charts 2 and 3), branched isomeric oligosaccharides derived from human milk, were examined by CID. The two oligosaccharides differ only by the position of the two fucoses. In LNDFH-I, both are positioned toward the nonreducing end, while in LNDFH-II, one of the fucoses is on the reducing end. The MALDI-FTMS spectrum of the two isomers are nearly identical in the positive ion mode (spectra not shown). The loss of fucose is the most dominant product in the MALDI spectra. CID in the cation mode only promotes further fucose loss, and little structural information is obtained until all fucose residues are dissociated

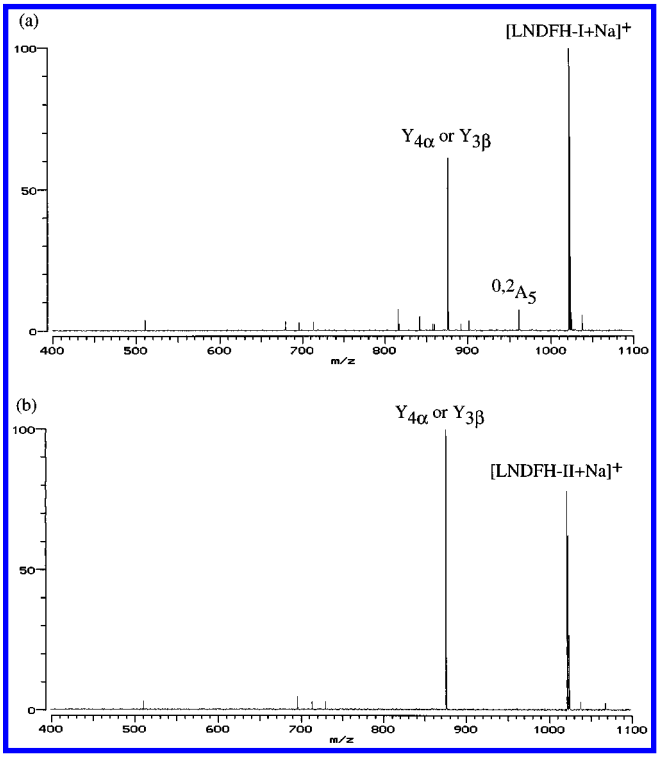


Figure 5. CID of sodiated isomers of (a) LNDFH-I and (b) LNDFH-II. The quasimolecular ions were isolated with CID performed under SORI conditions. The loss of fucose is the dominant fragment in both spectra.

(Figure 5). CID was performed on the sulfate derivatives [LNDFH + HSO_4 − H_2O]$^-$ (m/z 1078.32) to determine whether the two isomers can be differentiated in the negative ion mode. The CID spectra of the isolated derivatives are shown in Figure 6. The spectra clearly differentiate the two isomers. The spectrum of LNDFH-I shows extensive cross-ring cleavages at the reducing end (see also Chart 2 and Table 3) corresponding to $^{0,2}A_5$, $^{0,3}A_5$, and $^{2,4}A_5$. The glycosidic bond cleavages at $B_{2\alpha}$ and B_3 determine the position of the fucose residues. The cross-ring cleavages in LNDFH-II are less abundant and only observed with the accompanying loss of fucose on the reducing ring ($Y_{1\beta}/^{2,4}A_4$, $Y_{1\beta}/^{0,3}A_4$, $Y_{1\beta}/^{0,2}A_4$). The glycosidic bond cleavages corresponding to $B_{2\alpha}$ and $Y_{2\alpha}$ distinguish the position of the fucose residues. It should be emphasized that both B- and Y-type cleavages are observed giving complementary reducing end and nonreducing end ions. The presence of complementary pairs aids in determining the sequence more precisely.

Two acidic oligosaccharides, LS-tetrasaccharide a (LSTa, Chart 4) and monosialylated, galactosylated biantennary N-linked oligosaccharide (A1, Chart 5), were examined with the sulfate dopant. LSTa is derived from human milk while A1 is obtained from human fibrinogen. Both oligosaccharides contain one sialic acid residue at the nonreducing end. Neither oligosaccharide forms the adduct or the derivative with HSO_4^-. LSTa produces predominantly the deprotonated species, [LSTa − H]$^-$ (m/z 997.33). Both sulfated and nonsulfated fragments are observed in the mass spectrum (Figure 7a). Peaks corresponding to B_1 [Neu5Ac − H]$^-$ (m/z 290.09) and B_2 [Gal + Neu5Ac − H]$^-$ (m/z 452.14) cleavages are present as nonsulfated fragments. The sulfated fragments detected are [Y_4 + HSO_4]$^-$ (m/z 804.21) and

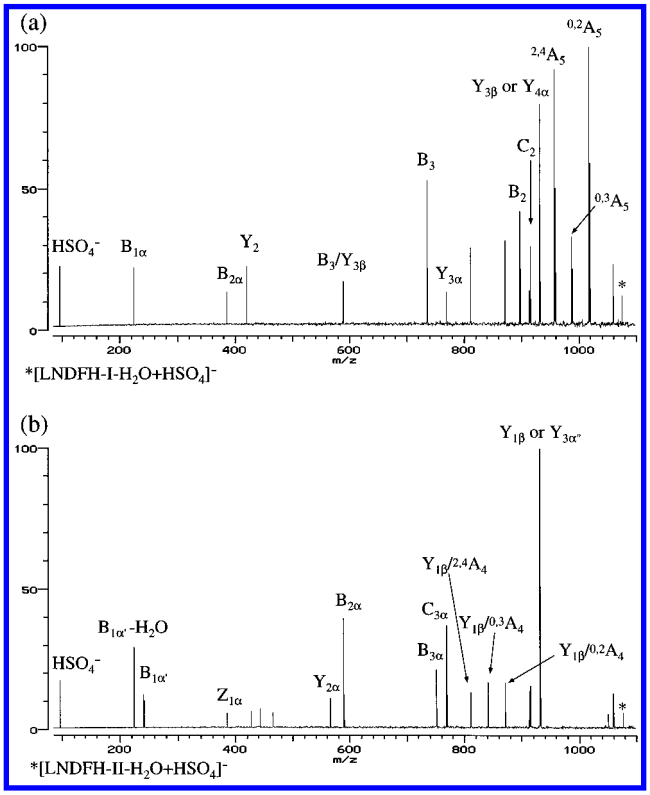


Figure 6. CID of isomers isolated sulfate derivatives of (a) LNDFH-I $[LNDFH\text{-}I + HSO_4 - H_2O]^-$ and (b) LNDFH-II $[LNDFH\text{-}II + HSO_4 - H_2O]^-$. The position of the fucose is readily determined in the two compounds. The two isomers produce nearly identical mass spectra in the cation mode.

Table 3. Fragmentation Assignments of the Peaks from SORI CID of Lacto-*N*-difucohexaose I and II (LNDFH-I and -II)

m/*z*	LNDFH-I	LNDFH-II
1018.30	$^{0,2}A_5$	
988.29	$^{0,3}A_5$	
958.28	$^{2,4}A_5$	
932.27	$Y_{3\beta}$ or $Y_{4\alpha}$	$Y_{3\alpha''}$ or $Y_{1\beta}$
916.27	C_4	$Y_{3\alpha'}$
898.26	B_4	
872.25	$Y_{3\beta}/^{0,2}A_5$	$Y_{1\beta}/^{0,2}A_4$
842.23		$Y_{1\beta}/^{0,3}A_4$
812.22	$Y_{3\beta}/^{2,4}A_5$	$Y_{1\beta}/^{2,4}A_4$
770.21	$Y_{3\alpha}$	$C_{3\alpha}$
736.20	B_3	
752.20		$B_{3\alpha}$
590.14	$Y_{3\beta}/B_3$	$B_{2\alpha}$
567.13		$Y_{2\alpha}$
444.09		$Y_{3\alpha''}/B_{2\alpha}$
421.07	Y_2	
387.06	$B_{2\alpha}$	$Z_{1\alpha}$
243.02		$B_{1\alpha''}$ or $B_{3\beta}$
241.00		$B_{1\alpha'}$
225.01	$(B_{1\alpha}$ or $B_{2\beta}) - H_2O$	$(B_{1\alpha''}$ or $B_{3\beta}) - H_2O$
96.96	HSO_4^-	HSO_4^-

[a] See also Figure 5a,b and Charts 2 and 3.

$[B_1 + HSO_4]^-$ (*m*/*z* 388.06). These fragments result from the coordination of HSO_4^- with the desialylated LSTa and, surprisingly, to a uncharged sialic acid, respectively. When the oligosaccharide and the sulfate dopant are allowed to sit for a longer time (up to 2 h), the intensity of $[Y_4 + HSO_4]^-$ is found to increase,

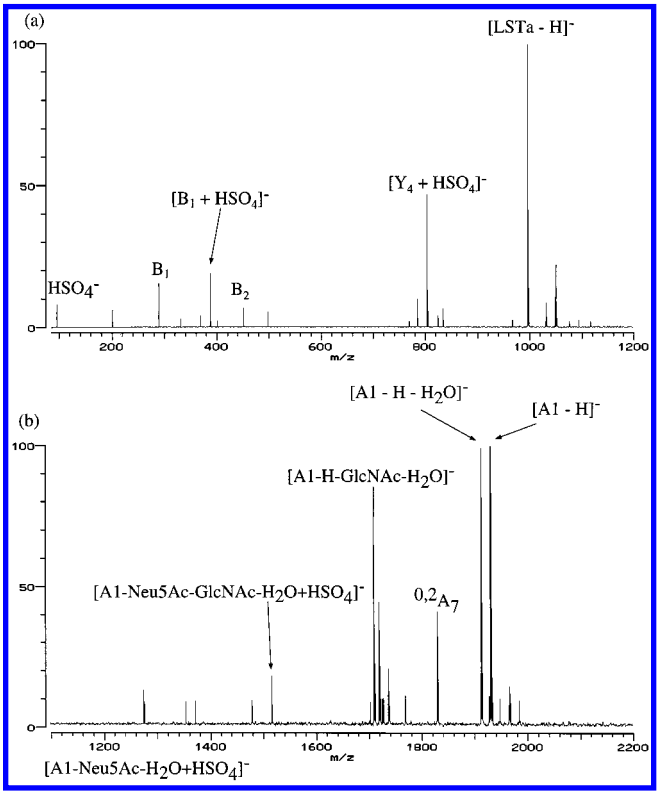


Figure 7. MALDI-FTMS spectrum of two acidic oligosaccharide (a) LSTa and (b) A1 both doped with 0.01 M H_2SO_4.

Chart 4

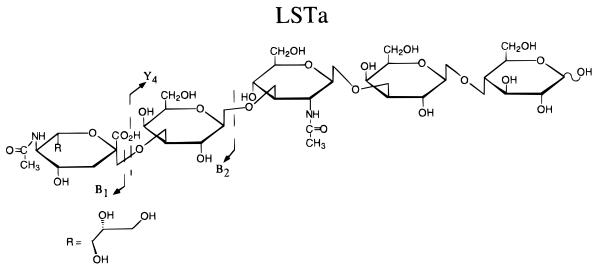
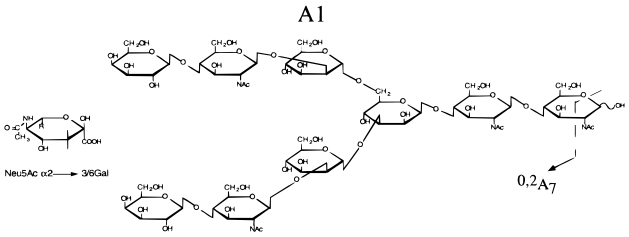


Chart 5



suggesting that the desialylation most likely occurs in solution and this fragment is already formed prior to ionization.

The deprotonated species $[A1 - H]^-$ (*m*/*z* 1930.70) is present as the base peak in the MALDI-FTMS spectrum (Figure 7b). The two most abundant nonsulfated fragment ions correspond to the loss of water $[A1 - H - H_2O]^-$ (*m*/*z* 1912.69) and the loss of GlcNAc $[A1 - H - GlcNAc - H_2O]^-$ (*m*/*z* 1709.61). Two sulfated fragments due to the loss of sialic acid $[A1 - Neu5Ac - H_2O + HSO_4]^-$ (*m*/*z* 1719.57) and the loss of both GlcNAc and sialic acid $[A1 - Neu5Ac - GlcNAc - H_2O + HSO_4]^-$ (*m*/*z* 1516.49) are observed. Cross-ring cleavage in the reducing end GlcNAc ($^{0,2}A_7$) (*m*/*z* 1829.66) is also present.

With the acidic oligosaccharides, $[M - H]^-$ is the only "molecular ion" observed. There are no adducts resulting from a neutral (uncharged) carboxylic acid group. Only fragments that lose the sialic acid residue are adducted. Given that both sulfate and carboxylic acid groups are potential sources for the negative charge, electrostatic factors may play the key role in determining the features of the mass spectra. If the complex is produced in the gas phase, then combining a hydrogen sulfate anion with a neutral oligosaccharide is more energetically favorable than combining it with a deprotonated acidic oligosaccharide. If the complex is preformed in the crystal and is desorbed intact, then again electrostatic repulsion may prevent the complex from persisting until detection. In both cases, it is assumed that the carboxylic acid group is deprotonated throughout the ionization process.

Simultaneous Analysis of Neutral and Acidic Oligosaccharide Mixture. Oligosaccharides from biological sources often occur as mixtures of neutral and acidic oligosaccharides. Unfortunately, the simultaneous analysis of mixtures composed of both acidic and neutral oligosaccharides is a difficult task. Neutral oligosaccharides do not produce abundant ions in the negative mode, while acidic oligosaccharides fragment readily in the positive mode with MALDI. An anionic dopant offers the possibility of observing neutral and acidic oligosaccharides simultaneously in the anion mode.

A mixture of neutral and acidic oligosaccharides were doped with H_2SO_4. Equimolar of LSTa + LNDFH-I doped with 0.01 M H_2SO_4 produces two major ionic species corresponding to deprotonated LSTa $[LSTa - H]^-$ and sulfated adduct LNDFH-I $[LNDFH\text{-}I + HSO_4]^-$ (m/z 997.33 and 1096.32, respectively, Figure 8) where few fragment ions observed. The quasimolecular ions from both oligosaccharides are clearly observed with excellent signal-to-noise ratios. In the absence of the anion dopant, only the acidic oligosaccharide is observed as the deprotonated species $[M - H]^-$. The two oligosaccharides exhibit similar sensitivities; the relative abundances of the quasimolecular ions are nearly equal. Although similar sensitivities are desirable, there is some variability observed with other neutral–acidic pairs. For example, when a mixture of A1 and NA4 is doped with 0.01 M H_2SO_4, the relative ratio of $[A1 - H]^-/[NA4 + HSO_4]^-$ is 5:1. We find that the acidic oligosaccharides generally will produce slightly stronger signals even when equal concentrations are used. Apparently, deprotonated acidic oligosaccharides are slightly more easily formed than the sulfate–neutral adduct. We are currently investigating a series of alkyl sulfates to find more strongly bound adducts so that the sensitivity differences between neutral and acidic oligosaccharides will be less. This topic will be the subject of future publications.

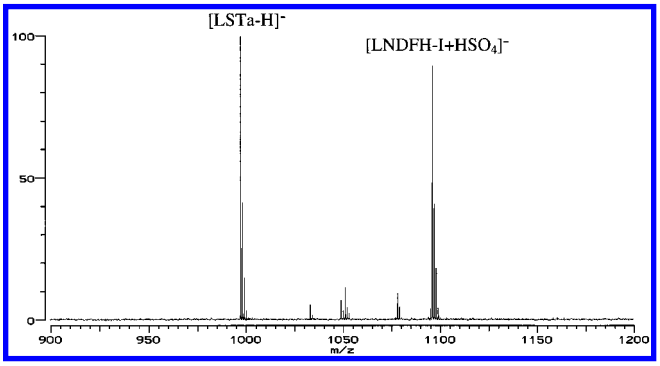


Figure 8. MALDI-FTMS spectrum of mixture containing acidic LSTa and neutral LNDFH-I doped with 0.01 M H_2SO_4.

CONCLUSION

Hydrogen sulfate is potentially a useful dopant for the analysis of neutral oligosaccharides. Both the sulfate adduct $[M + HSO_4]^-$ and the sulfate derivative $[M + HSO_4 - H_2O]^-$ are readily formed under the proper ionization conditions. The adduct provides no structural information but the derivative provides sequence and linkages. It provides structural information that is not readily obtained by the use of cationic dopants such as the alkali metal ions. The formation of the sulfate derivative is in effect an in situ derivatization method requiring no additional sample workup. However, it provides similarly high sensitivity and significantly more information than alkali coordinated complexes. It further extends the repertoire of derivatization methods that have proven to be very useful for the analysis of oligosaccharides.

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