Atmospheric Pressure MALDI Fourier Transform Mass Spectrometry of Labile Oligosaccharides

Jinhua Zhang, LaTasha LaMotte, Eric D. Dodds, and Carlito B. Lebrilla*

Department of Chemistry and School of Medicine, Biochemistry and Molecular Medicine, University of California Davis, Davis, California 95616

An atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI) source coupled to Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) under UV laser and solid matrix conditions has been demonstrated to analyze a variety of labile oligosaccharides including O-linked and N-linked complex glycans released from glycoproteins. Spectra were acquired by both AP MALDI and vacuum MALDI and directly compared. The results presented here confirm that AP MALDI can generate significantly less energetic ions than vacuum MALDI and is able to produce the intact molecular ions with little or no fragmentation in both positive and negative ion mode analyses. Under certain conditions, noncovalent complexes of sialylated oligosaccharides were observed. The sensitivity attainable by AP MALDI was found to be comparable to conventional MALDI, and tandem mass spectrometry of oligosaccharides ionized by AP MALDI was shown to allow detailed structural analysis. Analysis of *N*-glycan mixtures derived from human fibrinogen further demonstrated that AP MALDI-FT ICR MS is ideal for the study of complex glycan samples as it provides high-accuracy, high-resolution mass analysis with no difficulty in distinguishing sample constituents from fragment ions.

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has been extensively used for the rapid and sensitive analysis of oligosaccharides. MALDI has shown higher sensitivity than electrospray ionization (ESI) due to the hydrophilic character of underivatized oligosaccharides.[1,2] In addition, the analyte signals produced by ESI are distributed among singly and multiply charged ions as well as species with different associated cations, further reducing the sensitivity and complicating the mass spectral interpretation. MALDI yields predominant $[M + Na]^+$ ions for underivatized oligosaccharides. Usually, fragmentation of the sodiated species produces a higher percentage of cross-ring cleavage products than $[M + H]^+$ ions, thereby providing linkage information on the oligosaccharides.[3]

The implementation of MALDI with Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS) has demonstrated high performance with regard to mass accuracy, sensitivity, resolution, and tandem MS capabilities for various oligosaccharides and glycoconjugates as reported by this laboratory[4–19] and others.[20–22] However, the metastable decay of ions generated by conventional MALDI is sometimes problematic in the characterization of mixtures, as it is at times difficult to determine whether a particular ion is an independent species or merely a fragment. The metastable decay problem is more pronounced for carbohydrates and glycoconjugates containing labile groups, such as sialic acid, sulfate, fucose, and lipids, thus hindering the observation of the intact molecular ions in the mass spectrum. Extensive work has been conducted in the past several years on the development of MALDI methods that reduce the

* Corresponding author. Fax: 1-530-754-5609. Tel: 1-530-752-6364. E-mail: cblebrilla@ucdavis.edu.

(1) Burlingame, A. L.; Boyd, R. K.; Gaskell, S. J. *Anal. Chem.* **1994**, *66*, 634R–683R.

(2) Reinhold, V. N.; Reinhold, B. B.; Costello, C. E. *Anal. Chem.* **1995**, *67*, 1772–1784.

(3) Harvey, D. J.; Bateman, R. H.; Green, M. R. *J. Mass Spectrom.* **1997**, *32*, 167–187.

(4) Cancilla, M. T.; Penn, S. G.; Carroll, J. A.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1996**, *118*, 6736–6745.

(5) Penn, S. G.; Cancilla, M. T.; Lebrilla, C. B. *Anal. Chem.* **1996**, *68*, 2331–2339.

(6) Penn, S. G.; Cancilla, M. T.; Green, M. K.; Lebrilla, C. B. *Eur. Mass Spectrom.* **1997**, *3*, 67–79.

(7) Cancilla, M. T.; Penn, S. G.; Lebrilla, C. B. *Anal. Chem.* **1998**, *70*, 663–672.

(8) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 205–211.

(9) Cancilla, M. T.; Wang, A. W.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3206–3218.

(10) Penn, S. G.; Cancilla, M. T.; Lebrilla, C. B. *Int. J. Mass Spectrom.* **2000**, *196*, 259–269.

(11) Wong, A. W.; Wang, N.; Lebrilla, C. B. *Anal. Chem.* **2000**, *72*, 1419–1425.

(12) Tseng, K.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3747–3754.

(13) Tseng, K.; Wang, H.; Lebrilla, C. B.; Bonnell, B.; Hedrick, J. L. *Anal. Chem.* **2001**, *73*, 3556–3561.

(14) Franz, A. H.; Molinski, T. F.; Lebrilla, C. B. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 1254–1261.

(15) Tseng, K.; Xie, Y.; Seeley, J.; Hedrick, J. L.; Lebrilla, C. B. *Glycoconjugate J.* **2001**, *18*, 309–320.

(16) Xie, Y.; Lebrilla, C. B. *Anal. Chem.* **2003**, *75*, 1590–1598.

(17) An, H. J.; Peavy, T. R.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **2003**, *75*, 5628–5637.

(18) Xie, Y.; Liu, J.; Zhang, J.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **2004**, *76*, 5186–5197.

(19) Zhang, J.; Lindsay, L. L.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **2004**, *76*, 5990–6001.

(20) O'Connor, P. B.; Mirgorodskaya, E.; Costello, C. E. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 402–407.

(21) Solouki, T.; Reinhold, B. B.; Costello, C. E.; O'Malley, M.; Guan, S.; Marshall, A. G. *Anal. Chem.* **1998**, *70*, 857–864.

(22) Gaucher, S. P.; Cancilla, M. T.; Phillips, N. J.; Gibson, B. W.; Leary, J. A. *Biochemistry* **2000**, *39*, 12406–12414.

problem of metastable decay, particularly in FT ICR MS. These include the use of large alkali cations[6] and collisional cooling with high pressure.[20,23-25]

Atmospheric pressure MALDI (AP MALDI) was first developed by Laiko et al.[26] to analyze peptide mixtures. During the past several years, AP MALDI has been implemented with a number of mass analyzers such as orthogonal acceleration time-of-flight,[26] ion trap,[27-36] and FT ICR.[37] The AP MALDI interface not only allows the quick interchange with conventional atmospheric pressure ionization sources such as ESI and APCI but also facilitates high-throughput analysis since the sample is manipulated under atmospheric pressure. For AP MALDI, samples are prepared in a manner similar to that for conventional MALDI, with solid matrix cocrystallized with analyte. There is considerable evidence that the ions generated by AP MALDI are less energetic than those produced by vacuum MALDI and are thus subject to significantly less metastable decay.[27-37] The advantages of high pressure and AP MALDI have been utilized to analyze a variety of thermally labile oligosaccharides. Costello et al.[20] detected the molecular ion species of gangliosides without loss of the sialic acid residue using a high-pressure MALDI source coupled with FT ICR MS. Recently, Cotter et al.[27,33,34] and Doroshenko et al.[32] have analyzed and fragmented the sialylated oligosaccharides and standard N-linked oligosaccharides, respectively, using liquid matrix infrared AP MALDI. AP MALDI has not been widely implemented with FT ICR MS. There has only been one example by Fabris et al.[37] showing that complex peptide mixtures can be analyzed by AP MALDI-FT ICR MS. There has been no example of AP MALDI coupled to FT ICR MS to analyze oligosaccharides; however, the ultrahigh mass resolution and mass accuracy provided by FT ICR MS combined with the soft ionization feature offered by AP MALDI would appear to be particularly well suited to the analysis of complicated oligosaccharide mixtures.

In this work, we have applied UV laser AP MALDI-FT ICR MS to analyze various oligosaccharides, including sulfated, sialylated, O-linked, and N-linked oligosaccharides as well as gangliosides. These mass spectra were compared with those produced by vacuum MALDI-FT ICR MS. AP MALDI resulted in diminished fragmentation for all oligosaccharides studied, versus the extensive fragmentation produced by vacuum MALDI. AP MALDI interfaced with FT ICR MS has been shown to be a powerful tool for MS analyses of labile oligosaccharides and complex oligosaccharide mixtures with great sensitivity and speed as well as tandem MS capability.

EXPERIMENTAL SECTION

Materials. Maltohexose and gangliosides (GM1 and GT1b) were purchased from Sigma (St. Louis, MO). Neocarrahexose 2,4[1],4[3],4[5]-tetra-O-sulfate (Na$^+$) was obtained from Dextra Laboratories (Reading, U.K.). 6′-Sialyl-N-acetyllactosamine (6′-SLN), 6′-siallactose (6′-SL), and monosialylated, galactosylated biantennary (A1) N-glycan (Neu5Ac)(Gal)$_2$(Man)$_3$(GlcNAc)$_4$ were purchased from Oxford GlycoSciences (Abingdon, Oxfordshire, U.K.). Matrixes were obtained from Sigma and used without further purification.
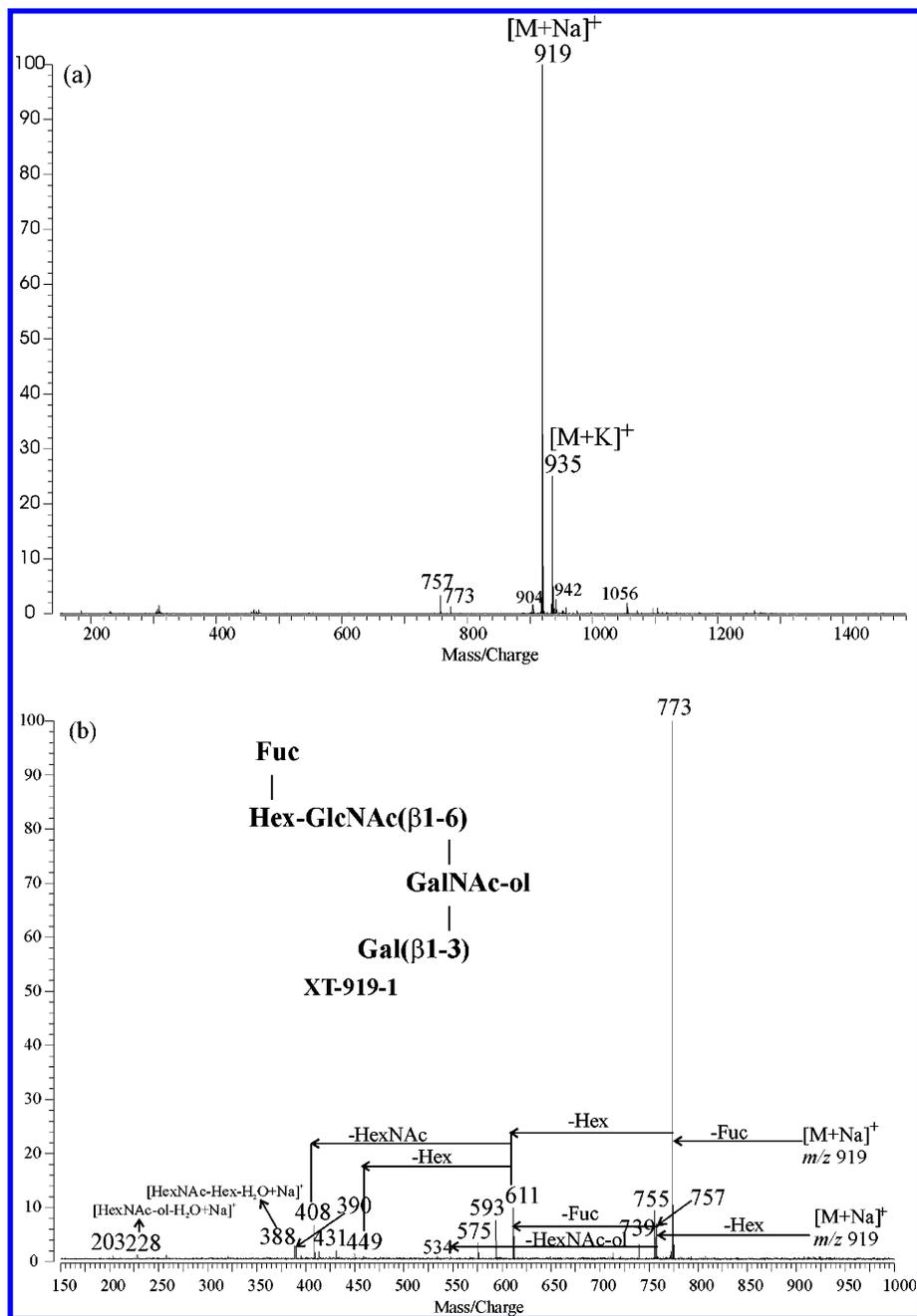
O-Linked neutral and anionic oligosaccharide alditols were released from the egg jelly of the amphibian *Xenopus tropicalis*. This procedure was described in detail in a previous publication.[19]

N-Linked oligosaccharides were released from human fibrinogen (Calbiochem, San Diego, CA) by incubating 1 mg of the glycoprotein with 10 units of PNGase F (Calbiochem) in 100 mM NH$_4$HCO$_3$ buffer (pH 7.5) overnight at 37 °C. Protein was precipitated from the preparation by addition of 100% ethanol and chilling at −20 °C for 30 min. After centrifugation, the supernatant was collected, dried in a vacuum centrifuge, and reconstituted in water. The oligosaccharide solution was purified on a porous graphitized carbon solid-phase extraction cartridge (PGC-SPE) (Alltech Associates Inc., Deerfield, IL) as described elsewhere.[19]

Sample Preparation. Samples for AP MALDI were prepared on the gold-plated target by mixing 1 μL of aqueous analyte solution with 1 μL of matrix solution. Unless otherwise stated, the amount of material for the analysis was in the range of a few picomoles. The matrix solution was composed of equal volumes of 0.4 M 2,5-dihydroxybenzoic acid in acetonitrile/water (50:50) and saturated 2,5-dihydroxyacetophenone in acetonitrile/water (50:50). For positive mode analyses, 1 μL of 0.01 M NaCl in acetonitrile/water solution (50:50) was added to enrich the Na$^+$ concentration and produce primarily sodiated species. The samples on the target were air-dried at room temperature.

AP MALDI-FT ICR MS. All experiments were performed on a commercial HiResESI Fourier transform mass spectrometer (IonSpec, Irvine, CA) using a 9.4-T actively shielded superconducting magnet. This instrument, equipped with a Z-spray source, may be interfaced with interchangeable AP MALDI and ESI external ion sources. For this work, the MassTech AP MALDI source (MassTech Inc., Columbia, MD) previously described by Fabris et al. was used.[37] Photons are produced by a nitrogen laser (337 nm) contained in the control unit and are transmitted to the ion source via a fiber-optic cable. UV light pulses are focused by a quartz lens and directed onto the target surface with a mirror. The 96-spot sample plate is loaded magnetically onto an X, Y translational stage. The position of the sample plate can be automatically controlled by the Target software (MassTech, Inc.). This software is also used to control the AP MALDI target motion and laser firing. The plate moves in a spiral pattern from the center of the sample spot once the acquisition is begun.

(23) Baykut, G.; Jertz, R.; Witt, M. *Rapid Commun. Mass Spectrom.* **2000**, *14* (14), 1238−1247.

(24) O'Connor, P. B.; Costello, C. E. *Rapid Commun. Mass Spectrom.* **2001**, *15* (19), 1862−1868.

(25) Ivleva, V. B.; Elkin, Y. E.; Moyer, S. C.; O'Connor, P. E.; Costello, C. E. *Anal. Chem.* **2004**, *76,* 6484−6491.

(26) Laiko, V. V.; Baldwin, M. A.; Burlingame, A. L. *Anal. Chem.* **2000**, *72* (4), 652−657.

(27) Von Seggern, C. E.; Zarek, P. E.; Cotter, R. J. *Anal. Chem.* **2003**, *75* (23), 6523−6530.

(28) Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2002**, *74* (17), 468A−476A.

(29) Laiko, V. V.; Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2000**, *72* (21), 5239−5243.

(30) Moyer, S. C.; Marzilli, L. A.; Woods, A. S.; Laiko, V. V.; Doroshenko, V. M.; Cotter, R. J. *Int. J. Mass Spectrom.* **2003**, *226* (1), 133−150.

(31) Creaser, C. S.; Reynolds, J. C.; Harvey, D. J. *Rapid Commun. Mass Spectrom.* **2002**, *16,* 176−184.

(32) Tan, P. V.; Taranenko, N. I.; Laiko, V. V.; Yakshin, M. A.; Prasad, C. R.; Doroshenko, V. M. *J. Mass Spectrom.* **2004**, *39,* 913−921.

(33) Von Seggern, C. E.; Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2003**, *75* (23), 3212−3218.

(34) Von Seggern, C. E.; Cotter, R. J. *J. Am. Soc. Mass Spectrom.* **2003**, *14,* 1158−1165.

(35) Von Seggern, C. E.; Cotter, R. J. *J. Mass Spectrom.* **2004**, *39,* 736−742.

(36) Mehl, J. T.; Cummings, J. J.; Rohde, E.; Yates, N. N. *Rapid Commun. Mass Spectrom.* **2003**, *17,* 1600−1610.

(37) Kellersberger, K. A.; Tan, P. V.; Laiko, V. V.; Doroshenko, V. M.; Fabris, D. *Anal. Chem.* **2004**, *76(14),* 3930−3934.

Figure 1. Positive mode AP MALDI-FT ICR MS analyses of O-linked neutral oligosaccharide alditol XT-919-1. (a) MS analysis. (b) CID spectrum of m/z 919. The deduced structure is inset.

During typical operation, the MALDI sample plate was held at high potential (3.5 kV) relative to the sampling cone to facilitate transfer of the MALDI plume into the skimmer region. The source temperature was maintained at 80 °C. Each spectrum was obtained with one scan and a hexapole accumulation time from 30 to 90 s, during which the laser was continuously fired using a 10-Hz repetition rate.

For tandem MS experiments, sustained off-resonance irradiation collision-induced dissociation (SORI-CID) experiments were performed to obtain structural information.[38] The desired ion was

isolated in the ICR cell with the use of an arbitrary waveform generator. Isolated ions were excited at +1000 Hz of their cyclotron frequencies for 1000 ms at an amplitude of 2−8 V (base to peak), depending on the desired level of fragmentation and the size of the oligosaccharide. Two argon pulses were used during the CID event to maintain a pressure of 10^{-6} Torr in the ICR cell.

MALDI (Vacuum)-FT ICR MS. All vacuum MALDI-FT ICR experiments were performed on a commercial HiResMALDI Fourier transform mass spectrometer (IonSpec) with a 7.0-T superconducting magnet. Sample preparation for the oligosaccharide analyses and instrumental conditions are described in earlier publications.[16−19]

(38) Gauthier, J. W.; Trautman, T. R.; Jacobson, D. B. *Anal. Chim. Acta* **1991**, *246*, 211−225.
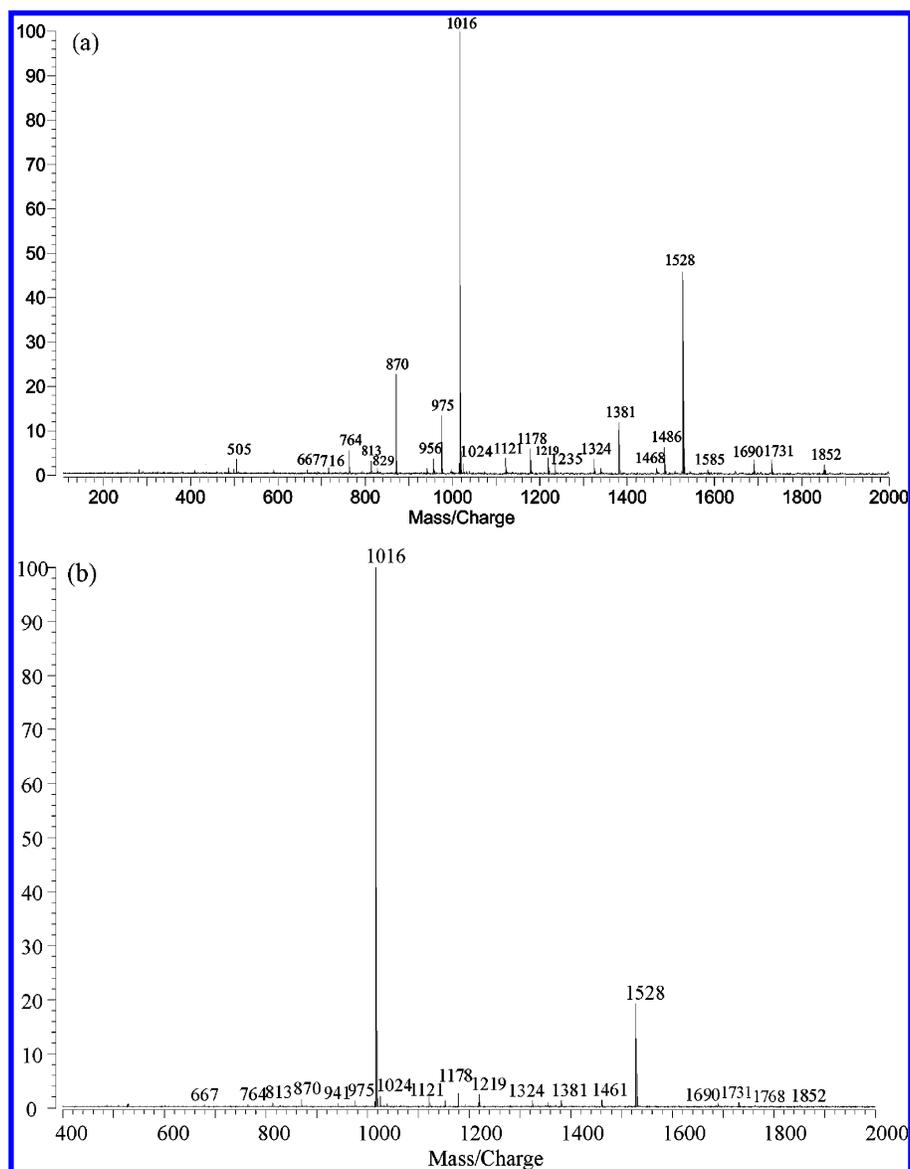
Figure 2. Negative mode MALDI-FT ICR MS analyses of O-linked anionic oligosaccharide mixtures. (a) Vacuum MALDI. (b) AP MALDI.

RESULTS AND DISCUSSION

AP MALDI-FT ICR MS of Labile Oligosaccharides. *O-Linked oligosaccharides.* In general, the time scale between ion formation and ion detection in MALDI-FT ICR MS is relatively long compared to MALDI-TOF, ranging from 0.3 to 30 s depending on the desired resolution and the system's pumping efficiency. The metastable ions formed in the MALDI process are subject to extensive fragmentation, particularly for labile compounds in FT ICR MS. In oligosaccharide analysis, the fragment ions resulting from loss of specific residues and functional groups, such as fucose, sialic acid, sulfate, and phosphate groups, are commonly observed and can cause difficulty in assigning quasimolecular ions.

To investigate the application of AP MALDI-FT ICR MS to the analysis of oligosaccharides, we tested the oligosaccharide alditols released from the egg jelly coat of amphibians. Figure 1a shows the AP MALDI-FT ICR MS of a neutral oligosaccharide alditol XT-919-1 (m/z 919.338) after HPLC separation as described in a previous publication.[19] The monosaccharide composition is calculated to be one fucose, two hexose, and two HexNAc based on

the theoretical mass (m/z 919.337). The base peak at m/z 919 represents the sodium adduct while the peak at m/z 935 corresponds to the potassium adduct. The peaks at m/z 757 and 773 are one hexose and one fucose less than the quasimolecular ions, respectively. One might assume that these ions are fragments due to the decomposition of m/z 919; however, based on our experience, the loss of fucose is always more abundant than the loss of hexose due to the more labile nature of the fucose residue compared to hexose. On the basis of this reasoning, we believe that the peaks at m/z 757 and 773 are not fragments of m/z 919, but minor components in this HPLC fraction. Therefore, AP MALDI allows us to quickly assign the parent ions without the additional step, for example, of doping with large alkali metals to decrease metastable decomposition.[15]

With the cooler ions produced by AP MALDI, there is a concern that tandem MS, specifically CID, would not yield abundant fragment ions. Figure 1b shows the CID mass spectrum of m/z 919. Fragment ions corresponding to cleavage of every

glycosidic bond are observed. The losses of fucose (*m/z* 773) and hexose (*m/z* 757) from the quasimolecular ion indicate that fucose and one of hexoses are present at the nonreducing ends. The group of ions at *m/z* 611, 449, 431, 413, 408, and 390 are characteristic fragments of the known trisaccharide core, Gal(β1−3)[GlcNAc(β1−6)]GalNAc-ol.[12,13] The ion at *m/z* 228 is the terminal monosaccharide residue corresponding to [GalNAc-ol − H$_2$O + Na]$^+$. The inset structure is proposed based on the CID fragmentation.

The lack of fragment ions in AP MALDI makes it ideal for rapid profiling of mixtures. Figure 2 shows the comparison of vacuum MALDI and AP MALDI negative mode FT ICR MS of an anionic oligosaccharide mixture released from mucin-type glycoproteins. The spectra are highly reproducible in both instruments. Repeat analyses of the same samples yield nearly identical spectra. Figure 2a is the mass spectrum produced through vacuum MALDI with our best attempt at minimizing the fragmentation. Figure 2b is the mass spectrum of the same sample produced by AP MALDI. Both spectra show two major sulfated oligosaccharide components present in the mixture corresponding to *m/z* 1016 and 1528, whose presence are supported by HPLC.[19] However, the fragment peaks at *m/z* 870 and 1381 are significantly reduced in AP MALDI spectra. These two peaks are fragments corresponding to the loss of fucose from each of the two species at *m/z* 1016 and 1528. Similarly, the peaks at *m/z* 505 and 1235, which are observed only in the vacuum MALDI spectra, are also fragments. This example illustrates the utility of AP MALDI in the analyses of anionic (sulfated) oligosaccharide mixtures.

Gangliosides. Gangliosides constitute a class of sialylated glycosphingolipids that are particularly labile, often yielding the loss of sialic acid during ionization. They are often isolated as mixtures with minor structural variations. This class of compounds has posed a significant challenge for mass spectrometry. The metastable fragmentation is of particular concern and interferes with the compositional determination of mixtures extracted from biological samples. Costello et al. have employed "high-pressure" MALDI-FT ICR MS by introducing pulsed cooling gas into the source during ionization to analyze the gangliosides.[20,24] Their results nicely demonstrated minimal fragmentation as a result of the cooling effect of the collision gas during the ionization in a vacuum MALDI. Figure 3a is a typical MALDI-FT ICR mass spectrum of a monosialylated ganglioside (GM1) in the negative mode. The base peak is the deprotonated sialic acid ([Neu5Ac − H]$^-$, *m/z* 290) produced by the loss of sialic acid from the quasimolecular ion. The quasimolecular ion [M − H]$^-$ corresponds to *m/z* 1572.911 (theoretical *m/z* 1572.901) with the peak at *m/z* 1545 corresponding to the low-mass homologue and differing from the high-mass homologue by a C$_2$H$_4$ unit due to the ceramide moiety.[24] Figure 3b shows the AP MALDI mass spectra of GM1 in the negative mode. Only quasimolecular ions are obtained with no evidence of fragmentation.

The vacuum MALDI-FT ICR MS of GT1b, a trisialylated ganglioside, in the negative mode (Figure 4a) does not yield the quasimolecular ions. Two sets of fragment ions are obtained corresponding to the loss of one and two sialic acids. The base peak at *m/z* 563 corresponds to a species composed of two sialic acids while a lone sialic acid is observed at *m/z* 290. These small ion fragments point to the abundant loss of sialic acids during
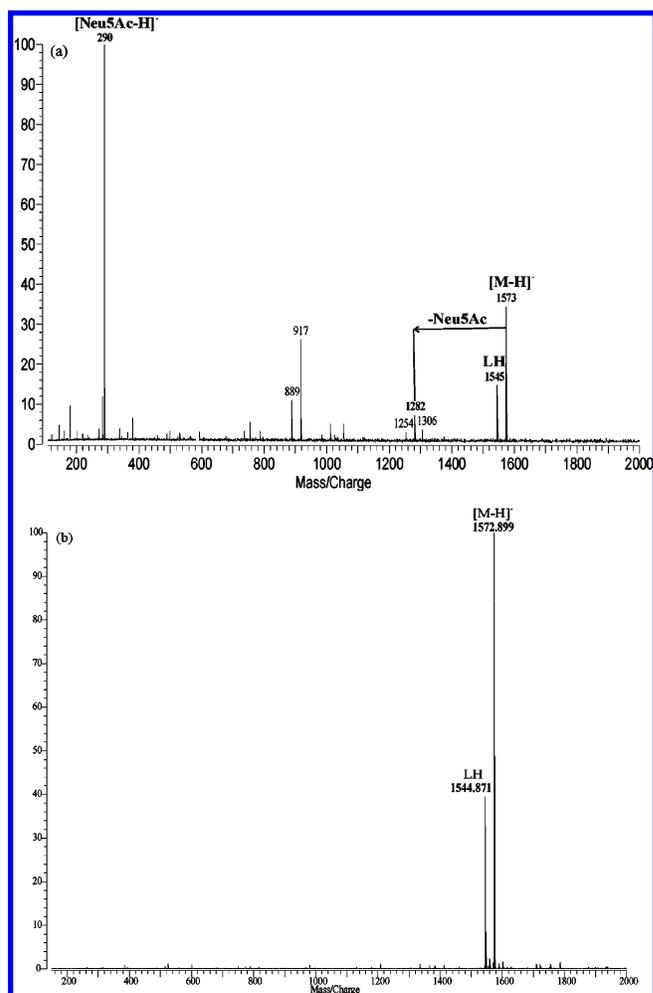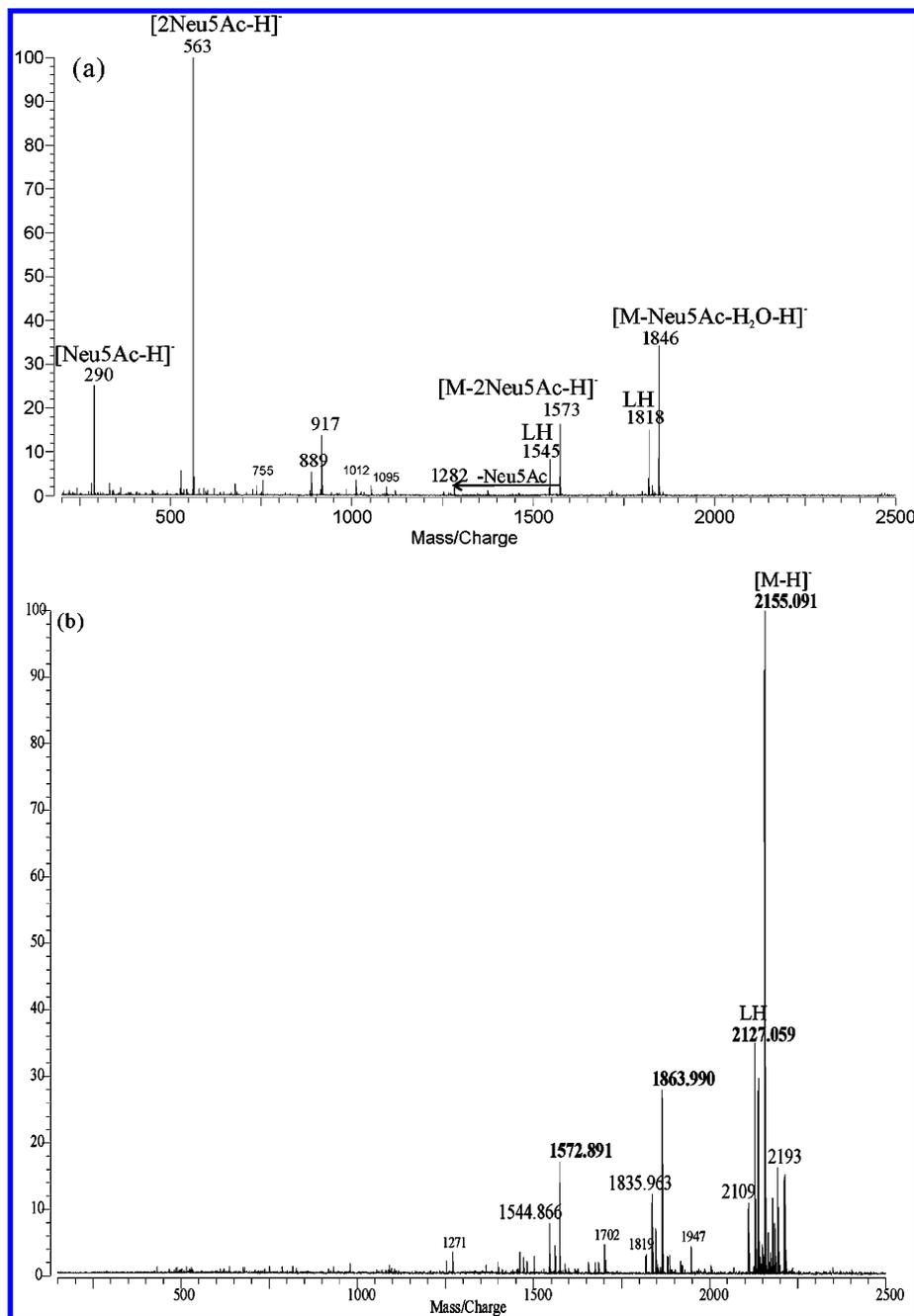

Figure 3. Negative mode MALDI-FT ICR MS analyses of ganglioside GM1. (a) Vacuum MALDI. (b) AP MALDI.

the ionization process. Figure 4b shows the AP MALDI-FT ICR mass spectrum of GT1b. The base peaks are the intact molecular ion [M − H]$^-$ (*m/z* 2155.091) and its lower homologue. Although there are peaks that correspond to the sequential losses of sialic acids, *m/z* 1863.990 and 1572.891, it is more likely that they are impurities. This notion is supported by the absence of *m/z* 290, which is the major fragmentation product of sialylated oligosaccharides in the negative mode.

Carrageenans. Multiply sulfated oligosaccharides and polysaccharides are difficult to study by MALDI because of the labile nature of the sulfate groups.[39] Furthermore, the tendency of the multiply sulfated groups to produce multiple anionic charges is not favorable for analyses with MALDI, which has the tendency of producing singly charged species. Carrageenans constitute a class of oligosaccharides found in seaweed and consisting of oligomers of sulfated residues. Neocarrahexose-2,4^{4}1,4^3,4^5-tetra-*O*-sulfate (Na$^+$) (monoisotopic mass 1344.051 Da), with four sulfate groups, does not yield the quasimolecular ion with vacuum MALDI (spectra not shown); however, AP MALDI yields the negative mode mass spectrum of this compound with the quasimolecular ion [M − Na$^+$]$^-$ at *m/z* 1321.098 (Figure 5, structure inset).

(39) Harvey, D. J. *Mass Spectrom. Rev.* **1999**, *18,* 349−451.

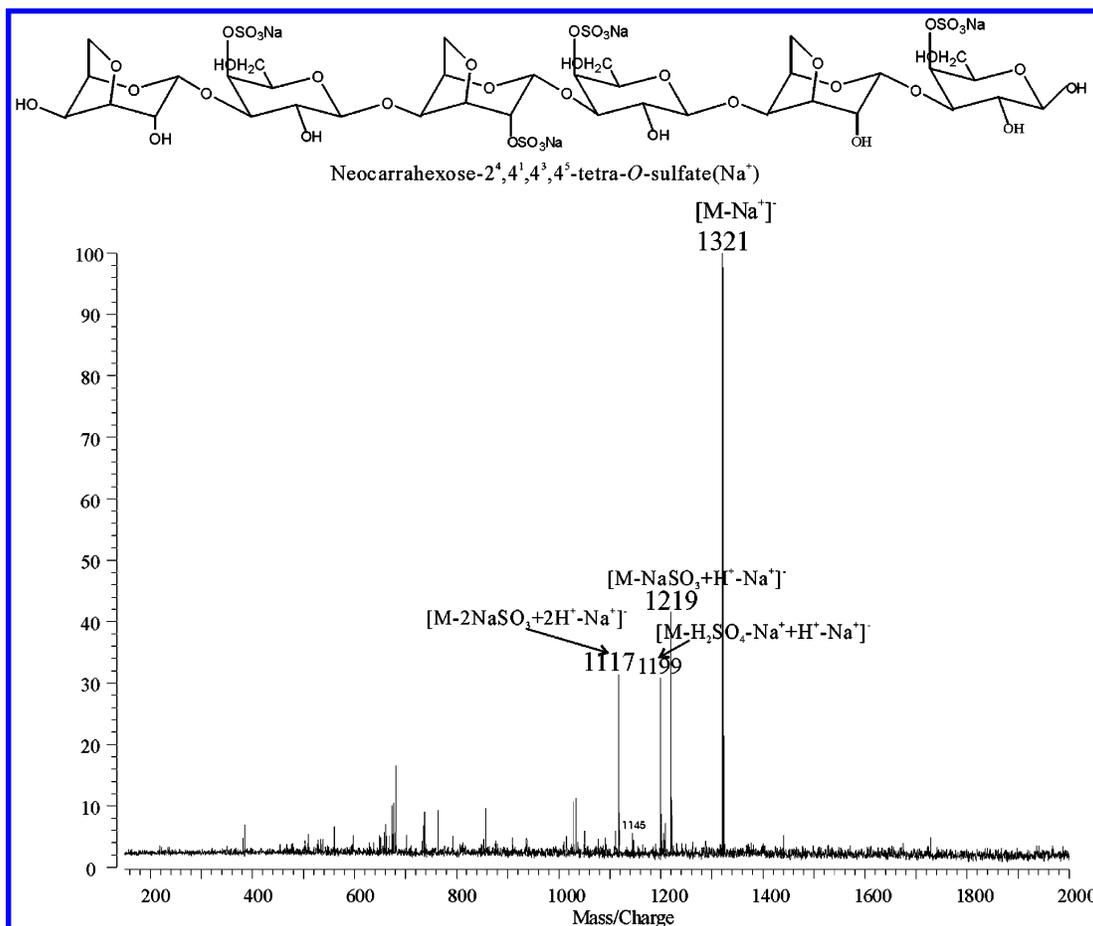**Figure 4.** Negative mode MALDI-FT ICR MS analyses of ganglioside GT1b (a) Vacuum MALDI. (b) AP MALDI.

The low-abundance ions at m/z 1219 and 1117 correspond to the stepwise loss of 102 mass units from the molecular ions, possibly through fragmentation. These two peaks can be assigned as $[M - NaSO_3 + H^+ - Na^+]^-$ and $[M - 2NaSO_3 + 2H^+ - Na^+]^-$, respectively. This fragmentation mechanism, proposed by Ackloo et al.,[40] is thought to occur first with the exchange of the Na^+ with an H^+ [$X-OSO_3Na \rightarrow X-OSO_3H$] followed by the immediate loss of the SO_3 group. According to those authors, ions such as m/z 1199 arise from a similar process whereby Na^+/H^+ exchange is now followed by loss of H_2SO_4.

These results demonstrate the detection of the base peak of molecular ions with little fragmentation due to desulfation of this tetrasulfated oligosaccharide. AP MALDI may provide even less fragmentation than MALDI-TOF MS, where metastable decay is mitigated by the short detection time and the fact that the fragments retain the velocity of the precursor ions when fragmentation occurs in the field-free region. Previous reports have shown that the most intense peak in the MALDI-TOF-MS spectrum of this compound does not correspond to the molecular ion, but to desulfated anions.[41] An additional feature of the AP MALDI FTICR MS is the lack of matrix clusters that tend to populate the low-mass regions of nearly all MALDI-TOF mass spectra.[41]

(40) Ackloo, S.; Terlouw, J. K.; Ruttink, P. A.; Burgers, P. C. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1152−1159.

(41) Fukuyama, Y.; Ciancia, M.; Nonami, H.; Cerezo, A. S.; Erra-Balsells, R.; Matulewicz, M. C. *Carbohydr. Res.* **2002**, *337*, 1553−1562.

Figure 5. Negative mode AP MALDI-FT ICR MS analysis of neocarrahexose- $2^4,4^1,4^3,4^5$-tetra-*O*-sulfate (Na$^+$). The structure is inset.

N-Linked Oligosaccharides. N-Linked oligosaccharides constitute a class of compounds that are often significantly larger than O-linked oligosaccharides. In MALDI FT ICR MS, high molecular weight oligosaccharides tend to produce more fragments than smaller oligosaccharides. This can be explained by the capability of higher mass ions to have greater internal energy. Producing molecular ions of N-linked oligosaccharides is therefore more challenging than in the case of O-linked oligosaccharides. The problem is more significant when the oligosaccharides contain sialic acid residues. It is often necessary to derivatize the carboxylic acid prior to analysis simply to obtain the quasimolecular ion.[42] The use of IR lasers with AP MALDI has recently been reported for the analysis of N-linked oligosaccharides using an ion trap analyzer.[32]
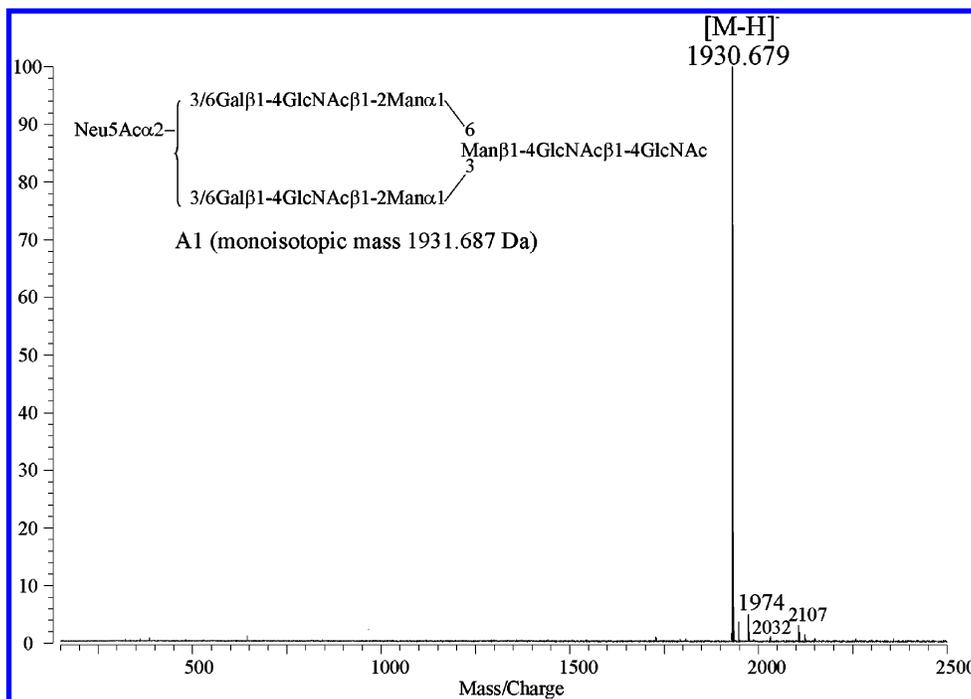
Figure 6 shows the negative mode AP MALDI-FT ICR MS spectrum of the monosialylated, galactosylated biantennary N-linked oligosaccharide A1. Again, no fragmentation is observed due to the loss of sialic acid, which is abundant in a vacuum MALDI (data not shown). The lack of fragment ions makes AP MALDI ideal for profiling N-linked oligosaccharides. To further illustrate this, *N*-oligosaccharides were released from fibrinogen and analyzed by AP MALDI-FT ICR MS. Fibrinogen is a human plasma glycoprotein that plays an important role in the final stages of blood clotting and contains five potential N-linked glycosylation sites (Swiss-Prot/TrEMBL). The *N*-glycans were released by

PNGase F and then purified by solid-phase extraction to remove the majority of the salts. The mixture was analyzed by AP MALDI-FT ICR MS in the positive mode to yield the spectrum shown in Figure 7. The two most abundant peaks at *m/z* 1976.678 and 1663.591 correspond to the doubly sodiated A1 ([M − H + 2Na]$^+$ = 1976.659 Da) and sodiated NA2 (Asialo, galactosylated biantennary, [M + Na]$^+$ = 1663.582 Da), respectively. The assignments are based on their accurate masses. The peak at *m/z* 2289.803 is the triply sodiated A2 glycan (disialylated, galactosylated biantennary, [M − 2H + 3Na]$^+$ = 2289.737 Da). The ion at *m/z* 1460.512 is a homologue of NA2 with one GlcNAc residue less. That this is in fact an NA2 homologue and not a fragment ion can be argued based on the fact that the sialic acid residues are significantly more labile than GlcNAc, and no fragment ions corresponding to the loss of sialic acid were observed. The *N*-glycan structures obtained by this MS analysis are consistent with those previously assigned by NMR.[43] The sialylated glycans A1 and A2 give rise to strong signals in the positive mode among the other neutral glycans without fragmentation, which eliminates the need for derivatization. AP MALDI therefore appears ideally suited for the analysis of complicated mixtures of *N*-glycans from biological sources.

Noncovalent Sugar−Sugar Complex Formation in AP UV-MALDI. AP IR-MALDI has been reported to be ideal for the study of weak noncovalent interactions in sugar−sugar and peptide−

(42) Powell, A. K.; Harvey, D. J. *Rapid Commun. Mass Spectrom.* **1996**, *10,* 1027−1032.

(43) Townsend, R. R.; Hilliker, E.; Li, Y.; Laine, R. A.; Bell, W. R.; Lee, Y. C. *J. Biol. Chem.* **1982**, *257,* 9704−9710.
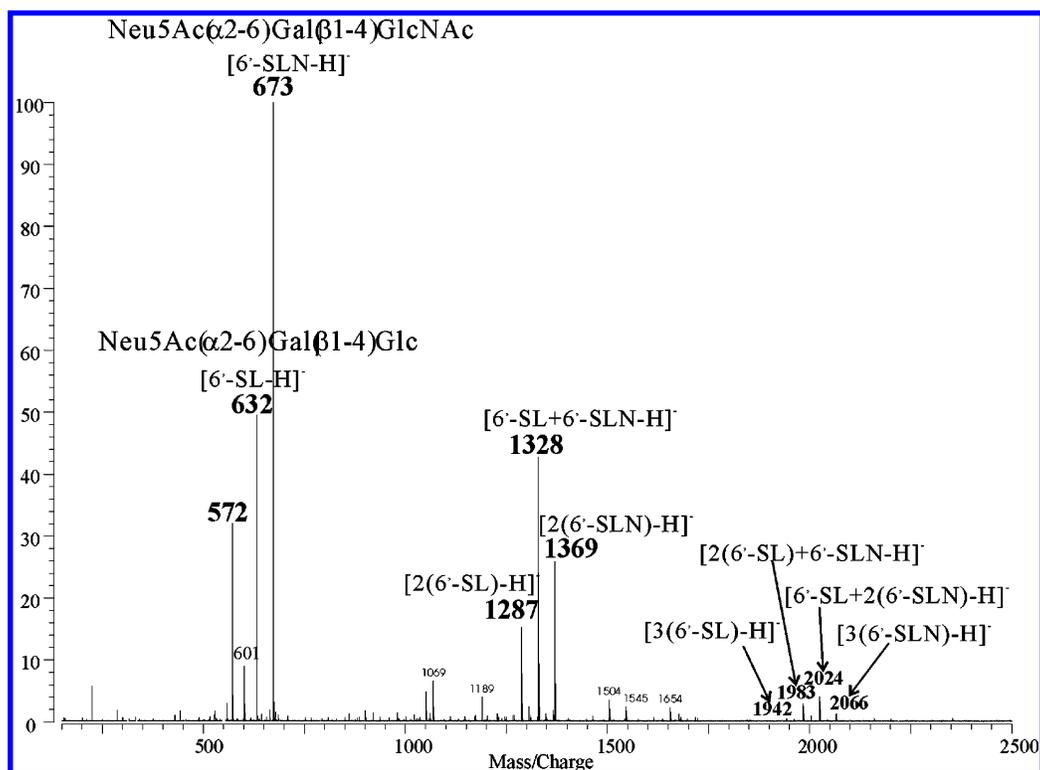
**Figure 6.** Negative mode AP MALDI-FT ICR MS analysis of A1 *N*-glycan. The structure is inset.
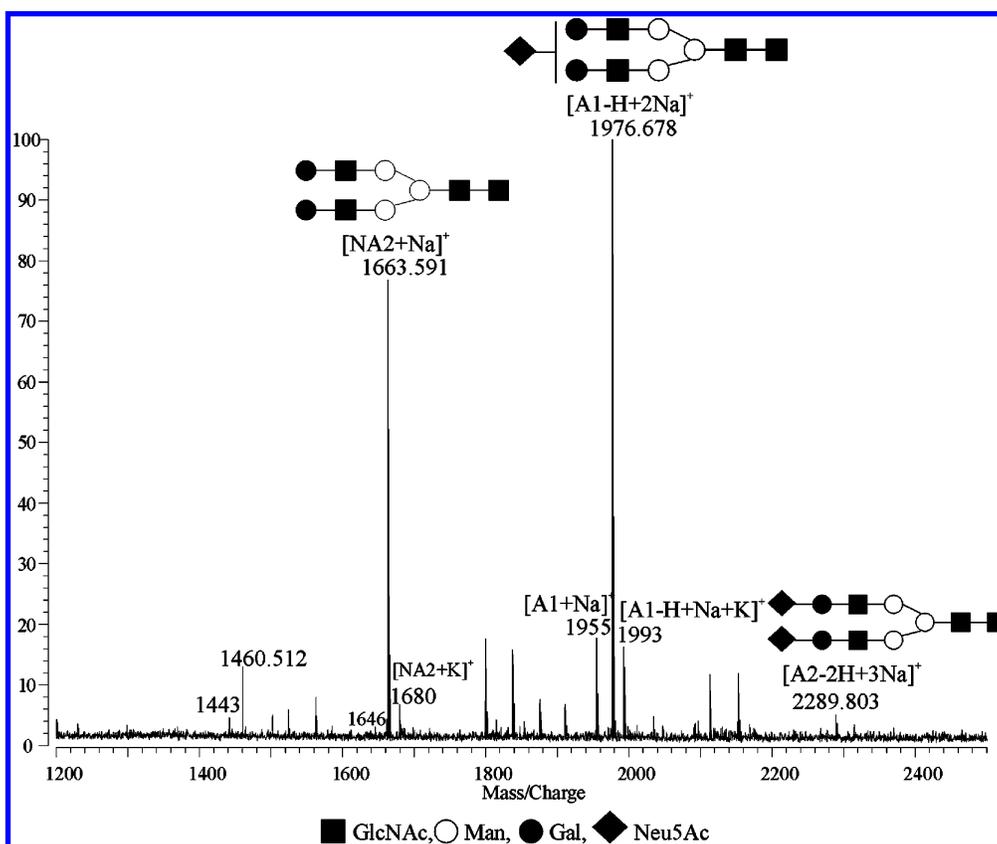
Figure 7. Positive mode AP MALDI-FT ICR MS analysis of an N-linked glycan mixture released from human fibrinogen. The corresponding structures are inset.

sugar complexes.[34,35] In this study, we examined whether the sugar−sugar complexes formed by AP IR-MALDI are similarly produced by a UV laser with two sialylated oligosaccharides: 6′-SL and 6′-SLN. Figure 8 illustrates the negative mode analysis of a mixture of 6′-SL and 6′-SLN. We find that similar complexes are in fact obtained with the UV laser (337 nm). Sugar−sugar

complexes consisting of two homodimers and one heterodimer are all formed in AP UV-MALDI-FT ICR MS. Remarkably, even trimers are formed. IR lasers are believed to produce cooler ions than UV lasers;[44] however, it appears that the collisional cooling in AP MALDI compensates for the differences in internal energy imparted during ionization.

Figure 8. Negative mode AP MALDI-FT ICR MS of 6′-SL and 6′-SLN mixture.
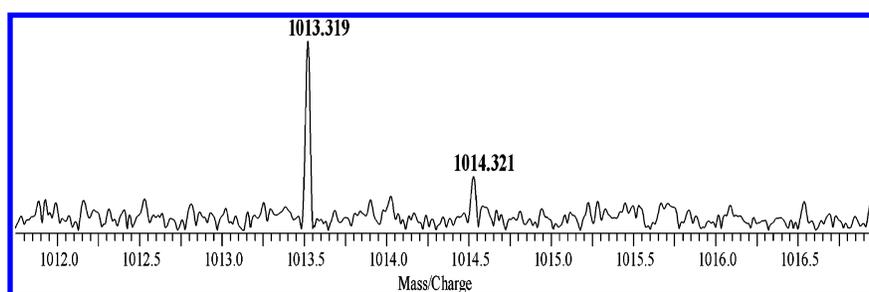
Figure 9. AP MALDI-FT ICR MS analysis of maltohexose (20 fmol deposited on the probe) with sodium dopant.

Detection Sensitivity of Oligosaccharides by AP MALDI-FT ICR MS. Attomole detection limits have been reported for AP MALDI-ion trap MS of peptides using electrospray sample deposition.[45] For oligosaccharides, the analysis of branched and labile sialylated oligosaccharides has been previously conducted with nanomole analyte quantities by both AP UV-MALDI[31] and AP IR-MALDI.[27,33,34] Recently, the subpicomole MS analysis of N-linked glycans by atmospheric pressure infrared laser ionization from solution was performed on an ion trap mass spectrometer.[32]

To determine the detection limit of AP MALDI-FT ICR MS, a standard hexasaccharide, maltohexose, was used. Figure 9 shows the AP MALDI-FT ICR mass spectrum resulting from analysis of 20 fmol of maltohexose applied to the probe. The sodiated species was the only quasimolecular ion obtained. The expanded molecular ion peak clearly shows the isotope pattern with a signal-to-noise ratio of >10. Even at the threshold of detection, 2 ppm mass accuracy was obtained with external calibration, and broadband resolution of 30 800 ($m/\Delta m_{fwhm}$) was attained.

CONCLUSION

Atmospheric pressure MALDI-FT ICR MS has been performed on a number of oligosaccharides as well as O-linked and N-linked glycan mixtures derived from biological samples. The oligosaccharides chosen for this study are particularly labile, containing fucoses, sialic acids, and sulfates. These oligosaccharides have posed significant challenges to conventional MALDI mass spectrometry due to their extensive fragmentation. The comparison between AP MALDI and conventional MALDI reveals that AP MALDI results in minimized fragmentation for both positive and negative mode mass analysis of labile oligosaccharides. The difficulties in differentiating the molecular ions from the fragment ions often encountered in a vacuum MALDI-FT ICR MS are eliminated. In addition, no evidence of matrix cluster formation was observed.

Due to the structural heterogeneity of glycans and glycoconjugates in biological systems, samples purified for MS analysis frequently represent complex mixtures, with components varying

(44) Berkenkamp, S.; Menzel, C.; Karas, M.; Hillenkamp, F. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1399−1406.
(45) Wei, H.; Nolkrantz, K.; Powell, D. H.; Woods, J. H.; Ko, M.-C.; Kennedy, R. T. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1193−1200.

in molecular weight by a few to several hundred mass units. The coupling of AP MALDI with FT ICR MS provides a powerful tool to obtain high mass accuracy and mass resolution analysis on complex carbohydrate analytes. The mass spectra yield readily interpretable molecular ion peaks with few or no fragment ions present. Therefore, AP MALDI-FT ICR MS has been demonstrated to be ideally suited for analysis of highly heterogeneous glycan and glycoconjugate mixtures.