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Negative-Ion Electrospray Mass Spectrometry of Neutral Underivatized Oligosaccharides

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Negative-ion electrospray mass spectrometry (ES-MS) with collision-induced dissociation (CID) and MS/MS scanning on a quadrupole-orthoganal time-of-flight instrument provide a sensitive means for structural analysis of neutral underivatized oligosaccharides. Molecular mass information can be readily obtained from the dominant $[M - H]^-$ ions in the ES mass spectrum formed with subnanomole amounts of oligosaccharides, and similar sensitivity is available from CID-MS/MS to give structural details. The CID spectra of 14 oligosaccharides demonstrated that sequence and partial linkage information can be derived and isomeric structures can be differentiated. Series of C-type fragment ions give sequence information while the double glycosidic D-type cleavage of a 3-linked GlcNAc or Glc and the saccharide ring fragmentation of the ^{0,2}A-type from 4-linked GlcNAc or Glc can provide partial linkage information. The distinctive D- and A-cleavages are important for differentiation of oligosaccharide type 1 and type 2 chains and to define the blood group H, Le^a, Le^x, Le^b, and Le^y determinants carried by their fucosylated analogues.

Mass spectrometry (MS) has been a primary technique in carbohydrate structural analysis for more than three decades with the information available depending strongly on instrumentation and ionization methods. The early application of electrospray (ES) ionization[1] and matrix-assisted laser desorption/ionization (MALDI)[2] in carbohydrate research was mainly for molecular mass determination of oligosaccharides. However, in the past few years, numerous publications have described oligosaccharide sequence, branching pattern, and linkage information deduced from MALDI[3,4] and ES[5−16] mass spectra.

Neutral underivatized oligosaccharides have been frequently analyzed directly by MALDI.[3,4] On the other hand, derivatization (e.g., permethylation[5−8] or reducing-terminal derivatization[9−14]) or metal ion complexation/cationization[15] has been employed for ES-MS. It is generally considered that derivatization is a prerequisite for ES-MS in order to generate useful structural information and to achieve high-sensitivity detection.[16] However, Bahr et al. were able to demonstrate that positive-ion nanospray sensitivity of underivatized oligosaccharides is comparable to the high-sensitivity detection of peptides.[17] We have found that the low chemical background noise[18] and the low level of cation adduct formation[19] in negative-ion mode is advantageous for oligosaccharide analysis, and in this report, we present some preliminary results of negative-ion ES-MS of neutral tetra- to heptasaccharides to assess the sensitivity and structural information that can be derived.

Oligosaccharide sequences of blood group-related antigens, such as those containing fucose and bearing the H, Lewis^a (Le^a) and Lewis^b (Le^b), and Lewis^x (Le^x) and Lewis^y (Le^y) determinants, occur naturally on the carbohydrate chains of glycoproteins and glycolipids and are associated with various biological functions.[20,21] The Le^x antigen appears at specific stages of embryonic development, cellular differentiation, and in oncogenesis,[21] and the important roles of Le^x/Le^a, and related sialylated and sulfated oligosaccharide sequences, as ligands for the selectins have been

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(1) Duffin, K. L.; Welply, J. K.; Huang, E.; Henion, J. D. *Anal. Chem.* **1992**, *64*, 1440−1448.
(2) Mock, K. K.; Davey, M.; Cottrell, J. *Biochem. Bionphys. Res. Commun.* **1991**, *177*, 644−51.
(3) Harvey, D. J.; Küster, B.; Naven, T. J. P. *Glycoconjugate J.* **1998**, *15*, 333−338.
(4) Tseng, K.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3747−3754.
(5) Reinhold, V. N.; Reinhold, B. B.; Costello, C. E. *Anal. Chem.* **1995**, *67*, 1772−1784.
(6) Weiskopf, A. S.; Vouros, P.; Harvey, D. J. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1493−504.
(7) Viseux, N.; de Hoffmann, E.; Domon, B. *Anal. Chem.* **1997**, *69*, 3139−3198.
(8) Weiskopf, A. S.; Vouros, P.; Harvey, D. J. *Anal. Chem.* **1998**, *70*, 4441−4447.
(9) Yoshino, K.; Takao, T.; Murata, H.; Shimonshi, Y. *Anal. Chem.* **1995**, *67*, 4028−4031.
(10) Ahn, Y. H.; Yoo, J. S. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 2011−2015.
(11) Li, D. T.; Her, G. R. *J. Mass Spectrom.* **1998**, *33*, 644−652.
(12) Charlwood, J.; Langridge, J.; Tolson, D.; Birrell, H.; Camilleri, P. *Rapid Commun. Mass* Spectrom. **1999**, *13*, 107−112.
(13) Saba, J. A.; Shen, X.; Jamieson, J. C.; Perreault, H. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 704−711.
(14) Shen, X.; Perreault, H. *J. Mass Spectrom.* **1999**, *34*, 502−510.
(15) Konig, S.; Leary, J. L. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 1125−1134.
(16) Viseux, N.; de Hoffmann, E.; Domon, B. *Anal. Chem.* **1998**, *70*, 4951−4959.
(17) Bahr, U.; Pfenninger, A.; Karas, M.; Stahl, B. *Anal. Chem.* **1997**, *69*, 4530−4535.
(18) Lawson, A. M.; Chai, W.; Cashmore, G. C.; Stoll, M. S.; Hounsell, E. F.; Feizi, T. *Carbohydr. Res.* **1990**, *200*, 47−57.
(19) Chai, W.; Luo, J.; Lim, C. K.; Lawson, A. M. *Anal. Chem.* **1998**, *70*, 2060−2066.
(20) Feizi, T. *Immunol. Rev.* **2000**, *173*, 79−88.
(21) Feizi, T. *Nature* **1985**, *314*, 53−57.

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recognized.[22−28] Assignment of the specificities of carbohydrate-recognizing receptors for their oligosaccharide ligands is a challenging topic in modern cell biology, and rapid and sensitive methods are required to determine oligosaccharide sequence in the biological context in order to derive structure/function relationships. In the present study, variously fucosylated oligosaccharides based on the tetrasaccharide backbones, Galβ1−3/4GlcNAcβ1−3Galβ1−4Glc, with either the type 1 (Galβ1−3GlcNAc−) or type 2 (Galβ1−4GlcNAc−) chains have been used to assess the feasibility of distinguishing blood group antigenic determinants by negative-ion ES-MS.

EXPERIMENTAL SECTION

Materials. Oligosaccharides LNT, LNnT, pLNH, pLNnH, LNFP I/II/III/V, and LNDFH I/II were purchased from Dextra Laboratories (Reading, U.K.). LNnDFH I/II, LNTFH I, and LNnTFH I were isolated from human urine and purified by chromatographic procedures, including gel filtration and normal and reversed-phase HPLC, and their sequences determined by ^{1}H NMR. Detailed isolation and characterization of LNnDFH I and LNTFH I will be published elsewhere. Ion-exchange resins AG50W-X8 and AG1-X8 were from Bio-Rad (Hemel Hempstead, U.K.).

Mass Spectrometry. Negative-ion ES-MS and collision-induced dissociation (CID) MS/MS was carried out on a Micromass (Manchester, U.K.) quadrupole-orthoganal time-of-flight Q-TOF mass spectrometer. Cone voltage was varied between 30 and 60 eV for different oligosaccharides while capillary voltage was maintained at 3 kV for conventional-scale electrospray and 700 V for nanospray using a capillary needle. Higher cone voltage, normally 10−30 V higher than the optimized condition for maximum intensity of the deprotonated molecule, was also used to increase ion energy and induce fragmentation. Source temperature was at 80 °C and the desolvation temperature at 150 °C. CID-MS/MS spectra were obtained using argon as the collision gas at a pressure of 1.7 bar. The collision energy between 13 and 28 eV was adjusted for optimal fragmentation. A scan rate of 1.5 s/scan was used for both ES-MS and MS/MS experiments and the acquired spectra were summed for presentation.

For analysis, oligosaccharides were dissolved in ACN/H_2O 1:1, typically at a concentration of 10 pmol/μL, of which 5 μL was loop-injected. Solvent (ACN/H_2O 1:1) was delivered by a Harvard syringe pump (Harvard Apparatus, Holliston, MA) at a flow rate of 5 μL/min, and for nanospray, ~0.5 μL of the sample solution was placed in the capillary needle. For oligosaccharides larger than pentasaccharides, a mixed-bed minicolumn of AG50W-X8 (H-form, 50 μL of gel, lower bed) and AG1-X8 (OH-form, 50 μL of gel, upper bed) was used to remove any possible cation and anion contamination.

RESULTS AND DISCUSSION

CID-MS/MS spectra were acquired of the $[M - H]^-$ ions of each oligosaccharide after optimizing their intensities by adjustment of cone voltage. The nonfucosylated isomeric tetrasaccharides, LNT and LNnT (Table 1), were analyzed first to determine their general fragmentation, and the main features were compared with those of the hexasaccharides pLNH and pLNnH. Variously fucosylated analogues of LNT and LNnT, which display the blood group antigenic determinants, were then investigated and the fragmentation of isomeric structures was compared. The numbering of monosaccharide residues to indicate the cleavage site[29] is based on the tetrasaccharide backbone. Glycosidic bonds of fucose residues are denoted by the residue number of the tetrasaccharide with an α suffix, e.g., 3α indicating the bond between fucose and the tetrasaccharide backbone at residue 3.

Tetrasaccharides with Type 1 and Type 2 Chains. The structural difference between tetrasaccharides LNT and LNnT is the linkage of the nonreducing terminal Gal to GlcNAc, with a β1−3 linkage in LNT and a β1−4 linkage in LNnT (Table 1). Their CID-MS/MS spectra (Figure 1) clearly identify this linkage difference (see below). In both spectra, the sequence can be derived from a complete set of C-type[29] fragment ions (C_1, m/z 179; C_2, m/z 382; C_3, m/z 544; Figure 1) while only a single B-type ion is present from the nonreducing terminal (B_1 at m/z 161). The A-type ring fragmentation occurs at 4-linked GlcNAc or 4-linked Glc residues. As indicated in Figure 1, both LNT and LNnT give $^{0,2}A_4$ ions at m/z 646 from cleavage of the reducing terminal −4Glc. More usefully, an $^{0,2}A_2$ ion (m/z 281, together with its dehydrated ion m/z 263) is produced from the −4GlcNAc in LNnT but not from the −3GlcNAc in the spectrum of LNT. However, this 3-linked GlcNAc residue in LNT gives a unique ion at m/z 202. This is assigned as a C_2−Z_2 double cleavage, designated as D_{1-2}, due to favorable fragmentation at the reducing side of the glycosidic oxygen. Thus, the −3GlcNAc type 1 linkage in LNT and a −4GlcNAc type 2 linkage in LNnT can be readily differentiated by the $^{0,2}A_2$ ion (m/z 281) and D_{1-2} ion (m/z 202), respectively.

Hexasaccharides with Type 1 and Type 2 Chains. Hexasaccharides pLNH and pLNnH (Table 1) comprise the LNT and LNnT sequences, respectively, extended by an internal lactosamine disaccharide. The CID-MS/MS spectra (Figure 2) of both pLNH and pLNnH are dominated by an intense ion at m/z 646 arising from $^{0,2}A_4$ cleavage of the additional internal −4GlcNAc residue. Similar to CID fragmentation of LNT/LNnT, the complete sets of C-type ions are observed and can be used to derive the sequences. The unique cleavages $^{0,2}A_2$ and D_{1-2} to give the ion doublet m/z 281/263 and an ion at m/z 202 identify the nonreducing terminal type 1 and type 2 chains in pLNH and pLNnH, respectively. The m/z 202 and 281/263 ions are of low intensity when compared with those from LNT and LNnT, but can be produced also by CID-MS/MS of their m/z 646 fragment ions generated by high-voltage-induced fragmentation (data not shown). As shown in the Figure 2, all the $^{0,2}A$ ions, derived from cleavage at either −4GlcNAc or −4Glc residues, are accompanied by ions from dehydration, e.g., $^{0,2}A_2$, 281/263; $^{0,2}A_4$, 646/628; $^{0,2}A_6$, 1011/993).

Monofucosylated Tetrasaccharide Backbone with Blood Group H, Le^a, and Le^x Determinants. Isomeric monofucosy-

(22) Larkin, M.; Ahern, T. J.; Stoll, M. S.; Shaffer, M.; Sako, D.; O'Brien, J.; Yuen, C.-T.; Lawson, A. M.; Childs, R. A.; Barone, K. M.; Longer-Safer, P. R.; Hasegawa, A.; Kiso, M.; Larsen, G. R.; Feizi, T. *J. Biol. Chem.* **1992**, *267*, 13661−13668.
(23) Bevilacqua, M. P.; Nelson, R. M. *J. Clin. Invest.* **1993**, *91*, 379−387.
(24) Feizi, T. *Curr. Opin. Struct. Biol.* **1993**, *3*, 701−710.
(25) Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679−691.
(26) Rosen, S. D.; Bertozzi, C. R. *Curr. Biol.* **1996**, *6*, 261−264.
(27) Feizi, T.; Galustian, C. *Trans. Biochem. Soc.* **1999**, *24*, 369−371.
(28) Handa, K.; Withers, D. A.; Hakomori, S. *Biochem. Bionphys. Res. Commun.* **1998**, *243*, 199−204.
(29) Domon, B.; Costello, C. E. *Glycoconjugate J.* **1988**, *5*, 397−409.

Table 1. Structures of Oligosaccharides Used for Negative-Ion ES-MS

Designation	Sequence	Chain type	Blood group determinant
non-fucosylated			
LNT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	1	-
LNnT	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	2	-
pLNH	Galβ1-3GlcNAcβ1-3Galβ1−4GlcNAcβ1-3Galβ1-4Glc	1, 2	-
pLNnH	Galβ1-4GlcNAcβ1-3Galβ1−4GlcNAcβ1-3Galβ1-4Glc	2	-
mono-fucosylated			
LNFP I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | Fucα1,2	1	H
LNFP II	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | Fucα1,4	1	Le^a
LNFP III	Galβ1-4GlcNAcβ1-3Galβ1-4Glc | Fucα1,3	2	Le^x
LNFP V	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | Fucα1,3	1	-
di-fucosylated			
LNDFH I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | | Fucα1,2 Fucα1,4	1	Le^b
LNnDFH I	Galβ1-4GlcNAcβ1-3Galβ1-4Glc | | Fucα1,2 Fucα1,3	2	Le^y
LNDFH II	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | | Fucα1,4 Fucα1,3	1	Le^a
LNnDFH II	Galβ1-4GlcNAcβ1-3Galβ1-4Glc | | Fucα1,3 Fucα1,3	2	Le^x
tri-fucosylated			
LNTFH I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc | | | Fucα1,2 Fucα1,4 Fucα1,2	1	Le^b, H
LNnTFH I	Galβ1-4GlcNAcβ1-3Galβ1-4Glc | | | Fucα1,2 Fucα1,3 Fucα1,2	2	Le^y, H

lated pentasaccharides LNFP I, II, and III, each give a unique CID fragment ion spectrum (Figure 3a−c, respectively) that permits identification of their respective blood group H, Le^a, and Le^x epitopes. All three pentasaccharides contain a 3-linked GlcNAc (Table 1) that readily undergoes double cleavage to produce D-type fragments. LNFP I has an unbranched −3GlcNAc, and hence, m/z 202 is observed as in the spectra of LNT and pLNH. In the spectrum of LNFP II (Figure 3b), the major fragment at m/z 348 results from D_{1-2} double cleavage of the 3-linked GlcNAc, indicating a Fuc residue linked at the 4-position of the −3GlcNAc (202 + 146). Similarly, the $D_{2-2\alpha}$ at m/z 364 in LNFP III (Figure 3c) indicates a Gal at the 4-position (202 + 162). Thus, LNFP I, II, and III are readily differentiated by the distinctive ions at m/z 202, 348, and 364, respectively.

The linear sequence of LNFP I can be deduced from the complete set of C-type ions (C_1, m/z 325; C_2, m/z 528; C_3, m/z 690) with the C_1 indicating Fuc linked to the terminal Gal. The C_1 ion at m/z 179 is not observed in LNFP II with the Le^a structure but occurs in LNFP III with the Le^x epitope. This indicates the Gal1−4GlcNAc glycosidic bond can readily cleave while a Gal1−3GlcNAc linkage is more resistant. However, when the Gal is substituted by a Fuc as in the case of LNFP I, this Gal1−3GlcNAc

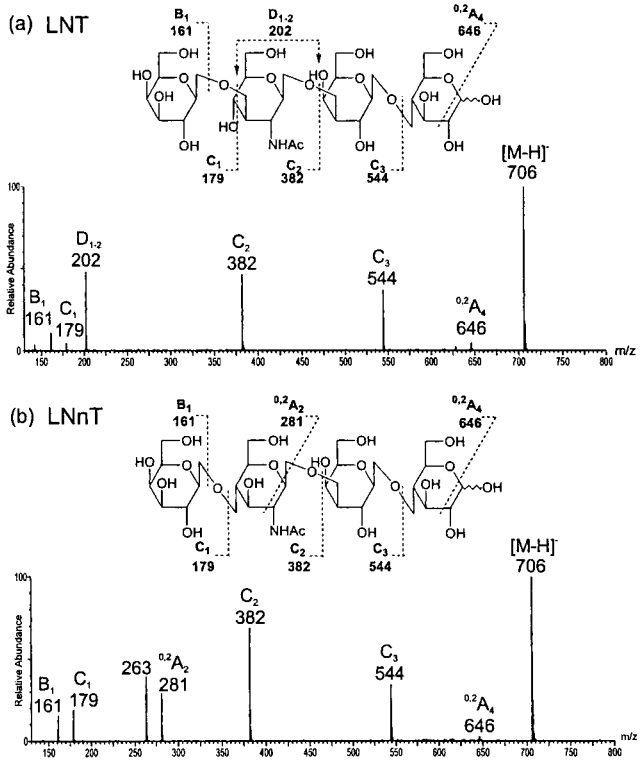

Figure 1. Electrospray CID-MS/MS spectra of LNT (a) and LNnT (b) with their structures shown to indicate the fragmentation.

linkage can be cleaved. Thus, the C_1 ions provide additional information about the terminal Gal, such that an ion at m/z 325 indicates a Fuc linked to Gal, and at m/z 179 a Gal1−4 linked to GlcNAc, while the absence of C_1 suggests a Gal1−3. Similar interpretation is also applicable to multiply fucosylated Le^a- and Le^x-containing oligosaccharides discussed later.

LNFP V does not carry a blood group antigen but contains a fucose at the reducing terminal. As expected, a complete set of C-ions (m/z 179, 382, and 544, Figure 3d) is observed and the double cleavage D_{1-2} ion at m/z 202 indicates a nonsubstituted and 3-linked GlcNAc. The Fuc at the reducing terminal Glc can be inferred by the mass difference of 308 (146 + 162, Fuc + Glc) between the ion C_3 and $[M - H]^-$. Interestingly, the Fuc linkage at the 3-position of the terminal −4Glc is also labile and can undergo fragmentation consistent with a double cleavage of D-type similar to a 3-linked GlcNAc. The resulting $D_{4-4\alpha}$ fragment ion at m/z 688 serves to define the fucose 3-linked to the Glc. As GlcNAc and Glc have the same stereoconfiguration, the favorable D-type fragmentation occurs in both 3-linked GlcNAc and Glc but not in a 3-linked Gal. For the same reason, ^{0,2}A-type cleavage can be observed in both 4-linked GlcNAc and Glc as discussed above.

Difucosylated Tetrasaccharide Backbone with Blood Group Le^a, Le^x, Le^b, and Le^y Determinants. Difucosylated LNDFH II and LNnDFH II (Table 1), carrying the Le^a and Le^x determinants, respectively, have the second fucose at the reducing terminal −4Glc. Their sequences can be defined by CID-MS/MS spectra. The C_2 fragment ion at m/z 528 in both spectra (Figure 4) identifies a nonreducing terminal trisaccharide of a composition of Fuc.Gal.GlcNAc. The ion at m/z 348 in LNDFH II (Figure 4a) can be deduced as a D_{1-2} fragment that indicates the −3GlcNAc with a 4-linked Fuc of the Le^a epitope (cf. Figure 3b). In the same way for LNnDFH II, the ion of m/z 364 can be accounted for by

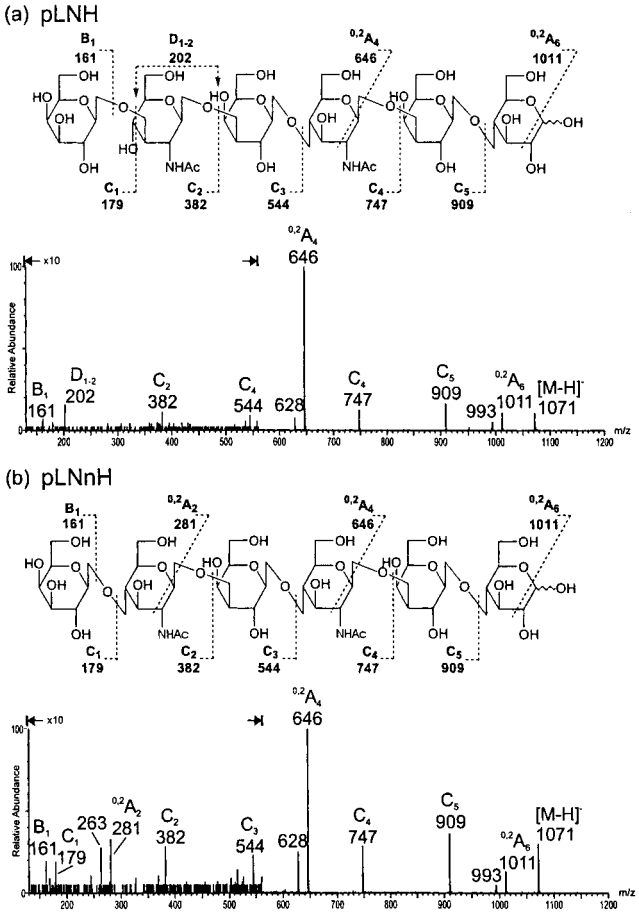

Figure 2. Electrospray CID-MS/MS spectra of pLNH (a) and pLNnH (b) with their structures shown to indicate the fragmentation.

a $D_{2-2\alpha}$ fragment ion indicating the −3GlcNAc bearing a Gal at the 4-position of the Le^x epitope (cf. Figure 3c). The C_1 ion at m/z 179 is present in the spectrum of LNnDFH II, but not in that of LNDFH II, and a similar situation can be found in the spectra of Le^x/Le^a-containing pentasaccharides LNFP III and II (Figure 3c and b). Hence, the presence or absence of a C_1 at m/z 179 ion serves to corroborate the evidence of D-type fragmentation for differentiating the respective Le^x and Le^a sequences. The second fucose at the 3-position of the reducing terminal Glc in both LNDFH II and LNnDFH II is deduced from the mass difference of 308 (Fuc + Glc) between C_3 (m/z 690) and $[M - H]^-$ (m/z 998) and the presence of $D_{4-4\alpha}$ at m/z 834 (Figure 4) as in the case of LNFP V (Figure 3d).

LNDFH I and LNnDFH I have type 1 and 2 chains, respectively, with Fuc on each of Gal and GlcNAc at the nonreducing terminal (Table 1) and hence exhibit the respective the Le^b or Le^y determinants. The fucoses on the terminal Gal and GlcNAc of LNDFH I are reflected by the C_1 (m/z 325) and C_2 (m/z 674) ions (Figure 5a). The ion at m/z 348 from D_{1-2} fragmentation further indicates an internal −3GlcNAc substituted at the 4-position by Fuc. Taken together, the C_1, C_2, and D_{1-2} ions define the Le^b epitope. In the spectrum of LNnDFH I (Figure 5b), the ion at m/z 510 (202 + 162 + 146) is deduced to be the double cleavage $D_{2-2\alpha}$ ion that indicates the internal −3GlcNAc substituted at the 4-position by Fuc-Gal. A complete set of C-type ions is present in the spectrum of LNDFH I (Figure 5a), but the C_2 fragment is missing from the series in the spectrum of LNnDFH I (Figure

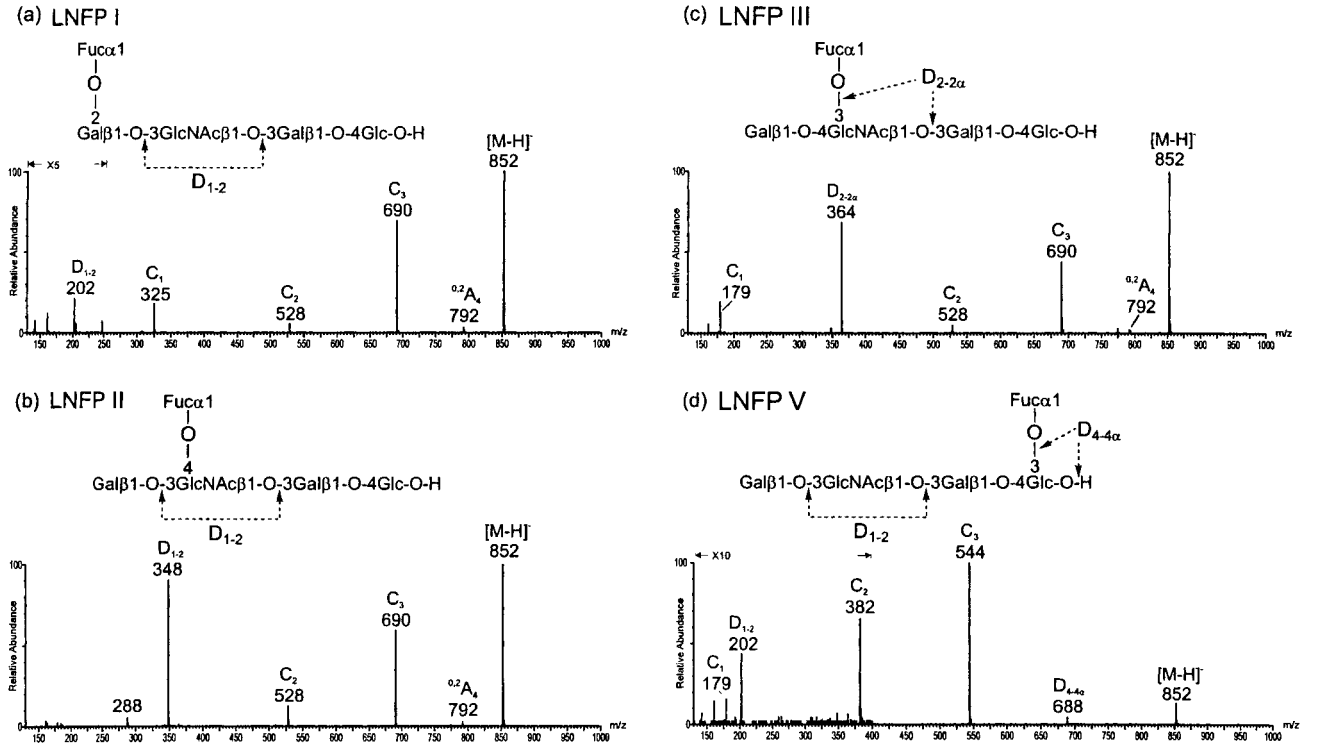

Figure 3. Electrospray CID-MS/MS spectra of LNFP I (a), LNFP II (b), LNFP III (c), and LNFP V (d) with their oligosaccharide sequence shown to indicate the D-type cleavage, (For A- and C-type cleavages, refer to Figures 1 and 2.).

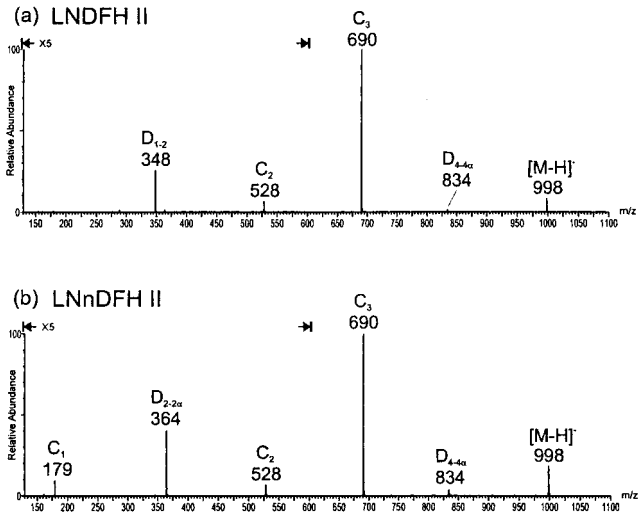

Figure 4. Electrospray CID-MS/MS spectra of LNDFH II (a) and LNnDFH II (b).

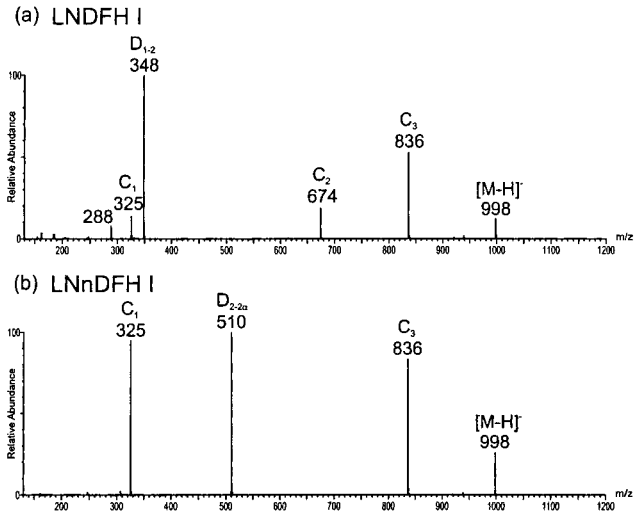

Figure 5. Electrospray CID-MS/MS spectra of LNDFH I (a) and LNnDFH I (b).

5b) for which the reason is unclear. As deduced above, either 1−4 or 1−3 glycosidic bonds between terminal Gal and the GlcNAc can be cleaved when the Gal is substituted by a Fuc. As fragmentation of the 1−4 linkage is more facile, the C_1 fragment at m/z 325 in the CID spectrum of LNnDFH I (Figure 5a) is much more intense than in that of LNDFH I (Figure 5b).

Trifucosylated Tetrasaccharide Backbone with Blood Group Le^b and Le^y Determinants. Trifucosylated LNTFH I and LNnTFH I are sequences containing the Le^b and Le^y epitopes, respectively, with a further Fuc at the internal Gal with a α1−2 linkage exhibiting the blood group H determinant (Table 1). The Le^b and Le^y determinants can be differentiated from the lower mass region of their spectra (Figure 6) from the D_{1-2} ion at m/z 348 in LNTFH I and $D_{2-2\alpha}$ at m/z 510 in LNnTFH I (cf. spectra of LNDFH I and LNnDFH I, Figure 5). The complete C-type ion series in the CID spectrum of LNTFH I (Figure 6a) clearly indicates the location of each fucose. In the spectrum of LNnTFH I, the C_2 ion is absent as already pointed out for the Le^y-containing LNnDFH I. However, the location of the three fucose residues can still be determined. The C_3 ion at m/z 982 indicates three Fuc residues on the nonreducing terminal trisaccharide sequence, with C_1 at m/z 325 defining one Fuc on the terminal Gal, and the $D_{2-2\alpha}$ ion indicating the second Fuc at 3-position of GlcNAc, leaving the remaining Fuc to be inferred as on the internal Gal.

Cone Voltage Fragmentation versus CID. Isomeric series of fucosylated oligosaccharides can be differentiated from the

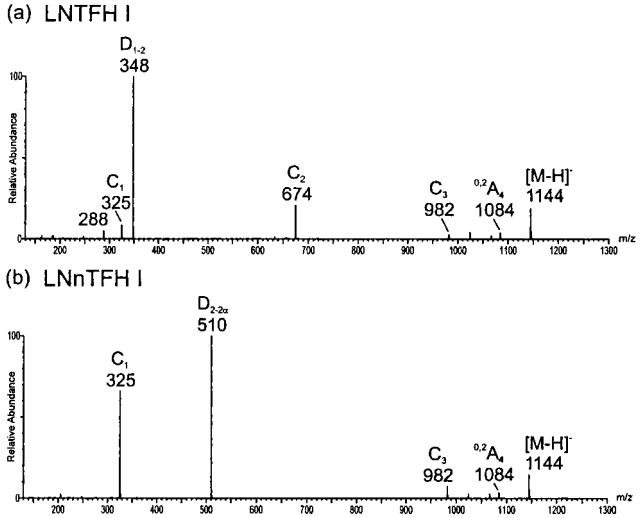

Figure 6. Electrospray CID-MS/MS spectra of LNTFH I (a) and LNnTFH I (b).

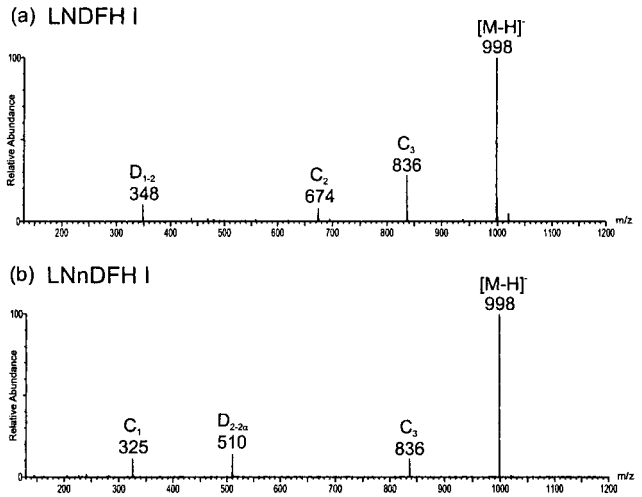

Figure 7. Electrospray mass spectra of LNDFH I (a) and LNnDFH I (b).

fragment ions generated either by collision-induced dissociation or by optimized cone voltage fragmentation (CVF), which give similar spectra. As an example, CVF spectra of the difucosylated LNDFH I and LNnDFH I are shown in Figure 7 that can be compared with their CID-MS/MS spectra (Figure 5). Generally 10−20 V above their optimized cone voltage is sufficient to obtain useful fragmentation. However, nonfucosylated tetra- and hexasaccharides, LNT/LNnT and pLNH/pLNnH, fragment more readily by CID (data not shown). CID-MS/MS of fragment ions generated from CVF in electrospray on the Q-TOF instrument can provide a sensitive "quasi" MS^n experiment as was employed in the case of pLNH and pLNnH (MS^3: $[M - H]^- \rightarrow m/z$ 646 → 202 or 281) discussed above. Quasi MS^3 can be valuable to obtain further structural information particularly when a limited number of diagnostic fragments are observed or a primary fragment ion is of high intensity, such as the fragment ion m/z 646 in pLNH and pLNnH.

Effect of Anion Contaminant. Neutral oligosaccharides up to pentasaccharide generally give rise to intense singly charged deprotonated ions. Oligosaccharides larger than pentasaccharides frequently require being fully desalted as otherwise the doubly

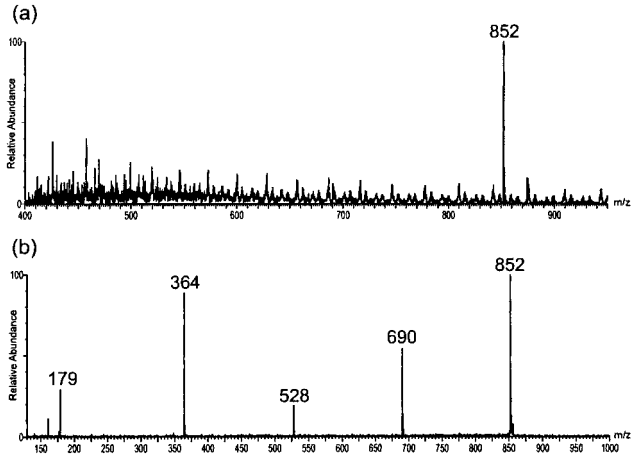

Figure 8. Electrospray mass spectrum (a) and CID-MS/MS spectrum (b) of LNFP III obtained with 0.5 pmol of sample (0.5 μL of 1 pmol/μL solution) under nanospray conditions.

charged anion adduct $[M + 96]^{2-}$ dominates the spectrum (data not shown) when low cone voltage (e.g., 30 V) is used and poses a problem for CID. The mass increment of 96 Da implies an attachment of either SO_4^{2-} or HPO_4^{2-}, but doping experiments with both acids indicated that this is $[M + SO_4]^{2-}$. Similar sulfate adduction preferably with larger oligosaccharides has been also observed in MALDI mass spectrometry.[30] The source of SO_4^{2-} is not known but trace amounts can be responsible for the adduction due to the extremely high sensitivity of detection of SO_4^{2-} in negative-ion ES-MS. Use of a minicolumn of mixed-bed anion−cation exchanger was successful in removing sufficient sulfate contaminant to avoid adduction; however, this could not be achieved by other methods including reversed-phase HPLC (e.g., on a porous graphitized carbon column).

Sensitivity. Good-quality spectra can be obtained from low-picomole amounts of oligosaccharides using flow injection on normal-scale electrospray, and only ~100-fmol amounts are consumed by nanospray. In both cases, normal scan and CID-MS/MS spectra are recorded with similar sensitivity with the Q-TOF configuration as demonstrated in Figure 8. This shows the nanospray MS and MS/MS spectra of LNFP III obtained with 0.5 pmol (0.5 μL of 1 pmol/μL of sample solution). The actural consumption of material is much less, and unused sample solution can be readily recovered from the capillary needle with a microsynringe. A good signal-to-noise ratio (greater than 6:1) was observed on conventional-scale ES-MS with 5 pmol (1 pmol/μL and 5-μL injection) of LNFP III and LNnH (data not shown).

CONCLUSIONS

Negative-ion electrospray mass spectrometry with CID and MS/MS scanning on a Q-TOF instrument provides a sensitive means for structural analysis of neutral underivatized oligosaccharides. Molecular mass information can be readily obtained from the dominant $[M - H]^-$ in the ES spectrum with subnanomole amounts of material, and similar sensitivity with CID-MS/MS provides structural details.

Fragmentations obtained from 14 oligosaccharides in the present study can be summarized as follows: (1) All fragment

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

ions produced from single cleavage are from the nonreducing terminal. (2) Oligosaccharide sequence information can be deduced unambiguously from a complete set of C-type fragments. (3) Partial linkage information can be deduced from D- and ^{0,2}A-type fragmentations. A 3-linked GlcNAc or Glc produces a unique D-type ion by double C−Z cleavages. If the 3-linked GlcNAc is not substituted, a D-ion is produced at m/z 202. If the 3-linked GlcNAc is substituted by a 4-linked Fuc, the D-ion shifts to m/z 348 (202 + 146). Similarly, if there is a Gal at the 4-position of −3GlcNAc, the D-ion is at m/z 364 (202 + 162). A mono-4-substituted GlcNAc or Glc gives ^{0,2}A-type cleavage. Further substitution at the 3-position of GlcNAc or Glc prevents this fragmentation.

Thus, the distinctive D- and ^{0,2}A-type fragmentations are important for differentiating oligosaccharide type 1 and type 2 chains and to define the blood group H, Le^a/Le^x and Le^b/Le^y determinants: fragment ions at m/z 348/528 define a Le^a antigen while m/z 364/528 indicate a Le^x; a Le^b shows fragment ions at m/z 348 and 674, and a Le^y give an unique fragment ion at m/z 510.

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