Californium-252 Plasma Desorption Mass Spectrometry of Oligosaccharides and Glycoconjugates: Control of Ionization and Fragmentation

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The fragmentation of underivatized and peracetylated maltooligosaccharides, ranging in length from four to seven glucose residues, has been investigated by ^{252}Cf plasma desorption mass spectrometry (PDMS). Investigations are made of the effects on the mass spectra of (1) peracetylation, (2) sample preparation by electrospray deposition onto metallic substrates versus adsorption onto nitrocellulose films, and (3) sodium addition to or elimination from the sample. Peracetylation enhances the mass spectrometric response of the oligosaccharides and also enables the efficient removal from the sample of water-soluble components such as sodium salts. A rapid and simple method has been developed for controlling the amount and type of fragmentation of peracetylated maltooligosaccharides. The method involves control of the amount of sodium in the sample introduced into the mass spectrometer. When a large molar excess of sodium is added to the sample, in the form of sodium chloride, the positive-ion mass spectrum is dominated by a peak corresponding to the sodium-cationized molecule. On the other hand, when the sample is completely depleted of sodium, the spectrum shows no quasimolecular ions [$(M + Na)^+$ or $(M + H)^+$] whatsoever and is instead totally dominated by fragment ion species. Thus, it proves feasible to alternate between a spectrum dominated by the $(M + Na)^+$ ion peak and one dominated by fragment ions. This method has also been found to be useful in controlling the fragmentation of permethylated gangliosides.

INTRODUCTION

Some of the more recently developed mass spectrometric ionization techniques are proving to be of considerable utility for the analysis of complex oligosaccharides and glycoconjugates (*1*). One powerful class of techniques involves desorption and ionization of condensed-phase samples by bombardment with energetic atoms or ions. The desorption and ionization is effected either by kiloelectronvolt energy particles as in liquid-matrix secondary ionization mass spectrometry (LSIMS) (*2*) or by megaelectronvolt energy particles as in ^{252}Cf plasma desorption mass spectrometry (PDMS) (*3*).

LSIMS has been extensively applied to the study of both underivatized (*4–9*) and derivatized (*9–23*) carbohydrates. For underivatized oligosaccharides, the technique often yields reliable molecular masses but only limited information concerning primary structure (*4–9*). Derivatization of the oligosaccharides by permethylation (*10–17*), acetylation (*17–19*), or the attachment of moieties such as trimethyl(*p*-aminophenyl)ammonium chloride (*20*) or ethyl *p*-aminobenzoate (*21–23*) to the reducing termini has been shown to enhance considerably the sensitivity for measuring the mass of the intact derivatized molecule as well as the amount of structural information that can be derived from the LSIMS mass spectra. Informative fragmentation has also been observed in the mass spectrum of mycobacterial 6-*O*-methyl-D-glucose polysaccharide, which is naturally partially methylated (*24–26*), and glycoconjugates such as glycosphingolipids in both derivatized (*9*) and underivatized (*8*) forms.

^{252}Cf PDMS is also a useful method for analyzing carbohydrates and glycoconjugates, although it has been used to a more limited extent than LSIMS. Jardine et al. (*27–29*) have demonstrated that peracetylated glycoconjugates and oligosaccharides adsorbed on a thin layer of nitrocellulose (NC) produce plasma desorption mass spectra consisting of relatively intense quasimolecular ion peaks and structurally informative fragment ion peaks. Peracetylation was found to increase the mass spectrometric sensitivity over that obtained for the underivatized materials. Kamensky and Craig (*30*) compared the plasma desorption mass spectrum of a sample of peracetylated maltoheptaose prepared by electrospray deposition with that obtained from the sample adsorbed to a film of NC. They observed an enhanced yield of the $(M + Na)^+$ ion for the sample deposited on NC over that from the electrosprayed layer. A comparison was also made between the spectra obtained from a sample of peracetylated maltoheptaose sorbed on NC prior to and after rinsing with 0.1% trifluoroacetic acid. Although a significant reduction in the $(M + Na)^+$ ion peaks was observed after rinsing, no difference was noted in the absolute abundances and types of fragment ions. Furukawa et al. (*31*) incorporated PDMS to assist in the structural elucidation of gangliosides obtained from cat and sheep erythrocytes. Electrosprayed samples of the permethylated derivatives yielded spectra that were particularly useful for elucidating the sialic acid composition of the gangliosides and the sequence of their disialosyl side chains. Cotter et al. have used PDMS to assess the heterogeneity of an underivatized high-mannose glycopeptide GP 432 from the variant surface glycoprotein of trypanosomes (*32*) and to assist in the elucidation of the structures of the lipid A portion of the lipopolysaccharides from the rough (R_e) mutant of *E. coli* (*33*).

The plasma desorption mass spectrometric studies described above indicate that sample preparation plays a critical role in determining the sensitivity and the quality of the mass spectra obtained. In order to better understand the effects of sample preparation on the spectra, we undertook a systematic study of a series of maltooligosaccharides ranging in length from four to seven glucose residues in which we investigated the effects on the spectra of (1) peracetylation, (2) sample preparation by electrospray deposition onto metallic substrates versus adsorption onto nitrocellulose films, and (3) sodium addition to or elimination from the sample.

In agreement with previous studies using PDMS (*27–30*), we find that peracetylation enhances the sensitivity for the measurement of oligosaccharides and yields mass spectra rich in informative fragmentation. We have also developed a new method for controlling the amount and type of fragmentation observed with peracetylated oligosaccharides. The method involves control of the amount of sodium in the sample introduced into the mass spectrometer. When a large molar excess of sodium is added to the sample, in the form of sodium chloride, the positive-ion mass spectrum is dominated by a peak corresponding to the sodium-cationized molecule. On

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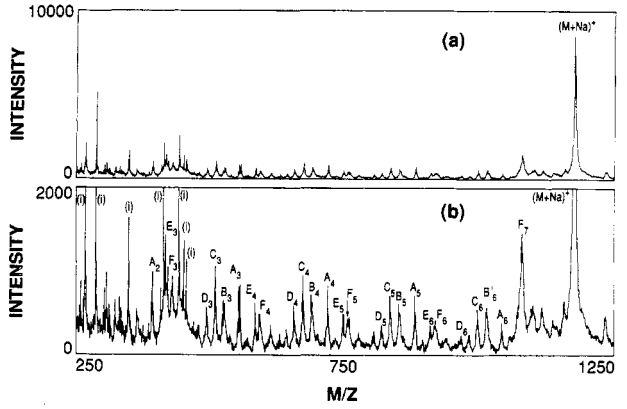

Figure 1. (a) Positive-ion ^{252}Cf plasma desorption mass spectrum of a sample of underivatized maltoheptaose prepared by electrospray deposition. (b) Same spectrum shown expanded vertically by a factor of 5 to accentuate the relatively weak fragment-ion peaks. Peaks labeled "i" result from impurities present in the sample.

the other hand, when the sample is completely depleted of sodium, the spectrum shows no quasimolecular ions [$(M + Na)^+$ or $(M + H)^+$] whatsoever and is instead totally dominated by fragment ion species. Thus, it proves feasible to alternate between a spectrum dominated by the $(M + Na)^+$ ion peak and one dominated by fragment ion peaks. This control over the fragmentation was also achieved with permethylated glycosphingolipids and provides a useful means for regulating the amount of fragmentation in oligosaccharides and glycoconjugates.

EXPERIMENTAL SECTION

Maltooligosaccharides containing from four to seven glucose residues were obtained from Sigma Chemical Company (St. Louis, MO). Peracetylation was performed at room temperature by adding a 2:1 (v:v) mixture of trifluoroacetic anhydride and glacial acetic acid to the underivatized oligosaccharide (*34*). After 20–30 min, the solution was evaporated to dryness and the acetylated sugar was promptly dissolved in trifluoroethanol. Permethylated gangliosides prepared as described previously (*31*) were provided by Drs. Kenneth O. Lloyd and Koichi Furukawa of the Sloan Kettering Institute, New York.

Samples were prepared for insertion into the mass spectrometer by two different methods. The first involved electrospray deposition (*35*) of the oligosaccharides dissolved in trifluoroethanol (0.2 nmol/μL) onto aluminized Mylar foils. The second method involved adsorption of the sugar onto a nitrocellulose film with a thickness of 1 μm (*36*). The majority of the results given here were obtained with sample amounts in the range 1–5 nmol.

The mass spectra were obtained by using the ^{252}Cf fission fragment ionization time-of-flight mass spectrometer constructed at The Rockefeller University and described previously (*37*, *38*). The flux of fission fragments through the 1-cm^2-area sample foil was 3×10^3/s, and the spectra were acquired for periods of 30–60 min. The accuracy of the mass determinations was generally better than 200 ppm.

RESULTS AND DISCUSSION

Underivatized Oligosaccharides. The four underivatized maltooligosaccharides, when prepared by electrospray deposition on metallized Mylar foils, each produced a mass spectrum consisting of a dominant $(M + Na)^+$ ion peak and an extensive series of relatively weak fragment ion peaks. The mass spectrum from underivatized maltoheptaose is shown in Figure 1a. The quasimolecular $(M + Na)^+$ ion (calculated m/z = 1176.0) is observed at m/z = 1176.1. The fragmentation involves a large number of reaction channels. The most intense fragment-ion peaks are identified and marked in Figure 1b (a vertically expanded version of Figure 1a). The assigned identities and measured and calculated masses of these fragment ions are provided in Table I. Six series (series A, B, B′, C, D, and E) of sodium-containing fragment ions and one series (series F) of sodium-free fragment ions are observed. The A series appears to result from ring cleavage with retention of HCO on the glycosidic oxygen of the charged, reducing-end fragment (i.e., the fragment ion containing the reducing end of the oligosaccharide), as previously suggested by Dell (*9*). The B series results from cleavage at the glycosidic oxygen with retention of this oxygen on the charged fragment and hydrogen transfer to the neutral fragment. A related series B′, whose members have masses 2 u higher than those of the B series, arises from hydrogen transfer to the charged fragment. The D series appears to result from a cleavage analogous to that observed in the B series with the additional elimination of the elements of CH_3OH from the charged fragment. Two fragmentation routes appear to be possible for the C series ions. The first involves cleavage at the glycosidic oxygen with retention of this oxygen on the charged fragment, transfer of hydrogen to the charged fragment, and

Table I. Calculated and Measured Masses of the Fragment Ions from Underivatized Maltoheptaose

ion	calcd mass	measd mass
A_1	231.0	*a*
A_2	393.2	393.3
A_3	555.5	555.3
A_4	717.5	717.5
A_5	879.6	879.5
A_6	1041.8	1041.4
B_1	201.0	*a*
B_2	363.2	*a*
B_3	525.3	525.2
B_4	687.5	687.3
B_5	849.6	849.5
B_6	1011.8	1011.7
B'_1	203.0	*a*
B'_2	365.2	*a*
B'_3	527.3	527.3
B'_4	689.5	687.4
B'_5	851.6	851.5
B'_6	1013.8	1013.5
C_1	185.0	*a*
C_2	347.1	*a*
C_3	509.3	509.3
C_4	671.5	671.3
C_5	833.6	833.5
C_6	995.8	995.5
C_7	1158.0	1158.3
D_1	169.0	*a*
D_2	331.1	*a*
D_3	493.1	493.2
D_4	655.4	655.2
D_5	817.6	816.3
D_6	979.7	980.3
E_1	97.0	*a*
E_2	259.1	*a*
E_3	421.2	421.2
E_4	583.4	583.3
E_5	745.5	745.3
E_6	907.7	907.5
F_1	104.9	*a*
F_2	267.0	*a*
F_3	429.1	429.4
F_4	591.4	591.4
F_5	753.6	753.4
F_6	915.7	916.7
F_7	1077.9	1078.2

[a] The mass could not be obtained unambiguously because of the presence of interferring impurity ions and/or insufficient intensity of the fragment ions. $A_i = [M + Na + 29 - \{(7 - i)G + H\}]^+$, $B_i = [M + Na - H - \{(7 - i)G + H\}]^+$, $B'_i = [M + Na + H - \{(7 - i)G + H\}]^+$, $C_i = [M + Na - 17 - \{(7 - i)G + H\}]^+$, $D_i = [M + Na - H - 32 - \{(7 - i)G + H\}]^+$, $E_i = [M + Na - 105 - \{(7 - i)G + H\}]^+$, and $F_i = [M + H - 75 - \{(7 - i)G + H\}]^+$ where M = mass of maltoheptaose and G = mass of a glucose residue.

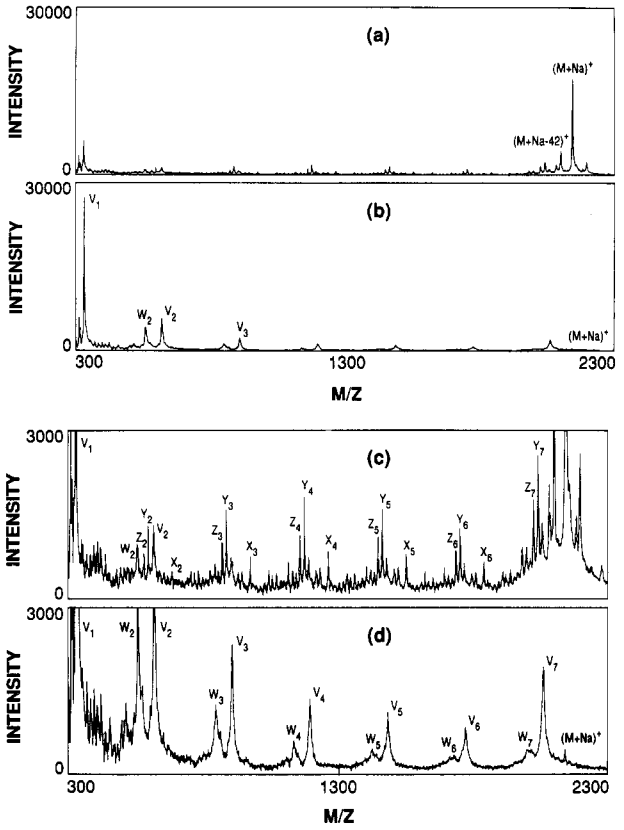

Figure 2. Positive-ion ^{252}Cf plasma desorption mass spectra obtained from 5 nmol of electrospray-deposited, peracetylated maltoheptaose. (a) Prior to water washing. (b) After water washing. Better inspection of weak fragment ions is facilitated by c, which is a vertically expanded version (×10) of panel a, and d, which is a vertically expanded version (×10) of panel b.

elimination of water from the charged fragment. The second involves cleavage at the glycosidic oxygen with retention of this oxygen on and transfer of hydrogen to the neutral fragment. The E and F series ions are formed by cleavage of the sugar rings. In contrast to series A–E, fragment ions of the F series do not contain sodium.

Because the component sugar moieties of the maltooligosaccharides are identical, these mass spectrometric data do not generally give unambiguous information as to whether the charge is retained on the reducing or the nonreducing end fragment. Because of this ambiguity and because many of the fragment ions contain sodium, no attempt was made in the present work to align our nomenclature for the fragment ions with that used previously (*39*).

Peracetylated Oligosaccharides. Peracetylation was found to enhance the $(M + Na)^+$ ion intensities obtained from electrospray-deposited samples of the four oligosaccharides studied by a factor of 2–3 when compared to the underivatized compounds.

The mass spectrum of electrospray-deposited peracetylated maltoheptaose, containing the sodium impurities normally present in our preparations, comprises an intense $(M + Na)^+$ ion peak at $m/z = 2143.1$ (calculated $m/z = 2142.9$) and a large number of relatively weak fragment-ion peaks (Figure 2a). Although the identities and amounts of the adventitious sodium-containing impurities have not been determined, an almost identical spectrum was obtained from peracetylated maltoheptaose that was repeatedly extracted to remove trace amounts of the sodium-containing impurities and to which, subsequently, a 10:1 molar excess of NaCl was added. A relatively intense peak is also observed 42 u below the $(M + Na)^+$ peak. In order to determine whether this peak arises from incomplete acetylation or mass spectrometric loss of an acetyl group, an electrospray ionization mass spectrum was obtained from the sample (*40*). The dominant quasimolecular ion species observed with this technique resulted from the attachment of two sodium cations to the molecule. We observed a similar ratio of $(M + 2Na - 42)^{2+}$ to $(M + 2Na)^{2+}$ in the electrospray ionization mass spectrum to the ratio of $(M + Na - 42)^+$ to $(M + Na)^+$ observed in the PD mass spectrum. Because electrospray ionization is a considerably softer technique than PDMS, we conclude that mass spectrometric loss of an acetyl group from the fully protected molecule does not contribute strongly to the $(M + Na - 42)^+$ ion peak. Rather, this peak is likely the result of incomplete acetylation of maltoheptaose or loss of a labile acetyl group during sample handling (*34*). The observed fragmentation involves a large number of reaction channels as seen in Figure 2c, which is a vertically expanded version of Figure 2a. The fragment-ion peaks are relatively sharp, indicating that a large proportion of these ions are stable on the time scale of their transit through the flight-tube (ca. 10^{-4} s). The most intense fragment ions are identified and indicated in Figure 2c, and the assigned identities and measured and calculated masses are provided in Table II. The major fragment ions from peracetylated maltoheptaose, labeled X, Y, and Z, are formed by dissociation of the $(M + Na)^+$ ion to give sodium-containing products. The X series ions result from ring cleavage, with retention of HCO on the glycosidic oxygen of the charged, reducing end fragment as in the case of the A series ions from the underivatized compound. The Y series ions are formed by cleavage at a glycosidic oxygen, retention of this oxygen on and transfer of a hydrogen to the charged fragment, and the loss of the elements of CH_3COOH from the charged fragment. The Z series ions are also formed by cleavage at the glycosidic oxygen but involve the retention of this oxygen on the neutral fragment, transfer of a hydrogen from the neutral to the charged fragment, and the loss of the elements of CH_3COOH from the charged fragment.

Table II. Calculated and Measured Masses of the Most Intense Fragment Ions from a Sample of Peracetylated Maltoheptaose Containing Sodium

ion	calcd mass	measd mass
X_1	399.4	*a*
X_2	687.6	687.8
X_3	975.8	975.8
X_4	1264.1	1264.5
X_5	1552.4	1552.6
X_6	1840.7	1840.5
Y_1	311.1	312.1
Y_2	599.5	599.2
Y_3	887.8	888.1
Y_4	1176.0	1176.1
Y_5	1464.3	1464.1
Y_6	1752.6	1752.6
Y_7	2040.9	2040.7
Z_1	295.3	*a*
Z_2	583.5	583.6
Z_3	871.8	872.3
Z_4	1160.0	1160.3
Z_5	1448.3	1448.4
Z_6	1736.6	1737.0

[a] The mass could not be obtained unambiguously because of the presence of interferring impurity ions. $X_i = [M + Na + 29 - \{(7 - i)G' + CH_3CO\}]^+$, $Y_i = [M + Na - 59 - \{(7 - i)G' + CH_3CO\}]^+$, and $Z_i = [M + Na - 59 - 16 - \{(7 - i)G' + CH_3CO\}]^+$, where M = mass of peracetylated maltoheptaose and G′ = mass of a peracetylated glucose residue.

Effects of Sodium Removal on the Fragmentation of Peracetylated Oligosaccharides. We discovered that it is possible to remove the sodium-containing impurities, which we normally observe in our preparations, from electrospray-

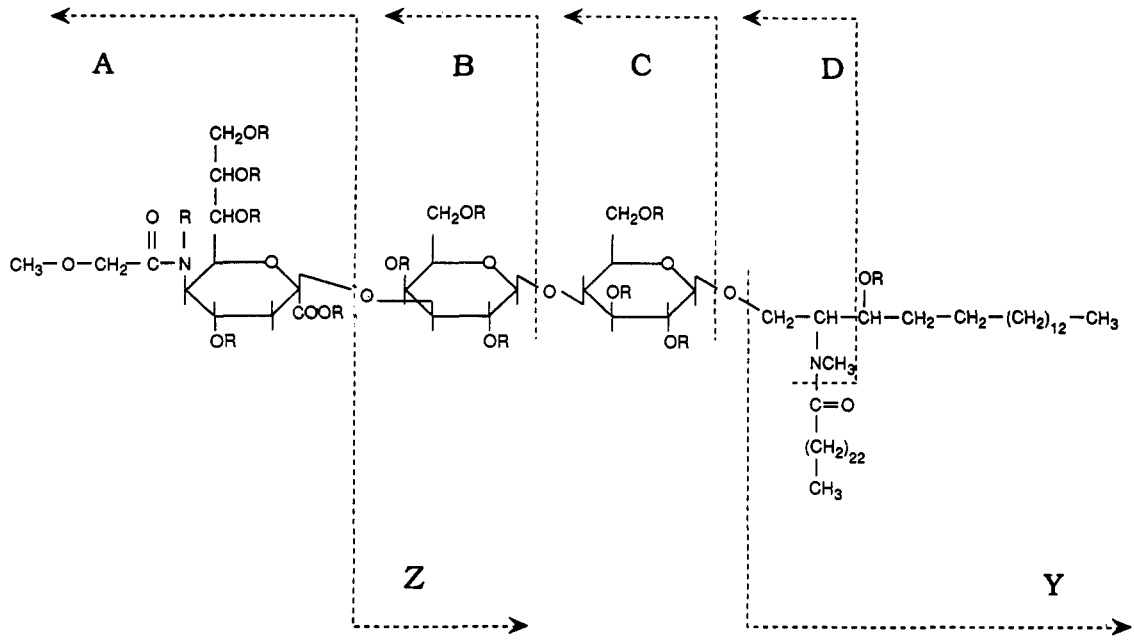

Figure 3. Structure of permethylated, reduced *N*-glycolylneuraminic acid G_{M3} showing proposed fragmentation pattern in PDMS. "R" represents a methyl group.

deposited peracetylated oligosaccharide films by simply dipping the sample foil in deionized water. The peracetylated compounds are sufficiently insoluble in water so that the films can be rendered sodium-free by a 1–2-min immersion without incurring significant sample loss from the foil. The effect on the spectra of sodium removal by this water washing procedure can be seen by comparing Figure 2a (unwashed) and Figure 2b (washed). Because only a very small fraction (<0.01%) of the sample is consumed during the acquisition of a spectrum, the same sample film is used for both measurements. The differences between these two spectra are dramatic. In contrast to the sodium-containing sample, the sodium-depleted sample yields virtually no $(M + Na)^+$ ions. The fragmentation is also strikingly different—compare the spectra given in Figure 2c (unwashed) and 2d (washed), which are vertically expanded versions of Figure 2a and 2b, respectively. In contrast to the fragments observed from the sodium-containing sample, the fragment ions from the water-washed sample do not contain sodium, are broad, indicating that they are unstable on the timescale of their transit through the flight tube (ca. 10^{-4} s), and are comprised of only two series of fragment-ion peaks designated V and W (Table III). These fragment ions appear to be formed by dissociation at the glycosidic linkages of the protonated species, $(M + H)^+$. The $(M + H)^+$ ion is, however, not observed, implying that it is unstable on a timescale shorter than that required to accelerate the protonated intact molecule to full energy ($\leq 2 \times 10^{-7}$ s).

It is noteworthy that the sodium-containing fragment ions from the unwashed sample are formed by ring as well as glycosidic cleavages. On the other hand, sodium-free fragment ions from the water-washed, sodium-depleted sample are formed exclusively by glycosidic cleavages. This finding is in agreement with the earlier observations of Cotter and coworkers (*41*) on the fragmentation observed in laser desorption and fast atom bombardment of oligosaccharides.

Adsorption on Nitrocellulose. Underivatized maltooligosaccharides deposited on NC yielded low-intensity mass spectra consisting of very weak $(M + Na)^+$ ions, indicating poor adsorption to the NC film. The peracetylated compounds, on the other hand, adsorbed relatively strongly to the NC and yielded spectra consisting of an intense $(M + Na)^+$ ion peak and weak sodiated fragment ion peaks, which were similar to those observed from the electrospray-deposited samples. When these peracetylated oligosaccharide samples adsorbed on NC were washed by dipping in deionized water, the results were closely similar to those obtained with the water-washed electrospray-deposited samples.

Although the responses of the electrosprayed and NC-bound peracetylated compounds were similar for amounts of sample that completely cover or saturate the 1-cm^2 sample probe surface (>2 nmol), the NC-bound samples yielded cleaner, more intense spectra for lower sample amounts. For unwashed NC-bound peracetylated maltoheptaose, the intensity of the $(M + Na)^+$ ion peak decreased linearly as a function of the amount of sample applied below 1 nmol but still yielded a 10:1 signal-to-noise fluctuation ratio for a 1-h acquisition from a 50-pmol sample.

Effect of Sodium Addition and Depletion on the Fragmentation of a Permethylated Ganglioside. We have previously investigated the ^{252}Cf plasma desorption mass spectrometric fragmentation of a number of different permethylated gangliosides (*31*). In this earlier study, no attempt was made to remove adventitious sodium so that the spectra all contained relatively intense $(M + Na)^+$ ion peaks. Here, we have reinvestigated the effect on the fragmentation of the addition and deletion of sodium to a sample of permethylated, reduced *N*-glycolylneuraminic acid G_{M3}. The structure and sites of cleavage are indicated in Figure 3. Figure 4a shows the mass spectrum obtained from an electrospray-deposited sample containing 1 nmol of the ganglioside and 5 nmol of NaCl. The prominent features of this spectrum are a relatively intense $(M + Na)^+$ ion peak and a number of fragment ions yielding structural information on both the carbohydrate and ceramide portions of the molecule. The same sample, subjected to sodium removal by immersion in deionized water, produces a plasma desorption mass spectrum that is strikingly different (Figure 4b). Water washing reduces drastically the intensity of the $(M + Na)^+$ ion peak and the sodiated carbohydrate containing fragment ion peaks. At the same time, there is a striking enhancement of the sodium-free ceramide peak, Y^+, and the pair of *N*-glycolylneuraminic acid fragment ion peaks, A^+ and $(A - ROH)^+$. It should be emphasized that the effects described above are reversible in that sodium can be added back into a thin film sample deposit to once again obtain an intense $(M + Na)^+$ ion peak. Thus, it proves possible to readily alternate between a spectrum containing an intense

Table III. Calculated and Measured Masses of the Fragment Ions from a Sample of Peracetylated and Water-Washed (Sodium-Depleted) Maltoheptaose

ion	calcd mass	measd mass
V_1	331.0	331.1
V_2	619.6	619.7
V_3	907.8	908.1
V_4	1196.1	1196.6
V_5	1484.4	1484.7
V_6	1772.6	1772.3
V_7	2060.9	2060.9
W_1	271.0	*a*
W_2	559.5	560.4
W_3	847.8	848.4
W_4	1136.0	1137.0
W_5	1424.3	*b*
W_6	1712.6	*b*
W_7	2000.9	*b*

[a] The mass could not be unambiguously assigned because of the presence of interferring impurity ions. [b] The mass could not be assigned accurately because the peaks are too broad and weak. $V_i = [M + H - 17 - \{(7 - i)G' + CH_3CO\}]^+$ and $W_i = [M + H - 60 - 17 - \{(7 - i)G' + CH_3CO\}]^+$ where M = mass of peracetylated maltoheptaose and G′ = a peracetylated glucose residue.

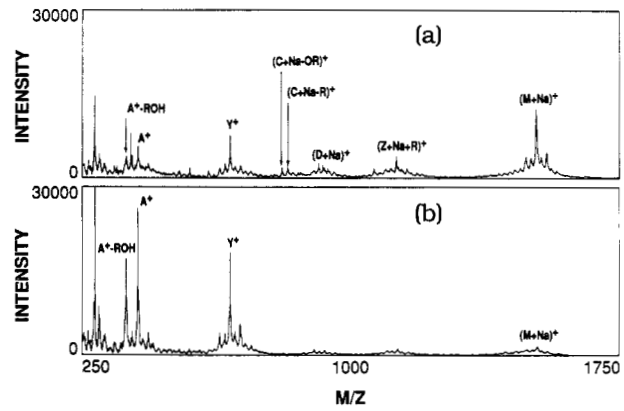

Figure 4. Positive-ion ^{252}Cf plasma desorption mass spectra obtained from permethylated, reduced *N*-glycolylneuraminic acid G_{M3} prepared by electrospray deposition. (a) Spectrum obtained from a sample containing 1 nmol of ganglioside and 5 nmol of NaCl. (b) Spectrum from the same sample after water washing to remove sodium. "R" represents a methyl group.

$(M + Na)^+$ ion and one in which certain fragment-ion peaks are maximized.

CONCLUSION

Electrospray-deposited as well as NC-adsorbed, peracetylated maltooligosaccharide samples produce intense $(M + Na)^+$ ions and a large number of relatively weak sodiated fragment ions. Washing these samples with water causes sodium depletion without significant loss of the sample. These washed samples produce mass spectra consisting of neither $(M + Na)^+$ nor $(M + H)^+$ ion peaks but a smaller number of intense sodium-free fragment ion peaks that are structurally informative. Equivalent results are obtained with samples prepared by electrospray desorption and adsorption to NC. Thus, it appears that the amount of sodium present in the sample strongly influences the nature and extent of fragmentation in the plasma desorption mass spectrum rather than the method of preparation for peracetylated maltooligosaccharides. It is expected that the enthalpy of association of H^+ with the peracetylated oligosaccharides will be greater than that for the association of Na^+, and if the association occurs in the gas phase under low enough density conditions that the energy release is not removed by collisions, more fragmentation will result from H^+ addition than from Na^+ addition (*42*). This method of controlling fragmentation has also been successfully applied in the case of a permethylated ganglioside, indicating a wider range of application for the technique. The method, therefore, provides a useful means to obtain selectively either molecular mass information or structural information from the same sample.

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LITERATURE CITED

(1) Burlingame, A. L.; Millington, D. S.; Norwood, D. L.; Russel, D. H. *Anal. Chem.* **1990**, *62*, 268R–303R.
(2) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, *7*, 325–327.
(3) Macfarlane, R. D.; Torgerson, D. F. *Science* **1976**, *191*, 920–925.
(4) Kamerling, J. P.; Heerma, W.; Vliegenthart, J. F. G.; Green, B. N.; Lewis, J. A. S.; Strecker, G.; Spik, G. *Biomed. Mass Spectrom.* **1983**, *10*, 420–425.
(5) Bosso, C.; Defaye, J.; Heyraud, A.; Ulrich, J. *Carbohydr. Res.* **1984**, *125*, 309–317.
(6) Carr, S. A.; Reinhold, V. N.; Green, B. N.; Hass, J. R. *Biomed. Mass Spectrom.* **1985**, *12*, 288–295.
(7) Adinolfi, M.; Mangoni, L.; Parilli, M.; Self, R. *Biomed. Mass Spectrom.* **1984**, *11*, 310–314.
(8) Domon, B.; Costello, C. E. *Biochemistry* **1988**, *27*, 1534–1543.
(9) Dell, A. *Adv. Carbohydr. Chem. Biochem.* **1987**, *45*, 19–72.
(10) Karlsson, H.; Carlstedt, I.; Haensson, G. *FEBS Lett.* **1987**, *226*, 23–27.
(11) Dell, A.; York, W. S.; McNeil, M.; Darvill, A. G.; Albersheim, P. *Carbohydr. Res.* **1983**, *117*, 185–200.
(12) Dell, A.; Oates, J.; Lugowski, C.; Romanowska, E.; Kenne, L.; Lindberg, B. *Carbohydr. Res.* **1984**, *133*, 95–104.
(13) Angel, A.; Lindh, F.; Nilsson, B. *Carbohyr. Res.* **1987**, *168*, 15–31.
(14) Weintraub, A.; Lindberg, A. A.; Lipniunas, P.; Nilsson, B. *Glycoconjugate J.* **1988**, *5*, 207–213.
(15) Dell, A.; Rogers, M. E.; Thomas-Oates, J. E.; Huckerby, T. N.; Sanderson, P. N.; Nieduszynski, I. A. *Carbohydr. Res.* **1988**, *179*, 7–19.
(16) Sasaki, H.; Ochi, N.; Dell, A.; Fukuda, M. *Biochemistry* **1988**, *27*, 8618–8626.
(17) Dell, A.; Thomas-Oates, J. E.; Rogers, M. E.; Tiller, P. R. *Biochimie* **1988**, *70*, 1435–1444.
(18) Dell, A.; Morris, H. R.; Egge, H.; Von Nicolai, H.; Strecker, G. *Carbohydr. Res.* **1983**, *115*, 41–52.
(19) Egge, H.; Dell, A.; Von Nicolai, H. *Arch. Biochem. Biophys.* **1983**, *224*, 235–253.
(20) Dell, A.; Carman, N. H.; Tiller, P. R.; Thomas-Oates, J. E. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 19–24.
(21) Poulter, L.; Earnest, J. P.; Stroud, R. M.; Burlingame, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6645–6649.
(22) Poulter, L.; Earnest, J. P.; Stroud, R. M.; Burlingame, A. L. *Biomed. Environ. Mass Spectrom.* **1987**, *16*, 25–30.
(23) Weber, J. W.; Jiang, K.; Gillece-Castro, B. L.; Tarentino, A. L.; Plummer, T. H.; Byrd, J. C.; Fisher, S. J.; Burlingame, A. L. *Anal. Biochem.* **1988**, *169*, 337–349.
(24) Forsberg, L. S.; Dell, A.; Walton, D. J.; Ballou, J. *Biol. Chem.* **1982**, *257*, 3555–3563.
(25) Dell, A.; Ballou, C. *Biomed. Mass Spectrom.* **1983**, *10*, 50–56.
(26) Dell, A.; Ballou, C. *Carbohydr. Res.* **1983**, *120*, 95–111.
(27) Jardine, I.; Hunter, S. W.; Brennan, P. J.; Macfarlane, R. D. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 273–276.
(28) Jardine, I. The Analysis of Peptides and Proteins by Mass Spectrometry. *Proceedings of the Fourth Texas Symposium on Mass Spectrometry*; McNeal, C. J., Ed.; Wiley: College Station, TX, 1988; pp 41–54.
(29) Jardine, I.; Scanlon, G.; McNeil, M.; Brennan, P. J. *Anal. Chem.* **1989**, *61*, 416–422.
(30) Kamensky, I.; Craig, A. G. *Anal. Instrum.* **1987**, *16*, 71–91.
(31) Furukawa, K.; Chait, B. T.; Lloyd, K. O. *J. Biol. Chem.* **1988**, *163*, 14939–14947.
(32) Bean, M. F.; Bangs, J. D.; Doering, T. L.; Englund, P. T.; Hart, G. W.; Fensalau, C.; Cotter, R. *J. Anal. Chem.* **1989**, *61*, 2686–2688.
(33) Cotter, R. J. *Anal. Chem.* **1988**, *60*, 781A–793A.
(34) Tsai, P.; Dell, A.; Ballou, C. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *82*, 4119–4123.
(35) McNeal, C. J.; Macfarlane, R. D.; Thurston, E. L. *Anal. Chem.* **1979**, *51*, 2046–2039.
(36) Chait, B. T.; Field, F. H. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 420–426.
(37) Chait, B. T.; Agosta, W. C.; Field, F. H. *Int. J. Mass Spec. Ion Phys.* **1981**, *39*, 339–366.
(38) Chait, B. T.; Field, F. H. *Int. J. Mass Spectrom. Ion. Phys.* **1981**, *41*, 17–29.
(39) Domon, B.; Costello, C. *Glycoconjugate J.* **1988**, *5*, 397–409.
(40) Aduru, S.; Chait, B. T. 1990, The Rockefeller University, unpublished work.
(41) Martin, W. B.; Silly, L.; Murphy, C. M.; Raley, T. J.; Cotter, R. J.; Bean, M. F. *Int. J. Mass Spectrom. Ion Processes* **1989**, *92*, 243–265.
(42) Chait, B. T.; Shpungin, J.; Field, F. H. *Int. J. Mass Spectrom. Ion Processes* **1984**, *58*, 121–137.

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