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High-sensitivity structural analyses of oligosaccharide probes (neoglycolipids) by liquid-secondary-ion mass spectrometry

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ABSTRACT

The sensitivity of detection and extent of information on structure obtainable by liquid-secondary-ion mass spectrometry (l.s.i.-m.s.) of neoglycolipids on the conventional target probe and directly from the surface of silica plates following t.l.c. has been assessed. Neoglycolipids were derived from malto-oligosaccharides, chitin oligosaccharides, and a range of deoxyhexose-, hexose-, 2-acetamido-2-deoxyhexose-, and sialic acid-containing mammalian oligosaccharides by reductive amination using phosphatidylethanolamine dipalmitoate (PPEADP). Sub-pmol amounts of the maltopentaose–PPEADP derivative applied directly to the target probe provided information on molecular weight, whereas ~ 1 pmol was required when analysed on the silica gel t.l.c. plate. With a biantennary octasaccharide derivative, the sensitivity of detection was 20–50 times lower and the other oligosaccharides had intermediate sensitivities. Information on composition and sequence was obtained readily from fragment ions, using 5 pmol of the maltopentaose derivative and 50 pmol of the octasaccharide derivative on the target probe, and 50 and 200 pmol, respectively, on the silica gel chromatogram. The optimised conditions formed the basis for characterising the structures of the components of mixtures of oligosaccharides generated from glycoproteins.

INTRODUCTION

As a general approach to the study of the bioactivities of oligosaccharides, a new micro-technology has been introduced whereby oligosaccharides released from glycoproteins and proteoglycans, or synthesised chemically, may be conjugated to a lipid such as phosphatidylethanolamine dipalmitoate (PPEADP) and used as solid phase probes in ligand-binding assays with antibodies and with carbohydrate-binding proteins of plant, animal, and microbial origins[1–8]. An important aspect of the procedure is that neoglycolipids derived from mixtures of oligosaccharides can be used. Therefore, it is highly desirable to identify individual neoglycolipids resolved by the t.l.c. systems employed in ligand-binding assays. L.s.i.-m.s. has great potential for this purpose, as has been shown for natural glycolipids[9,10]. We now report on the sensitivity of l.s.i.-m.s.

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in the analysis of neoglycolipids and on the information on composition and sequence that can be obtained.

EXPERIMENTAL

Oligosaccharides. — Malto-triose, -pentaose, and -heptaose, and chitin oligosaccharides were purchased from Sigma. Lacto-*N*-neotetraose (LNT), β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc; lacto-*N*-tetraose (LNNT), β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc; lacto-*N*-fucopentaose III (LNFP III), β-Gal-(1→4)[α-Fuc-(1→3)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc; and lacto-*N*-difucohexaose I (LNDFH I), α-Fuc-(1→2)-β-Gal-(1→3)[α-Fuc-(1→4)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc were prepared from human milk and were gifts of Professor W. M. Watkins and Dr. A. S. R. Donald. α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc and α-NeuAc-(2→3)-β-Gal-(1→4)-Glc were provided by Dr. G. Strecker. The GM_1-octasaccharide, β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)[β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)]-β-Man-(1→4)-GlcNAc, was isolated from the urine of a patient with GM_1-gangliosidosis and was a gift from Dr. A. Lundblad.

Neoglycolipids. — Unless otherwise stated, oligosaccharides were conjugated to PPEADP as follows[4]. A mixture of PPEADP (400 nmol), oligosaccharide (50 nmol), and 1:1 chloroform–methanol (55 μL) was sonicated (10 min) and heated (2 h, 50°). Sodium cyanoborohydride (200 nmol) in methanol (1.3 μL) was added, and reaction was continued for 16 h at 50° before the addition of water (6 μL) and sonication (10 min). For tetra-*N*-acetylchitotetraose, maltoheptaose, and GM_1-oligosaccharide, 3 μL of water was added to the oligosaccharide before the addition of PPEADP, in order to increase the solubility in the reaction mixture. Pure derivatives for analysis directly from the l.s.i.-m.s. target were obtained by elution from columns of phenylboronic acid[11] (Jones Chromatography). Salts were removed prior to t.l.c. by dissolving the derivatives in chloroform–methanol–water (15:70:30, 600 μL) and applying to a pre-equilibrated column of octadecylsilica (100 mg, Jones Chromatography). The absorbed lipids were washed with the initial solvent mixture (3 x 200 μL), eluted with chloroform–methanol–water (60:35:8, 2 x 1 mL), and recovered by concentration. T.l.c. was performed on Silica Gel 60 (HPTLC, aluminium-backed plates, Merck) with detection by orcinol or primulin and u.v. illumination (Fig. 1).

In order to determine whether derivatisation and detection by l.s.i.-m.s. *in situ* could be carried out on pmol amounts, samples of maltopentaose (3, 10, 30, 100, 300, and 1000 pmol) were conjugated by the method above, but employing PPEADP (100 nmol) and cyanoborohydride (160 nmol) in 1:1 chloroform–methanol (20 μL) for each sample. Each total reaction mixture was subjected to t.l.c. The area containing the PPEADP derivative was excised and analysed.

Mass spectrometry. — A VG Analytical ZAB2-E mass spectrometer, fitted with a caesium gun operated at 35 keV and an emission current of 0.5 μA, was used for l.s.i.-m.s. Following t.l.c. and primulin staining, bands were excised from the plate

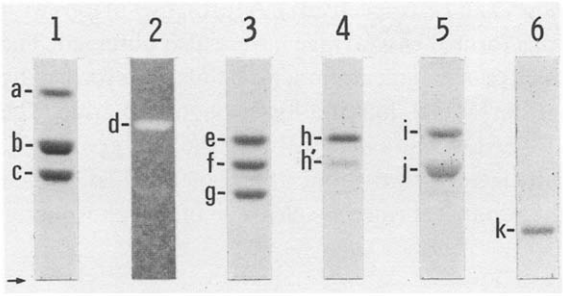


Fig. 1. T.l.c. (chloroform–methanol–water, 105:100:25) on silica gel of PPEADP derivatives (~ 1 nmol) of maltotriose (a), maltopentaose (b), maltoheptaose (c), tetra-*N*-acetylchitotetraose (d), LNNT (e), LNFP III (f), LNDFH I (g), LNT (h), sialyl-lactose (i), sialyl-LNNT (j), and GM_1-octasaccharide (k). The additional band (h') in lane 4 is the PPEADP derivative of a contaminant (LNFP III). All lanes were detected with orcinol, with the exception of lane 2 which was detected with primulin–u.v. light; → indicates the origin.

together with the aluminium backing foil. Alternatively, samples were run in duplicate lanes, one of which was sprayed with orcinol reagent, and an area corresponding to the orcinol-positive band was removed from the second lane. The t.l.c. strips (~ 1.5 x 5 mm) were attached to the target probe tip by a 1:6 mixture of a water-soluble glue and glycerol. A liquid matrix (3 μL) consisting of diethanolamine, tetramethylurea, and *m*-nitrobenzyl alcohol (DEA–TMU–NBA 2:2:1) was added to the silica-absorbed sample after the initial addition of 2 μL of methanol–chloroform–water (25:25:8).

Purified derivatives were also analysed directly from the stainless steel l.s.i.-m.s. target, using the DEA–TMU–NBA matrix. L.s.i.-m.s. spectra of free oligosaccharides were obtained from a thioglycerol matrix.

Mass spectra were acquired in the positive and negative modes from single scans at 30 s.decade^{-1}, using the VG Analytical 11-250J data system in continuous multi-channel analysis mode at resolutions up to 2500 RP.

RESULTS

Malto- and chito-oligosaccharides. — Malto-triose, -pentaose, and -heptaose [(1→4)-linked α-D-gluco-oligosaccharides] and chitin oligomers [(1→4)-linked 2-acetamido-2-deoxy-β-D-gluco-oligosaccharides] were used for initial investigation of the l.s.i.-m.s. of PPEADP derivatives in order to assess the sensitivity of their detection by analysis directly from silica gel chromatograms or from the normal target.

T.l.c. of the PPEADP derivatives of the malto-oligosaccharides is illustrated in Fig. 1, and the negative-ion mass spectra acquired from the material (1 nmol) in each band directly from the silica surface are shown in Fig. 2. Each derivative gave an abundant $[M-H]^-$ ion (*m/z* 1178.7, 1502.8, and 1826.9 for the triose, pentaose, and heptaose, respectively). The fragment ions differing in mass by − 162 Da (*e.g.*, *m/z* 1016 and 854 for the triose, Fig. 2a) were those expected from cleavage *a* in Scheme 1, as were the accompanying ions at *m/z* 998 and 836 and *m/z* 1044 and 882 which corresponded to

rearrangement fragmentation (cleavages *b* and *c*, respectively). A further set of ions with masses 2 Da less than those of the ions formed by cleavage *a* were also observed. The presence of these ion quartets is distinctive of fragmentation, and, thus, ions formed by cleavage *a* can be distinguished from the $[M-H]^-$ ion of a lower oligosaccharide. The glucose residue attached directly to PPEADP was associated with the ions at *m/z* 854, whereas the PPEADP group was associated with the ions at *m/z* 690 and 647 which represented the intact moiety and the product formed by cleavage of the phosphate–ethylamine bond, respectively.

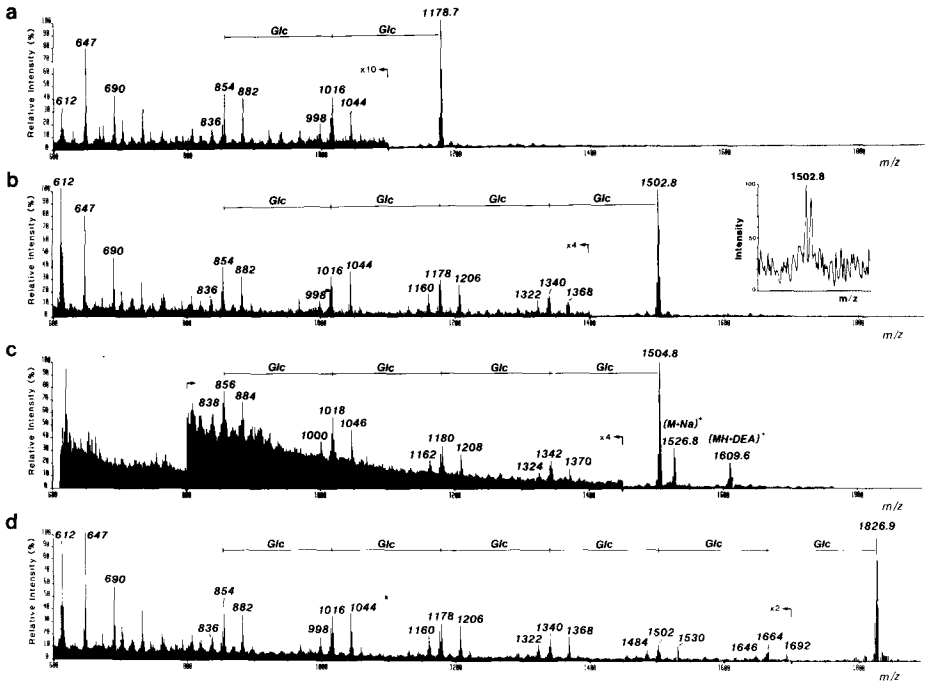
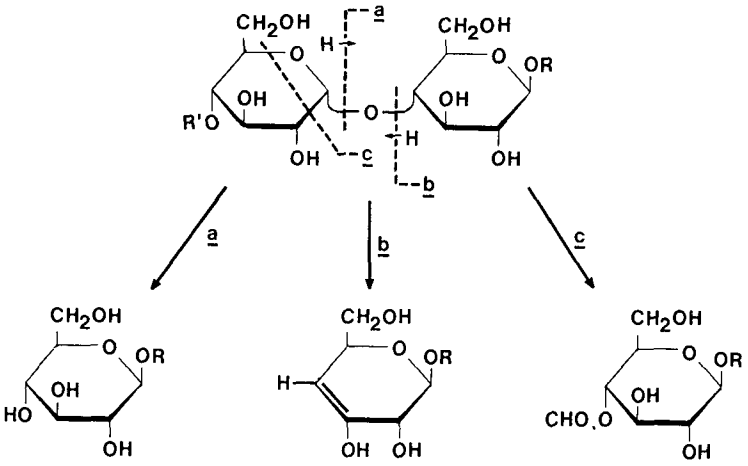


Fig. 2. The negative-ion spectrum obtained from 1 nmol of the PPEADP derivatives of maltotriose (a), maltopentaose (b), and maltoheptaose (d) recorded directly from the silica surface following the addition of matrix and solvent. The positive-ion spectrum of maltopentaose–PPEADP is also shown (c). The inset in (b) shows the region for $[M-H]^-$ ions from the spectrum acquired from 3 pmol of maltopentaose after derivatisation and ionisation *in situ* from the t.l.c. surface. Integral mass values are given for ions other than $[M-H]^-$ ions.

The positive-ion spectra of the malto-oligosaccharide–PPEADP derivatives (see Fig. 2c for the spectrum of maltopentaose derivative, 1 nmol) were similar to the negative-ion spectra. The $[M + H]^+$ ion was accompanied by $[M + Na]^+$ due to contamination with sodium, a matrix adduct ion at *m/z* 1609.6, and fragment ions that had masses 2 Da higher than those of the equivalent negative ions. The same order of sensitivity was obtained from either negative-ion or positive-ion l.s.i.-m.s. However, due to the lower background in the negative mode and the reduction in sensitivity by the formation of adduct ions in the positive mode, negative-ion scans were used to measure the limits of detection of PPEADP derivatives.



Scheme 1. Principal cleavages which provide information on the sequence in the spectra of oligosaccharide–PPEADP conjugates and occur equally for the neutral saccharides shown and for those containing a HexNAc group: R = reducing end of molecule including the PPEADP moiety, R′ = H or non-reducing end of molecule. The cleavages are analogous to those observed in l.s.i.-m.s. of oligosaccharides[12] and their derivatives formed by reductive amination[13–17].

Analysis of a 50-pmol aliquot of the maltopentaose derivative directly from the surface of the silica gel plate coated with matrix and solvent produced a spectrum with an $[M-H]^-$ ion having a signal-to-noise ratio of ~20:1 in addition to fragment ions, whereas only the $[M-H]^-$ ion was present at the 1-pmol level (signal-to-noise ratio 3:1; spectrum not shown). When the reactions were carried out with pmol amounts throughout (*i.e.*, 3, 10, 30, 100, 300, and 1000 pmol) and the total sample was subjected to t.l.c. for detection *in situ* by l.s.i.-m.s., $[M-H]^-$ and sequence ions were present in spectra from 100–1000 pmol, whereas the $[M-H]^-$ ions were clearly apparent above background down to the 3-pmol sample (signal-to-noise ratio ~4:1; Fig. 2b inset). Charging of the sample was prevented by maintaining good electrical contact with the holder of the l.s.i.-m.s. target at ion-source potential. This contact was made possible by using an electrical conducting adhesive to bind the aluminium-backed silica strip to the target. The limit of detection for visualising maltopentaose–PPEADP on the chromatogram with primulin–u.v. light or orcinol was ~100 pmol and, hence, l.s.i.-m.s. provided greater sensitivity for detection of absorbed sample bands.

When the PPEADP derivatives were added directly to the stainless steel target, the sensitivity of detection was substantially greater. The signal-to-noise ratio for the molecular ion species of maltopentaose–PPEADP from a complete mass-range scan of a 100-fmol sample was ~12:1 (Fig. 3a). At this concentration, fragment ions were not normally visible above the background. However, these sequence-determining ions were clearly apparent from 1 pmol of material placed on-target, and a 5-pmol sample was a practical amount for analysis (Fig. 3b).

The PPEADP derivatives of di-*N*-acetylchitobiose, tri-*N*-acetylchitotriose, and tetra-*N*-acetylchitotetraose each gave an intense $[M-H]^-$ ion (*m/z* 1098.7, 1301.8, and

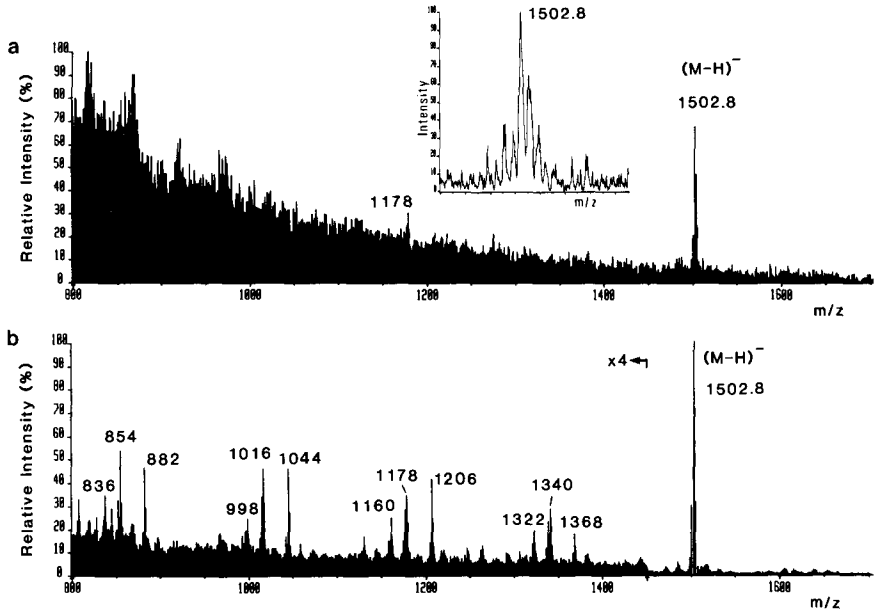


Fig. 3. The negative-ion spectrum obtained from 100 fmol (a) and 5 pmol (b) of maltopentaose–PPEADP placed directly on the target probe. An expanded view of the region for molecular ions from the 100-fmol sample is shown as an inset in (a).

1504.8, respectively) and cleavage ions of lower intensity as illustrated in Fig. 4 for the tetraose–PPEADP run directly from the t.l.c. plate. The regular difference of 203 Da of the ions with *m/z* 1504.8, 1301, 1098, and 895 (corresponding to cleavage *a*, Scheme 1) was in accord with the linear sequence of 2-acetamido-2-deoxyhexose residues, as were the series of ions with *m/z* 1283, 1080, and 877 (cleavage *b*) and *m/z* 1319, 1116, and 913 (cleavage *c*). The ion with *m/z* 895 for the core acetamido sugar linked to PPEADP is distinct from the corresponding ion (*m/z* 854) for core hexose linked to PPEADP (as shown above). The formation of neoglycolipids of chitosaccharides was accompanied by partial dehydration of the linkage sugar, giving the $[M-H-18]^-$ ion, but this ion was of low abundance in the spectrum shown in Fig. 4.

Mammalian oligosaccharides. — Mass spectra of the PPEADP derivatives of several different oligosaccharides of mammalian origin were acquired directly from the silica surface after t.l.c.

(a) Tetrasaccharide isomers, β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc (LNNT) and β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc (LNT). The PPEADP derivative of LNNT gave a spectrum with $[M-H]^-$ at *m/z* 1381.8 consistent with the composition of one HexNAc and three Hex units. The Gal–GlcNAc–Gal–Glc sequence was reflected by the presence of ions at *m/z* 1219, 1016, and 854 as expected from respective losses of Gal, Gal–GlcNAc, and Gal–GlcNAc–Gal (Fig. 5). The PPEADP portion of this neoglycolipid underwent limited fragmentation with rearrangement losses of RCO–H and RCO_2H, where $R = CH_3(CH_2)_{14}$, to give ions with *m/z* 1143 and 1125, respectively.

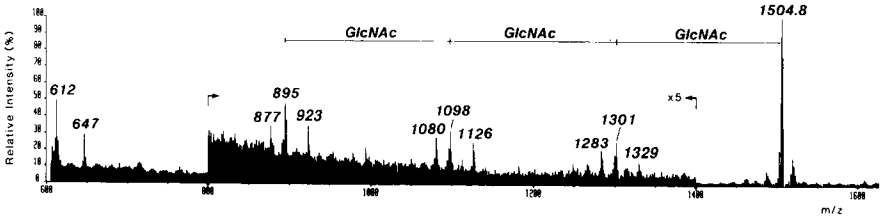


Fig. 4. The negative-ion spectrum of tetra-*N*-acetylchitotetraose–PPEADP (1 nmol) from the t.l.c. plate.

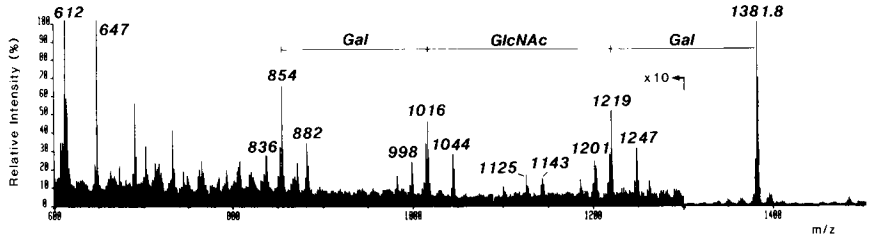


Fig. 5. The negative-ion spectrum of lacto-*N*-neotetraose, β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc-PPEADP (1 nmol), from the t.l.c. plate.

The spectrum (not shown) of the PPEADP derivative of LNT (differing from LNNT only in the Gal–GlcNAc linkage) was almost identical to that shown in Fig. 5; hence, it was not possible to differentiate between oligosaccharides having the β-Gal-(1→3)-GlcNAc and β-Gal-(1→4)-GlcNAc sequences.

(b) Fucosylated oligosaccharides, β-Gal-(1→4)[α-Fuc-(1→3]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc (LNFP III) and α-Fuc-(1→2)-β-Gal-(1→3)[α-Fuc-(1→4)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc (LNDFH I). These oligosaccharides, when conjugated to PPEADP, gave bands on t.l.c. (Fig. 1) which produced intense $[M-H]^-$ ions consistent with their monosaccharide composition, *i.e.*, at *m/z* 1527.8 and 1673.9 for LNFP III and LNDFH I, respectively (Fig. 6). LNFP III gave fragment ions with *m/z* 1381, 1365, 1016, and 854 arising from [M–H] by loss of Fuc (–146 Da), Gal (–162 Da), Fuc(Gal)–GlcNAc (–511 Da), and Fuc(Gal)–GlcNAc–Gal (–673 Da), respectively, as expected for this branched sequence. In agreement with all of the spectra studied, ions formed by glycosidic cleavage did not undergo further fragmentation, *i.e.*, no fragment was found for GlcNAc–Gal–Glc–PPEADP. Similarly, in the spectrum of LNDFH I, the ion at *m/z* 1527 corresponded to elimination of only one fucose [either from α-Fuc-(1→2)-Gal or α-Fuc-(1→4)-GlcNAc linkages] and not two consecutive losses of fucose. The fragmentation ions in the spectrum of LNDFH I that gave information on the sequence were those at *m/z* 1527, 1365, 1016, and 854 consistent with the non-reducing end structures, Fuc– (–146 Da), Fuc–Gal– (–308 Da), $(Fuc)_2$–Gal–GlcNAc– (–657 Da), and $(Fuc)_2$–Gal–GlcNAc–Gal (–819 Da). The absence of an ion with *m/z* 1219 accorded with the lack of $(Fuc)_2$–Gal and, therefore, is consistent with the presence of Fuc and Fuc–Gal branches on the GlcNAc of this oligosaccharide. The ions at *m/z* 1016 and 854 corresponded to the Gal–Glc–PPEADP and Glc–PPEADP

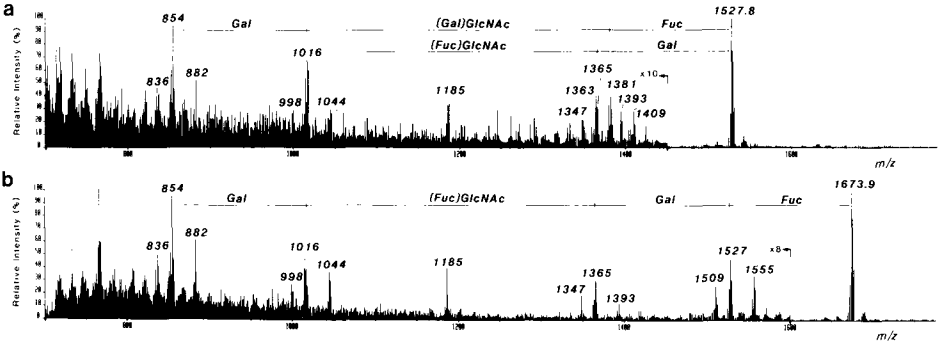


Fig. 6. The negative-ion spectrum of 1 nmol each of lacto-*N*-fucopentaose III, β-Gal-(1→4)[α-Fuc-(1→3)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc-PPEADP (a), and lacto-*N*-difucohexaose I, α-Fuc-(1→2)-β-Gal-(1→3)[α-Fuc-(1→4)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc-PPEADP (b), from the t.l.c. plate.

fragments, respectively, in both LNPF III and LNDFH I. The origin of the ion at *m/z* 1185 was not determined.

(c) Sialylated oligosaccharides. The spectrum of the PPEADP derivative of sialyllactose, α-NeuAc-(2→3)-β-Gal-(1→4)-Glc, is shown in Fig. 7a. In addition to the $[M-H]^-$ ion, small Na and K adduct ions (*m/z* 1329.7 and 1345.7, respectively) and losses of 28 and 44 Da from $[M-H]^-$ were noted. The Gal–Glc–PPEADP sequence was apparent from the ions formed by glycosidic cleavage at *m/z* 1016 and 854, and the difference in mass of 291 Da (–NeuAc + H) between the ions at *m/z* 1307.7 and 1016 was expected in the presence of the non-reducing terminal NeuAc residue. Fragmentation of the NeuAc ring (cleavage *c*, Scheme 1) for non-ionic oligosaccharides would produce an ion at *m/z* 1060, *i.e.*, 1016 + 44. Its absence suggested this to be an unfavourable cleavage in sialylated oligosaccharides and this was supported by the spectrum of sialyl-LNNT, α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc–PPEADP (Fig. 7b) where this fragment was also absent. The sequence of the pentasaccharide NeuAc–Gal–GlcNAc–Gal–Glc is clearly apparent from the ions at *m/z* 1381, 1219, 1016, and 854 (Fig. 7b).

(d) GM_1-Octasaccharide. The PPEADP derivative of GM_1-octaosaccharide gave the negative-ion spectrum shown in Fig. 8a. The $[M-H]^-$ ion at *m/z* 2112.0, consistent with the composition $(Gal)_2$–$(GlcNAc)_3$–$(Man)_3$–PPEADP, was accompanied by a small amount of an $[M-H]^-$ ion at *m/z* 2094.0 reflecting loss of the elements of water from the linkage sugar. The occurrence of the three series of fragment ions accords with Scheme 1, the first at *m/z* 1977, 1774, and 1612, the second at *m/z* 1949, 1746, and 1584, and the third at *m/z* 1931, 1728, and 1566 are ascribed to a Gal–GlcNAc–Man sequence. A fourth series of ions at *m/z* 1947, 1744, and 1582 is also present (Fig. 8a) and analogous to the series of ions in the malto-oligosaccharide–PPEADP spectra described above. Other fragment ions of appreciable intensity were at *m/z* 923, 895, and 877, which are diagnostic of HexNAc linked to PPEADP. The absence of additional fragmentation together with the known composition from the $[M-H]^-$ ion were those expected for two Gal–GlcNAc–Man sequences occurring as branches on a Man–GlcNAc–PPEADP

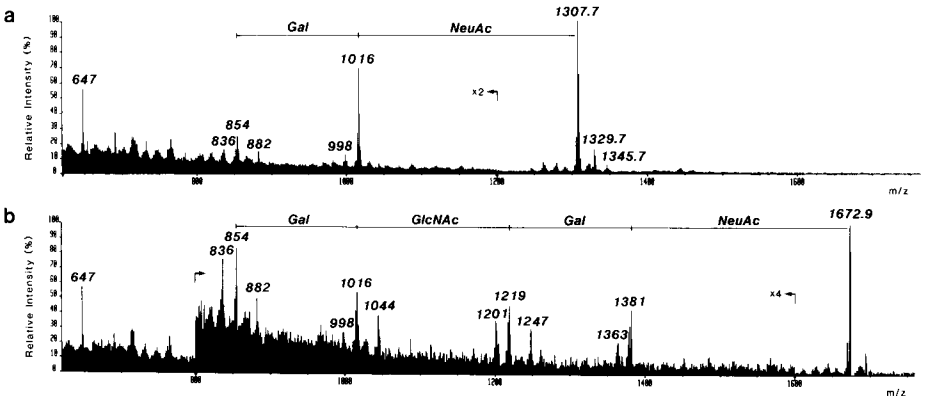


Fig. 7. The negative-ion spectrum of 1 nmol each of α-NeuAc-(2→3)-β-Gal-(1→4)-Glc-PPEADP (a) and α-NeuAc-(2→6)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc-PPEADP (b) from the t.l.c. plate.

core. The ion at m/z 2094.0 arose from partial dehydration of the linkage sugar as found also in the derivatives of other oligosaccharides having a HexNAc at the reducing end. Reaction conditions to minimise this dehydration or to carry it to completion are under investigation.

In separate experiments to test the sensitivity of the $[M-H]^-$ ion produced from 2 pmol of GM_1–PPEADP, there was a signal-to-noise ratio of 8:1 when ionisation occurred from the normal target (Fig. 8b), but ~50 pmol were required for the production of $[M-H]^-$ from the silica surface (not shown). In order to obtain both $[M-H]^-$ and sequence-determining fragment ions readily from the target and directly from t.l.c., ~50 and ~200 pmol, respectively, of derivative were required.

DISCUSSION

The foregoing results show the sensitivity with which saccharide composition, sequence, and branching points of oligosaccharide–PPEADP derivatives can be determined by negative-ion l.s.i.-m.s. Intense molecular ions enable the molecular weight to be established, whereas the fragment ions, although of lower relative abundance, give clear information of composition and sequence. The major fragment ions contain the derivative end of the molecule which simplifies interpretation of the spectra, although the possibility of non-reducing terminal fragments or multiple cleavages in larger structures remains to be investigated.

The sensitivity of detection by l.s.i.-m.s. of the purified malto-oligosaccharide–PPEADP derivatives, analysed from the normal sample target (detection limit of 100 fmol for maltopentaose–PPEADP), is higher than that reported for any other derivative in terms of information on molecular weight, despite the increase in mass created by the PPEADP moiety, and may be attributed to the favourable surface activity of these neoglycolipids and the charged phosphate group. However, the detection sensitivity

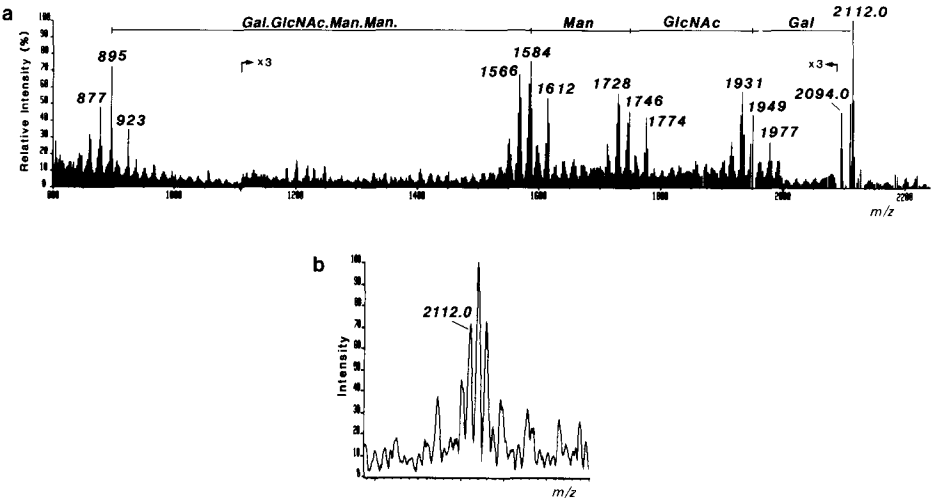


Fig. 8. The negative-ion spectrum of 2 nmol of the PPEADP derivative of the GM_1-oligosaccharide, β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→3)[β-Gal-(1→4)-β-GlcNAc-(1→2)-α-Man-(1→6)]-β-Man-(1→4)-GlcNAc-PPEADP, from the t.l.c. plate (a). An expanded view of the region for molecular ions, obtained from 2 pmol of GM_1–PPEADP, analysed directly on the target is shown in (b).

with individual oligosaccharides needs to be assessed separately, as mass and surface activity will influence ionisation. For the GM_1-oligosaccharide derivative, for example, the limit of detection for the $[M-H]^-$ ion was ~20 times greater than for the maltopentaose conjugate. High sensitivity has been achieved for other derivatives formed by reductive amination. Thus, for the ethyl *p*-aminobenzoate derivative of maltoheptaose, a limit of detection of 4 pmol for $[M-H]^-$ has been reported and of 40 pmol for sequence-ion fragmentation[15]. A limit of detection of 30 pmol for the 2-amino-6-methylpyridine derivative of maltoheptaose has been recorded[18]. Improvements in detector systems, such as the introduction of multichannel array devices, should increase sensitivities by up to 100 times[19].

The exceptional ionisation sensitivity from the normal target of derivatives formed by reductive amination can be compared with the limits of detection of underivatised oligosaccharides in either positive or negative mode which, depending on size and type of structure, require at least 1–5 nmol to give a molecular ion. For example, in a separate study (not shown), a signal-to-noise ratio of 2:1 was observed for the $[M-H]^-$ ion from 1 nmol of maltopentaose in thioglycerol matrix. This sensitivity was at least 4 orders of magnitude less than that of the PPEADP derivative and, as reported[20], quasi-molecular ions are present whereas ions providing information on sequence are generally absent from the spectra of underivatised malto-oligosaccharides.

Of great importance is the ability to analyse, by l.s.i.-m.s., mixtures of neoglycolipids which have been resolved by chromatography on silica gel. The sensitivity of detection on the silica surface depends on the molecular size of the oligosaccharide and also largely on the extent to which the sample can be brought into solution at the surface

of the matrix. Depending on structure, between 1 and 50 pmol (*i.e.*, ~1–70 ng) of oligosaccharide was required for detection of $[M-H]^-$, whereas 50 and 200 pmol (40–300 ng) was needed to produce the additional informative sequence ions. This high sensitivity was maintained when only pmol amounts of oligosaccharide (maltopentaose) were derivatised and analysed from the t.l.c. surface despite the presence of large molar excesses of the reagents in the reaction mixture. The molecular ion species was readily detected from a 3-pmol sample of maltopentaose taken through the entire procedure. The limit at which the PPEADP derivatives could be detected on t.l.c. by orcinol or primulin–u.v. light was ~100 pmol; hence, detection of $[M-H]^-$ ions by l.s.i.-m.s. was superior for all the oligosaccharides studied.

The combination of neoglycolipid ligand-binding and l.s.i.-m.s. is a powerful approach for identifying reactive structures in oligosaccharide mixtures. This capability has been exploited in the construction of a library of neoglycolipids from mixtures of oligosaccharides derived from *N*-glycosylated proteins, and studies of oligosaccharide recognition by mammalian[7,8,21] and bacterial[6] carbohydrate-binding proteins.

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