Fast-atom-bombardment mass spectrometry and electron-impact direct-probe mass spectrometry in the structural analysis of oligosaccharides with special reference to the poly(*N*-acetyl-lactosamine) series

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A comparison has been made of positive- and negative-ion fast-atom-bombardment (FAB) and electron-impact (EI) mass spectrometry for analysis of oligosaccharides and alditols containing alternating and consecutive sequences of neutral and acetamido sugars. Among these were novel chemically synthesized tetrasaccharides with *Ii* antigen activities. FAB ionization has the advantage that it is applicable to non-derivatized oligosaccharides and it can determine M_r . However, the abundance of fragment ions providing structural information and the amount of material required for analysis (1–50 nmol) varied from sample to sample. In contrast, EI mass spectrometry of 5 nmol of permethylated or peracetylated oligosaccharides reliably gives all the fragment ions formed by cleavage across the glycosidic bonds.

FAB mass spectrometry has recently been applied to the structural analysis of oligosaccharides as a method for determining the sequence and composition of their constituent monosaccharides (Dell & Ballou, 1983; Dell *et al.*, 1983a; Kamerling *et al.*, 1983). Before the application of this technique the physico-chemical analysis of oligosaccharide sequence had been achieved by EI direct-probe mass spectrometry of permethylated derivatives (Karlsson *et al.*, 1974; Watanabe *et al.*, 1978; Kochetkov *et al.*, 1976; Hounsell *et al.*, 1981; McNeil *et al.*, 1982). An advantage of the FAB ionization method is that it can be used for the analysis of non-derivatized material. However, further studies by Dell *et al.* (1983a) and Egge *et al.* (1983) have suggested that derivatized oligosaccharides are required to obtain FAB mass spectra with an abundance of the fragment ions which provide sequence information.

In the present study we have compared the information obtained from permethylated and peracetylated oligosaccharides by EI and FAB mass spectrometry and that obtained from underivatized oligosaccharides by positive and negative FAB ionization. We have particularly investigated reduced di- to tetra-saccharides of the poly(*N*-

acetyl-lactosamine) series. This family of structures are of special interest for they are components of differentiation antigens termed *I* and *i* (Feizi, 1981). They occur in glycoproteins as backbone regions of *N*- or *O*-linked oligosaccharide chains, in glycolipids and as free oligosaccharides with reducing-end hexose or hexosamine residues, for example in milk and urine. Oligosaccharides with consecutive neutral and acetamido sugars were also investigated to determine whether such sequences would be identified in unknown samples. Oligosaccharides were studied as their alditols because these derivatives are often produced during oligosaccharide isolation steps in order to: (a) increase their stability to base, for example, under the conditions used to release *O*-glycosidically linked chains from protein (Iyer & Carlson, 1971); (b) introduce a ^3H label for ease of detection; (c) prevent α - and β -anomerization at the reduced end and for ease of purification (Hounsell *et al.*, 1984); and (d) determine the composition with respect to the reduced-end residue.

Experimental

Materials

The tetrasaccharide methyl glycoside Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-OMe, the free tetrasaccharide Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc and the disaccharides Gal β 1-4GlcNAc and

Abbreviations used: FAB, fast atom bombardment; EI, electron impact; LNT, lacto-*N*-tetraose; LNT-ol is the corresponding alditol.

Fig. 1. Positive-ion FAB mass spectrum (a) and the negative-ion FAB mass spectrum (b) of the tetrasaccharide methyl glycoside (Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-OMe), the EI mass spectrum (c) and the positive-ion FAB mass spectrum (d) of the permethylated tetrasaccharide

Gal β 1-3GlcNAc were chemically synthesized (Veyrières, 1981) and kindly provided by Dr. A. Veyrières, Dr. C. Augé and Professor S. David, Université de Paris Sud, Orsay, France. LNT (Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc) was kindly provided by Dr. W. M. Watkins of this Institute. Stachyose (Gal α 1-6Gal α 1-6Glc α 1-5Fru) was obtained from Sigma. Chitotriose (GlcNAc β 1-4GlcNAc β 1-4GlcNAc) was obtained by acetolysis of chitin as described by Barker *et al.* (1958).

Methods

Free oligosaccharides were reduced with NaBH₄ (BDH) by the method of Tomana *et al.* (1978). Acetylation was achieved by heating at 100°C for 1.5 h in pyridine/acetic anhydride (1:1, v/v), followed by evaporation with toluene and extraction of the peracetylated derivatives into chloroform. Non-acetylated samples were deionized on SCX and SAX Bond Elut columns (Analytichem International, Harbor City, CA, U.S.A.). Permethylation and subsequent derivatization to partially methylated alditol acetates was achieved by the method of Hakomori (Stellner *et al.*, 1973).

Mass spectrometry

Mass spectrometry was carried out on a Varian (Finnigan) MAT-731 double-focusing mass spectrometer. EI spectra of peracetylated and permethylated oligosaccharides were obtained by using an ionizing voltage of 70 eV, an accelerating voltage of 8 kV and at resolving powers between 1000 and 2500 depending on the compound analysed. Samples introduced on the direct-insertion probe were heated from 50 to 450°C at 24°C/min.

A commercial electron-impact ion source was modified for FAB ionization by replacement of the ion block and repeller plate with a single assembly of open construction. Samples for analysis were dissolved in the matrix on a stainless-steel target stage and bombarded by a xenon atom beam (at 10 kV, 10 μ A) from an atom gun supplied by M-Scan Ltd., Ascot, Berks., U.K. Permethylated and non-derivatized oligosaccharides for positive-ion FAB were dissolved in an appropriate solvent (e.g. methanol, water or methanol/water) and 2.5–5 μ g introduced into a thin film of glycerol coated on the target. Peracetylated samples were dissolved in methanol and analysed in a thioglycerol/glycerol (1:1, v/v) matrix.

methyl glycoside (Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-OMe), and the EI mass spectrum (e) and the positive-ion FAB mass spectrum (f) of the permethylated reduced tetrasaccharide (Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-ol)

Negative-ion FAB spectra were acquired from non-derivatized oligosaccharides from either a glycerol or poly(ethylene glycol) (recommended by Rose *et al.*, 1983) matrix.

The Varian (Finnigan) SS200 data system was used for acquisition and processing of mass-spectral data. Mass calibration was established with a mixture of tris(perfluorononyl)-*s*-triazine and perfluoroalkane in the EI mode and permitted calibration up to mass 1500 at full accelerating voltage. Tris(perfluoroheptyl)-*s*-triazine and CsI₂ were employed for calibration under FAB conditions.

Results and discussion

Figs. 1(a) and 1(b) show the positive- and negative-ion FAB mass spectra respectively of the tetrasaccharide methyl glycoside consisting of alternating hexose and hexosacetamido sugars. The most informative fragment ion in the positive-ion spectrum (Fig. 1a) is the oxonium ion (m/z 366), which arises from cleavage of the glycosidic bond at the acetamido sugar:

The equivalent fragment ion m/z 731 formed by cleavage at the methyl glycoside end of the molecule occurs in low abundance:

Fragment ions formed by cleavage between the hexose and hexosacetamido residues, which would result in the charge being in the hexose ring, are of low intensity (m/z 163, 528 in Fig. 1a), and no fragment ions are formed that contain the methyl glycoside end of the molecule. Thus the positive-ion FAB spectrum gives limited structural information. In contrast, the negative-ion mass spectrum of the tetrasaccharide methyl glycoside (Fig. 1b) has three readily identifiable fragment ions, m/z 234, 396 and 599, formed by cleavage at the

glycosidic oxygen of both neutral and acetamido sugars, e.g.:

Hence a combination of positive- and negative-ion FAB spectra gives most of the fragment ions defining the sequence of this tetrasaccharide. Its M_r is inferred from the ion m/z 763 ($M + H$)⁺ in the positive-ion spectrum, which changes to m/z 761 ($M - H$)⁻ in the negative-ion spectrum.

The direct-probe EI spectrum of the permethylated tetrasaccharide methyl glycoside (Fig. 1c) has fragment ions of high abundance resulting from both ends of the molecule and it also contains characteristic ions formed by loss of methanol, m/z 912 ($M - 32$), m/z 432 (m/z 464 - 32) and m/z 187 (m/z 219 - 32). This single spectrum therefore gives more extensive structural information than the positive- and negative-ion FAB spectra of the underivatized molecule.

The positive-ion FAB spectrum of the permethylated tetrasaccharide methyl glycoside (Fig. 1d)

shows three ions related to the M_r , m/z 945 ($M + H$)⁺, m/z 969 ($M + Na$)⁺ and m/z 913 ($M + H - MeOH$)⁺, and two fragment ions, m/z 668 and m/z 464, formed by cleavage across the glycosidic bonds. The latter ion can be obtained by cleavage at either side of the middle glycosidic oxygen atom, although the presence of m/z 668 and the absence of m/z 260 (given in Fig. 1c in large abundance) might suggest that m/z 464 arises from the non-reducing end of the molecule. In comparison, the EI spectrum (Fig. 1e) of the oligosacchar-

ide Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-ol reduced before methylation has the fragment ions m/z 276, 480 and 725 arising from the reducing end of the molecule and the three ions m/z 219, 464 and 668 from the non-reducing end of the molecule, whereas only one ion from this last set (m/z 464) is readily identified in the positive-ion FAB spectrum (Fig. 1f).

The EI spectrum of the acetylated, reduced tetrasaccharide Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-ol (LNT-ol) shown in Fig. 2(a) also had all six fragment ions formed by cleavage at the glycosidic bonds (m/z 331, 618 and 906 from the non-reducing end of the molecule and m/z 375, 663, 950 including the reducing end). The positive-ion FAB spectrum of this alditol, as reported by Dell *et al.* (1983a), lacks the fragment ions 906 and 663 and has a molecular ion m/z 1298 ($M+H$)⁺.

The highest mass ions m/z 1179 and 1119 in Fig. 2(a) probably represent rearrangement ions involving loss of acetyl groups from the reduced terminal sugar ($M-58$ and $M-118$ respectively), but no molecular ion was observed. The EI spectrum in Fig. 1(e) also shows ions that appear to be related to

the M_r (m/z 915 and 830). These ions are proposed to be formed by the loss of one and two carbon fragments respectively as follows:

Ions of this type are useful for determining structural elements of the reduced terminal sugar and may provide information on M_r ; however, the principal advantage of the EI spectra is the predictable and abundant formation of ions from cleavage across the glycosidic bonds. Complete sequence information has been obtained on all types of oligosaccharides studied by EI of 2.5–5 μg of reduced permethylated or peracetylated deriva-

Fig. 2. Positive-ion FAB mass spectrum (a) of the peracetylated derivative of Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-ol and the negative-ion FAB mass spectrum (b) of its non-derivatized alditol

tives, whereas FAB mass spectrometry gives a variable abundance of diagnostic fragmentations, and in some cases increased sample size is required. For example, the negative-ion FAB spectra of the reduced tetrasaccharides Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-ol and LNT-ol (that for LNT-ol is shown in Fig. 2b) gave only a molecular ion ($M-H$)⁻ in comparison to the abundant fragment ions shown for the tetrasaccharide methyl glycoside (Fig. 1b). The positive-ion FAB mass spectra of the reduced tetrasaccharides were similar to that for the methyl glycoside (Fig. 1a) with a molecular ion and one diagnostic fragment ion (m/z 366) bearing the positive charge on the acetamido group (spectra not shown). Oligosaccharides having consecutive rather than alternating neutral and acetamido sugars, e.g. stachyose, chitotriose and chitotri-itol (spectra not shown), gave positive- and negative-ion FAB mass spectra that together had the possible fragment ions from both ends of the molecules formed by cleavage across the glycosidic bonds. However, these latter spectra could only be obtained on analysis of a 20 μ g sample, whereas 2.5–5 μ g was used for the other FAB mass spectra described. It can be deduced from these experiments that mass spectrometry of 5 nmol or less of certain unknown oligosaccharides, e.g. reduced samples or those containing alternating and consecutive neutral and acetamido sugars, may give inadequate information for structural elucidation.

An increased sample size for FAB mass-spectral analysis of stachyose has been reported previously (Clay *et al.*, 1983). Another documented case of sample-to-sample variability in FAB analysis involves oligosaccharides of the lacto-*N* series; whereas LNT-ol was analysed successfully as its acetylated derivative (Dell *et al.*, 1983a), the peracetylated mono- and di-fucosylated derivatives of LNT (fucopentaose I and difucohexose) were reported by those authors to require target heating to obtain positive-ion FAB spectra. Thioglycerol has since been recommended for FAB mass spectrometry of derivatized material (Dell *et al.*, 1983b). We have studied two smaller oligosaccharides related to LNT: Gal β 1-4GlcNAc and Gal β 1-3GlcNAc, as their reduced acetylated derivatives, using thioglycerol as matrix. A molecular ion and two fragment ions (m/z 331 and 374) were obtained on positive-ion FAB mass spectrometry of 3 μ g of material (results not shown).

Although further investigations are required before FAB mass spectrometry achieves general applicability, when successfully applied it can provide information additional to that achieved by EI. Importantly, FAB ionization usually gives ions related to the M , that in the positive-ion mode is a protonated molecular ion at mass ($M+H$)⁺ or a

cationized molecular ion such as ($M+Na$)⁺ or ($M+K$)⁺ and in the negative-ion mode a molecular ion at mass ($M-H$)⁻. The difference in the positive- and negative-ion FAB spectra confirms the assignment of M . FAB mass spectrometry is also important for the analysis of larger oligosaccharides (non-derivatized), where the increased M , resulting from derivatization may exceed the mass range of the instrument used. It is also appropriate for the analysis of oligosaccharides having substituents such as phosphate, sulphate and methyl groups which could be lost on derivatization. However, when peracetylated or permethylated derivatives are obtained during oligosaccharide purification (Valent *et al.*, 1980; Hounsell *et al.*, 1983, 1984; Egge *et al.*, 1983) or during the preparation of partially methylated alditol acetates for analysis of the position of glycosidic linkages (Björndal *et al.*, 1970; Stellner *et al.*, 1973), small samples of these (less than 5 nmol) will reliably give EI mass spectra with complete sequence information.

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