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Note

A rapid mass-spectrometric procedure for probing the non-reducing structures of lactosaminoglycan-containing glycoconjugates*

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Lactosaminoglycan is a novel type of carbohydrate structure present in both glycoproteins[1–3] and glycolipids[4]. It is composed of long chains of the β-Gal-(1→4)-GlcNAc unit, which may be modified by sialylation, fucosylation, branching etc. to afford determinants for a variety of antigens including[5,6] ABH, Ii and SSEA-1. Structural studies on erythroid and myeloid (granulocyte-monocyte) cells are indicating that different cell lineages and different stages of differentiation may be characterised by specific lactosaminoglycan structures[6–9]. We now report a rapid fast-atom-bombardment mass-spectrometric (f.a.b.m.s.) procedure for identifying the presence of these structures in intact glycopeptides and glycolipids.

The method is as follows: (*i*) intact glycoconjugates, irrespective of size, are converted into their permethylated derivatives; (*ii*) positive f.a.b. spectra in the 3000 mass range are obtained from the derivatised, intact glycoconjugate by using a high-field mass spectrometer operated at full sensitivity; no attempt is made to record the molecular ion, which may be as great as 15,000–20,000 mass units; and (*iii*) spectra are interpreted by assigning compositions to all major fragment-ions present in the 300–3000 mass range.

It is now well established that f.a.b.m.s. can be a valuable adjunct to classical chemical procedures in the structural analysis of oligosaccharides (see, *e.g.*, refs. 10–14). The novelty of the work described in this paper is that it allows the analysis of very much larger glycoconjugates than previously studied. There is no requirement for the molecular ion to be within the mass range of the mass spectrometer, and substances of molecular weight significantly greater than 10,000 daltons are amenable to analysis. Further, the method is designed to yield readily interpretable spectra containing structural information derived only from the non-reducing ends of the molecule. The latter is achieved by exploiting the known[10,13] fragmentation

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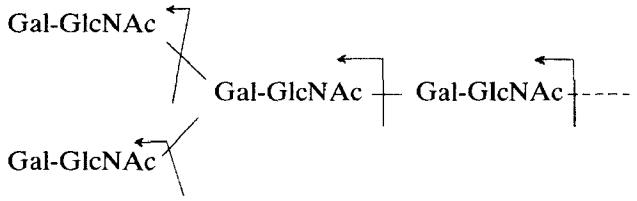
behaviour of permethylated carbohydrates containing amino sugars, *i.e.*, cleavage occurs preferentially at the aminoglycosidic linkages with charge retention on the non-reducing fragments. Thus, permethylated lactosaminoglycans cleave at each GlcNAc residue affording a "fingerprint" of fragment ions to each of which a unique composition of sialic acid, fucose, hexose, and hexosamine residues may be assigned.

We made the unexpected discovery that permethylated molecules as large as 15,000 daltons give excellent fragmentation data in the 3000 mass range during our studies on the Band 3 glycoproteins of human adult and fetal erythrocytes[8,9] and the lactosaminoglycan from human granulocytes[6]. From these studies, we have defined routine running conditions (see Experimental) and have established that very large molecules give reproducible fragmentation-patterns. The types of non-reducing structures present, *e.g.*, whether the chains are branched, the extent of sialylation, fucosylation etc., may be deduced from the spectra by noting which fragment ions are present and which are absent. The masses of the non-reducing termini present in lactosaminoglycans are 464, 638, ad 825 for HexHexNAc, FucHexHexNAc, and NeuNAcHexHexNAc, respectively. Increments of 174, 361, and 449 mass units are added to these non-reducing termini for additional Fuc, NeuNAc, and HexHexNAc, respectively. For example, an unbranched, monofucosylated lactosaminoglycan chain will give a series of ions at *m/z* 638, 1087, 1536, 1985, 2434 etc., whereas a non-substituted linear lactosaminoglycan affords the series *m/z* 464, 913, 1362, 1811, 2260 etc. Branching is indicated by the absence of signals from the relevant series. For example, structure I cleaves as shown to give the series *m/z* 464, 1362, 1811 etc., with *m/z* 913 being absent:



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We illustrate the technique with data obtained from sample A-1 examined during our studies on human granulocyte lactosaminoglycans[16]. The f.a.b. "map" of permethylated A-1 contained four series of ions, indicating that four types of lactosaminoglycan chain are present. These are (*i*) unsubstituted linear, giving *m/z* 464 ($HexHexNAc^+$), 913 ($Hex_2HexNAc_2^+$), and 1362 ($Hex_3HexNAc_3^+$); (*ii*) monofucosylated linear giving *m/z* 638 ($FucHexHexNAc^+$), 1087 ($FucHex_2HexNAc_2^+$), and 1536 ($FucHex_3HexNAc_3^+$); (*iii*) difucosylated linear giving *m/z* 1261 ($Fuc_2Hex_2HexNAc_2^+$), and 1710 ($Fuc_2Hex_3HexNAc_3^+$); and (*iv*) sialylated linear giving *m/z* 376 ($NeuNAc^+$), 825 ($NeuNAcHexHexNAc^+$), 1274 ($NeuNAcHex_2HexNAc_2^+$), and 1723 ($NeuNAcHex_3HexNAc_3^+$). The absence of

signals in the 2000–3000 mass range suggests that each branch is short, containing no more than three lactosaminyl groups. This conclusion was confirmed by a complete structure-determination of the lactosaminoglycan[16]. Further, the absence of signals at *m/z* 999, 1448, and 1897 indicated that chains containing both NeuNAc and Fuc are not present in sample A-1. Such ions were present in other samples from the glycoprotein[16].

Our f.a.b. procedure for "mapping" the antigenic moieties in glycoconjugates has the following key features:

(*1*) No prior knowledge is required of glycoconjugate size or structure.

(*2*) The method is rapid; after permethylation a complete m.s. analysis and interpretation can be completed in a few hours.

(*3*) Information is obtained on all non-reducing structures and heterogeneity can be readily recognised.

(*4*) Provided permethylation can be successfully performed, the size of the molecule remote from the non-reducing ends does not appear to affect the results. We anticipate that the procedure will be applicable to molecules considerably in excess of 15,000 daltons and may prove valuable for rapidly defining the type of structure present in polysaccharides made up of repeating units.

(*5*) Mixtures of glycoconjugates can be examined, although in such experiments it will not be possible to determine which component gives rise to which fragment ion.

EXPERIMENTAL

Glycopeptides and oligosaccharides were obtained as described[6,8,9,15,16] and permethylated by the Hakomori procedure[6,8].

F.a.b.m.s. was performed by using a VG Analytical ZAB-HF mass spectrometer fitted with an M-Scan f.a.b. gun. Xenon was used as the bombarding gas and the gun was operated at 10 kV, 18 μA. The mass spectrometer was operated at the full 8-kV accelerating voltage, thus ensuring maximum sensitivity. Linear, mass-controlled scans were obtained in the 3000–300 mass range at a scan rate set to cover the complete instrumental mass-range of 3300 m.u. in 300 sec. Spectra were recorded on oscillographic paper and manually counted.

Samples were dissolved in methanol (~10–20 μg/μL) and 1 μL of solution was carefully loaded into a 1:1 mixture of glycerol–monothioglycerol (1–2 μL) on the stainless-steel target. The probe was inserted into the ion-source region and the methanol allowed to evaporate. This was normally accompanied by co-evaporation of the monothioglycerol and, for successful f.a.b. results, it was necessary to withdraw the probe at this stage and add an additional 0.5–1.0 μL of monothioglycerol. After re-insertion of the probe, a scan was initiated from *m/z* 3000 and careful visual monitoring of the scope display was carried out while the spectrum was being recorded. With experience, it was possible to recognise when the quality of the spectrum began to deteriorate. At this point the scan was stopped, the probe

removed, and an additional 0.5–1.0 μL aliquot of monothioglycerol added. If sufficient sample was available, an additional aliquot was also added. After re-insertion of the probe, a new scan was started 50–100 m.u. above the most prominent ion present at the low-mass end of the first scan. If necessary, further re-loads were carried out until the complete mass range had been spanned.

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