## Note

# Fast-atom bombardment-mass spectrometry for the characterization of cello- and malto-oligosaccharides

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(Received July 8th, 1983; accepted for publication, September 7th, 1983)

Mass-spectrometric characterization of carbohydrates is routinely performed using electron impact or chemical ionization modes on the permethylated or peracetylated derivatives. These techniques, however, have a drawback, because they artificially increase the molecular weights and induce multifragmentation processes that frequently preclude their use for the direct characterization of mixtures of oligomers. The fast-atom bombardment-molecular beam, solid analysis technique (f.a.b.-m.b.s.a.), introduced by Devienne and Roustan<sup>1</sup> in 1966, and further developed through the work of Benninghoven<sup>2</sup> and Barber<sup>3</sup> and their coworkers for the characterization of labile and nonvolatile biopolymers<sup>4</sup>, has recently found application in the field of oligosaccharides, small polysaccharides, and glycolipids $^{5-8}$ . The underivatized, reducing oligosaccharides usually show with this technique in the positive recording mode, signals for the protonated, cationized  $(Na^+, K^+)$  species and, in some instances, for the terminal glycosyloxycarbenium ion, a signal at  $(M - 17)^+$ , together with fragment ions related to this positive species; whereas the ready acquisition of the molecular anion in negative-mode recording makes this technique particularly attractive for charged or acylated polysaccharides.

We now report the use of the f.a.b./m.b.s.a.-m.s. technique, in both the positive and negative modes, for the characterization of cello- and malto-oligosaccharides of interest in studies related to the chemical or enzymic hydrolysis of glycans, a main research activity of this laboratory<sup>9,10</sup>. In view of the importance of the cationized species in the appearance of pseudomolecular ions, a systematic study was further made with several metal salts.

### **RESULTS AND DISCUSSION**

Cello- and malto-oligosaccharides, originating from acetolysis<sup>11</sup> or enzymic hydrolysis of the corresponding biopolymers, were fractionated according to chain length on a Biogel P-2 column, leading to homogeneous oligosaccharides having d.p. 3 to 9.

Positive f.a.b./m.b.s.a. mass spectra of underivatized oligosaccharides in both series (see Figs. 1 and 2, respectively), recorded using a glycerol matrix, uniformly exhibited a weak pseudomolecular ion  $(M + H)^+$  with, in some instances, the simultaneous appearance of the corresponding cationized species  $(M + K)^+$  or  $(M + Na)^+$  that presumably arose from traces of metal salts in the sam-



Fig. 1. Mass spectra (f.a.b.<sup>+</sup>) of cello-oligosaccharides (d.p. 3–7) in a glycerol matrix (G), with and without addition of sodium iodide  $([G_n + H]^+ \text{ ions result from glycerol clusters.})$ 



Fig. 2. Mass spectra (f.a.b.<sup>+</sup>) of malto-oligosaccharides (d.p. 3-5, and 9) in a glycerol matrix (G), with and without addition of sodium iodide.

ple. Addition of sodium iodide to the matrix enhanced the latter signal and, in every case, this signal was predominant in all spectra.

Reduction of the free anomeric end-group in malto-oligosaccharides did not distinctly affect the quality of the resulting spectra and their previously described, characteristic ions (see Fig. 3), although the sensitivity appeared to be slightly enhanced. The pseudomolecular ions  $(M + H)^+$  for the investigated series (d.p. 4, 5, and 7–9) are well resolved in each case. Negative ion detection led, as well, to characterization of the expected pseudomolecular ions  $(M - H)^-$  in both the reduced and unreduced malto- or cello-oligosaccharide series in the absence of metal salts (see Fig. 4).

To ascertain the importance of adduct formation in the appearance of pseudomolecular, cationized ions, a systematic study was made for cellotriose (see Fig. 5) and maltopentaitol (see Fig. 6), using defined ratios of sodium or am-

Maltotetraitol



Fig. 3. Mass spectra  $(f.a.b.^+)$  of malto-oligosaccharide-alditols (d.p 4, 5, and 7-9) in a glycerol matrix (G).

monium chloride or both, in addition to the glycerol matrix. In both series, presence of the ammonium salt resulted in an enhancement of the pseudomolecular ion as its cationized species  $(M + NH_4)^+$  (see Figs. 5b and 6b). When both ammonium and sodium cations were added in equal proportions to the matrix, the signal arising from the pseudomolecular sodium adduct was, however, preponderant (see Fig. 5c) or even stood alone (see Fig. 6c). Negative-ion recording allowed, in the oligosaccharide as well as the oligosaccharide-alditol series, detection of the (M + Cl)<sup>-</sup>, in addition to the  $(M - H)^-$ , pseudomolecular ion (see Fig. 7a). Addition of cuprous chloride to cellopentaose, in the presence of ammonium hydroxide, led to detection of  $(M + H)^+$ ,  $(M + NH_4)^+$ , and  $(M + Cu)^+$  ions in the positive mode, with, however, predominance of the protonated species  $(M + H)^+$  (see Fig. 7b). This association behavior was also found in the negative recording mode for maltopentaitol with pseudomolecular ions at  $(M - H)^-$ ,  $(M + Cl)^-$ ,  $(M + CuCl)^-$ , and  $(M + CuCl + Cl)^-$ , and it may be of help in characterization of the molecular ions.

Application of these results to a mixture of malto-oligosacchariditols of d.p. 2 to 8, previously characterized by 3.5-MPa liquid chromatography (see Fig. 8a),

Cellotriose



Fig. 4. Mass spectra  $(f.a.b.^{-})$  of cello-oligosaccharides (d.p. 3 and 4) and malto-oligosaccharide-alditols (d.p. 4, 8, and 9) in a glycerol matrix (G).



Fig. 5. Comparative mass spectra (f.a.b.<sup>+</sup>) of cellotriose in a glycerol matrix (G) with variations in the nature of the added cation. [a, Cellotriose ( $20 \ \mu g$ ); b, cellotriose ( $20 \ \mu g$ ) + sodium chloride ( $10 \ \mu g$ ); and c, cellotriose ( $20 \ \mu g$ ) + sodium chloride ( $10 \ \mu g$ ) + ammonium chloride ( $10 \ \mu g$ ).]



Fig. 6. Comparative mass spectra (f.a.b.<sup>+</sup>) of maltopentaitol in a glycerol matrix (G) with variations in the nature of the added cation. [a, Maltopentaitol ( $20 \ \mu g$ ); b, maltopentaitol ( $20 \ \mu g$ ) + ammonium chloride ( $10 \ \mu g$ ); and c, maltopentaitol ( $20 \ \mu g$ ) + ammonium chloride ( $10 \ \mu g$ ) + sodium chloride ( $10 \ \mu g$ ).]

led to the spectrum in Fig. 8b which distinctly shows the pseudomolecular ions  $(M + H)^+$  up to d.p. 5. Addition of sodium iodide to the matrix enhances the intensity of the pseudomolecular ions  $(M + Na)^+$ , and allows characterization of components up to d.p. 7.

These results confirm the potential of the f.a.b./m.b.s.a.-m.s. technique for the characterization of homo-oligosaccharides, even as mixtures. The importance of adducts in the formation of pseudomolecular ions is emphasized, and variations that depend on the nature of the salt that is added to the glycerol matrix may be of interest for the precise determination of molecular weights. Although, in the present application of f.a.b./m.b.s.a.-m.s. to cello- and malto-oligosaccharides and their corresponding reduced end-group derivatives, no fragmentation pertinent to the carbohydrate sequence could be observed, it is noteworthy that this behavior is not a general rule in oligosaccharide series, as cleavage at interglycosidic oxygen atoms has been reported to occur in some instances<sup>5-8</sup>. This points up the need for further systematic work, in order to delineate more thoroughly the extent of application of these results to other types of oligosaccharide.

#### EXPERIMENTAL

*Materials.* — Cello-oligosaccharides were prepared from cotton linters (Schleicher–Schuell, Dassel, Germany; d.p.<sub>w</sub> 2700) by successive acetolysis and *O*-deacetylation according to ref. 11. Malto-oligosaccharides were a commercial preparation (Roquette S.A., Lille, France; ref. MDO5) arising from acid and enzymic hydrolysis of corn starch. Reduced malto-oligosaccharides were prepared by reduc-



Fig. 7. Comparative mass spectra  $(f.a.b.^+ \text{ or } f.a.b.^-)$  of a cello-oligosaccharide and three maltooligosaccharide-alditols in a glycerol matrix (G) with, accordingly, variation in composition (of ammonium, copper, and sodium salts). [a, With addition of ammonium chloride and ammonium hydroxide, and b, with addition of cuprous chloride and ammonium hydroxide.]

tion with sodium borohydride (20 mg) of the commercial malto-oligosaccharide sample (100 mg) in water (10 mL) during 12 h, followed by demineralization with Amberlite IR-120 ion-exchange resin, concentration, and co-evaporation with methanol containing 1% of hydrochloric acid (M).

Oligosaccharides were fractionated by gel-permeation chromatography in a column (4.5 × 150 cm) of Biogel P-2 (Biorad, U.S.A.; 200–400 mesh) at 65° with water<sup>12</sup>. The analytical liquid chromatography<sup>13</sup> of oligosaccharide-alditols (see Fig. 8a) used a Waters model 201 U/6000 apparatus equipped with a  $\mu$ Bondapak C<sub>18</sub> column (Waters); eluant, water; flow rate, 1 mL/min at room temperature; a differential refractometer detector (Waters model R 401); and a pressure of 3.5 MPa.





Fig. 8. F.a.b.<sup>+</sup> analysis (glycerol matrix) of a mixture of malto-oligosaccharide-alditols. [a, L.c. identification ( $\mu$ Bondapak C<sub>18</sub> column in water at 3.5 MPa); b. mass spectrum without added salt; and c, mass spectrum with addition of sodium iodide ]

Mass spectrometer. — Spectra were recorded by using a double-focusing Kratos-AEI MS50 mass spectrometer (Manchester) fitted with the usual 1.2-T magnet, having a mass range of 1-1300 u.m.a., and operated at the full accelerating potential (6 kV); an f.a.b. 11NF, Ion Tech atom-gun; and a MAT SS200 Finnigan (DEC-PDP 11-34) computer. The samples  $(5 \mu g)$  were usually dissolved in water (0.5  $\mu$ L), and the solution added to a drop of glycerol on a copper target. The gas used was xenon, and the gun was set to have a  $40-\mu A$  equivalent atom-beam of 7–8 keV (1 mA total current at the anode). In the systematic study of the influence of adducts on the formation of the pseudomolecular ions (see Figs. 5 and 6), the sample (20  $\mu$ g) in water (1  $\mu$ L) was added to the glycerol matrix (5  $\mu$ L), and a first spectrum was recorded; sodium chloride  $(10 \,\mu g)$  in water  $(0.5 \,\mu L)$  was then added, with recording of a new spectrum; then, ammonium chloride  $(10 \ \mu g)$  in water (0.5  $\mu$ L) was added, and a final spectrum was recorded. For the results on maltotriose (see Fig. 6), the sample (20  $\mu$ g) in water (1  $\mu$ L) was added to glycerol; a spectrum was recorded; then ammonium chloride (10  $\mu$ g) in water (0.5  $\mu$ L) was added, and another spectrum was recorded; sodium chloride (10  $\mu$ g) in water (0.5  $\mu$ L) was now added, and a final spectrum was recorded. Positive-ion mass spectra were recorded in the same sequence, but the target was cleaned between each recording.

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