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Note

Polysaccharide–lipid interaction analyzed by fast-atom-bombardment mass spectrometry

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Fast-atom-bombardment mass spectrometry[1,2] (f.a.b.–m.s.) is a convenient method for the determination of the molecular sizes and sequences of relatively large biological molecules[3], including oligosaccharides[4,5]. As demonstrated in the early studies and in more recent applications in which glycerol is used as the matrix, ion clusters of large mass are often observed, and a useful method of instrument calibration is based on the formation of cesium iodide cluster ions $[(CsI)_nCs^+]$ (ref. 3).

For several years, one of us has been interested in the interaction of long chain fatty acids with partially methylated lipophilic polysaccharides from mycobacteria[6]. These polymethylpolysaccharides coil up in the presence of appropriate fatty acids to form inclusion complexes with the lipid[7,8] that have dissociation constants on the order of 0.1 μM[9]. It seemed to us that such a complex might be observable by f.a.b.–m.s., and that this technique could be used to confirm the molecular order of the complex and to obtain further information about the specificity of the interaction. This report describes our investigations on the complex formed between the 3-*O*-methylmannose polysaccharide homolog having 12 hexose units (MMP-III) and alkyltrimethylammonium ions having decyl and hexadecyl alkyl chains.

RESULTS AND DISCUSSION

F.a.b. mass spectra were determined in the positive mode because a complex of MMP-III with alkyltrimethylammonium ion would carry the charge of the quaternary ammonium group, whereas the uncomplexed MMP-III can yield a positive ion, $M + H^+$, by protonation. The partial spectrum reproduced in Fig. 1A shows ions for MMP-III + H^+ at *m/z* 2131 and for the complex of MMP-III with hexadecyltrimethylammonium ion at *m/z* 2414, the latter ion having an even

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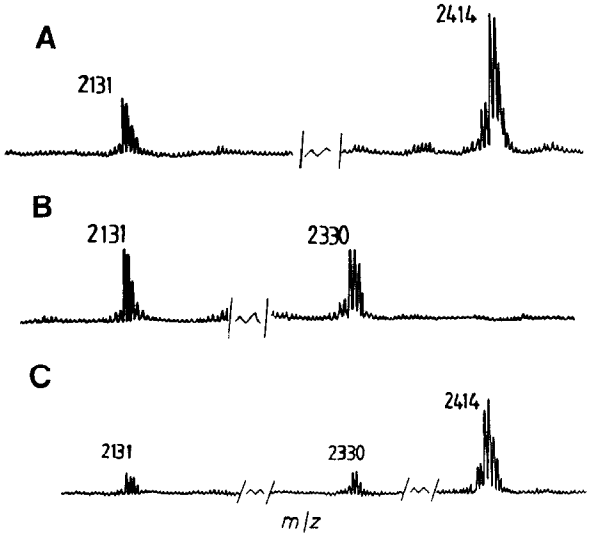

Fig. 1. Partial f.a.b. mass spectra for MMP-III/alkyltrimethylammonium mixtures. A, MMP-III and hexadecyltrimethylammonium bromide; B, MMP-III and decyltrimethylammonium bromide; C, MMP-III with decyltrimethylammonium bromide and hexadecyltrimethylammonium bromide. In all mixtures, each component was present at 1mM concentration.

number owing to the presence of a single nitrogen. The ratio of relative abundance of the two ions is about 7 to 1, in favor of the complex, although this value may not reflect the true ratio of the two components in the mixture because it is unlikely that the two ions would be formed with equal ease. The polysaccharide–lipid complex carries a charge even in the glycerol matrix, whereas the uncomplexed MMP-III must be protonated in order to develop a charge. No ion was observed at higher or lower mass that would be consistent with higher order complexes, which confirms the earlier conclusion that the interaction is highly specific for the formation of a 1:1 molar complex[9].

A partial spectrum for the MMP-III/decyltrimethylammonium mixture is shown in Fig. 1B, and again ions for uncomplexed MMP are observed. A major difference is seen in the ratio of the two, however, which suggests that the complex with the C_{10} alkyltrimethylammonium ion is weaker than that with the C_{16} derivative. This result was expected, because it was previously demonstrated by fluorimetric titration with parinaric acid that MMP-III forms a tighter complex with the C_{16} than with the C_{10} acyl-coenzyme A derivative[9]. Thus, there is a clear lipid chain-length dependence for the interaction. A polysaccharide chain-length dependence has also been demonstrated[9], but that feature was not investigated in the present study.

Under appropriate conditions, MMP would be expected to form an adduct with tetramethylammonium ion to yield an ion at $[M + 74]^+$ since adducts with ammonium ion itself are often observed in the positive-ion spectra of such compounds. In fact, at a 1:1 molar ratio of MMP-III to tetramethylammonium chloride

no such ion was seen, and only when the ratio was raised to 1:10 was an ion observed at *m/z* 2204 equivalent in intensity to about 10% of the ion at $[M + H]^+$. However, the important point is not that ammonium ion adducts with carbohydrates occur, but rather that in the present example the intensity of the complex ion relative to the ion at $[M + H]^+$ is dependent on the chain length of the alkyl group of the alkyltrimethylammonium ligand. That this observation is significant is supported by the fact that the data are consistent with previous studies on the dissociation constants of MMP-III and other lipids[9].

Competition between C_{10} and C_{16} alkyltrimethylammonium ions for MMP was investigated by analyzing a mixture of the three components at a concentration of 1mM each. In this study, it seemed probable that the MMP–lipid complexes with the two different alkyl derivatives would be very similar in their properties and should produce ions with equal ease although not in equal amounts. The spectrum

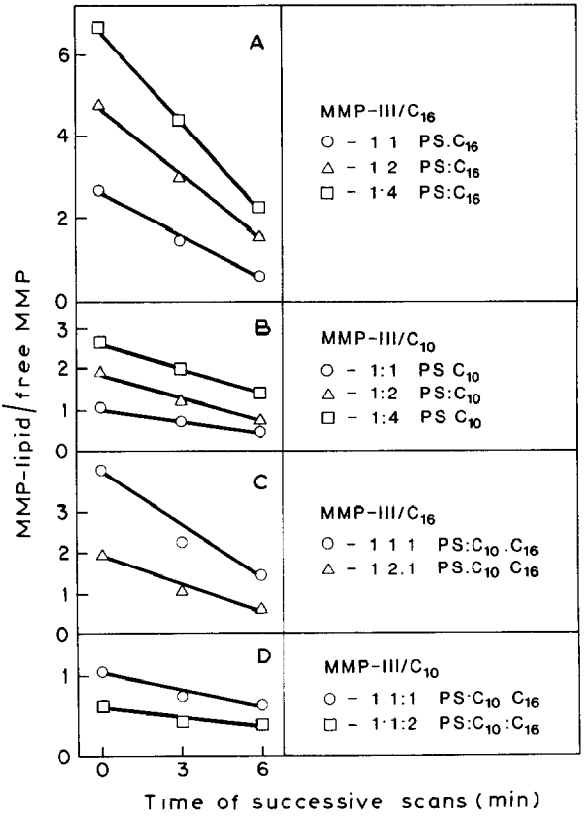

Fig. 2. Partial f.a.b. mass spectra showing time-dependent changes in the ratio of MMP-III–lipid to MMP ion abundance. A, Decrease in the ratio of MMP-C_{16} to MMP ions in mixtures of MMP and C_{16} derivative; B, decrease in the ratio of MMP-C_{10} to MMP ions in mixtures of MMP and C_{10} derivative; C, decrease in the ratio of MMP-C_{16} to MMP ions in mixtures of MMP, C_{10} and C_{16} derivatives; and D, decrease in the ratio of MMP-C_{10} to MMP ions in mixtures of MMP, C_{10} and C_{16} derivatives. Symbols are identified on the figure. PS = polysaccharide.

in Fig. 1C shows that the signal for the complex with the C_{16} derivative preponderates, a result that is consistent with the studies on the individual mixtures and with the fluorimetric titration[9]. Because the concentrations are far above the known dissociation constant of the MMP-III/palmitate complex, a valid constant can not be derived from these data. F.a.b.–m.s. has been used, however, to determine the dissociation constants of weak acids[10].

Time-dependent changes in the ratio of MMP–lipid complexes to uncomplexed MMP were investigated by making scans at three minute intervals of samples on the probe as it was kept in the atom beam within the instrument source. In all instances, the ion ratio for the MMP–C_{16} complex decreased slightly faster (12%/min) than that for the MMP–C_{10} complex (8%/min) (Fig. 2). It is commonly observed that the ions for substances that differ greatly in mass or in chemical structure may decay at different rates, but in this instance the structures and sizes of the MMP-lipid complexes are similar. Some difference in structure may exist, however, if the weaker complex of MMP–C_{10} reflects a less tightly coiled polysaccharide and the stronger complex with the longer lipid chain a more tightly coiled polysaccharide. Such a difference in structure could affect the interaction of the complexes with the glycerol matrix and, thus, the rates at which the two different complex ions leave the surface of the matrix, the rates at which the ions are replaced at the surface of the matrix, and the rates at which the uncomplexed MMP reacts with free lipids to replace the ionized species.

EXPERIMENTAL

Materials. — Decyltrimethylammonium bromide (M_r = 280) was from Kodak and hexadecyltrimethylammonium bromide (M_r = 364) was from Sigma. The first compound was recrystallized repeatedly from acetone. *Anal.* Calc. for $C_{13}H_{30}NBr$: C, 55.71; H, 10.79; N, 5.00. Found: C, 55.70; H, 10.60; N, 4.91. The latter compound was recrystallized from water. *Anal.* Calc. for $C_{19}H_{42}NBr$: C, 62.62; H, 11.62; N, 3.84. Found: C, 62.62; H, 11.49; N, 3.80. Each compound gave a single positive molecular ion in f.a.b.–m.s. that corresponded to the calculated mass. Methylmannose polysaccharide isomer III (MMP-III), which has the structure Man-(3MeMan)$_{11}$-OCH_3 (M_r = 2130)[11], was isolated from *Mycobacterium smegmatis* and purified by gel filtration and by high-pressure liquid chromatography[11,12].

Methods. — Aqueous solutions (~20 mL) of both lipids were prepared at a concentration of 2.0 μmol/mL. The MMP-III solution was prepared by dissolving the polysaccharide (~7 mg) in water so that the solution contained 1 μmol/mL, based on determination of the sugar by the phenol–sulfuric acid method[13], with methyl α-D-mannoside as the standard.

Complex formation between polysaccharide and lipid occurred immediately after mixing the two solutions, as evidenced by the disappearance of the detergent effect of the free lipid. Portions of these solutions, 1–2 μL, were loaded into about

2 μL of glycerol on the probe target, and the positive-ion f.a.b. mass spectra were recorded on an oscillograph coupled to a VG Analytical ZAB 1F High-Field Magnet mass spectrometer[4]. Masses were determined by counting the spectral lines on the print-out. The first scan was made as soon as possible, usually within 1 min after inserting, and repetitive scans were made at 3-min intervals over the range 2500 to 2000 mass units. For more detailed analysis, some spectra were scanned from 3100 to 0 mass units.

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