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Dear Sir,

Fast Atom Bombardment Mass Spectrometry of Carbohydrates Contaminated With Inorganic Salts Using a Crown Ether

Carbohydrates represent an important class of biological molecules that serve numerous biological functions in both the plant and animal worlds. There has been great interest for a long time in elucidating the structures of carbohydrates, which requires knowledge of several parameters such as sugar sequence, type of linkage between monosaccharide subunits, anomeric configuration and identification of the reducing end. The invention of the fast atom bombardment (FAB) ionization technique[1] in the early 1980s revolutionized the mass spectrometric (MS) analysis of polar, non-volatile and thermally fragile carbohydrate molecules.[2,3] Although FABMS does not give complete structural information on the analyte in many instances, the intact molecular ions generated by FAB enable one to perform tandem MS on molecular ions to probe the primary structures of carbohydrates.[4]

One of the primary difficulties typically encountered in FABMS analysis of oligosaccharides results from the presence of inorganic salts introduced during isolation of the oligosaccharides. For example, the closely related oligosaccharides obtained from the enzymatic digestion of complex carbohydrates found in the cell walls of plants and the glycans released from glycoproteins are often separated by high-pH anion-exchange chromatography (HPAEC), a technique that involves dissolving the oligosaccharides in concentrated inorganic salt solutions.[5] These contaminating salts suppress the molecular ion, $[M + H]^+$, and promote the formation of alkali metal ion adducts, i.e. $[M + Na]^+$, $[M + K]^+$, $[M + 2Na - H]^+$, etc.[6] Consequently, a signal from the protonated molecule may not be observed in the spectrum. These adduct ions may lead to incorrect molecular mass assignments for the analyte carbohydrates and reduce the signal-to-noise ratio of the sample ions by spreading their ion current over multiple peaks. The absence of the $[M + H]^+$ ion from the sample forces the researcher to perform MS/MS on one of the alkali metal adducts, even though such tandem mass spectra are complicated and difficult to interpret owing to the double-bond cross-ring cleavages that are promoted by the alkali metal ion.[7]

Several techniques have been developed to minimize the difficulties arising from the presence of contaminating salts in carbohydrate samples prior to FABMS analysis, including the use of an ion inclusion resin (Kryptofix),[8] gel filtration[9] and chemical derivatization[10] methods to remove salts. However, these techniques are time consuming and prone to sample loss. A simple method has been reported to alleviate the detrimental effects of inorganic salts during the MS analysis of biological samples by adding crown ethers to the FAB matrix.[11,12] Here we report the results of our investigation on using a crown ether with FAB matrices to desalt carbohydrates directly on the FAB probe tip and discuss the advantage of this technique in the MS/MS analysis of carbohydrates.

The FAB mass spectrum of maltoheptaitol is depicted in Fig. 1(a). The presence of dominant $[M + Na]^+$, $[M + 2Na - H]^+$, $[M + 3Na - 2H]^+$ and $[M + 2Na + K - 2H]^+$ adduct ions in the FAB spectrum reveals the extent of contamination of the maltoheptaitol sample with inorganic salts. When 18-crown-6 was added to the FAB matrix, the abundance of alkali metal adduct ions was substantially decreased and the $[M + H]^+$ ion dominates the spectrum [Fig. 1(b)]. This is attributed to the fact that 18-crown-6 has a higher alkali metal ion affinity than carbohydrates have, facilitating the formation of the $[M + H]^+$ ion. The addition of 18-crown-6 improves the signal-to-noise ratio of the $[M + H]^+$ ion by increasing this ion's abundance by a factor of approximately five compared with its abundance before the addition of 18-crown-6 [Fig. 1(a)]. Similar enhancements of the signal-to-noise ratio of $[M + H]^+$ ions by the use of a crown ether with the FAB matrix were observed with various underivatized, permethylated and peracetylated carbohydrate oligomers.

In the course of our experiments to desalt the contaminated carbohydrates on the FAB probe tip, we noted that the abundances of the $[M + H]^+$ ions produced upon addition of 18-crown-6 allows one to perform MS/MS on the $[M + H]^+$ ion to determine the carbohydrate sequence. Studies of the MS/MS analysis of carbohydrates revealed that the spectra obtained from alkali metal adduct ions are more informative than those obtained from the $[M + H]^+$ ions owing to cross-ring cleavages, but it is also very difficult to interpret such spectra if the compound is an unknown. In contrast to the alkali metal adducts, the $[M + H]^+$ ions undergo simple glycosidic bond cleavages from which the non-stereochemical sequence can be deduced readily, and this complements the data obtained from the alkali metal adducts.

To demonstrate the advantage of using a crown ether in the MS/MS analysis of carbohydrates we chose an unknown carbohydrate moiety derived from the enzymatic digestion of xyloglucan, a complex carbohydrate found in plant cell walls.[13] The FAB mass spectrum of peracetylated xyloglucan oligoglycosyl alditol (pxoa) moiety is shown in Fig. 2. Although the xyloglucan moiety is peracetylated, abundant sodiated ions are detected in the FAB mass spectrum of pxoa [Fig. 2(a)]. The addition of a crown ether produced an abundant $[M + H]^+$ ion [Fig. 2(b)] whose signal-to-noise ratio is approximately ten times higher than that in Fig. 2(a) and allowed the molecule to be sequenced by MS/MS of the $[M + H]^+$ ion of the xyloglucan moiety. For comparison, the tandem mass spectra of both the $[M + Na]^+$ and $[M + H]^+$ ions of pxoa are shown in Fig. 3(a) and (b), respectively. The facile cleavage of glycosidic bonds in the case of the $[M + H]^+$ ion yields structurally informative ions from which the glycosyl sequence of the xyloglucan oligomer can be readily deduced [Fig. 3(b)]. The fragment ions at *m/z* 1267 and 547 in Fig. 3(b) unambiguously locate the positions of the monopentosyl and dipentosyl side chains in the pxoa. On the other hand, the $[M + Na]^+$ ion gives many fragment ions that are concentrated in the high-mass region of the spectrum and are difficult to interpret in terms of the sequence of pxoa [Fig. 3(a)].

Thus, we have demonstrated that the use of a crown ether with the FAB matrix is a powerful technique for removing contaminating salts that complicate the FABMS analysis of carbohydrates. The ease with which this technique can be used gives it wide applicability in the analysis of those carbohydrates that may become contaminated with alkali metal

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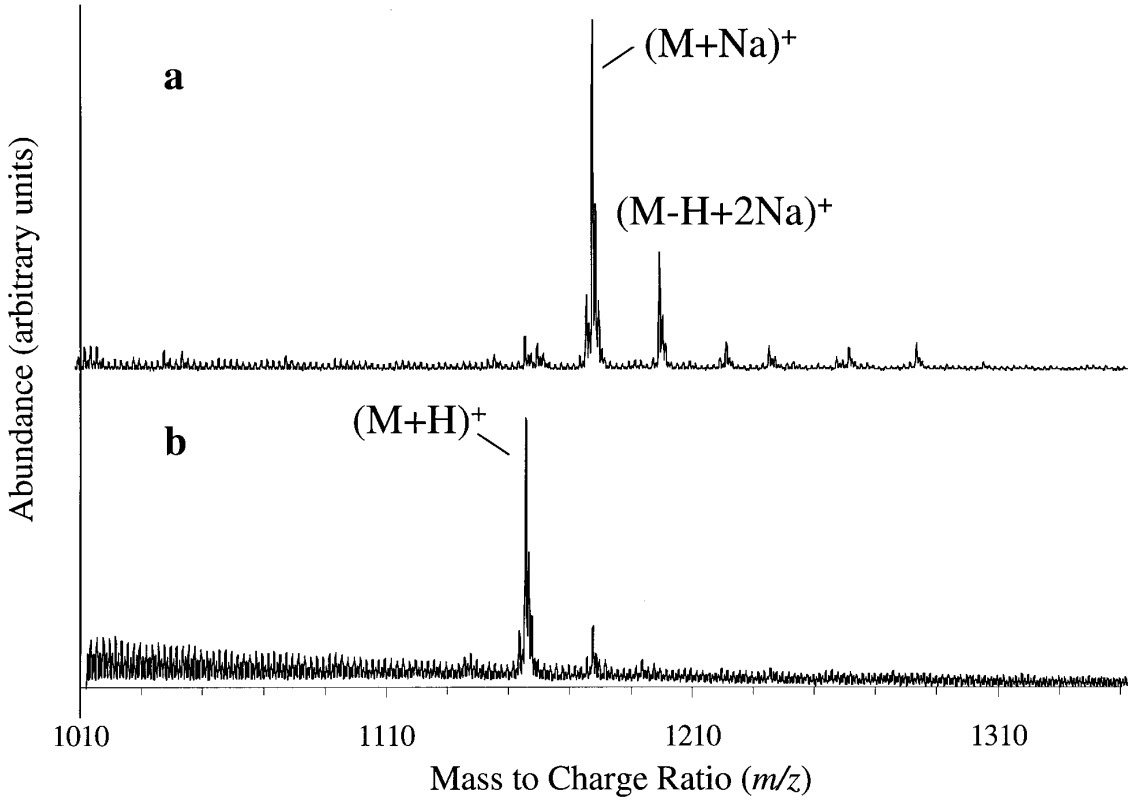

Figure 1. FAB mass spectra of maltoheptaitol (a) without and (b) with crown ether.

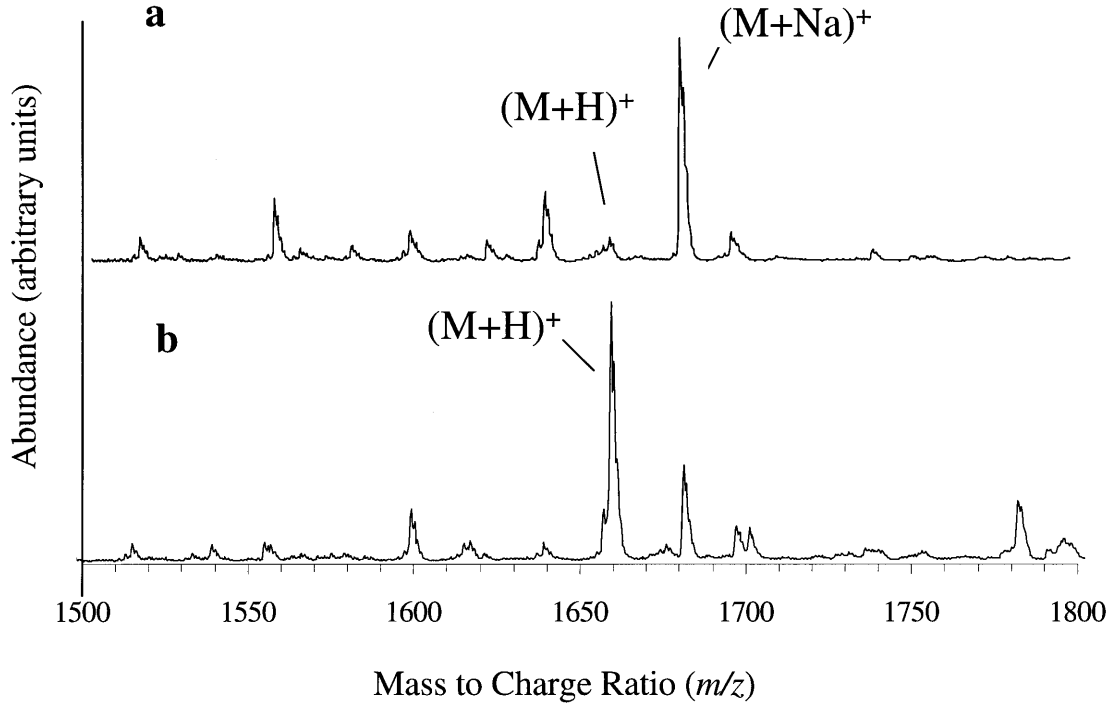

Figure 2. FAB mass spectra of a peracetylated xyloglucan oligoglycosyl alditol (a) without and (b) with crown ether.

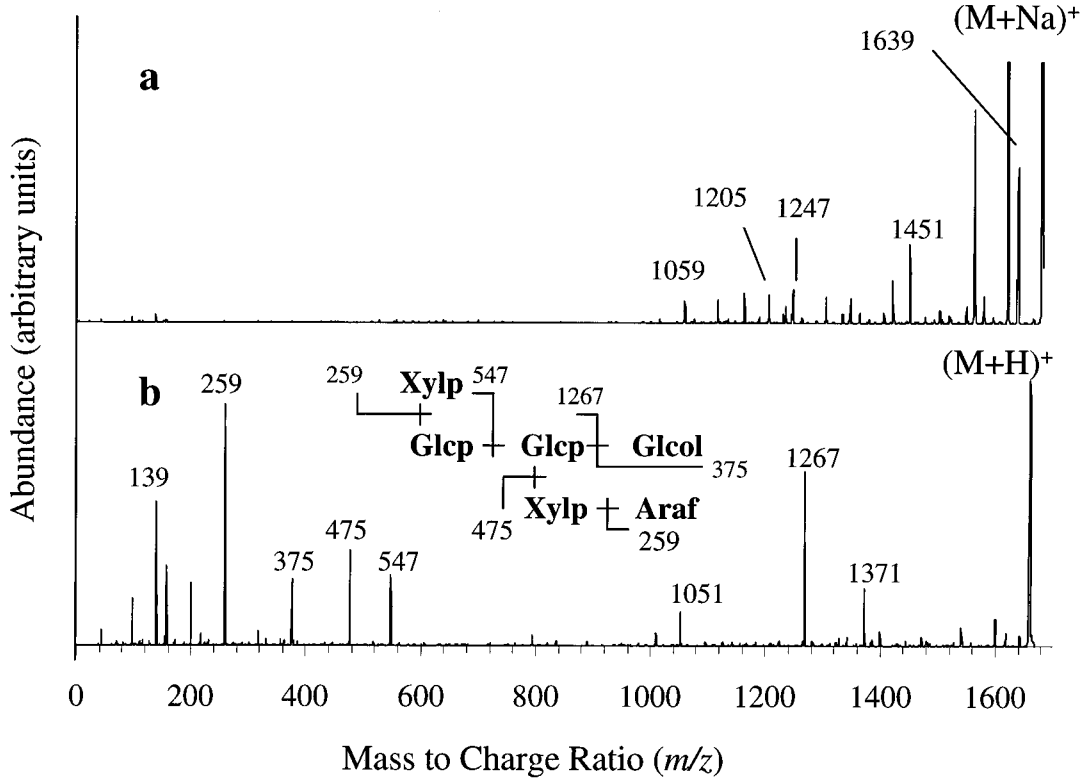

Figure 3. FAB tandem mass spectra of the (a) $[M + Na]^+$ and (b) $[M + H]^+$ ions of a peracetylated xyloglucan oligoglycosyl alditol.

salts during sample preparation. As this technique does not involve additional purification procedures typical of the conventional desalting approach, it reduces the time required for analysis and keeps sample losses to a minimum. The FAB mass spectra obtained before and after the addition of the crown ether can assist in assigning the correct molecular masses to the carbohydrates, and both spectra can be recorded with one sample loading. The facile formation of $[M + H]^+$ ions accomplished by adding a crown ether to the FAB matrix makes it possible to perform MS/MS on the $[M + H]^+$ ion, providing a fragment ion spectrum that can be readily interpreted in terms of the primary structure of the biomolecule.

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Yours,

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