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Inclusion of Alkali Metal Ions by Permethylated Inulin Studied by Electrospray Ionization Mass and Multinuclear Nuclear Magnetic Resonance Spectrometric Approaches

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Inclusions of alkali metal ions by permethylated inulin (Melu) were examined by electrospray ionization (ESI) mass spectrometry and multinuclear nuclear magnetic resonance (NMR) spectroscopy. Complexes of Melu with metal ions were observed as multicharged ions in ESI mass spectra. The number of fructofuranose units of Melu binding to one metal ion was 12, 6, and 7 for Li⁺, K⁺, and Cs⁺ respectively, on the basis of ESI mass spectra. However, the number estimated on the basis of multinuclear NMR spectra was 2 and 3 for Li⁺ and K⁺ respectively. The binding ability of Melu to Li⁺ is very weak, so metal ions dissociate from the complex in the ionization process. As a result, the ratio of fructofuranose units to Li⁺ ions might have been underestimated in excess. In the case of K⁺ and Cs⁺, the number of fructofuranose units binding a metal ion is found to be in good agreement with that of the oxyethylene units of crown ethers in solution.

1. Introduction

Electrospray ionization (ESI) is one of the most useful ionization methods in mass spectrometry (MS), since solution-state samples are directly injected into the instruments, and biomacromolecules and supramolecular complexes involving non-covalent bonds are ionized easily. However, it is not clear whether binding ability or selectivity in non-covalent complexation by multiple species is reflected in the peak intensity of the corresponding complex ions in ESI mass spectra. Nonetheless, the binding abilities of biomacromolecules such as proteins and deoxyribonucleic acid (DNA) have often been examined by ESI mass spectrometry (ESIMS). Researchers have also reported that the relative association constants in solution agreed with the relative peak intensity in ESI mass spectra of complexes of small molecules such as crown ethers and cryptands with metal ions. In good agreement with the relative peak intensity of small chiral molecules in the solution state was estimated in excess. In the case of K⁺ and Cs⁺, the number of fructofuranose units binding a metal ion is found to be in good agreement with that of the oxyethylene units of crown ethers in solution.

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in ESI mass spectra was nevertheless not reflected in the concentration ratio of complex ions in the solution state.

In this study, permethylated inulin (MeInu, Chart 1) was selected as a biomacromolecule derivative, and its complexes with alkali metal ions were observed by ESIMS to examine the intermolecular interactions. When using ESIMS, it is very important to consider the selectivity of complex ions in biomacromolecules to clarify whether disagreements of the molecular recognition ability in solution with that estimated by ESIMS in the chiral recognition complexation systems are one of exceptional cases.

Inulin is one of the storage polysaccharides and is found in the roots and rhizomes of more than 36,000 species of plants such as chicory and agaves. The polysaccharide consists of β(2→1)-linked d-fructofuranose units and α-d-glucopyranose at the reduced end, and the backbone includes a polyethylene glycol structure, which is known to have high binding affinity for cations via charge–dipole electrostatic interactions. In general, polyethylene glycols have a helical structure, and cations bind to the oxyethylene units of the backbone including a polyethylene glycol complete proton-decoupling 13C-NMR technique under pulse sequence conditions to avoid the nuclear Overhauser effect. The 13C-NMR spectra of a MeInu solution in chloroform-d (45.2 mM, 0.600 mL) were measured under the following conditions: instrument, JEOL JMN Lambda 600; NMR frequency (FR), 150.02 MHz; acquired time (AQ), 3.277 s; acquired data points (ADP), 32768; resonance line width (RLW), 10,000 Hz; accumulation times (AT), 2048. This DP value was applied in later experiments.

2. Experimental

2.1 Materials

Commercial products of alkali metal salts, LiSCN·(H2O)2 (Kisida), KSCN (Wako), and CsSCN (Katayama Chemicals) were used without further purification. These metal thiocyanates contain a small amount of ammonium salt (<0.003%) as an impurity.

Permethylated inulin (MeInu) was prepared from free inulin (Wako) by the Hakomori method. A solution of inulin (0.6 g) in dimethylsulfoxide (DMSO) was added to a slight excess of dimethylsulfinyl carbamions, which had been prepared from DMSO and NaH, under Ar with stirring. After stirring for 4 h, the solution was cooled below 10°C using an ice bath, and methyl iodide (3 mL) was added dropwise. The mixture was allowed to stand at room temperature, and stirring was continued overnight. After extraction with CHCl3, the organic layer was washed with a Na2S2O3 aqueous solution and water; then, it was dried with a Na2S2O3 powder (0.64 g, 81% with a MW = 7378, see 82.2). 1H-NMR (400 MHz, CDCl3) δ 4.08 (d, 1H, J = 7.0 Hz, F3), 3.85 (m, 1H, J = 16.7 Hz, F5), 3.74 (s, 2H, F1, 1), 3.71 (t, 1H, J = 7.0 Hz, F4), 3.54 (m, 2H, F6, 6'), 3.48 (s, 3H, CH3), 3.43 (s, 3H, CH3), 3.35 (s, 3H, CH3). 13C-NMR (100 MHz, CDCl3) δ 83.9, 84.9, 78.2, 73.2, 64.4, 58.9, 58.3, 58.0. The proton signals related to the glucopyranose moiety were relatively very small and overlapped with the signals related to the fructofuranose moieties to be assigned, except the anomeric position (1H-NMR, δ 5.46 ppm; 13C-NMR, δ 89.1 ppm).

Polyethylene glycol 1540 (degree of polymerization, 34) was also permethylated in the same manner.

2.2 Determination of the average degree of polymerization (DP) of MeInu

As MeInu is a mixture of several polymers having different degrees of polymerization (DP), the averaged DP must be determined first in all experiments. The average DP was estimated to be 36 (average molecular weight, 7378) on the basis of the relative integral value of the C2 signal of the fructofuranose moieties to the C1 signal of the glucopyranose moiety in the NMR spectrum. The spectrum was measured using a complete proton-decoupling 13C-NMR technique under pulse sequence conditions to avoid the nuclear Overhauser effect. The 13C-NMR spectra of a MeInu solution in chloroform-d (45.2 mM, 0.600 mL) were measured under the following conditions: instrument, JEOL JMN Lambda 600; NMR frequency (FR), 150.02 MHz; acquired time (AQ), 3.277 s; acquired data points (ADP), 32768; resonance line width (RLW), 10,000 Hz; accumulation times (AT), 2048. This DP value was applied in later experiments.

2.3 ESIMS

2.3.1 ESIMS conditions

Mass spectra were obtained using a Mariner HM Biospectrometry instrument (Applied Biosystems) and the data were processed using Biospec Data Exp software. Calibration was carried out with reserpine (m/z, 609). The measurement conditions were as follows: spray tip voltage, 3 kV; scan interval, m/z 0.1; spray flow rate, 3.0 μL min⁻¹; mass range m/z, 200–4000; capillary tempera-
ture, 140°C; nebulizer gas N₂, 0.2 L min⁻¹.

2.3.2 Preparation of sample solutions The sample solutions were prepared by the following procedure. (1) A 0.77 mM solution of a host (H) in chloroform was prepared (solution A). (2) A 20 mM solution of a metal thiocyanate (M⁺SCN⁻) in methanol was prepared (solution B). (3) 200 μL of solution A, 100 μL of solution B, and 1.00 mL of chloroform were mixed and these mass spectra were measured. The concentrations of the species in the final mixed solution were as follows: [H] = 0.119 mM, [M⁺SCN⁻] = 1.54 mM, [M⁺SCN⁻]/[H] = 13.

2.4 Multinuclear NMR experiments

2.4.1 NMR measurement conditions ¹H, ⁷Li, and ¹³C-NMR spectra were taken with a Brucker ARX 400 NMR spectrometer. Each NMR spectrum was measured under the following conditions: (1) ¹H-NMR, FR 400.1 MHz, AQ 3.834 s, ADP 32768, RLV 4132.23 Hz, AT 16, pulse width 5.000 s; (2) ⁷Li-NMR, FR 155.5 MHz, AQ 1.049 s, ADP 16384, RLV 7812.50 Hz, AT 128; (3) ¹³C-NMR, FR 18.7 MHz, AQ 0.1721 s, ADP 4096, RLV 11904 Hz, AT 2246.

2.4.2 Preparation of sample solution for ⁷Li and ¹³C-NMR measurements A solution of a complex of Melnu with a metal ion in chloroform-d was prepared by treating metal thiocyanate in the host solution with a supersonic homogenizer for over 5 min. In this treatment, metal thiocyanates, which are insoluble in chloroform, are extracted with chloroform for complexation with Melnu. The concentration of metal thiocyanate in the prepared solution was estimated on the basis of the concentration of the standard solution in the inner tube of the double-layer external reference system tube (610 mm, Wilmad). A typical example of a solution of Melnu with LiSCN is as follows: LiSCN·middot; (H₂O)₆ (61.2 mg) was added to a 2.76 mM solution of Melnu in chloroform-d, and the suspension was treated with a supersonic homogenizer. The supernatant was dried over a molecular sieve 4A and its ¹H-NMR spectra was measured. A 1.02 M solution of LiSCN in D₂O was used as the external standard for quantitative analysis. The relative peak area between the external standard in the inner tube and a 2.72 mM solution of LiSCN in acetonitrile in the outer tube was referred to estimate the concentration of the sample solution. A solution to measure the ¹³C-NMR spectra was prepared in same manner ([H] = 2.52 mM).

2.4.3 ³⁵K-NMR titration A 0.216 M KSCN solution in pyridine-d₅ was prepared. A 2 mL volume of this solution was placed into a 10 mm NMR tube. Melnu was added stepwise to the solution, and ³⁵K-NMR spectrum was measured six times at 243 K. The concentration ratios [H]/[KSCN] were 0, 0.016, 0.029, 0.052, 0.081, 0.12, and 0.16. The concentration of free K⁺ in each solution was estimated directly from the peak area. The concentration of the complexed K⁺ in each solution was calculated from the difference in concentration of free K⁺ between the initial solution and each solution.

3. Results and Discussion

3.1 ESI mass spectra of Melnu complex with metal ions

3.1.1 Dependency of complex ion peak types on metal ions Typical mass spectra are shown in Fig. 1. The complex ions of the host (H) with the guest (M⁺SCN⁻) were observed as three typical types of peaks, (H+nM⁺)⁺, (H+nM⁺+SCN⁻)ⁿ⁻, and (H+(n−1)M⁺+2SCN⁻)ⁿ⁻⁻. The number of included metal ions in a complex ion peak. Each peak was assigned on the basis of peak intervals and the relative abundance of isotope peaks. The natural abundance of isotopes was calculated using the software package Bunshiro. The numerator and denominator in Fig. 1 represent the DP of the host and the number of included metal ions, respectively. The total peak intensities of each type of complex ion is shown in Fig. 2.

In the case of a LiSCN guest, a small amount of complex ions of Melnu with ammonium ions, which exist in the commercial products of metal thiocyanates as an impurity, was observed. In the case of LiSCN and KSCN guests, complex ions, including one or two counter anions (SCN⁻), were observed. In the case of a CsSCN guest, these complex ions were barely observed. The ionic radii of the given alkali metal ions are in the following order: Cs⁺ (1.87Å) > K⁺ (1.33Å) > Li⁺ (0.60Å). The charge densities of the metal ions are in the inverse order. The larger the charge density is the stronger the electrostatic interaction with the counter anion. Therefore, complex ions including counter anions are produced in the case of Li⁺ and K⁺ guests.

As shown in Fig. 3, cyclic polyethers (Chart 2) show ion size selectivity toward alkali metal ions depending upon the cavity size of their molecular centers. Small cavities in macromolecules such as Melnu with conformational changes, are very rigid and their formation is entropically unfavorable. Thus, larger metal ions bind more strongly to Melnu. With respect to the strong binding ability to Melnu and small interactions with the counter anion, only complex ions consisting of Melnu and metal ions were observed in the case of a Cs⁺ guest. The NH₄⁺ ion is of the same size as K⁺. The binding ability of NH₄⁺ to Melnu is expected to be larger than that of Li⁺. Therefore, the complex ions of Melnu with NH₄⁺, which is present as an impurity in the commercial product of LiSCN, were observed in the mass spectra.

3.1.2 Influence of side chains upon complexation In order to examine the influence of side chains (the furanose ring moieties) on the complexation, the ESI mass spectra of Melnu with KSCN were compared with those of MePeg with KSCN (Chart 3). The peak intensity of the complex ions with K⁺ is plotted against the DPs of Melnu at each charge of the complex ion (Fig. 4). The intensities are the simple sum of intensities for all types of complex ions. The complex ions of Melnu with a maximum of 8 K⁺ ions were observed, and the DP was wide and abnormal distribution at each charged complex ion. On the other hand, the mass spectrum of MePeg with KSCN was very simple, as shown in Fig. 1 (d). The complex ions of MePeg included a maximum...
Fig. 1.  ESI mass spectra of complex ions of hosts with metal ions. (a) Host, \textit{MeInu}; metal ion, K\textsuperscript{+}. (b) Host, \textit{MeInu}; metal ion, Cs\textsuperscript{+}. (c) Host, \textit{MeInu}; metal ion, Li\textsuperscript{+}. (d) Host, \textit{MePeg}; metal ion, K\textsuperscript{+}. The counter anion was thiocyanate (SCN\textsuperscript{−}). Each expanded spectrum is shown in each second row. The assignment of each complex ion is represented as X/Y. X is the degree of polymerization of the host, and Y is the charge. (1), (2), and (A) are (H\textsubscript{n}M\textsuperscript{+}SCN\textsubscript{n−1})\textsuperscript{+}, (H\textsubscript{n}M\textsuperscript{+}2SCN\textsubscript{n−2})\textsuperscript{+}, and (H\textsubscript{n−1}M\textsuperscript{+}NH\textsubscript{4})\textsuperscript{+}, respectively.
of 4 K$^+$ ions, and the DP had a normal distribution (Fig. 5). The reason for the wide DP is that \textit{MeInu} itself has a wide DP by nature. The inclusion of a larger number of metal ions and the abnormal distribution of the DP is because of the complicated complexation of \textit{MeInu} with the fructofuranose ring moieties in comparison with those of \textit{MePeg}. Thus, the binding points of the host to one guest increase with the contribution of the side chain toward complexation to stabilize the complex ions of \textit{MeInu} with the multiple cationic guests.

### 3.2 Multinuclear NMR spectra of \textit{MeInu} complex with metal ions

#### 3.2.1 Complexation of \textit{MeInu} with metal ions in solution

The $^7$Li and $^{39}$K-NMR spectra of the complexes of \textit{MeInu} with each metal salt are shown in Fig. 6. Each sharp signal was assigned to an external reference. The signals of the complexed metal ions become broad because of the high anisotropy present.
Therefore, the broad signals were assigned to the complexed metal ions. On the basis of the integral ratio of the signals, the concentrations of the complexed host and guest were estimated as follows: (a) $[\text{Li}]/[\text{MeInu}] = 2.76 \text{ mM}$ and $[\text{Li}]/[\text{K}]/[\text{MeInu}] = 65.7 \text{ mM}$ and (b) $[\text{K}]/[\text{MeInu}] = 2.52 \text{ mM}$ and $[\text{K}]/[\text{MeInu}] = 23 \text{ mM}$. As the averaged DP of MeInu is 36, the number of fructofuranose units binding to one metal ion is calculated to be 2 and 4 for Li and K, respectively.

3.2.2 Complexation of MeInu with K studied by NMR

As shown in Fig. 6(b), the peak of the complexed K was too broad to estimate the exact stoichiometry of the MeInu•K complexation. In the $^{39}$K-NMR spectra, the signal of the complexed K in pyridine-5 was not observed because of broadening due to the suppression of relaxation by complexation with the host in that solvent. KSCN is soluble in pyridine, and the signal is observed clearly. Therefore, the population of the complexed K is estimated by calculating the reduction in the free KSCN peak area induced by the addition of MeInu. These spectral changes are shown in Fig. 7. The concentration of the free K shows a linear relationship to that of MeInu, as shown in Fig. 8. The slope ($=3.0$) represents the number of the fructofuranose units binding to one K ion. The number of fructofuranose units binding to one K ion was estimated to be 3.

3.3 Stoichiometry of MeInu complexes with metal ions estimated by ESIMS and NMR

The peak intensity of the complex ions related to MeInu with DP = 36 in the ESI mass spectra is shown in Fig. 9 as per their charge. $^{6}$Li, $^{8}$K, $^{10}$Cs gave the highest peak intensity of the complex ions at 3, 6, and 5 charges, respectively. The number of fructofuranose units binding to one metal ion of $^{6}$Li, $^{8}$K, and $^{10}$Cs is calculated to be 12, 6, and 7, respectively. These results are summarized along with the results from the multinuclear NMR spectra and the reported data of crown ethers in Table 1. In the case of K, the result by
ESIMS (6 units) was slightly larger than that by NMR (3-4 units). This discrepancy may be ascribed to the contribution of the side chain to the complexation reaction. Nevertheless, as the sensitivity of $^{39}$K-NMR for the complexation with Melnu was very low, these results can be considered to be in agreement. In the case of K$^+$ and Cs$^+$, number of oxyethylene units of crown ethers having a cavity size fitting a metal ion agrees with the results by ESIMS. We reported that the hexamer and octamer of permethylated cyclofructans, which are cyclic fructooligosaccharides, showed the same alkali metal ion-selectivity as their corresponding crown ethers.$^{26,27}$ The hexamer and octamer associated with K$^+$ and Cs$^+$ showed the strongest complexation among all the alkali metal ions, respectively. The best cavity size for each metal ion is constant and independent of the structure of the host, cyclic or acyclic. In regard to the above results, it is reasonable to conclude that the stoichiometry estimated by ESIMS adequately reflects the complexation behavior in solution, in the case of both K$^+$ and Cs$^+$.

In the case of Li$^+$, the results by ESIMS showed disagreement with those obtained by $^7$Li-NMR and from the stoichiometry of crown ethers. As described above, the binding affinity of Melnu for Li$^+$ is small. Thus, the reason for disagreement may be the dissociation of Li$^+$ or LiSCN from the complex ions containing Melnu in the ESI process.

4. Conclusion

In conclusion, we clarified that the complexation behavior of Melnu with metal ions with strong intermolecular interactions can be observed in ESIMS. Further, in the case of weak complexes, such as between Melnu and Li$^+$, the stoichiometry cannot be evaluated by ESIMS. Extensive study of intermolecular interactions should be conducted using other methods in combination with ESIMS. The accumulation of information on intermolecular interactions by ESIMS is very useful to extend the applicable coverage of ESIMS.

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