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 (**MeInu**) were examined by electrospray ionization (ESI) mass spectrometry and multinuclear nuclear magnetic resonance (NMR) spectroscopy. Complexes of **MeInu** with metal ions were observed as multicharged ions in ESI mass spectra. The number of fructofuranose units of **MeInu** 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 3–4 for Li^+ and K^+, respectively. The binding ability of **MeInu** 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 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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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.[1), 2)] 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).[3), 4)] 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.[5)–7)] On the other hand, we previously reported that enantioselective intermolecular interactions could not be evaluated by ESIMS.[8), 9)] Until now, we had found that the chiral recognition ability (ratio of association constants, K_R/K_S) of small chiral molecules in the solution state was in good agreement with the relative peak intensity [$I(H+G_R)^+/I(H+G_{S-dn})^+ = I_R/I_{S-dn}$; n, number of deuterium atoms] of diastereomeric complex ions consisting of a chiral host (H) with a deuterium-labeled enantiomeric guest ($G_{S-dn}{}^+$) or with another unlabeled enantiomeric guest ($G_R{}^+$) in fast atom bombardment (FAB) mass spectra.[10)–13)] However, when the diastereomeric complex ions in the chiral recognition complexation system were examined, the relative peak intensity (I_R/I_{S-dn}) in ESI mass spectra was depressed in comparison with that in FAB mass spectra, and the degree of depression particularly depended on the chemical structure of the host and the guest, and the distribution and proportion of the hydrophilic and hydrophobic groups in the molecular structure.[8), 9)] When the internal labeled reference was exchanged from one enantiomeric guest to another, the correlation of $I_R/I_{S-dn} = I_{R-dn}/I_S$ was detected. Further, when the chirality of the host was changed, the chiral cross-correlation, I_R/I_{S-dn} (host: one enantiomer) $\times$ I_R/I_{S-dn} (host: another enantiomer) $= 1.0$, was also detected. Moreover, in those studies, chiral ammonium salts or chiral carboxylate salts, which are preionized, were selected as the guests.[9)] Therefore, the differences in molecular weight, ionization efficiency, and transferability to the gas phase of the sample from those of the reference were disregarded in those chiral recognition complexation systems. Although these differences such as molecular weights, ionization efficiency, and transferability to the gas phase were eliminated as described above, the relative peak intensity of the complex ions

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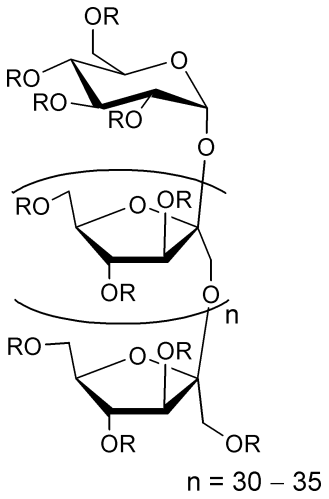

Chart 1. Permethylated inulin (**MeInu**)

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.[14] 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 polyethylene glycols at regular positions along the helix.[15–18] As the electrostatic intermolecular interaction is weak in highly polar solvents such as water, inulin was permethylated to dissolve in low-polar solvents and to enhance its binding ability to alkali metal ions.

Finally, the binding characteristics of permethylated inulin (**MeInu**) to alkali metal ions in solution were examined by multinuclear nuclear magnetic resonance (NMR) spectroscopy. In particular, the focus of the analysis was to determine the number of D-fructofuranose units of permethylated inulin contributing to form a complex with one cation and the stoichiometry of the complexation reaction.

2. Experimental

2.1 Materials

Commercial products of alkali metal salts, LiSCN•$(H_2O)_2$ (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.[19–21] A solution of inulin (0.6 g) in dimethylsulfoxide (DMSO) was added to a slight excess of dimethylsulfinyl carbanions, which had been prepared from DMSO and NaH, under Ar with stirring. After stirring for 4 h, the solution was cooled below 10℃ 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 $CHCl_3$, the organic layer was washed with a $Na_2S_2O_3$ aqueous solution and water; then, it was dried and evaporated *in vacuo* to afford **MeInu** as a white powder (0.64 g, 81% with a MW=7378, see §2.2). ^{1}H-NMR (400 MHz, $CDCl_3$) 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, 1 H, J=7.0 Hz, F4), 3.54 (m, 2H, F6, 6′), 3.48 (s, 3H, CH_3), 3.43 (s, 3H, CH_3), 3.35 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 85.9, 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 (^{1}H-NMR, d, δ 5.46 ppm; ^{13}C-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 ^{13}C-NMR technique under pulse sequence conditions to avoid the nuclear Overhauser effect. The ^{13}C-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*, 200–4,000; capillary tempera-

ture, 140℃; nebulizer gas N_2, 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 ^{1}H, ^{7}Li, and ^{39}K-NMR spectra were taken with a Brucker ARX 400 NMR spectrometer. Each NMR spectrum was measured under the following conditions: (1) ^{1}H-NMR, FR 400.1 MHz, AQ 3.834 s, ADP 32768, RLW 4132.23 Hz, AT 16, pulse width 5.000 s; (2) ^{7}Li-NMR, FR 155.5 MHz, AQ 1.049 s, ADP 16384, RLW 7812.50 Hz, AT 128; (3) ^{39}K-NMR, FR 18.7 MHz, AQ 0.1721 s, ADP 4096, RLW 11904 Hz, AT 2246.

2.4.2 Preparation of sample solution for ^{7}Li and ^{39}K-NMR measurements A solution of a complex of **MeInu** 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 **MeInu**. 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 (ϕ10 mm, Wilmad®).

A typical example of a solution of **MeInu** with LiSCN is as follows: LiSCN•middot; $(H_2O)_2$ (61.2 mg) was added to a 2.76 mM solution of **MeInu** in chloroform-*d*, and the suspension was treated with a supersonic homogenizer. The supernatant was dried over a molecular sieve 4A and its ^{7}L-NMR spectra was measured. A 1.02 M solution of LiSCN in D_2O was used as the external standard for quantitative analysis. The relative peak area between the external standard in the inner tube and a 7.20 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 ^{39}K-NMR spectra was prepared in same manner ([H] = 2.52 mM).

2.4.3 ^{39}K-NMR titration A 0.216 M KSCN solution in pyridine-d_5 was prepared. A 2 mL volume of this solution was placed into a 10 mm NMR tube. **MeInu** was added stepwise to the solution, and ^{39}K-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 MeInu 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, $(H+nM)^{n+}$, $(H+nM+SCN)^{(n-1)+}$, and $(H+(n-1)M+2SCN)^{(n-2)+}$ (n, 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.[22] The numerator and denominator in Fig. 1 represent the DP of the host and the number of included metal ions, respectively. The total peak intensity 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 **MeInu** 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^+ (1.67Å) > K^+ (1.33Å) > Li^+ (0.60Å).[23] 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.[24] Small cavities in macromolecules such as **MeInu** with conformational changes, are very rigid and their formation is entropically unfavorable. Thus, larger metal ions bind more strongly to **MeInu**. With respect to the strong binding ability to **MeInu** and small interactions with the counter anion, only complex ions consisting of **MeInu** and metal ions were observed in the case of a Cs^+ guest. The NH_4^+ ion is of the same size as K^+. The binding ability of NH_4^+ to **MeInu** is expected to be larger than that of Li^+. Therefore, the complex ions of **MeInu** with NH_4^+, 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 **MeInu** 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 **MeInu** at each charge of the complex ion (Fig. 4). The intensities are the simple sum of intensities for all complex ion types. The complex ions of **MeInu** 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

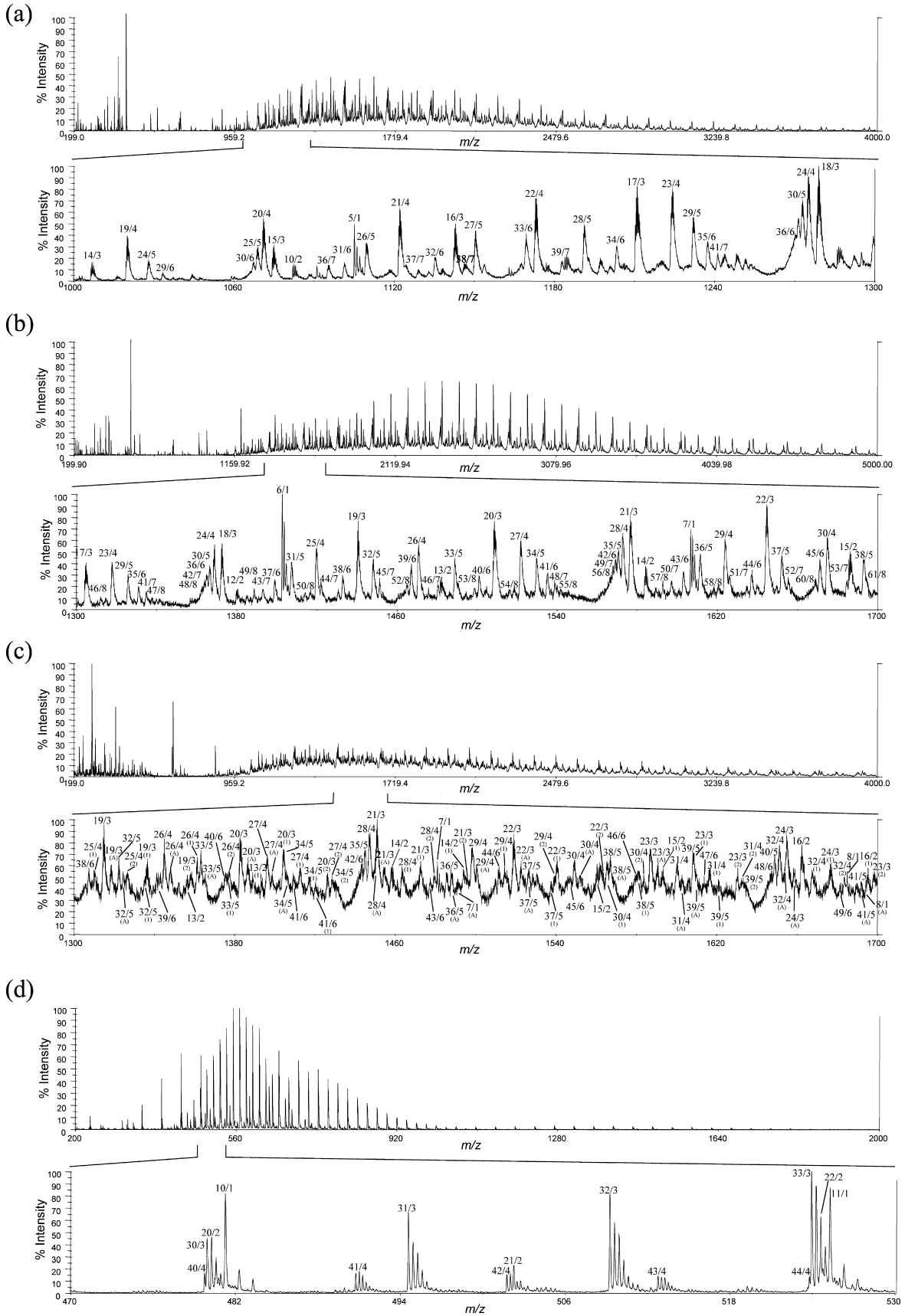

Fig. 1. ESI mass spectra of complex ions of hosts with metal ions. (a) Host, **MeInu**; metal ion, K^+. (b) Host, **MeInu**; metal ion, Cs^+. (c) Host, **MeInu**; metal ion, Li^+. (d) Host, **MePeg**; metal ion, K^+. The counter anion was thiocyanate (SCN^-). Each expanded spectrum is shown in each second 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 $(H+nM+SCN)^{(n-1)+}$, $(H+nM+2SCN)^{(n-2)+}$, and $(H+(n-1)M+NH_4)^{n+}$, respectively.

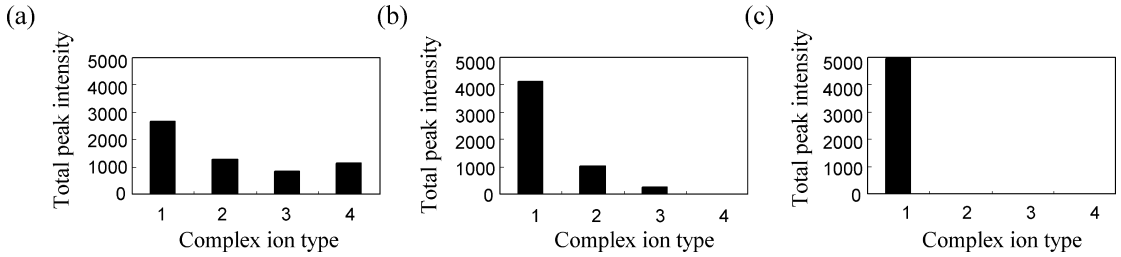

Fig. 2. Complex ion types observed in ESI mass spectra: Type 1, $(H+nM)^{n+}$; 2, $(H+nM+SCN)^{(n-1)+}$; 3, $(H+nM+2SCN)^{(n-2)+}$; 4, $(H+(n-1)M+NH_4)^{n+}$, and the peak intensity. (a) Li^+, (b) K^+, and (c) Cs^+. H is **MeInu**, and the counter ion is thiocyanate (SCN^-).

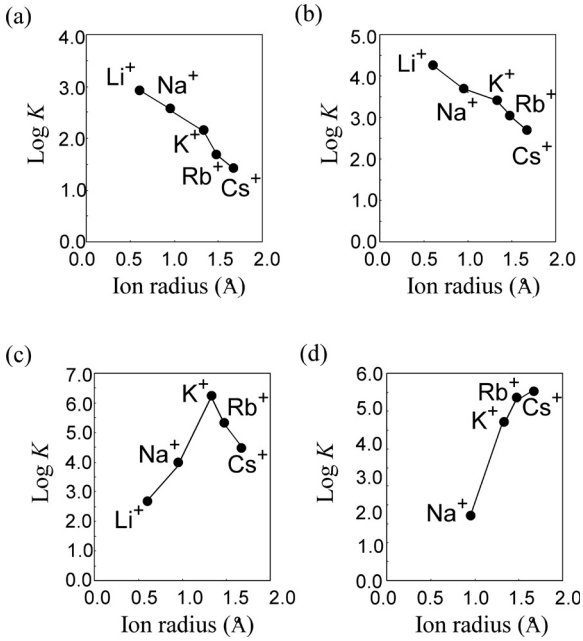

Fig. 3. Plot of the association constants of crown ethers with alkali metal ions *versus* ionic radii of metal ions. Host, solvent: (a) 12C4, propylene carbonate, (b) 15C5, propylene carbonate, (c) 18C6, propylene carbonate, (d) 21C6, methanol.

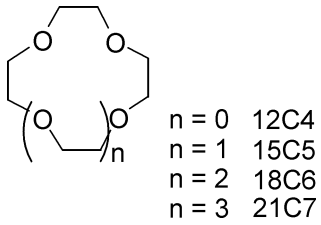

Chart 2. Cyclic polyethers (crown ethers)

of 4 K^+ ions, and the DP had a normal distribution (Fig. 5). The reason for the wide DP is that **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 **MeInu** with the fructofuranose ring moieties in comparison with those of **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 **MeInu** with the multiple cationic guests.

3.2 Multinuclear NMR spectra of MeInu complex with metal ions

3.2.1 Complexation of MeInu with metal ions in solution The ^7Li and ^{39}K-NMR spectra of the complexes of **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

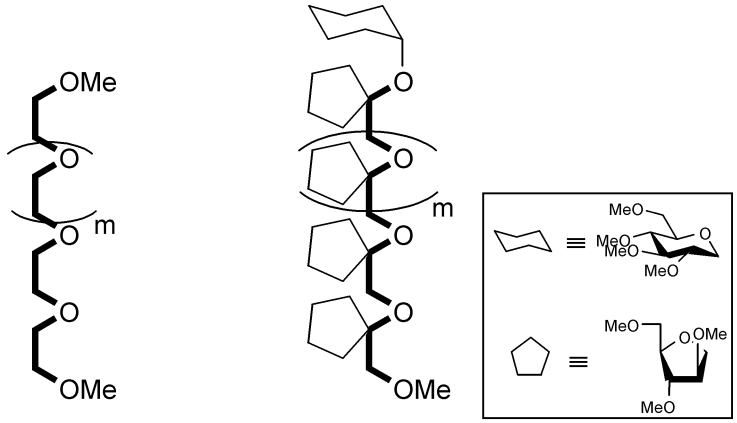

Chart 3. Dimethyl polyethylene glycol 1540 (**MePeg**)

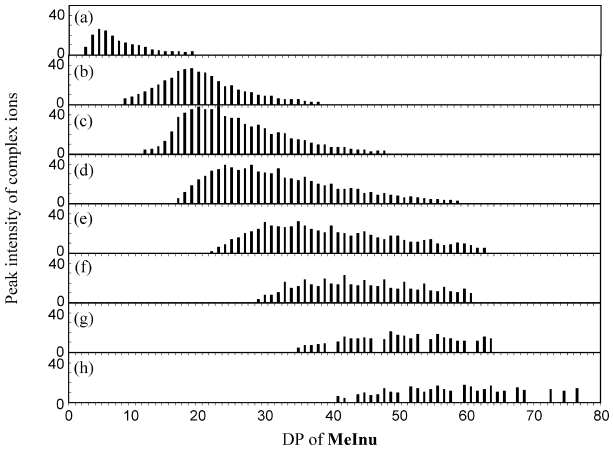

Fig. 4. The DP distribution of **MeInu** in the complex ion with K^+ by the charges in the ESI mass spectrum. (a) +1, (b) +2, (c) +3, (d) +4, (e) +5, (f) +6, (g) +7, and (h) +8. All types of complex ions were included.

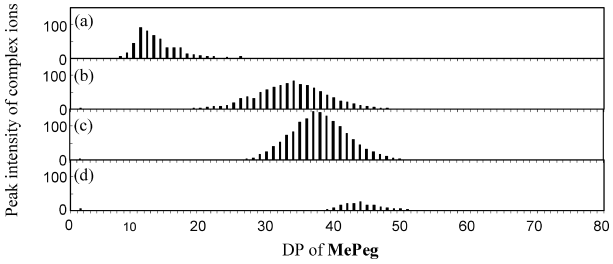

Fig. 5. The DP distribution of **MePeg** in the complex ion with K^+ by the charges in the ESI mass spectrum. (a) +1, (b) +2, (c) +3, and (d) +4. The complex ion peaks with charge numbers >+5 were not observed. All types of complex ions were included.

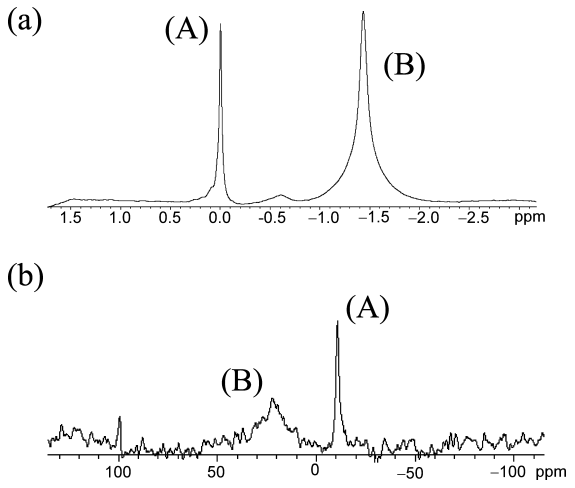

Fig. 6. Multinuclear NMR spectra of complex ions of **MeInu** with metal ions. (a) ^{7}Li-NMR, [**MeInu**]= 2.76 mM in chloroform-*d*. (b) ^{39}K-NMR, [**MeInu**] =2.52 mM in chloroform-*d*. A is the reference free metal ion peak. B is the complexed metal ion peak. The reference solutions of (a) and (b) were 0.0657 M LiSCN solution in H_2O and 0.009 M KSCN solution in H_2O, respectively.

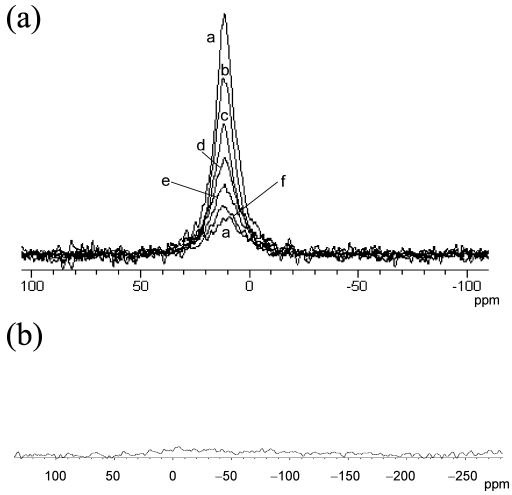

Fig. 7. ^{39}K-NMR spectral changes of free K^+ by addition of **MeInu** in pyridine-d_5 at 243 K. (a) [H]/[KSCN]: a, 0; b, 0.016; c, 0.029; d, 0.052; e, 0.081; f, 0.12; g, 0.16. (b) [H]/[KSCN]: large excess (>2).

in the coordination of the host molecule.[25] 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) ^{7}Li-NMR, [**MeInu**]= 2.76 mM and [Li^+]=65.7 mM and (b) ^{39}K-NMR, [**MeInu**] =2.52 mM and [K^+]=23 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-d_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 complexed with 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. Li^+, K^+, and 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 Li^+, K^+, and 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

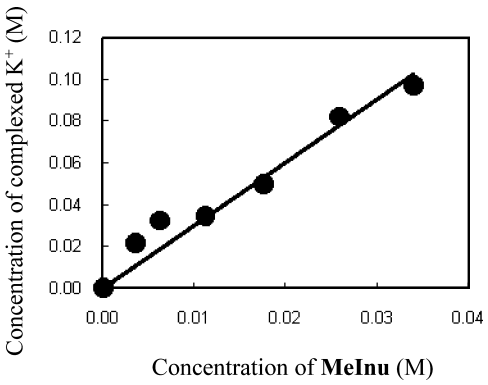

Fig. 8. Correlation between concentration of complexed K^+ and that of **MeInu** in pyridine-d_5 at 243 K.

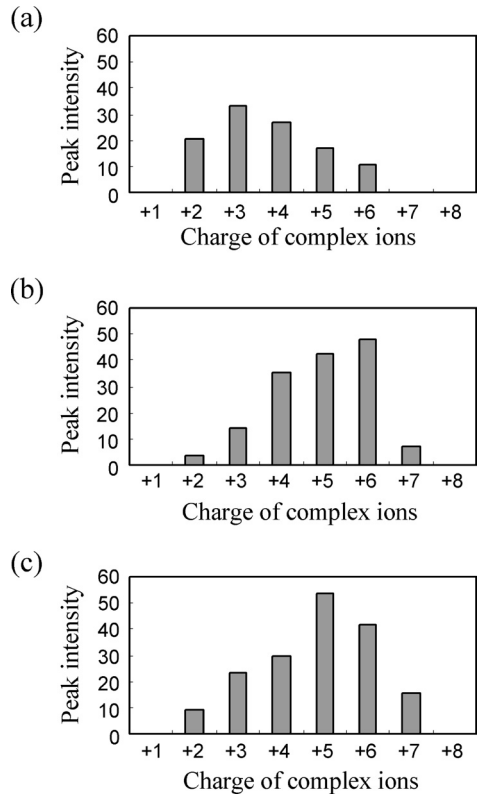

Fig. 9. Summation of peak intensities of complex ions of **MeInu** (DP=36) with metal ions by their charges. (a) Li^+, (b) K^+, and (c) Cs^+.

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 **MeInu** 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^+.

Table 1. Number of Fructofuranose Units of **MeInu** and Oxyethylene Units of Crown Ethers for Complexation with One Metal Ion

Metal ion	Number of fructofuranose units for complexation with one metal ion		Number of oxyethylene units of crown ethers for complexation with one metal ion[a]
	ESIMS	NMR	
Li^+	12	2	<5
K^+	6	3–4	6
Cs^+	7	—	>7

[a]Ref. 24.

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 **MeInu** for Li^+ is small. Thus, the reason for disagreement may be the dissociation of Li^+ or LiSCN from the complex ions containing **MeInu** in the ESI process.

4. Conclusion

In conclusion, we clarified that the complexation behavior of **MeInu** with metal ions with strong intermolecular interactions can be observed in ESIMS. Further, in the case of weak complexes, such as between **MeInu** 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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