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A High Resolution ^{23}Na NMR Study of Sodium Complexes with Ionophores in Solution. Stability of Complexes as Viewed from Displacements of ^{23}Na Chemical Shifts as Referred to Those in the Solid State

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We have recorded ^{23}Na NMR spectra of sodium complexes with naturally occurring and synthetic ionophores in chloroform and methanol solution, to gain insight into conformational stability of these complexes as examined from the displacements of ^{23}Na chemical shifts with respect to those in the solid state. Interestingly, the ^{23}Na chemical shifts of sodium complexes with monensin and tetranactin obtained in chloroform solution are very close to those in the solid, as a result of adopting similar conformation between the solid and solution. For the rest of complexes, however, the ^{23}Na NMR signals are displaced downfield in solution as compared with those of the solid state. Such downfield shifts are well interpreted in terms of the presence of conformational fluctuation and/or sodium ions interacting with solvent or anions. Therefore, downfield displacement is more significant in methanol which can be good ligand molecules. The presence of fluctuation-induced quadrupole coupling constants is consistent with our view about ^{23}Na chemical shifts. These quadrupole interactions were reduced in methanol solution, owing to the presence of exchange with solvents.

It is well-recognized that ionic permeability of alkaline cations in biological or artificial membranes is strongly enhanced by the presence of naturally occurring or synthetic ionophores.[1-4] This phenomenon can be interpreted in terms of a mobile carrier model of ionophores in which most important step is complexation of metal ions to these ionophores at interface and interior of membrane lipids.[2] These ligand molecules should undergo marked conformational change to achieve stability and ion selectivity for particular metal ions. Nevertheless, it has not yet been fully explored whether or not conformations of these ionophores in the crystalline state are retained in the liquid phase or membrane-bound state because of lack in suitable experimental means. One of the most promising new approach to this end is to examine displacements of the conformation-dependent ^{13}C chemical shifts (up to 8 ppm) which are varied upon particular conformations, as manifested from our previous high-resolution solid-state ^{13}C NMR studies of a variety of crystalline free and complexed ionophores.[5-8]

Alternatively, we can utilize ^{23}Na chemical shifts of sodium complexes in solution as compared with those of the solid state. For this purpose, we have recently demonstrated that ^{23}Na chemical shifts of solid sodium salts and complexes are significantly displaced (up to 60 ppm) depending on a variety of anions or ligand molecules and the Na–O interatomic distances,[9,10] as determined by magic angle spinning (MAS) method. The advantage of recording ^{23}Na NMR spectra in the solid state is that NMR signals of the individual species of the ion–ion, ion-solvent, and ion-ligand interactions were observed separately in the solid state, free from any chemical exchange process as encountered in solution. For the latter, extensive conventional high-resolution ^{23}Na NMR studies have been performed to obtain the kinetic and thermodynamic data of complexation in solution[11-25] although separate observation of such species is difficult owing to the presence of fast chemical exchange. Accordingly, use of these solid-state ^{23}Na NMR data provides one unambiguous reference data to assess whether or not conformations achieved in the solid state are retained in solution or membrane-bound state. For this purpose, it seems to be essential to utilize solvent of lower polarity such as chloroform to reproduce hydrophobic environment as in interior of biomembranes, although previous ^{23}Na NMR study by Haynes et al.[12] was mainly conducted for sodium complexes in methanol solution owing to the encountered difficulty in recording substantially broadened spectra in chloroform.

In this paper, we aimed to compare ^{23}Na chemical shifts of sodium complexes with some naturally occurring (valinomycin, nonactin, tetranactin, monensin, and lasalocid) and synthetic (cryptands 22, 221, 222, and 18-crown-6) ionophores (see Fig. 1) in chloroform and methanol solution with those obtained in the solid state previously reported.[10] Surprisingly, we noted that the linewidths of ^{23}Na NMR signals in these systems are substantially increased (up to 2.2 kHz for the complex with valinomycin). In addition, the ^{23}Na chemical shifts of monensin and tetranactin in chloroform solution were very close to the data of solid state, indicating the similarity of conformations between the solid and solution. Nevertheless, solid-state conformations are not retained for the rest of complexes in chloroform and methanol solution, because of the presence of rapid conformational fluctuation and ligand ex-

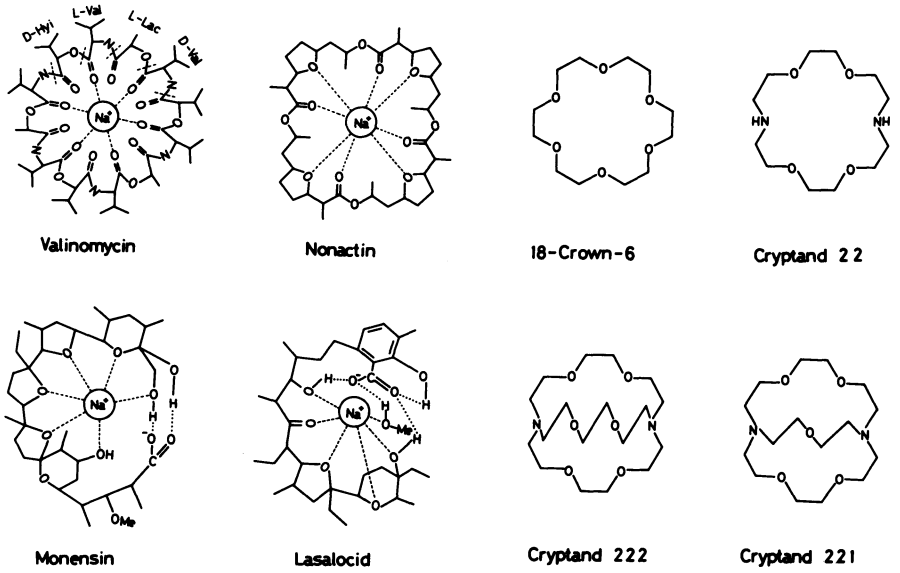

Fig. 1. Schematic representation of some sodium complexes with naturally occurring and synthetic ionophores.

change between the ionophores and solvent molecules.

Experimental

Sodium complexes of valinomycin (Sigma Chemical Company, MI, USA), nonactin (Boehringer-Mannheim GmbH, Germany), and tetranactin (Chugai Pharmaceutical Co. Ltd., Tokyo) (see Fig. 1) were prepared from ethyl acetate solution containing 10% excess NaSCN as described previously.[7] Complex formation was confirmed by the characteristic changes of ^{13}C NMR signals in the crystalline complexes as determined by the cross polarization-magic angle spinning (CP-MAS) NMR method.[7,8] Monensin (Na salt) and lasalocid (Na salt) were purchased from Sigma Chemical Company, MI, USA. These samples were recrystallized from diethyl ether-petroleum ether (1:1) containing small amount of acetone or methanol. Cryptands 22, 221, and 222 were purchased from Aldrich Chemical Company, WI, USA. 18-Crown-6 (18C6) was purchased from Aldrich Chemical Company, WI, USA. Sodium complexes with these cryptands and 18C6 were prepared by the method previously reported.[8]

High-resolution ^{23}Na NMR spectra were recorded on a Bruker CXP-300 spectrometer operating at 79.35 MHz. The crystalline complexes obtained by the procedure described above were dissolved (10 mg ml^{-1}) in deuterated chloroform (Merck Company, Germany) or methanol solution and transferred to 10 mm o.d. quartz NMR sample tubes (Shigemi Standard Joint Company, Tokyo), to avoid undesirable intense signal arising from standard Pyrex sample tubes. Spectral width and data points were usually taken as 10 kHz and 4 K, respectively. To record extremely broadened ^{23}Na NMR signal from the sodium complex with valinomycin, we utilized spectral width 25 kHz and data point 1 K. Free induction decays were accumulated usually 100—500 times. We further confirmed that the observed ^{23}Na signals did not arise from empty sample tube. ^{23}Na chemical shifts were referred to the resonance peak of 1 M† NaCl solution. Proton-decoupled ^{13}C NMR spectra were recorded on the same spectrometer operating at 75.46 MHz. ^{13}C spin-lattice relaxation times were measured for the same samples observed for ^{23}Na NMR spectra by the standard pulse-sequence of the inversion recovery.

Results

Figure 2 illustrates high-resolution ^{23}Na NMR spectra of some sodium complexes with naturally occurring ionophores in chloroform solution. Generally, the ^{23}Na NMR peaks of these sodium complexes were resonated at higher field region than the reference peak of 1 M NaCl solution. In particular, the ^{23}Na NMR peak of Na^+-tetranactin complex appears at the highermost peak-position (−27.8±0.3 ppm). Obviously, ^{23}Na NMR signals of complexes with lasalocid, monensin and valinomycin in chloroform solution are significantly broadened (up to 2200 Hz) but those of nonactin and tetranactin are not. By contrast, these signals were considerably narrowed in methanol solution and the extent of displacements of peaks was significantly reduced in methanol solution, as shown in Fig. 3. Note that relative peak position was also altered between chloroform and methanol solution. In a similar manner, Figs. 4 and 5 summarize ^{23}Na NMR spectra of some sodium complexes with synthetic ionophores in chloroform and methanol solution, respectively. In chloroform solution, a ^{23}Na NMR signal of very low intensity is clearly seen at the peak position of 1 M NaCl solution (Fig. 4). Undoubtedly, this peak arose from the ^{23}Na

† 1 M=1 mol dm^{-3}.

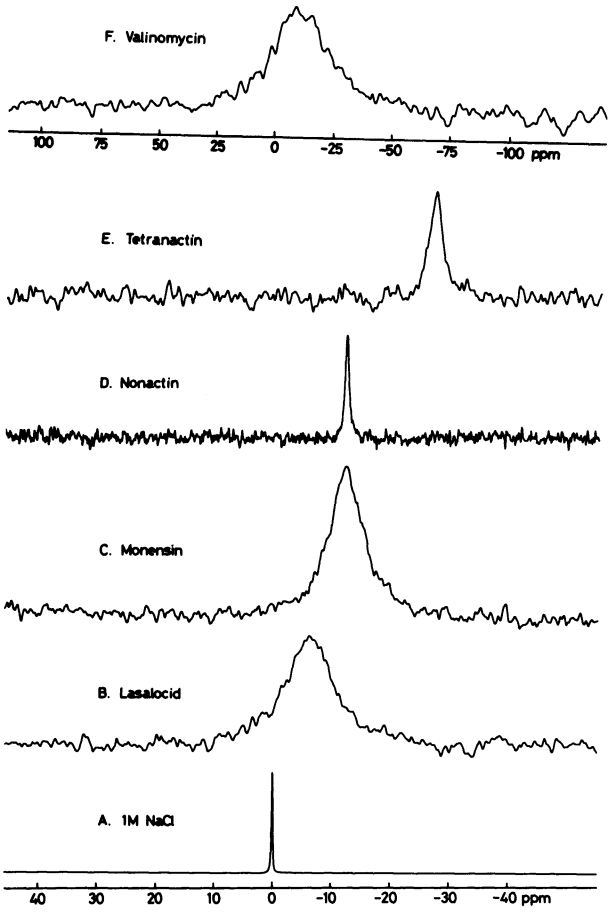

Fig. 2. 79.35 MHz ^{23}Na NMR spectra of some sodium complexes with naturally occurring ionophores in chloroform solution. Note that horizontal scale of F is expanded 2.5 times as that of A–E.

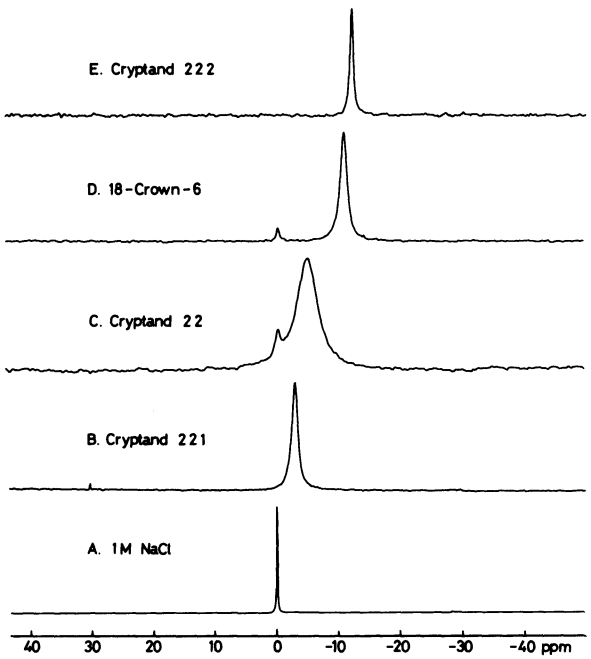

Fig. 4. 79.35 MHz ^{23}Na NMR spectra of some sodium complexes with synthetic ionophores in chloroform solution.

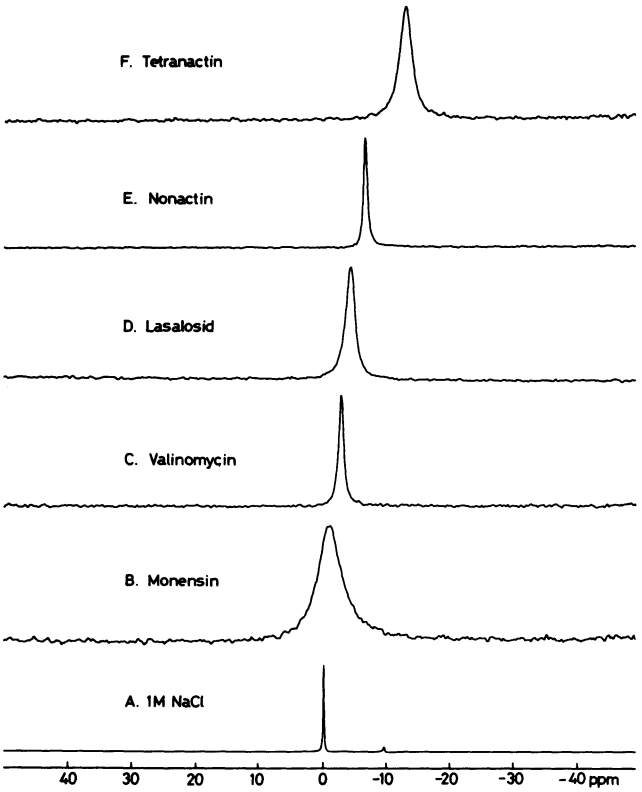

Fig. 3. 79.35 MHz ^{23}Na NMR spectra of some sodium complexes with naturally occurring ionophores in methanol solution.

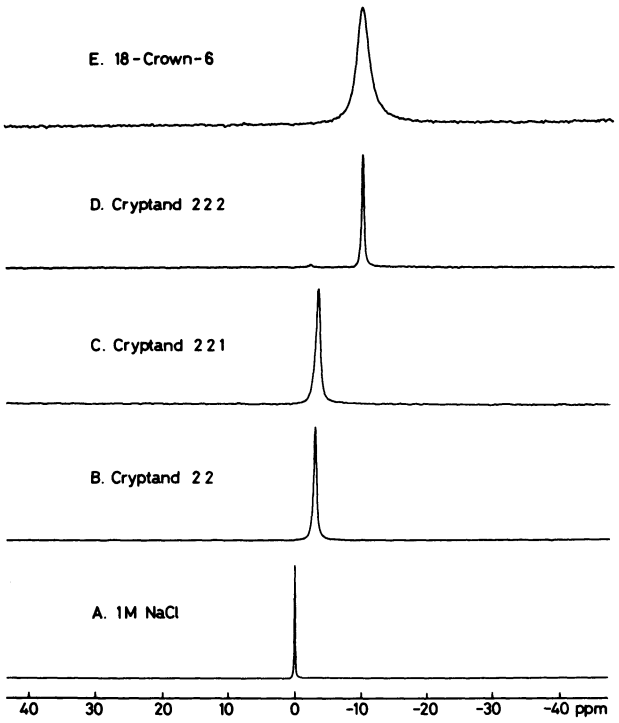

Fig. 5. 79.35 MHz ^{23}Na NMR spectra of some sodium complexes with synthetic ionophores in methanol solution.

Table 1. ^{23}Na Chemical Shifts of Some Sodium Complexes with Naturally Occurring and Synthetic Ionophores in the Solid and Solution (Referred to 1 M NaCl)

	Solid[a]		Solution			
	Chemical Shift	Linewidth	in $CDCl_3$		in CH_3OH	
	ppm	Hz	Chemical Shift	Linewidth	Chemical Shift	Linewidth
			ppm	Hz	ppm	Hz
Valinomycin	−23.3±4.7	3000	−7.7±3.5	2200	−2.6±0.1	70
Lasalocid	−11.6±3.3	2100	−6.2±1.1	710	−4.0±0.2	130
Monensin	−14.2±3.4	2800	−12.4±0.8	520	−0.7±0.6	370
Nonactin	−18.2±1.0	680	−12.1±0.1	60	−6.3±0.1	50
Tetranactin	−24.0±1.2	740	−27.8±0.3	210	−12.7±0.3	170
22	−18.3±3.5	2200	−5.0±0.5	320	−3.0±0.1	40
222	−16.2±1.7	1040	−12.2±0.1	50	−10.3±0.1	28
221	−16.4±2.8	1700	−2.9±0.1	90	−3.6±0.1	60
18C6	−16.0±0.9	530	−10.9±0.1	98	−10.3±0.1	160

a) Data taken from Ref. 10.

Table 2. Effective Quadrupole Coupling Constants, Correlation Times, and ^{13}C Spin-Lattice Relaxation Times of Some Sodium Complexes with Naturally Occurring and Synthetic Ionophores

	e^2qQ/h (MHz)		τ_C	^{13}C Spin-Lattice Relaxation Times (T_1's) (ms)[a]	
	$CDCl_3$	CH_3OH	s	CH	CH_2
Valinomycin	4.7	0.86	7.8×10^{-11}	605±72	
Lasalocid	2.2	0.94	1.2×10^{-10}	400±16	249±23
Monensin	2.0	1.7	1.0×10^{-10}	460±39	295±14
Nonactin	0.82	0.75	7.1×10^{-11}	668±63	356
Tetranactin	1.4	1.2	9.0×10^{-11}	527±13	216
22	3.3 (4.4)[b]	1.2 (1.6)[b]	2.3×10^{-11}		1025±67
222	1.4 (1.7)[b]	1.0 (1.3)[b]	2.1×10^{-11}		1145±53
221	2.1 (2.4)[b]	1.7 (1.9)[b]	1.7×10^{-11}		1370±83
18C6	1.9 (2.4)[b]	2.4 (3.1)[b]	2.2×10^{-11}		1094

a) Averaged value of several T_1 values from different peak positions. b) Calculated based on the T_1 value of 1.85 s (Ref. 18).

ion coordinated to either anion or water molecules present in trace amount in chloroform (slow chemical exchange). No such a peak was seen in methanol solution, because of the presence of rapid ligand exchange between the ionophores and solvents, if any. The relative peak positions in both the solvent systems were also altered. Table 1 summarizes these ^{23}Na chemical shifts and linewidths, together with those obtained in the solid state. We summarize ^{13}C spin-lattice relaxation times of these complexes measured in chloroform solultion in Table 2 (spectra not shown).

Discussion

^{23}Na Chemical Shifts. As demonstrated in our previous papers,[9,10] the ^{23}Na chemical shifts of sodium salts and complexes in the solid state are strongly influenced by a variety of anions and ligand moieties (C=O, OH, or ether), and Na–O distance. Interestingly, the Na–O distances of the sodium complexes with ionophores studied in this paper are usually longer than those of open-chain ligand molecules to be able to take optimal configuration.[26] As a result, it appears that the ^{23}Na NMR signals of these complexes are resonated at a higher field position by about 10—20 ppm, as compared with those complexes with open-chain ligand molecules.[10] As a clue for an explanation of this trend, it has been mentioned that the ^{23}Na chemical shifts are strongly influenced by the extent of electron transfer from the ligand molecule to a 3p orbital of the central metal cation, which in turn induces downfield displacement of peaks through contribution of paramagnetic shielding effect.[11a] Otherwise, it is impossible to account for the displacement of peaks as large as 60 ppm by means of the diamagnetic shielding term alone which is caused by 2s electrons of Na atom. In fact, we showed on the basis of an ab initio molecular orbital calculation that the extent of electron transfer

from ligand molecule to a 3p orbital could be increased when the Na–O distance is decreased until 2.5 Å: under these conditions, ^{23}Na NMR signal would be displaced downfield together with a decrease of the Na–O distance.[10]

By contrast, interpretation of the ^{23}Na chemical shifts in solution state is much complicated, because the observed ^{23}Na shift, δ_{obsd}, consists of time-averaged values of the following several contributions:

$$\delta_{obsd} = p_1\delta_{complex} + p_2\delta_{solvent} + p_3\delta_{ion}$$
$$p_1 + p_2 + p_3 = 1 \quad (1)$$

where $\delta_{complex}$, $\delta_{solvent}$, and δ_{ion} stand for the ^{23}Na chemical shifts of the sodium ions interacting with the ionophores, solvent molecules, and anions, respectively. To be used for reference data of $\delta_{complex}$, $\delta_{solvent}$, and δ_{ion} values, we have already recorded ^{23}Na NMR spectra of a variety of corresponding crystalline samples by high-resolution solid-state ^{23}Na NMR method, as pointed out previously.[9,10] It is cautioned, however, that these data obtained in the solid state are not necessary the same as those in solution, if molecular conformation achieved in the solid is not always retained in solution state. This will happen for a variety of complexes in which the Na–O distance of the complex is much larger than the sum of crystallographic radii of sodium (0.99 Å) and oxygen (1.40 Å) atoms as in the case of Na$^+$-valinomycin[10] and/or resultant conformational fixation of ligand molecule by metal-binding is not sufficient in solution. In such cases, it is conceivable that the Na–O bond distance would be instantaneously decreased to gain more effective ion-dipolar interaction as a result of thermal fluctuation of ligand moiety, leading to the downfield displacement of the ^{23}Na NMR peaks in view of the data as mentioned above. Then, the $\delta_{complex}$ value obtained in the solid could be modified to some extent. At the same time a plausible instantaneous decrease in the cation–anion distance during thermal fluctuation of ligand molecules would also cause downfield displacement of ^{23}Na peaks, although anions in crystalline sodium complexes are not in the vicinity of sodium ions and do not affect the ^{23}Na NMR peak position in the complexes.[10]

In chloroform solution, contribution from $\delta_{solvent}$ can be neglected because chloroform is not served as ligand molecules for the cation. It is emphasized that peaks from the remaining two factors, $\delta_{complex}$ and δ_{ion}, are independently observed or at least in slow exchange in chloroform solution, as seen as two separate peaks in Fig. 4. Therefore, it is expected that the ^{23}Na chemical shifts of the complexes in chloroform solution should be the same as those observed in the solid, if there exists no conformational change between these two systems, as in the sodium complexes with monensin and tetranactin (Table 1). This finding is consistent with our previous data concerning with the similarity of the conformation-dependent ^{13}C chemical shifts of ligand molecules between the solid and solution.[7,27]

Nevertheless, it is obvious from Table 1 that the ^{23}Na NMR peaks are displaced downfield in chloroform solution for the rest of complexes (valinomycin, lasalocid, nonactin, 22, 221, 222, and 18C6). This observation strongly suggests that molecular conformation of these complexes could be effectively distorted by thermal fluctuation of ligand molecules in solution, because downfield displacements of peaks were noted in chloroform solution, consistent with our view as described above. In fact, such a downfield displacement of ^{23}Na NMR peaks in solution is most significant for the complex with valinomycin (15.6 ppm). As to Na$^+$-valinomycin complex, no detailed data of molecular conformation in the solid state are available from X-ray diffraction study. However, we previously demonstrated on the basis of the conformation-dependent ^{13}C chemical shifts[7] that conformation of Na$^+$-valinomycin complex in the solid state is not significantly different from that of K$^+$-valinomycin.[26] Nevertheless, the size of cavity with the Na–O distances[28] 2.69—2.83 Å, which is optimal for K$^+$ ion (1.33 Å), seems to be too large to accommodate smaller sodium ion (0.99 Å). As a result, acquired stabilization energy of the ion-dipolar interaction will be decreased for the sodium complex than that of the potassium complex. This is one of reasons why ionic selectivity of K$^+$ ion is larger than that of Na$^+$ ion by 1.7×10^4.[3] Accordingly, the above-mentioned conformation achieved in the solid state might be easily deformed to gain more favorable interaction energy by the metal-ligand interaction. Obviously, such a deformation of ligand molecule from the octahedral symmetry[28] would result in creation of a large electric field gradient around the sodium ion, thermal fluctuation of which is responsible for the extensive line broadening of the ^{23}Na NMR signal in solution.

On the contrary, it was previously shown by X-ray diffraction studies of K$^+$- and Na$^+$-complexed nonactin and tetranactin[29] that the cubic coordination by eight equidistant oxygen atoms obtained in the K$^+$ complexes (K$^+$–O: 2.8—2.9 Å) is deformed in the Na$^+$ complexes (Na–C=O: 2.40—2.45 Å. Na–O(ether): 2.74—2.83 Å).[30–32] In spite of such a close conformational similarity between the two types of sodium complexes, there appears a substantial difference in stability of such complexes in chloroform solultion, as manifested from the difference of ^{23}Na chemical shifts between the solid and solution: Na$^+$-tetranactin is stable, while Na$^+$-nonactin is not. This observation is again consistent with our previous high-resolution solid-state ^{13}C NMR study of Na$^+$-nonactin complex in which ^{13}C chemical shifts of the C=O carbons are substantially displaced upfield in chloroform solution

as compared with those of the solid, by reflecting instability of the complex.[7] In a similar manner to that of Na^+-valinomycin complex, the downfield displacement of ^{23}Na signal could be explained by the presence of conformational flexibility which would modify either the Na–O or Na-anion distances. Consistent with this view, nonactin molecule is more flexible than tetranactin in the sodium complexes, as manifested from the observed differences in the ^{13}C spin-lattice relaxation times (and resultant correlation times), as described later (see Table 2).

Lasalocid[33–35] and monensin[36,37] are charged carboxyl ionophores in which there is no symmetry around the sodium ion, because sodium ion is coordinated by ether, hydroxyl or carboxyl groups, as depicted schematically in Fig. 1. In addition to the monomeric form[35] of the sodium complex of lasalocid in which methanol is also complexed, dimeric form is also known to exist both in the solid[33,34] and nonpolar solvent[38,39] such as cyclohexane. The structure of the dimeric form consists of a sandwich type in which the cation resides in a cavity between the two ionophores.[33,34] Thus, a plausible conversion from the monomeric crystalline form to the dimeric form or thermal fluctuation of ligand molecules in chloroform solution could nicely account for the downfield displacement (by 5.0 ppm) of peaks by going from the solid to chloroform solution (see Table 1).

In a similar manner, ^{23}Na chemical shifts of sodium complexes with cryptands and 18C6 in chloroform are resonated downfield as compared with those of the solid state. Obviously, similar explanation to these downfield shifts applies straightforwardly to these samples by taking into account of flexibility of this class of compounds. In this connection, it is interesting to note that deviation of the ^{13}C chemical shifts in chloroform solution from those in the solid is most significant in Na^+-221 complex,[8] as compared with the sodium complexes with 22 and 222. In parallel with this observation, the downfield displacement of ^{23}Na NMR signal is most pronounced in the complex with 221.

In methanol solution, the ^{23}Na NMR signal of the above-mentioned complexes are substantially displaced downfield, due to the presence of ligand-exchange process with solvent molecules. Among them, significant displacements of ^{23}Na NMR signals in the complexes with monensin and tetranactin are noteworthy (11.7 and 15.1 ppm, respectively). Naturally, the original conformations of these complexes achieved both in the solid and chloroform solution are not any more retained in methanol solution. In this connection, it is interesting that the ^{23}Na chemical shifts of sodium complexes with cryptands and 18C6 are not strongly different between solutions of chloroform and methanol.

^{23}Na NMR Linewidths. Surprisingly, the linewidth of ^{23}Na NMR signal of Na^+-valinomycin complex in chloroform solution is in the same order of magnitude observed in the solid state, as summarized in Table 1. On the contrary, the linewidths of the other complexes are reduced to 1/3—1/20 of the solid state. As pointed out in the previous section, location of the sodium ion within the cavity of asymmetric electric field gradient creates strong field gradient eq. Thus, the interaction of this field gradient with the nuclear quadrupole moment eQ produces the efficient pathway of nuclear relaxation.

Under the condition of extreme narrowing ($w^2\tau_C^2 \ll 1$), the linewidth $W_{1/2}$ is written as,[40]

$$W_{1/2} = 1/\pi T_2 = 2\pi/5(e^2qQ/h)^2\tau_C \quad (2)$$

where w and τ_C are Larmor frequency and the correlation time which characterizes the fluctuation of the electric field gradient, respectively, e^2qQ/h is the quadrupole coupling constant. The correlation time τ_C can be independently determined by measurement of the ^{13}C spin-lattice relaxation time (T_1),[41]

$$1/T_1 = N\hbar^2\gamma_C^2\gamma_H^2 r^{-6}\tau_C \quad (3)$$

where N is number of proton(s) attached to carbon nuclei in question, γ_C and γ_H are gyromagnetic ratio of the carbon and proton, respectively, and r is the C–H distance.

By combination of Eqs. 2 and 3,[42] it is now possible to calculate the quadrupole coupling constant e^2qQ/h, which is a very important parameter to determine the linewidths of ^{23}Na NMR signals, from a knowledge of the correlation time τ_C, as deduced by the ^{13}C spin-lattice relaxation times. As listed in Table 2, the quadrupole coupling constants of some complexes in chloroform solution are in parallel with the degree of asymmetry in the electric environment of sodium atoms. It is now possible to estimate ^{23}Na NMR linewidths of these complexes under various situation, if correlation times of molecular tumbling are known. For instance, ^{23}Na NMR linewidths of these complexes incorporated in biological membranes are estimated as 0.5—50 kHz on the basis of Eq. 2 using the quadrupole coupling constant 2 MHz and correlation times 10^{-8}—10^{-10} s.[43] Accordingly, it appears that ^{23}Na NMR spectra of these complexes might be substantially broadened in biomembranes. To make this situation worse, presence of slower collective motion tends to further broaden linewidth ($T_2 \ll T_1$).[43] In fact, we were not successful in recording ^{23}Na NMR spectra of these ionophores incorporated into sonicated lipid bilayers of egg phosphatidylcholine (unpublished finding).

Strictly speaking, the correlation times τ_C in methanol solution might be different from those in

chloroform. However, we neglected such a difference to qualitatively estimate the quadrupole coupling constants. Further, it is also desirable to remove residual oxygen molecules, by freezing-and-thawing, to obtain reliable spin-lattice relaxation times longer than about 1000 ms. Instead, we used published T_1 value[18] of 18C6 under the conditions of degassing to evaluate the quadrupole coupling constants of some sodium complexes with cryptands and 18C6, as shown in the parentheses of Table 2. Interestingly, the estimated quadrupole coupling constants of sodium complexes with cryptands and 18C6 (in parentheses of Table 2) by this treatment became larger than those of the naturally occurring ionophores, reflecting inherent asymmetric nature of the complexes.

When ligand-exchange process occurs as in methanol solution, the ^{23}Na NMR linewidths are substantially decreased, as summarized in Table 1. Analogous to the expression of the ^{23}Na chemical shifts given in Eq. 1, the observed quadrupole coupling constant $(e^2qQ/h)_{obsd}$ is given by Eq. 4.

$$(e^2qQ/h)_{obsd} = p_1(e^2qQ/h)_{complex} + p_2(e^2qQ/h)_{solvent} + p_3(e^2qQ/h)_{ion}, \quad (4)$$

where subscripts, complex, solvent and ion, have the same meaning as those described already. If we can neglect fractions of sodium complexes of mixed ligands with ionophores and solvents, the second and third terms $(e^2qQ/h)_{solvent}$ and $(e^2qQ/h)_{ion}$ become nearly zero because of symmetric property. Therefore, the linewidths will be reduced in proportion to the fraction of the complexed species. In particular, such reduction of the linewidth is remarkable from 2200 Hz to 70 Hz in methanol for the complex with valinomycin. Thus, it is likely that sodium ion in this case is not mainly coordinated to valinomycin but to methanol. For the complexes with other ionophores, this trend is not drastic as in the case of the above-mentioned valinomycin but is still significant.

In conclusion, we have demonstrated that comparison of high-resolution ^{23}Na NMR spectra of sodium complexes in solution with those obtained in the solid is very useful to reveal conformational stability of these complexes in solution, as a complementary means to ^{13}C NMR spectroscopy utilizing the conformation-dependent ^{13}C chemical shifts.

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