

Structural Evidence for an Independent Inclusion of Hexyl and Octyl Chains of Hexyldimethyloctylammonium Bromide in an α-Cyclodextrin Cavity

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Proton NMR spectroscopy was applied to determine the macroscopic binding constants and the solution structures of the 1:1 and 1:2 complexes between *N*,*N*,*N*-hexyldimethyloctylammonium bromide (HDOAB) and α-cyclodextrin (α-CD). The terminal methyls of the hexyl and octyl groups of HDOAB are distinguishable from each other in the NMR spectrum. The chemical-shift variations of these methyls, compared with the α-CD complexes of hexyltrimethylammonium (HTAB) and octyltrimetylammonium (OTAB) bromides, indicated that this system formed two 1:1 complexes (10% hexyl-in complex and 90% octyl-in complex). The chemical-shift variations of HDOAB and α-CD protons with the 1:1 and 1:2 complexations were quantitatively explained based on the assumption that the geometry of the hexyl and octyl groups of the HDOAB complexes with α-CD is identical to that of the HTAB and OTAB. This finding indicated that the hexyl and octyl groups bind to α-CD independently. Based on the assumption that the microscopic binding constants of the hexyl and octyl groups of HDOAB are identical to the macroscopic 1:1 binding constants of HTAB and OTAB with α-CD, the mole fraction of the hexyl-in complex in the 1:1 complex was estimated to be 0.11. This is very close to the above-mentioned estimation from the chemical-shift variations of the terminal methyls. The intensities of intermolecular cross-peaks on the ROESY spectrum of a 3 mM HDOAB and 3 mM α-CD solution indicated that the octyl-in complex is the predominant 1:1 complex. The present method and result will serve to estimate noncovalent interactions, binding equilibria, and solution structures of other multiple supramolecular complexes.

Cycloamyloses (cyclodextrins, CDs) are doughnut-shaped molecules, formed from D(+)-glucose units linked in a cycle. Cyclohexaamylose (α-CD) has a diameter of approximately 0.45 nm. Because the interior of the doughnut predominantly contains CH groups, it provides a relatively hydrophobic environment into which nonpolar molecules can be trapped.[1] This CD cavity, therefore, can accommodate surfactants very well. Complex formation between surfactants and CDs has been extensively investigated by spectroscopic, thermodynamic, surface chemical, and electrochemical methods.[2–8]

Most of these extensive studies are concerned with complex formation between single-chain surfactants and CDs. For instance, the NMR chemical shift method using an internal reference has recently been employed for determining binding constants and chemical shift variations of α-CD complexes with hexyltrimethylammonium (HTAB) and octyltrimethylammonium (OTAB) bromides. The binding constants are consistent with those obtained by other methods, and the chemical-shift variations are correlated with the geometry of the complexes with HTAB and OTAB.[8] The relationship between the chemical shift and the stereo structure remains almost unexplored for CD inclusion complexes,[9] although it has very recently been reported for α-CD complexes with short chain surfactants.[8,10]

However, little is known about the interaction between double-chain surfactants and CDs.[2,3] Because double-chain surfactants have two binding sites to CDs, they can form two or more complexes. This complicates data analysis, and provides us with challenging subjects: the determination of the binding constants and structures of a few complexes, the preference of the binding sites, and inhibitory or cooperative interactions between the binding sites. The complex formation of diheptanoyllecithin with α-, β-, and γ-CD was investigated by NMR and molecular-mechanics calculations.[11,12] These systems were too complicated to estimate any detailed structures of the complexes and microscopic binding constants. The complex formation of didecyldimethylammonium bromide with α-, β-, and γ-CD was investigated by electromotive-force measurements.[13] From a comparison between the binding constant for a single chain surfactant and didecyldimethylammonium bromide, it is suggested that the two decyl chains almost independently bind to α-CD, and are simultaneously incorporated in a γ-CD cavity. However, very few structure-chemical studies on these multiple complexes have yet been made. The detailed structure of such a complex will provide information about the preference of the binding sites and inhibitory or cooperative interactions between them.

Because CDs are rather nontoxic, they are added to foods and pharmaceuticals, for example, for the stabilization of labile compounds, the suppression of bitter tastes and hemolysis, and the long-term preservation of color, odor, and flavor.[2] However, because CDs are hemolytic, the parental administration of CDs is restricted by health authorities. This hemolysis is caused by the extraction of erythrocyte membrane components (phospholipid and cholesterol) by CDs.[14] Phospholipid has two acyl chains that can interact with α-CD. On the other

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hand, α-CD suppresses hemolysis induced by surfactants, because it can incorporate the surfactants inside the cavity, instead of phospholipid.[6]

Dialkyldimethylammonium bromide could be regarded as a model compound of phospholipid. *N*,*N*,*N*-Hexyldimethyloctylammonium bromide (HDOAB) has asymmetric alkyl chains, and these chains may be distinguishable from each other by proton NMR spectroscopy. Because their alkyl chain lengths are close to the depth of α-CD, the number of plausible structures of the complexes will be limited. Furthermore, complex formation of α-CD with HTAB and OTAB has been investigated by proton NMR; their chemical-shift data and solution structures are available.[8] For these reasons, we chose the HDOAB and α-CD system in this work. The 1:1 and 1:2 macroscopic binding constants and the chemical-shift variations with full binding were determined by NMR. From the chemical-shift variations of the terminal methyls of the hexyl and octyl chains, the populations of two 1:1 complexes (the hexyl-in and octyl-in complex) were estimated, and were compared with the values predicted from the microscopic binding constants of the hexyl and octyl groups. The ROESY intensity data on the 1:1 complex were obtained in order to directly investigate the preferential binding of the octyl group over the hexyl group. The solution structures of the hexyl-in and octyl-in complex and the 1:2 complex were estimated from the chemical-shift variations of HDOAB and α-CD. These NMR and binding data were used to investigate the independent binding of the hexyl and octyl groups to α-CD.

Experimental

Materials. Commercial samples of α-CD (Ensuiko Research Laboratories, Yokohama), tetramethylammonium chloride (TMA, Nacalai Tesque, Kyoto), and 99.9 at.% D deuterium oxide (Aldrich) were used as received. A sample of HDOAB, specially prepared by Tokyo Kasei Organic Chemicals Co., was used after its purity had been confirmed by NMR.

NMR Measurements. All ^{1}H NMR experiments were carried out in deuterium oxide at 298.2 ± 0.1 K. The NMR spectra were obtained with a JEOL Lambda 500 spectrometer. The proton chemical shift was determined by Nuts data processing software. The proton chemical shifts of HDOAB and α-CD were determined as a function of the α-CD concentration, up to 42.09 mmol dm^{-3} (mM), in the presence of 3.02 mM HDOAB. These chemical shifts were referenced to the internal 0.5 mM TMA signal at 3.176 ppm.[8]

The observed chemical shifts of HDOAB and α-CD were used to determine macroscopic binding constants and chemical-shift variations by two nonlinear least-squares methods. The Excel method yields the best-fit values alone, whereas the Multi method, developed by Yamaoka, gives the best-fit values and standard deviations.[15] These two methods gave very close values for the binding constants and chemical-shift variations.

Two-dimensional rotating-frame Overhauser enhancement spectroscopy (ROESY) for a solution containing 3.02 mM HDOAB and 3.24 mM α-CD was performed at 500 MHz with the JEOL standard pulse sequences; the data consisted of 8 transients collected over 2048 complex points. A mixing time of 250 ms, a repetition delay of 1.2 s, and a 90° pulse width of 11.0 μs were used. The ROESY data set was processed by applying an exponential function in both dimensions and zero-filling to 2048 × 2048 real data points prior to a Fourier transformation. Small cross-peaks were neglected, because their magnitude was close to that of the noise. The volume of a ROESY cross-peak was measured by summing the spectrum intensities with a certain region around the cross-peak, and slightly depended on the region of integration, peak overlap, and signal-to-noise ratio. The ROESY spectrum of 3.02 mM HDOAB in the absence of α-CD was also recorded in two chemical-shift ranges of $\delta = 0.6$–1.7 and 0.6–5.0 to obtain evidence for intramolecular association between the hexyl and octyl groups.

All of the NMR experiments were carried out below the critical micelle concentration (cmc) of HDOAB.[16,17]

Results

Chemical Shifts and Macroscopic Binding Constants. The 500 MHz ^{1}H NMR spectrum of a 3.02 mM HDOAB solution is displayed in Fig. 1, together with the NMR spectra of 3 mM HTAB and 3 mM OTAB and the sum of these spectra. The α- (Hα) and β-methylene (Hβ) protons of HTAB and OTAB exhibit complicated multiplet signals around 3.25 and 1.7 ppm, respectively, and resonate at a lower field than those of HDOAB, because of the difference in number of *N*-methyl groups. The chemical shifts δ of *N*-methyls (HN), middle methylenes (Hmid), and terminal methyls (Hω) of HDOAB are close to those of HTAB and OTAB. As shown in Fig. 1, the sum of the spectra of 3 mM HTAB and 3 mM OTAB is very close in shape to that shown for 3 mM HDOAB. This similarity allows us to distinguish between the ω-methyls for the hexyl and octyl group: δ(Hω6) = 0.867 and δ(Hω8) = 0.856 ppm. Although the signals of Hα for the hexyl and octyl group were distinguishable from each other in the absence of α-CD, they became a broad, indistinguishable signal with an increase in the α-CD concentration.

In Fig. 2 the apparent chemical-shift variations ($\Delta\delta = \delta - \delta_{\mathrm{HDOAB}}$) of protons HN (circles), Hω6 (triangles), and Hω8 (squares) of 3.02 mM HDOAB are shown as a function of the α-CD concentration. It is notable that the apparent chemical-shift variation of Hω8 is saturated at a lower α-CD concentration than that of Hω6.

The H1 proton of α-CD appeared near 5.1 ppm; protons H3,

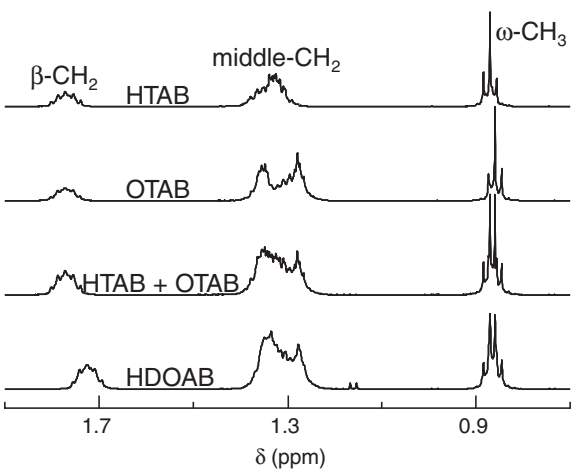

Fig. 1. NMR spectra of 3 mM HTAB, 3 mM OTAB, and 3.02 mM HDOAB and the sum of the spectra of 3 mM HTAB and 3 mM OTAB.

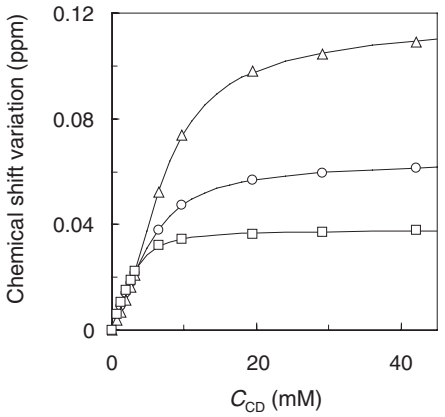

Fig. 2. Chemical-shift variations of protons HN (circles), Hω6 (triangles), and Hω8 (squares) of 3.02 mM HDOAB as a function of the α-CD concentration. The solid lines are calculated using the values of K_1, K_2, $\Delta\delta_1$, and $\Delta\delta_2$ for HDOAB given in Table 1.

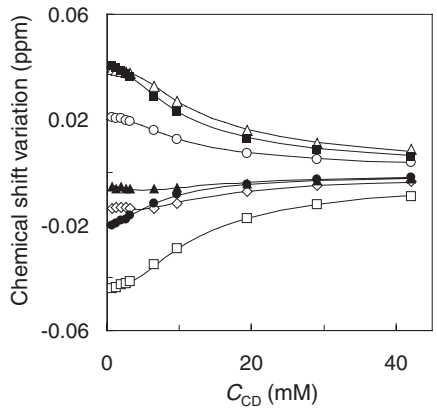

Fig. 3. Chemical-shift variations of all α-CD protons as a function of the α-CD concentration in the presence of 3.02 mM HDOAB: H1, ○; H2, △; H3, □; H4, ■; H5, ◇; H6*S*, ●; H6*R*, ▲. The solid lines are calculated using the values of K_1, K_2, $\Delta\delta_1$, and $\Delta\delta_2$ for α-CD given in Table 1.

H5, and H6 resonated around 3.85 ppm; and protons H2 and H4 were assigned to the peaks centered at 3.6 ppm. Because two protons, H6*R* and H6*S*, of H6 were distinguishable from each other, we dealt with these signals separately. The chemical shifts and the vicinal spin–spin coupling constants for all α-CD protons were determined by computer simulations of the NMR spectra in the presence 3.02 mM HDOAB. In Fig. 3 the apparent chemical-shift variations ($\Delta\delta = \delta - \delta_{CD}$) of all α-CD protons are shown as a function of the α-CD concentration. The outer protons (H1, H2, and H4) exhibit low-field shifts, whereas the inner protons (H3, H5, H6*S*, and H6*R*) exhibit high-field shifts. It is also noted that the inner protons exhibit large variations. These tendencies have already been reported for many aliphatic guest molecules.[8,18]

As expected from the chemical structures of HDOAB and α-CD, they can form the 1:1 complex (HDOAB–CD) and 1:2 complex (HDOAB–CD_2). The macroscopic binding constant (K_1) for the 1:1 complex is defined as

$$K_1 = [\text{HDOAB–CD}]/[\text{HDOAB}][\text{CD}]. \quad (1)$$

Here, [HDOAB–CD], [HDOAB], and [CD] denote the molarities of the 1:1 complex, HDOAB in the free state, and CD in the free state, respectively. The macroscopic binding constant (K_2) for the 1:2 complex is defined as

$$K_2 = [\text{HDOAB–CD}_2]/[\text{HDOAB–CD}][\text{CD}]. \quad (2)$$

The total concentration (C_{HDOAB}) of HDOAB is written as

$$\begin{aligned} C_{\text{HDOAB}} &= [\text{HDOAB}] + [\text{HDOAB–CD}] + [\text{HDOAB–CD}_2] \\ &= [\text{HDOAB}] + K_1[\text{HDOAB}][\text{CD}] \\ &\quad + K_1K_2[\text{HDOAB}][\text{CD}]^2. \end{aligned} \quad (3)$$

The total concentration (C_{CD}) of α-CD is written as

$$\begin{aligned} C_{\text{CD}} &= [\text{CD}] + [\text{HDOAB–CD}] + 2[\text{HDOAB–CD}_2] \\ &= [\text{CD}] + K_1[\text{HDOAB}][\text{CD}] \\ &\quad + 2K_1K_2[\text{HDOAB}][\text{CD}]^2. \end{aligned} \quad (4)$$

In the present system, complex formation is rapid on the NMR time scale. Under this condition, the chemical shift of HDOAB protons can be written as

$$\begin{aligned} \delta &= ([\text{HDOAB}]\delta_{\text{HDOAB}} + [\text{HDOAB–CD}]\delta_1 \\ &\quad + [\text{HDOAB–CD}_2]\delta_2)/C_{\text{HDOAB}} \\ &= ([\text{HDOAB}]\delta_{\text{HDOAB}} + K_1[\text{HDOAB}][\text{CD}]\delta_{\text{HDOAB–CD}} \\ &\quad + K_1K_2[\text{HDOAB}][\text{CD}]^2\delta_{\text{HDOAB–CD}_2})/C_{\text{HDOAB}}. \end{aligned} \quad (5)$$

Here, δ_{HDOAB}, δ_1, and δ_2 stand for the proton chemical shifts of HDOAB in the free state, the 1:1 complex, and the 1:2 complex, respectively. The chemical shift of an α-CD proton can be written as

$$\begin{aligned} \delta &= ([\text{CD}]\delta_{\text{CD}} + K_1[\text{HDOAB}][\text{CD}]\delta_1 \\ &\quad + 2K_1K_2[\text{HDOAB}][\text{CD}]^2\delta_2)/C_{\text{CD}}. \end{aligned} \quad (6)$$

Here, δ_{CD}, δ_1, and δ_2 stand for the chemical shifts of the CD proton in the free state, the 1:1 complex, and the 1:2 complex, respectively.

Once the binding constants, K_1 and K_2, and the chemical shifts, δ_1 and δ_2, of a proton are given, one can calculate theoretical chemical shifts of HDOAB and CD from Eqs. 3–6. The theoretical chemical shifts were simultaneously fitted to all of the observed ones shown in Figs. 2 and 3 to determine the 22 best-fit values of the binding constants, K_1 and K_2, and the chemical shifts, δ_1 and δ_2 of 3 HDOAB protons and 7 α-CD protons. Table 1 summarizes these binding constants, chemical shifts, and their standard deviations obtained by a nonlinear least-squares program, named Multi. The chemical shifts are given as their variations at full binding, namely, $\Delta\delta_1$ ($= \delta_1 - \delta_{free}$) and $\Delta\delta_2$ ($= \delta_2 - \delta_{free}$), where δ_{free} denotes δ_{HDOAB} or δ_{CD}. The standard deviation of these chemical shift variations of 0.001 ppm is very close to the experimental errors of the chemical shifts.

Microscopic Binding Constants. From a microscopic viewpoint, HDOAB and α-CD can simultaneously form two 1:1 complexes, the hexyl-in complex (HH-CD) and the octyl-in complex (HO-CD). Their microscopic 1:1 binding constants can be defined as

Table 1. Macroscopic Binding Constants and Chemical-Shift Variations (ppm) for Binary and Ternary Complexes of α-CD with HTAB, OTAB, and HDOAB

	HTAB[a]	OTAB[a]	HDOAB			HDOAB	
	obsd	obsd	obsd	calcd		obsd	calcd
K_1/mM^{-1}	0.436	3.61	2.009 ± 0.062[b]	4.05[c]	K_2/mM^{-1}	0.306 ± 0.007	0.389[d]
$\Delta\delta_1$(HN)	0.049	0.027	0.025 ± 0.001	0.029[e]	$\Delta\delta_2$(HN)	0.065 ± 0.001	0.076[f]
$\Delta\delta_1$(Hω6)	0.117		0.012 ± 0.001		$\Delta\delta_2$(Hω6)	0.118 ± 0.001	0.117[g]
$\Delta\delta_1$(Hω8)		0.036	0.032 ± 0.001		$\Delta\delta_2$(Hω8)	0.038 ± 0.001	0.036[h]
$\Delta\delta_1$(H1)	0.025	0.025	0.025 ± 0.001	0.025[i]	$\Delta\delta_2$(H1)	0.027 ± 0.001	0.025[j]
$\Delta\delta_1$(H2)	0.066	0.044	0.046 ± 0.001	0.046[i]	$\Delta\delta_2$(H2)	0.059 ± 0.001	0.055[j]
$\Delta\delta_1$(H3)	−0.079	−0.045	-0.051 ± 0.001	−0.048[i]	$\Delta\delta_2$(H3)	-0.063 ± 0.001	−0.062[j]
$\Delta\delta_1$(H4)	0.038	0.048	0.049 ± 0.001	0.047[i]	$\Delta\delta_2$(H4)	0.047 ± 0.001	0.043[j]
$\Delta\delta_1$(H5)	−0.042	−0.010	-0.015 ± 0.001	−0.013[i]	$\Delta\delta_2$(H5)	-0.027 ± 0.001	−0.026[j]
$\Delta\delta_1$(H6*S*)	−0.006	−0.020	-0.025 ± 0.001	−0.019[i]	$\Delta\delta_2$(H6*S*)	-0.015 ± 0.001	−0.013[j]
$\Delta\delta_1$(H6*R*)	−0.021	−0.005	-0.006 ± 0.001	−0.007[i]	$\Delta\delta_2$(H6*R*)	-0.014 ± 0.001	−0.013[j]

a) Observed values for HTAB and OTAB, taken from Ref. 8. b) Standard deviation. c) Calculated from Eq. 9. d) Calculated from Eq. 12. e) Calculated from Eq. 18. f) Calculated from Eq. 19. g) Equal to $\Delta\delta_1$(Hω6, HTAB). h) Equal to $\Delta\delta_1$(Hω8, OTAB). i) Calculated from Eq. 20. j) Calculated from Eq. 21.

$$k_1(\mathrm{H}) = [\mathrm{HH\text{-}CD}]/[\mathrm{HDOAB}][\mathrm{CD}], \quad (7)$$

$$k_1(\mathrm{O}) = [\mathrm{HO\text{-}CD}]/[\mathrm{HDOAB}][\mathrm{CD}]. \quad (8)$$

From Eqs. 1, 7, and 8, these microscopic binding constants can be connected with the macroscopic 1:1 binding constant by

$$K_1 = k_1(\mathrm{H}) + k_1(\mathrm{O}). \quad (9)$$

Similarly, two microscopic 1:2 binding constants can be defined as

$$k_2(\mathrm{H}) = [\mathrm{HDOAB\text{–}CD_2}]/[\mathrm{HO\text{-}CD}][\mathrm{CD}], \quad (10)$$

$$k_2(\mathrm{O}) = [\mathrm{HDOAB\text{–}CD_2}]/[\mathrm{HH\text{-}CD}][\mathrm{CD}]. \quad (11)$$

These microscopic binding constants can be connected with the macroscopic 1:2 binding constant by

$$K_2 = k_2(\mathrm{H})k_2(\mathrm{O})/[k_2(\mathrm{H}) + k_2(\mathrm{O})]. \quad (12)$$

If the complexations of the hexyl and octyl group with α-CD occur independently, we can expect the following equations:

$$k_1(\mathrm{H}) = k_2(\mathrm{O}) = K_1(\mathrm{HTAB}), \quad (13)$$

$$k_1(\mathrm{O}) = k_2(\mathrm{H}) = K_1(\mathrm{OTAB}). \quad (14)$$

Here, K_1(HTAB) and K_1(OTAB) denote the macroscopic 1:1 binding constants of HTAB and OTAB with α-CD and are 0.436 and 3.61 mM^{-1} (Table 1), respectively.[8] Substitution of these values into Eqs. 9 and 12 yielded theoretical 1:1 and 1:2 macroscopic binding constants of 4.05 and 0.389 mM^{-1} (Table 1). Then, the theoretical mole fraction (x_H) of the hexyl-in complex in the 1:1 complex can be calculated from

$$\begin{aligned} x_\mathrm{H} &= k_1(\mathrm{H})/[k_1(\mathrm{H}) + k_1(\mathrm{O})] \\ &= K_1(\mathrm{HTAB})/[K_1(\mathrm{HTAB}) + K_1(\mathrm{OTAB})] \\ &= 0.108. \end{aligned} \quad (15)$$

The theoretical mole fraction (x_O) of the octyl-in complex in the 1:1 complex is 0.892.

Microscopic Analysis of Chemical Shift Variations. The signals of two ω-methyls of HDOAB are distinguishable from each other (Figs. 1 and 2). In Table 1 it is notable that $\Delta\delta_1$(Hω6) for HTAB and $\Delta\delta_1$(Hω8) for OTAB are very close to $\Delta\delta_2$(Hω6) and $\Delta\delta_2$(Hω8) for HDOAB, respectively. This finding allows us to assume that the geometry of the hexyl ω-methyl group in the HTAB complex is the same as that in the HDOAB complex, and that its chemical-shift variation at full binding is also equal to each other. This holds true for the proton Hω8 of OTAB and HDOAB.

This finding gives us a clue to analyze the $\Delta\delta$ values given in Table 1. First, the $\Delta\delta_1$(Hω6) and $\Delta\delta_1$(Hω8) values for HDOAB are smaller than $\Delta\delta_1$(Hω6) for HTAB and $\Delta\delta_1$(Hω8) for OTAB. These smaller $\Delta\delta_1$(Hω) values for HDOAB are ascribed to partial binding of the hexyl and octyl group for the 1:1 complex of HDOAB and α-CD. From this viewpoint, we can estimate the mole fractions of the hexyl-in and octyl-in complex in the 1:1 complex of HDOAB and α-CD:

$$x_\mathrm{H} = \Delta\delta_1(\mathrm{H}\omega6, \mathrm{HDOAB})/\Delta\delta_1(\mathrm{H}\omega6, \mathrm{HTAB}) = 0.102, \quad (16)$$

$$x_\mathrm{O} = \Delta\delta_1(\mathrm{H}\omega8, \mathrm{HDOAB})/\Delta\delta_1(\mathrm{H}\omega8, \mathrm{OTAB}) = 0.902. \quad (17)$$

The sum of these mole fractions yields 1.004, which is very close to the theoretical value of 1. Furthermore, these mole fractions are close to those predicted from the binding constants ($x_\mathrm{H} = 0.108$ and $x_\mathrm{O} = 0.892$). Hereafter, we will employ rough values of $x_\mathrm{H} = 0.10$ and $x_\mathrm{O} = 0.90$.

For the $\Delta\delta_1$(HN) and $\Delta\delta_2$(HN) values of HDOAB, we can expect the following theoretical values:

$$\begin{aligned} \Delta\delta_1(\mathrm{HN}, \mathrm{HDOAB}) = {} & x_\mathrm{H}\Delta\delta_1(\mathrm{HN}, \mathrm{HTAB}) \\ & + x_\mathrm{O}\Delta\delta_1(\mathrm{HN}, \mathrm{OTAB}), \end{aligned} \quad (18)$$

$$\begin{aligned} \Delta\delta_2(\mathrm{HN}, \mathrm{HDOAB}) = {} & \Delta\delta_1(\mathrm{HN}, \mathrm{HTAB}) \\ & + \Delta\delta_1(\mathrm{HN}, \mathrm{OTAB}). \end{aligned} \quad (19)$$

These theoretical chemical shifts are given in Table 1. The agreement between theory and experiment for $\Delta\delta_1$(HN, HDOAB) is excellent, whereas it is not very good for $\Delta\delta_2$(HN, HDOAB). The reason for the poor agreement of $\Delta\delta_2$(HN, HDOAB) is due to the difference in the chemical

structure between HDOAB and HTAB or OTAB: HDOAB has the dimethylammonium group, whereas HTAB and OTAB have the trimethylammonium group. When one applies Eq. 18 to Hω6, one obtains the equation $\Delta\delta_1$(Hω6, HDOAB) = $x_H\Delta\delta_1$(Hω6, HTAB). This equation was used to estimate x_H. The chemical shift of Hω6 of HDOAB is not changed upon the formation of an octyl-in complex. This is the physical meaning of the independent binding of the hexyl and octyl chains. The HN proton is influenced by the formation of both the hexyl-in and octyl-in complexes. This is taken into consideration in Eq. 18.

For the $\Delta\delta_1$(HDOAB) and $\Delta\delta_2$(HDOAB) values of the CD protons, one can expect the following equations:

$$\Delta\delta_1(\text{HDOAB}) = x_H\Delta\delta_1(\text{HTAB}) + x_O\Delta\delta_1(\text{OTAB}), \quad (20)$$

$$\Delta\delta_2(\text{HDOAB}) = [\Delta\delta_1(\text{HTAB}) + \Delta\delta_1(\text{OTAB})]/2. \quad (21)$$

Equation 20 is equivalent to Eq. 18, but Eq. 21 is different from Eq. 19. Because the 1:2 complex contains two CD molecules, the calculated value in Eq. 21 is the average over these CD molecules. As is evident from Table 1, the agreement between theory and experiment for $\Delta\delta_1$(HDOAB) and $\Delta\delta_2$(HDOAB) is very good.

ROESY Spectra and Structures of Complexes. An HDOAB molecule has hexyl and octyl chains. These chains may be associated by hydrophobic interactions to adopt a folded conformation in aqueous solutions. To detect this conformation, we recorded two ROESY spectra (data not shown) of a 3.02 mM HDOAB solution in two chemical-shift ranges of δ = 0.6–1.7 and 0.6–5.0. The former gave a better resolved spectrum than the latter, because of enhanced data points in the former spectrum. Cross-peaks between protons proximal to each other on one of these chains were actually observed in this better spectrum. However, we could not find any clear cross-peaks between protons on the different chains, although one of the reasons for this failure is some overlapping of the signals. This result, therefore, does not exclude a folded conformation of HDOAB in aqueous solution.

A partial ROESY spectrum of a solution containing 3.02 mM HDOAB and 3.24 mM α-CD is shown in Fig. 4. Under this condition, the concentration of the 1:1 complex HDOAB–CD is 1.59 mM. Therefore, the cross-peaks between protons of HDOAB and α-CD are due to the 1:1 complex. The volumes (ROE intensity) of the cross-peaks were determined by integration, and are given in Table 2. The signals of Hα, Hβ, and HN are distant from the signals of the other methylenes (Fig. 1). Because these protons did not have cross-peaks with the protons of α-CD, they are omitted from Table 2. Protons H1 and H2 do not have cross-peaks with the protons of HDOAB. The ROE intensity of a cross peak is proportional to the number (n) of equivalent protons. For instance, the middle methylene signals of HDOAB were regarded as a single signal (Hmid) with equivalent protons of n_{AB} = 16. The ROE intensity (ROE/$n_{AB}n_{CD}$) is a measure of the inter-proton distance, where n_{CD} denotes the number of equivalent protons of α-CD.

The ROE intensity is proportional to a negative 6-th power of the inter-proton distance: as two protons become closer to each other, the ROE intensity of the cross-peak between them increases rapidly. Second, the ROE intensity is proportional to the concentration of a complex. Namely, the observed ROE intensities are the sum of the contributions of the hexyl-in and octyl-in complexes. The ROE intensities for the cross-peaks of Hω with protons H3, H5, and H6 in the 1:1 complexes of α-CD with HTAB and OTAB are given in Table 2. For these three systems, the ROE intensity of the cross-peak between Hω and H5 is larger than those of the others. The ROE intensities of H3 and H6 relative to this strongest peak are given in Table 2. These relative intensities for HDOAB are closer to those for OTAB than those for HTAB. This finding indicates that the octyl-in complex is the major 1:1 complex.

As is evident in Table 1, the theoretical chemical shift variations for all of the protons are very close to the observed ones: Eqs. 16–21 hold true for the HDOAB–α-CD system. This finding indicates that the solution structures of the hexyl-in and octyl-in complex are very close to those of the α-CD complexes with HTAB and OTAB, respectively. We have already determined the solution structures of the 1:1 α-CD complexes with HTAB and OTAB.[8] The solution structures of the 1:1 and 1:2 complexes of HDOAB with α-CD are

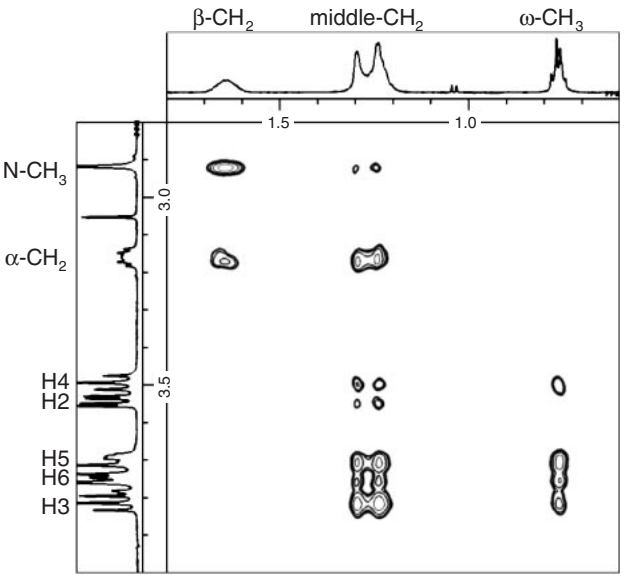

Fig. 4. Partial ROESY spectrum of a solution containing 3.02 mM HDOAB and 3.24 mM α-CD.

Table 2. Intensities, ROE/$n_{AB}n_{CD}$, and Relative Intensities of ROESY Cross-Peaks between Protons Hω and Hmid of HDOAB, HTAB, and OTAB and Three Kinds of α-CD Protons

		H3	H5	H6
HDOAB	Hω	0.4	1.7	0.4
		(25%)	(100%)	(24%)
	Hmid	1.7	0.94	0.11
HTAB[a)]	Hω	2.2	4.2	1.7
		(52%)	(100%)	(40%)
OTAB[a)]	Hω	1.2	5.6	1.7
		(21%)	(100%)	(30%)

a) Original data on HTAB and OTAB were taken from Ref. 8.

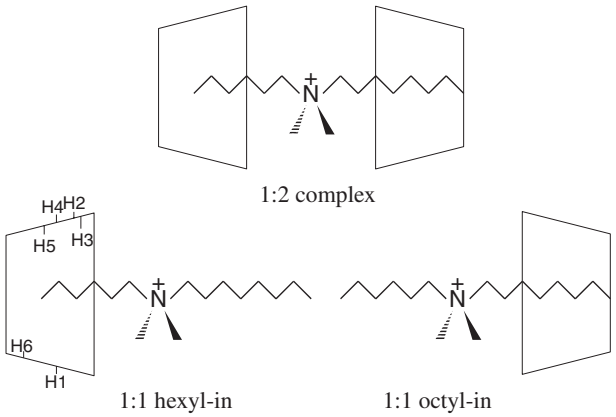

Fig. 5. Solution structures of the 1:1 octyl-in complex, the 1:1 hexyl-in complex, and the 1:2 complex of HDOAB and α-CD, estimated from the present chemical-shift data and the solution structures of the 1:1 α-CD complexes with HTAB and OTAB.[8]

shown in Fig. 5, where the geometry of the bound hexyl and octyl chains of HDOAB is identical to that in the 1:1 α-CD complexes with HTAB and OTAB.[8] These will be time-averaged structures, instead of rigid bodies. Thus, the hexyl and octyl groups of HDOAB bind to α-CD independently from the structure-chemical viewpoint.

Discussion

Most of the conclusions of the present investigation were derived from accurate chemical-shift determinations. In the literature, external and internal standard methods have been both employed. The merits and demerits of these methods have been investigated for systems of CD with various guest molecules. The advantage of an internal reference lies in the fact that the effective field experienced by the nuclei of both the reference and sample molecules is exactly the same. However, the compounds used as references must be inert with respect to the sample as regards intermolecular effects. External referencing avoids the difficulty of dealing with the solute–solvent reference interactions, but the problem of differential shielding now arises.[19–21] We employed 0.5 mM TMA as the internal standard for chemical shifts, because it is an excellent internal standard for CDs and cationic guests in aqueous solutions.[20,21] Because HDOAB forms micelles above cmc, the concentration of HDOAB was kept at a constant concentration of 3.02 mM below cmc.[16,17] The concentration of α-CD was changed over a wide range of concentrations below and above the HDOAB concentration. This allowed us to determine accurate chemical shift variations (at full binding) for some HDOAB protons as well as all α-CD protons. The separate determinations of Hα, as well as Hβ, for the hexyl and octyl chains were very hard because of an overlap of the signals.

The chemical-shift variation at full binding has not fully used to estimate the solution structures of the CD complexes with various guest molecules, except for aromatic molecules.[9,21,23] The chemical-shift variation of the alkyl group fully complexed with α-CD is likely to be a function of the position in the α-CD cavity. For instance, the chemical-shift variation of proton Hω of the hexyl group is much larger than that of the octyl group (Table 1), because the former proton is closer to the O4 atoms of α-CD than the latter (Fig. 5).[8] The chemical-shift variations for HDOAB and α-CD allowed us to estimate the structures of the 1:1 and 1:2 complexes and the mole fraction of the hexyl-in complex in the 1:1 complexes. These structures were estimated based on the assumption that the positions of the hexyl and octyl groups of HDOAB in the α-CD cavity are identical with those in the 1:1 α-CD complexes with HTAB and OTAB. This assumption means that the hexyl and octyl groups of HDOAB bind to α-CD independently (Eqs. 16–21). The excellent agreement between the observed and calculated chemical-shift variations for all protons demonstrates the validity of this assumption. If this assumption did not hold true, the calculated chemical shift variations would be different from the observed ones.[23] Furthermore, the chemical-shift variations for HDOAB and α-CD give a more reliable estimation of the mole fraction of the hexyl-in complex in the 1:1 complexes than the binding constants (Eqs. 16 and 17). To our knowledge, this is a first report on the determination of the molar ratio of the coexisting 1:1 complexes by NMR.

From the chemical-shift variations for the systems of diheptanoyllecithin and α-, β-, and γ-CD, we attempted to estimate the structures of complexes and the populations of the coexisting complexes.[11,12] Although these systems are more complicated than the HDOAB–α-CD system, the structures of the complexes of diheptanoyllecithin and α-CD are qualitatively consistent with those of α-CD with HDOAB, HTAB, and OTAB. Oxyphenonium bromide and α-CD form two 1:1 complexes the cyclohexyl-in and phenyl-in complexes. Based on the ROE intensities, we estimated rather detailed solution structures of these complexes.[23] A rough mole fraction of the phenyl-in complex was estimated from the chemical-shift variations of the phenyl protons, as compared with those of the 1:1 complex of α-CD and benzenesulfonate.[21,23]

Electromotive-force measurements,[5,13] surface tension measurements,[4] visible-light and fluorescence probe measurements, and calorimetry give reasonable binding constants for systems of CDs and surfactants.[5,6] If the appropriate attentions mentioned above are paid, the NMR chemical-shift method gives reasonable binding constants, even for multiple complexes. This method requires estimations of the chemical-shift variation and the binding constant for a complexation, although the chemical-shift variation affords a clue to estimate the structure of the complex.

The observed macroscopic 1:1 binding constant (K_1) is the sum of the binding constants of α-CD with the hexyl and octyl groups (Eq. 9). There are two methods for estimating the microscopic binding constant. The first method is to employ the macroscopic binding constant of a very similar guest molecule (Eqs. 13 and 14). The second method is to use the equations $k_1(\mathrm{H}) = x_\mathrm{H} K_1(\mathrm{HDOAB})$ and $k_1(\mathrm{O}) = x_\mathrm{O} K_1(\mathrm{HDOAB})$. These equations predict microscopic binding constants of $k_1(\mathrm{H}) = 0.10 \times 2.01 = 0.201\ \mathrm{mM}^{-1}$ and $k_1(\mathrm{O}) = 0.90 \times 2.01 = 1.81\ \mathrm{mM}^{-1}$. These are comparable to the observed macroscopic binding constants for HTAB and OTAB (Table 1). The uncertainties in this method are how to estimate x_H and x_O and the assumption of independent binding of the hexyl and octyl groups. The assumption of independent binding holds true for all data, except for the K_1 value given in Table 1. Apart

from the accuracy of the observed macroscopic constants for HTAB, OTAB, and HDOAB, some populations of HDOAB molecules may adopt a folded structure in aqueous solutions. This conformation can result in a decrease of the binding constant (K_1), because the folded conformer will have a lower affinity than the extended conformer. The ROESY spectrum of 3.02 mM HDOAB in the absence of α-CD did not provide clear evidence for distinguishing between the interchain and intrachain proximities of protons. Double-alkyl chain compounds can associate intramolecularly by hydrophobic interactions in aqueous solutions, so that their hydrophobicity is smaller than expected from single-chain compounds.[24–26]

In conclusion, most of the present data can be explained based on the independent binding of the hexyl and octyl group to α-CD. The chemical-shift variation with full binding is explicable in terms of the structure of a complex. The population and stoichiometry of the complex can be estimated from the chemical-shift variations, if those variation data for related guest molecules are available. The present method and result will serve to estimate non-covalent interactions, multiple binding equilibria, and solution structures of other supramolecular complexes.[27–29]

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