Formation of Through-Ring α-Cyclodextrin Complexes with α,ω-Alkanedicarboxylate Anion. Effects of the Aliphatic Chain Length and Electrostatic Factors on the Complexation Behavior

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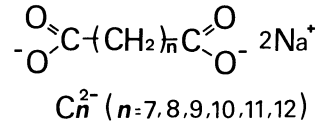
α,ω-Alkanedicarboxylic acid anions with a long spacer methylene chain were observed to form dynamically and statically stable complexes with α-cyclodextrin (α-CD) at an NMR time scale in both neutral and basic media. The structures of these complexes were confirmed to be a "through-ring" type CD complex by measurements of the intermolecular NOESY and NOE difference spectra. From the thermodynamic data in basic media (pD 13), the dynamical stability of these type complexes were attributed not to the steric interaction, but to an electrostatic interaction between the dicarboxylate anion and ionized-CD.

Cyclodextrin (CD) has been well known to incorporate guest compounds into its hydrophobic cavity, and the complexation behaviors have been investigated in order to construct model systems for molecular recognition. Aromatic compounds have been mainly used as guest compounds, and thermodynamic data have been discussed in terms of hydrophobicity, size, and shape of the guest molecules.[1] However, only a few papers have been published concerning the CD complexes of aliphatic compounds, probably because the usual spectrophotometric methods (e.g. UV or fluorescence spectra) are not useful for elucidating the complexation behaviors.

We recently reported that through-ring CD complexes could be obtained when the guest molecule possessed a polymethylene chain terminated with a positively charged bulky aromatic group, such as viologen or pyridinium units.[2,3] It was also shown that CD includes the guest molecule, not at the aromatic parts but at the polymethylene chain. Large equilibrium constants were observed for compounds having a long methylene chain (statically stable). Furthermore, the exchange rates of the CDs-complexes were sufficiently slow to be observed in the ^{1}H NMR time scale (dynamically stable). The process was extremely retarded by an energy barrier which may be raised by a steric effect of the bulky aromatic group passing through the CD cavity. In these studies, ^{1}H NMR has become a useful method for analyzing not only the kinetics of CD complexation, but also the structures of the complexes.

In the present study we found that the α,ω-alkanedicarboxylate anion (C_n^{2-}), which does not have a very bulky group on both terminals, but has anionic charges, forms a statically and dynamically stable complex with α-CD. The effects of the terminal carboxylate groups were elucidated by studying the NMR spectra in basic D_2O solutions, where the dissociation of secondary hydroxyl groups of α-CD was also expected.



C_n^{2-} (n=7,8,9,10,11,12)

Experimental

Materials. α,ω-Alkanedicarboxylic acid disodium salts (Na_2C_n: n=6—12) were prepared by neutralization of the corresponding free acid (Tokyo Kasei, Wako Chemicals, and Aldrich Chemicals) by sodium hydroxide, and were purified in the usual manner. Guaranteed-grade alpha-, beta-, and gamma-CD were used as received (Tokyo Kasei) without further purification.

NMR Spectra. ^{1}H and ^{13}C NMR spectra were obtained with a JNM-GSX 400 NMR spectrometer (JEOL) using a variable-temperature unit with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external standard. Since CD induces a shift of the reference signal (probably due to complexation with CD), a sealed glass capillary containing D_2O solution of DSS was placed in the sample tube in order to obtain a reference signal. Samples were prepared at pD 8 with a phosphate buffer and at pD 13 with NaOD, respectively. H–H COSY, C–H COSY, NOESY, and NOE difference spectra were also measured with the spectrometer.

Evaluation of Thermodynamic Parameters. Complex dissociation (k_d; s^{-1}) and association rates (k_a; $M^{-1}\,s^{-1}$) (M=mol dm^{-3}) were defined as follows:

$$\alpha\text{-CD} + C_n^{2-} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \alpha\text{-CD}\cdot C_n^{2-} \qquad (1)$$

In Eq. 1, the formation constant (K; M^{-1}) of the complex was also defined as follows:

$$K = \frac{[\alpha\text{-CD}\cdot C_n^{2-}]}{[\alpha\text{-CD}][C_n^{2-}]}. \quad (2)$$

Here,

$$K = k_a/k_d. \quad (2')$$

The formation constant (K) and exchange rates (k_d, k_a) at each temperature were estimated by simulations of the dynamic ^{1}H NMR spectra.[4]

The free-energy difference ($\Delta G°$) between the free and complexed species were calculated according to

$$\Delta G° = -RT \ln K. \quad (3)$$

The activation free energy for complex association ($\Delta G_0^{\ddagger}$) and the dissociation ($\Delta G^{\ddagger}$) were also calculated using eqs. (4) and (5), respectively:

$$\Delta G_0^{\ddagger} = -RT \ln (k_a \cdot h/k_B T) \quad (4)$$

and

$$\Delta G^{\ddagger} = -RT \ln (k_d \cdot h/k_B T). \quad (5)$$

Here, h and k_B are the Planck and Boltzmann constants, respectively; the transmission coefficient was assumed to be unity. The enthalpy ($\Delta H°$, $\Delta H_0^{\ddagger}$, and $\Delta H^{\ddagger}$) and entropy ($\Delta S°$, $\Delta S_0^{\ddagger}$, and $\Delta S^{\ddagger}$) changes for each kinetics were evaluated from the temperature dependencies of the respective free-energy differences.

Results and Discussion

Complexation at Neutral Medium. The NMR spectra of protons in methylene groups of C_{12}^{2-} in neutral media (pD 8) at room temperature are shown in Fig. 1. When α-CD was added into a C_n^{2-} solution, new sets of signals for the methylene groups appeared. They were attributed to one of the complexed species (which are indicated by letters with a single prime (a′—f′) and also by letters with a double prime (c″—f″)); many of them were observed at lower field with respect to those of free species. A structural assignment of these complexes was made from their ^{13}C NMR and C–H COSY spectra. The structure of the complex was shown to be asymmetric (Fig. 3) (vide infra). The assignment suggested that other sets of signals for the complex (H-a″ and b″) were superimposed on signals H-a and b for the free species, respectively. The signal intensities of the complexed species increased with α-CD concentration. These observations show that the lifetime of the complexed species is sufficiently long to give separated signals, apart from the uncomplexed one, at the NMR time scale. Similar observations were made for complexation with polymethylene compounds with pyridinium as bulky head groups at both terminals, the transverse size (d=6.5 Å) of which is larger than the cavity of α-CD (d=4.5 Å), and was enough to become a barrier for dissociation of the complex.[2] As shown

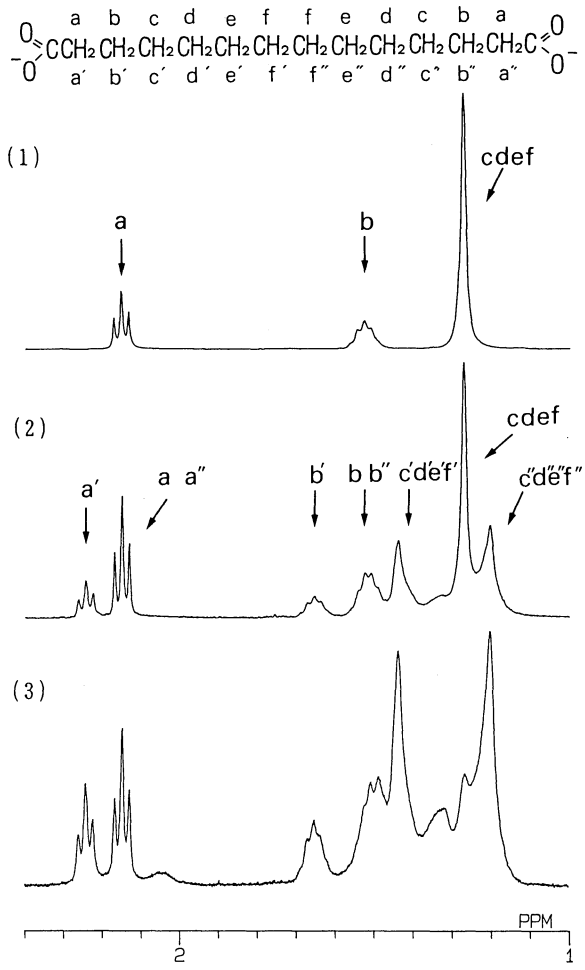


Fig. 1. ^{1}H NMR spectra of the methylene part of C_{12}^{2-} (5 mM[#]) in D_2O at 30 °C. (1) without α-CD, (2) with α-CD (2.5 mM), (3) with α-CD (5 mM). ([#]1 M=1 mol dm^{-3}).

above, this dicarboxylate compound also forms a stable complex with α-CD, suggesting that this carboxylate anion acts as a similar barrier, even if this type of guest molecule does not have very bulky head groups (d=4.8 Å for carboxylate) with respect to the cavity size.

The same complexation behavior was also observed in the ^{1}H NMR spectra of other dicarboxylates with shorter methylene chains (Fig. 2). C_{11}^{2-}, C_{10}^{2-}, and C_8^{2-} also showed distinct signals of CD complexes apart from those of the respective free species. However, the signals became broader with a decrease in the methylene chain length. In the case of C_7^{2-}, only the coalescent spectra were observed at room temperature; finally, dicarboxylate with six methylene chains did not give any evidence of complexation with α-CD.

A similar complexation behavior was also confirmed by the ^{13}C NMR spectra. Figure 3 shows the ^{13}C NMR spectra of the methylene parts of C_{12}^{2-}. In Figs. 3-(2),(3) new signals appeared apart from the peaks for the free

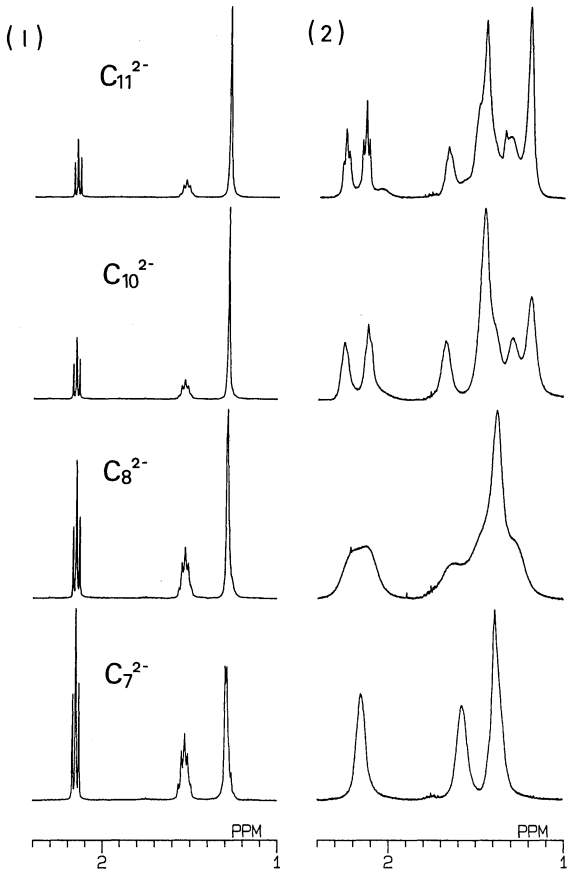


Fig. 2. ^{1}H NMR spectra of the methylene part of C_n^{2-} (n=7, 8, 10, 11) in D_2O at 30°C. (1) C_n^{2-} (5 mM) only, (2) addition of α-CD (5 mM).

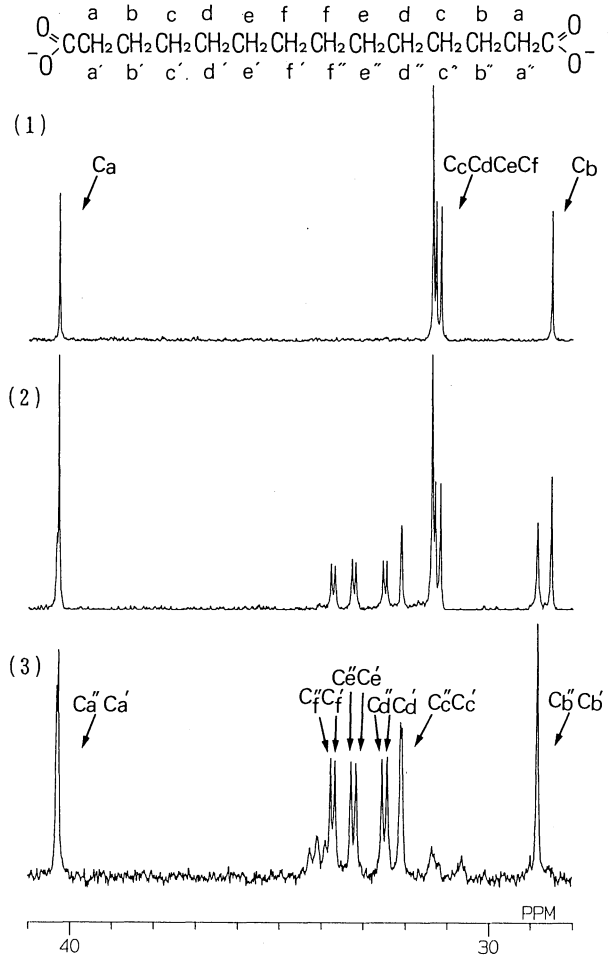


Fig. 3. ^{13}C NMR spectra of the methylene part of C_{12}^{2-} (100 mM) in D_2O at 30°C. (1) without α-CD, (2) with α-CD (50 mM), (3) with α-CD (100 mM).

species, when α-CD was added. These signals were assigned to the α-CD complex of C_{12}^{2-}, as is shown in the figure. Since these peaks are a doublet, except for the β-methylene carbon (C-b′ and b″), it is concluded that each pair of the methylene carbon is located in an asymmetric environment, which may be induced by complexation with α-CD. This induced asymmetry by complexation with CD was also reported in a previous paper.[2]

On the other hand, β- and γ-CD gave no evidence of complexation with dicarboxylates. The suitability of the size in both the cavity of α-CD and the guest molecule is likely to be a factor for stable complex formation.

Considering that the symmetric dicarboxylate became asymmetric upon complexation with α-CD, we assumed that α-CD exists near the center of the methylene chain and induces a different magnetic environment. To confirm this, intermolecular NOEs were measured. Both NOESY and NOE difference spectra of the C_{12}^{2-}–α-CD complex are illustrated in Fig. 4. NOE correlation peaks between methylene protons in the near central parts of carboxylate (H-c′—f′ and H-c″—f″) and H-3 and H-5 of α-CD were clearly observed in the NOESY spectrum. Since the H-3 and H-5 protons are located inside of the CD, it was suggested that the methylene of C_{12}^{2-} is wrapped by a CD molecule. This was further confirmed by the NOE difference spectra. When protons at H-3 of α-CD were irradiated, an appreciable amplitude of NOE for the near central part of the methylene proton (H-b′—f′ and H-b″—f″), and a small NOE for protons at H-a′ and H-a″ were observed. On the other hand, only the center parts were responsible for NOE by irradiation at H-5 and H-6, whose chemical shifts were too close to be irradiated individually. From these observations it was concluded that α-CD incorporates C_{12}^{2-} into its hydrophobic cavity with a relatively long lifetime on the NMR time scale, and that the mean position of α-CD is a near central part of C_{12}^{2-}.

For analyzing the complexation behavior quantitatively, we should determine thermodynamic data. However, there were several difficulties, as follows: (1) The chemical shifts of α-CD for the free and the complex species overlapped each other in neutral media. (2) Under the condition shown in Fig. 1 (5 mM each), the

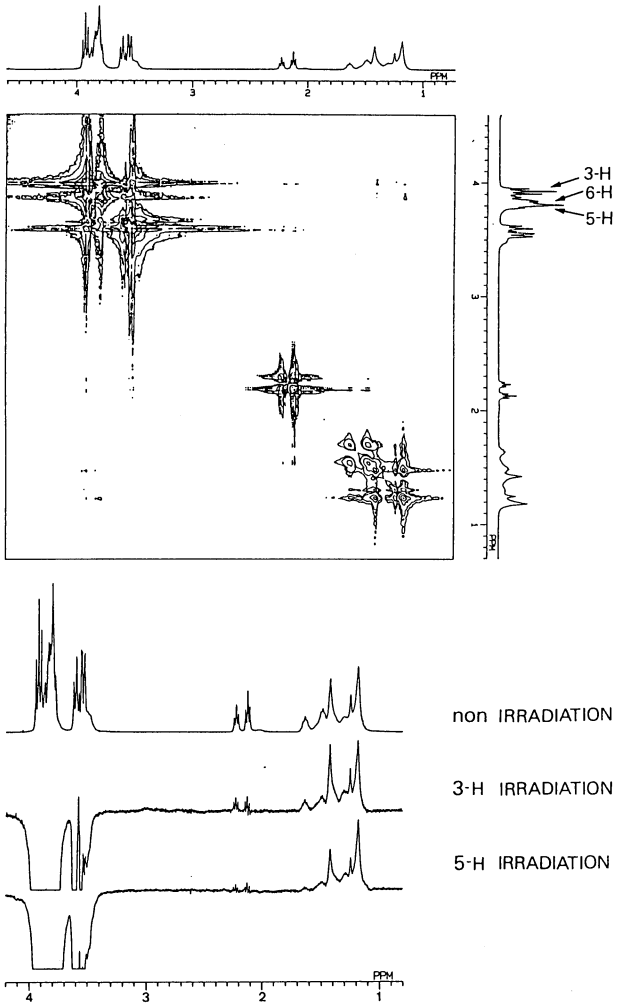


Fig. 4. NOESY (above) and NOE difference spectra (below) of the C_{12}^{2-}-α-CD complex in D_2O at 30 °C.

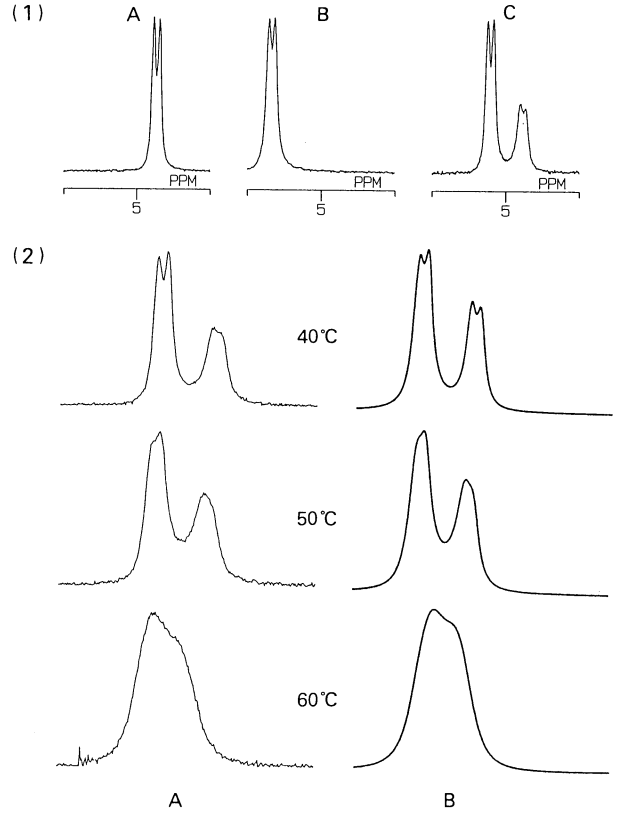


Fig. 5. ^1H NMR spectra for the H-1 protons of α-CD. (1) A. CD only (pD 8), B. CD–C_{12}^{2-} complex (pD 8), C. CD–C_{12}^{2-} complex (pD 13). (2) H-1 signal of the CD–C_{12}^{2-} complex (pD 13) at various temperatures. A. observed spectra, B. simulated spectra.

concentrations of free species of α-CD ([α-CD]), which were estimated from the total concentration of α-CD and the concentration of the complexed species, were too low to be detected. This may be due to a large formation constant for the complex ($K>10^4$ M^{-1}). These difficulties could not be solved by measurements at low concentrations (less then 1 mM).

Complexation at Basic Condition. Since these dicarboxylate compounds have anionic moieties on both terminals, the complexation properties would be expected to be affected by the dissociation of the hydroxyl group of α-CD. Since the reported pK_a value of secondary hydroxyl group is about 12,[5,6] in basic media (pD 13) the dissociation of secondary hydroxyl groups of CD would occur. Under this condition, it is expected that the electrostatic repulsion between the guest molecule and the ionized secondary hydroxyl group on CD reduces the exchange rate of free and complex species.

In the presence of α-CD, the ^1H NMR spectra of C_{12}^{2-} at pD 13 are shown in Fig. 5. There was not much difference from those at neutral pD for the methylene region (1—2.3 ppm) of C_{12}^{2-}. However, the H-1 signal of α-CD was clearly split into those for the free and complexed species at low temperatures (<40 °C). Even under this basic condition, the structure of this complex was confirmed to be the same as that at neutral pD by measurements of the NOESY and NOE difference spectra.

When the temperature was increased to more than 60 °C, this H-1 proton of α-CD coalesced. A simulation of these temperature-dependent spectra of H-1 was available for the determination of the dissociation rates for the complex (k_d) and the concentrations of free and complexed species at each temperature. The concentration of free C_n^{2-} was estimated from the signal intensities of a, a′, and a″, which appeared around at 2.2 ppm. Thus, the $\Delta G°$ value for complexation and activation free energies for the association ($\Delta G_0^{\ddagger}$) and dissociation ($\Delta G^{\ddagger}$) processes were calculated, and the enthalpy and entropy for each process were evaluated from the temperature dependence of the free-energy differences in the usual manner. These thermodynamic parameters are listed in Table 1. In Table 1, the ΔG

Table 1. Thermodynamic Parameters for Complexation of Alkane Dicarboxylate Anion with α-CD under Basic Condition
(1) Free-Energy Difference for Complexation Equilibria
(2) Activation Energies for Association Process
(3) Those for Dissociation Process

(1)

	C_n^{2-}				PyC_n[a]
	K/M^{-1}	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J K^{-1} mol^{-1}	$\Delta G°$/kJ mol^{-1}
n=8	310	−14.5	−14.9	−1	−10.9
n=9	630	−16.2	−22.6	−21	−14.9
n=10	1400	−18.2	−18.2	0	−17.8
n=11	1500	−18.4	−25.1	−22	—
n=12	5400	−21.6	−25.7	−14	−19.6

(2)

	C_n^{2-}			PyC_n[a]
	$\Delta G_0^{\ddagger}$/kJ mol^{-1}	$\Delta H_0^{\ddagger}$/kJ mol^{-1}	$\Delta S_0^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G_0^{\ddagger}$/kJ mol^{-1}
n=8	51.5	42.8	−28	50.1
n=9	53.2	46.1	−23	51.7
n=10	52.5	50.7	−6	51.9
n=11	54.4	48.2	−21	—
n=12	50.9	47.4	−17	49.5

(3)

	C_n^{2-}			PyC_n[a]
	$\Delta G^{\ddagger}$/kJ mol^{-1}	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta G^{\ddagger}$/kJ mol^{-1}
n=8	66.0	57.7	−27	62.0
n=9	69.4	68.7	−2	66.6
n=10	70.7	68.9	−6	69.7
n=11	72.8	73.3	1	—
n=12	72.5	73.1	−3	69.1

a) The data were calculated from Ref. 2.

values which were calculated from previously reported data[2] are also listed.

The free-energy difference between the free and complexed species tended to become more negative (becoming more statically stable) with an increase in the methylene chain length. These values are not much different from the $\Delta G°$ values for α,ω-bis(pyridinium) compounds. Although there may be a charge repulsion between the host and guest compounds, the carboxylate complexes were only 2—3 kJ mol^{-1} statically more stable than the respective α-CD complexes of α,ω-bis(pyridinium) compounds with the same methylene chain length. This may be caused by the high ionic strength (μ=0.1), which reduces the charge repulsion, and the pyridinium moiety may be more sterically bulky than a carboxylate for the CD existing in the methylene chain. In any case, the similarity in the dependence of $\Delta G°$ on the chain length to the previous one shows that the stability of the C_n^{2-}–α-CD complex is dependent on the hydrophobic interaction between the methylene chain and the cavity of α-CD. On the other hand, since the $\Delta H°$ and $\Delta S°$ values are negative, the contribution of the van der Waals interaction to the stability of the complexes may be larger than that of the hydrophobic interaction. As shown in Table 1-(1), $\Delta G°$ depended on $\Delta H°$ rather than $\Delta S°$, and these complexation processes were exothermic. This tendency was also reported for complexation with an aliphatic compound with α-CD.[7]

In contrast to $\Delta G°$, the activation free energies for the formation processes of the complexes ($\Delta G_0^{\ddagger}$) did not change so much with respect to the length of methylene chain, and the reaction of these through-ring CD complex formations were predominantly governed by the enthalpy ($\Delta H_0^{\ddagger}$), as is shown in Table 1-(2). These results suggest that the activation energy of complex formation is dependent on an ionic repulsion between the guest molecule and anionic CD, since the steric bulkiness would not act as a barrier, as mentioned before. Alternatively, a hydration sphere on the carboxylate anion was suggested to play a role as a steric barrier, which may be much larger than a cavity of α-CD. These activation energies were almost the same as those of bis(pyridinium) compound–α-CD.[2] This suggests that the charge repulsion between the carboxylate and the α-CD anion is almost equal to the energy which comes from the steric bulkiness.

On the other hand, the activation free energy for dissociation ($\Delta G^{\ddagger}$) was dependent on the methylene chain length (Table 1-(3)). This is mainly caused by the free-energy dependence of the statical stabilities of the α-CD

complex ($\Delta G°$), as mentioned before. Furthermore, these activation free energies were also close to those of the complex of α,ω-bis(pyridinium) compounds with α-CD, with the same chain length dependence. The activation free energy was 1—4 kJ mol^{-1} larger than those for the latter compounds with a corresponding methylene chain length. Since these differences are almost equal to those for $\Delta G°$, the difference of the dynamical stabilities between the two cases could be attributed to a difference of the statical stabilities of respective complexes.

As described above, the two carboxylate anions at both terminals of the methylene chain play a significant role in slow complexation and dissociation with α-CD. This was also confirmed by the following facts: The complexes with nonionized α-CD exchanged faster than those with ionized α-CD. Though α-CD formed a complex with a saturated fatty acid anion which has one carboxylate anion, the complex showed a rapid chemical exchange, giving no distinct signals of complexed and uncomplexed species (not dynamically stable).

In conclusion, the present paper first demonstrated the formation of a stable complex at the NMR time scale between an anionic molecule and α-CD. The stability of the complex is dependent not only on the chain length of the methylene groups and the size of CD cavity, but also on the ionic property of the functional group of the guest molecule. The bulkiness of the functional group is not likely to be involved in this case.

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