Guest-Induced Excimer Formation of β-Cyclodextrin Modified with a Branched Arm Possessing Two Naphthyl Moieties

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The β-cyclodextrin derivative (DN-β-CyD) having a branched arm derived from L-ornitine and two molecules of 2-naphthoic acid was synthesized for exploring the conformational change associated with guest binding. When DN-β-CyD was alone in a 10% ethylene glycol aqueous solution, one of the naphthoyl residues in the branched arm was accommodated in the DN-β-CyD cavity. This intramolecular self complex was stabilized by the other naphthoyl moiety located outside of the cavity on the basis of a hydrophobic capping effect. Upon guest binding, the naphthoyl residue residing in the cavity was expelled from the cavity, forming an excimer with the other naphthoyl residue outside of the cavity. DN-β-CyD was not able to bind the guests strongly, owing to the stability of the intramolecular complexation form. Although DN-β-CyD was proven to be a poor host in guest binding, the guest recognition ability of DN-β-CyD could be estimated by the use of guest-induced fluorescence variations. DN-β-CyD could bind both monoterpenes and cholic acid derivatives to nearly the same extent. However, variations in fluorescence intensity induced by guest binding indicated, as a fluorescent sensing system, that DN-β-CyD was able to detect cholic acid derivatives with higher sensitivity than terpenoids.

Key words modified β-cyclodextrin; excimer; host–guest complex; molecular recognition

Molecular recognition by synthetic and semi-synthetic hosts is an important subject for understanding of which and how non-covalent weak interactions participate in assembling biological macromolecules as well as in expressing their molecular recognition ability.[1] Among synthetic and semi-synthetic hosts, cyclodextrin (CyD) derivatives[2] are interesting materials because CyD can bind guests into its cavity, mainly due to hydrophobic and van der Waals interactions which are regarded as major forces in molecular association events by biological macromolecules.

Although CyD itself has a relatively rigid structure which limits its molecular recognition ability, the introduction of hydrophobic residues at the narrower or wider rim of CyD may alter its molecular recognition ability,[3] changing the shape and volume of the hydrophobic region into which neutral organic guest molecules can be adequately incorporated. In cases in which the hydrophobic moiety which is introduced to a CyD molecule has spectroscopic properties sensitive to an environmental change, the insertion of a guest into the cavity can alter the spectroscopic properties of the introduced moiety. This phenomenon allows us to use modified CyDs as sensing probes for detecting organic molecules.[4] Along with this strategy, we and others have paid a lot of effort to synthesizing many CyD derivatives possessing hydrophobic aromatic residues, and we have investigated their guest recognition ability by the use of their spectroscopic variations upon guest binding.[4]

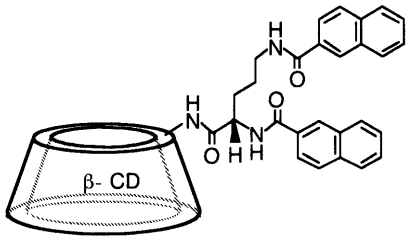
Of the spectroscopically active CyDs, doubly modified CyDs were particularly interesting entities because they would be able to attain conformations suitable to individual guest compounds, and as a consequence, to lead to diverse spectroscopic changes associated with guest binding.[5] Thus, doubly modified CyDs would be useful in sensing neutral organic molecules. Contrary to this, there has been a very few report on guest binding or the molecular recognition behavior of CyD modified with a branched arm possessing two aromatic moieties.[6] It seemed interesting, therefore, to explore whether the two aromatic moieties on the same "arm," which connects them to the CyD framework, affect the guest binding independently or cooperatively.

Here, we would like to report the intra- and intermolecular inclusion phenomena of β-CyD modified with a branched arm consisting of L-ornitine and two 2-naphthoyl groups (DN-β-CyD). Our results indicate that the two naphthyl moieties in the branched arm stabilize the "intramolecular self complexation" form, where one naphthyl moiety was bound in the β-CyD cavity with the other one, regarded as a hydrophobic cap, thus stabilizing the self complex conformation.

Experimental

Materials β-CyD was purchased from Wako Chemical Co. (Tokyo, Japan) and recrystallized from water. Guest compounds used in this study were of the best grade reagents available commercially and were used as received. Ethylene glycol used for spectroscopic measurements was distilled under reduced pressure from a guaranteed grade reagent.

Measurements UV-visible, fluorescence, and circular dichroism (CD) spectra were recorded on a Shimadzu UV-250 spectrophotometer, Jasco FP-770 spectrofluorophotometer, and Jasco J-720 spectropolarimeter, respectively. Unless otherwise noted, all measurements were performed in a 10% ethylene glycol aqueous solution, due to the poor solubility of DN-β-CyD in pure water, at 25 °C. The temperature was maintained by passing thermostated water through cell holders. For characterization, we used JEOL GX-500 and Hitachi R-3000 spectrometers for obtaining the ^{1}H-NMR spectra, a Jasco A-302 spectrophotometer for infrared



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spectra, and a JEOL JMSDX303 mass spectrometer for fast atom bombardment mass spectra.

Syntheses: *N,N'*-Bis(2-naphthoyl)-L-ornitine 2-Naphthoic acid (0.84 g, 4.88 mmol) was suspended in thionyl chloride (5 ml) and the resulting suspension was heated at reflux for 3 h. During progression of the reaction, the suspension became a clear solution. After the solution was cooled to room temperature, the excess thionyl chloride was removed by rotary evaporation and the residue was dried further *in vacuo*. The residual yellow syrup was redissolved in 1,4-dioxane (2 ml), and this solution was added to a mixture of L-ornitine hydrochloride (0.41 g, 2.44 mmol), 1,4-dioxane (10 ml), and 1 N NaOH (10 ml) over 30 min at 0 °C. The resulting mixture was stirred at 5 °C for 2 h, and then at room temperature for 8 h. After 1 N HCl (2 ml) was added to the mixture to adjust the pH to around 3, water (50 ml) was added. The precipitates which formed were collected, washed with a small amount of ice cold water, and dried *in vacuo*. Crystallization from 1,4-dioxane-hexane gave the title compound as fine colorless needles (0.56 g, 52%). FAB-MS *m/z*: 441 $[M+1]^+$. IR (KBr) cm^{-1}: 1640 ($\nu_{C=O}$), 1540 (δ_{NH}). ^{1}H-NMR (DMSO-d_6), δ: 1.65—1.80 (2H, m, $CH(COOH)CH_2C\underline{H}_2CH_2$)), 1.84—2.00 (2H, m, $CH(COOH)C\underline{H}_2CH_2CH_2$)), 3.38 (2H, quintet, $J=6.0$ Hz, $CH(COOH)CH_2CH_2C\underline{H}_2$), 4.50—4.66 (1H, m, $NHC\underline{H}(COOH)CH_2$), 7.90—8.84 (16H, m, aromatics and amides).

***N*-[(2*S*)-*N'*,*N''*-Bis(2-naphthoyl)-2,5-diaminopentanoyl]-6-amino-6-deoxy-β-cyclodextrin (DN-β-CyD)** The above diamide (230 mg, 0.53 mmol) was dissolved in anhydrous *N,N*-dimethylformamide (DMF, 2 ml). To this solution, *N,N'*-dicyclohexylcarbodiimide (146 mg, 0.71 mmol) and *N,N*-dimethyl-4-aminopyridine (8.6 mg, 0.071 mmol) were added under N_2 atmosphere at −4 °C, and the resulting mixture was stirred for 30 min at this temperature. After the addition of 6-amino-6-deoxy-β-CyD[7] (200 mg, 0.176 mmol), the mixture was stirred for another 2 h at the same temperature and for 1 h at room temperature. Insoluble materials were removed by filtration and the filtrate was concentrated nearly to dryness. A small amount of DMF (*ca.* 5 ml) was added to the syrup, and the remaining insoluble materials were removed again. The filtrate was then dropped into acetone (150 ml) under vigorous stirring. The precipitates which formed were collected, washed with a small amount of ice-cold water followed by acetone and ether, and dried *in vacuo*. The title compound was obtained as colorless powder (82 mg, 30%). The purity of this compound was checked by HPLC using an octadecyl silica (ODS) column, and no compounds having an absorption at 254 nm, other than the desired compound, was detected. Also, TLC showed a single spot regarded as the desired compound. FAB-MS *m/z*: 1556 $[M+1]^+$, 1578 $[M+Na]^+$. IR (KBr) cm^{-1}: 1640 ($\nu_{C=O}$), 1540 (δ_{NH}). ^{1}H-NMR (DMSO-d_6) δ: 1.63—1.75 (2H, m, $CH(COOH)CH_2C\underline{H}_2CH_2$)), 1.81—1.90 (2H, m, $CH(COOH)C\underline{H}_2CH_2CH_2$)), 3.33—3.63 (m, others, overlapped with H_2O), 4.45—4.67 (7H, m, primary hydroxyls and $NHC\underline{H}(COOH)CH_2$), 4.78—4.91 (7H, m, anomeric H), 5.68—5.73 (14H, m, secondary hydroxyls), 7.80—8.66 (16H, m, aromatics and amide). *Anal.* Calcd for $C_{69}H_{93}N_3O_{37}\cdot 6H_2O$: C, 49.79; H, 6.36; N, 2.52%. Found: C, 49.60; H, 6.38; N, 3.09%.

Results and Discussion

Intramolecular Complexation of DN-β-CyD In the beginning of this study, we checked the concentration dependence of the fluorescence and CD spectra of DN-β-CyD in a 10% ethylene glycol aqueous solution because some modified CyDs have a tendency to form association dimers[3d,6,8] or even higher aggregated species.[9] Neither spectra showed any significant concentration dependence in the concentration ranging from 3×10^{-4} to 3×10^{-6} M. Thus, we anticipated that DN-β-CyD would exist as a monomeric form in a 10% ethylene glycol aqueous solution at the concentration below 3×10^{-4} M.

Figure 1 shows the UV-visible spectrum together with the CD spectrum of DN-β-CyD in a 10% ethylene glycol aqueous solution. The CD spectra of DN-β-CyD obtained in various contents of ethylene glycol in solutions are also shown in Fig. 1. DN-β-CyD has an intense absorption

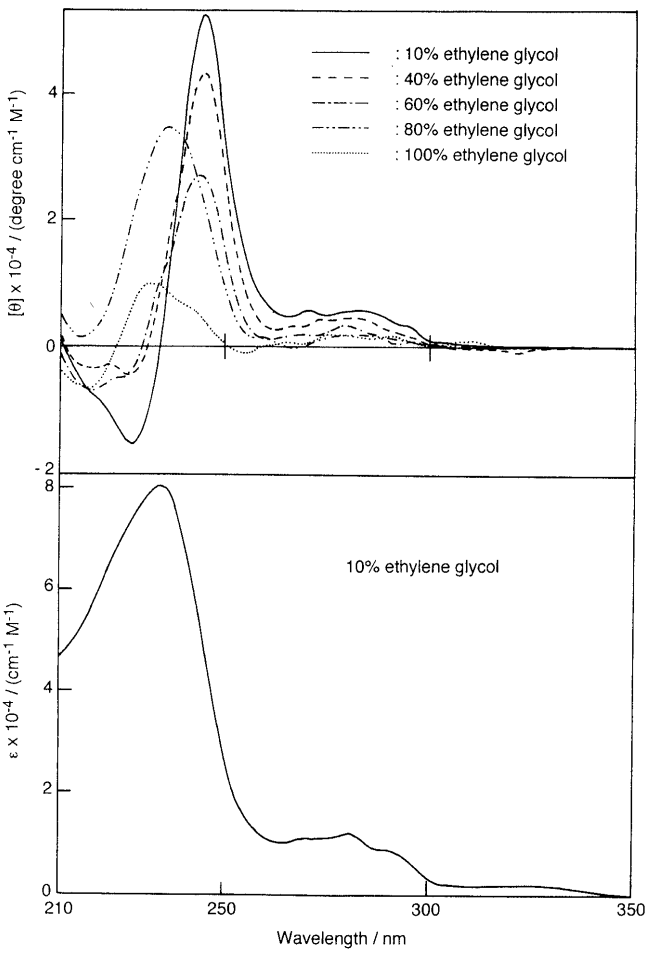

Fig. 1. UV Absorption Spectrum of DN-β-CyD (1.49×10^{-5} M) in a 10% ethylene glycol aqueous solution at 25 °C (Bottom), CD Spectra of DN-β-CyD (*ca.* 1.5×10^{-5} M) in 10, 40, 60, 80, and 100% Ethylene Glycol Aqueous Solutions at 25 °C (Top)

around 220—240 nm with the peak maximum at 235 nm and weak absorptions in the region of 250—330 nm. These are characteristic absorptions for naphthalene derivatives. In the CD spectrum obtained in a 10% ethylene glycol aqueous solution, a set of positive and negative bands appeared around 235 nm with the peak maximum and trough minimum at 244 and 225 nm, respectively. These split type CD bands are attributed to an exciton coupling pattern[10] which indicates that the orientation of the two chromophores existing in close proximity to each other is fixed, with their transition moments having a certain orientation other than parallel. Thus, the two naphthalene chromophores of DN-β-CyD should be in close proximity, and their transition moments along the longitudinal axis[11] of the naphthalene ring should be skewed from complete parallel orientation.

The exciton coupling pattern diminished with increasing the content of ethylene glycol, and a new positive peak with a peak maximum at 235 nm appeared in an 80% ethylene glycol aqueous solution. The disappearance of the exciton coupling pattern and the appearance of a positive CD band around 235 nm is probably due to the locational change of the naphthyl moieties. The appearance of the positive CD band at 235 nm may be attributable to the fact that CD bands are observed when

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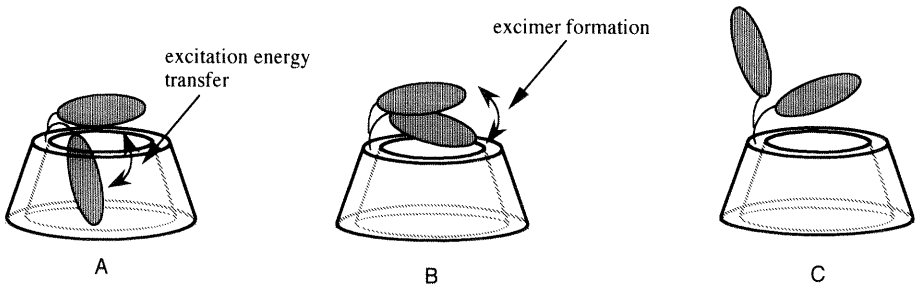

Chart 1

a chromophore is located in or around a CyD cavity.[12] It is well known that CyD can bind an organic compound into its cavity more strongly in an aqueous media than in an organic medium such as ethylene glycol, even if the polarity and other properties of the latter as a solvent are similar to water. In addition, the β-CyD cavity is too small to accommodate two naphthalene molecules simultaneously, particularly from the narrower primary hydroxyl group side. Therefore, the exciton coupling pattern seen in the CD spectrum of DN-β-CyD in an aqueous rich solution containing less than 60% ethylene glycol may be derived from conformation A drawn in Chart 1 in which one naphthyl moiety is incorporated in the cavity of DN-β-CyD and the other one is located atop the rim of the primary hydroxyl group side, acting as a hydrophobic cap[13] to stabilize the intramolecular complexation. The cap residue may not move freely because of the hydrophilic nature of the outside of the cavity, and it is possible that the hydrogen bonding participating between the two amide groups in the branched arm and primary hydroxyl groups takes part in the stabilization of the intramolecular complexation. However, since conformation A is dominant in an aqueous solution, the hydrogen bonding is presumed to be less pronounced in stabilizing the self complexation form. Thus, an increase in the ethylene glycol content in a solution of DN-β-CyD loosens the hydrophobic interaction engaging the two naphthyl moieties and the cavity, resulting in weakening the exciton coupling pattern.

It is noted that we could not determine a precise conformation of DN-β-CyD in the absence of a guest with regard to which naphthyl moiety in the branched arm was predominantly accommodated in the cavity. However, Corey–Pauling–Koltun (CPK) molecular model considerations enabled us to say that, owing to steric hindrance and the stereochemistry of the branched arm, the naphthyl residue attached at the δ-amino group of the L-ornitine-derived branched arm may be preferably accommodated in the DN-β-CyD cavity, and the other naphthyl residue attached at the α-amino group may be located atop the primary hydroxyl side. If this conformer is an actual dominated species, the transition moments for the longitudinal axis of the naphthyl groups would be arranged in a clockwise manner, which satisfies the requirement for the exciton coupling pattern[10] seen in Fig. 1.

In a pure ethylene glycol solution, the CD band around 235 nm became weaker than those observed in solutions containing water. This suggests that the interaction integrating the naphthyl moieties with the hydrophobic cavity, namely, a hydrophobic interaction, nearly disappeared in a pure ethylene glycol solution. It is noteworthy that even in an organic medium such as dimethyl sulfoxide and ethylene glycol, native CyDs can accommodate some organic compounds having a suitable size and shape to the cavity, but the binding strength was extremely weak as compared to that in an aqueous solution.[14] As a consequence, the remaining CD band in an ethylene glycol solution may be derived from the weak interaction between the naphthyl moieties and the cavity.

Figure 2 shows the fluorescence spectra of DN-β-CyD obtained for solutions containing various amounts of ethylene glycol. In a pure ethylene glycol solution, DN-β-CyD exhibited nearly excimer-free fluorescence attributable to the monomer fluorescence from the naphthyl moieties (368 nm). The absence of the excimer fluorescence indicates that the two naphthyl moieties cannot attain a conformation in which they exist close enough to form an excimer within their lifetimes in the singlet excited state. When combining this speculation with the conclusion drawn from the CD spectral behavior, we can infer a conformation of DN-β-CyD dominating in a pure ethylene glycol solution, as depicted in Chart 1, conformation C. In an ethylene glycol solution, hydrophobic interactions integrating the two naphthyl moieties and cavity are remarkably weak, owing to the relatively hydrophobic nature of the exterior of the cavity, and thus, the intramolecular complexation or association of the two naphthyl moieties scarcely occurs.

The fluorescence behavior of DN-β-CyD in solutions containing various amounts of ethylene glycol was complicated, as seen in Fig. 2. Up to 60% of ethylene glycol content in a solution, the excimer fluorescence (*ca.* 450 nm) keeps its intensity as large as that observed in a 10% ethylene glycol aqueous solution, whereas the monomer fluorescence intensity monotonously increases with the increasing content of ethylene glycol in a solution. As seen in the CD spectra (Fig. 1), the exciton coupling band remains for solutions containing up to 60% ethylene glycol, but the intensity of the exciton coupling is weakened with increasing ethylene glycol content. Although the conformation attributable to the appearance of the exciton coupling pattern (conformation A in Chart 1) prohibits excimer formation, the distance between the two naphthyl groups is close enough to attain an excitation energy transfer which quenches the monomer fluorescence of the naphthyl moieties.[15] In solutions with a medium amount of ethylene glycol (*i.e.*, 60%), although

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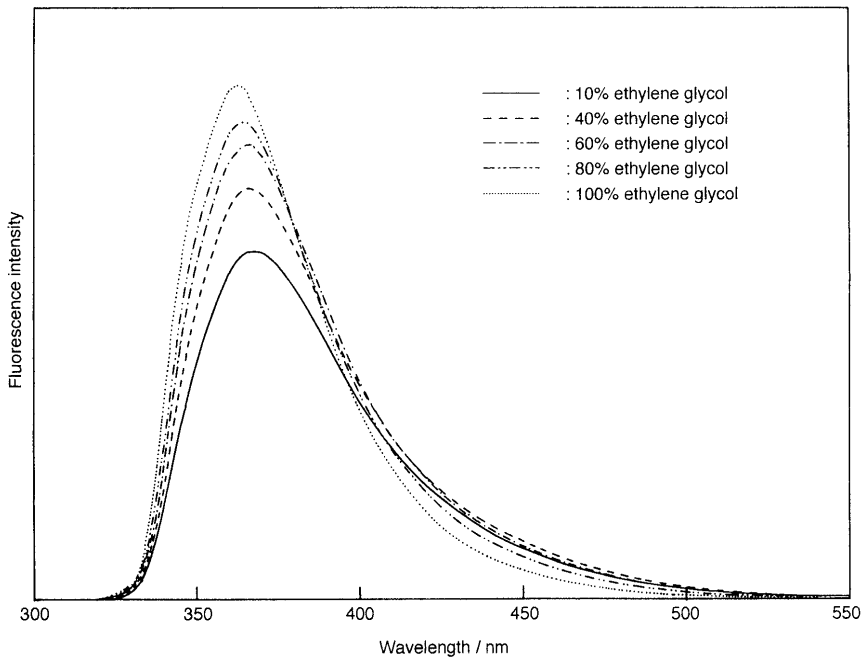

Fig. 2. Fluorescence Spectra of DN-β-CyD (1.49×10^{-5} M) in 10, 40, 60, 80, and 100% Ethylene Glycol Aqueous Solutions at 25 °C, Excited at 280 nm

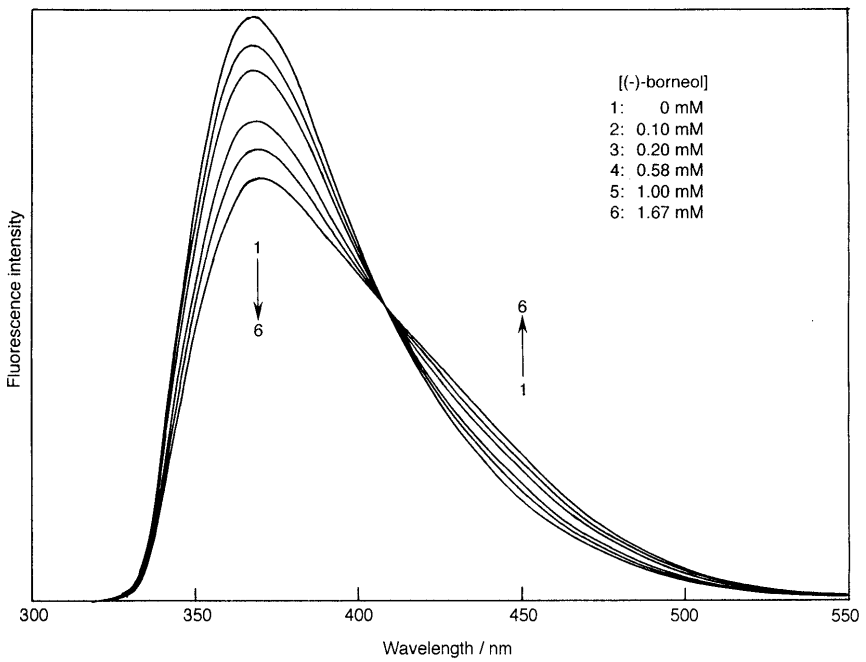

Fig. 3. Fluorescence Spectra of DN-β-CyD (1.49×10^{-5} M), Alone or in the Presence of 0.10, 0.20, 0.58, 1.0, and 1.67 mM of (−)-Borneol at 25 °C, Excited at 280 nm

DN-β-CyD can take the conformation A, the increased hydrophobicity as compared to that in a 10% ethylene glycol aqueous solution breaks the conformation A to some extent, and thus, the excitation energy transfer becomes less pronounced. However, the hydrophobicity of the medium may not be strong enough to break the "microenvironmental hydrophobic effect" (or π–π interaction) with which two naphthyl moieties can associate to form an excimer. Conformation B in Chart 1 represents this situation.

The excimer fluorescence of DN-β-CyD substantially decreases in an 80% ethylene glycol aqueous solution, where nearly no exciton coupling band is observed in the CD spectrum. At this level of ethylene glycol content, the "microenvironmental hydrophobic interaction" seems to vanish abruptly, but the hydrophobic interaction between the naphthyl groups and the cavity still seems to be alive.

Guest Binding Behavior of DN-β-CyD It is expected that if a guest compound is inserted into the hydrophobic cavity of DN-β-CyD occupied by a naphthyl moiety, an increase in the excimer fluorescence should be observed,

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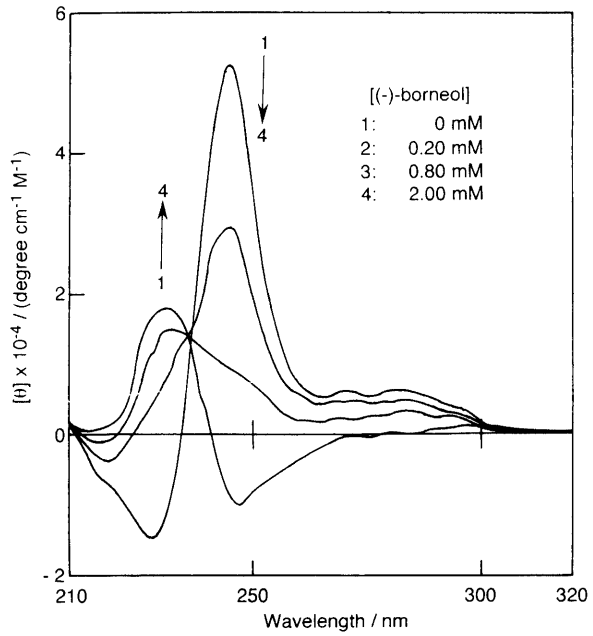

Fig. 4. CD Spectra of DN-β-CyD (1.49×10^{-5} M), Alone or in the Presence of 0.20, 0.80, and 2.00 mM of (−)-Borneol at 25 °C, Excited at 280 nm

because the possibility of the two naphthyl moieties interacting would be enhanced in terms of attaining close proximity positions, which would be a crucial factor in the formation of an excimer. Indeed, as shown in Fig. 3, the fluorescence spectra of DN-β-CyD alone in a 10% ethylene glycol aqueous solution exhibited weak excimer fluorescence around 450 nm, whereas the addition of (−)-borneol as a guest increased the excimer fluorescence by increasing the monomer fluorescence intensity.

Figure 4 shows guest-induced CD spectral variations in a 10% ethylene glycol aqueous solution. The exciton coupling pattern observed in a solution without a guest was diminished upon the addition of (−)-borneol, and further addition of (−)-borneol induced a new exciton coupling pattern with the peak maximum and trough minimum at 230 and 247 nm, respectively. The complete change in the signs of the exciton coupling patterns, namely, from plus to minus around 245 nm and from minus to plus around 230 nm, associated with guest binding, indicates that the guest of (−)-borneol changed the mutual orientation of the naphthyl moieties. We have already figured out that the exciton coupling pattern observed in a 10% ethylene glycol aqueous solution in the absence of a guest species resulted from conformation A in Chart 1. Molecular model (CPK) considerations also revealed that a β-CyD cavity was not capable of accommodating both the naphthyl moiety and a guest (*e.g.* (−)-borneol). In addition, as mentioned above, the addition of (−)-borneol enhanced the excimer fluorescence. Therefore, we can reasonably assume that one of the naphthyl moieties incorporated in the cavity of DN-β-CyD was excluded by the insertion of (−)-borneol, and the excluded naphthyl moiety was able to get into a position to interact with the other naphthyl moiety to form an excimer outside of the cavity. The resulting excimer is likely to take a relatively rigid orientation

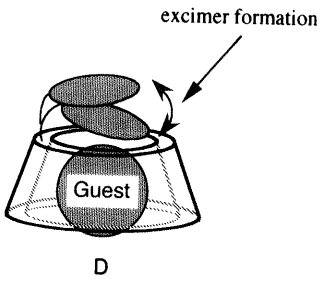

Chart 2

with respect to the transition moments of the naphthyl moieties, exhibiting an exciton coupling pattern opposite to that observed without a guest. Although the naphthyl groups were located outside of the cavity, a face-to-face interaction essential for the formation of an excimer between the naphthyl moieties could be achieved because of their hydrophobic nature and the extremely hydrophilic nature of the aqueous environment surrounding them. Conformation D in Chart 2 depicts a schematic representation of the guest binding behavior of DN-β-CyD.

1 : 1 Host-Guest Binding Constants Since we observed both isoemissive and isoellipticity points in the guest-induced spectral variations of fluorescence and CD spectra of DN-β-CyD, respectively, we reasonably assumed that 1 : 1 host-guest complexation occurred for cyclohexanol (CH), (+)-menthol ((+)-ML), (−)-menthol ((−)-ML), (−)-borneol ((−)-BL), 1-adamantanol (1-AL), and 1-adamantanecarboxylic acid (1-ACA). Although the presence of a high concentration of lithocholic acid (LCA), chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA) perturbed the isoemissive point in the fluorescence spectra of DN-β-CyD, probably due to the formation of host–guest complexes other than the 1:1 stoichiometry, the fluorescence spectra measured with a low concentration of the guests exhibited isoemissive points. Thus, we were able to calculate the host–guest binding constants for complexation between DN-β-CyD and the guests cited above from guest-induced fluorescence spectral variations obtained under a low guest concentration region, assuming only 1 : 1 complex formation.

Table 1 contains the calculated values of binding constants for the guests, together with the sensing parameters of $\Delta I_{(368)}/I_{0(368)}$ and $\Delta I_{(450)}/I_{0(450)}$ where I_0 stands for the fluorescence intensity of DN-β-CyD alone and ΔI stands for an amount of change in the fluorescence intensity at a given concentration of a guest (negative and positive values represent a decrease and increase in the intensity, respectively). Numbers in the parentheses denote the wavelength at which the data were collected. The sensing parameters were useful for estimating the molecular recognition ability of chromophore-appended CyDs when they were used as sensing components.[4)]

The binding constant of unmodified β-CyD for CH in an aqueous solution was reported as $474\,M^{-1}$,[16)] and we previously obtained $930\,M^{-1}$ for the same host–guest combination in a 20% ethylene glycol aqueous solution.[17)] Although binding constants for any host–guest complexa-

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Table 1. 1 : 1 Host–Guest Binding Constants (K) and Sensitivity Factors ($\Delta I/I_0$) of DN-β-CyD for Various Guests in a 10% Ethylene Glycol Aqueous Solution[a)]

Guest	K	$\Delta I_{(368)}/I_{0(368)}$	$\Delta I_{(450)}/I_{0(450)}$	Conc.[b)]
	M^{-1}			mM
CH	55	−0.022	0.033	1.0
1-AL	1090	−0.071	0.126	0.1
		−0.295	0.437	1.0
(−)-BL	1380	−0.053	0.074	0.1
		−0.230	0.336	1.0
(−)-ML	1950	−0.041	0.074	0.1
		−0.182	0.312	1.0
(+)-ML	2850	−0.051	0.091	0.1
		−0.197	0.336	1.0
1-ACA	495	−0.079	0.171	0.1
LCA	2090	−0.224	0.210	0.1
CDCA	2360	−0.141	0.198	0.1
UDCA	4210	−0.226	0.333	0.1

a) All measurements were performed at 25 °C. b) A guest concentration at which $\Delta I_{(x)}/I_{0(x)}$ values were collected.

tion process are likely to depend somewhat on the methods applied, it seems reasonable to assume that the binding constant for combination in a 10% ethylene glycol aqueous solution resides in the range of 100—1000 M^{-1}. Thus, the small binding constant of 55 M^{-1} means that the branched arm in DN-β-CyD prevents, more or less, insertion of the guest into the cavity. Smaller binding constants of DN-β-CyD for the other guests were also obtained. These values were similar to those obtained with β-CyD derivatives possessing two naphthyl moieties at primary hydroxyl groups of different glucopyranose units: in a 10% ethylene glycol aqueous solution, β-CyD modified with 2-naphthylacetyl residues, mainly at A and D glucopyranose units, had binding constants for CH and (−)-BL of 90 and 1900 M^{-1}, respectively.[18] In this host, one of the naphthyl groups predominantly accommodated to form an intramolecular complex was excluded from the cavity by the insertion of a guest, and the two naphthyl moieties were able to attain a conformation to interact with each other to form an excimer. This guest-induced conformational behavior is similar to that proposed for DN-β-CyD. Thus, taking into consideration the small binding constants of DN-β-CyD, the naphthyl moiety existing outside of the cavity before guest insertion would greatly stabilize the intramolecular self complexation, the form A in Chart 1, preventing the guest binding.

Regarding the ability of DN-β-CyD in sensing organic compounds, large $\Delta I/I_0$ values were obtained for cholic acid derivatives which were bound strongly by DN-β-CyD, although the binding strength of DN-β-CyD for the guests seems weaker than those of other β-CyD derivatives. Detailed inspection of the relationship between $\Delta I/I_0$ values and corresponding binding constants, however, suggests that the two parameters of binding constants and $\Delta I/I_0$ values do not entirely correlate.

According to the binding constants, among the guest compounds examined here, (+)-ML was the secondary guest to be bound strongly by DN-β-CyD, lead by UDCA. However, both $\Delta I_{(X)}/I_{0(X)}$ values for (+)-ML at 0.1 mM of the guest concentration were extremely small compared to those for LCA and CDCA, for which the binding constants were smaller than that for (+)-ML. This fact indicates that cholic acid derivatives (LCA, CDCA, and UDCA) induced more drastic conformational changes with respect to the naphthyl moieties of DN-β-CyD when it bound them. Although the precise conformations of host-guest complexes between DN-β-CyD and the guests are still unclear, deep penetration of the cholic acid derivatives may greatly exclude the naphthyl moiety which was bound by the DN-β-CyD cavity, and as a consequence, the naphthyl moieties may be able to get into a position to form an emissive excimer outside of the cavity.

When the $\Delta I_{(X)}/I_{0(X)}$ values for (+)-ML obtained at 1.0 mM of the guest concentration are compared with those for 1-AL and (−)-BL, for which the binding constants were also smaller than that for (+)-ML, results similar to the cases of cholic acid derivatives were obtained. Thus, although (+)-ML (and also (−)-ML) was bound strongly by DN-β-CyD, (+)-ML and (−)-ML do not largely move the naphthyl moieties, leading to a small variation in fluorescence spectra as compared to the cholic acid derivatives, 1-AL and (−)-BL.

Regarding chiral discrimination in guest binding, we checked the binding constants and $\Delta I_{(X)}/I_{0(X)}$ values for (−)-ML and (+)-ML. We found that (+)-ML was a more suitable guest than (−)-ML in terms of binding constants. $\Delta I_{(X)}/I_{0(X)}$ values for the guests, however, were not so different, regardless of the guest concentration or wavelength. This indicates that discrimination in the chirality of the guests was partly achieved by DN-β-CyD in binding strength, but (−)-ML was likely to cause a larger conformational change to DN-β-CyD than (+)-ML. This conformational change would cancel the difference in binding constants, and as a result, the apparent $\Delta I_{(X)}/I_{0(X)}$ values for the (−)-ML and (+)-ML had similar values.

On the other hand, for CDCA and UDCA, which are diastereomers on the hydroxyl group at the C-7 position, DN-β-CyD bound UDCA more strongly than CDCA, and the $\Delta I_{(X)}/I_{0(X)}$ values nearly correlated with the binding constants. This indicates that the difference in the orientation of the hydroxyl group at the C-7 position in the steroidal framework was reflected in binding constants and $\Delta I_{(X)}/I_{0(X)}$ values. The lack of that hydroxyl group (LCA) resulted in a smaller binding constant and larger $\Delta I_{(X)}/I_{0(X)}$ values compared to CDCA.

The difference in binding constants or $\Delta I_{(X)}/I_{0(X)}$ values corresponding to the small variation in guest shape may relate to hydrogen bonding[19] between DN-β-CyD and a guest upon complexation. A smaller binding constant for 1-ACA, which exists as a carboxylate form under the condition applied here, than that for 1-AL, may be explained by the participation of the hydrogen bonding, because the hydroxyl group of 1-AL can act as a donor and acceptor for the hydrogen bonding, whereas the carboxylate of 1-ACA can act only as an acceptor for the hydrogen bonding. The hydrophobic interaction may also take part in the larger binding constant for 1-ACA as compared to that for 1-AL, because the anionic form of 1-ACA is more hydrophilic than 1-AL. However, β-CyD can bind the anionic form of 1-ACA

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more strongly than 1-AL.[20] This is opposite to our results on DN-β-CyD. Therefore, although the hydrophobic interaction primarily promotes the formation of complexes between DN-β-CyD and 1-AL or 1-ACA, further participation of hydrogen bonding in stabilizing the complexes may be more effective in the DN-β-CyD–1-AL complex, with the hydroxyl group of 1-AL acting as both a hydrogen donor and acceptor.

Comparison with Other CyD Derivatives As described above, although DN-β-CyD was a rather poor host in binding a guest, the fluorescence change associated with complexation was sensitive enough to discriminate CDCA and UDCA. This situation is different from the other β-CyD derivatives possessing two naphthyl moieties. For instance, the three regioisomers of β-CyD bearing two 2-naphthylsulfonyl groups[5c] cannot discriminate between CDCA and UDCA in fluorescence output, regardless of monomer or excimer fluorescence. The difference in molecular recognition ability between DN-β-CyD and the bis-naphthyl-β-CyDs may be attributable to the stability of the self complexation form of DN-β-CyD; the stable self complexation would not permit a guest of small or medium size to penetrate into the cavity, but a large guest such as CDCA or UDCA could penetrate deep, changing the location of the naphthyl moieties. This deep penetration and large change in the location of the naphthyl moieties may provide DN-β-CyD with the ability to distinguish CDCA, both in binding strength and fluorescence output. As in the complexes between DN-β-CyD and 1-AL and 1-ACA, hydrogen bonding would participate in discriminating between CDCA and UDCA.

In addition, since DN-β-CyD bears the naphthyl moieties at the "same" glucopyranose unit, the possibility of forming an excimer between them outside of the cavity may occur when DN-β-CyD binds cholic acid derivatives. This may result in the large fluorescence changes induced by cholic acid derivatives, especially by UDCA. In contrast to DN-β-CyD, the bis-naphthyl-β-CyDs bear naphthyl moieties at "different" glucopyranose units. When these hosts bind a large guest, their naphthyl moieties cannot take conformations suitable for forming excimers because the large guest may push the naphthyl moieties apart from each other. This may relate to the lesser ability to discriminate between CDCA and UDCA in the bis-naphthyl-β-CyDs.

Conclusion

The branched arm of DN-β-CyD was found to be rather ineffective in guest binding because of the stability of the intramolecular complex in which one of the naphthyl moieties was accommodated in the cavity and the other acted as a hydrophobic cap. This conformation was similar to that found in disubstituted β-CyD, having two naphthyl groups on different glucopyranose units.[5c,18] Although the guest binding ability of DN-β-CyD was relatively weak, the host can detect some organic compounds, especially cholic acid derivatives, on the basis of changes in florescence intensity induced by guest binding, as in the case of disubstituted β-CyD having two naphthyl moieties.[5c] Moreover, DN-β-CyD was an effective host for discriminating between the epimers of CDCA and UDCA, which cannot be discriminated by the bis-naphthyl-β-CyDs. This suggests the advantage of providing a branched arm over disubstitution on a CyD framework in constructing sensing devices based on host-guest complexation.

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