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Fluorescence and Circular Dichroism Studies on Host–Guest Complexation of γ-Cyclodextrin Bearing Two 2-Naphthyl Moieties

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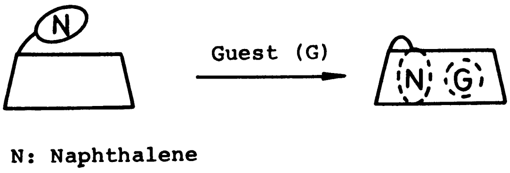
Host-guest complexation of γ-cyclodextrin bearing two 2-naphthyl moieties (**1**) has been studied by circular dichroism and fluorescence spectroscopic methods. The exciton coupling band observed in the naphthalene ^1L_a transition region shows that the two naphthyl moieties of **1** take a chiral orientation in the cavity. The guest binding of **1** changes the circular dichroism pattern, finally affording a negative band, but hardly influences the fluorescence pattern in which excimer emission is predominant. These phenomena suggest that a guest molecule is included in the cavity of **1** by extruding two naphthyl moieties from the cavity so as to make the moieties to act as hydrophobic caps. Dimethyl sulfoxide(DMSO) was found to be effective to change both fluorescence and circular dichroism patterns, increasing monomer-like emission and converting the exciton coupling pattern to a simple negative band. These results suggest that the two naphthyl moieties are not in proximal positions in DMSO-rich solutions.

Cycloamyloses (cyclodextrins, CDs) are a series of cyclic oligomers consisting of six or more α-1,4-linked D-glucopyranose units. The number of glucopyranose unit is designated by a Greek letter: α for six, β for seven, γ for eight, and so on. The smaller α- and β-CDs have been widely utilized for various purposes because of their remarkable property of forming inclusion complexes with a variety of small molecules.[1] However, the use of γ-CD has not been frequent due to its large cavity size in addition to the factor of its commercial unavailability. In recent reports of spectroscopic studies,[2–4] it was suggested that γ-CD can include two molecules of benzene or naphthalene derivatives in marked contrast to α-and β-CDs which form 1:1 host-guest complexes. The unique property of γ-CD places the basis for using γ-CD as a molecular flask which facilitates reactions by involving two reactants as shown by excimer[2] or charge-transfer complex[3] formation and dimerization.[5]

As a new extension of our works, we have prepared several γ-CD derivatives, which bear a covalently attached aromatic moiety,[6] and observed that the moiety acts as a spacer which enables the γ-CD derivatives to form 1:1 host-guest complexes by narrowing the large γ-CD cavity (Scheme 1). The complexation behavior of the γ-CD derivatives may be regarded as an induced-fit type of complexation since the host changes the location of the appended moiety so as to be suited for guest binding.

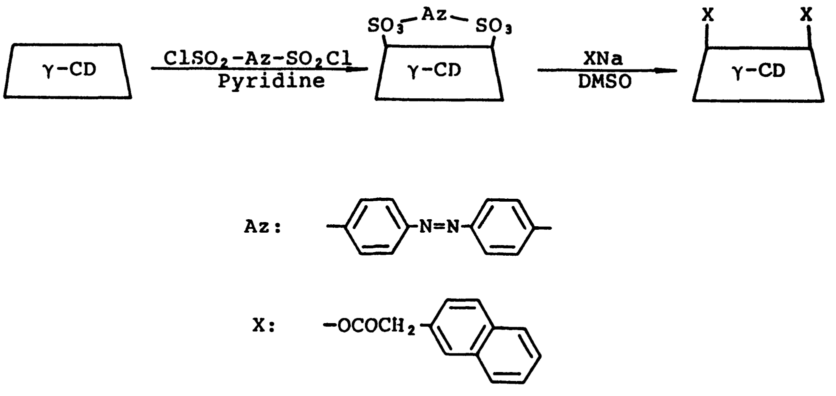


Scheme 1.

We now wish to describe here another induced-fit type of complexation which was observed for γ-CD bearing two 2-naphthyl moieties (**1**). The preliminary study on the complexation behavior of **1** showed that orientation and location of the appended naphthyl moieties changed by guest binding.[7] In addition to the detailed features of the binding behavior, this work is concerned with the conformational changes induced by dimethyl sulfoxide (DMSO).

Results

We prepared the compound **1** via azobenzene-capped γ-CD as shown in Scheme 2. Figure 1 shows the absorption and circular dichroism spectra of **1** in an aqueous 10% DMSO solution. The circular dichroism spectrum reveals a peak around 282 nm and a trough around 258 nm in the naphthalene ^1L_a transition region. The exciton coupling pattern indicates that the two 2-naphthyl moieties of **1** form a pair with a chiral geometry in the cavity of **1**. The spectral pattern in shorter wavelengths (^1B_b transition region) may be a decisive indicator for determining the direction of the chirality since the ^1B_b transition occurs along the long axis of naphthalene ring. However, as the measurements in the shorter wavelengths could not be achieved due to the absorption of DMSO, we used aqueous 10% ethylene glycol solutions for the purpose. The circular dichroism spectrum of this solution shows a trough at 234 nm with the molar ellipticity of 29000 and a peak at 217 nm with the molar ellipticity of 158000 associated with the naphthalene ^1B_b transition. Although the spectrum has unballanced intensities between positive and negative bands, the pattern may be regarded as that of the exciton coupling of counterclockwise orientation of the two naphthyl moieties.[8] Its deformed shape might be due to the electronic interaction between ^1B_b and ^1L_a transitions within the naphthyl pair. The circular dichroism pattern in the ^1L_a transition region of this aqueous 10% ethylene glycol solution is the same as that of the aqueous 10%



Scheme 2.

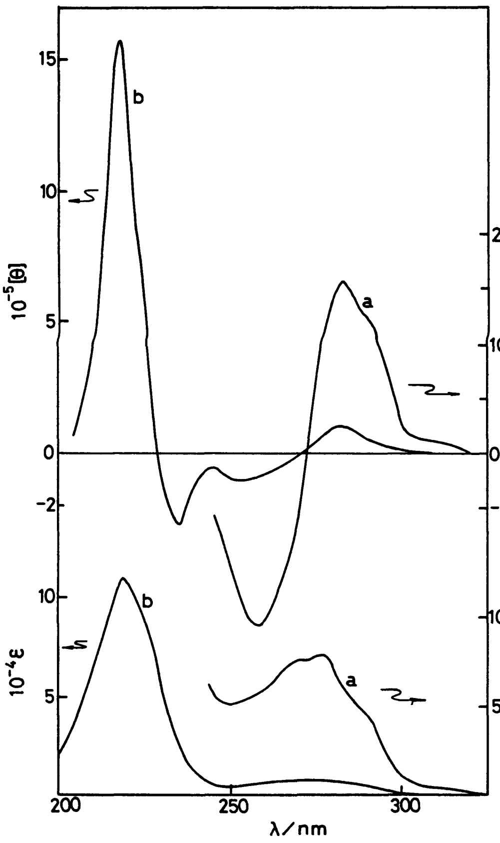


Fig. 1. Circular Dichroism (top) and absorption (bottom) spectra of **1** in aqueous 10% DMSO solution (a) and in aqueous 10% ethylene glycol solution (b). Concentrations of **1** were 6.53×10^{-5} and 7.66×10^{-6} mol dm^{-3} for the spectra a and b, respectively.

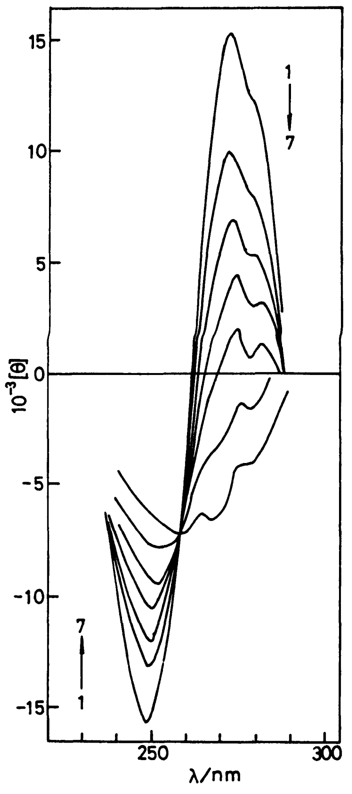


Fig. 2. Circular dichroism spectra of **1** in aqueous 10% DMSO solutions (6.53×10^{-5} mol dm^{-3}) at various cyclododecanol concentrations (1, 0.0; 2, 2.02×10^{-5}; 3, 4.04×10^{-5}; 4, 6.05×10^{-5}; 5, 8.07×10^{-5}; 6, 1.21×10^{-4}; 7, 1.81×10^{-4} mol dm^{-3}).

DMSO solution. Therefore, the orientation of the two naphthyl moieties involved in the cavity of **1** should be the same in both solutions.

The intensity of the positive peak of the exciton coupling band in the naphthalene ^1L_a transition region diminishes with increasing guest concentration. Figure 2 shows the variation of the circular dichroism pattern in the case of cyclododecanol. It is noted that the spectra have an isoellipticity point at 268 nm, this confirming that two forms of **1**, free and complexed ones are present in the present system.

The guest-induced variations of the molar ellipticity were used to obtain binding constants (K) of **1**. It was reported that the binding constant is given by Eq. 1 when circular dichroism intensity changes by guest.[9]

$$K = \frac{\theta_h - \theta_x}{(\theta_x - \theta_c)\left[C_g - C_h\dfrac{\theta_h - \theta_x}{\theta_h - \theta_c}\right]} \tag{1}$$

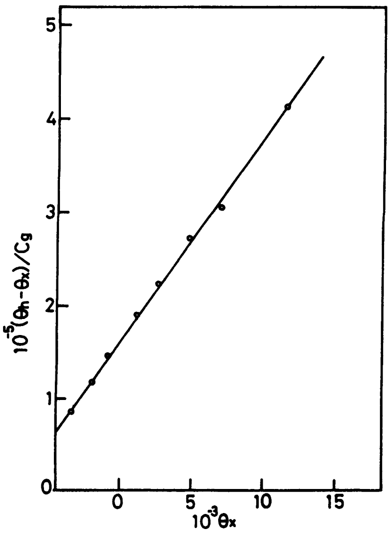


Fig. 3. The plot of $(\theta_h-\theta_x)/C_g$ as a function of θ_x in the system of **1** and cyclohexanol.

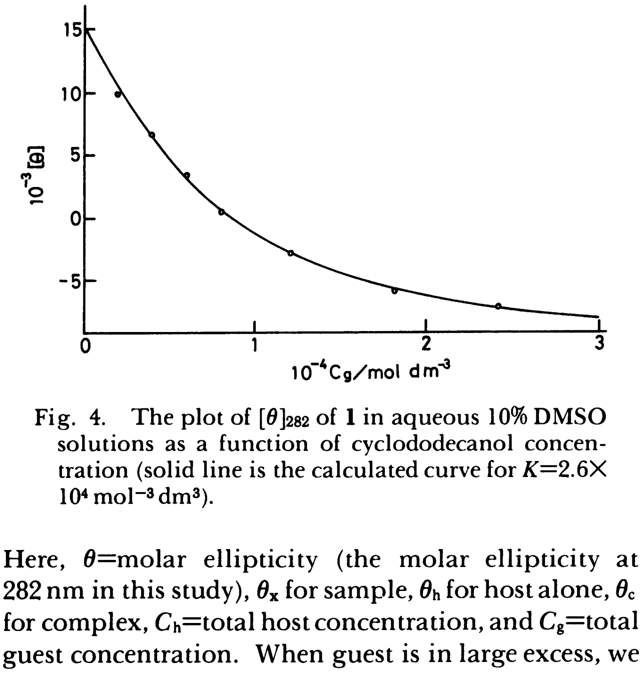


Fig. 4. The plot of $[\theta]_{282}$ of **1** in aqueous 10% DMSO solutions as a function of cyclododecanol concentration (solid line is the calculated curve for $K=2.6\times 10^4$ mol^{-3}dm^3).

Here, θ=molar ellipticity (the molar ellipticity at 282 nm in this study), θ_x for sample, θ_h for host alone, θ_c for complex, C_h=total host concentration, and C_g=total guest concentration. When guest is in large excess, we used Eq. 2 which derives from Eq. 1.

$$\frac{\theta_h-\theta_x}{C_g} = K\theta_x - K\theta_c \qquad (2)$$

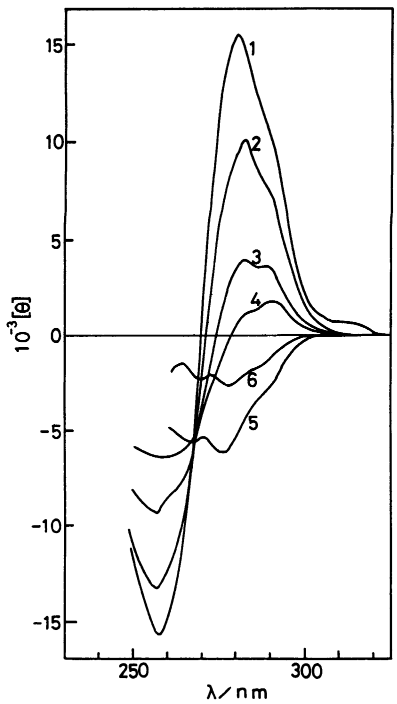


Fig. 5. Circular dichroism spectra of **1** (6.53×10^{-4} mol dm^{-3}) at various DMSO contents (1, 10; 2, 15; 3, 20; 4, 25; 5, 70; 6, 100%).

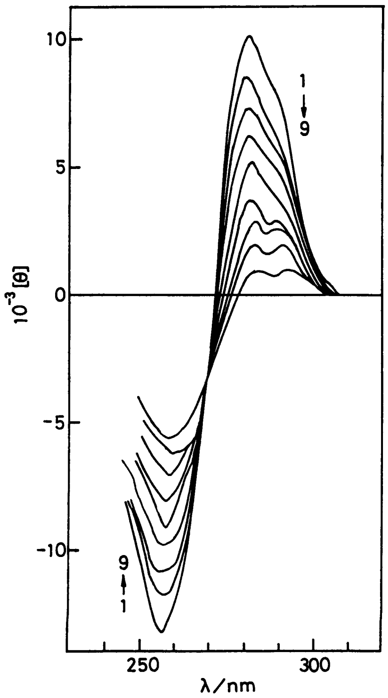


Fig. 6. Circular dichroism spectra of **1** in aqueous 15% DMSO solutions (7.54×10^{-5} mol dm^{-3}) at various (−)-borneol concentrations (1, 0.0; 2, 1.00×10^{-4}; 3, 2.00×10^{-4}; 4, 3.00×10^{-4}; 5, 4.00×10^{-4}; 6, 5.39×10^{-4}; 7, 6.98×10^{-4}; 8, 8.97×10^{-4}; 9, 11.9×10^{-4} mol dm^{-3}).

Figure 3 shows the plot of $(\theta_h-\theta_x)/C_g$ againtst θ_x in the case of cyclohexanol guest. When guest is not in large excess, a curve-fitting method was used as an alternative method. Figure 4 shows the curve-fitting data for cyclododecanol guest. The binding constants obtained by these methods are listed in Table 1. The binding constants in aqueous 10% DMSO solutions are roughly in the order of guest sizes, ranging from 21.7 mol^{-1} dm^3 for cyclohexanol to 26000 mol^{-1}dm^3 for cyclododecanol. DMSO was found to influence the circular dichroism pattern of **1**, decreasing the intensity of the peak around 282 nm and finally giving a negative band around 275 nm (Fig. 5). The effect of DMSO content on guest binding was checked by using (−)-borneol as guest. Figure 6 shows the (−)-borneol-

Table 1. Binding Constant of **1** at 25 °C[a)]

Guest	Solvent	K/mol^{-1}dm^3 [b)]
Cyclohexanol	10% DMSO	21.7
(−)-Menthol	10% DMSO	329
1-Adamantanecarboxylic acid	10% DMSO	507
(−)-Borneol	10% DMSO	795
	15% DMSO	1000
	20% DMSO	1500
	25% DMSO	1500
Cyclododecanol	10% DMSO	26000[c)]

a) Values were obtained by the plot of $(\theta_h-\theta_x)/C_g$ vs. θ_x except for the value for cyclododecanol which was obtained by the curve-fitting method. b) Errors are within ±15%. c) 9730 mol^{-1}dm^3 in Ref. 7.

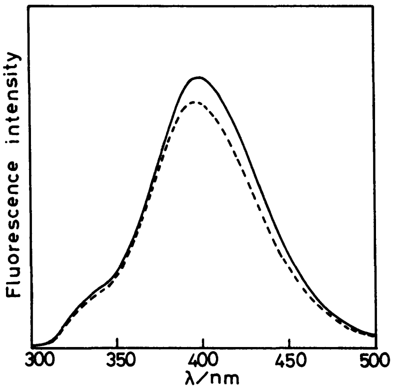


Fig. 7. Fluorescence spectra of **1** in aqueous 10% DMSO solutions (6.53×10^{-5} mol dm^{-3}) in the absence (——) and presence (----) of cyclodoecanol (2.41×10^{-4} mol dm^{-3}). Excitation wavelength is 290 nm.

dependent variation of the circular dichroism pattern of **1** in aqueous 15% DMSO solutions. The binding constans obtained in the range of 10—25% of DMSO are also shown in Table 1. The binding increased wtih increasing DMSO content until 20% DMSO. It should be noted that the analysis was limited to the solutions below 25% DMSO because of the depressed circular dichroism intensity.

Figure 7 shows the fluorescence spectra of **1** in aqueous 10% DMSO solutions, alone and in the presence of cyclododecanol. The spectra exhibit a predominant excimer emission around 400 nm and a shoulder of normal fluorescence around 330 nm. In contrast to the drastic circular dichroism changes, the fluorescence pattern was found to be hardly influenced by guest binding. However, marked changes of the fluorescence pattern was caused by increasing DMSO content (Fig. 8). This result suggests that the two naphthyl moieties of **1** in the DMSO-rich solutions are difficult to be held in the γ-CD cavity of **1** due to the weakened hydrophobic driving force.

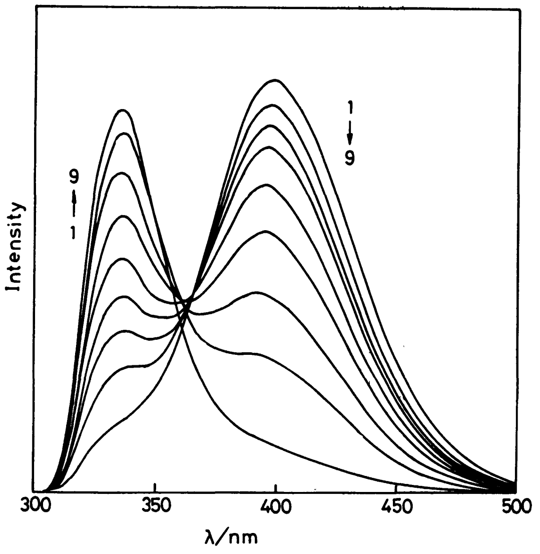
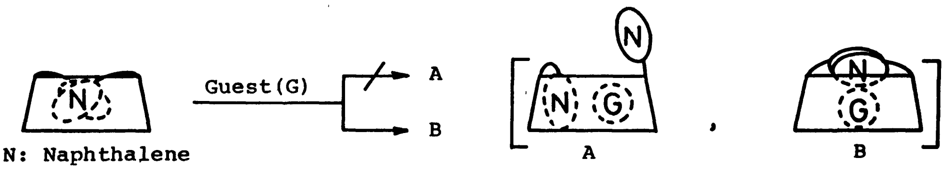


Fig. 8. Fluorescence spectra of **1** (7.54×10^{-5} mol dm^{-3}) at various DMSO contents (1, 10; 2, 20; 3, 30; 4, 40; 5, 50; 6, 60; 7, 70; 8, 80; 9, 100%).

Discussion

The synthesis of **1** was performed via γ-CD capped by azobenzene-4,4′-disulfonate moiety. Therefore, the positions of the two naphthyl moieties are those of primary hydroxyls sulfonated by azobenzene-4,4′-disulfonyl dichloride. When glucose units of CD ring are denoted by A, B, C, D, etc., in the order, primary hydroxyl groups of A and D glucopyranose units of β-CD were reported to be selectively sulfonated by stilbene-4,4′-disulfonyl dichloride.[10)] Similar selectivity may be expected here. The examination of Corey-Pauling-Koltun models of the present system shows that substitution is most likely to occur at A-D and A-E positions of γ-CD. However, in spite of our efforts to observe the isomeric forms of **1**, there was no indication of mixing of the isomers. Furthermore, the circular dichroism spectra of **1** at various guest concentrations had an isoellipticity point and could be analyzed based on a simple 1:1 stoichiometry, so the physical nature of the isomers should be very similar even if the present sample is composed of two isomeric forms.

We have examined guest-induced circular dichroism variations for aqueous 10% DMSO solutions. Guest binding of **1** cancelled the exciton coupling in the 250—300 nm region (Fig. 2), this phenomenon indicating that the chiral orientation of the naphthyl pair was lost by forming inclusion complexes. In contrast to this marked variation of the circular dichroism pattern, the excimer emission was hardly influenced by guest binding. These results indicate that the two naphthyl moieties are still close in the complexes. It was reported that γ-CD bearing a naphthyl moiety showed an induced-fit type of complexation (Scheme 1).[6a)] In the system, the naphthyl moiety was fixed in the cavity together with a guest molecule. If similar type of complexation occurs in the present system, the complex

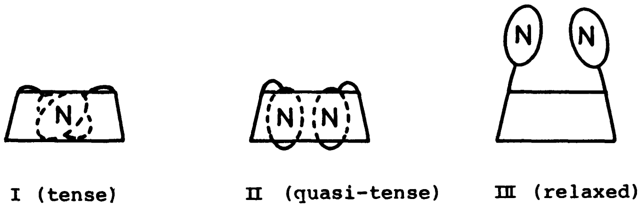


Scheme 3.

A should be formed (Scheme 3). However, the excimer emission from the complexes is not consistent with the structure A in which the face-to-face interaction between the two naphthyl moieties is not allowed. Thus, the structure B is the form which may be supported by both circular dichroism and fluorescence data. The two naphthyl moieties are likely to contact with the included guest molecule as flexible caps. It is interesting that **1** accommodates only one guest molecule in the cavity in spite of the fact that the γ-CD cavity itself is large enough to include two molecules of smaller guests. The hydrophobic naphthyl caps seem to contribute to form such 1:1 complexes.

The DMSO-enhanced binding ability of **1**, which was observed below 25% DMSO, is worthy to note since DMSO in aqueous solutions usually tends to decrease the binding ability of CDs. In the present system, the two naphthyl moieties seem to be so tightly involved in the cavity when water content is high that they are hard to be replaced by a guest molecule. Consequently, the proposed mechanism for the DMSO-enhanced binding is that DMSO increased the mobility of the naphthyl moieties so as to make the cavity suited for guest accommodation.

The fluorescence data indicate that the two naphthyl moieties are close to each other both in free and complex forms of **1** when DMSO content is low, but are located apart in pure DMSO. Figure 9 shows the plots of excimer-monomer ratio and circular dichroism intensity at 282 nm against DMSO content. It is obvious that exciton coupling is more sensitive to DMSO than excimer emission. This result suggests that the two naphthyl moieties are located still in the cavity after loosing the chiral orientation and then extruded outward from the cavity with increasing DMSO content. Thus, three forms with different geometries as illustrated below are likely to be present.



The two naphthyl moieties are tightly and loosely bound to the cavity in the tense and the quasi-tense forms, respectively, whereas not bound in the relaxed form.

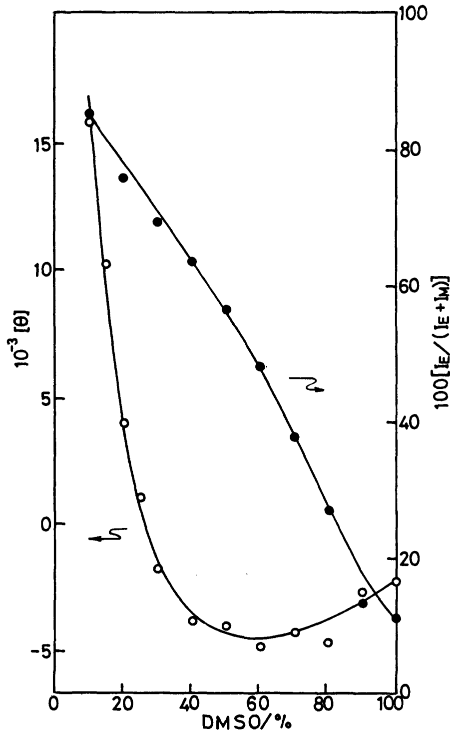


Fig. 9. Plots of excimer-monomer fluorescence ratio (I_M and I_E are intensities at 335 and 400 nm, respectively) and the molecular ellipticity at 282 nm of **1** (7.54×10^{-5} mol dm^{-3}) as a function of DMSO content.

The important aspect of the modified γ-CDs such as those bearing one and two naphthyl moieties is that native γ-CD, which is a rigid host, can be converted to flexible hosts. On this basis, various modified γ-CDs with different guest-selectivity may be designed and construction of new enzyme models, which are capable of induced-fit type of substrate binding, is now in progress.

Experimental

Materials. γ-CD was kindly gifted from Nihon Shokuhin Kako Ltd. The following commercially available guaranteed reagents were used without further purification; *p*-nitrobenzenesulfonic acid, cyclohexanol, (−)-menthol, 1-adamantanecarboxylic acid, (−)-borneol, and cyclododecanol. DMSO was Dotite-spectrosol grade.

Measurements. The circular dichroism, UV and fluorescence spectra were measured at 25 °C using a JASCO J-400X spectrodichrometer, a Shimadzu UV-250 spectrophotometer and a Shimadzu RF-500 spectrofluorophotometer, respectively. The circular dichroism intensities were expressed as molar ellipticity (in degree cm^2 dmol^{-1}) on the basis of the total concentration of **1**. Due to the poor solubility of **1** in pure water, DMSO-containing aqueous solutions were used as solvents.

Disodium Azobenzene-4,4′-disulfonate (2). Sodium hydroxide solution (63.2 g in 150 ml H_2O) was added dropwise to the solution of *p*-nitrobenzenesulfonic acid (20.0 g, 0.0985 mol) and water (135 ml). The mixed solution was heated at 50 °C and then D-glucose solution (126 g, 0.699 mol and 150 H_2O) was added dropwise. Air was introduced into the resultant solution at room temperature for 10 h. The precipitates were collected by filtratioin, washed with 15% NaCl solution (100 ml), and recrystallized from 50% ethanol solution to give 9.6 g (25%) of **2**: ^{1}H NMR (D_2O) δ=8.14 (8H, m, Ar). Found: C, 36.46; H, 1.87; N, 7.23%. Calcd for $C_{12}H_8N_2O_6S_2Na_2$: C, 36.65; H, 2.05; N, 7.13%.

Azobenzene-4,4′-disulfonyl Dichloride (3). Thionyl chloride (8 ml) was added dropwise to the stirred suspension of **2** (9.0 g, 0.0233 mol) in *N,N*-dimethylformamide (150 ml). After vigorous stirring for 1 h, the solution was poured into ice water (280 ml). The precipitates were collected, washed with ether, and recrystallized from benzene to give 6.4 g of **3** (73%): mp 223—225 °C; ^{1}H NMR ($CDCl_3$) δ=8.20 (8H, m, Ar); IR (KBr) 1377, 1183, 655, 582, and 528 cm^{-1}; R_f=0.5 (2:1 hexane-acetone). Found: C, 38.19; H, 1.84; N, 7.24. Calcd for $C_{12}H_8S_2O_4N_2Cl_2$: C, 38.00; H, 2.13; N, 7.39.

***trans*-Azobenzene-4,4′-disulfonyl γ-CD (4).** To a stirred solution of dry γ-CD (4.0 g, 3.08 mmol) in dry pyridine (100 ml), azobenzene-4,4′-disulfonyl dichloride (1.40 g) 3.7 mmol) was added, and then heated at 60 °C for 1 h. After cooling the reaction mixture, pyridine was removed by evaporation under reduced pressure. Water addition to the residue followed by evaporation was repeated several times until no pyridine odor was detected. The resultant oily residue was dissolved in a small amount of water and subjected to Sephadex G-15 column chromatography. Recrystallization from methanol afforded the product (0.69 g, 13%) as orange powder: R_f=0.40 (5:1 CH_3CN-H_2O); Found: C, 42.93; H, 5.89; N, 1.45. Calcd for $C_{60}H_{86}N_2O_{44}S_2 \cdot 4H_2O$: C, 43.01; H, 5.66; N, 1.67.

Bis(2-naphthylacetyl) γ-CD. A mixture of **4** (0.515 g, 0.307 mmol), sodium 2-naphthylacetate (0.265 g, 1.28 mmol), and DMSO (10 ml) was stirred at 80 °C for 5 h. After cooling, the reaction mixture was poured into 500 ml of acetone. The precipitates were collected by filtration and subjected to Sephadex G-15 column chromatography. Recrystallization from water gave the desired product (0.12 g, 23%): R_f=0.48 (5:1 CH_3CN-H_2O); ^{1}H NMR (DMSO-d_6, 60 °C) δ=3.1—3.8 (48H, m, CD protons other than C_1H and OH), 4.0—4.5 (6H, m, O_6H), 4.8—4.9 (8H, br. s, C_1H), 5.8 (16H, br. s, O_2H, O_3H), 7.2—7.9 (14H, m, Ar); UV (9:1 H_2O-DMSO) (ε) 277 nm (7900); IR (KBr):1735 cm^{-1} (CO). Found: C, 50.73; H, 6.12. Calcd for $C_{72}H_{96}O_{42} \cdot 4H_2O$: C, 50.70; H, 6.15.

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