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Fluorescent Molecular Recognition and Sensing System of Bis-Dansyl Modified γ-Cyclodextrins

MIYUKI NARITA[1], FUMIO HAMADA[1,⋆], MAYUMI SATO[1], IWAO SUZUKI[2] and TETSUO OSA[2]

[1]*Department of Materials-Process Engineering and Applied Chemistry for Environments, Faculty of Engineering and Resource Sciences, Akita University, Tegata, Akita 010-0852, Japan*

[2]*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980-8578, Japan*

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Abstract. Flexible hosts, 6A,6B-; 6A,6C-; 6A,6D-; and 6A,6E-bis dansylglycine-modified γ-cyclodextrins (γ-1, γ-2, γ-3, and γ-4, respectively) have been synthesized as a sensing molecule for organic guests including terpenoids and bile acids. These host compounds show a pure monomer fluorescence whose intensity is decreased or enhanced upon addition of guest species. The value $\Delta I/I_0$, where I and I_0 are fluorescence intensities in the presence and absence of a guest and ΔI is $I_0 - I$, was used as a parameter of sensitivity. These hosts exhibit highly sensitive and selective molecular recognition ability, particularly, for lithochoic acid, chenodeoxycholic acid, and ursodeoxycholic acid. The behaviors of the appended moieties of these hosts when host–guest complexation occurs are studied by induced circular dichroism (ICD) spectra and fluorescence spectral change on accommodation of a guest. The ICD pattern of these hosts alone or on accommodation of a guest is very similar, indicating that the behavior of the appended moieties are very similar. The guest-induced variations in the fluorescence or ICD intensity suggest that the appended moieties act as a hydrophobic cap that enables the cyclodextrin to form 1 : 1 host–guest complexes.

Key words: modified γ-cyclodextrin, dansylglycine, fluorescent sensory, lithocholic acid, bile acid.

1. Introduction

Dansyl and its derivatives are frequently used as fluorescent probes, in which the fluorophore displays phenomena such as fluorescence, spectral shifts, fluorescence quenching, fluorescence polarization, and induced circular dichroism. The resulting information leads to a deeper insight of events at the cellular and molecular levels in a variety of biological systems [1–2]. On this basis, we prepared dansylglycine-modified β- and γ-cyclodextrins, in which the dansyl moiety acts as a probe to describe the host–guest binding behavior of the cyclodextrins [3–4]. Cyclodextrins, which are torus-shaped cyclic oligomers of D-glucopyranose and named α-, β-, and γ- for the hexamer, heptamer, and octamer, respectively, can include a variety of organic compounds in the cavities in aqueous solution [1–2]. The fluorescent cyclodextrins have recently received increasing attention

⋆ Author for correspondence.

because these compounds show remarkable variations in their fluorescence spectra associated with the formation of inclusion complexes; because of this, they have been used as sensors or indicators of molecules in aqueous solution [5–9]. We studied the binding abilities of these derivatives with terpenoids and bile acids as guest molecules, because they are biological substances produced by plants or animals and are utilized as crude drugs. These compounds exhibit a decrease or increase in their fluorescence intensity upon guest binding, which varies depending on the nature of the guest, even at a common concentration. Some mono-dansyl modified cyclodextrin analogues have been reported [3–4, 10–13]. The studies showed that β-analogues have a much higher sensitivity for organic guest such as bile acids than those of the γ-analogues. The modification position of the dansyl moiety on the cyclodextrin cavity is also discussed and the primary side of cyclodextrin is shown to be better than the secondary side. Recently, we described four analogues of γ-cyclodextrin derivatives modified with bis-fluorescent active moieties, in positions 6A,6B-; 6A,6C-; 6A,6D-; and 6A,6E- of the glucose units of the cyclodextrins, which show high selectivity and sensitivity for guest molecules as compared with those of mono-derivatives [6, 14–16]. This indicates that the position of the modification and the number of modified moieties affect the sensing ability of the cyclodextrin. In this paper, we would like to describe the host–guest binding system and molecular recognition ability of 6A,6B-; 6A,6C-; 6A,6D-; and 6A,6E-bis dansylglycine-modified γ-cyclodextrins (γ-1, γ-2, γ-3, and γ-4, respectively). These compounds show much higher sensitivity and selectivity for the guests examined than those of the mono-γ-dansyl modified analogue.

2. Experimental

2.1. PREPARATIONS OF 6A,6B-; 6A,6C-; 6A,6D-; AND 6A,6E-BIS DANSYLGLYCINE-MODIFIED γ-CYCLODEXTRINS (γ-1, γ-2, γ-3, AND γ-4, RESPECTIVELY)

A mixture of 6A,6B-bis *p*-tosyl γ-cyclodextrin (800 mg, 0.50 mM) [17] and sodium dansylglycine (363 mg, 1.10 mM) in 20 mL of DMF was heated at 80 °C for 24 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitates were filtered and dissolved in 5 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lober column LiChroprep RP18). After stepwise elution from 500 mL of 10 vol.-%, 300 mL of 20 vol.-%, 300 mL of 30 vol.-%, 300 mL of 40 vol.-%, 300 mL of 50 vol.-%, 300 mL of 55 vol.-%, and 500 mL of 60 vol.-% aqueous MeOH were applied to give γ-1. Compounds γ-2, γ-3, and γ-4 were prepared by the same procedure as for γ-1 as shown in Figure 1.

γ-1: Yield 12.9%. R_f 0.55 (butanol : ethanol : water = 5 : 4 : 3 by volume, TLC; silica gel 60F254) and 0.62 (methanol : water = 2 : 1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) = 2.87 (12H, s, N-CH_3), 3.0–3.8 (52H, M, CH_2 and C_2-C_6H of cyclodextrin), 3.9–4.2 (22H, br, O_2H, O_3H and O_6H), 4.8–

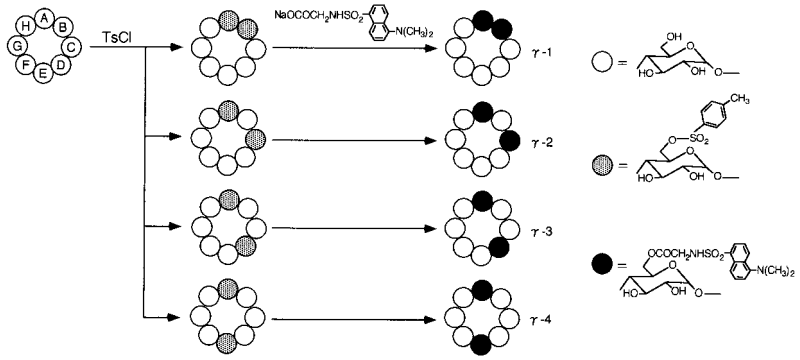


Figure 1. Preparations of γ-1, γ-2, γ-3, and γ-4.

4.95 (8H,M, C_1H of cyclodextrin), 7.31 (2H, d, J = 7.2 Hz, aromatic-H), 7.57 (4H, q, J = 7.7 Hz, aromatic-H), 8.09 (2H, d-d, J = 6.9 Hz, aromatic-H), 8.30 (2H, d, J = 6.6 Hz, aromatic-H), 8.47 (2H, d, J = 7.2 Hz, aromatic-H). Calcd. for $C_{76}H_{108}O_{46}N_4S_2 \cdot 4H_2O$: C, 46.82; H, 6.00; N, 2.87%. Found: C, 46.65; H, 6.30; N, 2.70%. MS(FAB): 1876 (M^+).

γ-2: Yield 20.2%. R_f 0.56 (butanol : ethanol : water = 5 : 4 : 3 by volume, TLC; silica gel 60F254) and 0.64 (methanol : water = 2 : 1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) = 2.83 (12H, s, N—CH_3), 3.1–3.7 (52H, M, CH_2 and C_2—C_6H of cyclodextrin), 3.9–4.2 (22H, br, O_2H, O_3H and O_6H), 4.8–4.95 (8H, M, C_1H of cyclodextrin), 7.25 (2H, d, J = 7.2 Hz, aromatic-H), 7.58 (4H, M, aromatic-H), 8.09 (2H, d, J = 7.8 Hz, aromatic-H), 8.26 (2H, d, J = 8.7 Hz, aromatic-H), 8.47 (2H, M, aromatic-H). Calcd. for $C_{76}H_{108}O_{46}N_4S_2 \cdot 5H_2O$: C, 46.39; H, 6.04; N, 2.84%. Found: C, 46.20; H, 6.34; N, 2.88%. MS(FAB): 1876 (M^+).

γ-3: Yield 14.0%. R_f 0.57 (butanol : ethanol : water = 5 : 4 : 3 by volume, TLC; silica gel 60F254) and 0.64 (methanol : water = 2 : 1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) = 2.85 (12H, s, N—CH_3), 3.1–3.8 (52H, M, CH_2 and C_2—C_6H of cyclodextrin), 3.9–4.2 (22H, br, O2H, O_3H and O_6H), 4.8–4.95 (8H, M, C_1H of cyclodextrin), 7.28 (2H, d-d, J = 7.5 Hz, aromatic-H), 7.57 (4H, M, J = 7.8 Hz, aromatic-H), 8.07 (2H, t, J = 8.1 Hz, aromatic-H), 8.26 (2H, d, J = 8.4 Hz, aromatic-H), 8.47 (2H, d-d, J = 7.8 Hz, aromatic-H). Calcd. for $C_{76}H_{108}O_{46}N_4S_2 \cdot 5H_2O$: C, 46.39; H, 6.04; N, 2.84%. Found: C, 46.38; H, 6.35; N, 2.61%. MS(FAB): 1876 (M^+).

γ-4: Yield 14.5%. R_f 0.56 (butanol : ethanol : water = 5 : 4 : 3 by volume, TLC; silica gel 60F254) and 0.81 (methanol : water 2 : 1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) = 2.85 (12H, s, N—CH_3), 3.1–3.7 (52H, M, CH_2 and C_2—C_6H of cyclodextrin), 3.8–4.2 (22H, br, O_2H, O_3H and O_6H), 4.8-4.95 (8H, M, C_1H of cyclodextrin), 7.27 (2H, d, J = 8.1 Hz, aromatic-H), 7.59 (4H,M, J = 7.9 Hz, aromatic-H), 8.09 (2H, d, J = 7.5 Hz, aromatic-H), 8.27 (2H, d, J = 8.7 Hz, aromatic-H), 8.47 (2H, d, J = 8.7 Hz, aromatic-H). Calcd. for

$C_{76}H_{108}O_{46}N_4S_2{\cdot}5H_2O$: C, 46.39; H, 6.04; N, 2.84%. Found: C, 46.11; H, 5.88; N, 2.56%. MS(FAB): 1876 (M^+).

2.2. MEASUREMENTS

Ultraviolet, fluorescence, and circular dichroism spectra were measured at 25 °C, with a Perkin Elmer Lambda 40 UV/Vis spectrophotometer, a Perkin Elmer LS 40B fluorescence spectrometer, and a JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 340 nm and excitation and emission slits were 10 nm. Ethylene glycol aqueous solution (10 vol.-%) was used as solvent for hosts for the spectroscopic measurements because their solubility in pure water is poor. 5 μL of guest species (0.5, 0.05 and 0.005 M) in dimethyl sulfoxide (DMSO) or MeOH were injected into a 10 vol.-% ethylene glycol aqueous solution of host (2.5 mL) to make a sample solution with a host concentration of 1×10^{-6} M and guest concentration of 0.01, 0.1 and 1.0 mM, respectively.

3. Results and Discussion

3.1. INDUCED CIRCULAR DICHROISM (ICD) SPECTRA AND FLUORESCENCE SPECTRA

Figure 2 show ICD spectra and UV-spectra of γ-1, γ-2, γ-3, and γ-4 alone or in the presence of ursodeoxycholic acid in a 10 vol.-% ethylene glycol aqueous solution. The ICD and UV-spectral patterns of the compounds are similar. The ICD spectra of the compounds alone show a positive shoulder at around 320 nm and a negative band at around 365 nm, whose intensities are decreased upon guest addition. These results suggest that the dansyl moieties moved from the interior of the hydrophobic cyclodextrin cavity toward the outside bulk water environment while simultaneously a guest is included in the cyclodextrin cavity [4]. Figure 3 shows fluorescence spectra of γ-2 in a 10 vol.-% ethylene glycol aqueous solution in the presence and absence of ursodeoxycholic acid. The fluorescence spectra of these hosts are composed of almost pure monomer emission with a peak around 526 nm, and the fluorescence intensity decreases with increasing ursodeoxycholic acid. It is reported that the guest-induced fluorescence enhancement means that the appended moiety is moving into the cyclodextrin cavity deeply and a decrease means that the appended moiety is moving out of the cavity [4]. The results obtained from ICD and fluorescence spectral changes of these hosts suggest that the dansylglycine moieties are excluded from the cyclodextrin cavity upon guest binding and act as a hydrophobic cap as illustrated in Figure 4.

As reported previously, the extent of the variation of the fluorescence intensity of these hosts is dependent on the nature of the guest, even at a common concentration; these hosts can be used as sensing molecules as seen for dansylglycine-modified cyclodextrin analogues. To display the sensing ability of modified cy-

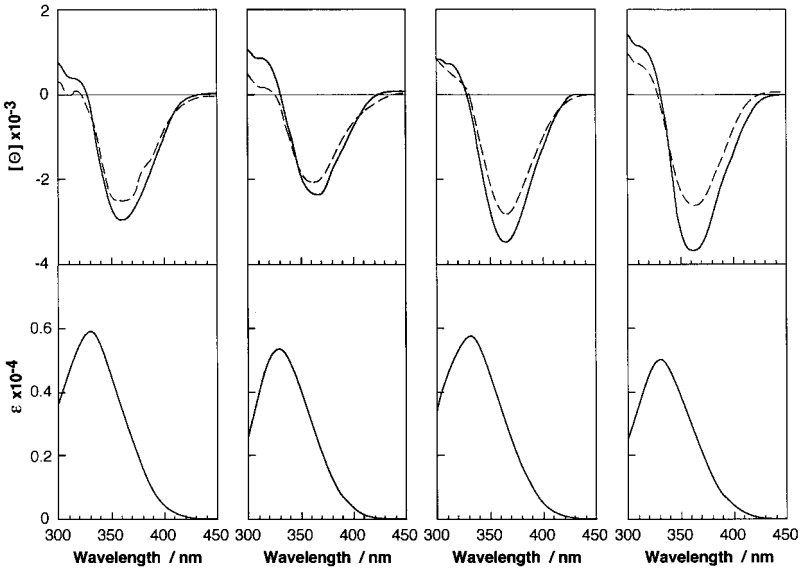


Figure 2. Induced circular dichroism spectra and UV-spectra of γ-1, γ-2, γ-3, and γ-4 in 10 vol.-% ethylene glycol aqueous solution (10^{-4} M: ———) and containing ursodeoxycholic acid (10^{-4} M: – – –).

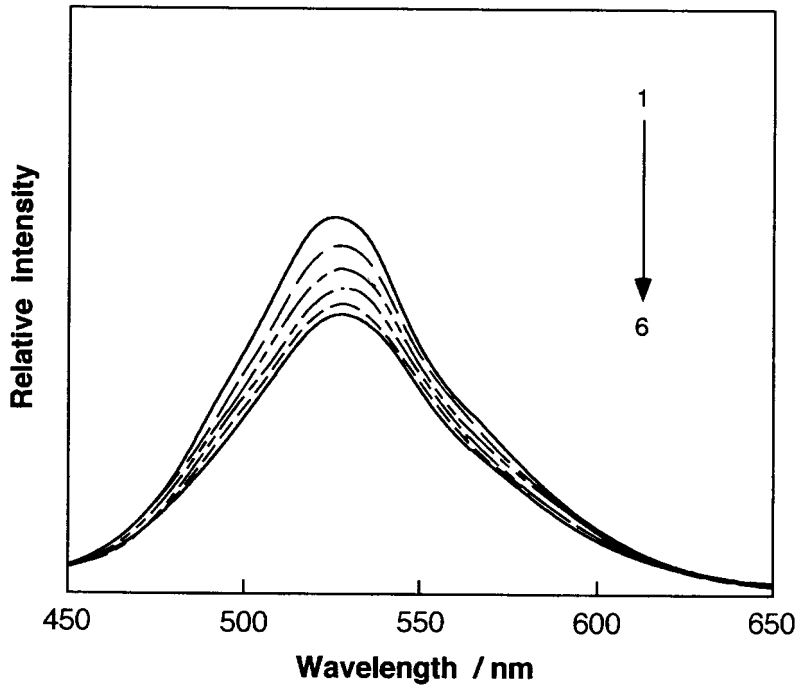


Figure 3. Fluorescence spectra of γ-2 (10^{-6} M) in 10 vol.-% ethylene glycol aqueous solution at various concentrations of ursodeoxycholic acid (1: 0, 2: 2.0×10^{-5}, 3: 4.0×10^{-5}, 4: 6.0×10^{-5}, 5: 8.0×10^{-5}, 6: 1.0×10^{-6} M).

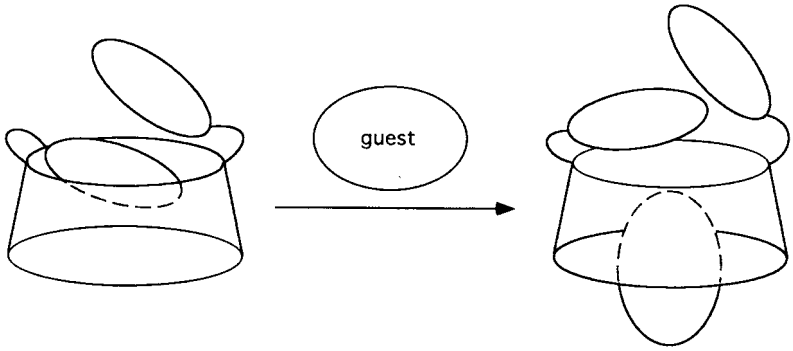
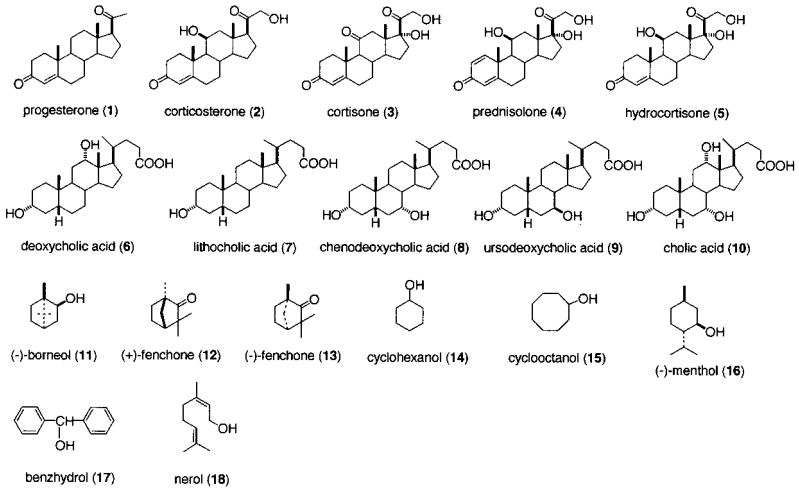


Figure 4. Host–guest complexation mechanism of bis dansylglycine-modified γ-cyclodextrins.



Scheme 1. Guest molecules.

clodextrins, the $\Delta I/I_0$ value as a sensitivity parameter was used. Here ΔI is I_0-I, where I_0 is the fluorescence intensity for the host alone, and I is the fluorescence intensity for a complex. Figure 6 shows the parameter values of γ-1, γ-2, γ-3, and γ-4 with steroids at 0.1 mM except for lithocholic acid (**7**), which was examined at 0.01 mM because 0.1 mM of lithocholic acid is not soluble in 10 vol.-% ethylene glycol aqueous solution and terpenoids at 1.0 mM. It is evident that chenodeoxycholic acid (**8**) and ursodeoxycholic acid (**9**) are detected with remarkably high sensitivity, exhibiting values of 0.212, 0.161, 0.149, and 0.126 for γ-2, γ-4, γ-3, and γ-1 and 0.250, 0.231, 0.206, and 0.191 for γ-2, γ-4, γ-1, and γ-3, respectively. Lithocholic acid (**7**) was detected with high sensitivity, even at one tenth concentration, exhibiting values of 0.235, 0.189, 0.145, and 0.108 for γ-4, γ-3, γ-2, and γ-1, respectively. Deoxycholic acid (**6**), which is different from the other steroids only in the position of one hydroxyl group, was detected with lower sensitivity. Cholic

Table I. Binding constants (K/mol^{-1} dm^3) of γ-1, γ-2, γ-3, and γ-4.

Guest	γ-1	γ-2	γ-3	γ-4
Progesterone (**1**)	26,500 ± 2,600	7,550 ± 570	2,100 ± 210	7,430 ± 550
Deoxycholic acid (**6**)	6,000 ± 440	11,000 ± 630	4,600 ± 170	3,000 ± 260
Lithocholic acid (**7**)	116,000 ± 8,300	122,000 ± 7,100	101,000 ± 9,800	93,900 ± 3,800
Chenodeoxycholic acid (**8**)	9,500 ± 500	6,700 ± 400	13,000 ± 1,100	10,400 ± 470
Ursodeoxycholic acid (**9**)	5,200 ± 190	5,200 ± 380	4,900 ± 350	1,100 ± 90
Borneol (**11**)	2,370 ± 160	550 ± 30	1,340 ± 90	230 ± 30

acid (**10**), which bears one more hydroxyl group than **8** and **9**, was hardly detected, probably due to its increased polarity. The sensing factors of bile acids by γ-1 and γ-2 decrease in the sequence; **9** > **8** > **7** > **6** > **10** and γ-3 and γ-4 decrease in the sequence; **9**=**7** > **8** > **6** > **10**, respectively. These guests were detected by the four hosts in the approximate order γ-4 = γ-2 > γ-3 > γ-1. This indicates that modifying the cyclodextrin cavity affects the sensing ability as shown in the case of bis-sodium anthranilate or bis-naphthalene modified analogues [9]. All hosts show only low sensitivity for ketosteroids which have two and three hydroxyl groups. Progesterone (**1**), which bears no hydroxyl group and is more hydrophobic than the other ketosteroids, was detected with values of 0.086, 0.073, 0.063, and 0.059 for γ-1, γ-4, γ-2, and γ-3, respectively, which is higher than those of other ketosteroids. The observation suggests that the complex formations are affected by hydrophobic interaction between a guest and a host. The complexation behaviors of the four hosts are affected by the molecular structure and size because (−)-borneol (**11**), (+)-fenchone (**12**), and (−)-fenchone (**13**), which are bicyclic derivatives, are detected with positive sensitivity factors, while monocyclic derivatives such as cyclohexanol (**14**), cyclooctanol (**15**), and (−)-menthol (**16**) are detected with negative sensitivity factors. Bicyclic derivatives show $\Delta I/I_0$ values ranging from 0.089 to 0 and monocyclic show $\Delta I/I_0$ values ranging from −0.036 to 0. Nerol (**18**), which is a noncyclic compound, was hardly detected by the system.

The guest-induced fluorescence variation at 526 nm was employed to deduce the binding constants of these hosts by using Equation (1) as reported previously [15].

$$\frac{1}{I_f - I_{f0}} = \frac{1}{a[CD]_0} + \frac{1}{a[CD]_0 K} \cdot \frac{1}{[G]_0} \tag{1}$$

Here, I is the fluorescence intensity at 526 nm (I_f for complex, I_{f0} for the host alone), $[CD]_0$ is the total host concentration, $[G]_0$ is the total guest concentration, a is a constant. The binding constants of γ-1, γ-2, γ-3, and γ-4 for several guests were obtained to examine the correlation between the fluorescence variations and

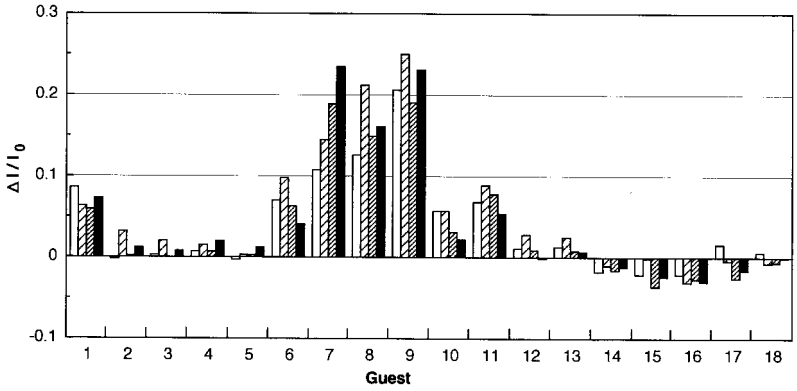


Figure 5. Sensitivity factors of γ-1 (□), γ-2 (▨), γ-3 (▨), and γ-4 (■) for all guests examined.

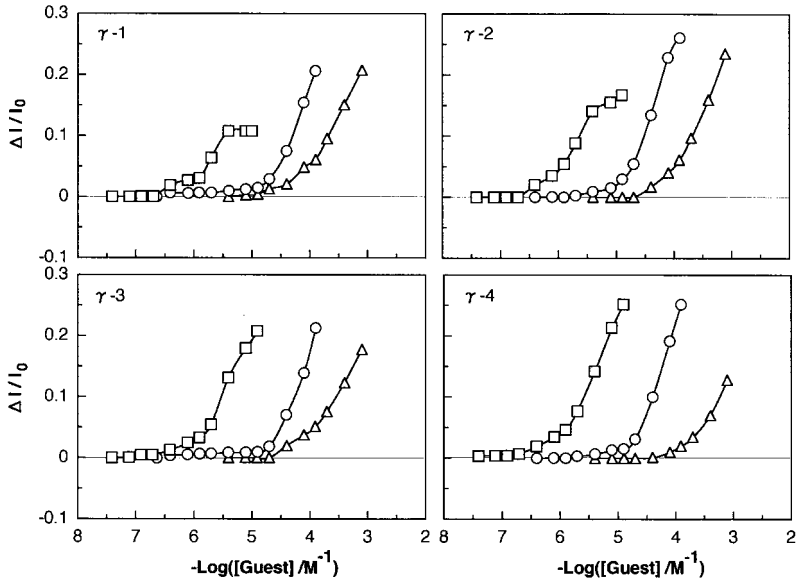


Figure 6. Fluorescence variations of γ-1, γ-2, γ-3, and γ-4 for lithocholic acid (□), ursodeoxycholic acid (○), and cholic acid (△) as a function of guest concentration.

the binding abilities of the hosts. The binding constants are roughly parallel with the sensitivity factors as shown in Table I.

3.2. RESPONSE RANGES

The response curves of γ-1, γ-2, γ-3, and γ-4, for some guests such as lithocholic acid, ursodeoxycholic acid, and cholic acid, are shown in Figure 6. Since these guests were detected with remarkably different responses by the hosts in the order lithocholic acid < ursodeoxycholic acid < cholic acid, they are expected to have

different response ranges when the guest concentration is varied. All hosts give clear concentration dependency for the guests, reflecting the sensitivities of the system for the guests with response ranges of $10^{-6.5}$–10^{-5} M, $10^{-4.5}$–10^{-4} M, and above 10^{-4} M for lithocholic acid, ursodeoxycholic acid, and cholic acid, respectively. When lithocholic acid was used as a guest, saturation phenomena by γ-1 and γ-2 was observed; it is suggested that the formation of a complex between the host and lithocholic acid is very strong.

4. Conclusion

Four analogues of bis dansylglycine-modified γ-cyclodextrins have been prepared to investigate their sensing ability for organic guests including steroids and terpenoids, which are biologically significant substances. These hosts show a pure monomer fluorescence, whose intensity variation was used as a parameter to describe the sensing ability. The position of modification affects the sensing ability of these hosts, it is probably caused by the difference in the behavior of the appended moieties of each host when host–guest complexation occurs. It is recognized that the appended moieties of these hosts act as a hydrophobic cap to elevate the binding ability. Using the fluorescent-sensory system of modified cyclodextrins seems to be a very convenient and useful method, because the chemical modification of a guest, which is spectroscopically inert is not necessary; a guest can be examined directly in this system.

References

1. (a) M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag, New York (1977).
2. J. Szejtli: *Cyclodextrin Technology*, Kluwer, Dordrecht (1988).
3. A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada, and K. Murai: *Chem. Lett*. 605 (1990).
4. F. Hamada, Y. Kondo, and R. Ito: *J. Incl. Phenom. Mol. Recognit. Chem.* **15**, 273 (1993).
5. A. Ueno: in A. W. Czarnik (ed.), *Fluorescent Chemosensors for Ion and Molecule Recognition*, ACS symposium series 538; American Chemical Society: Washington, DC,1992; Chapter 6, pp. 74–84.
6. F. Hamada, Y. Kondo, K. Ishikawa, H. Ito, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **17**, 267 (1994).
7. M. Eddaoudi, H. Parrot-Lopez, S. F. de Lamotte, D. Ficheux, P. Prognon, and A. W. Coleman: *J. Chem. Soc., Perkin Trans. 2* 1711 (1996).
8. R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia, and G. Sartor: *J. Org. Chem.* **62**, 6283 (1997).
9. F. Hamada, S. Minato, T. Osa, and A. Ueno: *Bull. Chem. Soc. Jpn.* **70**, 1339 (1997).
10. Y. Wang, T. Ikeda, A. Ueno, and F. Toda: *Chem. Lett.* 863 (1992).
11. Y. Wang, T. Ikeda, H. Ikeda, A. Ueno, and F. Toda: *Bull. Chem. Soc. Jpn.* **67**, 1598 (1994).
12. H. F. M. Nelissen, F. Venema, R. M. Uittenbogaard, M. C. Feiters, and R. J. M. Nolte: *J. Chem. Soc., Perkin Trans. 2* 2045 (1997).
13. K. Hamasaki, S. Usui, H. Ikeda, T. Ikeda, and A. Ueno: *Supramol. Chem.* **8**,125 (1997).

14. F. Hamada, K. Ishikawa, R. Ito, H. Shibuya, S. Hamai, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom. Mol. Recognit. Chem.* **20**, 43 (1995).
15. F. Hamada, K. Ishikawa, I. Tamura, and A. Ueno: *Anal. Sci*. **11**, 935 (1995).
16. F. Hamada, K. Ishikawa, I. Tamura, K. Murai, Y. Akagami, and A. Ueno: *Int. J. Soc. Mat. Eng. Res.*, **5**, 69 (1997).
17. K. Fujita, H. Yamamura, T. Imoto, T. Fujioka, and K. Mihashi: *J. Org. Chem.* **53**, 1943 (1988).