Journal of Inclusion Phenomena and Macrocyclic Chemistry **50**: 79–85, 2004.

Mini Review

Fluorescent Molecular Sensing System Based on Tri-Pyrenes-Labeled γ-Cyclodextrin at the Hetero Rim

NARANCHIMEG DORJPALAM, MASAYA TODA, HIDEAKI ITOH and FUMIO HAMADA*

Department of Materials-Process Engineering and Applied Chemistry for Environments, Faculty of Engineering and Resource Science, Akita University, Tegata, Akita 010-8502, Japan

(Received: 2 October 2003; in final form: 10 November 2003)

Key words: γ-cyclodextrin, excimer emission, fluorescent molecular sensing, hetero rim, intermolecular complex, intramolecular complex

Abstract

Fluorescent active host labeled at the upper and lower rims of γ-cyclodextrin, namely, mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-$6^{X,Y}$-*O*-bis-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-3) has been synthesized as a chemosensor for steroidal guests using fluorescence spectra change upon a guest addition. The γ-3 shows monomer and excimer fluorescence, which results in an increase of the intensity of monomer and decrease of excimer fluorescence with a host–guest binding in the cyclodextrin cavity. The extent of monomer and excimer fluorescence variations of γ-3 was used as an indication for the sensing ability for the guests examined. The guest-induced fluorescence changes were measured for 10^{-7} M solutions of γ-3. The sensing parameters ($\Delta I_{ex}/I^0_{ex}$ and $\Delta I_{mon}/I^0_{mono}$) were used to describe the sensing ability of γ-3. The values of $\Delta I_{ex}/I^0_{ex}$ describe that γ-3 shows less selectivity for guests than that of mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-*O*-mono-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-2), and the values of $\Delta I_{mon}/I^0_{mono}$ show that γ-3 shows higher sensitivity and selectivity than that of γ-2.

Introduction

Cyclodextrins (CyDs) are basically spectroscopic inert, however, CyDs can be transformed into spectroscopic-active hosts by an introduction with a fluoroscopic unit. Fluoroscopic CyDs exhibit fluorescence spectra changes when guest-inclusion occurs and this phenomenon is applied for molecular sensing system [1–3], particularly in the area of environmental hormones detecting [4–9], because detection of these guests is needed with very high sensitivity. Recently, we have reported that γ-cyclodextrin derivative (γ-1) which is selectively labeled with pyrene and tosyl on the hetero rims of the CyD cavity, in which the pyrene moiety acts to elevate the host–guest binding ability and the tosyl unit acts as a spacer to regulate the cyclodextrin cavity size [10], after which we reported the synthesis of mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-6^{X}-*O*-mono-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-2), of which the spectroscopic behavior is interesting because there are two kinds of fluorescence spectra produced by a formation of intramolecular or intermolecular interaction of pyrene units of γ-2 [11]. For further extension of the work, we prepared a new spectroscopic-active host based on γ-cyclodextrin, which is mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-$6^{X,Y}$-*O*-bis-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-3).

* Author for correspondence. E-mail: hamada@ipc.akita-u.ac.jp

Experimental

Preparation of mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-$6^{X,Y}$-O-bis-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-3, X, Y = A, B, C, D, E, F, G and H, X ≠ Y)

A mixture of *p*-toluenesulfonyl chloride (2504 mg, 13.14 mmol) and mono-3-deoxy-3-pyrenebutylamido-mono-altro-γ-CD (I) (2600 mg, 1.642 mmol) [11] in 170 mL of dry pyridine was stirred for 7 h at room temperature. The reaction mixture was concentrated *in vacuo* to give an oily residue, which was treated with 200 mL of acetone. The resulting precipitates were filtered and dissolved in 10 mL of DMF. The DMF soluble fraction was separated in a reverse-phase column (Lobar column LiChroprep RP-18). Stepwise elution in 1 L of aqueous MeOH at concentrations of 10, 20, 30, 40, 50 vol.% aqueous MeOH were used to obtain mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-$6^{X,Y}$-*O*-bis-(*p*-tosyl) mono-altro-γ-CD (II). Mono-3^{A}-deoxy-3^{A}-pyrenebutylamido-$6^{X,Y}$-*O*-mono-(*p*-tosyl) mono-altro-γ-CD (γ-1) was isolated as a minor product.

II: yield 33.8%. R_f 0.60 (methanol–water 4:1 by volume, TLC; RP-18F_{254S}) ^{1}H-NMR (DMSO-d_6): 2.38 (6H, s, CH_3 of *p*-tosyl), 3.1–3.8 (46H, m, C^2—C^3H of glucose unit without pyrene and C^4—C^6H of CyD), 3.85–3.95 (1H, m, C^3H of glucose unit with pyrene), 4.1–4.3 (1H, m, C^2H of glucose unit with pyrene), 4.4–4.65 (6H, m, O^6H of CyD), 4.7–5.05 (7H, m, C^1H of glucose unit without pyrene), 5.4–5.5 (1H, m, C^1H of glucose unit with pyrene), 5.6–5.9 (15H, m, O^2H and O^3H of CyD), 7.36–7.47 (4H, m, aromatic-H of *p*-tosyl), 7.71–7.79 (4H, m, aromatic-H of *p*-tosyl), 7.96 (1H, d, $J = 7.5$Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.5$ Hz, aromatic-H of pyrene) 8.13 (2H, s, aromatic-H of pyrene), 8.20–8.30 (4H, m, aromatic-H of pyrene), 8.41 (1H, d, $J = 9.3$ Hz, aromatic-H of pyrene).

A mixture of II (100 mg, 0.053 mmol) and sodium 4-pyrenylbutanoate (49 mg, 0.159 mmol) in 15 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was concentrated *in vacuo* to give an oily residue, which was treated with 100 mL of acetone. The resulting precipitates were filtered and dissolved in 1 mL of DMF. The DMF soluble fraction was separated in a reverse-phase column (Lobar column LiChroprep RP-18). Stepwise elution in 700 mL of aqueous MeOH at concentrations of 10, 30, 50, 70, 80, and 90 vol.% aqueous MeOH were used to obtain mono-3^A-deoxy-3^A-pyrenebutylamido-$6^{X,Y}$-*O*-bis-(pyrenebutylate) mono-altro-γ-CD (γ-3).

γ-3: yield 59.6%. R_f 0.64 (1-butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F_{254}), R_f 0.35 (methanol–water 4:1 by volume, TLC; RP-18F_{254S}).

^{1}H-NMR (DMSO-d_6): 3.2–3.8 (46H, m, C^2—C^3H of glucose unit without pyrene and C^4—C^6H of CyD), 3.8–3.95 (1H, Br, C^3H of glucose unit with pyrene), 4.12–4.2 (1H, Br, C^2H of glucose unit with pyrene), 4.4–4.65 (6H, Br, O^6H of CyD), 4.7–5.1 (7H, Br, C^1H of glucose unit without pyrene), 5.4–5.5 (1H, m, C^1H of glucose unit with pyrene), 5.6–6.0 (15H, m, O^2H and O^3H of CyD), 7.85–8.00 (3H, m, aromatic-H of pyrene), 8.00–8.10 (3H, m, aromatic-H of *p*-tosyl), 8.13 (6H, s, aromatic-H of pyrene), 8.15–8.20 (12H, m, aromatic-H of pyrene), 8.20–8.45 (3H, d, $J = 9.3$ Hz, aromatic-H of pyrene). TOF-MS (m/z): 2130 ($[M + Na]^+$).

Measurements

Fluorescence and circular dichroism spectra were measured at 25 °C using a Perkin-Elmer LS 40B fluorescence spectrophotometer and JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 345 nm and the excitation and emission slits were 4 nm wide. Ethylene glycol aqueous solution (10 vol.%) was used as a solvent for the spectroscopic measurements because the solubility of the hosts in pure water is poor. Five μL of guest species (0.05 and 0.005 M) in dimethyl sulfoxide (DMSO) or MeOH were injected into a 10 vol.% ethylene glycol aqueous solution of the host (2.5 mL) to make a sample solution with a host concentration of 1.0×10^{-7} M and guest concentration of 0.1 and 0.01 mM.

Results and discussion

The preparation of mono-3^A-deoxy-3^A-pyrenebutylamido-$6^{X,Y}$-O-bis-pyrenebutylate-mono-altro-γ-cyclodextrin (γ-3, X, Y = A, B, C, D, E, F, G and H, X ≠ Y)

As reported previously [10], γ-1 would be a mixture of isomers bearing the pyrene unit at 3A-position of glucose units and tosyl unit at 6A- to 6H-position of glucose units. Therefore, γ-1 was named as mixture γ-1. The synthetic procedure for γ-3 is shown in Figure 1. It is reasonable to decide that compound II is also a mixture of isomers, even though it is hardly separated by a reverse-phase column chromatography and the existing ratio of the isomers is unable to be determined by ^{1}H-NMR analysis. The γ-3 seems to be a mixture of isomers, too, because II was starting material for γ-3, of which the structure is mono-3^A-deoxy-3^A-pyrenebutylamido-$6^{X,Y}$-*O*-bis-pyrenebutylate-mono-altro-γ-cyclodextrin (*X*, *Y* = A, B, C, D, E, F, G and H, $X \neq Y$). This is because γ-3 was named as mixture γ-3 as described in the cases of γ-1 and γ-2. In this paper, the names of mixture γ-1, γ-2, and γ-3 are fixed as γ-1, γ-2, and γ-3, respectively.

Fluorescence spectra

The fluorescence spectra of γ-3 in 10 vol.% ethylene glycol aqueous solutions in the absence and presence of lithocholic acid are shown in Figure 2. The concentration of γ-3 in the fluorescence measurement is 5.0×10^{-8} M. The spectra of γ-3 are composed of monomer and excimer fluorescence with peaks at around 377, 397 and 480 nm, respectively. These peaks appeared at almost same wavelength as those of γ-2.

As reported previously [11], γ-2 shows two kinds of excimer emissions depending on the concentration of γ-2, which is produced by an intramolecular or intermolecular interaction of γ-2. In order to clarify whether the pyrene excimer of γ-3 is produced in an intramolecular or intermolecular interaction, a ratio of monomer fluorescence *versus* excimer fluorescence intensities depending on the concentration of γ-3 is studied. The results are shown in Figure 3. The values of I_{ex}/I_{m1} and I_{ex}/I_{m2} were used as parameters, where I_{m1} and I_{m2}, and I_{ex} are intensities of monomer fluorescence at 377 and 397 nm and intensity of excimer fluorescence at 480 nm, respectively. The values of I_{480}/I_{377} and I_{480}/I_{397} of γ-3 were almost the same in the range from 1.0×10^{-9} to 1.0×10^{-8} M, and then changed in over 1.0×10^{-8} M. As shown in Figure 3, it is estimated that the pyrene excimer is produced by intermolecular interaction of γ-3, which should occur over at

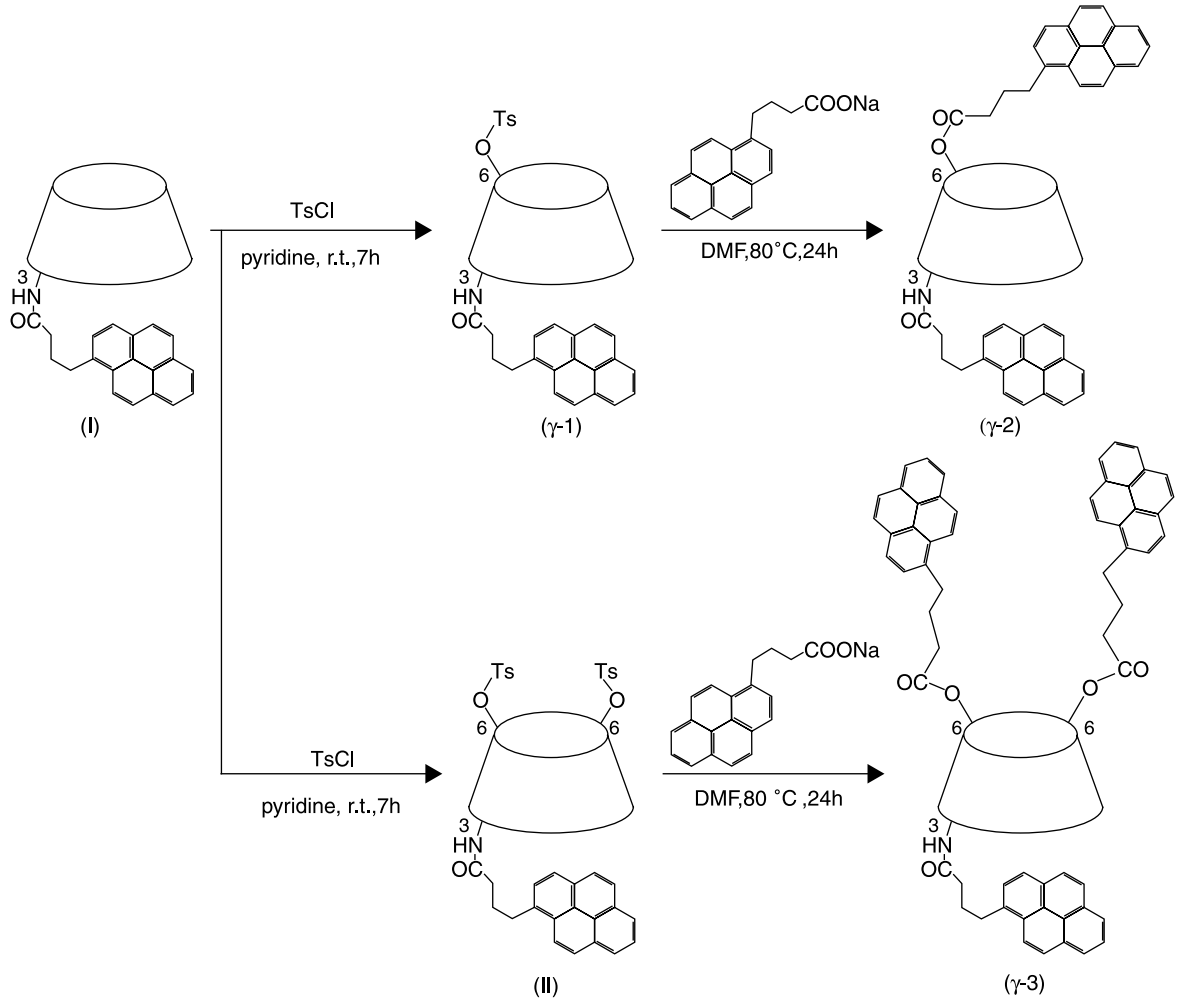


Figure 1. Preparation of γ-2 and γ-3.

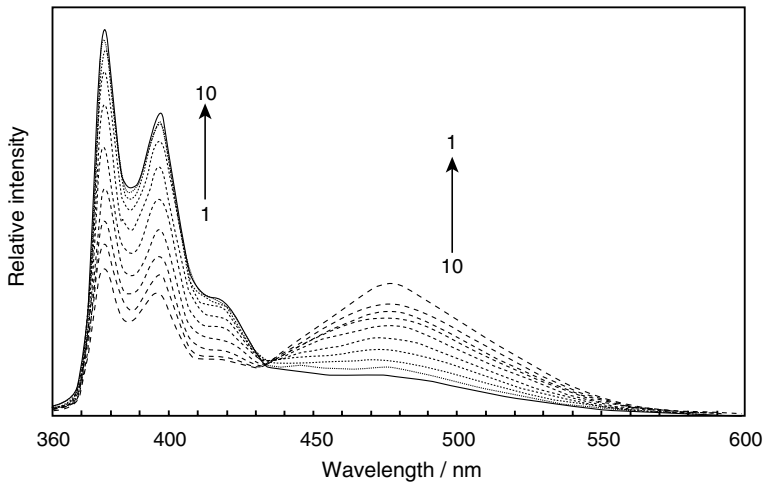


Figure 2. Fluorescent spectra of γ-3 in a 10 vol.% ethylene glycol aqueous solution (5.0×10^{-8} M, 25 °C) at various concentrations of lithocholic acid (1:0, 2:4.0×10^{-7}, 3:1.2×10^{-6}, 4:2.4×10^{-6}, 5:4.0×10^{-6}, 6:6.0×10^{-6}, 7:8.3×10^{-6}, 8:1.1×10^{-5}, 9:1.4×10^{-5}, 10:1.8×10^{-5} M.)

1×10^{-8} M concentration, where γ-3 makes an association dimer. In comparison with excimer intensities between γ-2 [11] and γ-3, the value of γ-3 is larger than that of γ-2, as shown in Figure 3. The association dimers of γ-2 and γ-3 are illustrated in Scheme 1. In the case of γ-3, four pyrene units are associated together, which contributes to make higher fluorescence intensity than that of γ-2. The monomer and excimer fluorescence

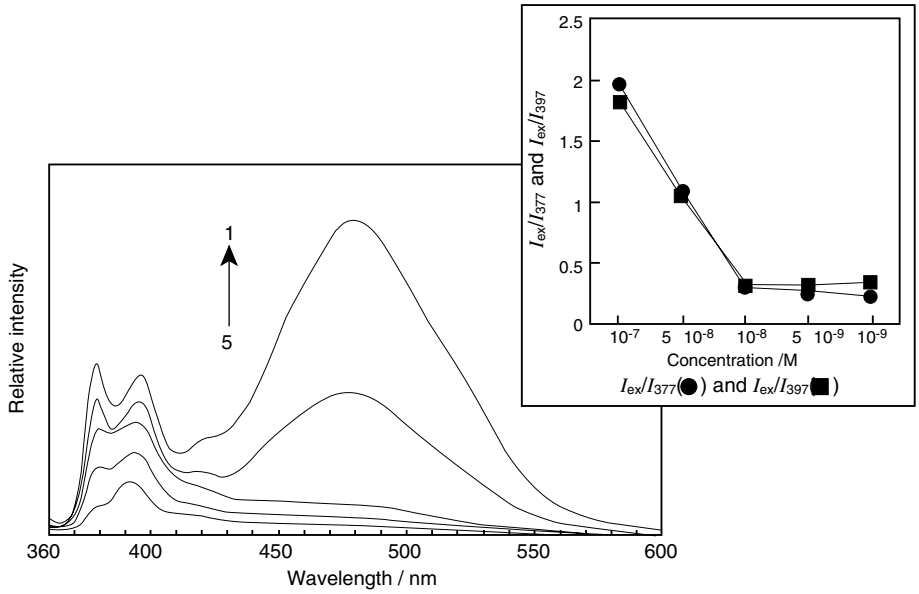
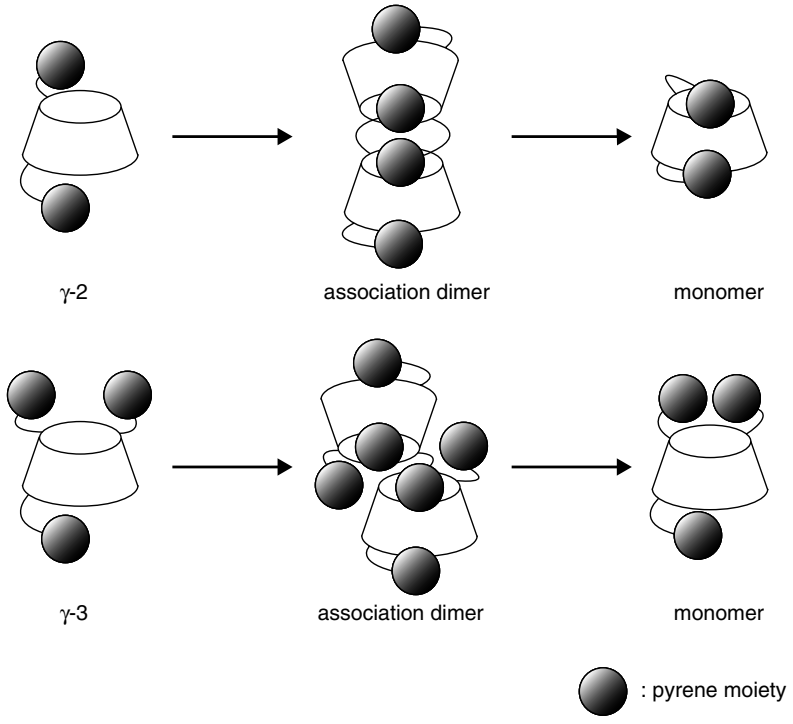


Figure 3. Monomer and excimer emissions depending on concentration of γ-3 in a 10 vol.% ehtylene glycol aqueous solution (1:1.0×10^{-7}, 2:5.0×10^{-8}, 3:1.0×10^{-8}, 4:5.0×10^{-9}, 5:$1.0 \times {}^{-9}$ M).



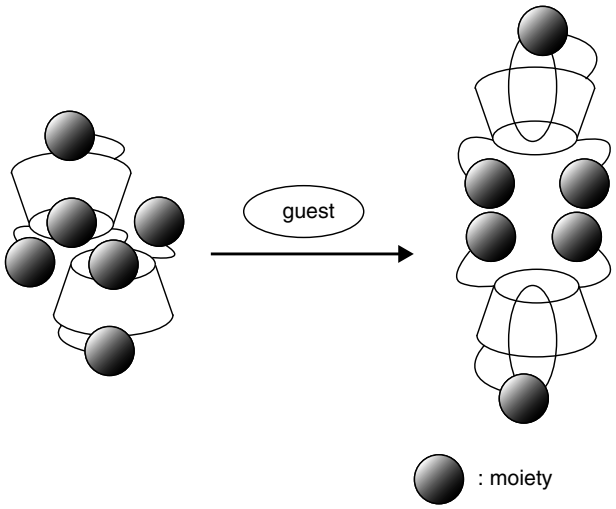
Scheme 1. Self-inclusion types of γ-2 and γ-3.

spectra of γ-3 increases and decreases, respectively, with increasing lithocholic acid concentration, which indicates that dimer associations are cancelled as illustrated in Scheme 2 when a host–guest complexation occurs.

Induced circular dichroism (ICD) spectra

Figure 4 shows the ICD spectra of γ-1, γ-2, and γ-3, alone and in the presence of lithocholic acid in 10 vol.% ethylene glycol aqueous solutions. The ICD spectrum of γ-3 shows positive Cotton peaks at around 278, 333, and 349 nm and negative Cotton peaks at around 255, 286, and 359 nm. When lithocholic acid was added to the solution of γ-3, the ICD pattern did not basically change, which suggests that dimer formation is still kept even in addition of a guest molecule.

On the other hand, the spectra patterns of γ-1 and γ-2 changed with decrease of the intensities of Cotton effect



Scheme 2. Estimated host–guest complexation of γ-3.

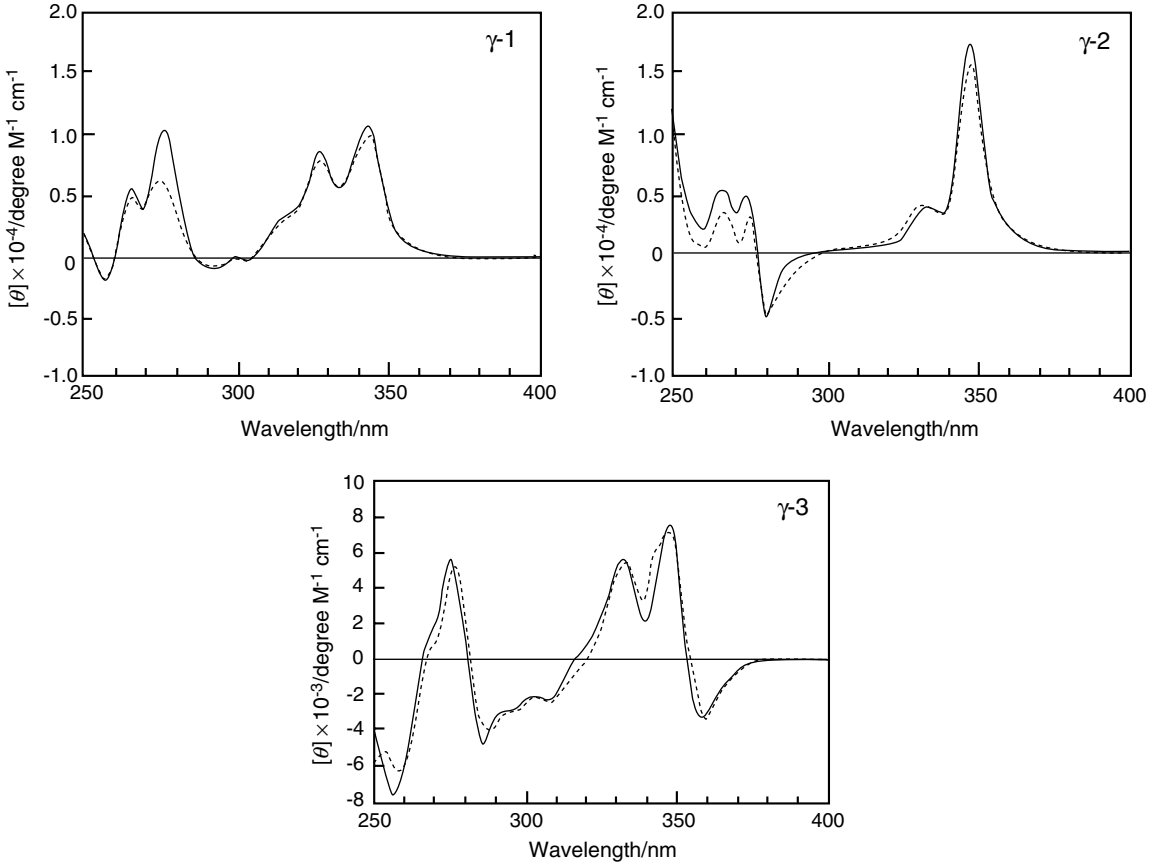


Figure 4. ICD spectra of γ-1, γ-2 and γ-3 in a 10 vol.% ehtylene glycol aqueous solution (γ-1:1.0×10^{-4} M, γ-2:1.0×10^{-5} M γ-3:5.0×10^{-5} M: ——, 25 °C) and containing lithocholic acid (1.0×10^{-5} M for γ-1 and γ-2, 5.0×10^{-5} M for γ-3: - - - -).

at 277, 328, and 345 nm, and 267, 275, and 348 nm, respectively, which indicates that the dimer formations of γ-1 and 2 are not so strong as that of γ-3.

Sensing ability of γ-3 for bile acids

In order to evaluate the sensing ability of γ-3, the $\Delta I_{ex}/I^0_{ex}$ and $\Delta I_{mon}/I^0_{mono}$ values were used as sensitivity parameters. Here, ΔI_{ex} is $I^0_{ex}-I_{ex}$, where I^0_{ex} is the intensity of excimer at 480 nm for the host, and I_{ex} is the intensity of excimer emission in the presence of a guest, and ΔI_{mono} is $I^0_{mono}-I_{mono}$, where I^0_{mono} is the intensity of monomer emission at 377 nm for the host, and I_{mono} is the intensity of monomer emission in the presence of a guest. We used five bile acids as guests: deoxycholic acid (**1**), lithocholic acid (**2**), chenodeoxycholic acid (**3**),

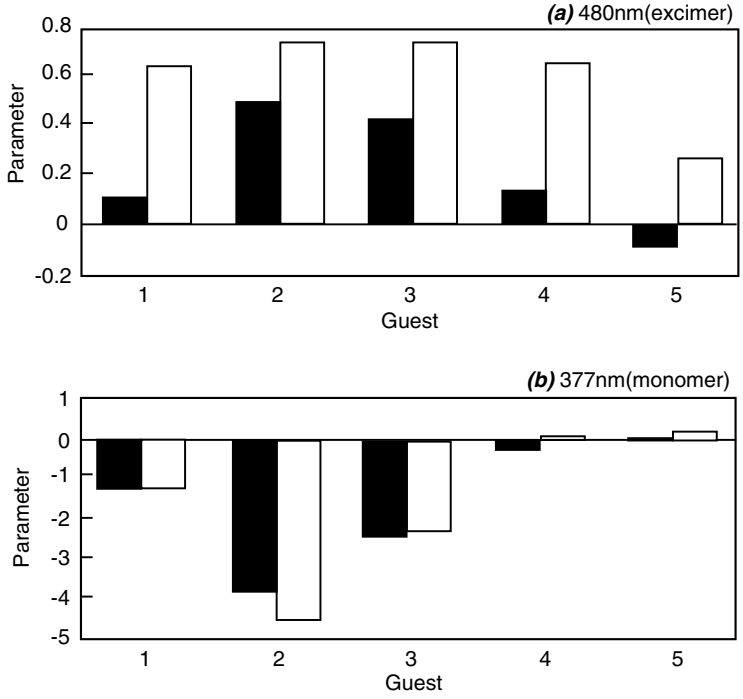


Figure 5. (a)–(b) Sensitivity factors of γ-2 (■) and γ-3 (□) in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-7} M, 25 °C) for steroids guests examined.

ursodeoxycholic acid (**4**), and cholic acid (**5**). Figures 5a and b show the parameters of γ-3 together with γ-2 obtained using bile acids at 0.1 mM except for lithocholic acid, which was examined at 0.01 mM because 0.1 mM of lithocholic acid is not soluble in 10 vol.% ethylene glycol aqueous solutions. Lithocholic acid (**2**) and ursodeoxycholic acid (**3**) are detected by γ-3 with the highest value $\Delta I_{ex}/I^0_{ex}$ of 0.725. Deoxycholic acid (**1**) and ursodeoxycholic acid (**4**) are detected by γ-3 with the next highest value $\Delta I_{ex}/I^0_{ex}$ of 0.624. The $\Delta I_{ex}/I^0_{ex}$ values of γ-2 for all guests range from 0.469 to −0.085, which suggests that the selectivity of γ-2 for the guests is higher than that of γ-3, because $\Delta I_{ex}/I^0_{ex}$ values of γ-3 for the guests range from 0.725 to 0.274. From the point of view of the molecular sensing system, γ-2 works much more efficiently than that of γ-3, because of the low selectivity even with higher sensitivity for guest molecules. The parameter values $\Delta I_{mon}/I^0_{mono}$ of γ-2 and γ-3 obtained from monomer emission variations upon a guest addition are similar, as shown in Figure 5b. The parameter values of γ-2 and γ-3 range from −3.871 to 0.097 and from −4.582 to 0.279, respectively. These results suggest that γ-3 shows higher sensitivity and selectivity than γ-2.

Conclusion

The sensor system based on three kinds of γ-cyclodextrin analogs modified with mono-, di-, and tri-pyrene units have been studied. Among them, γ-2 and γ-3 form an association dimer, which can give much more information such as the intensity change based on monomer together with excimer emissions for fluorescent molecular sensing system. The modification with three pyrenes at the hetero rims of γ-CyD will contribute to an increase of the hydrophobic environment around the CyD cavity more than that of γ-2, which results in higher sensitivity of molecular sensing for the guest molecules examined. Unfortunately, the positions of pyrene introduced at lower and upper rims of CyD cavity have not been determined, so far. We are now trying to make a good crystal of these host molecules for X-ray analysis.

Acknowledgements

This study was supported by a Grant-in Aid for Specially Promoted Research (No. 404: Molecular Synchronization for Design of New Materials System) from the Ministry of Education Science, Sports and Culture of Japan.

References

1. M. Eddaoudi, H.-P. Lopez, S.F. de Iamotte, D. Ficheux, P. Probnon, and A.W.Coleman: *J. Chem. Soc., Perkin Trans.* **2**, 1711 (1996).
2. B.K. Hubbard, L.A. Beilstein, C.E. Heath, and C.J. Abelt: *J. Chem. Soc., Perkin Trans.* **2**, 2751 (1996).
3. H. Nakashima, Y. Takenaka, M. Higashi, and N. Yoshida: *J. Chem. Soc., Perkin Trans.* **2**, 2096 (2001).

4. M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **16**, 37 (2000).
5. M. Narita, N. Ogawa, and F. Hamada: *Anal. Sci.* **16**, 701 (2000).
6. M. Narita, A. Makabe, K. Kinoshita, K. Endo, and F. Hamada: *Int. J. Soc. Mater. Eng. Resour.* **9**, 6 (2001).
7. M. Narita, S. Mima, N. Ogawa, and F. Hamada: *Anal. Sci.* **17**, 379 (2001).
8. M. Narita, E. Tashiro, and F. Hamada: *Anal. Sci.* **17**, 1453 (2001).
9. M. Narita, N. Dorjpalam, K. Teranishi, and F. Hamada: *Anal. Sci.* **18**, 711 (2001).
10. M. Narita, E. Tashiro, and F. Hamada: *J. Incl. Phenom. Macrocycl. Chem.* **42**, 137 (2002).
11. M. Narita and F. Hamada: *J. Incl. Phenom. Macrocycl. Chem.* **44**, 335 (2002).