Molecular Recognition of Deoxycholic Acids by Pyrene-Appended γ-Cyclodextrin Connected with a Rigid Azacrown Spacer

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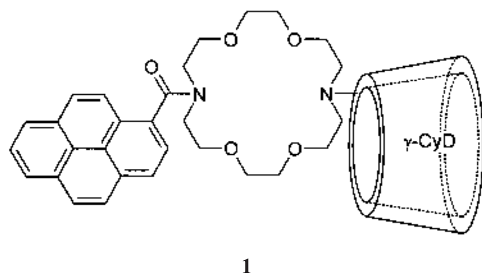
A γ-cyclodextrin (γ-CyD) derivative (1) having a pyrene moiety, connected through a 4,13-diaza-18-crown-6 ether moiety to γ-CyD, was synthesized and evaluated for guest binding and sensing properties. In aqueous solution, 1 existed as an association dimer in which the secondary hydroxyl sides faced each other to accommodate two pyrene moieties. Photo-induced electron transfer (PET) between the amino group and the excited pyrene moiety regulated the monomer fluorescence intensity of 1. The monomer–dimer equilibrium and the PET indicated that 1 may be used as a host capable of detecting guest complexation by changes in fluorescence intensity from the pyrene moiety. Deoxycholic acids were found to be good guests for detection by 1, and deoxycholic acid itself induced different fluorescence changes compared to the other deoxycholic acids. This indicated that 1 could recognize the position of the hydroxy groups on the steroidal framework. The azacrown part may participate in the guest selectivity for the deoxycholic acids by regulating the distance between the amino group and the pyrene moiety, modifying PET efficiency.

Key words γ-cyclodextrin; azacrown ether; pyrene; photo-induced electron transfer; fluorescent chemosensor

Cyclodextrins (CyDs) are cyclic oligomers of D-(+)-glucopyranoside with torus shapes. The most attractive feature of CyDs is that they can form host-guest complexes in aqueous solution with a wide variety of organic compounds.[1—3] This feature makes CyDs useful as enzyme model compounds.[4] In addition, since CyD has little toxicity for living systems, CyDs can be used as a solubilizer, stabilizer, and so on, through drug formulation in the pharmaceutical field.[5] In the last decade, CyDs modified with a hydrophobic aromatic residue have been studied with respect to their potential as molecular sensing devices for photometric determination of spectroscopically inert compounds.[6,7] The key concept of this molecular sensing is that the fluorescence, UV-visible absorption, and circular dichroism of the modified CyDs are changed upon binding an external guest (target) compound. In other molecular sensing systems based on the supramolecular concept, photo-induced electron transfer (PET) plays an important role.[8—10] The action of PET results in a large change in fluorescence from aromatic moieties appended to the host moiety. In principal, the efficiency of PET is regulated by the distance between the electron donor and acceptor.[11] In general, modified CyDs which form intramolecular self-complexes change their conformations to intermolecular complexes when they bind an externally added guest compound. This process is accompanied by a large change in the location of the modified residue. Therefore, combining PET phenomenon and host-guest interaction of modified CyDs, we can construct a detecting system for hydrophobic organic compounds on the basis of modified CyDs having an electron donating group and an electron accepting group. Keeping this concept in mind, we synthesized a new host γ-CyD derivative having 4,13-diaza-18-crown-6 ether and pyrene moieties (**1**). The azacrown moiety has two amino groups. One amino group bears a pyrene moiety introduced as an electron acceptor through an amide bond, and a γ-CyD moiety is attached at the other amino group. Although the electron donating capability of the former amino group is hindered by the amide function, the later amino group can act as an electron donor because its lone pair is not masked. Another important feature of the use of the azacrown moiety is that it has a rigid structure as compared to a flexible alkyl chain. This structural rigidity may be crucial in discriminating structurally similar guest compounds, because a flexible alkyl group may hinder conformational difference in complexes of modified CyDs.[12,13] Herein we wish to report the molecular association and sensing properties of **1**.

Experimental

Materials γ-CyD was a kind gift from Nihon Shokuhin Kako., Co. Ltd., and used without further purification. 4,13-Diaza-18-crown-6 was syn-



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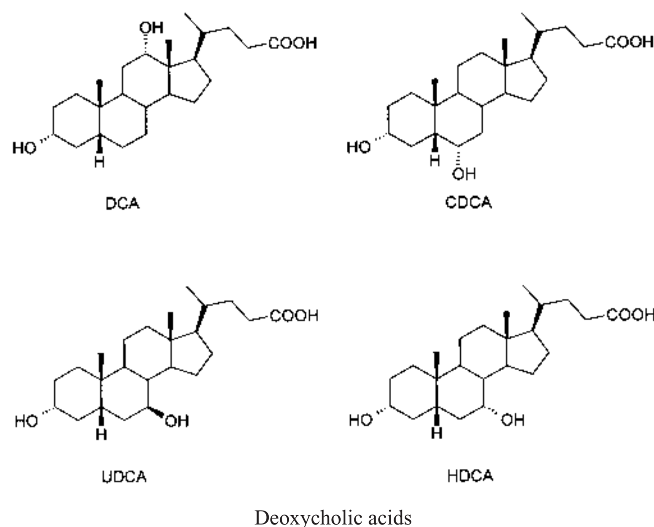

Deoxycholic acids

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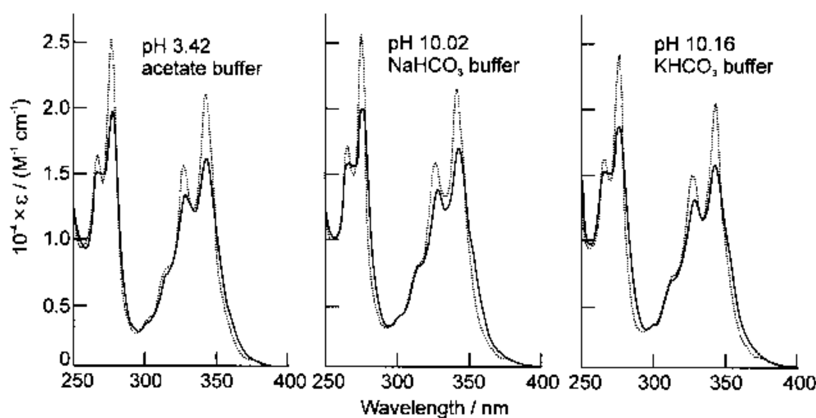

Fig. 1. UV-Visible Absorption Spectra of **1**

3.0×10^{-4} M, path length=0.1 cm, dotted lines; 3.0×10^{-5} M, path length=1.0 cm, solid lines, HOAc–NaOAc (pH 3.42; left), $NaHCO_3$–Na_2CO_3 (pH 10.02; middle), $KHCO_3$–K_2CO_3 (pH 10.16; right) buffered aqueous solutions.

thesized according to the literature.[14] 1-Pyrenecarboxylic acid was prepared from the corresponding aldehyde with Ag_2O oxidation in an alkaline ethanol solution, and recrystallized from ethanol.[15]

Syntheses ***N*-(1-Pyrenecarbonyl)-4,13-diaza-18-crown-6 (2)** 1-Pyrenecarboxylic acid (1.47 g, 5.96 mmol) was suspended in $SOCl_2$ (25 ml) and the suspension was heated at reflux for 1.5 h. During the reaction, the suspension turned to a clear, dark yellow solution. The excess $SOCl_2$ was completely removed by rotary evaporation to leave a dark, greenish yellow solid. The solid was re-dissolved in benzene (200 ml) and this solution was added dropwise during 6 h to a benzene solution (60 ml) of 4,13-diaza-18-crown-6 (1.53 g, 5.96 mmol) and Et_3N (2.10 g, 20.8 mmol) under vigorous stirring at room temperature. After stirring 14 h, the reaction mixture was washed with water (40 ml×3) and 10% HCl (80 ml×3), dried ($MgSO_4$), and the solvent was evaporated to dryness. The residue was subjected to Al_2O_3 column chromatography (basic form, deactivated to activity II to III). Elution with $CHCl_3$–benzene (2 : 3) gave the title compound as a yellow viscous oil (0.544 g, 19%). ^{1}H-NMR ($CDCl_3$) δ: 2.75—2.85 (m, 4H), 3.30—4.20 (m, 21H), 7.92—8.24 (m, 9H). IR (KBr) cm^{-1}: 3455, 3044, 2868, 1630, 1510, 1545, 1420, 1350, 1288, 1248, 1115, 1080, 964, 852, 766, 725, 682, 638, 511, 465: MS *m/z*: 490 (M^+). High resolution MS *m/z*: 490.2489 (Calcd for $C_{29}H_{34}N_2O_5$: 490.2466).

***N*-(1-Pyrenecarbonyl)-*N′*-[(6-deoxy-γ-cyclodextrin)-6-yl]-4,13-diaza-18-crown-6 (1)** A mixture of 6-deoxy-6-iodo-γ-cyclodextrin (350 mg, 0.249 mmol), which was prepared from 6-*O*-tosyl-γ-cyclodextrin and NaI in DMF, and **2** (280 mg, 0.571 mmol) in DMF (8 ml) was heated at 80 °C for 3 d. After concentration to 3 ml, the mixture was poured into acetone (200 ml) to form precipitates. The resulting precipitates were collected and washed with acetone, and dried *in vacuo*. Ion-exchange chromatography (SP Sephadex C-25, NH_4^+ form) followed by reprecipitation with acetone afforded pure **1** (66 mg, 15%). ^{1}H-NMR ($(CD_3)_2SO$) δ: 2.55—3.02 (m, 4H), 3.12—3.98 (m, overlapped with H_2O), 4.33—4.53 (m, 7H), 4.83—4.93 (m, 8H), 5.95 (bs, 16H), 7.97—8.49 (m, 9H). IR (KBr) cm^{-1}: 3395, 2926, 1632, 1420, 1157, 1111, 1080, 1028, 941, 853, 760, 706, 584, 530. FAB-MS *m/z* 1769 ($[M+H]^+$). *Anal*. Calcd for $C_{77}H_{112}O_{44}N_2\cdot13H_2O$; C, 46.15; H, 6.94; N, 1.44. Found; C, 46.07; H, 6.76; N, 1.10.

Measurements UV-visible spectra was recorded on a Shimadzu UV-250 spectrophotometer, using quartz cells with path length of 0.1, 0.5, 1.0, 2.0, and 5.0 cm. Fluorescence spectra were recorded on a Jasco FP-770 spectrofluorophotometer and measured with an excitation wavelength of 340 nm. All measurements were performed at 25 °C by circulating thermostated water through the cell holders. Most of the experiments were carried out in acetic acid (HOAc)–sodium acetate (NaOAc) (pH 3.42), $NaHCO_3$–Na_2CO_3 (pH 10.02), and $KHCO_3$–K_2CO_3 (pH 10.16) buffer solutions. Fluorometric pH titration experiments were conducted with LiOH and HCl aqueous solutions. pH values were measured on a Horiba pH meter (F-7_{II}) by immersing a glass electrode (Horiba 6029-10T) into a cell filled with a sample solution.

Results and Discussion

Figure 1 shows UV-visible absorption spectra of **1** in three different media. In *ca.* 3×10^{-4} M solutions, the pyrene ^1L_a band (300—400 nm) and ^1B_b band (250—300 nm) were broadened as compared with diluted (*ca.* 3×10^{-5} M) solutions. This indicates that the pyrene moiety of **1** formed a stacked structure, and thus **1** tends to form an association dimer.[12,13] The concentration dependent absorption spectral changes allowed us to estimate the association constants for dimer formation (K_a), and we obtained values of 1.0×10^4 M^{-1} in acidic solution at pH 3.42, and 1.5×10^4 M^{-1} in the pH 10 solutions.[14] The K_a values at around pH 10 were independent of the alkali metal cation present in solution.

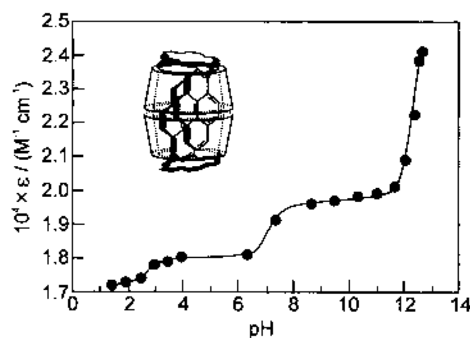

Fig. 2. pH Profile for Molar Absorptivity of **1** at 342 nm

Inset is a proposed structure for the association dimer of **1**.

The structure of the dimer of **1** was clarified from the pH titration experiments. The pH profile of the molar absorption of **1** at 342 nm is shown in Fig. 2, exhibiting a drastic increase in the absorptivity above pH 12 where secondary hydroxyl groups dissociate.[2] Since this increase in the absorptivity should be accompanied by dissociation of the secondary hydroxyl groups in the γ-CyD residue, the secondary hydroxyl sides must face each other in the association dimer.

Association constants for externally added guests (K_g) were also estimated from the guest induced absorption changes.[14] The K_g values for cyclohexanol (CyH), 1-adamantanol (1-AdOH), and 1-adamantanecarboxylic acid (1-AdCA), and four common deoxycholic acids (deoxycholic acid, DCA; chenodeoxycholic acid, CDCA; ursodeoxycholic acid, UDCA; hyodeoxycholic acid, HDCA) in the three different media are listed in Table 1. Despite the difference in medium, the obtained K_g values were remarkably small as compared with reported values for other γ-CyD derivatives.[16—19] This indicates an inhibitory effect of the modified

residue on guest binding and stability of the association dimer of **1** in aqueous solution.

Almost no effect of the alkali metal cations, as judged from the K_g values for 1-AdCA and deoxycholic acids in alkaline solutions, again suggests that the azacrown moiety scarcely participates in the guest recognition by **1**. This may be due to the amide function at one end of the azacrown moiety which can reduce the cation binding capability of the azacrown ether, and due to an inadequate conformation of the azacrown moiety for cation binding forced by intramolecular complexation between the γ-CyD cavity and the pyrene moiety. In addition to this conformational explanation, cation binding by crown ethers is known to be weakened in aqueous solution.[20]

The left panel of Fig. 3 shows the fluorescence spectrum of **1** in aqueous solutions at different pH values. The right panel of Fig. 3 shows the pH profile of the fluorescence intensity at 382 and 472 nm. As expected from the observation that **1** formed an association dimer, **1** exhibited red-shifted, broadened fluorescence around 470 nm assignable to excimer fluorescence, in addition to the monomer fluorescence of the pyrene residue. Exciplex fluorescence arising from the excited-state complex between the amino group and the pyrene moiety is another possibility for the origin of the red-shifted, broadened fluorescence. In a CyD cavity, formation of a fluorescing exciplex has been reported.[21] However, exciplex was not dominant species for the origin of the red-shifted fluorescence, because this fluorescence maintained intensity even in acidic conditions where the amino group should be in the protonated ammonium form, and have no capability of forming an exciplex, as shown in Fig. 3, right panel.

Although the excimer fluorescence intensity was independent of pH below 11, it decreased abruptly above pH 12, where the association dimer dissociated. Monomer fluorescence intensity was strong under acidic conditions while the alkaline conditions decreased monomer fluorescence intensity. This pH dependency of the monomer fluorescence is attributable to PET between the amino nitrogen atom and an excited pyrene moiety, because the monomer fluorescence intensity dramatically changed around the pK_a of the amino group. In addition, the efficient observation of PET indicates a favorable conformation around the lone pair of the amino nitrogen atom and the π-surface of the pyrene residue, since PET efficiency is regulated by the distance and degree of orbital overlap between an electron donor and electron acceptor.[11]

No pH dependency for excimer fluorescence below pH 10 suggests that PET was unfavorable for the excimer species. This may be due to a large difference in the energy level between HOMO (Highest Occupied Molecular Orbital) of the amino group and LUMO (Lowest Unoccupied Molecular Orbital) of the excimer.

Figure 4 shows fluorescence spectra of **1** in a pH 10.02 aqueous solution, alone and in the presence of CyH, 1-AdOH, and HDCA. The addition of guests induced a decrease in excimer fluorescence due to dissociation of the association dimer of **1**. The degree of decrease in excimer fluorescence intensity is simply related to the concentration of the association dimer, and hence the strength of binding guests. Contrary to the excimer fluorescence, changes in the monomer fluorescence intensity were strongly dependent on the guest species. HDCA remarkably intensified the monomer fluorescence. Since at this pH the amino group is in its neutral form, monomer fluorescence of the pyrene residue should be quenched by PET. Therefore, the observation of intensified monomer fluorescence in the presence of HDCA clarified that introduction HDCA increased the distance between the amino group and the pyrene residue. This means that HDCA deeply penetrates into the γ-CyD cavity of **1**, extruding the pyrene residue from the cavity to the outer aqueous phase.

Although the excimer fluorescence was reduced as 1-AdOH concentration was increased, the monomer fluores-

Table 1. 1 : 1 Host–Guest Association Constants (K_g) of **1** for Cyclohexanol (CyH), 1-Adamantanol (1-AdOH), and 1-Adamantanecarboxylic Acid (1-AdCA), and Deoxycholic Acids[a]

Guest	K_g (M^{-1})		
	pH 3.42[b]	pH 10.02[c]	pH 10.16[d]
CyH	34 (23600)	55 (25500)	54 (25300)
1-AdOH	120 (23000)	255 (25200)	275 (24200)
1-AdCA	—[e]	330 (24700)	315 (24500)
DCA	—[e]	11000 (25300)	12000 (24500)
CDCA	—[e]	16000 (24900)	16000 (24100)
UDCA	—[e]	16000 (24500)	16500 (23100)
HDCA	—[e]	22000 (23400)	21000 (24200)

a) Determined from UV-visible spectral variations. All measurements were carried out at 25 °C. The molar absorptivity (in $\text{M}^{-1}\,\text{cm}^{-1}$) for 1 : 1 host–guest complexes are represented in parentheses. *b*) Measured in an acetate buffer solution. *c*) Measured in a sodium carbonate buffer solution. *d*) Measured in a potassium carbonate buffer solution. *e*) K_g values could not be determined owing to the poor solubility of these guests at pH 3.42.

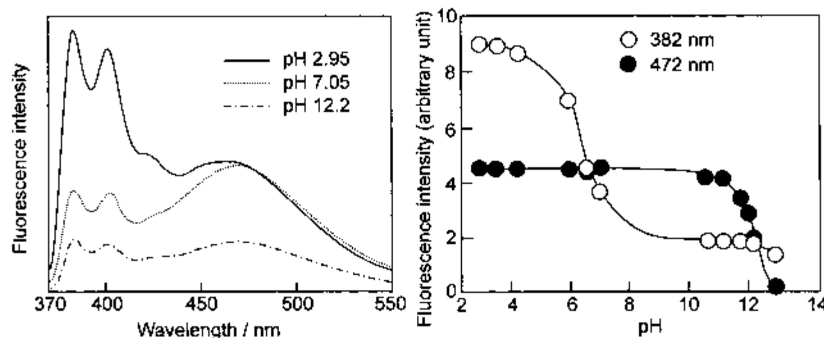

Fig. 3. (Left) Fluorescence Spectra of **1** (3.0×10^{-5} M) in Aqueous Solution with Different pH (2.95, Solid Line; 7.05, Dotted Line; 12.2, Chain Line), (Right) pH Profiles for Fluorescence Intensity at 382 nm (Open Circle) and 472 nm (Closed Circle)

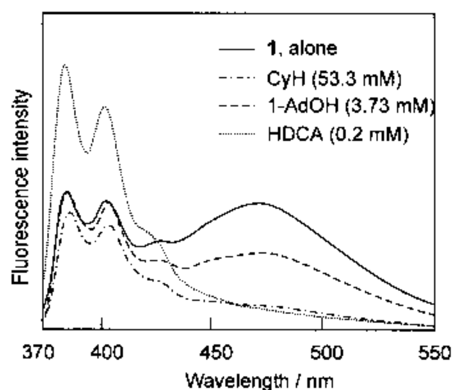

Fig. 4. Fluorescence Spectra of **1** (3.0×10^{-5} M in pH 10.02 $NaHCO_3$–Na_2CO_3 Buffered Aqueous Solutions, Alone (Solid Line), or in the Presence of HDCA (0.20 mM; Dotted Line), 1-AdOH (3.73 mM; Broken Line), and CyH (53.3 mM; Chain Line)

cence intensity was virtually unchanged. Without PET, the excimer fluorescence intensity should be related to the decrease and increase in the concentration of dimer and monomer species, respectively, and should accompany an increase in monomer fluorescence intensity. The large decrease in excimer fluorescence apparently resulted from decomposition of the association dimer of **1**. The unchanged monomer fluorescence intensity, despite the decrease in the association dimer concentration, strongly indicates that the monomer fluorescence of **1** was quenched in a complex with 1-AdOH. Thus, in the complex, the pyrene moiety would exist closer to the amino group to undergo efficient PET than that of free **1**.

Slight, but apparent decreases in the monomer fluorescence intensity caused by CyH showed that the distance between the pyrene group and the amino group became closer in a complex of **1** with CyH than in that with 1-AdOH. Smaller CyH would allow the pyrene moiety to penetrate deeper into the γ-CyD cavity. This conformation promotes efficient PET. On the other hand, the large HDCA molecule prohibits the insertion of the pyrene ring into the cavity when HDCA penetrates into it. Thus, the pyrene ring may be exposed to the aqueous phase and the distance to the amino group should become large.

The fluorescence behavior of **1** in conjunction with the guest binding strength demonstrates a potential use of **1** for sensing deoxycholic acids. In Table 2, we listed the sensing parameters $\Delta I_e/I_e^0$ and $\Delta I_m/I_m^0$, which are fluorescence intensity differences caused by the presence of a guest normalized by the initial fluorescence intensity, I^0. I_e and I_m mean excimer and monomer fluorescence, respectively. Negative and positive values in Table 2 refer to a decrease and increase in the fluorescence intensity, respectively. The data listed in Table 2 were acquired in pH 10 carbonate buffer solutions with the concentrations of guest and **1** being 0.1 and 0.03 mM, respectively.

At 0.1 mM, CyH, 1-AdOH, and 1-AdCA caused slight decreases in the excimer fluorescence and eventually no changes in the monomer fluorescence intensity. On the other hand, deoxycholic acids induced large decreases in the excimer fluorescence with concomitant increases in the monomer fluorescence. These guest-induced fluorescence changes, especially the monomer fluorescence changes,

Table 2. Sensing Parameters of **1** for Several Guests Obtained at 0.1 mM Guest Concentration[a)]

Guest	Na_2CO_3+$NaHCO_3$		K_2CO_3+$KHCO_3$	
	$\Delta I_m/I_m^0$	$\Delta I_e/I_e^0$	$\Delta I_m/I_m^0$	$\Delta I_e/I_e^0$
CyH	−0.002	−0.005	−0.002	−0.005
1-AdOH	0.003	−0.040	0.003	−0.038
1-AdCA	0.003	−0.052	0.002	−0.053
DCA	0.373	−0.712	0.383	−0.702
CDCA	1.031	−0.757	1.068	−0.821
UDCA	1.040	−0.755	1.100	−0.828
HDCA	0.831	−0.834	0.844	−0.840

a) Data were taken in sodium carbonate and potassium carbonate buffer solutions (pH 10.02 and pH 10.16, respectively) at 25 °C. Excitation wavelength was 337 nm.

demonstrates that **1** can be regarded as a selective host for deoxycholic acids.

Interestingly, of the deoxycholic acids, DCA induced a relatively small increase in the monomer fluorescence intensity as compared to the other deoxycholic acids (CDCA, UDCA, and HDCA). The smaller increase in the monomer fluorescence despite the large decrease in the excimer fluorescence is attributable to occurrence of modestly effective PET, as seen in the case of 1-AdOH. Therefore, DCA was bound by **1** with a different conformation from the other deoxycholic acids, being shallowly accommodated in the cavity. The pyrene residue may act as a hydrophobic cap in this conformation. However, the binding constants clearly indicate that DCA has the weakest affinity to **1**. Hence, a pyrene residue is not an effective cap, rather exerting an inhibitory effect upon binding by DCA. The other deoxycholic acids may deeply penetrate the cavity. In this conformation, the hydrophobic steroidal part was mostly sequestered from an aqueous phase by the γ-CyD framework. This conformation is entropically favorable for guest binding by CyD because the hydration shell around the steroidal framework is not formed. Some studies have demonstrated that γ-CyD complexation is driven by hydrophobic interactions (entropically favorable) while van der Waals interactions (enthalpically favorable) governed β-CyD complexation.[22)]

The structural difference between DCA and the other deoxycholic acids is the position of a hydroxyl group other than the C-3 hydroxyl group. DCA bears an additional hydroxyl group at the C-12 position. CDCA and UDCA bear a hydroxyl group at C-7 position. HDCA bears a hydroxyl group at C-6 position. Only DCA has the hydroxyl group near to the steroidal side chain. The presence of the hydroxyl group probably prohibits DCA from penetrating deeply in the γ-CyD cavity of **1**. In this regard, **1** can recognize the position of hydroxyl groups on the deoxycholic acids upon complexation. A recent two-dimensional NMR study of a β-CyD–UDCA complex has revealed that the steroidal side chain played an important role in binding by the β-CyD cavity.[23)]

Figure 5 shows plots of sensing parameters obtained from the monomer fluorescence variations for the guests used in this study, as a function of guest concentration. The plots demonstrate that CyH, 1-AdOH, and 1-AdCA do not perturb the fluorescence response of **1** for deoxycholic acids, if they are contaminated at concentrations below 1 mM. From the plots, we can conclude that **1** can discriminate DCA from the other deoxycholic acids. Structural rigidity served by the

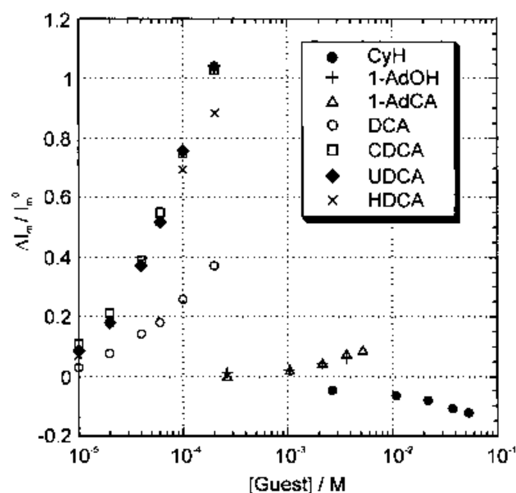

Fig. 5. Plots of $\Delta I_m/I_m^0$ Induced by as a Function of Guest Concentration

azacrown moiety may result in the conformational difference, and hence the fluorescence signal difference by regulating the efficiency of PET.

Conclusion

We synthesized **1** to investigate its capability for sensing deoxycholic acids by fluorescence signal changes. Owing to the formation of the association dimer, **1** could detect guest species, especially deoxycholic acids, from guest-induced monomer and excimer fluorescence changes. The monomer fluorescence intensity was controlled by PET between the amino group of the azacrown moiety and the excited pyrene moiety. Although the azacrown moiety hardly participated in guest recognition through binding cations, its structural rigidity enabled **1** to discriminate DCA from the other deoxycholic acids. We have earlier reported that γ-CyD derivatives modified with two naphthyl groups could recognize the difference between CDCA and UDCA, based on different fluorescence spectral changes.[19] If we can construct other CyD-based sensing systems with different responses for HDCA, simultaneous detection of the four common deoxycholic acids can be made.

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