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Strong Binding Between Acidic Guests and Fluorescein Modified γ-Cyclodextrin via Hydrogen Bonding

FUMIO HAMADA,* KYOKO ISHIKAWA and YUTAKA HIGUCHI
Department of Materials Engineering and Applied Chemistry, Mining College, Akita University, Tegata, Akita 010, Japan.

YOUICHI AKAGAMI*
Akita Prefectural Industrial Center, 4-11 Sanuki, Araya, Akita 010, Japan.

AKIHIKO UENO*
Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Yokohama 227, Japan.

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Abstract. γ-Cyclodextrin with appended fluorescein (**1**) has been prepared as a sensor and a charge-changeable receptor for detecting organic compounds including terpenoids, carboxylic acids and bile acids. Compound **1** has cationic, neutral and anionic forms depending on the pH of the solutions. The anionic form of **1** at about neutral pH exhibits the highest sensing ability for carboxylic acids and bile acids, while at alkaline pH it detects hardly any of the guests examined. The high sensitivity and selectivity of the anionic form of **1** at around neutral pH for acidic guests seems to be caused by the hydrogen bond between the phenoxide anion moiety of fluorescein and acidic guests. The neutral form of **1** exhibits little sensing ability for all the guests, but the cationic form shows comparatively higher sensing ability for the guests examined.

Key words: Fluorescein modified γ-cyclodextrin, fluorescence spectroscopy, acidic guests.

1. Introduction

Fluorescein 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 the molecular levels in a variety of biological systems [1]. Cyclodextrins are torus-shaped cyclic oligomers of D-glucopyranose, which can accommodate a variety of guests in their cavity and have attracted much interest as model compounds for studies of enzyme [2]. The modification of cyclodextrin with fluorescein can lead to a charge changeable receptor [3], in which the fluorescein moiety can act as a fluorescent probe and as a charge changeable residue. For the last decade, we have

* Authors for correspondence.

reported the host–guest complexation properties of modified cyclodextrins, bearing chromophores such as naphthalene and anthranilate moieties [4, 5]. In those systems, the appended moiety can act as a probe of the host–guest binding behaviors and acts either as a spacer, which enables the cyclodextrin to form a 1 : 1 host–guest complex by narrowing the γ-cyclodextrin cavity, or as a hydrophobic cap to elevate the guest-binding ability of cyclodextrins by enlarging the hydrophobic environment around the cyclodextrin cavity. Recently, Ueno and coworkers reported the host–guest binding behavior of fluorescein modified β-cyclodextrin (**2**), which has cationic, neutral and anionic forms depending on the pH of the solution [6]. All three molecular forms of **2** change their absorption spectra upon guest addition with blue shifts each revealing an isosbestic point in the visible region, which demonstrates 1 : 1 complex formation. In many cases, γ-cyclodextrin shows different inclusion behavior in comparison to β-cyclodextrin because of its different molecular size. As an extension of our work, we prepared γ-cyclodextrin capped by fluorescein, and report here the fluorescent molecular recognition and host–guest complexation behavior of **1** in cationic, neutral and anionic forms, which are very different from that of the β-cyclodextrin analogue.

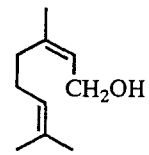
2. Experimental

2.1. Preparation of Fluorescein Modified γ-Cyclodextrin (**1**)

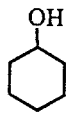
A mixture of 6-iodo-6-deoxy-γ-cyclodextrin (1.6 g, 1.14 mM) and fluorescein sodium salt (0.56 g, 1.49 mM) in 20 mL of DMF was heated at 80 °C for 24 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 500 mL of EtOH. The resultant precipitates were filtered and dried. The crude product was purified with a CM-Sephadex C-50 column (3 × 90 cm) to afford 0.13 g (8% yield) of pure **1** in the anionic form. R_f 0.48 (1-butanol : ethanol : water 5 : 4 : 3 by volume). ^{1}H-NMR(DMSO-d_6) = 8.14 (1H, d, J = 7.6, aromatic-H), 7.78 (1H, t, J = 7.6, aromatic-H), 7.69 (1H, t, J = 7.6, aromatic-H), 7.40 (1H, d, J = 7.6, aromatic-H), 6.46 (2H, m), 6.12 (4H, m), 5.20–5.80 (16H, m, O_2H, O_3H of cyclodextrin), 4.2–5.0 (15H, m, O_6H, C_1H of cyclodextrin), 3.0–3.9 (48H, m, C_2H—C_6H of cyclodextrin). *Analysis. Calcd.* for $C_{68}H_{89}O_{44}Na{\cdot}8H_2O$ C, 45.95; H, 5.95%. *Found* C, 45.71; H, 5.92. MS (FAB):1656 ([M + Na]$^+$).

2.2. Measurements

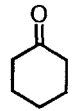
Ultraviolet, fluorescence and circular dichroism spectra were measured at 25 °C, with a Hitachi U-2000 spectrophotometer, a Hitachi F-3010 fluorescence spectrometer and a JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 450, 460 and 470 nm for cationic, neutral, and anionic forms, respectively. The excitation and emission slits were 5 nm. Five μL of guest (0.5, 0.05 and 0.005 M in dimethylsulfoxide (DMSO) or MeOH were injected into an aqueous solution of **1** (2.5 mL)



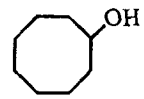
nerol(**3**)



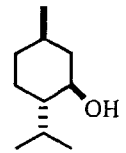
cyclohexanol(**4**)



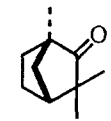
cyclohexanone(**5**)



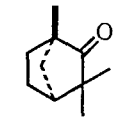
cyclooctanol(**6**)



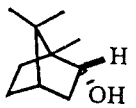
l-menthol(**7**)



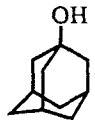
d(+)-fenchone (**8**)



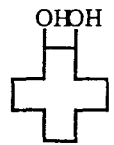
l(-)-fenchone(**9**)



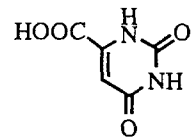
l-borneol(**10**)



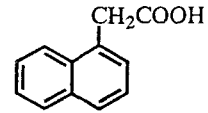
1-adamantanol (**11**)



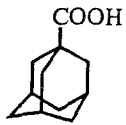
cis-1,2-cyclododecanediol (**12**)



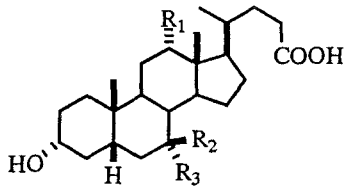
orotic acid (**13**)



1-naphtahleneacetic acid (**14**)



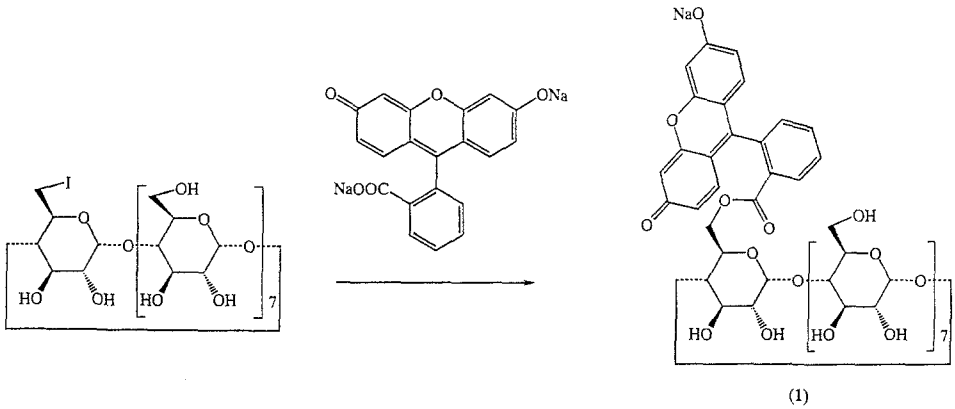
1-adamantanecarboxylic acid(**15**)



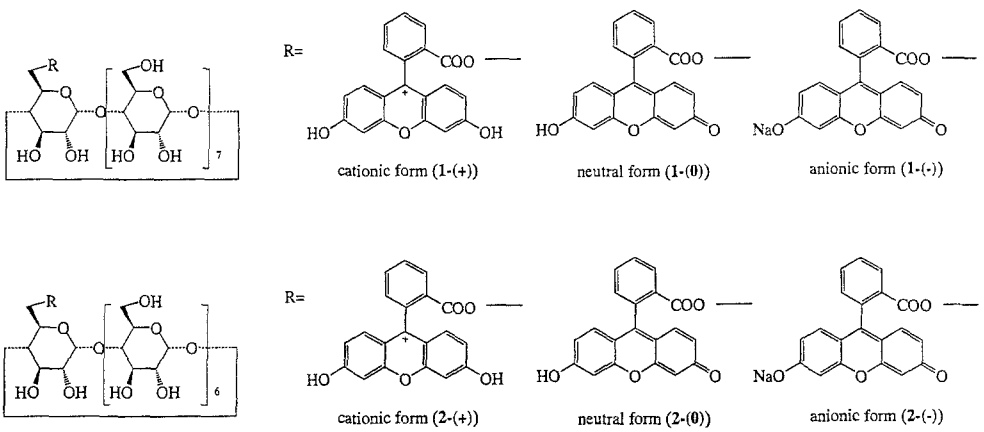
cholic acid (**16**) : R^1 = OH, R^2 = H, R^3 =OH

lithocholic acid (**17**) : R^1 = H, R^2 = H, R^3 = H

ursodeoxycholic acid (**18**) : R^1 = H, R^2 = OH, R^3 = H



Scheme 1. Preparation of **1** from iodo-γ-cyclodextrin.



Scheme 2. Molecular forms of **1** and **2**.

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. Hydrochloric acid aqueous solution of pH 1.40, 0.50 M phosphate buffer adjusted at pH 4.0, distilled water (pH 6.02) and 0.50 M carbonate buffer of pH 9.47 were used to make samples of cationic, neutral, and anionic forms of **1**, respectively.

3. Results and Discussion

Compound **1** which was prepared from an iodo-γ-cyclodextrin by treating it with fluorescein sodium salt, was isolated as the sodium phenoxide derivative as shown in Scheme 1. We also isolated a mixture of phenoxide and phenol derivatives

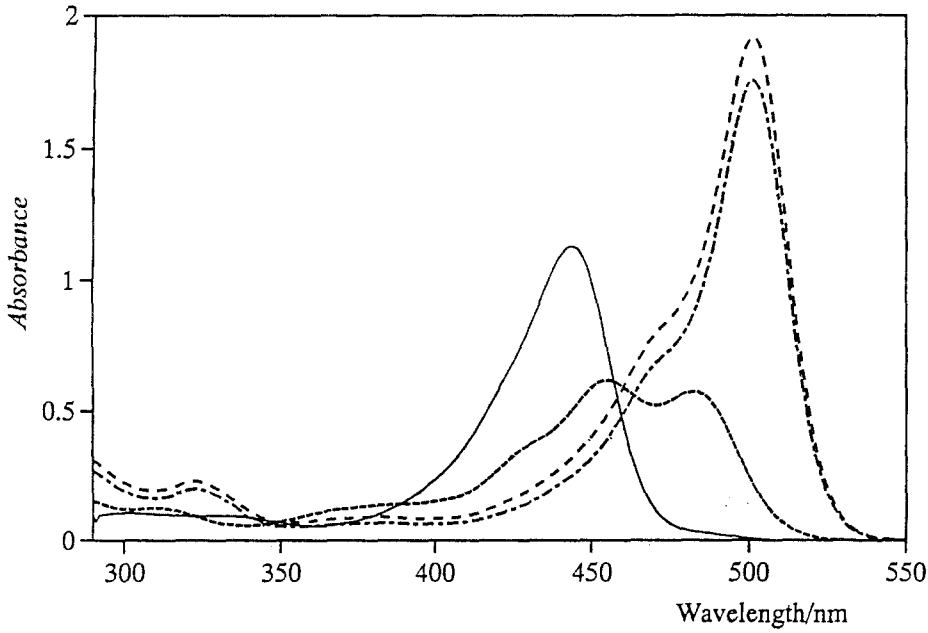


Figure 1. Absorption spectra of **1** (0.3×10^{-4} M, 25 °C) measured at pH 1.40 (——), 4.0 (- - - - -), 6.02 (— — —) and 9.47 (— - — - —).

Table I. Binding constants (K) of **1**-(−) at pH 6.02 and **1**-(+) at pH 1.40 in aqueous solution at 25 °C.

Guest	K/(mol^{-1} dm^{-3})	
	1-(−)	**1**-(+)
Nerol (**3**)	–	2 100 ± 300
Orotic acid (**13**)	2 800 ± 500	–
1-Napthalene acetic acid (**14**)	11 000 ± 1 900	1 100 ± 90
1-Adamantane carboxylic acid (**15**)	27 400 ± 1 500	13 900 ± 2 200
Cholic acid (**16**)	7 700 ± 400	–
Lithocholic acid (**17**)	24 000 ± 3 900	–
Ursodeoxycholic acid (**18**)	45 500 ± 1 500	122 100 ± 1 400

of **1** as a minor product. The mixture was difficult to separate. Compound **1**, as illustrated in Scheme 2, also has cationic, neutral and anionic forms depending on the pH of the solution, which are indicated as **1**-(+), **1**-(0) and 1-(−), respectively. The absorption spectra of the three different molecular forms of **1** are shown in Figure 1. They are similar to those of fluorescein except for a slight red shift. Compound **1** exists as the anionic form at around neutral pH, and is converted to cationic and neutral forms in strong acidic (pH 1.40) and weak acidic solution (pH 4.0), respectively. Compound **1** also exists as the anionic form at alkaline pH. The fluorescence spectra of **1** at different pH values are shown in Figure 2. The excitation

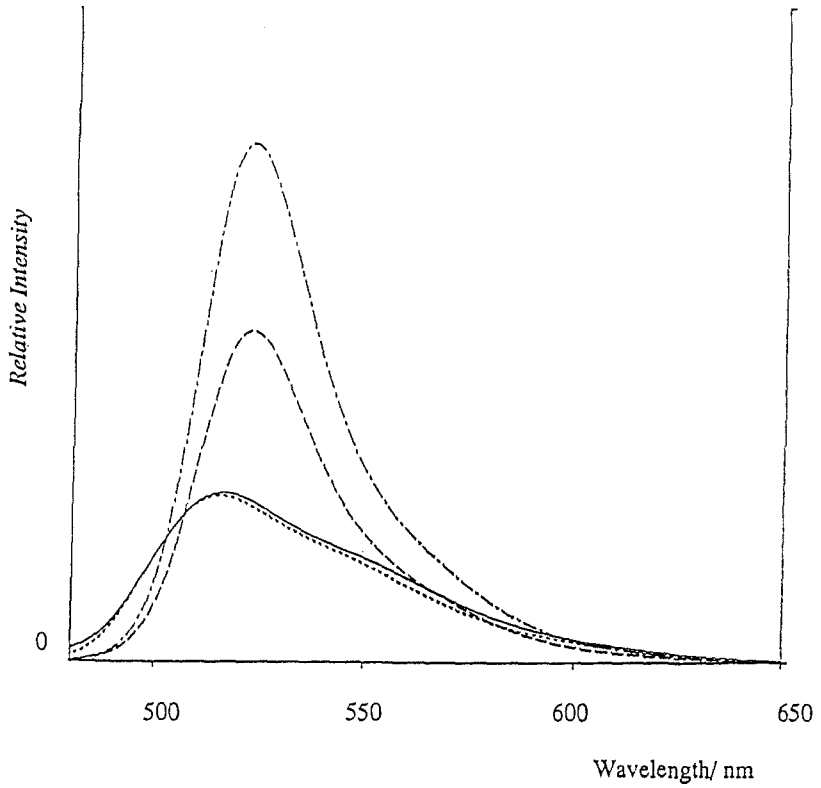


Figure 2. Fluorescence spectra of **1** (1×10^{-6} M, 25 °C) measured at pH 1.40 (——), 4.0 (- - - -), 6.02 (— — —) and 9.47 (— - — - —).

wavelengths are 450, 460 and 470 nm for the cationic, neutral and anionic forms, respectively. All three molecular forms of **1** change their fluorescence spectra upon addition of guest. The fluorescence spectra of **1** in an aqueous solution at pH 6.02, with and without adamantanecarboxylic acid, are shown in Figure 3. The spectrum of **1**, alone, exhibits a fluorescence peak at 521 nm, the fluorescence intensity of which decreases with increasing adamantanecarboxylic acid concentration. The guest-induced fluorescence change suggests a movement of the appended moiety into a different polarity environment [7]. Upon the binding of a guest to a host site, a signal associated with the probe may change in response to direct interaction of the host molecule. To clarify the behavior of the host, induced circular dichroism (ICD) spectra of **1** were recorded in an aqueous solution at pH 6.02, because an increase of ICD spectra is ascribed to the formation of a complex between achiral fluorescein and a chiral cyclodextrin [8]. The ICD spectra of **1** show a positive band at around 490 nm and a negative band at around 335 nm, as shown in Figure 4. When adamantanecarboxylic acid was added as a guest, a guest-induced decrease of the ICD bands in the 490 and 335 nm regions was observed. This suggests that the fluorescein moiety moved from the interior of the hydrophobic cyclodextrin

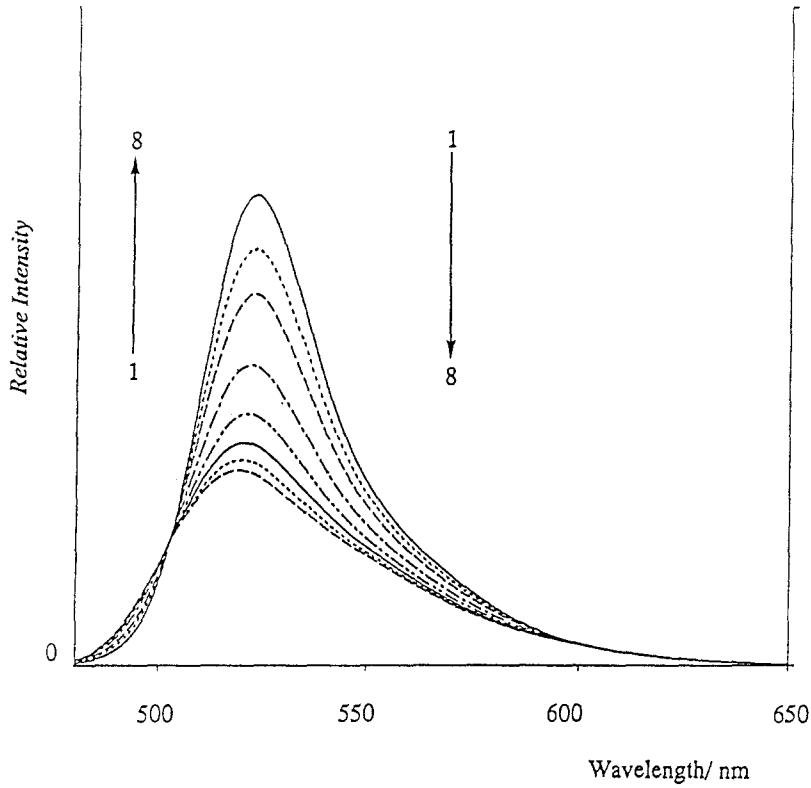


Figure 3. Fluorescence spectra of **1** (1×10^{-6} M, 25 °C) measured at pH 6.02 with various concentrations of adamantanecarboxylic acid: (1) 0; (2) 4×10^{-6}; (3) 1.2×10^{-5}; (4) 2.4×10^{-5}; (5) 4×10^{-5}; (6) 6×10^{-5}; (7) 8.4×10^{-5}; (8) 1.12×10^{-4} M.

cavity toward the outside bulk water environment while simultaneously a guest is included in the cyclodextrin cavity [8]. This binding mechanism could never happen in the case of a fluorescein capped β-cyclodextrin (**2**), because the cavity size is too small to include the fluorescein moiety. The extent of variation of the fluorescence intensity of **1**, in all three forms obtained with a guest is different when a different guest is used, even at the same concentration. This indicates that **1** can be used as a molecular sensor because of its sensitivity and selectivity for guests. To calculate the sensing ability of **1** in the three different forms, the $\Delta I/I^0$ value was used as the sensitivity factor [9]. Here ΔI is $I^0 - I$, where I^0 is the fluorescence intensity at each fluorescence peak of the three different forms for the host alone, and I is for a mixture of host and guest. The $\Delta I/I^0$ values of **1** in the cationic, neutral, and the anionic forms at pH 6.02 and 9.47 obtained with 16 guests are shown in Figures 5 and 6, respectively. Terpenoids and carboxylic acids were added at 1.0 mM (M=mol dm^{-3}), except for adamantanecarboxylic acid, which was examined at 0.1 mM because 1.0 mM of adamantanecarboxylic acid is not soluble in pure water.

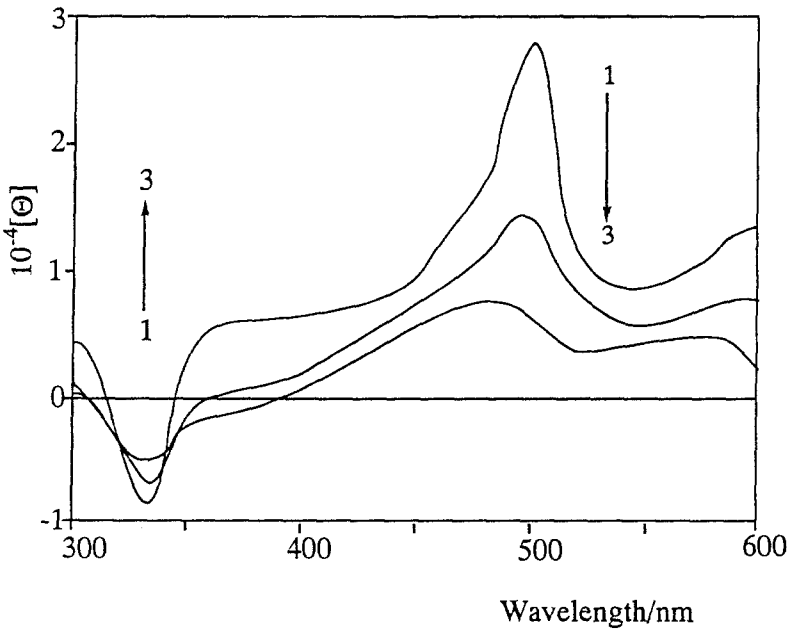


Figure 4. ICD spectra of **1** (1×10^{-5} M, 25 °C) measured at pH 6.02 with various concentrations of adamantanecarboxylic acid: (1) 0; (2) 10^{-4}; (3) 2×10^{-4} M.

Because of the poor solubility in pure water, bile acids were examined at 0.01 mM. The $\Delta I/I^0$ values obtained from **1**-(+) and **1**-(0) range from −0.04 to 0.39 and −0.06 to 0.26, respectively. The cationic form can detect nerol, 1-naphthaleneacetic acid and ursodeoxycholic acid with the highest sensitivity followed by *d,l*-fenchone and 1-adamantanol. On the other hand, the neutral form can only recognize *cis*-1,2-cyclododecanediol. The sensitivity factors obtained from **1**-(−) at pH 6.02 and 9.47 are absolutely different, and range from 0.02 to 0.73 and −0.06 to 0.05, respectively. It is evident that **1**-(−) at around neutral pH can detect acidic guests, such as orotic acid, 1-naphthaleneacetic acid and 1-adamantanecarboxylic acid with very high selectivity and sensitivity and also bile acids such as lithocholic acid and ursodeoxycholic acid even at 0.01 mM concentration. On the other hand, **1**-(−) at pH 6.02 can hardly detect the corresponding sodium carboxylate such as sodium 1-naphthylacetate. The highest binding ability of **1**-(−) at pH 6.02 could be ascribable to the effect of hydrogen bonding between the phenoxide moiety of **1** and the carboxylic acid, which is probably leading an acidic guest into the cyclodextrin cavity, as shown in Scheme 3. However, **1**-(−) in alkaline solution hardly detects any of these guest molecules. This suggests that there is no hydrogen bond between a host and a guest because an acidic guest probably exists as the corresponding anion in carbonate buffer at pH 9.47. Cholic acid was detected at lower sensitivity by **1**-(−) at neutral pH, which is probably due to its higher polarity than those of

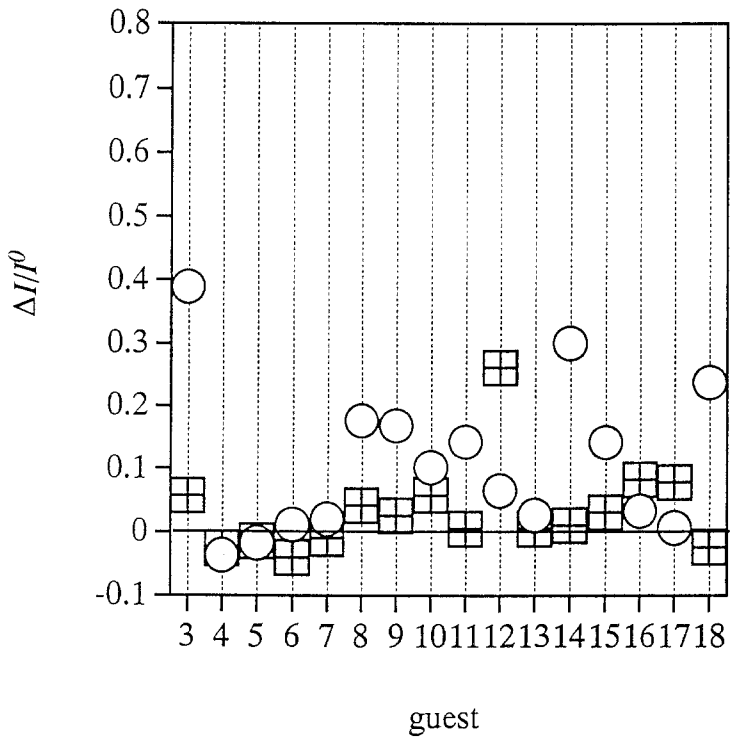


Figure 5. The sensitivity factors of **1**-(+) at pH 1.40 (○) and **1**-(0) at pH 4.0 (⊞).

ursodeoxycholic acid and lithocholic acid, because cholic acid bears one and two additional hydroxy groups in its structure compared to ursodeoxycholic acid and lithocholic acid, respectively. Ueno and co-workers reported the binding constants for a few guests by a fluorescein modified β-cyclodextrin (**2**) in three different forms [6]. Compound **2** in the cationic form displays a higher sensing ability than in its neutral and anionic forms, which is the same tendency as shown by **1** except in the case of the anionic form at neutral pH. Although, **1**-(−) in alkaline pH shows little sensitivity for guests, **2**-(−) at pH 9.3 detects 1-adamantanol, 1-adamantanecarboxylic and ursodeoxycholic acid with relatively high sensitivity. It seems inconceivable that **1** interacts less with ursodeoxycholic acid than **2** because γ-cyclodextrin easily forms a host–guest complex with bigger guest molecules such as bile acids compared with β-cyclodextrin. There is probably something different in the binding mechanism to cause the different binding ability. The movement of the appended moiety upon guest addition is different. As mentioned before, the appended moiety of **2** simply comes closer to the cavity, but for **1**, there are two possibilities: the appended moiety moves out of the cavity or it moves more deeply into the cavity. Consequently, the cavity size and the movement of the appended moiety should effect the binding ability of **1** and **2**. To determine

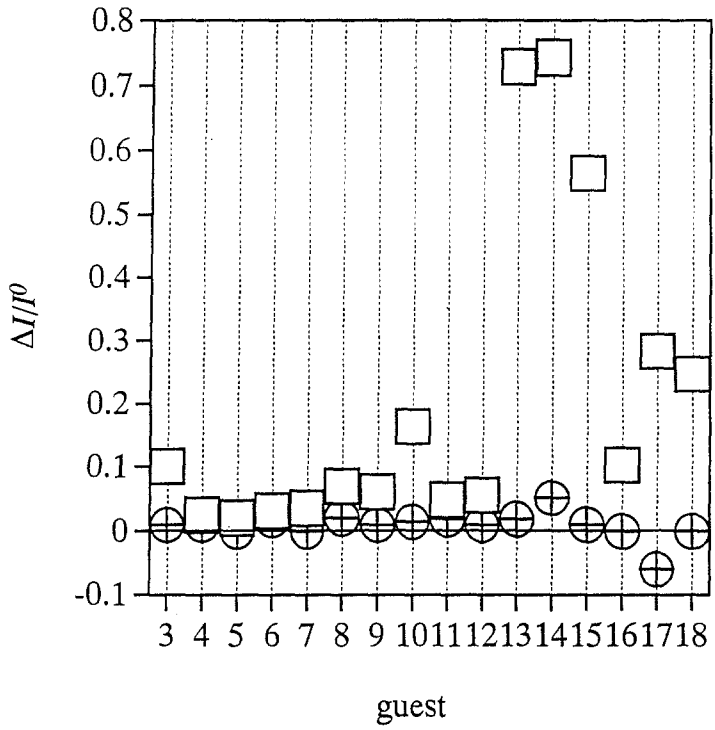
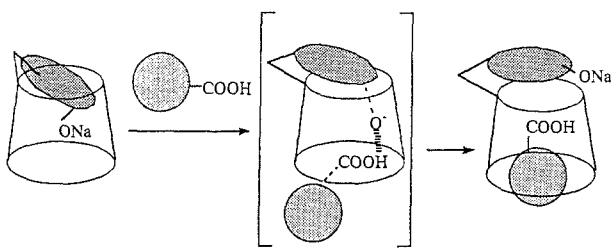
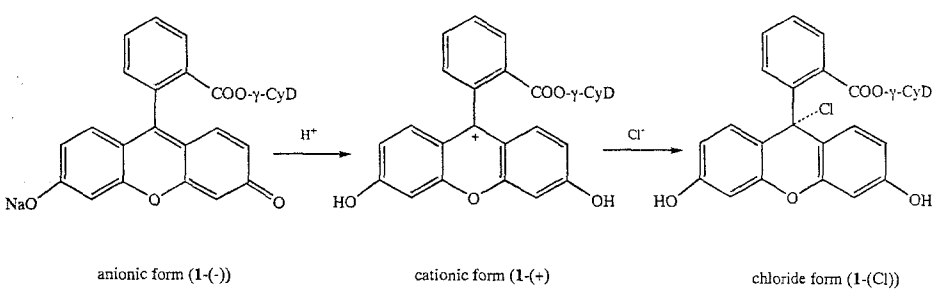


Figure 6. The sensitivity factors of **1**-(−) at pH 6.02 (□) and **1**-(−) at pH 9.42 (⊕).



Scheme 3. Host-guest complexation of an acidic guest with the anionic form of **1** via hydrogen bonding.



Scheme 4. Preparation of **1**-(Cl) from **1**-(−) via **1**-(+).

the accurate sensitivities of **1**-(−) at pH 6.02 for carboxylic and bile acids, the binding constants were obtained by the analysis of the guest-induced fluorescence variations using Equation (1) [5].

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

Here I is the fluorescence intensity at 470 nm (I_f for the complex, I_{f0} for the host alone), $[\mathrm{CD}]_0$ is the total host concentration, $[\mathrm{G}]_0$ is the total guest concentration, a is a constant. The binding constants of **1**-(−) at pH 6.02 for these guests are listed in Table I. Because of relatively high sensing factors of **1**-(+) at pH 1.40 for nerol, 1-naphthalene acetic acid and ursodeoxycholic acid, the binding constants for these guests were also obtained. In the **1**-(+) system, the cationic appended moiety should act as a much reduced hydrophobic cap compared to the neutral residue in the host–guest complexation system. Hydrophobic caps or floors were reported to promote the guest-binding ability of cyclodextrin by enlarging the hydrophobic environment around the cyclodextrin cavity [10]. However, contrary to our expectation, **1**-(+) at pH 1.40 exhibits higher sensitivity than **1**-(0) at pH 4.0, which suggests that the appended moiety of **1**-(+) is probably altered to another structure such as the chloride derivative (**1**-(Cl)) as depicted in Scheme 4, because **1**-(+) seems to be unstable in strong acidic conditions. Compound **1**-(Cl) is much more hydrophobic than **1**-(+). The configuration of the appended moiety of **1**-(Cl) is different from those of **1**-(+), **1**-(0), and **1**-(−), which are flat. That also should affect the sensitivity of those hosts for guest molecules.

4. Conclusion

The properties of cyclodextrins caused by their cavity structure caused rather unspecific formation of inclusion complexes with various guest molecules including sixteen compound mentions here. γ-Cyclodextrins possess the largest cavity, compared to α- and β-cyclodextrin, and can accommodate even bulky molecules, such as compound **13**. The hydrogen bond between negatively charged **1**-(−) at pH = 6.02 and various acids (**13**, **14**, **15**) plays an important role in forming these inclusion complexes.

References

1. M. Miki and C.G. dos Remedios: *J. Biochem.* **104**, 232 (1988).
2. M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Spring Verlag, New York (1978).
3. R. Markuszewski and H. Diehl: *Talanta* **27**, 937 (1980).
4. A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai: *J. Am. Chem. Soc.* **110**, 4323 (1988).
5. F. Hamada, K. Ishikawa, R. Ito, H. S. Hamai, I. Suzuki, T. Osa, and A. Ueno: *J. Incl. Phenom.* **20**, 43 (1995).
6. Y. Wang, T. Ikeda, A. Ueno, and F. Toda; *Tetrahedron Lett.* **34**, 4971 (1993).
7. D. G. Chung and P.N. Lewis: *Biochemistry* **25**, 5036 (1986).
8. A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai: *Bull. Chem. Soc. Jpn.* **59**, 465 (1986).

9. A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada, and K. Murai: *Chem. Lett.* 605 (1990).
10. F. Hamada, M. Fukushima, T. Osa, and A. Ueno: *Macromol. Chem., Rapid Commun.* **14**, 279 (1993).