Phenolphthalein-Modified β-Cyclodextrin as a Molecule-Responsive Colorless-to-Color Change Indicator

Tetsuo Kuwabara,*,† Makoto Takamura,‡ Akiko Matsushita,‡ Hiroshi Ikeda,‡ Asao Nakamura,‡ Akihiko Ueno,*,‡ and Fujio Toda§

Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi University, 4 Takeda, Kofu 400-8511, Japan, Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan, and Tokyo Polytechnic College, 2-32-1 Ogawa-nishi-machi, Kodaira, Tokyo 187-0035, Japan

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Phenolphthalein-modified β-CD, **1**, was synthesized for the purpose of developing a new type of guest-responsive color change indicator. The pH titration curve of **1** depends on its concentration, suggesting that **1** exists not only as an self-inclusion form but also as an association form at a concentration of 10^{-4} M in neutral aqueous solution. At pH 11.0, the association species dissociates into the monomer one, taking a dianion form in the phenolphthalein part. Upon the guest addition at pH 9.70, **1** exhibits the color change from colorless to purple at its concentration of 5.0×10^{-6} M due to the 1:1 host−guest complex formation. The guest-induced absorption changes were used for molecule sensing. The sensing abilities of **1** for various guests are roughly parallel to the binding constants.

Introduction

Cyclodextrins (CDs), which are cyclic oligosaccharides composed of D-(+)-glucopyranose units with α-1,4-glycosidic linkage, can form inclusion complexes with a variety of organic compounds in aqueous solution.[1] The complexation phenomenon often involves remarkable variations in photophysical and photochemical properties of guest molecules because of the environmental difference between the CD interior and aqueous medium. On this basis, the spectra of chromogenic guest molecules are usually perturbed in the presence of CDs.

One typical example for this type of the spectral change was reported for phenolphthalein.[2,3] Phenolphthalein is known as a pH indicator that changes color from colorless to purple when the neutral solution becomes alkaline one. However, phenolphthalein included in the β-CD cavity exhibits no absorbance even at pH 10.5 in the visible region. This was explained in terms of the constrained conformation of phenolphthalein in the β-CD cavity. Methyl orange also changes its color from red to yellow by complexation with α-CD in acidic medium due to the shift in the acid dissociation equilibrium.[3−5] In the case of the fluorophores such as naphthalene[6,7] and pyrene[8] as guests, the changes in their fluorescent spectra were observed upon complexation. Recently, Ueno et al. have shown that some fluorescently-modified CDs exhibit variations in fluorescence intensity on binding organic molecules and constructed the CD-based fluorescent sensors.[9−14] These systems are based on the guest-induced locational changes of the fluorophore, mostly from inside to outside of the CD cavity. When a dye was used as the substituent in place of the fluorophore, the binding of the guest species is transduced into the color change signals based on the same principle. Recently, we reported that modified CDs bearing methyl red[15,16] or *p*-nitrophenol[17,18] as a dye unit change colors or absorption intensities on guest binding under the acidic or neutral conditions.

To develop another type of CD-based sensor that works in alkaline solutions, we have designed a novel color-changeable CD (**1**), in which the phenolphthalein unit is attached to a primary side of CD (Chart 1). Here, we report that **1** exhibits absorption changes from colorless to purple upon guest binding in alkaline solutions and **1** is useful as a sensor for detecting organic molecules by color change.

Results and Discussion

Synthesis. The phenolphthalein unit was linked with β-CD through the amide bond, which was expected to be

* To whom correspondence should be addressed. Tel.: +81-552-20-8548. Fax: +81-552-20-8772. E-mail: kuwabara@ab11.yamanashi.ac.jp.
† Yamanashi University.
‡ Tokyo Institute of Technology.
§ Tokyo Polytechnic College.

(1) *Comprehensive Supramolecular Chemistry*; Szejtli, J., Osa, T., Eds.; Pergamon: Oxford, 1996; Vol. 3.
(2) Buvári, Á.; Barcza, L.; Kajtár, M. *J. Chem. Soc., Perkin Trans. 2* **1988**, 1687.
(3) Yoshida, N.; Shirai, T.; Fujimoto, S. *Carbohydr. Res.* **1989**, *192*, 291.
(4) Buvári, Á.; Barcza, L. *J. Inclusion Phenom.* **1989**, *7*, 313.
(5) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kindo: Budapest, 1982.
(6) Hamai, S. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2721.
(7) Hamai, S. *J. Phys. Chem.* **1990**, *94*, 2595.
(8) Hamai, S. *J. Phys. Chem.* **1989**, *93*, 6527.
(9) Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno, A. *J. Am. Chem. Soc.* **1996**, *118*, 10980.
(10) Wang, Y.; Ikeda, T.; Ikeda, H.; Ueno, A.; Toda, F. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1598.
(11) Hamasaki, K.; Ueno, A.; Toda, F.; Suzuki, I.; Osa, T. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 516.
(12) Hamasaki, K.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, F.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1993**, *115*, 5039.
(13) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Chem. Lett.* **1992**, 863.
(14) Ueno, A.; Minato, S.; Osa, T. *Anal. Chem.* **1992**, *64*, 1154.
(15) Ueno, A.; Kuwabara, T.; Nakamura, A.; Toda, F. *Nature* **1992**, *356*, 136.
(16) Kuwabara, T.; Nakamura, A.; Ueno, A.; Toda, F. *J. Phys. Chem.* **1994**, *98*, 6297.
(17) Kuwabara T.; Matsushita, A.; Nakamura, A.; Ueno, A.; Toda, F. *Chem. Lett.* **1993**, 2081.
(18) Matsushita, A.; Kuwabara, T.; Nakamura, A.; Ikeda, H.; Ueno, A. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1705.

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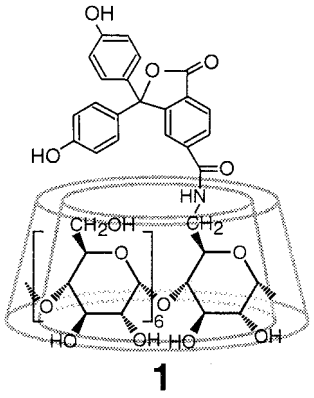
Chart 1. Structure of Phenolphthalein-Modified *β*-CD, 1



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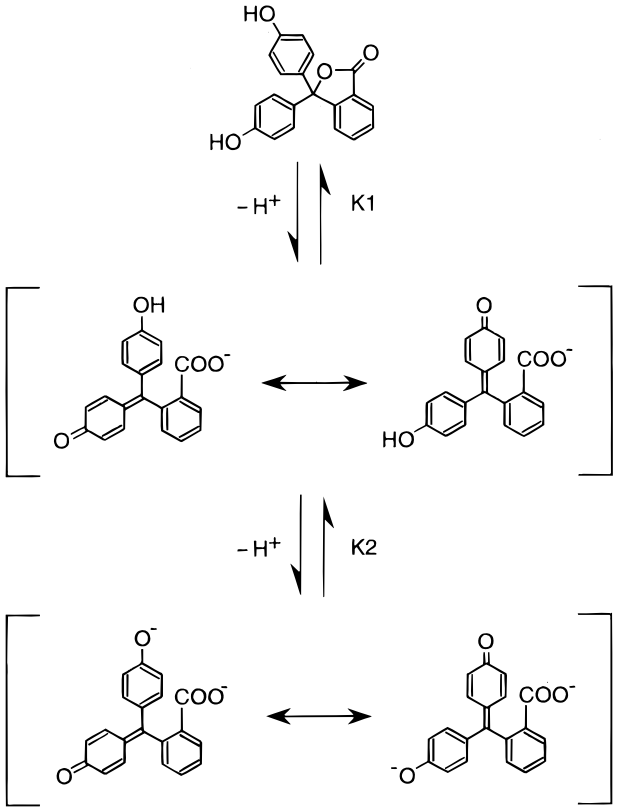
Scheme 1. Schematic Representation for Acid Dissociation Equilibria of Phenolphthalein



stable in the alkaline condition as compared with the ester one. The amide bond formation reaction was performed in dimethylacetamide by using dicyclohexylcarbodiimide (DCC) as a condensation reagent. Before this reaction, we checked the possibility of the reactivity of the lactonoid group in phenolphthalein with 6-deoxy-6-amino-*β*-CD and found that this reaction gave no product. The desired product was purified by ion-exchange chromatography on a QAE-Sephadex column and HPLC with an ODS column and characterized by TLC, ^{1}H NMR spectra, and elemental analysis.

Absorption Spectra of 1. The color change of phenolphthalein involves the structural change as shown in Scheme 1.[2] The colorless lactonoid form existing under acidic or neutral conditions dissociates two protons to form a purple dianion form when the solution becomes alkaline. The resonance between two phenol units is achieved in the phenolphthalein dianion form with one

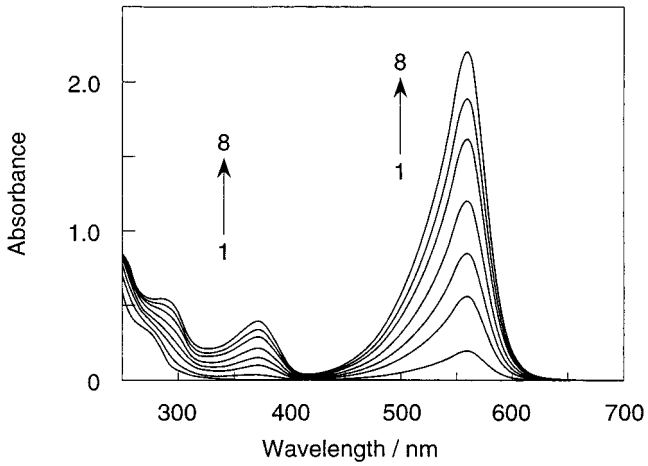


Figure 1. Absorption spectra of **1** (1.0×10^{-4} M) at different pH values. pH of the solution: (1) 8.51, (2) 9.53, (3) 9.82, (4) 9.96, (5) 10.10 (6) 10.29, (7) 10.46, (8) 11.22.

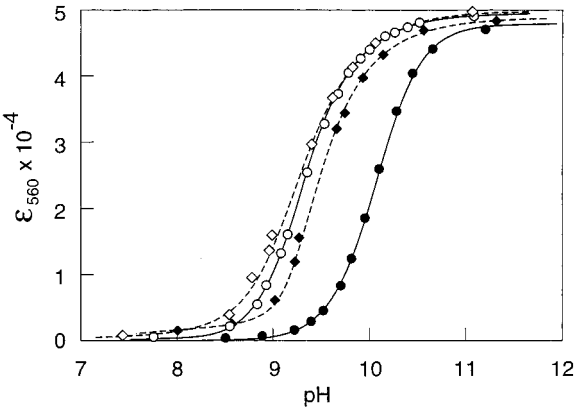


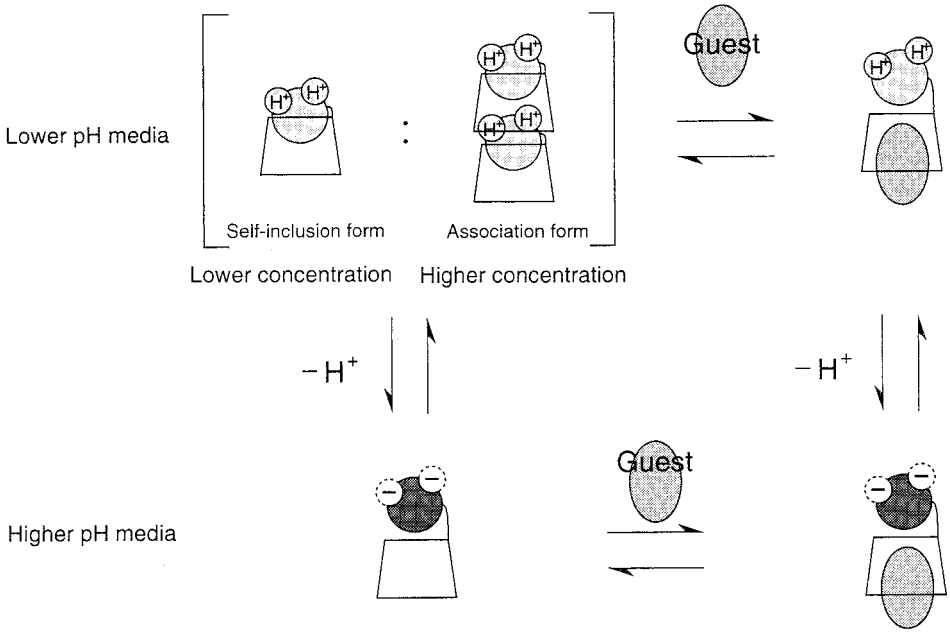
Figure 2. Plot of ϵ_{560} of **1** as a function of pH in the absence and the presence of 1-adamantanol (6.0×10^{-4} M): 1.0 μM solution of **1** in the absence (−◆−) and the presence (−◇−) of 1-adamantanol; 0.1 mM solution of **1** in the absence (−●−) and the presence (−○−) of 1-adamantanol.

of phenol units existing as a phenolate anion and the other as a quinoidal form.

Figure 1 shows the effect of pH on the absorption spectra of **1** (1.0×10^{-4} M). A strong peak at 560 nm at pH 11.32 indicates that the phenolphthalein unit of **1** exists as the dianion form. This peak decreased with lowering pH of the solution and disappeared at pH 8.02, indicating the conversion of the phenolphthalein unit into the lactonoid form from the dianion one. Since **1** exhibits the similar pH-induced spectral variation to that of phenolphthalein itself, the acid dissociation equilibrium behavior of **1** is attributed to that of the phenolphthalein unit, which is exposed in the bulk water.

Similar pH-induced absorption variations were observed when the concentration of **1** was lowered (1.0×10^{-6} M). It was found, however, that the pH titration curve of **1** was remarkably affected by the concentration of **1**. Figure 2 shows the plot of molecular extinction coefficients of **1** at 560 nm, ϵ_{560}, as a function of pH under various conditions. In the solution of the higher concentration of **1** (1.0×10^{-4} M), the pH titration curve of **1** was shifted toward the alkaline side as compared with that in the lower concentration (1.0×10^{-6} M), suggesting the existence of the association form of **1** in the solution of the higher concentration of **1**. The phenolphthalein

Scheme 2. Schematic Representation for Conformational and Acid Disociation Equilibria of 1 and Guest Binding



unit of **1** may be included in another CD cavity of **1** in the solution of higher concentration (1.0×10^{-4} M). However, the fact that there is no difference in the molecular extinction coefficients of **1** at pH 11.00 between the higher and lower concentration solutions indicates that **1** exists as a monomer form in both solutions with the phenolphthalein unit assuming the dianion form.

In the presence of the excess of 1-adamantanol as a guest, however, no concentration dependency in the pH titration curve was observed, indicating that the intermoleculer interaction of **1** is canceled by accommodation of the guest molecule into the CD cavity. Since the interaction between **1** and guest molecule is stronger than the intermolecular interaction of **1**, the cavities of all hosts are occupied by the guest molecule and cannot interact with the phenolphthalein unit, which is exposed to the bulk water solution in this condition. On the other hand, the shift of the pH titration curve toward the acidic side by the guest addition observed even in a highly diluted concentration of **1** (1×10^{-6} M) suggests the existence of the self-inclusion form, in which the phenolphthalein unit of **1** is included in its CD cavity. However, in the self-inclusion form, the phenolphthalein unit is not deeply included in the CD cavity as indicated by a slight shift of the curve occurring upon the guest addition. The shift may be interpreted by the facilitated dissociation of the hydroxyl proton of phenolphthalein unit by the guest-induced exposure of the unit to the solution.

The shifts of the pH titration curves are reflected in the shifts of the apparent pK_a values of **1**. The pK_a values estimated by fitting of the absorbance at 560 nm as a function of pH value using the equation reported by Yoshida et al. are summarized in Table 1.[3] In the absence of the guest, the pK_a2 values are hardly affected by the concentration of **1**, showing 9.05 (1.0×10^{-6} M) and 8.93 (1.0×10^{-4} M). It is obvious, however, that the pK_a1 values are strongly affected by the concentration of **1**. The pK_a1 estimated here for the lower concentration of **1** is 9.75, while that for higher one is 11.16. Such tremendously high pK_a1 value in the higher concentration of **1** indicates the difficulty of the first proton dissociation due to the formation of the association form of **1**.

Table 1. pK_a Values of the Acid Dissociation Equilibria of 1 and Phenolphthalein

compd	concn (M)	[1-adamantanol] (M)	pK_a1	pK_a2
1	1.0×10^{-6}	0	9.75	9.05
		6.0×10^{-4}	8.84	9.13
	1.0×10^{-4}	0	11.16	8.93
		6.0×10^{-4}	9.51	8.97
phenolphthalein	6.0×10^{-5}		9.22	9.51

The guest addition to the solution of **1** causes the shift in pK_a1 toward the acidic side from 9.75 to 8.84 in the lower concentration of **1** and from 11.16 to 9.51 in the higher concentration case. These results suggest that the dissociation of the proton occurring upon guest addition is facilitated in both cases of the self-inclusion form and the association one. Phenolphthalein shows 9.22 and 9.51 for pK_a1 and pK_a2, respectively.[3] Similar results were obtained in the case of the lower concentration of **1** in the presence of the guest, suggesting that the phenolphthalein unit is mostly exposed in the bulk water in the intermolecular (host−guest) complex of **1**. On the contrary, the solution of higher concentration (1.0×10^{-4} M) of **1** in the presence of guest gives larger value for pK_a1 than for pK_a2. The result may be due to the fact that **1** exists not solely as the host−guest complex and there still remains the self-inclusion form of **1**.

Such structural feature was summarized in Scheme 2. In the absence of the guest at pH 11.00, **1** exists as a dianion monomer. Under the conditions of high concentration of **1** at lower pH, the intermolecular interaction of plural molecules of **1** becomes stronger. However, it is not clear how many host molecules associate in this system. Taking account of the moderately high concentration (1.0×10^{-4}) employed in this system, **1** may exist predominantly as a dimer form. In the presence of the guest, **1** accommodates the guest molecule in its CD cavity, forming the host−guest inclusion complex, and the phenolphthalein unit is exposed to bulk water.

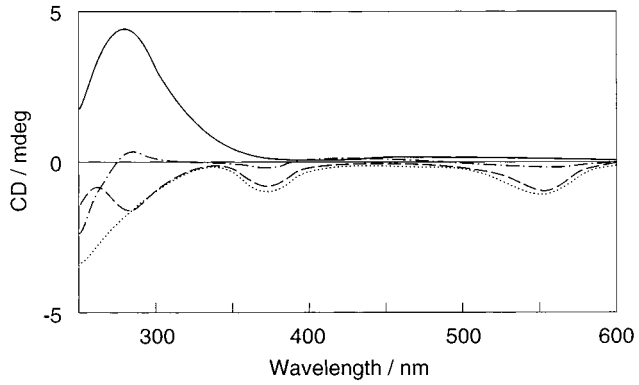


Figure 3. Induced circular dichroism spectra of **1** (1.0×10^{-5} M) alone at pH 7.24 (—) and at pH 11.45 (– –) and in the presence of 1-adamantanol (3.0×10^{-4} M) at pH 7.42 (–•–) and at 11.61 (•••).

Induced Circular Dichroism of 1. Since CD consists of the chiral D-glucose, chromophore-modified CD may exhibit induced circular dichroism in the wavelength region of the electronic transitions.[19−21]

Figure 3 shows the induced circular dichroism spectra of **1** under various conditions. In the neutral solution, **1** exhibits the positive dichroism band at 290 nm, associated with the $\pi-\pi^*$ transition of a phenol unit in the phenolphthalein of **1**. This indicates that **1** includes a phenol unit of the phenolphthalein unit in the CD cavity with an orientation parallel to the CD axisis. This positive dichroism band disappeared upon the guest addition, suggesting that the phenol unit included in the CD cavity was excluded to outside of the cavity. This observation is in good agreement with the results obtained by absorption measurements.

In an alkaline solution of **1** at pH 11.45, two weak negative dichroism bands at 560 and 375 nm were observed, indicating that the phenolphthalein unit existing as a dianion form interacts with CD cavity so as to cap the primary hydroxyl group side of **1**. This implies that the chromophore part is not included deeply in the CD cavity of **1**. Upon addition of the guest, the changes in dichroism spectrum are small, suggesting no remarkable conformational change of **1** occurs by guest accommodation. This result is also in good agreement with the result obtained by pH titration experiment.

Because no concentration dependency in the dichroism spectrum of **1** was observed in neutral condition as well as in the alkaline one, the basic features for interaction between phenolphthalein unit and CD cavity may be similar in monomer and associated forms of **1**.

^{1}H NMR Spectra of 1. The interaction between the phenolphthalein unit and the CD unit in **1** was clearly observed by using the visible spectrophotometric or the spectropolarimetric techniques. We also studied the conformation of **1** in the presence and the absence of the guest in aqueous solution by ^{1}H NMR spectroscopy in order to gain a better comprehension about this interaction.

Figure 4 shows the 1D and 2D ^{1}H NMR spectra of **1** in D_2O. Although the unmodified CDs show one set of ^{1}H resonances around 3.5−4.0 and 5.0 ppm in D_2O like a glucose monomer, each ascribing to H2−H6 and H1 protons of the glucopyranose units in CD, respectively, **1** shows the complex ^{1}H NMR spectrum with the remarkable shifted and splitted peaks of the CD unit. Four doublet peaks were observed in the upper-field in the range of 1.6−2.8 ppm. The resonances at 1.64 and 2.58 ppm were assigned to H6 protons connecting at the same C6 position by the ^{1}H−^{1}H-correlated spectroscopy (COSY) and the 2D total correlation spectroscopy (TOCSY) spectrum, and the resonances at 2.30 and 2.70 ppm were assigned to H6 protons connecting at the same C6 position of another glucose unit. Such shift of H6 protons to the upper-field indicates that the aromatic ring of the phenolphthalein unit faces to these H6 protons and that the phenolphthalein unit exists near the rim of the primary hydroxyl group, interacting to the CD cavity. The major conformation around the C5−C6 bond of the glucose units is the **gg** rotamer because both of the coupling constants $J_{5,6a}$ and $J_{5,6b}$ are less than 2 Hz.[22,23]

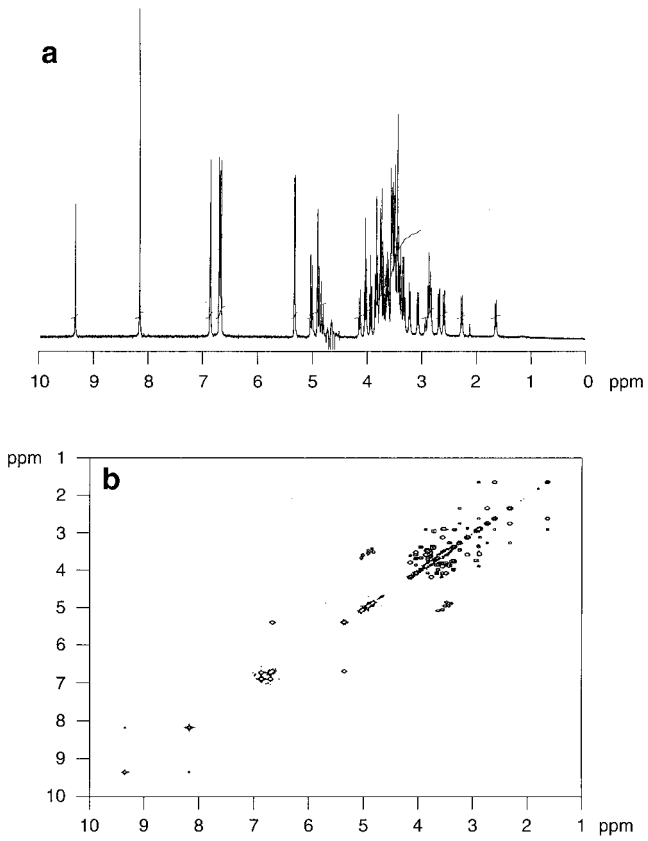


Figure 4. 1D (a) and 2D (b) ^{1}H NMR spectra of **1** in D_2O.

These upper-field-shifted resonances disappeared by the addition of 1-adamantanol as a guest or in the alkaline condition, suggesting the weaker interaction of the phenolphthalein unit with the CD cavity in the presence of the guest or in the alkaline condition. These ^{1}H NMR observations ensured the interaction between the CD cavity and the phenolphthalein unit and are consistent with the observation in the spectrophotometric or the spectropolarimetric studies.

Guest Binding and Sensor Ability of 1. At pH 9.70, the guest-induced hyperchromic effect of **1** (5.0×10^{-6}

(19) Harata, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375.
(20) Shimizu, H; Kaito, A.; Hatano, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2678.
(21) Kodaka, M.; Fukaya, T. *Bull. Chem. Soc. Jpn.* **1989** *62*, 1154.
(22) Streefkerk, D. G.; DE Bie, M. J. A.; Vliegenthart, J. F. G. *Tetrahedron* **1973**, *29*, 833.
(23) Ikeda, H.; Moon, H.-t.; Du, Y.-q.; Toda, F. *Supramol. Chem.* **1993** *1*, 337.

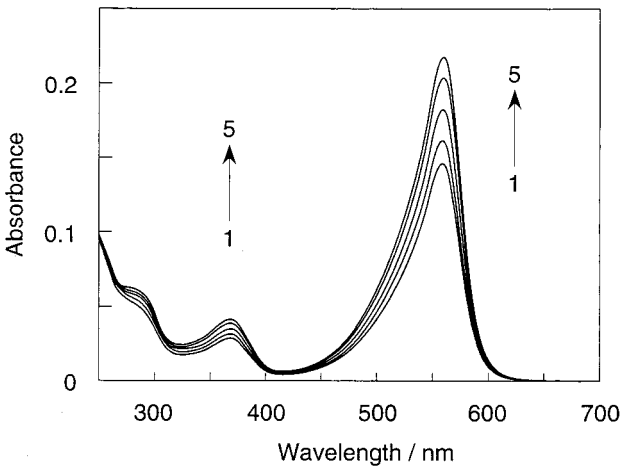


Figure 5. Absorption spectra of **1** (5.0×10^{-6} M) at pH 9.70 with various concentrations of 1-adamantanol. 1-Adamantanol: (1) 0, (2) 6.0×10^{-6}, (3) 1.2×10^{-5}, (4) 4.8×10^{-5}, (5) 9.6×10^{-5} M.

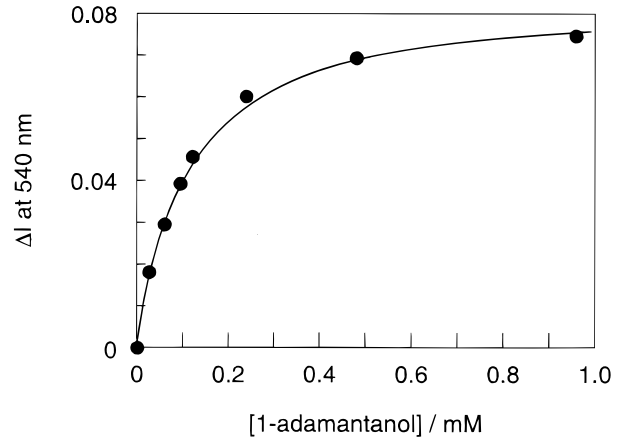


Figure 6. Curve-fitting data for the guest-induced absorption intensities of **1** at 540 nm as a function of 1-adamantanol concentration.

M) was observed as shown in Figure 5. The increase of the intensity around 560 nm with increasing concentration of 1-adamantanol is interpreted by the conversion of phenolphthalein unit of **1** from the lactonoid form to the dianion one, the deprotonation being due to the exclusion of the phenolphthalein unit from inside to outside of the cavity associated with the host−guest complex formation. Similar results were observed when the other guests were added to the solution of **1**.

We have investigated the molecule sensing ability of **1** and have determined binding constants for various guest compounds shown in Chart 2. The increase in the absorbance at 540 nm relative to its original value ($\Delta I/I°$) was used as a sensitivity parameter. The binding constant was estimated by a nonlinear square fitting analysis from the plot of the guest-induced absorbance change at 540 nm for **1** as a function of the guest concentration.

A typical example of the saturation curve for **1** and 1-adamantanol is shown in Figure 6. Similar fitting could be obtained for other guests, and the results are shown in Table 2. In Table 2, the binding constants of the parent β-CD with some guests and those of alizarin yellow-modified cyclodextrin (**13**)[24] were also shown for comparison. Among the steroidal compounds examined,

Table 2. Sensitivity Factor $\Delta I/I°$ and Binding Constants of 1 for Various Guest Compounds

	host		
	1		**13**
guest	$\Delta I/I°$	K/M^{-1}	K^b/M^{-1}
ursodeoxycholic acid (**2**)[a,d]	0.19	385000	93200
chenodeoxycholic acid (**3**)[a,d]	0.14	137000	9180
deoxycholic acid (**4**)[d]	0.026	16300	1360
1-adamantanecarboxylic acid (**5**)[a,d]	0.17	49800	9240
1-adamantanol (**6**)[d]	0.24	96100	8160
1-adamantanol (**6**)[e]	0.36		
d-fenchone (**7**)[e]	0.093	4470	*c*
l-fenchone (**8**)[e]	0.11	4430	*c*
d-camphor (**9**)[e]	0.087	6870	460
l-camphor (**10**)[e]	0.17	12300	393
d-menthol (**11**)[e]	0.48	22600	918
l-menthol (**12**)[e]	0.48	26700	1010

[a] Binding constants of β-CD: 32 000, 23 000, and 18 000 M^{-1} for **2**, **3**, and **5**.[25,26] [b] Binding constant of alizarin-yellow modified CD. [c] No data. [d] Guest concentration is 7.5×10^{-6} M. [e] Guest concentration is 7.5×10^{-5} M.

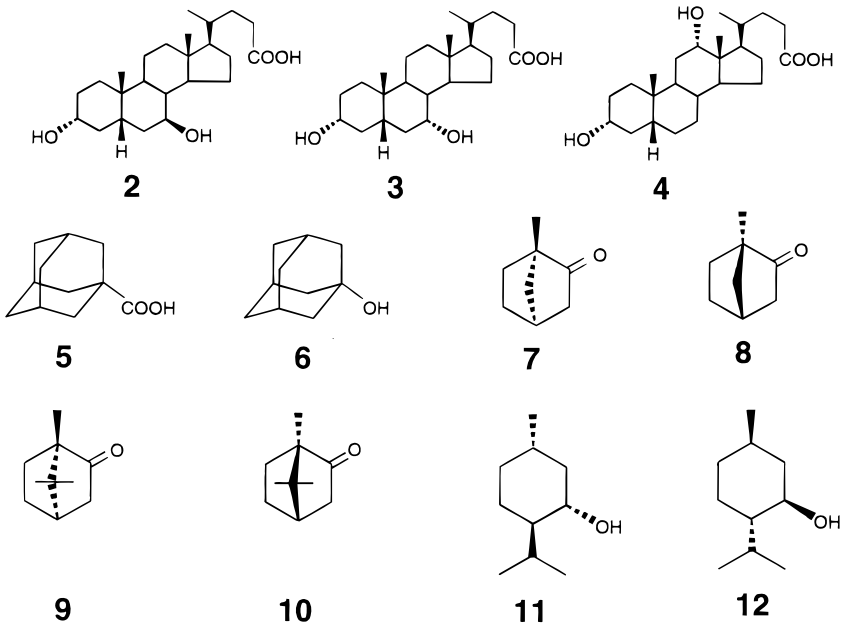
ursodeoxycholic acid (**2**) and chenodeoxycholic acid (**3**) were detected by **1** with higher sensitivity than deoxycholic acid (**4**), which is different from **2** and **3** only in the position of hydroxyl group. The order of the sensitivities of the three steroids is correlated with the binding constants with the values of 358 000, 137 000, and 16 000 M^{-1} for **2**, **3**, and **4**, respectively. Since the binding constants of the parent β-CD for **2** and **3** are 32 000 and 23 000 M^{-1}, respectively,[25] such larger binding constants of **1** for **2** and **3** suggest that **1** has an ability to bind the guest molecules strongly. This may be due to the phenolphthalein unit acting as a stabilizer for the complex formation of **1**. The similar corelation between the sensitivity and the binding constant was observed for adamantane compounds. The $\Delta I/I°$ value for 1-adamantanol (**6**) is larger than that for 1-adamantanecarboxylic acid (**5**), and the binding constant for **5** is about half the value for **6**. This may be due to the ionic nature that seems unfavorable for **5** to be included in a hydrophobic CD cavity. However the binding constant of **1** for **5** is larger than that of the parent β-CD (18 000 M^{-1} at pH 8.5[26]). This fact also indicates that the phenolphthalein unit plays an important role for guest binding of **1**.

Both *d* and *l* enantiomers of menthol, fenchone, and camphor were used for evaluating the sensor ability to the terpenoid compounds. Among these guests, *d*-menthol (**11**) and *l*-menthol (**12**), which are monocyclic compounds, were detected with the high sensitivity than the other bicyclic guests. Although similar $\Delta I/I°$ values for *d*-fenchone (**7**) and *d*-camphor (**9**) were observed, *l*-camphor (**10**) was detected with higher sensitivity than *l*-fenchone (**8**). Furthermore, a slightly favorable discrimination was observed for the *l*-enantiomer compared to the *d*-enantiomer for each enantiomer pair. For these guests also, the order of sensitivities is roughly correlated with the binding constants. The binding constants for **11** and **12** were more than 20 000, while those for **7** and **8** were ca. 4400. The binding constant for **10** is half the value for **9**.

All these binding constants of **1** for the guest molecules examined are more than 10 times higher than those of

(24) Aoyagi, T.; Nakamura, A.; Ikeda, H.; Ikeda, T.; Mihara, H.; Ueno, A. *Anal. Chem.* **1997**, *69*, 659.
(25) Tan, X.; Lindenbaum, S. *Int. J. Pharm.* **1991** *74*, 127.
(26) Eftink, M. R.; Andy, M. L.; Bystom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765.

Chart 2. Structure of the Guest Compounds



13 except for **2** and **5**, these binding constants of **1** being ca. 5 times higher than those of **13**. This implies that **1** is better host for detecting the guest species with high sensitivity. The order of binding constants is roughly parallel between host **1** and **13**. It was noted, however, that the binding constant of **13** for **5** is almost same as that of **6**, while that of **1** for **5** is almost half as that of **6**. This result indicates that **1** can be used for detecting the difference between the hydroxyl or the carboxyl group on the adamantane framework.

Conclusion

Phenolphthalein-modified β-CD, **1**, was prepared as a guest-responsive color change indicator. In the neutral medium in the higher concentration, **1** tends to form the association form as well as the self-inclusion form, causing the pK_a1 shift of the phenolphthalein unit of **1** toward the alkaline side. Upon the guest addition at pH 9.70, **1** was converted into 1:1 host−guest complexes accompanying color change from colorless to purple. The sensing ability of **1** is roughly parallel to the binding constant. All these results demonstrate that the host **1** is capable of detecting organic compounds by changing color in alkaline solution. Other systems can be constructed on the same basis.

Expermental Section

Measurements. Absorption and induced circular dichroism measurements were performed by using a 1 cm cell at 25 °C in an aqueous solution except for the pH titration experiment at 1.0×10^{-4} M of **1**, which was performed by using a 5 mm cell. The pH meter was calibrated at 25 °C with pH standard solutions of pH 4.01 ± 0.01, 6.86 ± 0.01, and 10.11 ± 0.01. Sodium hydroxide was used to set pH in the alkaline region in the pH titration experiment. Carbonate buffer was used in the measurements for binding constants of **1** (5.0×10^{-6} M) to set pH at 9.70. 1D and 2D NMR spectra were recorded in D_2O at 25 °C on a Varian VXR-500S operating at 499.843 MHz for ^{1}H. All the NMR spectra were measured by using pulse sequences and standard procedures offered by Varian.

The binding constants of **1** for several guests, K, were defined by the following equation:

$$K = \frac{C}{(G_0 - C)(H_0 - C)} \tag{1}$$

Here, H_0, G_0, and C represent the initial concentrations of host, guest, and the complex, respectively. In the condition of a large excess of G_0, eq 1 gives the following equation (eq 2).

$$K = \frac{C}{G_0(H_0 - C)} \tag{2}$$

The concentration of the complex, C, is reflected in the magnitude of ΔI, where ΔI is the guest-induced absorbance change at 540 nm and is equal to ΔI_{max} when every host exists as the inclusion complex. Using the values of H_0, G_0, ΔI, and ΔI_{max}, eq 2 may be led to the following representation:

$$K = \frac{\Delta I/\Delta I_{max}}{G_0(1 - (\Delta I/\Delta I_{max}))} \tag{3}$$

Therefore

$$\Delta I = \frac{KG_0\Delta I_{max}}{(1 + KG_0)} \tag{4}$$

The binding constant, K, is estimated by fitting eq 4 to the data obtained.

Materials. β-CD was a kind gift from Nihon Shokuhin Kako Co. Ltd. All chemicals were reagent grade and were used without further purification unless otherwise noted.

Synthesis. 6-Deoxy-6-[[6-[3,3′-bis-(4-hydroxyphenyl)-phthalide]carboxy]amino]-β-cyclodextrin (1). A dimethylacetamide (DMAc) solution of carboxyphenolphthalein (150 mg, 0.41 mmol), dicyclohexylcarbodiimide (DCC) (80 mg, 4.35 mmol), and hydroxybenzotriazole (HOBt) (60 mg, 4.45 mmol) was stirred at 0 °C for 10 min. To the mixture was added 6-deoxy-6-amino-β-cyclodextrin (1.0 g, 0.88 mmol), and the resultant solution was stirred at 0 °C for 2 h and then at room temperature for 2 days. To this solution was added additional DMAc solution of DCC (50 mg, 2.72 mmol), HOBt (30 mg, 2.22 mmol), and 6-deoxy-6-amino-β-cyclodextrin (1.0 g, 0.88 mmol), and the mixture was stirred at 0 °C for 10 min and then at room temperature for 2 days. After the insoluble material was

removed by filtration, filtrate was concentrated by a rotary evaporator. The resultant solution was poured into acetone (500 mL) to precipitate the product. After being washed with acetone (300 mL) several times, the crude product was charged on a column of QAE Sephadex and eluted with an aqueous solution of ammonium hydrogencarbonate (0 to 1 × 0.001 N). The fraction containing **1** was concentrated by a rotary evaporator and injected to HPLC with an ODS column (eluted with water/methanol 100/0 to 50/50). The eluted solution was concentrated to obtain the desired product as white powder (60 mg, 10%): R_f 0.67 (*n*-butanol−ethanol−water 5:4:3); ^{1}H NMR (D_2O; 500 MHz) δ 1.64 (1H, d), 2.30 (1H, d), 2.59 (1H, d), 2.71 (1H, d), 2.80−4.20 (majority, m) (protons of CD unit), 4.78−5.05 (7H, m, anomeric protons), 5.33 (2H, d), 6.65 (2H, d), 6.69 (2H, d), 6.85 (2H, d), 8.14 (2H, s), 9.32 (1H, s) (aromatic protons of phenolphthalein unit). Anal. Calcd for $C_{63}H_{83}NO_{39}\cdot 3H_2O$: C, 49.38; H, 5.85; N 0.91. Found: C, 49.75; H, 6.11; N, 0.92.

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