Molecular-Recognition and Sensory System of γ -Cyclodextrin Capped by Sodium Anthranilate

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γ -Cyclodextrin with a sodium anthranilate moiety (1) has been prepared as a sensing molecule for organic compounds including terpenoids and bile acids. The sensing and host-guest complexation properties are discussed here in comparison with those of β -cyclodextrin with a sodium anthranilate moiety (2) and γ -cyclodextrin with two sodium anthranilate moieties (3). Compound 1 shows a pure monomer fluorescence with an intensity which increases upon the addition of guest species. The extent of fluorescence variation with a guest is used to determine the sensing factor ($\Delta I/I^0$) of 1 for a guest. Compound 1 recognizes terpenoids better than 3 and bile acids better than 2.

Keywords Modified γ -cyclodextrin, sensor system, fluorescence spectroscopy, sodium anthranilate moiety

Cyclodextrin, torus-shaped cyclic oligomers of D-glucopyranose and named α -, β - and γ - for the hexa, hepta and octamers, respectively, can perform host-guest complexation with a variety of organic compounds in their cavity in aqueous solutions.¹ When the inclusion phenomena of cyclodextrins are investigated, spectroscopically active guests should be used because cyclodextrins are optical spectroscopically inert. The functionalization of cyclodextrins with chromophores has attracted much attention, because a modification can improve or alter their inclusion properties and spectroscopically inert guests can be used when a host-guest complexation study is made. In those cases, the appended moiety of the cyclodextrin acts as a probe of the host-guest complexation behavior, and at the same time as a cap to elevate the hydrophobic interaction with a guest or as a spacer to regulate the cyclodextrin cavity size. We have reported such a system as being an induced-fit type of host-guest complexation mechanism, as shown in Fig. 1.² During the last decade we prepared a number of cyclodextrins modified with chromophores, such as naphthalene³, anthracene⁴ and dancylglycine moieties.^{5,6} These molecules can be used to detect organic guest molecules, because they exhibit selective and sensitive recognition of guest molecules.

Recently, we described host-guest sensing systems using β - and γ -cyclodextrin modified with mono-sodium anthranilate (2)⁷ and bis-sodium anthranilate (3)⁸, respectively. These cyclodextrin derivatives show unique complexation behaviors because of the smaller

Fig. 1 Induced-fit type of host-guest complexation of a- and b-types for the inclusion of a guest molecule in the cyclodextrin cavity.

size of the sodium anthranilate moiety. We studied the binding abilities of these derivatives with terpenoids and bile acids as guest molecules, because they are biologically significant substances produced by plants or animals and are used for crude drugs. Compound 2 exhibits a higher sensitivity to terpenoids and lower sensitivity, but with higher selectivity for the latter, in which the appended moiety acts as a spacer to narrow the too large cavity size for a guest molecule. On the other hand, 3 recognizes bile acids with much higher sensitivity than terpenoids^{4,5}, in which the appended moieties act in two ways, namely, as a hydrophobic cap for terpenoids and as a spacer for bile acids.

In many cases, γ -cyclodextrins show different in-

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clusion behaviors in comparison to β -cyclodextrins because of their different molecular size. As an extension of our work, we prepared γ -cyclodextrin modified by a sodium anthranilate; we would like to report here on the fluorescent molecular recognition and host-guest complexation behavior of **1**, which is more similar to that of the β -cyclodextrin analogue than that of the γ -cyclodextrin analogue. The host-guest complexation properties of **1** for terpenoids and bile acids are described below.

Experimental

Preparation of 6-deoxy-6-[2-sodium benzene carboxylate-1-amino]- γ -cyclodextrin (**1**)

6-Iodo-6-deoxy- γ -cyclodextrin 2.16 g (1.54 mM) was added to a solution of 0.27 g (1.70 mM) of sodium anthranilate in 30 ml of DMF. The reaction mixture was heated at 80°C for 23 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 300 ml of acetone. The resultant precipitates were filtered and dried. The crude product was purified with a Sephadex G-15 column (3×90 cm) to afford 0.32 g (7.4% isolated yield) of **1**. R_f 0.38 (butanol/ethanol/water=5/4/3, v/v/v). $^1\text{H-NMR}$ (DMSO- d_6)=3.3–3.8 (48H, m, C₂–C₆H of cyclodextrin), 4.3–4.9 (15H, m, O₆H, C₁H), 5.8 (16H, br.s, O₂H, O₃H), 6.5 (1H, t, J =7.9 Hz, aromatic H), 6.6 (1H, s, -NH), 6.8 (1H, d, J =7.9 Hz, aromatic H), 7.2 (1H, t, J =7.9 Hz, aromatic H), 7.7 (1H, d, J =7.9 Hz, aromatic H). Calcd. for C₅₅H₈₄O₄₁NNa·6H₂O: C, 42.71; H, 6.26; N, 0.91%. Found C, 42.63; H, 6.15; N, 0.88%. MS(FAB):

1438([M+H]⁺).

Measurements

Ultraviolet, fluorescence and circular dichroism spectra were measured at 25°C with a Hitachi U-2000 spectrophotometer, a Hitachi F-3010 fluorescence spectrophotometer and a JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 330 nm, and excitation and emission slits were 5 nm. Five microliters of guest species (0.5 and 0.005 M) in dimethyl sulfoxide (DMSO) or methanol were injected into an aqueous solution of **1** (2.5 ml) to make a sample solution with a host concentration of 1×10^{-6} M and guest concentration of 0.01 and 1.0 mM.

Results and Discussion

Compound **1** was prepared from an iodo γ -cyclodextrin by treating it with sodium anthranilate, as shown in Fig. 2. The absorption and circular dichroism (CD) spectra of **1** and **3** in an aqueous solution are shown in

Fig. 2 Preparation of **1** from iodo- γ -cyclodextrin.

Fig. 3 Absorption spectra of 1 (—) and 3 (---) in an aqueous solution (10^{-4} M).

Fig. 4 Induced circular dichroism spectra of 1 (—) and 3 (---) in an aqueous solution (10^{-4} M).

Fig. 5 Induced circular dichroism of 1 (—) and 3 (---) in an aqueous solution (10^{-4} M) containing ursodeoxycholic acid (10^{-4} M).

Fig. 6 Fluorescence spectra of 1 (10^{-6} M) in an aqueous solution at various concentrations of ursodeoxycholic acid (1, 0; 2, 2.0×10^{-6} ; 3, 6.0×10^{-6} ; 4, 1.2×10^{-5} ; 5, 2.4×10^{-5} ; 6, 4.8×10^{-5} M).

Figs. 3 and 4, respectively. The CD spectrum of 1 shows a positive band at *ca.* 270 nm and negative bands at *ca.* 245 and 330 nm. On the other hand, the spectrum of 3 shows a negative band at *ca.* 270 nm and positive bands at 255 and 325 nm. These suggest that the locations of the anthranilate residues in the cavity of 1 and 3 are different from each other.^{9,10} The CD spectra of 1 and 3 in the presence of ursodeoxycholic acid are displayed in Fig. 5. The spectrum of 1 exhibits positive bands at 250 and 330 nm, and hence, is more similar to that of 3, with a higher $[\theta]$ value at *ca.* 330 nm. This suggests that the anthranilate residue in 1 is included in the cavity more deeply than in 3.

The fluorescence spectra of 1 in an aqueous solution in the presence and absence of ursodeoxycholic acid are given in Fig. 6. The spectrum of 1 alone exhibits a peak at 424 nm, and the intensity increases with increasing ursodeoxycholic acid concentration. This fluorescence enhancement has been observed when other terpenoids and bile acids were used as a guest, and is the same phenomenon as that for 3 by the addition of a guest. On

the other hand, 2 shows a decrease of fluorescence intensity with terpenoids and an increase with bile acids. The guest-induced fluorescence enhancement suggests that the sodium anthranilate moiety moves into the cyclodextrin cavity deeply, and the decrease means that the sodium anthranilate moiety moves out of the cavity.⁷

The extent of the variation in the fluorescence intensity of 1 depended on the nature of the guest, even at a common concentration; hence, 1 can be used for sensing molecules. To calculate the sensing ability of modified cyclodextrins in pure water, the $\Delta I/I^0$ value was used as a sensitivity factor. Here, ΔI is $I^0 - I$, where I^0 is the fluorescence intensity for the host alone, and I is for a host and guest mixture. Figure 7 illustrates the $\Delta I/I^0$ values of 1 for 19 guests including terpenoids and bile acids. The terpenoids and aromatic compounds (small type-guests) were used at 1.0 mM and bile acids (large type-guests) at 0.01 mM. The sign of $\Delta I/I^0$ was negative for all of the guests examined, as in the case of a β -cyclodextrin derivative (2). On the other hand, 3 shows a positive $\Delta I/I^0$ for small guests, except for (—)-

menthol and benzhydrol. The $\Delta I/I^0$ values of **1** for small guests range from -0.6 to -0.067 . This suggests that the behavior of **1** upon complexation with a guest molecule is affected by the molecular structure and size. However, the sensitivity of **1** was lower for the terenoids and aromatic compounds than **2**, while it was higher than **3**. It is known that β -cyclodextrin can recognize small molecules, such as terpenoids, with higher sensitivity than γ -cyclodextrin, because of their cavity size. In this case, the cavity size of **1** seems to be larger than that of **3**, because the bis-appended moieties of **3** should be included in the cavity, although the binding ability of **1** is higher than that of **3**.

These results show that a chemical modification alters the binding property of cyclodextrin. The sensitivity of **1** to small guests decreases in the sequence: *l*-borneol (**8**) > *l*-fenchone (**7**) > *d*-fenchone (**6**) > cyclohexanol (**5**)

Fig. 7 Sensitivity factors of **1** for guest molecules.

Table 1 Sensitivity factors and binding constants of **1** and **3** for steroidal compounds

Guest	1 : $\Delta I/I^0$	$K/\text{mol}^{-1} \text{ dm}^3$	3 : $\Delta I/I^0$	$K/\text{mol}^{-1} \text{ dm}^3$
13	-0.316	600000±22000	-1.840	1400000±90000
14	-0.210	79000±4100	-0.254	76000±14000
15	-0.320	120000±3000	-0.958	78000±2100
16	-0.298	270000±9900	-0.982	95000±2200
17	-0.325	610000±33000	-1.537	190000±5200
18	-0.068	13000±1300	-0.302	15000±1500
19	-0.179	120000±9600	-0.219	26000±610

>*cis*-1,2-cyclododecanediol (**10**)>nerol (**1**)>*l*-menthol (**4**)>1-adamantaneacetic acid (**9**)>cyclohexanone (**3**), 1-naphthaleneacetic acid (**12**)>benzhydrol (**11**)>cyclohexanol (**2**). Compound **1** recognizes bi-cyclic ring molecules such as **8**, **7** and **6** with highest sensitivity, and seven- or twelve-membered ring molecules, such as **5** and **10**, with second-highest sensitivity. The $\Delta I/I^0$ values of **1** for large guests range from -0.325 to -0.069, which is smaller than that for **3**, but larger than that for **2**.

To compare the sensitivity of **1** with **3** for bile acids more precisely, the sensitivity of **3** for bile acids at 0.01 mM was examined¹¹, and the binding constants (k) of **1** for these bile acids were obtained by the analyses of the guest-induced fluorescence variations using Eq. (1).¹²

$$\frac{1}{I-I_0} = \frac{1}{(b-a)} + \frac{1}{(b-a)[CD]_0 K} \cdot \frac{1}{[G]_0} \quad (1)$$

Here, I is the fluorescence intensity at 424 nm (I_f for complex, I_0 for the host alone), $[CD]_0$ is the total host concentration, $[G]_0$ is the total guest concentration, and a and b are constants. The sensitivity factors and the binding constants of **3**¹³ and **1** for those bile acids are listed in Table 1. The selectivity of **3** and **1** for bile acids decreases in the sequence: lithocholic acid>hyodeoxycholic acid>ursodeoxycholic acid>chenodeoxycholic acid>cholic acid>deoxycholic acid>dehydroepiandrosterone for **3**; hyodeoxycholic acid>lithocholic acid>ursodeoxycholic acid>chenodeoxycholic acid, dehydroepiandrosterone>deoxycholic acid>cholic acid for **1**. These show the difference in sensitivity between **3** and **1**. Hyodeoxycholic acid (**17**), having a binding constant that is twice that of ursodeoxycholic acid (**16**) and five-times that of chenodeoxycholic acid (**15**), was detected with high sensitivity. In those guest molecules, the structural difference is only the position and configuration of a hydroxy group. Cholic acid (**18**), which bears three hydroxy groups, was hardly detected, probably due to an increased polarity. The association constants of **1** for deoxycholic acid (**14**), ursodeoxycholic acid (**16**), hyodeoxycholic acid (**17**) and dehydroepiandrosterone (**19**) are larger than that of **3**, but the $\Delta I/I^0$ values are smaller. This means that the $\Delta I/I^0$ value

gives a relative, but not absolute, measure of the sensing ability.

In conclusion, **1** exhibits a middle degree of molecular recognition in detecting terpenoids and bile acids due to its fluorescence. The binding property of **1** is more similar to the β -cyclodextrin analogue (**2**) than to the γ -cyclodextrin analogue (**3**). This indicates that the modification of γ -cyclodextrin with a small residue can alter the binding property of the γ -cyclodextrin into that of the β -cyclodextrin.

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