Guest-induced colour changes and molecule-sensing abilities of *p*-nitrophenol-modified cyclodextrins

Akiko Matsushita,[a] Tetsuo Kuwabara,[b] Asao Nakamura,[a] Hiroshi Ikeda[a] and Akihiko Ueno*,[a]

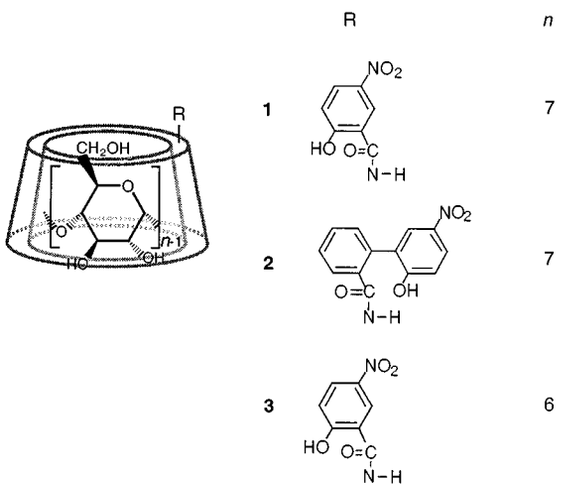
[a] *Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226, Japan*
[b] *Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi University, 4 Takeda, Kofu 400, Japan*

Two *p*-nitrophenol modified β-CDs (1, 2) were synthesized in order to assess colour changes induced by host–guest complexation as well as to investigate their conformations and binding properties as colour changeable indicators for the presence of organic molecules in pH neutral aqueous solutions. Host 3, which has an α-CD in place of the β-CD of 1, was also synthesized for comparison. The induced circular dichroism spectra of 1–3 suggest that the *p*-nitrophenol unit of 1 and 3 is not completely included in their CD cavities and that of 2 is fully included in its β-CD cavity. The spectra of 1–3 in the presence of guest supported these results, slightly enhancing the absolute intensities for 1 and 3 and decreasing the intensity for 2. The guest-induced conformation changes of 1–3 caused the colour to change from yellow to colourless, which is associated with the conversion of the appending moiety from phenolate anion to neutral phenol species at pH 5.10 for 1 and 3 and at pH 6.50 for 2. The binding constants of 1 were markedly larger than those for 2, reflecting that the *p*-nitrophenol moiety of 1 acts as an effective hydrophobic cap while that of 2 acts as an intramolecular inhibitor upon guest accommodation. These results demonstrate that various colour changeable indicators for molecules which work under neutral conditions may be constructed on the basis of the appropriate design of the appending part in dye-modified cyclodextrins.

Artificial receptors which transform binding of chemical species into spectroscopic signals are of current interest. The signal transduction of these systems usually occurs based on host–guest complexation. Although many artificial ionophores based on crown ethers,[1] cryptands[2] or calixarenes[3] have been shown to act as chemosensors which are responsive to metal or ammonium cations, there have been few examples of receptors which are responsive to organic molecules.[4]

Cyclodextrins (CDs) are typical hosts for organic compounds, forming inclusion complexes in aqueous solution.[5] Although CDs are spectroscopically inert, they can be converted into spectroscopically active hosts by modification with an appropriate chromophore. Ueno *et al*. have recently discovered that CDs can be used as starting materials for constructing fluorescent indicators for organic molecules.[6] They have prepared various types of modified CDs bearing one or two fluorophores: pyrene,[7] naphthalene,[8] dansyl[9] or *N*,*N*-dimethylaminobenzoyl[10] moieties, and found that they act as sensors for detecting organic compounds in aqueous solution. In these CD-based sensors, the binding of guest species is transduced into fluorescent signals through the guest-induced locational change of the chromophores, mostly from inside to outside the CD cavity. On the same basis, we have recently constructed colour-change indicators for detecting molecules using modified CDs bearing a methyl red unit.[11]

The methyl red modified systems work only in acidic solution, so in the present study, we have attempted to develop another type of CD-based colour-change sensor which works in neutral solution. From this point of view, *p*-nitrophenol is attractive as a unit to be attached since it changes from yellow to colourless when the neutral solution becomes acidic. Furthermore, the use of the *p*-nitrophenol unit seems promising since *p*-nitrophenol shifts its pK_a from 7.09 to 6.15 upon complexation with α-CD.[12] With this in mind, we have prepared CD derivatives **1**, **2** and **3**, which have a *p*-nitrophenol moiety at the primary side of the CD unit and examined their properties as colour-change indicators.



Results and discussion

Absorption spectra

Fig. 1 shows the absorption spectra of **1** alone (*a*) and in the presence of adamantan-1-ol (*b*) measured at various pH values. Compound **1** exhibits a strong peak at 389 nm at pH 8.06, indicating that the *p*-nitrophenol unit of **1** exists as the phenolate anion. When the pH of the solution was lowered, the absorbance decreased, while that at *ca*. 320 nm increased. This spectral change reflects the structural change of the *p*-nitrophenol unit from the phenolate anion form to the neutral phenol. The isosbestic point at 330 nm indicates the existence of an equilibrium between these two species. The pH dependency of **1** in the presence of adamantan-1-ol is similar to that of **1** alone, but differences exist as shown in Fig. 2.

* Fax: +81-45-923-0374; E-Mail: aueno@bio.titech.ac.jp

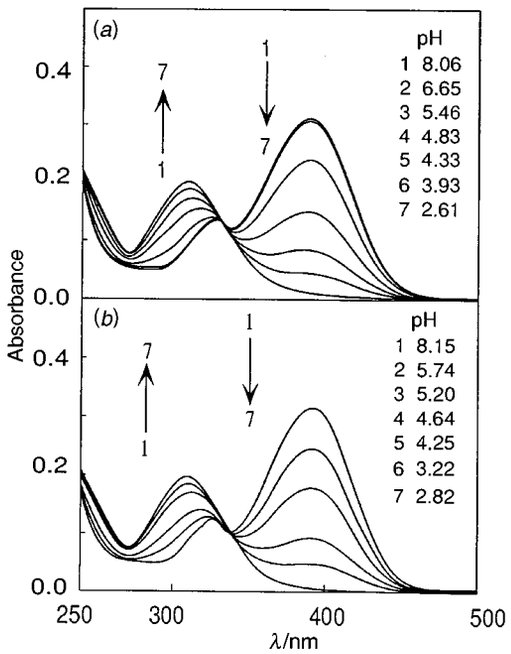


Fig. 1 Absorption spectra of **1** alone (*a*: 3×10^{-5} mol dm^{-3}) and in the presence of adamantan-1-ol (*b*: 6×10^{-4} mol dm^{-3}) at various pH values

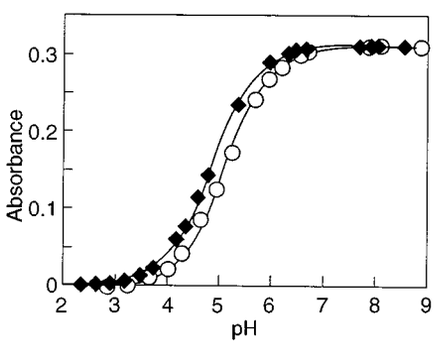


Fig. 2 pH dependence of the absorbance at 389 nm of **1** alone (◆) and in the presence of adamantan-1-ol (6×10^{-4} mol dm^{-3}) (○)

Fig. 2 shows that the pH titration curve of **1** is shifted to higher pH upon addition of adamantan-1-ol. These observations suggest that the pK_a values of the complex of the CD derivative are different from those of the individual components. Compound **2** exhibits an absorption maximum at 420 nm at pH 10.40, while that of **3** is at 390 nm at pH 9.50 [Fig. 4(*b*)]. A red shift in absorption maximum of the phenolate anion form of **2** may be due to the existence of the benzene ring as the spacer unit, which is absent in **1** and **3**. With a lowering of the pH of the solution of **2** and **3**, they display similar spectral variations to that of **1**. The existence of isosbestic points at 360 nm for **2** and at 330 nm for **3** indicates that there is also an equilibrium between the two forms in the *p*-nitrophenol unit for **2** and **3**. Compounds **2** and **3** showed similar shifts to that of **1** upon addition of adamantan-1-ol and hexan-1-ol, respectively. The pK_a values estimated by curve fitting analysis are summarized in Table 1.[11b] The guest-induced pK_a shifts for **1** and **2** are *ca*. 0.3, while that for **3** is 0.16. The environmental change around the *p*-nitrophenol unit may be reflected in the values of these guest-induced pK_a shifts. This argument is consistent with the reported result that the pK_a of *p*-nitrophenol is shifted from 7.09 to 6.15 by complexation with α-CD.[12] Therefore, the data of **1**–**3** suggest that their *p*-nitrophenol unit is partly or fully included in the hydrophobic CD cavities of **1**–**3** and is exposed to bulk water upon the addition of guest. The smaller guest-induced pK_a shift for **3** may be due to the fact that its

Table 1 pK_a of *p*-nitrophenol-modified CDs (**1**–**3**) alone and in the presence of guest[a]

	1	**2**	**3**
pK_a (alone)	4.82	6.40	4.98
pK_a (with guest)	5.11	6.73	5.14

[a] Guest are adamantan-1-ol (0.60 mmol dm^{-3}) for **1** and **2** and hexan-1-ol (3 mmol dm^{-3}) for **3**.

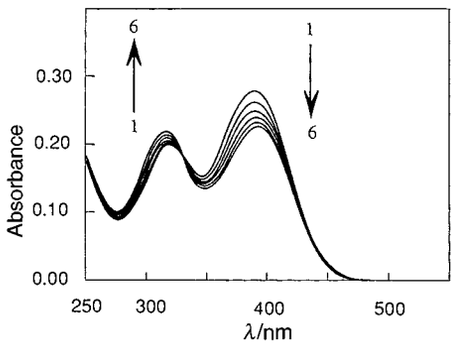


Fig. 3 Absorption spectra of **1** (3×10^{-5} mol dm^{-3}) with various concentrations of adamantan-1-ol at pH 5.00 guest concentration: (1) 0, (2) 6.0×10^{-6}, (3) 1.0×10^{-5}, (4) 1.4×10^{-5}, (5) 3.0×10^{-5}, (6) 6.0×10^{-5} mol dm^{-3}

p-nitrophenol unit is not deeply included in the α-CD cavity. The shallow inclusion of the appending moiety of **3** might imply that the ring size of the primary hydroxy side, where *p*-nitrophenol is attached, is too narrow or geometrically unfavourable to include the *p*-nitrophenol unit.

It is noted that spectral shapes of **1**–**3** are hardly influenced by the concentration itself in the range from 6×10^{-7} to 6×10^{-5} mol dm^{-3}, indicating that the inclusion of the *p*-nitrophenol unit in the CD cavity occurs not intermolecularly but intramolecularly.

At pH 5.00, it was observed that the yellow colour of **1** fades when adamantan-1-ol was added to the solution of **1**. Fig. 3 shows the absorption spectra of **1**, alone and in the presence of adamantan-1-ol. The absorbance at *ca*. 390 nm decreased upon addition of adamantan-1-ol, while that at *ca*. 320 nm increased. This observation suggests that the environment around the *p*-nitrophenol unit of **1** changes upon accommodation of adamantan-1-ol in the cavity, resulting in the structural conversion of the *p*-nitrophenol unit from the phenolate anion into the phenol form by protonation. Similar spectral variations were observed when other guests were added in place of adamantan-1-ol. The solution of **2** at pH 6.50 and that of **3** at pH 5.05 show similar spectral behaviour upon guest addition, suggesting the occurrence of the similar structural change in these systems. More detailed results and discussion on guest binding will be described below.

Induced circular dichroism spectra

Since CDs consist of chiral D-glucose units, the chromophore-modified CDs may exhibit induced circular dichroism in their absorption wavelength regions.[13] Fig. 4 shows the induced circular dichroism spectra of **1**–**3** in alkaline solutions in the absence and the presence of the guest. Compounds **1** and **3** exhibit a negative dichroism band at *ca*. 320 nm, while **2** exhibits positive ones at *ca*. 300 and 420 nm. The dichroism patterns of **1** and **3** suggest that **1** and **3** have similar structures which are different from the structure of **2**. The positive dichroism band of **2** at *ca*. 420 nm indicates that the *p*-nitrophenol unit is included in the cavity with an orientation parallel to the CD axis, being consistent with the reported result that the *p*-nitrophenol included in the β-CD exhibits a positive dichroism band at *ca*. 410 nm.[14] From the second derivative of the absorption spectra of **1** and **3**, it was found that they have transition peaks at 330 and 400

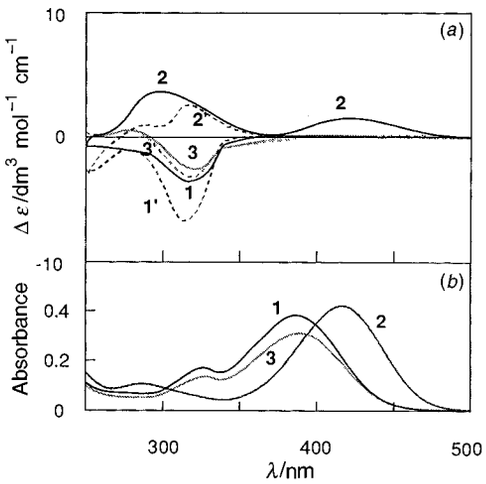


Fig. 4 Circular dichroism (*a*) and absorption (*b*) spectra of **1**–**3** (3.0×10^{-5} mol dm^{-3}) alone (**1**, pH 8.8; **2**, pH 10.4; **3**, pH 9.5) and in the presence of guests (**1′**, adamantan-1-ol, 0.60 mmol dm^{-3}; **2′**, adamantan-1-ol, 0.45 mmol dm^{-3}; **3′**, hexan-1-ol 3.0 mmol dm^{-3}) in alkaline solutions

nm, whose transition moments are coplanar with the aromatic ring. These results can be interpreted in terms of equatorial inclusion of the chromophore unit or shallow inclusion into its own CD cavity. The examination of CPK models indicates that the *p*-nitrophenol unit of **3** is unlikely to be fully included in its narrow α-CD cavity. The model examination also indicates that the equatorial inclusion of the *p*-nitrophenol unit of **1** is unlikely. Therefore, it is suggested that the *p*-nitrophenol unit of **1** and **3** is located near the rim of the CD ring with an orientation perpendicular to the CD axis. The larger absolute intensity of the Cotton effect of **1** than that of **3** indicates that the interaction between the *p*-nitrophenol unit and the CD cavity of **1** is stronger than that for **3**. This fact coincides with the result that the guest-induced pK_a shift of **1** is larger than that of **3**.

The induced circular dichroism spectra of **1**–**3** also change upon the guest addition (Fig. 4). Upon addition of adamantan-1-ol to the solution of **1**, the absolute intensity of the negative dichroism band at *ca*. 320 nm increased. Similar trends were observed for **3** when hexan-1-ol was added as a guest. These results suggest that the *p*-nitrophenol moiety, which may act as a hydrophobic cap, is located closer to the rim of β-CD in the complexes of **1** and **3** due to the hydrophobic interaction between the cap and the guest.[15]

On the other hand, the spectral behaviour of **2** is different from those of **1** and **3**. The positive dichroism band at *ca*. 410 nm disappeared upon adamantan-1-ol addition, indicating that the *p*-nitrophenol unit formerly included in the CD cavity is excluded from the cavity. These results demonstrate that the kind of the connecting group between CD and the appending moiety is important, determining the type of conformational change occurring associated with the guest binding.

NMR spectra

Current NMR techniques are powerful tools for the structural and conformational analysis of modified CDs. On this basis, we have investigated the structures of various modified CDs and determined the orientation of the pendant groups of several modified CDs by the combined use of 1D and 2D NMR techniques.[16] In this work, we studied the spectra of **1** and **3**. We estimated the orientation of the pendant group of **1** by the ^{1}H resonances for protons of the C6 position in the glucose unit **A** in which the pendant group was connected with an amide bond at the C6 position. It was difficult to assign all ^{1}H resonances of **1** because of the overlapping of the resonances for their anomeric protons. The partial ^{1}H NMR spectrum of **1** is shown in Fig. 5(*a*). The resonances for the protons of the C6 position in the

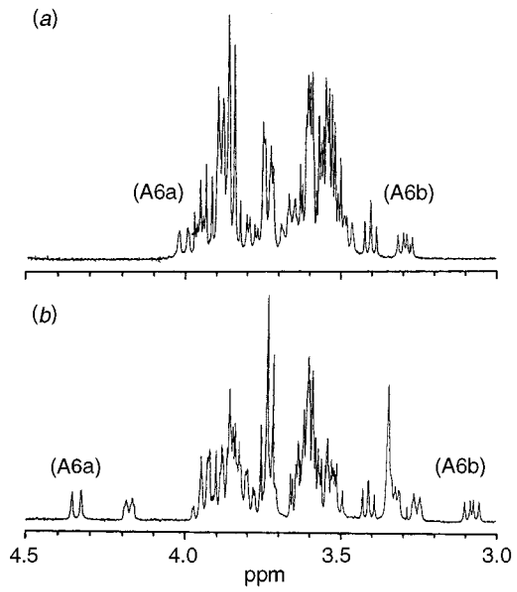


Fig. 5 (*a*) Partial 500 MHz ^{1}H NMR spectrum of **1** alone in D_2O (pD 4.8) at 25 °C; (*b*) partial 500 MHz ^{1}H NMR spectrum of **1** in the presence of adamantan-1-ol in D_2O (pD 4.8) at 25 °C

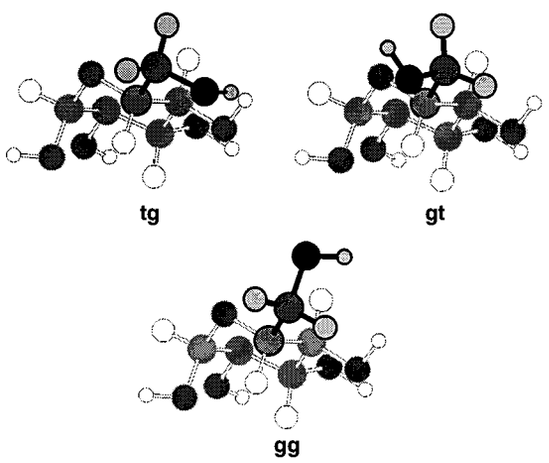


Fig. 6 The three rotamers about the C5–C6 bond of glucose

glucose unit **A** could be assigned to peaks at 3.28 and 4.03 ppm by the ^{1}H–^{13}C HSQC spectrum. This dispersion of the resonances may be attributed to the anisotropic shielding effect of the carbonyl group of the pendant moiety.[17] Before analysing the NMR spectra of **1** and **3**, we analysed the conformation around the C5–C6 bond of D-glucose. Coupling constants measured for D-glucose, of $J_{5,6a}$ and $J_{5,6b}$, are the averaged values of component coupling constants in three rotamers, **gg**, **gt** and **tg**, weighted by their fractional populations (Fig. 6).[18] On the basis of observed coupling constants, the populations of rotamers around the C5–C6 bond can be analysed. The coupling constants were estimated with the spin-simulation program on a Varian VXR500S system (Table 2). The rotamer **gt** is the major component. The rotamer **tg** scarcely existed, because of steric and/or stereoelectronic interaction. It is well known that the rotamer **tg** of D-glucopyranose monomer is unlikely to be present because of unfavourable parallel 1,3-interactions between C4–O and C6–O.[18] The orientation of the pendant group was estimated from the anisotropic shielding effect of the carbonyl group to the protons at the C6 position of glucose unit **A** and the most plausible rotamer around the C5–C6 bond is shown in Fig. 7.

The ^{1}H NMR spectrum of **1** in the presence of adamantan-1-ol (0.6 mol dm^{-3}) is shown in Fig. 5(*b*). The resonances for protons of the C6 position in the glucose unit **A** were found at 3.08 and 4.34 ppm and the dispersion of resonances became larger on addition of the guest. The conformation around the

Table 2 Calculated fractional populations (P) of rotamers of the C5–C6 bonds of modified glucose units and observed coupling constants ($J_{5,6a}$, $J_{5,6b}$) of **1** alone and in the presence of guest

	P_{gg}	P_{gt}	P_{tg}	$J_{5,6a}$/Hz	$J_{5,6b}$/Hz
1	14.4	78.7	6.9	2.5	9.1
1/adamantan-1-ol	19.0	81.0	~0	1.5	9.3

Table 3 The binding constants of **1** and **2** for various guests[a]

	K/dm^3 mol^{-1}	
	1	**2**
Adamantan-1-ol	700 000	15 500
Adamantane-1-carboxylic acid	140 000	3 880
(−)-Borneol	47 000	5 650
Cyclooctanol	16 000	1 530
Cyclohexanol	2 300	140
Nerol	3 400	60
Geraniol	2 500	480

[a] The binding constants are apparent values obtained at pH 5.0 for **1** and pH 6.5 for **2** at 25 °C. The binding constants of **3** for these guests are too small to be accurately determined.

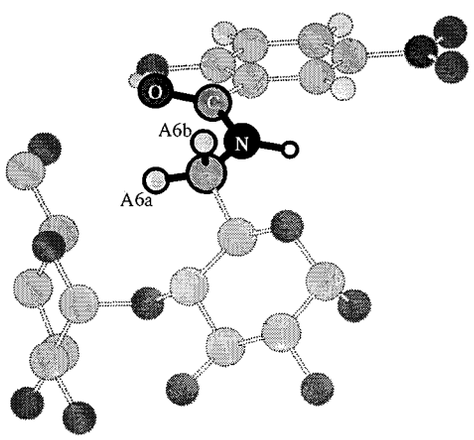


Fig. 7 3D-Conformation around the amide bond and a schematic representation of proximal glucose residues and the *p*-nitrophenol moiety of **1**

C5–C6 bond was also analysed by the coupling constants (Table 2). The populations of rotamers were scarcely changed by addition of the guest. This result indicates that the conformational change occurring upon addition of the guest is induced by the rotation around the C6–NHCO bond rather than the C5–C6 bond. Similar trends were observed for **3**.

Binding constants

The binding constants are determined by a nonlinear least-squares fitting procedure for the guest-induced changes of the absorbances at 389 and 420 nm for **1** and **2**, respectively.[11b] A typical saturation curve for **1** and adamantan-1-ol was obtained. The same curve fitting analysis could be applied to other guests with good fitting. The binding constants of **2** at pH 6.50 were also estimated in the same manner as the case of **1**. Table 3 shows the binding constants of **1** and **2** for various guest compounds. The binding constants of **1** for adamantan-1-ol **8** are markedly larger than that of **2**. All other guests examined are bound to **1** and **2** with the same tendency. These results indicate that the *p*-nitrophenol moiety of **1** acts as an effective hydrophobic cap which increases the hydrophobic nature of the environment around the cavity while that of **2** acts as an intramolecular inhibitor for guest accommodation. The binding ability of **3** is almost negligible for the guests shown in Table 3; however, it is noted that **3** can bind smaller guests, *e.g.* 9500 mol^{-1} dm^3 for hexan-1-ol.

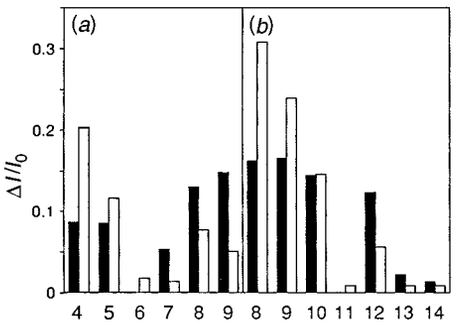
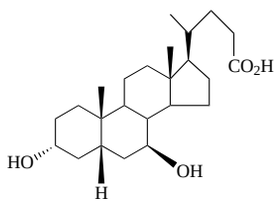
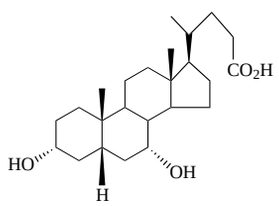


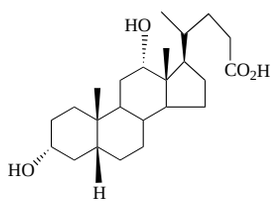
Fig. 8 The sensitivity factor $\Delta I/I_0$ of **1** (■) and **2** (□) (3×10^{-5} mol dm^{-3}) for guests **4**–**14** [(*a*) 3×10^{-5} mol dm^{-3}; (*b*) 1.5×10^{-4} mol dm^{-3}] at pH 6.50 for **2** and pH 5.00 for **1**



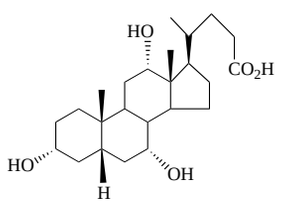
ursodeoxycholic acid (**4**)



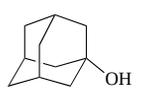
chenodeoxycholic acid (**5**)



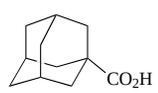
deoxycholic acid (**6**)



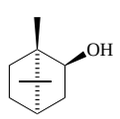
cholic acid (**7**)



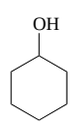
adamantan-1-ol (**8**)



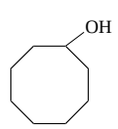
adamantane-1-carboxylic acid (**9**)



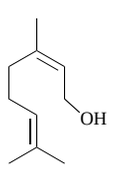
(–)-borneol (**10**)



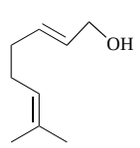
cyclohexanol (**11**)



cyclooctanol (**12**)



nerol (**13**)



geraniol (**14**)

Sensitivities and selectivity

The values of $\Delta I/I_0$ were used as the measure of the sensitivity of **1**, **2** and **3**, where $\Delta I = I_0 - I$, and I_0 and I are the absorbances in the absence and presence of guest, respectively, measured at 390 nm for **1** and **3** and at 410 nm for **2**. Fig. 8 shows the sensitivities of **1** and **2**. Fig. 8(*a*) shows the data obtained at 0.03 mmol dm^{-3} guest concentration. The sensitivity order of **1** for the steroids is **4** (ursodeoxycholic acid) ≈ **5** (chenodeoxycholic acid) > **7** (cholic acid) ≥ **6** (deoxycholic acid), while that of **2** is **4** > **5** > **6** ≈ **7**. Adamantane derivatives are known to be good guests in β-CD, and here **8** and **9** are compared with **4**. The sensitivities of **1** for **8** and **9** are larger than that for **4** while those of **2** are opposite, being smaller than that for **4**. The data

shown in Fig. 8(*b*) were obtained at 0.15 mmol dm^{-3} guest concentration. The use of a threefold higher concentration is due to the smaller responses of some of these guests. The sensitivities of **1** and **2** for **11** are much smaller than those for **12**. This result reflects the fact that the size of **12** fits the cavity of β-CD while **11** is too small to be accommodated in the cavity of β-CD. The bicyclic compound **10** was detected with higher sensitivity than the monocyclic **12**, but with a lower sensitivity than adamantane derivatives (**8** and **9**). These trends are unique for **2**. For the open chain monoterpene, the sensitivity to the *cis* form **13** is slightly better than the *trans* form, **14** with **1** while it is almost the same for both guests with **2**. On the other hand, the sensitivities of **3** for all these guest compounds were small or negligible, indicating that the sizes of these guests are unsuited to being accommodated in the small α-CD cavity of **3**. However, it is noted that **3** can be responsive for smaller linear guests as shown by the sensitivity value of 0.055 for hexan-1-ol (0.12 mmol dm^{-3}).

All these results demonstrate that the *p*-nitrophenol-modified CDs work under neutral conditions as sensors for molecules.

Experimental

Materials

β-CD and α-CD were kind gifts from Nihon Shokuhin Kako Co. Ltd. All reagents as guests were purchased from Tokyo Kasei and were used without further purification.

Syntheses

6-Deoxy-6-(2-hydroxy-5-nitrobenzoylamino)-β-CD 1. A solution of 2-hydroxy-5-nitrobenzoic acid (1.02 g, 5.57 mmol), hydroxybenzotriazole (0.80 g, 5.92 mmol) and dicyclohexylcarbodiimide (1.19 g, 5.77 mmol) in dimethylacetamide was stirred at 0 °C for 10 min. To the mixture was added 6-deoxy-6-amino-β-CD (1.51 g, 1.33 mmol) and the resultant solution was stirred at 0 °C for 18 h, and then at room temp. for 16 h. After removing the solid formed by filtration, the solution was poured into acetone (500 ml) to form a precipitate. The precipitate was collected by filtration and dried in a vacuum overnight. This procedure was repeated several times. After removing 6-deoxy-6-amino-β-CD by column chromatography on Sephadex CM-25, the solution was concentrated in a rotary evaporator and subjected to a HP-20 column which had been washed with methanol first, and then water before use. The column chromatography was performed by stepwise elution with 5, 10, 15 and 40–60% methanol–water solutions. The fractions obtained by elution with 40–60% methanol–water contained the desired product, giving a pale-yellow powder after removing the solvent (yield 14%).

The purity of the product was checked with TLC, ^{1}H NMR and elemental analysis: R_f 0.61 (*n*-butanol–ethanol–water, 5:4:3). Anal. calc. for $C_{49}H_{74}O_{38}N_2 \cdot 4H_2O$: C, 42.92; H, 6.03; N, 2.04. Found: C, 41.52; H, 5.94; N, 2.01%; δ_H(D_2O, 500 MHz) 3.28–4.00 (m), 4.96–4.99 (5H, m), 5.02 (1H, d), 5.07 (1H, d), 6.78 (1H, d), 8.12 (1H, dd), 8.63 (1H, d).

6-Deoxy-6-[2-(2-hydroxy-5-nitrophenyl)benzoylamino]-β-CD 2. A solution of 6-deoxy-6-amino-β-CD (2.44 g, 2.15 mmol) and 6-nitro-3,4-benzocoumarin (1.23 g, 5.08 mmol) in dimethylacetamide (30 ml) was stirred at 75 °C for 72 h. The reaction mixture was evaporated and concentrated in a vacuum, and acetone (1 l) was added to the residue. The crude product was collected, dried in a vacuum at room temp. overnight, and then dissolved in a small amount of water. After filtration, the solution was subjected to ion-exchange column chromatography on Sephadex QAE with Na_2CO_3–$NaHCO_3$ (0.03 mmol dm^{-3}), affording fractions that contained the desired product. The fractions were concentrated in a rotary evaporator and dried at 0 °C in a vacuum. The product was obtained as yellowish crystals which contained the sodium salt; it was desalted on an HP20 column (yield 10%). The purity of the product was checked with TLC, ^{1}H NMR and elemental analysis: R_f 0.58 (*n*-butanol–ethanol–water, 5:4:3). Anal. calc. for $C_{55}H_{77}O_{38}N_2 \cdot 6H_2O$: C, 44.54; H, 6.12; N, 1.89. Found: C, 44.31; H, 5.91; N, 1.84%; δ_H(D_2O, 500 MHz) 3.10–4.10 (m), 4.93–5.07 (7H, m), 6.76 (1H, br), 7.40 (1H, d), 7.50 (1H, t), 7.60 (2H, m), 7.22 (1H, d), 7.96 (1H, dd).

6-Deoxy-6-(2-hydroxy-5-nitrobenzoylamino)-α-CD 3. Compound **3** was prepared by the procedure employed for synthesis of **1**, using α-CD in place of β-CD. The purity of the product was checked with TLC, ^{1}H NMR and elemental analysis: R_f 0.56 (*n*-butanol–ethanol–water, 5:4:3). Anal. calc. for $C_{43}H_{64}O_{33}N_2 \cdot 5H_2O$: C, 42.09; H, 6.08; N, 2.27. Found: C, 41.58; H, 6.10; N, 2.31%; δ_H(D_2O, 500 MHz) 3.25–4.15 (m), 4.96–4.99 (4H, m), 5.02 (1H, d), 5.05 (1H, d), 6.65 (1H, d), 8.05 (1H, dd), 8.55 (1H, d).

Measurements

Absorption spectra were recorded on a Shimadzu UV-3100 spectrophotometer. Induced circular dichroism spectra were measured with a JASCO J-600 spectropolarimeter. All spectroscopic measurements were made at 25 °C in aqueous solution. The concentration of the solutions of **1**–**3** was 0.03 mmol dm^{-3}. ^{1}H NMR spectra were run on a Varian VXR-500S spectrometer in D_2O. The pH of the solution was measured on a TOA pH meter HM-60S, which had been calibrated at 25 °C with pH standard solutions of pH 4.01 ± 0.01, 6.86 ± 0.01 and 9.22 ± 0.01. Phosphate buffer was used for the measurements of binding constants of **2** at pH 6.50. Acetate buffer was used for the measurements of binding constants of **1** and **3** at pH 5.00. The ^{1}H NMR experiments were performed at pD 5.00 in DCl–D_2O solutions.

Acknowledgements

We are grateful to Nihon Shokuhin Kako Co. Ltd. for providing us with cyclodextrins. This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1 C. J. Pedersen, *J. Am. Chem. Soc.*, 1967, **89**, 2495; J.-M. Lehn and P. Vierling, *Tetrahedron Lett.*, 1980, **21**, 1323; D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 8190; *Comprehensive Supramolecular Chemistry, Vol. 1*, ed. G. W. Gokel, Pergamon, Oxford, 1996.

2 E. Graf, J.-P. Kintznger, J.-M. Lehn and J. LeMoigen, *J. Am. Chem. Soc.*, 1982, **104**, 1672; J.-C. Rodriguez-Ubis, B. Alpha, D. Plancherel and J.-M. Lehn, *Helv. Chim. Acta*, 1984, **67**, 2264; *Comprehensive Supramolecular Chemistry, Vol. 1*, ed. G. W. Gokel, Pergamon, Oxford, 1996.

3 C. D. Gutsche, *Calixarenes, Monograph in Supramolecular Chemistry*, ed. J. F. Stoddart, Royal Society of Chemistry, Cambridge, 1988; S. Shinkai, H. Koreishi, K. Ueda and O. Manabe, *J. Chem. Soc., Chem. Commun.*, 1986, 233; S. Shinkai, H. Koreishi, K. Ueda, T. Arimura and O. Manabe, *J. Am. Chem. Soc.*, 1987, **109**, 6371; *Comprehensive Supramolecular Chemistry, Vol. 2*, ed. F. Vögtle, Pergamon, Oxford, 1996.

4 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Supramol. Chem.*, 1995, **6**, 141; D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397; I. Aoki, Y. Kawahara, T. Sasaki, T. Harada and S. Shinkai, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 927.

5 G. Wenz, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 803; *Comprehensive Supramolecular Chemistry, Vol. 3*, ed. J. Szejtli and T. Osa, Pergamon, Oxford, 1996; M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1977; J. Szejtli, *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.

6 (*a*) A. Ueno, *Adv. Mater.*, 1993, **5**, 132; (*b*) A. Ueno, *Supramol. Sci.*, 1996, **3**, 31; (*c*) A. Czarnik, *Fluorescent Chemosensors for Ion and Molecule Recognition*, American Chemical Society, Washington DC, 1992, 74.

7 A. Ueno, I. Suzuki and T. Osa, *J. Am. Chem. Soc.*, 1989, **111**, 6391; *Anal. Chem.*, 1990, **62**, 2461; I. Suzuki, Y. Sakurai, M. Ohkubo, A. Ueno and T. Osa, *Chem. Lett.*, 1992, 2005.

8 A. Ueno, S. Minato and T. Osa, *Anal. Chem.*, 1992, **64**, 1154; 2562.

9 (*a*) A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada and K. Murai, *Chem. Lett.*, 1990, 605; (*b*) F. Hamada, Y. Kondo, R. Ito, I. Suzuki, T. Osa and A. Ueno, *J. Inclusion Phenom. and Mol. Recog. Chem.*, 1993, **15**, 273; (*c*) Y. Wang, T. Ikeda, H. Ikeda, A. Ueno and F. Fujio, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 1598; (*d*) H. Ikeda, A. Nakamura, N. Ise, A. Ueno and F. Toda, *J. Am. Chem. Soc.*, 1996, **118**, 10 980.
10 (*a*) K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki and T. Osa, *J. Am. Chem. Soc.*, 1993, **115**, 5035; (*b*) K. Hamasaki, A. Ueno and F. Toda, *J. Chem. Soc., Chem. Commun.*, 1993, 331; (*c*) K. Hamasaki, A. Ueno, F. Toda, I. Suzuki and T. Osa, *Bull. Chem. Soc. Jpn.*, 1994, **67**, 516.
11 (*a*) A. Ueno, T. Kuwabara, A. Nakamura and F. Toda, *Nature*, 1992, **356**, 136; (*b*) T. Kuwabara, A. Nakamura, A. Ueno and F. Toda, *J. Phys. Soc.*, 1994, **98**, 6297.
12 K. A. Connors and J. M. Lipari, *J. Pharm. Sci.*, 1976, **65**, 379.
13 K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 375; K. Fujita, T. Ueda, T. Imoto, I. Tabushi, N. Toh and T. Koga, *Bioorg. Chem.*, 1982, **11**, 108.
14 H. Shimizu, A. Kaito and M. Hatano, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2678.
15 J. Emert and R. Breslow, *J. Am. Chem. Soc.*, 1975, **97**, 670; I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata and K. Fujita, *J. Am. Chem. Soc.*, 1976, **98**, 7855.
16 H. Ikeda, Y. Nagano, Y-Q. Du, T. Ikeda and F. Toda, *Tetrahedron Lett.*, 1990, **31**, 5045; H. Ikeda, Y-Q. Du, A. Nakamura and F. Toda, *Chem. Lett.*, 1991, 1495; H. Ikeda, H-t. Moon, Y-Q. Du and F. Toda, *Supramol. Chem.*, 1993, **1**, 337.
17 Atta-ur-Rahman, *Nuclear Magnetic Resonance*, Springer-Verlag, New York, 1986.
18 D. G. Streefkerk, M. J. A. De Bie and J. F. G. Vliegenthart, *Tetrahedron*, 1973, **29**, 833.

Paper 7/01220I
Received 21*st February* 1997
Accepted 25*th April* 1997