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A Novel Color-Change Indicator for Molecules. Guest-Induced Color-to-Colorless Change of *p*-Nitrophenol-Modified β-Cyclodextrin

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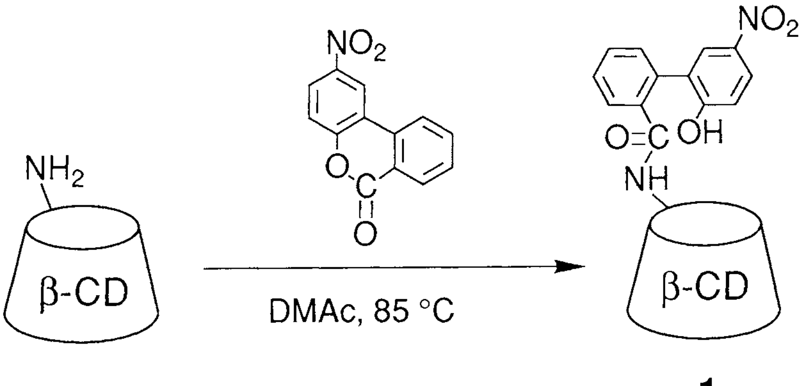
p-Nitrophenol-modified β-cyclodextrin changes its color from yellow to colorless upon guest binding at pH 6.50. This system was used to detect several organic molecules with remarkable molecular recognition, and the order of the guest-induced absorption changes was parallel with the order of the binding constants.

Many synthetic receptors are known to exhibit color changes on binding metal and ammonium cations.1) However, the attempt to detect neutral organic molecules have been rare.2) Recently, we have demonstrated that methyl red-modified cyclodextrin is useful for detecting organic species by color changes.3) Although this host acts as an indicator for molecules in aqueous solution, the pH of the solution must be very low. So it is desirable to develop another type of hosts which can act even in neutral solutions.

Here we report the synthesis and molecular recognition of a β-cyclodextrin derivative (**1**) which possesses a *p*-nitrophenol unit as a chromophore and exhibits a guest-induced color change from yellow to colorless under neutral conditions.

Host **1** was prepared by the reaction of 6-deoxy-6-amino-β-cyclodextrin with 6-nitro-3,4-benzocoumarin at 85 °C for 16 h in dimethylacetoamide as shown in Scheme 1. The product was purified by HPLC with an ODS column, a QAE-Sephadex and Diaion HP-20 column chromatography and identified by ^{1}H-NMR, TLC and elemental analysis.4)



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Scheme 1.

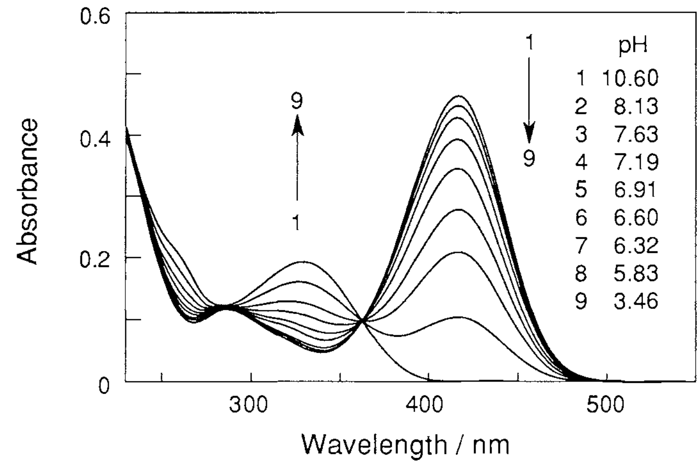

Fig. 1. Effect of pH on absorption spectrum of **1** (3×10^{-5} mol dm^{-3}) in an aqueous solution.

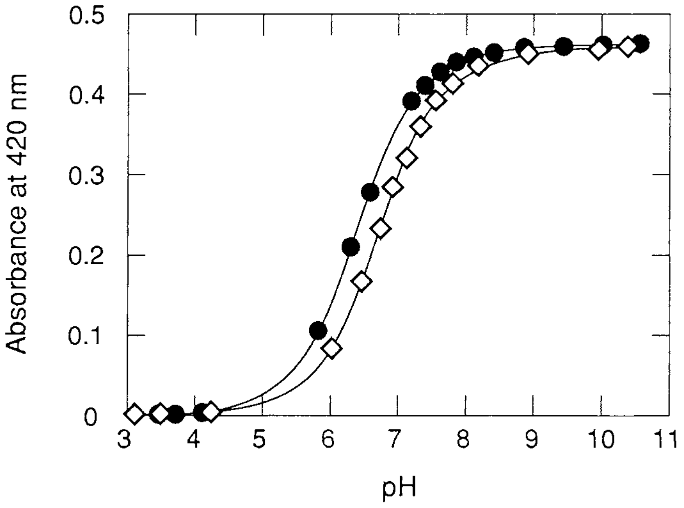

Fig. 2. pH-Dependence of the absorbance at 420 nm of **1** alone (●) and in the presence of 1-adamantanol (4.5×10^{-5} mol dm^{-3}) (◇).

Figure 1 shows the absorption spectra of **1** at various pH values in aqueous solution and Figure 2 shows the pH titration curve for the absorbance at 420 nm, alone and in the presence of 1-adamantanol as a guest. The absorption spectrum of **1** at pH 10.56 exhibits a strong peak at 417 nm, indicating that the chromophore of **1** exists as a phenolate anion. When pH was lowered, the absorbance around 420 nm decreased and finally disappeared at pH 3.56, while the absorbance around 330 nm increased, reflecting the conversion of the phenolate anion into the phenol form. The isosbestic point at 357 nm indicates the existence of the equilibrium between the two species. The pKa estimated here for **1** alone was 6.40, while in the presence of 1-adamantanol (4.5×10^{-4} mol dm^{-3}) as a guest, it became 6.73. This guest-induced pKa shift of **1** is related with the difference in the environment around *p*-nitrophenol unit of **1** and consistent with the reported result that the pKa of *p*-nitrophenol is lowered by complexation with α-cyclodextrin.$^{5)}$ The circular dichroism spectrum of **1** exhibits a positive band around 420 nm at pH 6.50 as well as at pH 10.60, indicating that the *p*-nitrophenol unit of **1** is included in the CD cavity with the orientation parallel to the CD axis.$^{6)}$ Upon addition of 1-adamantanol, however, the positive band disappeared. This result reveals that the *p*-nitrophenol unit of **1** is excluded from the cavity by 1-adamantanol. Thus, **1** undergoes an induced-fit type of conformational change in guest binding.$^{7)}$ It is noted that the absorption spectral band shape of **1** is hardly influenced by the concentration of itself in the range from 6×10^{-7} to 6×10^{-5} mol dm^{-3}, demonstrating that the inclusion of *p*-nitrophenol unit in CD cavity is not intermolecular but intramolecular.

We observed that yellow color of **1** fades upon addition of *l*-borneol at pH 6.50. Figure 3 shows the absorption spectra of **1**, alone and in the presence of *l*-borneol. The absorbance around 420 nm decreased

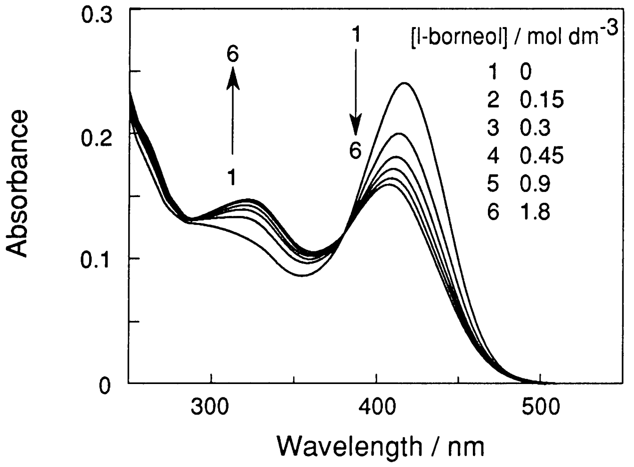

Fig. 3. Guest-induced absorption variations of **1**.

upon addition of *l*-borneol, while that around 330 nm increased. This result suggests that **1** excludes its *p*-nitrophenol unit from the cavity to bulk water to accommodate *l*-borneol in the cavity, resulting in the conversion of the molecular form of the *p*-nitrophenol unit from the phenolate anion into the acid form by protonation.

From the viewpoint of using **1** as the sensory system for detecting organic molecules,[8)] we have examined the sensing ability of **1** for several guests with different sizes. Table 1 shows variation in the absorbance at 405 nm of **1** (ΔI) induced by the guests (3 x 10^{-4} mol dm^{-3}) together with the binding constants. The decrease of the absorbance at 405 nm relative to its original value (ΔI/I^{o}) was used as a parameter which reflects the sensitivities of the host. It is obvious that 1-adamantanol was detected with a remarkably high sensitivity. The order of the sensitivities of four alcohol guests is 1-adamantanol > *l*-borneol > cyclooctanol > cyclohexanol. This result indicates that guests, which have a suitable size for the CD cavity of **1**, may be detected with high degrees of sensitivity. The order of the sensitivities is roughly parallel to that of the binding constants. Although the yellow color of **1** is not completely faded for the guests examined here, it is noted that the color change is basically color-to-colorless type. All these data demonstrate that this system and more sophisticated systems designed on the same principle may be applicable for detection of various kinds of organic molecules by color-to-colorless change in aqueous solution near neutral pH.

Table 1. Sensivity parameters and binding constants of **1** for various guests at pH 6.50

	AN[a)]	*l*-BN[b)]	C_8OH[c)]	C_6OH[d)]
ΔI/I^{o}	0.230	0.192	0.088	0.018
K (mol^{-1} dm^{3})	15500	5650	1530	140

a) adamantanol, b) *l*-borneol, c) cyclooctanol, d) cyclohexanol.

References
1) H.G. Löhr and F. Vögtle, *Acc. Chem. Res.*, **18**, 65 (1985); D. J. Cram, R. A. Carmack, and R. C. Helgeson, *J. Am. Chem. Soc.*, **110**, 571 (1988); T. Kaneda, S. Umeda, Y. Ishizaki, H. Kuo, and S. Misumi, *J. Am. Chem. Soc.*, **111**, 1881 (1989).

2) M. Inoue, K. Kim, and T. Kitao, *J. Am. Chem. Soc.*, **114**, 778 (1992).

3) A. Ueno, T. Kuwabara, A. Nakamura, and F. Toda, *Nature* , **356**, 136 (1992).

4) Key data for **1**: ^{1}H-NMR (D_2O; 500 MHz); d 3.10-4.10 (m), 4.93-5.07 (7H, m), 6.76 (1H, bs), 7.40 (1H, d), 7.50 (1H, t), 7.60 (2H, m),7.72 (1H, d), 7.96 (1H, dd); Anal. Found: C, 44.31; H, 5.91;N, 1.84%. Calcd for $C_{55}H_{78}N_2O_{38}\cdot6H_2O$: C, 44.54; H, 6.12; N 1.89%.

5) K. A. Connors and J. M. Lipari, *J. Pharm. Sci.*, **65**, 379 (1976).

6) K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn.*, **48**, 375 (1975); H. Shimizu, A. Kaito, and M. Hatano, *ibid*., **52**, 2678 (1979).

7) A. Ueno, I. Suzuki, and T. Osa, *J. Am. Chem. Soc.* , **111**, 6391 (1989); A. Ueno, F. Moriwaki, A. Azuma, and T. Osa, *Carbohyd. Res.* , **192**, 173 (1989); A. Ueno, M. Fukushima, and T. Osa, *J. Chem. Soc., Perkin Trans. 2* , **1990**, 1067; A. Ueno, S. Minato, I. Suzuki, M. Fukushima, M. Ohkubo, T. Osa, F. Hamada, and F. Murai, *Chem. Lett.*, **1990**, 605; S. Minato, T. Osa, M. Morita, A. Nakamura, H. Ikeda, F. Toda, and A. Ueno, *Photochem. Photobiol.*, **54**, 593 (1991); M. Fukushima, T. Osa, and A. Ueno, *Chem. Lett.*, **1991**, 709.

8) Y. Wang, T. Ikeda, A. Ueno, and F. Toda, *Chem. Lett.*, **1992**, 863; A. Ueno, S. Minato, and T. Osa, *Anal. Chem.*, **64**, 1154 (1992); K. Hamasaki, A. Ueno, and F. Toda, *J. Chem. Soc., Chem. Commun.*, **3**, 331 (1993).

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