Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 47 (2006) 4433–4436
Tetrahedron Letters

Synthesis and different molecular recognition of two dye-modified cyclodextrins with spacer of different length

Tetsuo Kuwabara,* Kazuyo Shiba, Mayumi Ozawa, Naoya Miyajima and Yasutada Suzuki

Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4 Takeda, Kofu 400-8511, Japan

Received 12 March 2006; revised 12 April 2006; accepted 14 April 2006
Available online 15 May 2006

This letter is dedicated to late Professor Akihiko Ueno (deceased on March 23rd, 2003) of Tokyo Institute of Technology

Abstract—Two β-cyclodextrin derivatives (**1** and **2**) bearing a hydroxyazobenzene unit, each having a butylene or a 4,7-dioxadecylene spacer between the cyclodextrin and the dye, were prepared, which showed guest-induced color changes with a marked difference in molecular recognition behavior in aqueous solution.

Chemical indicators that change color in response to the presence of organic compounds are of current interest. Host–guest complexation is a key phenomenon to develop such indicators for molecules.[1] Some synthetic indicators are known to exhibit color changes on binding molecules as well as metal ions in organic solvent.[2] However, there are few reports on indicators that act in aqueous solution. Cyclodextrins (CDs), which are cyclic oligosaccharides consisting of several glucose units and capable of forming inclusion complexes with a variety of organic compounds in aqueous solution, are useful for constructing such aqueous-based molecule-sensing systems.[3] During the past decade, we have shown that dye-modified β-CDs exhibit guest-induced color changes with interesting molecular recognition behaviors in aqueous solutions.[4] In these systems, the CD and the dye units act as the guest binding site and the transducer of the guest binding into the color change signals through the guest-induced location change of the dye unit from the inside to outside of the CD cavity, respectively. In this letter, we report the synthesis and unique molecular recognition behaviors of two β-CD derivatives, **1** and **2**, both bearing a hydroxyazobenzene (HAB) unit connected to the CD with a spacer of different length (Fig. 1). Compounds **1** and **2** have different flexibilities and are likely to form the inclusion complex

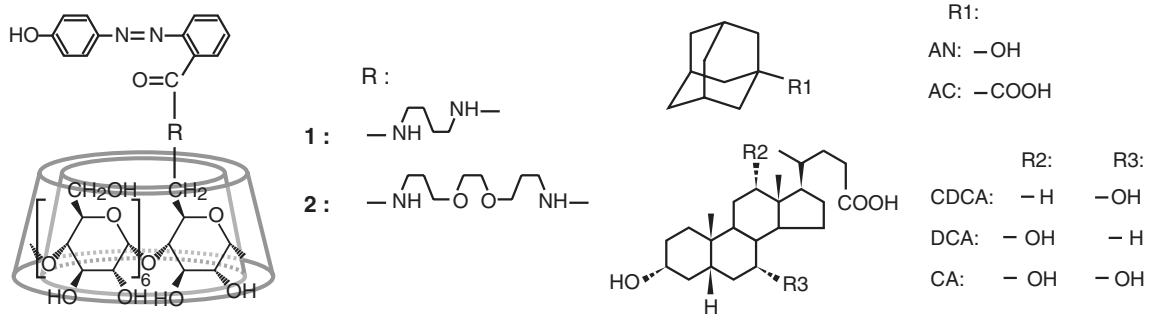


Figure 1. Structure of **1**, **2** and, guest compounds.

Keywords: Cyclodextrin; Molecular recognition; Dye; Inclusion complex; Color change.
* Corresponding author. Tel.:/fax: +81 55 220 8548; e-mail: kuwabara@yamanashi.ac.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.04.056

with different conformations. Such difference is expected to reflect in molecular recognition behaviors.

Compounds **1** and **2** were prepared by the reaction of 6-deoxy-6-tosylated β-CD and 1, 4-diaminobutane or ethylene glycol bis(3-aminopropyl) ether, respectively, followed by the DCC condensation reaction with 2-(4-hydroxyphenylazo)benzoic acid (HABA) in dimethylacetamide. These were purified by ion column chromatography on a QAE-Sephadex and Dianion HP-20, and identified by ^{1}H NMR, TLC, and elemental analysis.[5]

HABA changed from colorless to yellow when the solution became alkaline. This color change involves the structural change of HABA; the phenol form existing under weak acidic condition (pH 5.9) is converted into the phenolate anion form. Compound **1** exhibited the color change similar to HABA itself, as shown in Figure 2a. An absorption band around 350 nm was observed at pH 5.61. The band decreased with increasing pH and a band around 420 nm increased. This indicates the change in structure of the dye unit from the phenol form into the phenolate anion one with increasing pH. Compound **2** also exhibited the pH-induced spectral variation similar to **1**. It was observed that these spectral variations of **1** and **2** were accompanied with two isosbestic points at 381 and 383 nm for **1** and at 381 and 384 nm for **2**, respectively. The observation of two isosbestic points suggests the existence of two acid dissociation equilibria for both compounds.

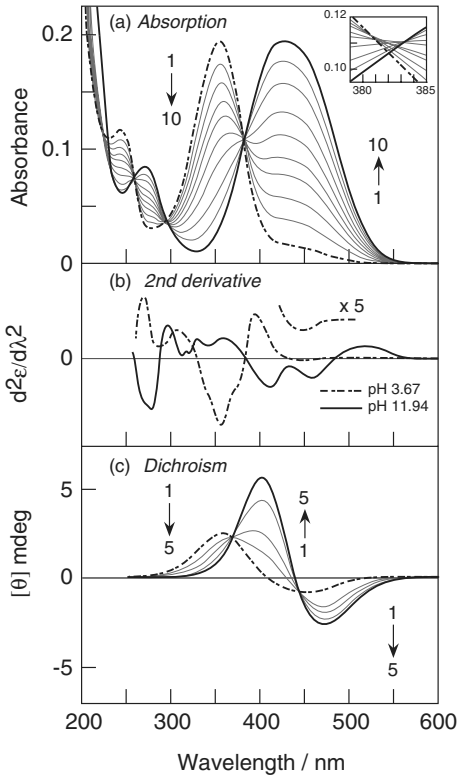
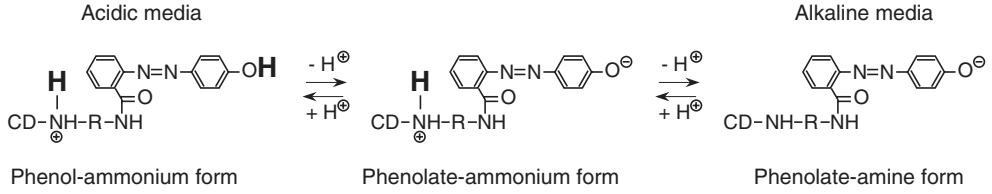


Figure 2. Absorption and the induced circular dichroism spectra of **1** at different pH values, and the second derivative curves of the absorption spectra at pH 3.67 and 11.94. The concentration of **1** is 0.01 and 0.03 mM for absorption and induced circular dichroism measurements, respectively. (a) pH of the solution: (1) 5.61, (2) 7.10, (3) 7.52, (4) 7.83, (5) 8.06, (6) 8.37, (7) 8.65, (8) 8.98, (9) 9.36, (10) 11.90; (c) pH of the solution: (1) 3.63, (2) 7.58, (3) 8.15, (4) 8.95, (5) 12.0.

The curve fitting analysis of the pH-titration curves of absorbance at 440 nm gave two apparent pK_a with the values of 7.44 and 8.67 for **1** and 7.62 and 8.76 for **2**, each corresponding to pK_{a1} and pK_{a2}, respectively. Taking account of the pK_a value for HABA itself (8.64) and diethylamine (11.1),[6] the pK_{a1} and pK_{a2} estimated for **1** and **2** should be associated with the equilibrium between the phenol and the phenolate anion forms in the HAB unit and that between the amine and ammonium forms of the secondary amine group located near the CD cavity, respectively. As shown in Scheme 1, therefore, **1** and **2** change their structures with increasing pH from the phenol–ammonium form into the phenolate–amine form via the phenolate–ammonium form as an intermediate.

In the presence of 1-adamantanol (AN) as the guest, the pH-induced spectral variations of **1** and **2** were similar to those in the absence of the guest. It was observed, however, that the pK_a values were shifted by the presence of the guest, as shown by the values estimated to be 7.39 and 8.56 for **1** and 6.90 and 9.10 for **2**, respectively. Such guest-induced shift in pK_a values are associated with the environmental change around the HAB and the secondary amine group. These results indicate the presence of the guest-induced conformational changes for **1** and **2**.

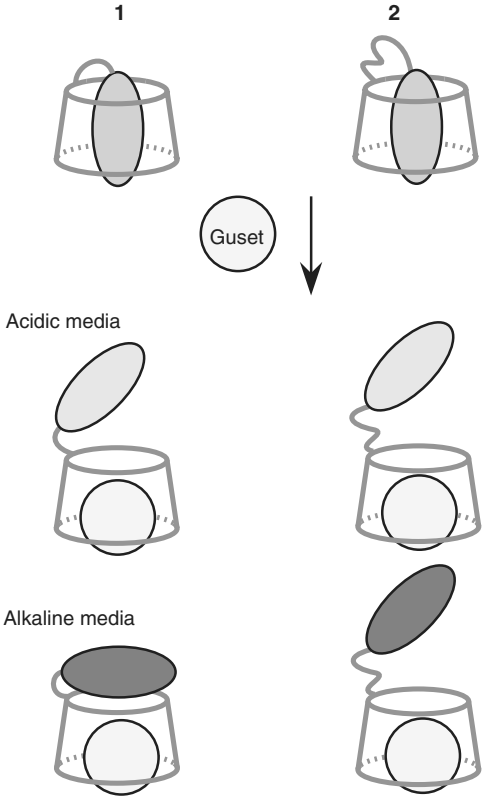
The conformational features were confirmed from the induced circular dichroism spectra.[7] Figure 2c shows the induced circular dichroism spectra of **1** under various pH conditions. The positive and negative dichroism bands were observed around 360 and 450 nm at pH 3.63 and around 400 and 470 nm at pH 12.0, respectively. The analysis of the absorption spectra of **1** gave the electronic transitions at 355 and 450 nm at pH 3.63 and 410 and 460 nm at pH 12.0 (Fig. 2b). These transitions are ascribed to π–π* and n–π* for the shorter and longer



Scheme 1. Schematic representation for acid base dissociation equilibria of **1** and **2**.

wavelengths in each condition, respectively.[8] Since the transition moments are parallel and perpendicular to the HAB unit for π–π* and n–π* transition, respectively, the HAB unit of **1** is included in the CD cavity with an orientation parallel to the CD axis. Under the condition not only at pH 3.63 and pH 12.0 but also in other pH, **1** accommodates the HAB unit in the CD cavity axially. A similar pH-induced dichroism variation was observed for **2**, indicating the similar conformation of **2** to that of **1**. Since the spectral shapes and the molecular extinction coefficients of **1** and **2** were not dependent on their concentrations in the range from 10^{-6} to 10^{-4} M, they exist as the intramolecular self-inclusion complex form, in which the HAB unit is included in their own CD cavity axially.

Upon addition of AN at pH 3.2, the dichroism intensities of **1** and **2** decreased and disappeared in the presence of a large amount of AN. These suggest that the HAB units are excluded to the outside of the cavities. Consequently, the dye units have no interaction with their CD cavities, as shown in Scheme 2. At pH 11.5, however, the guest-induced dichroism variations of **1** and **2** were different. Upon addition of AN, the dichroism pattern of **1** turned over oppositely, in which the positive dichroism band around 400 nm changed to the negative one and the negative band around 470 nm changed to the positive one. This indicates that the HAB unit of **1** changed the orientation from parallel to perpendicular to the CD axis by being excluded to the outside of the cavity (Scheme 2). On the contrary, the dichroism bands of **2** disappeared upon addition of AN, which is similar to the dichroism variation at pH 3.2. These results demonstrate that the guest-induced conformational change is different between **1** and **2**. The longer spacer of **2** can enhance the flexibility of the HAB unit, while the shorter one of **1** restricts the unit. Therefore, the HAB unit interacts with the guest with a different mode, which depends on the pH of the solution as well as the spacer length. Such guest-induced conformational difference observed for **1** and **2** are to be reflected in their molecular recognition abilities.



Scheme 2. Schematic representation of guest-induced conformational change for **1** and **2**.

When AN was added to the solution containing **1** at pH 8.4, the solution changed from colorless to yellow, as shown in Figure 3. The solution of **2** also exhibited a similar spectral variation upon addition of AN at pH 7.2. These indicate the conversion of the HAB unit from the phenol form to the phenolate anion one upon accommodation of AN into the CD cavities, accompanied with the exclusion of the dye unit from the inside to outside of the cavity.

By using this guest-induced color change, we have investigated the molecular recognition ability of **1** and **2**. The guest-induced change in absorbance at 440 nm that is normalized by the original absorbance ($\Delta I/I^0_{440}$) was used as the parameter to evaluate the molecular recognition ability. The results obtained are summarized in Table 1. Both **1** and **2** recognized 1-adamanatanecarboxilic

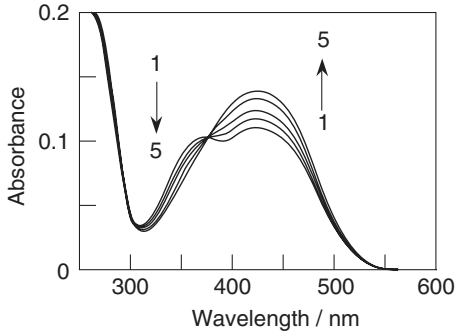


Figure 3. Absorption spectra of **1** (pH 8.4) in the presence of various concentrations of AN: (1) 0, (2) 0.07 mM, (3) 0.13 mM, (4) 0.5 mM, (5) 3 mM.

Table 1. Sensitivity parameter ($\Delta I/I^0$) and binding constants (K/M^{-1}) for **1** and **2**

	1		**2**	
	$\Delta I/I^0$	K/M^{-1}	$\Delta I/I^0$	K/M^{-1}
AN	0.119	4510	0.711	8180
AC	0.093	6380	0.854	25900
CDCA	0.214	46100	0.077	79800
DCA	0.093	2820	0.023	1220
CA	0.060	2990	0.046	1660

Sensitivity parameter ($\Delta I/I^0$) was estimated from the increase in the absorbance at 440 nm (ΔI) relative to its original value (I^0). [Host] = 0.01 mM, [Guest] = 0.2 mM. Binding constant was estimated from the guest-induced absorption variation at 440 nm except for **2** with steroid compounds, which is estimated from the analysis at 340 nm.

acid (AC), which has the carboxyl group instead of the hydroxyl group of AN, with lower affinities than AN because of the negative charge of AC. Interestingly, the different recognition pattern between **1** and **2** was observed for steroid compounds. Chenodeoxycholic acid (CDCA) was recognized by **1** with higher sensitivity than cholic acid (CA), deoxycholic acid (DCA), and AN. CDCA is different from CA only in the hydroxyl group at C12, while DCA is different from CA only in the hydroxyl group at C7. However, no color change of **2** was observed upon addition of CDCA as well as DCA and CA, namely, the steroid compounds could not be recognized by **2**. Bile acids form complex with compound **2**, which was detected with the change in absorbance at 340 nm. However, color change does not take place upon complexation. The reason of this may be the formation of a hydrogen bond between the hydroxyl group of HAB and the carboxyl group of the guest, which stabilizes the phenol form of the HAB, resulting in less change in color. This is an interesting phenomenon not only from the molecular sensing but also from host–guest chemistry viewpoint. The order of the recognition ability of **1** is roughly parallel to that of the binding constants, although the binding constant of **1** is smaller than that of **2**. The HAB unit that interacted with the CD cavity may act as the inhibitor for guest binding for **1**. Compound **2** detected the adamantine derivatives with higher sensitivity than **1**, but did not detect steroid guest at all.

In conclusion, the dye-modified CDs with spacers of different length showed unique molecular recognition, in which the dye unit acts not only as the signal generator but also as the molecule recognition site. Further works to clarify the mechanism of the molecular recognition involving the hydrogen bond in aqueous solution and the relationship between the spacer length and the molecular recognition ability is under way.

Acknowledgments

This work was supported by the Asahi Glass Foundation No. 02A-C01-P054 and a Grants-in-Aid No. 12750727 for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. The authors would like to thank Professor Masato Nanasawa (Yamanashi University) for helpful discussion.

References and notes

1. (a) Brzózka, Z.. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Suslick, K. S., Eds.; Pergamon: Oxford, 1996; Vol. 10, pp 187–212; (b) Pietraszkiewiez, M. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Suslick, K. S., Eds.; Pergamon: Oxford, 1996; Vol. 10, pp 255–266; (c) Lehn, J.-M. *Supramolecular Chemistry*; VCH Weinheim, 1995, p 90.
2. (a) Kubo, Y.; Maeda, S.; Tokita, S.; Kubo, M. *Nature* **1996**, *382*, 522–523; (b) Tsubaki, K.; Morimoto, T.; Otsubo, T.; Fuji, K. *Org. Lett.* **2002**, *4*, 2301–2304.
3. Szeijtli, J. *Cyclodextrin and Inclusion Compounds*; Academic Kiado: Budapest, 1982.
4. (a) Ueno, A.; Kuwabara, T.; Nakamura, A.; Toda, F. *Nature* **1992**, *356*, 136–137; (b) Kuwabara, T.; Nakajima, H.; Nanasawa, M.; Ueno, A. *Anal. Chem.* **1999**, *71*, 2844–2849; (c) Matsushita, A.; Kuwabara, T.; Nakamura, A.; Ikeda, H.; Ueno, A. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1705–1710; (d) Kuwabara, T.; Takamura, M.; Matsushita, A.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, T. *J. Org. Chem.* **1998**, *63*, 8729–8735.
5. Compound **1**: R_f: 0.07 (*n*-butanol/ethanol/water = 5:4:3). Anal. Calcd for $C_{59}H_{88}N_4O_{36}{\cdot}HCl{\cdot}4H_2O$: C, 46.08; H. 6.36; N, 3.64; Cl, 2.42. Found: C, 46.12; H, 6.24; N, 3.21; Cl, 2.05. ^{1}H NMR (D_2O, 400 MHz): δ 7.63 (2H, d), 7.45–7.62 (4H, m), 6.95 (2H, d), 4.88–5.0 (7H, m), 4.0–3.35 (m), 3.28 (2H, br d), 3.05 (2H, br t), 2.70–2.90 (4H, m), 1.58 (4H, br s). Compound **2**: R_f: 0.09 (*n*-butanol/ethanol/water = 5:4:3). Anal. Calcd for $C_{63}H_{96}N_4O_{38}{\cdot}9H_2O$: C, 45.05; H, 6.84; N, 3.34. Found: C, 44.95; H, 6.10; N, 3.23. ^{1}H NMR (D_2O, 400 MHz): δ 7.65 (2H, d), δ 7.50–7.40 (6H, m), 6.97 (2H, d), 4.83–4.95 (7H, m), 3.20–4.0 (m), 3.04–3.15 (4H, m), 2.91 (4H, m), 1.80–1.60 (4H, br s).
6. Dictionary of Organic Compounds, Chapman & Hall, Electronic Publishing Division.
7. Harada, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375–379.
8. Kronor, J.; Bock, H. *Chem. Ber.* **1968**, *101*, 1922.