Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **10**: 443–451, 1991.

The Conformational and Binding Properties of Ethylbipyridinio-Modified β-Cyclodextrin Using Induced Circular Dichroism

YI-QUN DU, ASAO NAKAMURA, and FUJIO TODA*
Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152, Japan

(Received 6 September 1990; in final form: 17 December 1990)

Abstract. The conformational and binding properties of mono-6-deoxy-6[4-(1-ethyl-4-pyridinio)-1-pyridinio]-β-cyclodextrin (**1**) in complex formation with some guest compounds were examined by induced circular dichroism (ICD) spectra in aqueous solution. Compound **1** showed much stronger binding ability for some guest compounds (1:1 complexes), compared with β-cyclodextrin (β-CDx) and a positively charged β-CDx [C-6-mono-pyridino-β-CDx (**2**)]. Marked conformational changes of **1** (the spatial position of the ethyl viologen (C_2V^{2+}) group relative to the cavity in **1**) were observed upon complex formation with some guests like 1-adamantanecarboxylic acid (ACA) and sodium cholate (SC).

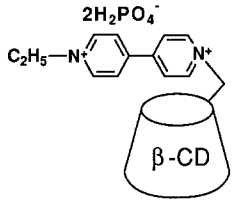
Key words. Cyclodextrin, ethyl viologen, binding ability, conformation, steroidal guest, complex.

1. Introduction

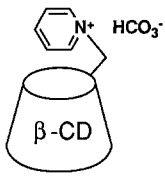
Cyclodextrins (CDxs) are torus-shaped cyclic oligomers of D-glucopyranose [1]. They have an ability to incorporate guest molecules in aqueous solution into their hydrophobic cavity. Utilizing this property, we intend to make a molecular device for photochemical charge separation with cyclodextrin inclusion complexes, and we have prepared viologen-appended CDx [mono-6-deoxy-6[4-(1-ethyl-4-pyridinio)-1-pyridinio]-β-cyclodextrin (**1**)] [2]. Viologens (4,4′-dipyridium salts) are good electron acceptors which act as mediators between the photosensitizer and the catalyst for hydrogen production [3], or between the photosensitizer and the enzyme–coenzyme system for NADH production [4]. As a basic study of such a molecular device, it is necessary to estimate quantitatively the ability of **1** to bind the guests in the cavity and the conformational properties of **1** in complex formation with the guests, because the spatial arrangement of the ethyl viologen (C_2V^{2+}) group relative to the cavity in **1** is important to control the rate of electron transfer in photochemical reactions.

We have previously reported the preparation of **1** and its conformation in aqueous solution [2]. In the present study we report the binding properties of **1** with some guests like 1-adamantanecarboxylic acid (ACA) or 1-adamantanol (ADN) and the conformational properties of **1** in the formation of each inclusion complex, as analyzed by induced circular dichroism (ICD) spectroscopy. The binding abilities of C-6-mono-pyridinio-β-CDx (**2**) and β-CDx with these guests are also described here for comparison. The inclusion phenomena between CDxs

* Author for correspondence.



1



2

and other spectroscopically inert guests have usually been studied by using spectroscopically active guests that exhibit changes in their absorption or fluorescence spectra upon complexation with CDxs as competitive ligands. To date there have been few reports which examine quantitatively the inclusion ability of CDxs with spectroscopically inert guests by ICD spectroscopy. Both the sign and the intensity of the ICD band provide the information on the extent of the complex formation as well as on the molecular structure of the complex. Moreover, the intensity of ICD spectra is proportional to the concentration of the CDx complex, so that the inclusion phenomena can be observed directly from the changes of ICD spectra and the measurement may become more accurate and easier.

2. Experimental

2.1. MATERIALS

β-Cyclodextrin (β-CDx) (Nihon Shokuhin Kako Co., Ltd.), *p*-toluenesulfonyl chloride (Kanto Chemical Co), *p*-nitrophenol (pNP$^-$), 1-borneol (BN), ursodesoxycholic acid (UCA) (Tokyo Kasei), 1-adamantanecarboxylic acid (ACA) (Tokyo Kasei.), 1-adamantanol (ADN) and sodium cholate (SC) (Wako Pure Chemical Ind Ltd.), were used without further purification.

C-6-Mono-tosylated-β-CDx was prepared in alkaline aqueous solution in the same manner as described in Ref. [6]. The crude product was purified by repeated recrystallization from water and the mixed solvent of methanol and water (5% by volume).

Mono-6-deoxy-6[4-(1-ethyl-4-pyridinio)-1-pyridinio]-β-cyclodextrin (**1**) was prepared as reported previously [2].

C-6-Mono-pyridinio-β-CDx (**2**) was prepared using a similar method to that described in Ref. [6]. C-6-Mono-tosylated-β-CDx (2 g) was added to pyridine (80 mL) and the solution was kept at 95°C for 48 h. After reprecipitation with acetone, the product was purified by gel chromatography on Sephadex G-10 and ion-exchange chromatography on a CM-Sephadex C-25 column with 0.05 M aqueous sodium bicarbonate buffer as an eluent. (0.3 g, 15% yield).

Anal. Found: C, 44.35; H, 6.16; N, 1.08. Calc. for $C_{47}H_{74}O_{34}N_1 \cdot HCO_3 \cdot 2\,H_2O$: C, 44.5; H, 6.1; N, 1.08.

2.2. MEASUREMENTS

Induced circular dichroism (ICD) spectra were recorded on a JASCO J-600 spectropolarimeter, using a 1 cm cell at 25°C.

Determination of the binding constant (K) of each host (**1**, **2** or β-CDx) with pNP$^-$ – various concentrations of each host were added to an aqueous buffer solution containing 5×10^{-5} M pNP$^-$ (pH 9.6 boric acid–NaOH buffer). The changes of the ICD spectra were recorded in the wavelength region of the absorption band for pNP$^-$ (400–420 nm).

Determination of the binding constant (K_1) of each host with other guests (ADC, ADN, BN, UCA or SC) – various concentrations of each guest were added to an aqueous buffer solution containing 5×10^{-5} M pNP$^-$ and 5×10^{-4} M of each host (pH 9.6 boric acid–NaOH buffer), respectively; then the changes of the relative ICD spectral intensity of pNP$^-$/host were recorded at 410 nm.

Determination of the conformation of **1** in the presence of each guest. Each guest (1.5×10^{-3} M) was added to an aqueous buffer solution containing 1×10^{-4} M **1** (pH 9.6 boric acid–NaOH buffer); then ICD spectra intensity were recorded at 255 nm.

3. Results and Discussion

3.1. DETERMINATION OF THE BINDING CONSTANT OF EACH HOST (1, 2 or β-CDx) WITH pNP$^-$

In Figure 1, the changes in the ICD intensity are shown on the ICD spectra in the presence of pNP$^-$ by adding various concentrations of **1** in the absorption region of pNP$^-$. An achiral guest molecule included in a chiral CDx cavity may exhibit an ICD in its absorption regions. The results in Figure 2 indicate that the complex was formed between **1** and pNP$^-$. A similar tendency was also seen in **2** and β-CDx with pNP$^-$.

Figure 2 shows the dependence of the ICD intensity of pNP$^-$ on the concentration of **1**, **2** or β-CDx at 410 nm as a function of the concentration of the complex formation of **1**, **2** and β-CDx with pNP$^-$. The curves were hyperbolic, which indicates that only 1 : 1 complex binding occurred in these systems. From the relation in Figure 2 we tried to estimate the binding constant for **1**, **2** and β-CDx with pNP$^-$, respectively.

When each host forms only a 1 : 1 complex with pNP$^-$ and each host is in large excess, the binding constant K can be approximately represented by the following equation.

$$K = x/h_0(p_0 - x) \tag{1}$$

Where p_0 = initial concentration of pNP$^-$ (5×10^{-5} M), h_0 = initial concentration of each host, and x = the concentration of the pNP$^-$–host complex.

The x value can be calculated from

$$x = p_0(\Delta\varepsilon/\Delta\varepsilon_{max}) \tag{2}$$

Where $\Delta\varepsilon$ = molar circular dichroism for the complex and $\Delta\varepsilon_{max}$ = the highest observed value of $\Delta\varepsilon$ at infinite concentration of each host.

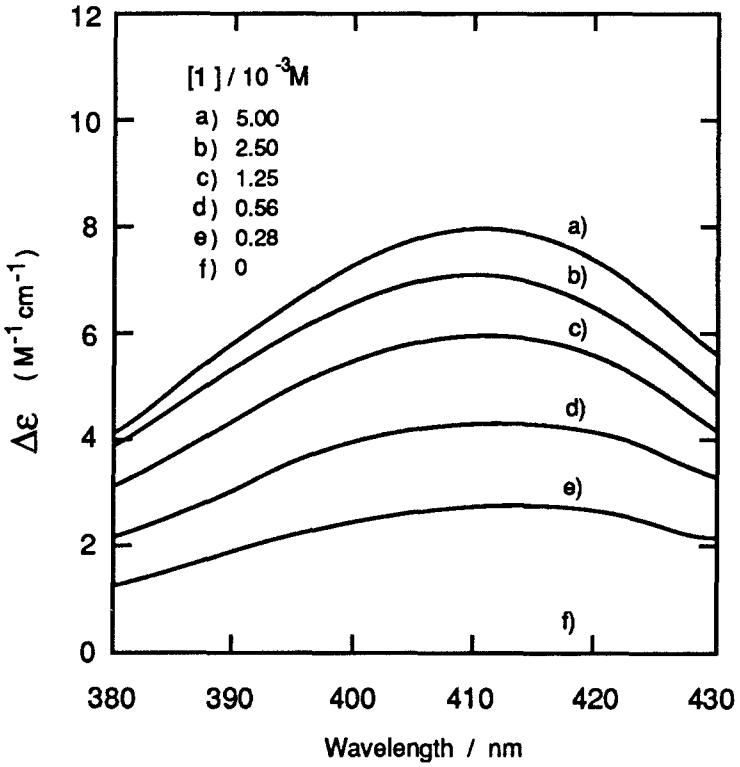


Fig. 1. ICD spectra of pNP$^-$ (5×10^{-5} M) in the presence of various concentrations of **1** at 25°C in the pH 9.6 borate buffer.

Equations 1 and 2 yield

$$\Delta\varepsilon = Kh_0\,\Delta\varepsilon_{max}/(1 + Kh_0) \tag{3}$$

The binding constants (K) and $\Delta\varepsilon_{max}$ values as shown in Table I were obtained by using Eq. 3. [plot of $\Delta\varepsilon$ vs h_0]. It indicates that the order of magnitude of K with pNP$^-$ was **1** > **2** > β-CDx.

3.2. DETERMINATION OF THE BINDING CONSTANT OF EACH HOST WITH OTHER GUESTS (ACA, ADN, BN, UCA OR SC)

Figure 4 shows the changes in the relative ICD intensity for the pNP$^-$–**1** complex with increasing concentration of ACA at 410 nm. It indicates that ACA undergoes complex formation with **1** in competition with pNP$^-$. Competition was also seen between pNP$^-$ and other guests. The binding constant for **1** with another guest (G) like ACA etc. may be obtained from a plot like that in Figure 5. When host (H) is in a larger excess than pNP$^-$, the two equilibria can be represented by the following two equations:

$$H + pNP^- \rightleftharpoons H\text{–}pNP^- \tag{4}$$

$$H + G \rightleftharpoons H\text{–}G \tag{5}$$

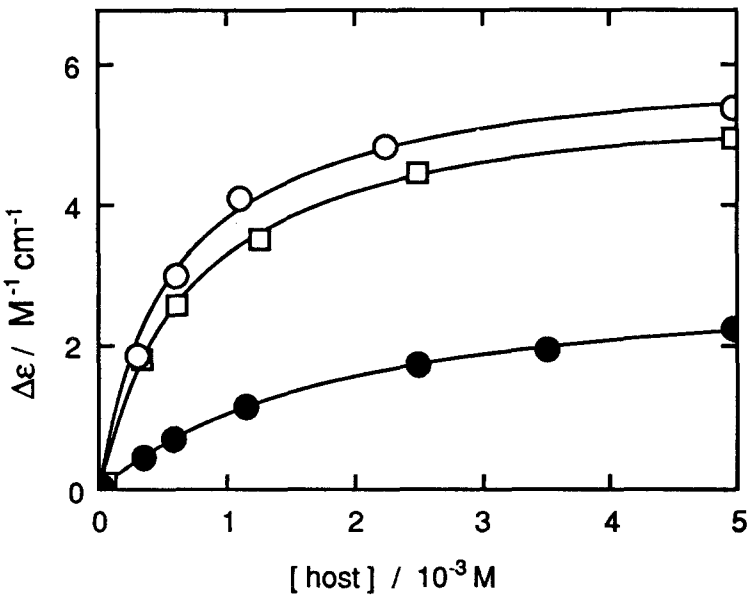


Fig. 2. Dependence of ICD intensity of pNP$^-$ on total concentration of **1** (○), **2** (□) and β-CDx (●) in pH 9.6 borate buffer. Total concentration of pNP$^-$ is 5×10^{-5} M. Wavelength = 410 nm.

Table I. Circular dichroism spectral data and the values of binding constants (K) for the inclusion complexes of **1**, **2** and β-CDx with pNP$^-$.

Host	K (M^{-1})	$\Delta\varepsilon_{max}$ ($M^{-1}\,m^{-1}$)
1	1690	6.01
2	1398	5.59
β-CDx	543	4.5

At 25°C in pH 9.6 borate buffer.

The binding constant with pNP$^-$ (K) and that of the other guest (K_1) are described as follows:

$$K = x/h(p_0 - x) \tag{6}$$

$$K_1 = x_1/h(g_0 - x_1) \tag{7}$$

The free concentration of host (h) is described as follows:

$$h = h_0 - x - x_1 \tag{8}$$

Where p_0 = initial concentration of pNP$^-$ (5×10^{-5} M), g_0 = initial concentration of another guest, x = concentration of pNP$^-$–H complex, x_1 = concentration of G–H complex.

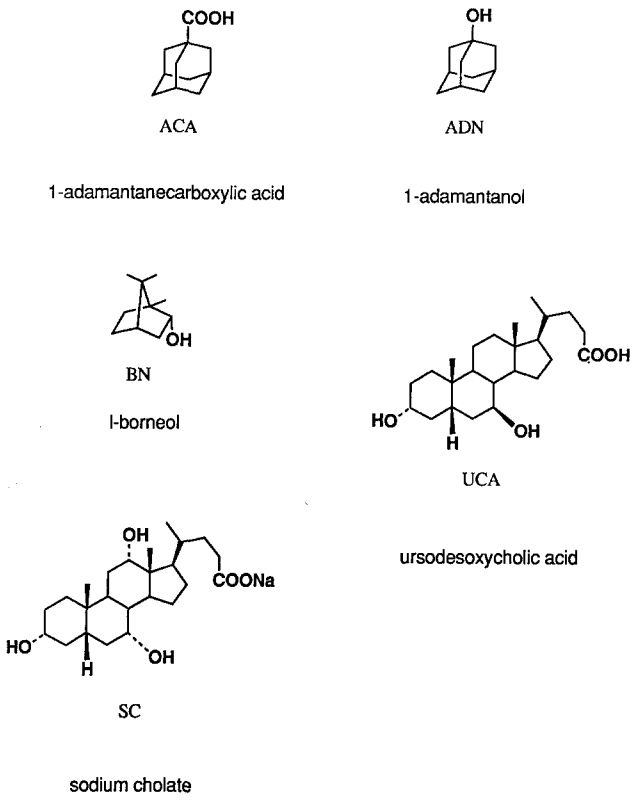


Fig. 3. The molecular structure of the guests.

Under the present conditions (h and $x_1 \gg x$), h can be approximately represented by the following expression:

$$h = h_0 - x_1 \tag{9}$$

From Eqs. 7 and 9, Eq. 10 can be derived.

$$h = [-\{1 + K_1(g_0 - h_0)\} + \{[1 + K_1(g_0 - h_0)]^2 + 4K_1h_0\}^{1/2}/2K_1 \tag{10}$$

Where h_0 = total concentration of each host (5×10^{-4} M), h may be calculated from Eqs. 6 and 2 where the values of $\Delta\varepsilon_{max}$ and K for **1**, **2** and β-CDx were shown in Table I. Binding constants (K_1) (Table II) were obtained by fitting Eq. 10 to the data with a non-linear least-square fitting procedure as shown in Figure 5.

As a result, compound **1** showed the strongest binding ability among the three hosts, although the binding ability of **2** was stronger than that of β-CDx. The magnitude of the binding constants (K_1) was in the order, ACA > UCA > ADN > BN > SC for each host.

These results show that the electrostatic interaction which acts between a doubly positively charged host like **1** and a negatively charged guest is stronger than that between a singly positively charged host like **2** and a negatively charged guest [6]. The binding ability of **1** for negatively charged guests like ACA was stronger than that for neutral guests like ADN (the K_1 value for **1** with ACA was 4.3 times larger

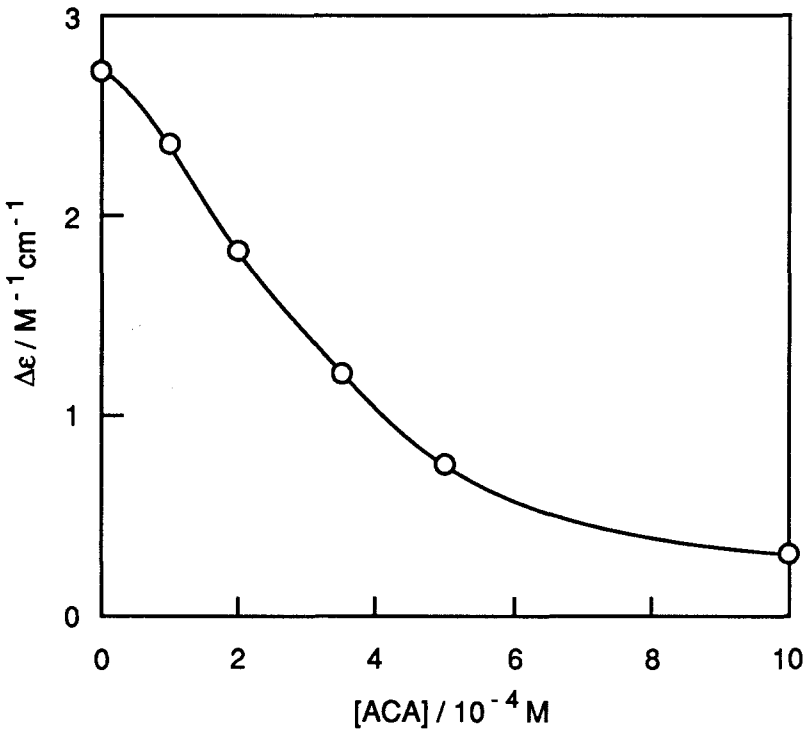


Fig. 4. Relative ICD intensity of pNP$^-$/1 (pNP$^-$ = 5 × 10^{-5} M) at various concentrations of ACA, at 25°C in pH 9.6 borate buffer. Wavelength = 410 nm.

than that of β-CDx and that of **1** for ADN was 2.3 times larger than that of β-CDx. The K_1 values of **1** for ACA and ADN were 1.5 and 2 times larger than those of **2** for ACA and ADN, respectively). Some changes in non-electrostatic interaction between host and guests also occurred. The K_1 values of **1** for some neutral guests like ADN or BN were larger than those of β-CDx. The results suggest that the C_2V^{2+} moiety protects the binding site of **1** from contact with a water molecule, to facilitate the stability of the inclusion complex of **1** for the guest. This capped effect was also seen in the results for **2**, but the effect in **2** was smaller than that in **1**. The differences in binding ability between **1** and **2** were regarded as the differences in the molecular charge values and the size between the bipyridinio group of **1** and the pyridinio group of **2**.

3.3. DETERMINATION OF THE CONFORMATION OF THE COMPLEX OF 1 WITH ACA, ADN, BN, UCA OR SC

Table III shows ICD spectral data of **1** in aqueous solution in the presence and absence of the various guests. Compound **1** gave a negative ICD sign in ICD spectra. According to the Kirkwood–Tinoco coupled oscillator model and the Kajtar *et al.* sector rule [7], the negative ICD sign of the band indicates that the transition dipole moment vector of an arene moiety included in the cavity of CDx is perpendicular to the symmetry axis of the host. On this basis, the conformation

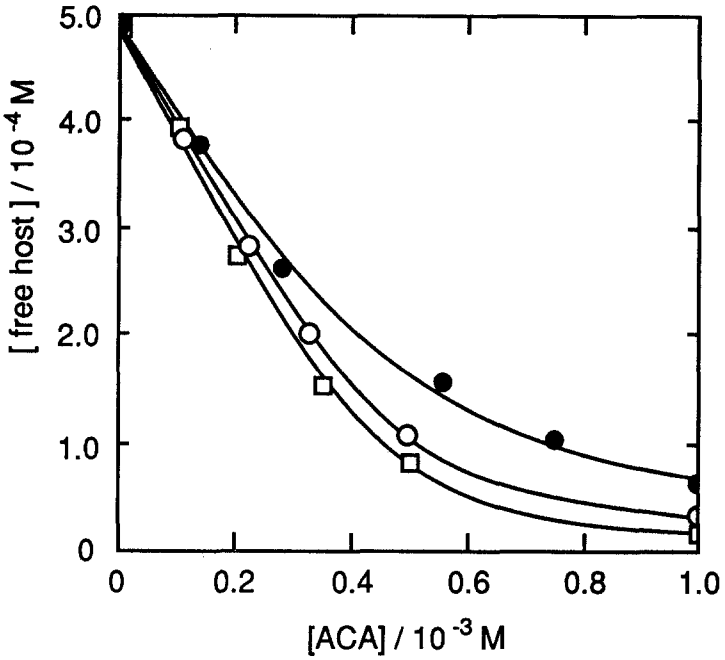


Fig. 5. Relation between concentration of free molecules of **1** (□), **2** (○) or β-CDx (●) and concentration of ACA, respectively, at 410 nm in pH 9.6 borate buffer solution (pNP$^-$ = 5×10^{-5} M, **1**, **2** or β-CDx = 5×10^{-4} M).

Table II. The values of the binding constant (K_1) for **1**, **2**, and β-CDx with ACA, ADN, UCA, SC and BN, respectively.

Host	K_1(M^{-1})				
	ACA	ADN	UCA	SC	BN
1	47600	11000	23700	3100	5000
2	31700	5360	17600	1830	2560
β-CDx	11100	4700	7840	925	1210

At 25°C, in pH 9.6 borate buffer.

Table III. Change in the circular dichroism for the C_2V^{2+} group of **1** in the presence and absence of the various guests.

	1	**1**/ACA	**1**/ADN	**1**/UCA	**1**/SC	**1**/BN
Δε (M^{-1} cm^{-1})	(−) 2.73	(−) 3.5	(−) 2.8	(−) 2.88	(−) 2.2	(−) 2.73
Δε/Δε**1**	1.00	1.30	1.03	1.05	0.81	1.00

[**1**] = 1×10^{-4} M; [each guest] = 1.5×10^{-3} M.
At 25°C in pH 9.6 borate buffer.

of **1** is suggested to be an equatorial inclusion, although there remains a doubt whether or not this simple rule may be applied to **1** because the C_2V^{2+} moiety is attached to the edge of the host cavity. When ADN, BN and UCA were added to the aqueous solution, little change was observed in the ICD spectral intensity. However, the increase in the absolute intensity of the ICD band was observed, when ACA was added to the aqueous solution of **1**. In contrast to ACA, a decrease in the intensity of the ICD band was observed by the addition of SC to the aqueous solution of **1**. One possible explanation is that the C_2V^{2+} group moves nearer to the cavity of **1** on complex formation with ACA but the C_2V^{2+} group becomes more distant from the cavity of **1** on complex formation with SC. Both ACA with ADN have the same adamatane unit, but the binding ability of **1** for ACA was 4.3 times larger than that of **1** for ADN, as shown in Table II. The difference in the location of the C_2V^{2+} moiety for the complexes may be reflected in the binding difference. The result that the C_2V^{2+} group of **1** is nearer to the cavity of **1** in **1**/ACA than in **1**/ADN suggests that the electrostatic, hydrophobic and other interactions between **1** and ACA become stronger than between **1** and ADN.

4. Conclusion

The method of examining the inhibitory effect of some spectroscopically inert compounds upon the ICD spectra of pNP$^-$ bound to CDxs was used to measure the binding constants of CDxs.

Compound **1** exhibits a much stronger binding ability for pNP$^-$ and some guest compounds than **2** and β-CDx. **1** exhibits conformational changes upon inclusion complex formation. The difference of the conformation of **1** was also reflected in the magnitude of the binding constants of **1** for the guests. The above results will be very useful for further work to make clear and to control the properties of **1** in photoreduction reactions.

References

1. M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag (1978).
2. Y. Du, A. Nakamura and F. Toda: *Bull. Chem. Soc. Jpn.* **63**, 3351 (1990).
3. M. Gratzel: *Ber. Bunsenges. Phys. Chem.* **84**, 981 (1980).
4. I. Willner, D. Mandler and A. Riklin: *J. Chem. Soc., Chem. Commun.* 1986, 1022.
5. K. Takahashi, K. Hattori and F. Toda: *Tetrahedron Lett.* **25**, 3331 (1984).
6. Y. Matsui, K. Ogawa, S. Mikami, M. Yoshimoto and K. Mochida: *Bull. Chem. Soc. Jpn.* **60**, 1219 (1987); Y. Matsui and A. Okimoto: *Bull. Chem. Soc. Jpn.* **51**, 3030 (1978).
7. K. Harata and H. Uedaira: *Bull. Chem. Soc. Jpn.* **48**, 375 (1975); T. Murakami, K. Harata and S. Morimoto: *Chem. Lett.* 1989, 341; M. Kajtar, Cs. Horvath-Toro, E. Kuthi and J. Szejtli: *Acta Chim. Acad. Sci. Hung.* **110**, 327 (1982).