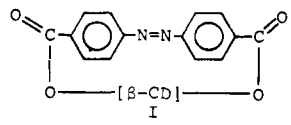
number of oxo vs. sulfur ligands. Quantitative aspects of these effects will be published in a subsequent paper.
(20) S. P. Cramer, H. B. Gray and K. V. Rajagopalan, *J. Am. Chem. Soc.*, accompanying paper in this issue.
(21) Recipient of a National Institutes of Health Postdoctoral Award No. GM 0657-02.
(22) Fellow of the Alfred P. Sloan Foundation, 1976–1978.

Thomas D. Tullius, D. M. Kurtz, Jr.[21]
S. D. Conradson, Keith O. Hodgson* [22]
Department of Chemistry, Stanford University
Stanford, California 94305
Received October 3, 1978

Photocontrol of Binding Ability of Capped Cyclodextrin

Sir:

Photosensitive systems are ubiquitous in nature. Many of the life processes of plants are regulated by the effect of light on the phytochrome system. The process of vision falls into the category of fundamental animal responses. These physiological changes are believed to be linked with light-induced structural changes.[1] The activity of biologically active macromolecules can be regulated by low-molecular-weight photochromic molecules capable of assuming (at least) two states.[1] As one approach to mimic such biological systems, we have examined the photoresponsive behavior of polypeptides containing azobenzene moieties in their side chains and have found the existence of light-induced conformational changes.[2] An additional refinement in our studies is the photoregulation of functions such as substrate binding and catalytic activity. From this strategy, we prepared azobenzene-capped β-cyclodextrin I to regulate the binding ability of β-cyclodextrin (β-CD) by light.[3] The parent β-CD itself represents a good enzyme model because of its ability to bind substrates into its cavity in aqueous solution. Compound I is expected to act as a photoregulated "switch" since the cap azobenzene undergoes cis–trans isomerization by photoirradiation, and the reversion of the cis isomer back to the trans takes place in the dark.[4] We now report (i) the preparation of I; (ii) changes in the circular dichroism spectra of I on addition of guest molecules; (iii) photoregulation of the binding ability of I, and (iv) the presence of 1:2 host–guest complexes[5] and photoregulation of their formation.



I

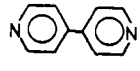
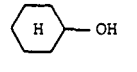
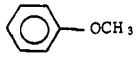
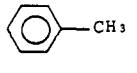
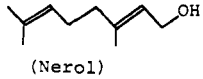
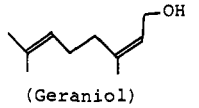
Compound I was obtained by condensation of 4,4′-bis-(chlorocarbonyl)azobenzene with β-CD in pyridine (20% yield). Recrystallization from water and Sephadex G-15 gel filtration[6] gave a pure sample of I.[7]

Figure 1 shows the circular dichroism spectra of a 5×10^{-5} M solution of I in water (Tris buffer, pH 7.2) before and after photoirradiation, alone and in the presence of excess cyclohexanol. Compound I presents an induced circular dichroism band in the azobenzene π–π^* region (355 nm) before irradiation, whereas it shows another band in the azobenzene n–π^* region (445 nm) after irradiation. Both induced circular dichroism bands nearly vanish on addition of guest molecules in large excess. This observation might reflect the transformation of β-CD residue from a "tense" conformation to a "relaxed" one upon inclusion of guest molecules as was reported by Saenger et al. for α-CD.[8] Formation constants K (or dissociation constants K_d) of *trans*-I and *cis*-I were obtained from the circular dichroism spectra (intensities at 355 and 445 nm were used for *trans*-I and *cis*-I, respectively) using the formula

$$K = \frac{\theta_I - \theta_x}{(\theta_x - \theta_s)\left[C_s - C_I \dfrac{\theta_I - \theta_x}{\theta_I - \theta_s}\right]}$$

which was reported by Mack et al. for 1:1 host/guest complex formation[9] where θ = molar ellipticity, θ_x for sample, θ_I for I alone, θ_s for highest substrate excess, C_I = total I concentration, and C_s = total substrate concentration. It was found that there are some cases which do not follow the formula but proceed according to the equation[10]

Table I. Dissociation Constants for Complexes of *trans*-I and *cis*-I with Various Substrates[a]

guest	host	$K_d(K_{d1})$, M	K_{d2}, M	$\frac{K_d(trans\text{-I})}{K_d(cis\text{-I})}$	$\frac{K_d(trans\text{-I or } cis\text{-I})}{K_d(\beta\text{-CD})}$
	β-CD	7.3×10^{-3}			
	trans-I	no complex formed			
	cis-I	2.2×10^{-3}			0.30
	β-CD	2.5×10^{-3} [b]			
	trans-I	3.9×10^{-3}		2.0	1.6
	cis-I	2.0×10^{-3}	1.6×10^{-3}		0.80
	β-CD	1.9×10^{-2}			
	trans-I	5.0×10^{-2}		3.8	2.6
	cis-I	1.3×10^{-2}	7.8×10^{-3}		0.68
	β-CD	1.5×10^{-1}			
	trans-I	4.9×10^{-2}		4.5	0.33
	cis-I	1.1×10^{-2}	1.2×10^{-2}		0.073
 (Nerol)	β-CD	1.7×10^{-3}			
	trans-I	3.2×10^{-3}		3.8	1.9
	cis-I	8.5×10^{-4}	1.5×10^{-3}		0.50
 (Geraniol)	β-CD	1.5×10^{-3}			
	trans-I	3.6×10^{-3}		8.6	2.4
	cis-I	4.2×10^{-4}	2.9×10^{-3}		0.28

[a] In 0.05 M Tris buffer (pH 7.2) at 25 °C. Substrates were added as CH_3CN solutions (total content of CH_3CN is smaller than 1% (v/v)). [b] Reported value, 2.0×10^{-3} M, in 0.05 M borate buffer (pH 10.0) at 25 °C.[3d]

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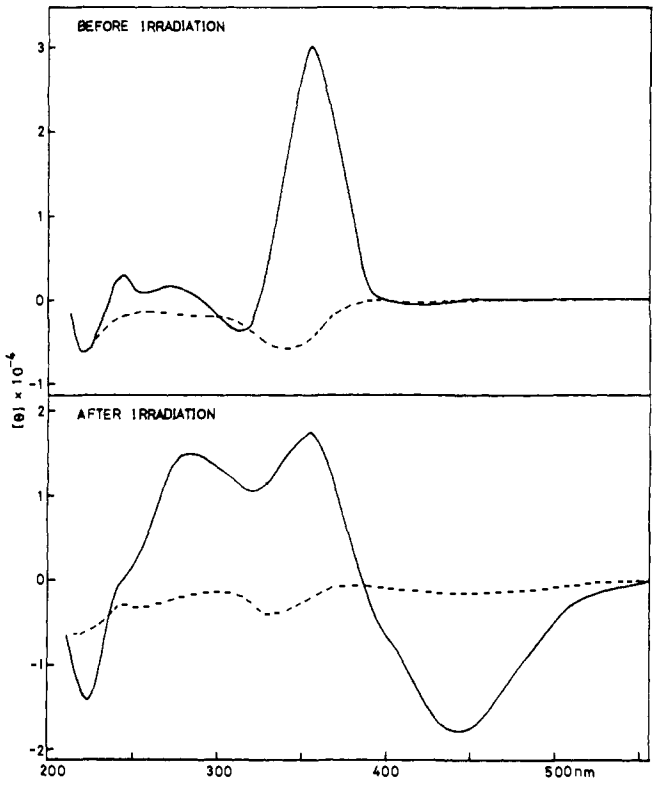
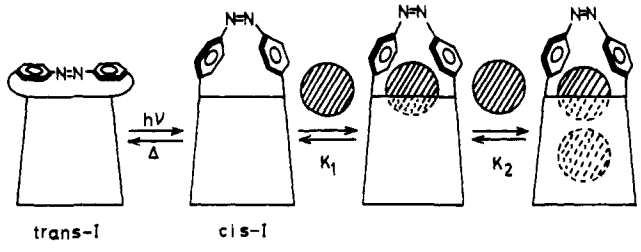


Figure 1. Circular dichroism of I before and after photoirradiation, alone (—) or in the presence of 2000-fold excess cyclohexanol (- - -). [I] was 5 × 10^{-5} M in Tris buffer (pH 7.2).

$$K = K_1 + K_1K_2C_s$$

assuming 1:2 host-guest[3a] stoichiometry (Figure 2) where K_1 and K_2 are formation constants (Scheme I). To compare the

Scheme I



binding ability of the capped CD with that of the parent β-CD, dissociation constants of β-CD–guest complexes were obtained from fluorescence measurements with 1-anilino-8-naphthalenesulfonate.[3b,d]

As shown in Table I, *cis*-I tends to include two guest molecules, whereas, *trans*-I includes one guest. Molecular models suggest that this is because the cavity of *cis*-I is large enough to accommodate two guest molecules. Another effect of photoirradiation is shown in the change in K_d. The values of K_d for *cis*-I are smaller than those for *trans*-I. The most striking result was obtained in the case of γ,γ′-dipyridyl; *cis*-I can bind the guest (K_d = 2.2 × 10^{-3} M), whereas *trans*-I cannot bind at all. This suggests that γ,γ′-dipyridyl is too large to be included in the cavity of *trans*-I, but can be included in the expanded cavity of *cis*-I. A comparison of the dissociation constants of *trans*-I with those of β-CD indicates that *cis*-I binds a guest molecule more strongly than does β-CD, whereas, *trans*-I binds more poorly than does β-CD except for toluene. This behavior is in accord with the "shallow floor" concept suggested by Emert and Breslow for their flexibly capped β-CD.[3a] Cyclodextrins modified by capping were reported to bind an

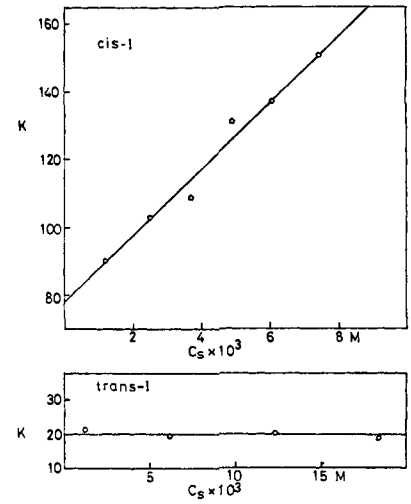


Figure 2. Plots of formation constants of *trans*-I and *cis*-I against anisole concentration.

aromatic guest more poorly[3a] or more strongly[3b] than does the parent CD. It is noted here that the binding ability of I depends on the structure of the cap and the kinds of guest molecules.

Photocontrol of catalytic activity of I in ester hydrolysis is now under way.

References and Notes

(1) (a) B. F. Erlanger, *Annu. Rev. Biochem.*, **45**, 267 (1976); (b) G. Montagnoli, *Photochem. Photobiol.*, **26**, 679 (1977), and references cited therein.
(2) (a) A. Ueno, J. Anzai, T. Osa, and Y. Kadoma, *J. Polym. Sci., Polym. Lett. Ed.*, **15**, 407 (1977); (b) A. Ueno, J. Anzai, T. Osa, and Y. Kadoma, *Bull. Chem. Soc. Jpn.*, **50**, 2995 (1977); (c) A. Ueno, J. Anzai, T. Osa, and Y. Kadoma, *ibid.*, **52**, 549 (1979).
(3) (a) J. Emert and R. Breslow, *J. Am. Chem. Soc.*, **97**, 670 (1975); (b) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakawa, and K. Fujita, *ibid.*, **98**, 7855 (1976); (c) I. Tabushi, K. Fujita, and L. C. Yuan, *Tetrahedron Lett.*, 2503 (1977); (d) I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura, *J. Am. Chem. Soc.*, **99**, 7100 (1977).
(4) Photoirradiation was done with a 500-W Xenon lamp using a Corning 7-37 filter to pass the light of 320–390 nm. The cis per cent of I at the photostationary state was calculated to be 30 using the absorbance at 335 nm.[2b] The half-life of *cis*-I is 255 min at 40 °C.
(5) Emert and Breslow reported the formation of a 2:1 complex between adamantanecarboxylic acid and β-CD.[3a]
(6) The recrystallized solid (200 mg) was dissolved in 20 mL water and passed through a Sephadex G-15 column (2.5 × 70 cm). The column was eluted with distilled water. Fractions of 5 mL were collected after 100-mL elution, and each fraction was checked with a UV spectrophotometer both before and after phenol–sulfuric acid treatment.[6a] Fractions 1–30 contained I, while fractions 20–50 contained β-CD (fractions 9 and 33 gave the maximum absorptions). Fractions 1–15 were lyophilized to yield orange solids of I (30 mg). (a) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
(7) 6,6′, *O,O′*-(4,4′-azobenzenedicarbonyl)cycloheptaamylose: R_f 0.57 (5:4:3 *n*-BuOH–EtOH–H_2O); NMR (D_2O) 8.4–7.4 (aromatic, 8 H), 5.1 (C_1 H, 7 H), 4.4–3.2 (others, 42 H); IR (KBr) 1710, 1285 cm^{-1}; UV (H_2O, Tris buffer, pH 7.2) 450 nm (ε 1000), 335 (28 000). Anal. Calcd for I·4H_2O): C, 46.66; H, 5.89; N, 1.94. Found: C, 46.56; H, 5.74; N, 1.74.
(8) (a) W. Saenger, M. Noltemeyer, P. C. Manor, B. Hingerty, and B. Klar, *Bioorg. Chem.*, **5**, 187 (1976). Strain relief as the main driving force of the formation of inclusion complexes of α-CD was recently opposed on the basis of calculations: (b) I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, *J. Am. Chem. Soc.*, **100**, 916 (1978). Alternatively it might be possible to interpret the observation by movement of the cap outward from the assymetric β-CD residue on inclusion of the guest. If it were the case for *trans*-I, it would not be compatible with the structure of *cis*-I since the cap of *cis*-I is situated apart from β-CD residue before complex formation (the induced circular dichroism band of *cis*-I may arise from the assymetric twist around the N=N bond).[8c] (c) J. L. Houben, O. Pieroni, A. Fissi, and F. Ciardelli, *Biopolymers*, **17**, 799 (1978).
(9) M. P. Mack, R. R. Hendrixson, R. A. Palmer, and R. G. Ghirardelli, *J. Am. Chem. Soc.*, **98**, 7830 (1976).
(10) This equation holds when $C_s \gg C_I$ and both 1:1 and 1:2 host–guest complexes have the same ellipticities.

Akihiko Ueno,* Hiromitsu Yoshimura
Rumiko Saka, Tetsuo Osa
Pharmaceutical Institute, Tohoku University
Aobayama, Sendai 980, Japan
Received November 29, 1978