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Trans-cis Photoisomerization of 1-Methyl-4-(4′-hydroxystyryl)pyridinium in Inclusion Complexes of β-Cyclodextrin and Its Derivatives

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Abstract. The effects of β-cyclodextrin (βCyD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCyD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCyD) on *trans-cis* photoisomerization of 1-methyl-4-(4′-hydroxystyryl)pyridinium (POH) have been studied in aqueous solutions. The ratio of [*cis*]/[*trans*] for POH in the photostationary state at pH 8.54 was remarkably reduced by the presence of βCyD or DMβCyD. The reduction of the [*cis*]/[*trans*] ratio in the photostationary state was explained in terms of the shift of the equilibrium of $POH^+_{trans} \rightleftharpoons PO_{trans} + H^+$ toward PO_{trans} formation. The binding constants of βCyD and DMβCyD for PO_{trans} were 2.00- and 1.36-fold larger than those for POH^+_{trans}, respectively. The binding constants of TMβCyD for both species are much smaller than those of βCyD and DMβCyD. This result indicates that PO_{trans}, which has a betain structure, forms stable complexes with βCyD and DMβCyD with its hydrophobic parts inside and the charged parts outside the CyD cavities.

Key words. 1-methyl-4-(4′-hydroxystyryl)pyridinium, *trans-cis* photoisomerization, betain, charge separation, cyclodextrin, inclusion complex.

1. Introduction

The photoisomerization of retinal in rhodopsin from 11-*cis* to all-*trans* acts as a trigg.. of the visual process. Similar on–off switching by light is seen in the photosynthesis of *Halobacterium halobium*, in which photoisomerization of retinal from all-*trans* to 13-*cis* causes charge separation, generating a gradient in proton concentration. These reactions are suggested to proceed via singlet excited states. In connection with these light-sensitive systems in nature, it seems interesting to study artificial systems which have a photosensitive unit incorporated. We wish to report here photoisomerization of 1-methyl-4-(4′-hydroxystyryl)pyridinium (POH^+), alone and in the presence of β-cyclodextrin (βCyD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCyD) or heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCyD). POH^+ and its derivatives have been intensively investigated recently and its photoisomerization is suggested to occur via singlet excited states within 5 ns [1–5]. Steiner investigated the photochemistry of POH^+ and various species relating to this compound, and clarified the following points. The trans form of POH^+ (POH^+_{trans}) is transformed photochemically into its cis isomer (POH^+_{cis}). When POH^+_{cis} is deprotonated to form PO_{cis}, this species can be isomerized to PO_{trans} either photochemically or thermally, but this isomerization is a one way process and the reversion from PO_{trans} to PO_{cis} does not take place directly. Shulten regarded

this cycle as a model of retinal, particularly as that of the protonated Schiff base of retinal that acts as an actual trigger in the light-driven proton pump in the purple membrane of *Halobacterium halobium*. On the other hand, cyclodextrins (CyDs) have been investigated as an essential part of models of many biological systems, for example, as in artificial enzymes [6], photoinduced reduction systems relating to photosynthesis [7] and microenvironments in which stereospecific reactions take place [8]. In all of these systems, the hydrophobic cavities of CyDs play substantially important roles. On the basis, we have investigated the effect of the hydrophobic environment of CyDs on the POH^+–PO deprotonation equilibrium as well as on *trans-to-cis* photoisomerization of the photosensitive species.

2. Experimental

βCyD, DMCyD and TMCyD were kindly donated by Nihon Shokuhin Kako Co., Ltd. 1-Methyl-4-(4′-hydroxystyryl)pyridinium iodide (POH-I) was purchased from Nippon Kankoh-shikiso Kenkyusho and used without further purification. Measurements have been performed with buffer solutions which were prepared with analytical grade reagents and deionized water made with a Millipore Milli 12 water purifier or deuterium oxide purchased from Merck for NMR study.

Photoirradiation was performed with a high-pressure mercury lamp. An emission line at 366 nm was selected using a combination of cut-off filters of Toshiba UV37 and UVD35 and an aqueous solution containing $NiSO_4$ (25 g/l). Absorption (UV) spectra were measured with a Shimadzu UV 3100 spectrophotometer. NMR spectra were measured at 500 MHz and 25°C (Varian VXR 5000 system) with 3-(trimethylsilyl)-propionic acid-d_6 sodium salt as an external standard, and the error limits in the chemical shifts were less than 0.002 ppm. The ratios of [*cis*]/[*trans*], where [*cis*] and [*trans*] are the total concentration of POH^+_{cis} and PO_{cis} and that of POH^+_{trans} and PO_{trans}, respectively, were estimated from the peak areas of ^{1}H-NMR signals of the C(3) proton in the pyridyl ring. The binding constants of these complexes of CyDs were determined by least-square curve fitting using the absorbance changes for PO_{trans} and the change in chemical shifts of the ^{1}H-NMR signals of the pyridyl-ring C(3) proton for POH^+_{trans} (δ 7.906–7.961 ppm) and PO_{trans} (δ 7.779–7.819 ppm). In this analysis, the concentration of POH^+_{trans} and PO_{trans} were both fixed at 10^{-5} M and the concentration of CyDs were in the range between 2.5×10^{-3} and 10^{-2} M. The acid dissociation constant (pK_a) was determined by measuring the absorbance of POH^+_{trans} and PO_{trans} at various pH values using citric, phosphate and borate buffer solution.

3. Results and Discussion

3.1. THE COMPLEX FORMATION AND THE ACID DISSOCIATION EQUILIBRIUM OF POH^+_{trans}

The pK_a value of POH^+_{trans} in the presence of βCyD was obtained from titration curves of POH^+_{trans} and PO_{trans}, monitoring the absorption maximum of POH^+_{trans} (372 nm) or that of PO_{trans} (450 nm), each titration curve giving 8.31 as the pK_a value. The titration curve for POH^+_{trans} is shown in Figure 1. The pK_a value of

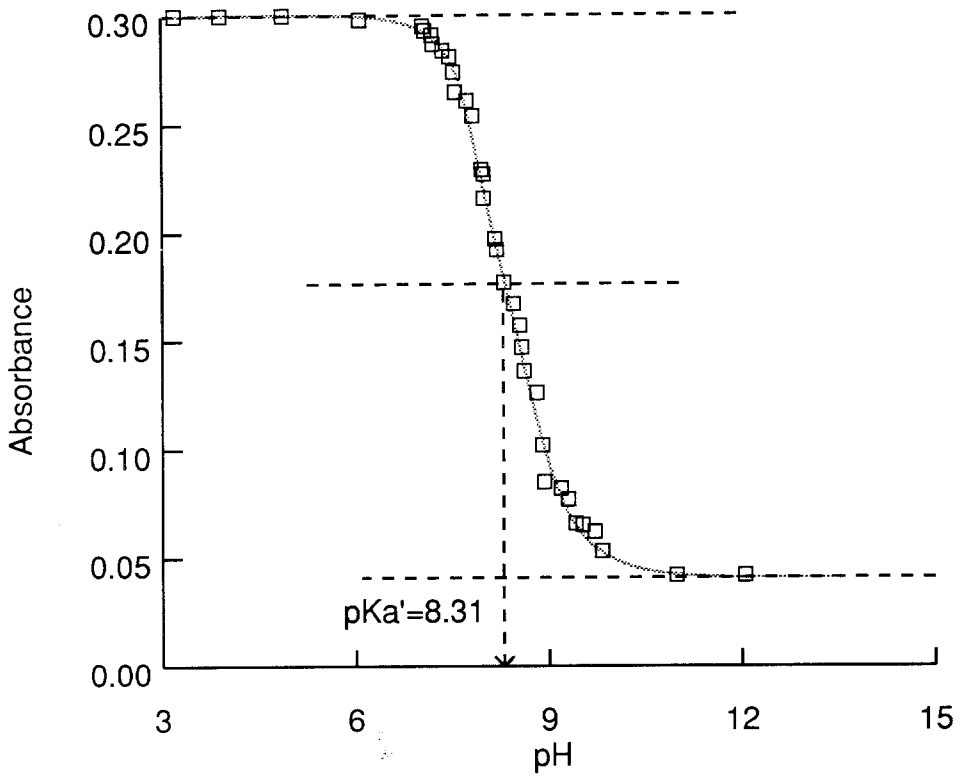

Fig. 1. Titration curve for POH^{+}_{trans}–PO_{trans} equilibrium in the presence of βCyD (10^{-2} M). The absorption maximum of POH^{+}_{trans} at 372 nm was monitored. Total concentration of POH^{+}_{trans} and PO_{trans} is 10^{-5} M.

POH^{+}_{trans} was reported to be 8.54 [5], and the pK_a value of 8.31 which was obtained here in the presence of βCyD is smaller than the reported one.

In the alkaline solution (pH 10.00), the absorbance maximum around 450 nm of PO_{trans} was shifted to longer wavelength upon addition of βCyD (Figure 2). Using this variation in absorption spectrum, the binding constants of CyDs for PO_{trans} were obtained. The absorbance at 500 nm was plotted as a function of concentration of each host and the curve fitting analysis of these data on the basis of 1:1 host:guest stoichiometry was performed based on the following modified Benesi–Hildebrand equation,

$$\text{Abs.}_{\text{obs}} = \frac{\text{Abs.}_{\text{min}} + \text{Abs.}_{\text{max}} K[\beta\text{CyD}]}{1 + K[\beta\text{CyD}]} \tag{1}$$

where, Abs._{obs} is the observed absorbance, Abs._{min} is the absorbance in the absence of βCyD, Abs._{max} is the absorbance when all the guest molecules were included into the cavities of βCyD, K is the binding constant of complex formation, and [βCyD] is the concentration of βCyD. Figure 3 shows an example of this fitting. The plots of experimental values fit well with the theoretical curve, giving 480M^{-1} as the binding constant. This result indicates that PO_{trans} forms a 1:1 complex with

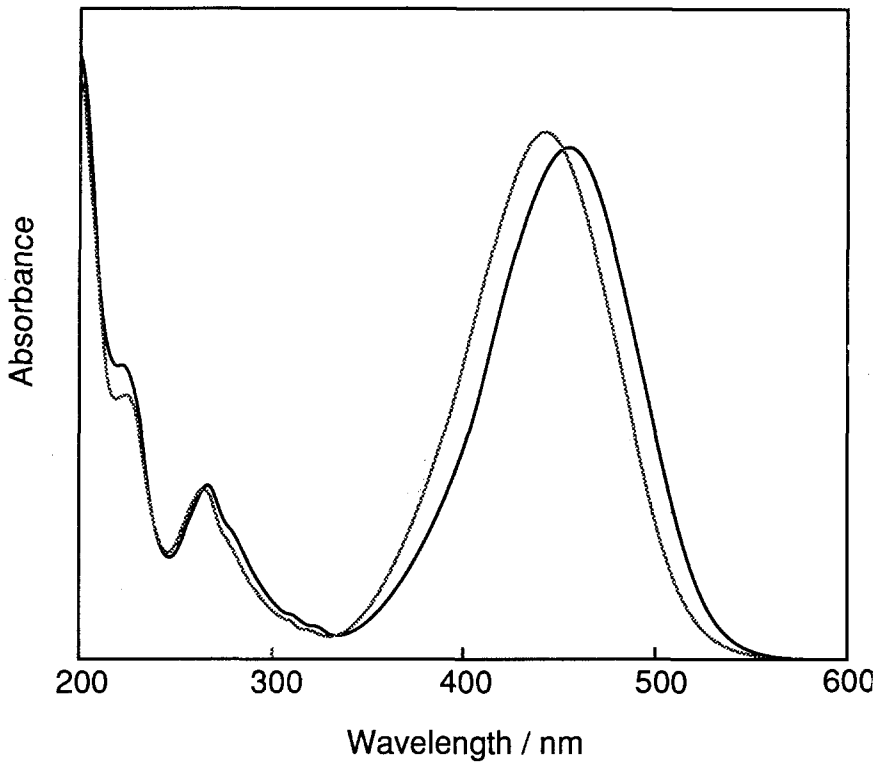

Fig. 2. Absorption spectra of PO_{trans} (10^{-5} M), in the absence (...) or in the presence of βCyD (10^{-2} M) (——).

βCyD. On the other hand, the absorption changes associated with the complex formation of POH^+_{trans} with βCyD were small, so the following equation was used to obtain the binding constants of βCyD for POH^+_{trans} at pH 8.54,

$$K'_a = \frac{[PO_{trans}] + [PO_{trans} - \beta CyD]}{[POH^+_{trans}] + [POH^+_{trans} - \beta CyD]}[H^+]$$
$$= \frac{[PO_{trans}]}{[POH^+_{trans}]}[H^+]\frac{1 + \dfrac{[PO_{trans} - \beta CyD]}{[PO_{trans}]}}{1 + \dfrac{[POH^+_{trans} - \beta CyD]}{[POH^+_{trans}]}} \quad (2)$$
$$= K_a \frac{1 + K_2[\beta CyD]}{1 + K_1[\beta CyD]}$$

where K_a (2.88×10^{-9}) and K'_a (4.90×10^{-9}) are the acid-dissociation constant of POH^+_{trans} and the apparent acid-dissociation constant of the mixture of POH^+_{trans} and POH^+_{trans}–βCyD complex, respectively. $[PO_{trans}]$, $[POH^+_{trans}]$, $[PO_{trans}$–$\beta CyD]$, $[POH^+_{trans}$–$\beta CyD]$ and $[H^+]$ are the concentrations of the corresponding species. K_1 and K_2 are binding constants of βCyD for POH^+_{trans} and PO_{trans}, respectively, and the value of K_2 has already been determined to be 480 M^{-1} from the curve-fitting

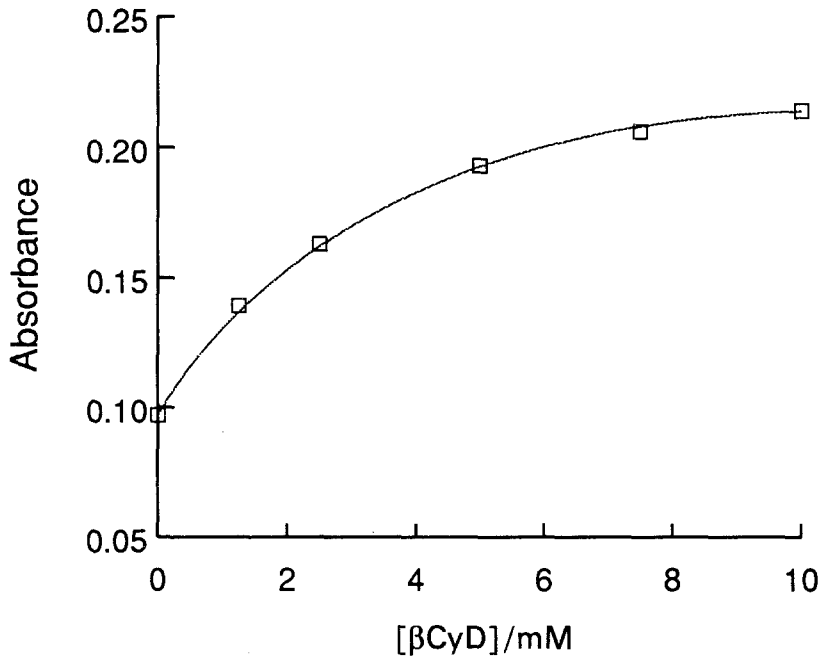

Fig. 3. The absorbance at 500 nm of PO_{trans} (10^{-5} M) as a function of βCyD concentration at pH 10.00 and the theoretical curve obtained from the modified Benesi–Hildebrand equation ($K = 480\ M^{-1}$).

analysis of the βCyD-induced absorption variations. The binding constant K_1 was calculated from the following equation and the value was 230 M^{-1}

$$K_1 = \frac{1}{[\beta\text{CyD}]}\left(\frac{K_a}{K'_a}(1 + K_2[\beta\text{CyD}]) - 1\right) \tag{3}$$

When was used the numbering of the protons of POH^+_{trans} defined by Steiner as shown in Figure 4 [1], the chemical shift for each proton of POH^+_{trans} and POH^+_{cis} varied with increasing concentration of βCyD. The data of the species, alone or in

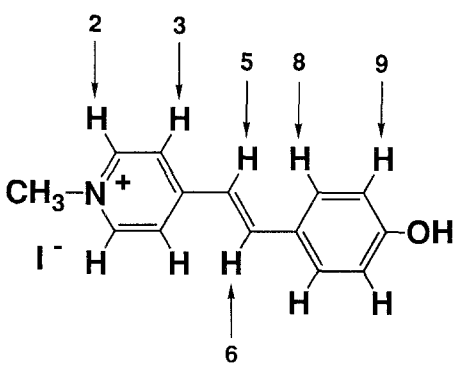

Fig. 4. The numbering of the aromatic protons of POH^+_{trans}.

Table I. Chemical shifts values of POH^{+}_{trans} and POH^{+}_{cis} at pH 3.00

		Chemical shift values δ (ppm)					
		C2	C3	C5	C6	C8	C9
POH^{+}_{trans}	none	8.441	7.906	7.136	7.700	7.594	6.914
	+βCyD[a]	8.492	7.961	7.114	7.697	7.577	6.881
POH^{+}_{cis}	none	8.391	7.714	6.581	7.110	7.169	6.784
	+βCyD[a]	8.477	7.782	6.679	7.116	7.192	6.747

[a]In the presence of βCyD (10^{-3} M).

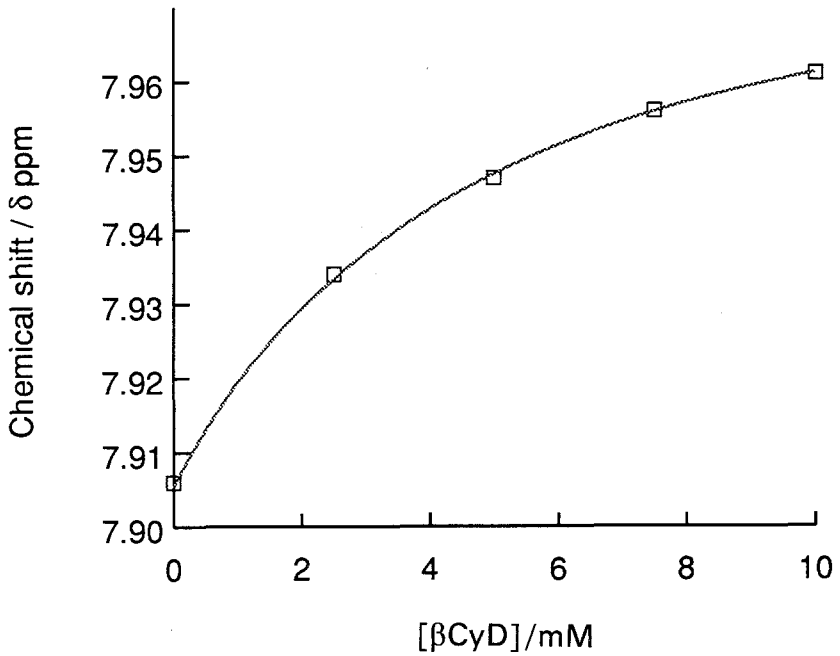

Fig. 5. The chemical shift value of the C(3) proton of POH^{+}_{trans} as a function of βCyD concentration and the theoretical curve obtained from the modified Benesi–Hildebrand equation ($K = 240\ M^{-1}$) (pH 3.0).

the presence of βCyD (10^{-3} M) are summarized in Table I. We used βCyD-induced chemical shift variations of pyridyl-ring C(3) proton to obtain the binding constant of βCyD for POH^{+}_{trans} and PO_{trans}, respectively. Figure 5 shows an example of this plot and curve fitting. Binding constants of βCyD, DMβCyD, and TMβCyD for POH^{+}_{trans} and PO_{trans} are summarized in Table II. The binding constants of βCyD for POH^{+}_{trans} and PO_{trans} were 240 M^{-1} and 470 M^{-1}, respectively, and are in good agreement with the K_1 value obtained from Equation (3) (230 M^{-1}) and the K_2 value obtained from βCyD-induced absorption variations for PO_{trans} (480 M^{-1}). The good agreement indicates that the model which leads to Equation (2) is reasonable. The variations in the chemical shift of POH^{+}_{cis} suggest

Table II. Binding constants of CyDs for POH^{+}_{trans} and PO_{trans} at 25°C.[a]

	Binding constants (M^{-1})		
	βCyD	DMβCyD	TMβCyD
POH^{+}_{trans}	240	220	50
PO_{trans}	480	300	60

[a]Values obtained from ^{1}H-NMR chemical shift variations and from absorbance variations at 500 nm for POH^{+}_{trans} and PO_{trans}, respectively.

that POH^{+}_{cis} also forms complexes with CyDs, but this complex formation does not affect the ratio of [*cis*]/[*trans*] in the photostationary state. This fact will be discussed in the next section.

3.2. THE EFFECT OF CyDs ON *trans-cis* PHOTOISOMERIZATION OF POH^{+}_{trans}

We observed that the ratio of [*cis*]/[*trans*] in the photostationary state is affected by the presence of CyDs. Figure 6 shows the time dependence of the absorbance of POH^{+}_{trans} at 372 nm in the neutral aqueous solution (0.02 M phosphate buffer pH 7.0), alone and in the presence of βCyD. It indicates that POH^{+}_{trans} is converted into POH^{+}_{cis}, the photostationary state being reached within 8 minutes under the experimental conditions. Although the effect of βCyD on the initial rate of this photoisomerization was small, the apparent ratio of [*cis*]/[*trans*] in the photostationary state was depressed by βCyD.

The accurate ratios of [*cis*]/[*trans*] in the photostationary state were estimated from the peak area of the signals of ^{1}H-NMR of POH^{+}_{cis} and POH^{+}_{trans}, and the results are summarized in Table III. It is known that the unprotonated form, PO_{trans}, does not isomerize into PO_{cis} photochemically or thermally [1], so that no isomerization proceeds in alkaline solution (pH 10.00, 0.05 M borate buffer) where solely PO_{trans} exists. In contrast to the behavior of PO_{trans}, POH^{+}_{trans} undergoes photoisomerization in acidic solution (pH 3.00, 0.035 M formate buffer), but the effects of CyDs on *trans*-to-*cis* photoisomerization were not appreciable at this pH. On the other hand, photoisomerization of azobenzene was reported to be retarded by the presence of βCyD [9], and consequently the result obtained here suggests that rotation of the carbon–carbon double bond of POH^{+}_{trans} in the complex is different from that of the nitrogen–nitrogen double bond of azobenzene in the complex with βCyD, probably existing near the rim of βCyD. There is an indication, described above, that POH^{+}_{cis} also forms a complex with CyDs, but the fact

Table III. Ratios of [*cis*]/[*trans*] in the photostationary state

pH	none	βCyD	DMβCyD	TMβCyD
3.00	60/40	58/42	57/43	60/40
8.54	29/71	14/86	17/83	25/75

Error limits are less than 2%. Total concentration 10^{-5} M

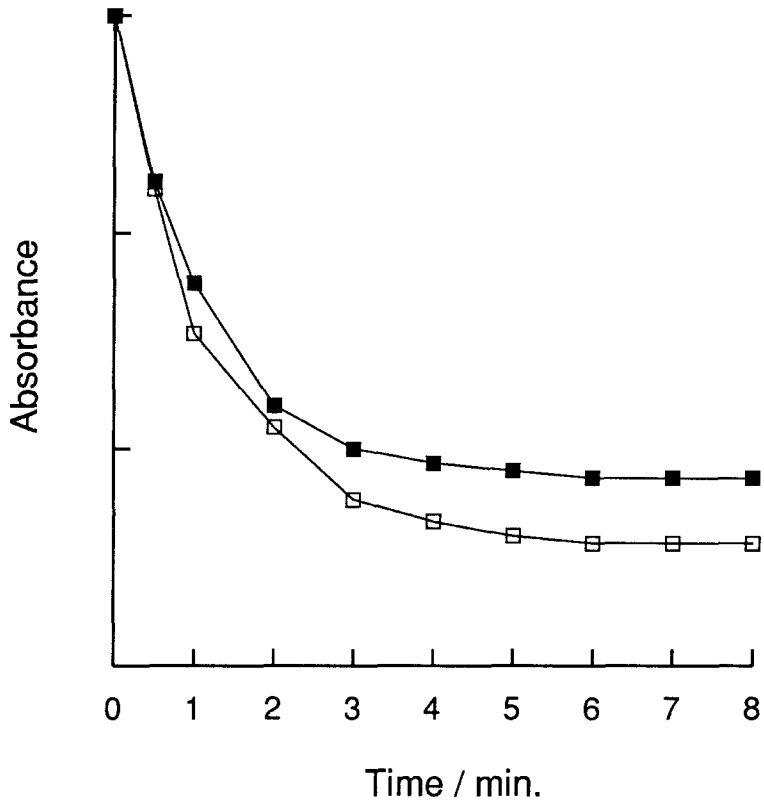

Fig. 6. The plots of absorbance at 372 nm of POH^+_{trans} (10^{-5} M) at 25°C as a function of irradiation time, alone (□) or in the presence of βCyD (10^{-2} M) (■) in a phosphate buffer solution (pH 7.0).

that the [*cis*]/[*trans*] ratio is not affected at pH 3.00 suggests that the *cis*-to-*trans* process of POH^+_{cis} is hardly influenced by the complexation. This may be related to the nonplanner structure of the *cis* isomer, which is not suited to deep inclusion in the cavity of CyDs. We found that in the solution having the pH value equal to pK_a of POH^+_{trans} (pH 8.54, 0.05 M borate buffer) where the concentrations of POH^+_{trans} and PO_{trans} are the same, the *trans*-to-*cis* photoisomerization was remarkably depressed upon addition of βCyD or DMβCyD. The pH dependence of the [*cis*]/[*trans*] ratio indicated that the shift of the equilibrium from POH^+_{trans} to PO_{trans} is the major factor that governs the ratio of *cis* isomer.

3.3. THE EFFECT OF CyDs ON THE REACTION CYCLE OF POH^+

The acid dissociation constant of the $POH^+_{trans}-\beta$CyD complax (K''_a) is obtained from the following equation

$$K''_a = \frac{[PO_{trans} - \beta CyD]}{[POH^+_{trans} - \beta CyD]}[H^+] = \frac{K_2}{K_1}[H^+] \qquad (4)$$

Table IV. Values of pK'_a and pK''_a obtained from Equations (2) and (4), respectively

	βCyD	DMβCyD	TMβCyD
pK'_a	8.32	8.45	8.52
pK''_a	8.25	8.42	8.47

K'_a: apparent acid-dissociation constant of the mixture of POH^+_{trans} and POH^+_{trans}–βCyD complex.
K''_a: acid-dissociation constant of the complex of POH^+_{trans} with CyDs.

The values of pK'_a and pK''_a were obtained from Equations (2) and (4), respectively, and the results are summarized in Table IV. In the case of βCyD, the pK'_a value obtained from Equation (2) was 8.32. The value is consistent with the one obtained by the titration method (Figure 1). In the case of βCyD, the pK_a value was 8.25, suggesting that the acid dissociation equilibrium of POH^+_{trans} was shifted toward PO_{trans} formation. The binding constants of PO_{trans}–βCyD and PO_{trans}–DMβCyD complexes are 2.00- and 1.36-fold larger than those of POH^+_{trans}–βCyD and POH^+_{trans}–DMβCyD, indicating that the former complexes are more stable than the latter ones. The binding features as well as the βCyD-induced shift of pK_a for POH^+_{trans} may be related to the reduced ratio of [*cis*]/[*trans*] of the species. The βCyD-induced conversion from POH^+_{trans} to PO_{trans} in the solution at pH 8.54 should decrease the *cis* concentration because POH^+_{trans} is the only species that undergoes photoisomerization from the trans to cis form. In contrast to the cases of βCyD and DMβCyD, TMβCyD did not affect the ratio of [*cis*]/[*trans*] in the photostationary state. This result seems reasonable because the binding constants of TMβCyD for POH^+_{trans} and PO_{trans} are much smaller than those of βCyD and DMβCyD (Table II). On the other hand, ratios of $\{[PO_{trans}] + [PO_{trans}\text{–CyDs}]\}/\{[POH^+_{trans}] + [POH^+_{trans}\text{-CyDs}]\}$ were obtained from Equation (2), and the results are given in Table V. In the case of TMβCyD, the ratio was not so different from that in the absence of CyDs.

The above results demonstrate that betain guests like PO_{trans} can form stable complexes with βCyD and DMβCyD in spite of charges existing at both ends of the molecules (Figure 7). This may be due to the fact that the charged guest molecule of PO_{trans} penetrates the cavities in the complexes of CyDs with its hydrophobic parts inside and charged part outside the cavities. In many biological systems, the hydrophobic or constrained microenvironment of enzyme or receptor cavities plays important roles in binding and reaction events. In the present system, the microenvironment of CyDs cavities shifts the protonation–deprotonation equilibrium as well as the *trans-cis* ratio in the photostationary state. On the other hand, charge separation is also important in many biological systems, and the

Table V. Ratios of $\{[PO_{trans}] + [PO_{trans}\text{–}\beta\text{CyD}]\}/\{[POH^+_{trans}] + [POH^+_{trans}\text{–}\beta\text{CyD}]\}$ before irradiation in borate buffer solution pH 8.54

none	βCyD	DMβCyD	TMβCyD
50/50	63/37	56/44	51/49

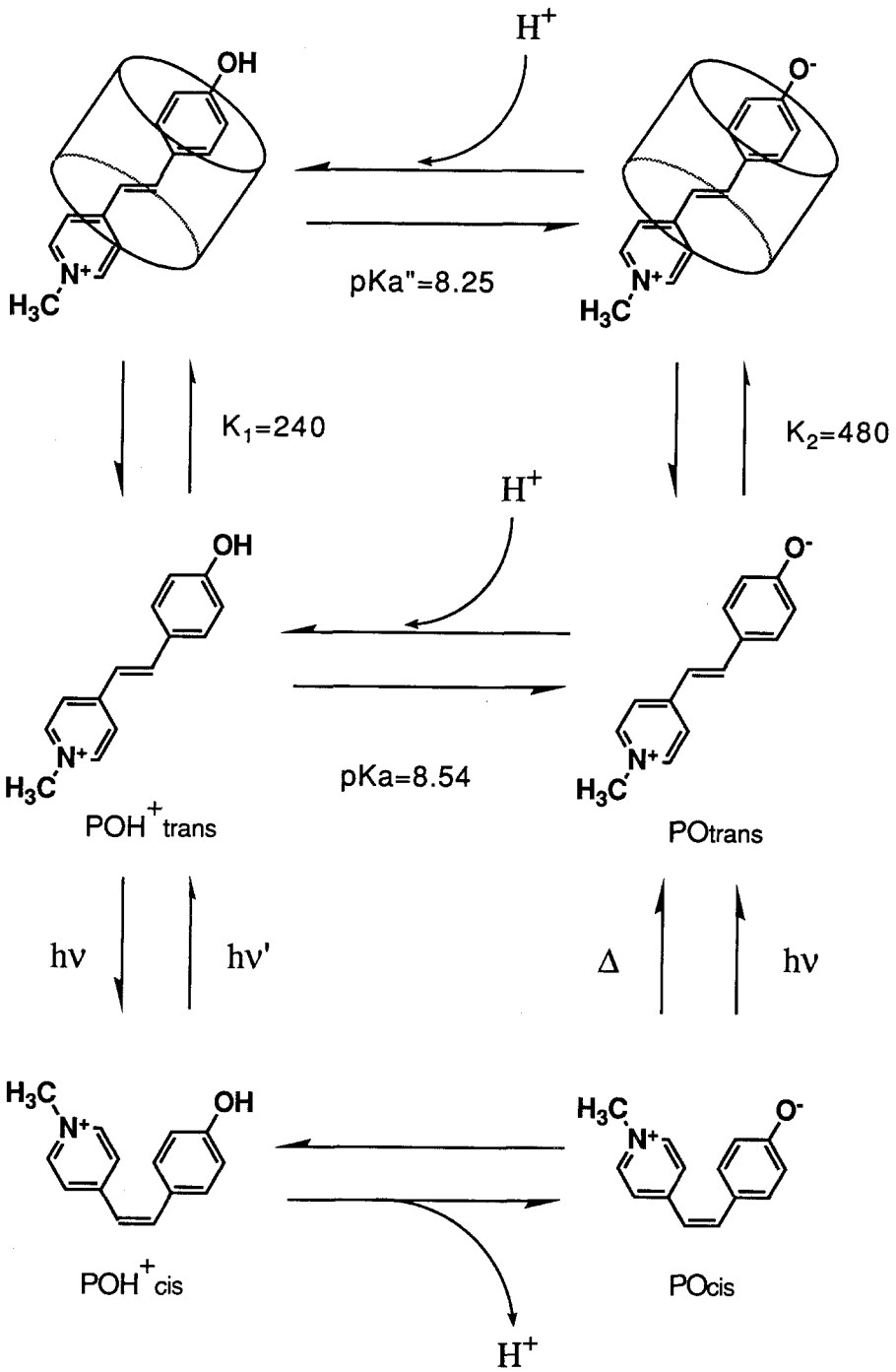

Fig. 7. Schematic illustration of *trans-cis* photoisomerization, acid dissociation and complex formation equilibrium. Binding constants and pK_a'' are for the case of βCyD.

present result suggests that the molecules with large electronic dipole may also be accommodated in the hydrophobic binding sites without appreciable difficulty if their charges are adequately positioned apart from each other.

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