

Interactions of Tetrakis(4-carboxyphenyl)porphyrin with Cyclodextrins in Aqueous Solutions Containing 1,1′-Diheptyl-4,4′-bipyridinium Dibromide

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In pH 10.1 buffers, the interactions of tetrakis(4-carboxyphenyl)porphyrin (TCPP) with γ-cyclodextrin (γ-CD), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), and 1,1′-diheptyl-4,4′-bipyridinium dibromide (HB) have been examined by studying absorption, fluorescence, and induced circular dichroism spectra of TCPP. TCPP has been found to form inclusion complexes with γ-CD and TM-β-CD. The stoichiometries of the γ-CD–TCPP and TM-β-CD–TCPP inclusion complexes are 1:1 and 2:1, respectively. Because the equilibrium constant for the formation of the 2:1 TM-β-CD–TCPP inclusion complex is exceedingly large (4.34×10^{18} mol^{-2} dm^6), the TM-β-CD–TCPP inclusion complex is quantitatively formed in TCPP solutions containing two equivalents of TM-β-CD. TCPP also forms an organic cation–organic anion complex with HB. In the presence of γ-CD, the TCPP–HB complex forms a 1:1:1 γ-CD–TCPP–HB inclusion complex, although TM-β-CD causes the dissociation of the TCPP–HB complex into its components. The equilibrium constant for the formation of the γ-CD–TCPP–HB inclusion complex from the TCPP–HB complex and γ-CD has been evaluated to be 905 mol^{-1} dm^3.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, and eight D-glucopyranose residues, which are called α-CD, β-CD, and γ-CD, respectively. Because CDs have hydroxy groups on their narrow and wide rims, they are soluble in water. In spite of the relatively high solubilities of CDs, they have a hydrophobic cavity in the molecular center. Consequently, many kinds of organic compounds can be incorporated into their cavities to form inclusion complexes in aqueous solutions.

Porphyrin derivatives are requisite substances in life, for they are related to photosynthesis and oxygen transportation. In photosynthesis, chlorophylls, which are Mg complexes of porphyrin derivatives, fulfil critical roles. Heme, which complexes with Fe, transports oxygen from its source in the organism, e.g., lungs or gills, to the sites where it is needed. Besides living systems, photochemical oxidation of water proceeds on the surface of colloidal RuO_2 and IrO_2 by using Zn tetrakis(4-sulfonatophenyl)porphyrin as a photosensitizer.[1,2]

Because the formation of inclusion complexes of CDs with porphyrin derivatives modifies the photochemical and photophysical properties of porphyrin derivatives, it is very important to examine the formation of inclusion complexes of CDs with porphyrin derivatives. The interactions between several porphyrin derivatives and CDs have hitherto been investigated. Equilibrium constants for the formation of 1:1 inclusion complexes of γ-CD with hematoporphyrin IX and coproporphyrin III have been estimated to be 73 and 64 mol^{-1} dm^3, respectively.[3] For 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid), a semi-closed complex with hydroxypropyl-β-CD has been reported.[4] A 2:1 heptakis(2,6-di-*O*-methyl)-β-CD–tetrakis[2,4-bis(pivaloyloxy)phenyl]porphyrin inclusion complex is formed in an ethanol/H_2O (2:1) mixture.[5] In the case of 5,15-diphenylporphyrin, a 2:1 heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD)–guest inclusion complex is formed in dimethyl sulfoxide.[6]

From NMR and spectrophotometric measurements, the formation of a 4:1 β-CD–Zn or Fe(III) tetrakis(4-sulfonatophenyl)porphyrin (Zn TSPP or Fe(III) TSPP) inclusion complex has been suggested.[7,8] Venema et al. have estimated the equilibrium constant for the formation of the 1:1 inclusion complex between β-CD and TSPP in pH 7.0 solutions to be 1400 mol^{-1} dm^3.[9] Ribo et al. have studied the complexation of TSPP with α-CD, β-CD, and γ-CD in aqueous solutions by means of electronic absorption and ^{1}H NMR spectroscopy.[10] They have concluded that, in neutral solutions, TSPP forms 2:1 host–guest inclusion complexes through its *meso*-sulfonatophenyl groups with β-CD and γ-CD, but not with α-CD. In acidic media where TSPP is protonated, it forms a 2:1 inclusion complex with β-CD alone.

Recently, by means of absorption, fluorescence, and circular dichroism spectroscopy, we have examined the formation of inclusion complexes of TSPP with CDs in alkaline solutions.[11] γ-CD forms a 1:1 inclusion complex with TSPP, whereas TM-β-CD forms a 2:1 host-guest inclusion complex with TSPP. α-CD and β-CD form both 1:1 and 2:1 host–TSPP inclusion complexes, although the concentration ratio of the 1:1 to 2:1 inclusion complexes depends on the CD concentration. In the case of Fe(III) TSPP, the stoichiometries of the CD-guest inclusion complexes are 1:1, except for TM-β-CD in pH 10.1 buffer, where the 1:1 TM-β-CD–Fe(III) TSPP inclusion complex as-

sociates with another TM-β-CD molecule to form a 2:1 inclusion complex at high TM-β-CD concentrations.[12]

In pH 7.0 buffers, a very strong binding of tetrakis(4-carboxyphenyl)porphyrin (TCPP) with TM-β-CD has been observed with a 2:1 host–guest stoichiometry.[13] The binding constants for the 2:1 inclusion complexes of the free and Zn TCPPs have been reported to be 1.4×10^{16} and 1.9×10^{16} mol^{-2} dm^6, respectively.

We have also examined the interactions between CDs and organic cation-organic anion complexes as well as the organic cation–organic anion interactions. Thionine forms an organic cation–organic anion complex with 2-naphthalenesulfonate.[14] Upon the addition of γ-CD to the aqueous solution containing thionine and 2-naphthalenesulfonate, a ternary 1:1:1 inclusion complex is formed among γ-CD, thionine, and 2-naphthalenesulfonate. In the case of β-CD, the thionine–2-naphthalenesulfonate complex is dissociated into the individual components, which form inclusion complexes with β-CD.

Methylene Blue forms complexes with 1- and 2-naphthalenesulfonates, 2-anthracenesulfonate, 2,7-naphthalenedisulfonate, and 1,3,6-naphthalenetrisulfonate in aqueous solutions.[15] Furthermore, the complexation of Methylene Blue with Acid Orange 7 or α-Naphthol Orange has been observed in aqueous solution.[16,17] In the presence of γ-CD, a 2:1:1 γ-CD–Methylene Blue–Acid Orange 7 (or α-Naphthol Orange) inclusion complex is formed. As in the case of the thionine–2-naphthalenesulfonate system, the addition of β-CD results in the dissociation of the Methylene Blue–Acid Orange 7 (or α-Naphthol Orange) complex to the individual components, with which β-CD forms inclusion complexes.

It is known that Zn TSPP forms an organic cation-organic anion complex with 1,1′-dimethyl-4,4′-bipyridinium dibromide.[18] Zn and Pd meso-tetraphenylporphyrintrisulfonic acids also form organic cation–organic anion complexes with 1,1′-dialkyl-4,4′-bipyridinium dibromides.[19,20]

Although TSPP forms an organic cation–organic anion complex with Methylene Blue in aqueous solution, the TSPP–Methylene Blue complex is dissociated by the addition of γ-CD.[21] The binding site of γ-CD towards TSPP is a sulfonatophenyl group, which is not too large.[10] However, a bulky porphyrin skeleton seems to obstruct the encapsulation of the TSPP–Methylene Blue complex into the γ-CD cavity. Consequently, both Methylene Blue and the sulfonatophenyl moiety of TSPP are not simultaneously incorporated into the γ-CD cavity.

When a less bulky organic cation and a less bulky organic anion (a porphyrin derivative) are used, there is the possibility that a ternary inclusion complex is formed among γ-CD, the organic cation, and the organic anion. Thus, we selected TCPP and 1,1′-diheptyl-4,4′-bipyridinium dibromide (HB) as an organic anion and an organic cation, respectively, and investigated the interactions among CDs, TCPP, and HB by means of absorption, fluorescence, and induced circular dichroism spectra.

Experimental

Tetrakis(4-carboxyphenyl)porphyrin (TCPP), 1,1′-diheptyl-4,4′-bipyridinium dibromide (HB), and 1,1′-dimethyl-4,4′-bipyridinium dichloride (MB), which were purchased from Tokyo Kasei Kogyo, were used as received. β-Cyclodextrin (β-CD), obtained from nakalai tesque, was twice recrystallized from water, although γ-CD and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), which were obtained from nakalai tesque, were used without further purification. Buffers of pH 10.1 were composed of 2.5×10^{-3} mol dm^{-3} of $NaHCO_3$ and 1.2×10^{-3} mol dm^{-3} NaOH solutions.

Absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were taken with a Shimadzu RF-501 spectrofluorometer equipped with a cooled Hamamatsu R-943 photomultiplier. The fluorescence spectra were corrected for the spectral response of the fluorometer. Induced circular dichroism spectra were recorded on a JASCO J-400X spectropolarimeter interfaced to a JASCO DP-500 data processor. Spectroscopic measurements were made at 25 ± 0.1 °C, except for the induced circular dichroism spectra which were measured at 25 ± 2 °C.

Results and Discussion

Inclusion Complex between γ-CD and TCPP in Basic Aqueous Solution. At 1.0×10^{-6} mol dm^{-3} of TCPP, its absorption bands at wavelengths longer than the 415-nm band (Soret band) have been very weak (the absorbance has been 0.014 at the 517-nm band). Consequently, we have focused on the Soret band of TCPP. Figure 1 shows absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. When γ-CD is added to TCPP solutions, the absorption maximum of TCPP is shifted to longer wavelengths, accompanied by the appearance of an isosbestic point at 416 nm. The absorption spectral changes indicate the formation of an inclusion complex of γ-CD with TCPP.

As the γ-CD concentration was increased, the fluorescence peaks of TCPP (5.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers were shifted to longer wavelengths, with a sharpening of the fluorescence bands. This fluorescence spectral change indicates the transfer of TCPP from a polar environment to a relatively nonpolar environment, i.e., the formation of the γ-CD–TCPP inclusion complex. In the case of TSPP, a 1:1 inclusion complex with γ-CD is formed.[11] For TCPP, the formation of a 1:1 in-

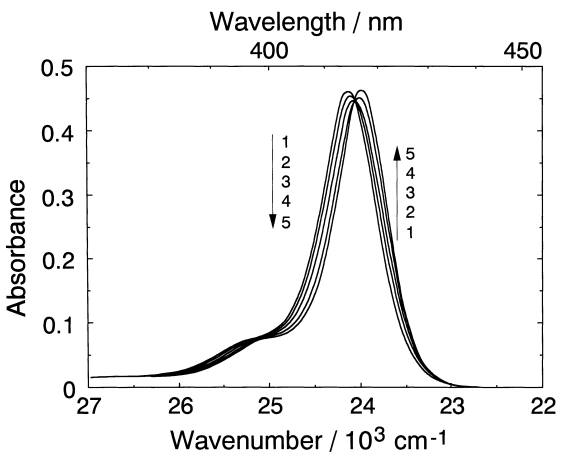

Fig. 1. Absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 3.0×10^{-5}, (3) 1.0×10^{-4}, (4) 3.0×10^{-4}, and (5) 1.0×10^{-3} mol dm^{-3}.

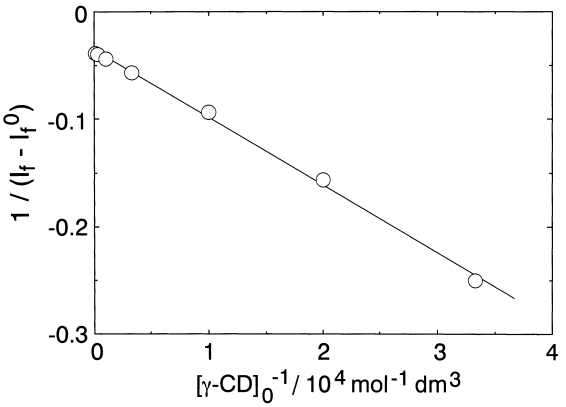

Fig. 2. Double reciprocal plot for the fluorescence intensities of TCPP (5.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. $\lambda_{ex} = 416$ nm. $\lambda_{obs} = 687$ nm.

clusion complex between γ-CD and TCPP is also most likely:

$$\gamma\text{-CD} + \text{TCPP} \overset{K_1}{\rightleftharpoons} \gamma\text{-CD}\cdot\text{TCPP} \quad (1)$$

where γ-CD·TCPP stands for the 1:1 γ-CD–TCPP inclusion complex, and K_1 is the equilibrium constant for the formation of γ-CD·TCPP. When the 1:1 γ-CD–TCPP inclusion complex is formed under the conditions that the γ-CD concentration is much higher than the TCPP concentration, K_1 can be estimated from the Benesi–Hildebrand type equation:[22]

$$1/(I_f - I_f^0) = 1/a + 1/(aK_1[\gamma\text{-CD}]_0) \quad (2)$$

Here, I_f and I_f^0 are the fluorescence intensities in the presence and absence of γ-CD, respectively, a is a constant, and $[\gamma\text{-CD}]_0$ is the initial concentration of γ-CD. As shown in Fig. 2, the plot of Eq. 2 exhibits a straight line, indicating the formation of the 1:1 γ-CD–TCPP inclusion complex. If a 2:1 γ-CD–TCPP inclusion complex is formed, a plot of $1/(I_f - I_f^0)$ against $1/[\gamma\text{-CD}]_0^2$ should afford a straight line. However, the curved line obtained for the plot provides additional evidence for no formation of the 2:1 γ-CD–TCPP inclusion complex (not shown). From the plot shown in Fig. 2, 5600 ± 300 mol^{-1} dm^3 is obtained as a K_1 value. This K_1 value for TCPP is about 3.5 times greater than that (1600 ± 200 mol^{-1} dm^3) for TSPP.[11] This is because a carboxyphenyl group in TCPP is more hydrophobic than a sulfonatophenyl group in TSPP is. From a similar analysis for the absorbance change, the K_1 value has been evaluated to be 10000 ± 400 mol^{-1} dm^3. This K_1 value is about two times greater than that evaluated from the fluorescence intensity change. The K_1 value obtained from the fluorescence intensity change is more reliable than that from the absorbance change, because the absorbance change in Fig. 1 is small compared to the fluorescence intensity change.

Inclusion Complex between TM-β-CD and TCPP in Basic Aqueous Solution. Upon the addition of β-CD to TCPP (1.0×10^{-6} mol dm^{-3}) solutions, no isosbestic point was observed, although the absorption spectrum of TCPP was changed. Consequently, 1:1 and 2:1 host–guest complexation seems to occur.

When TM-β-CD was added to TCPP (1.0×10^{-6} mol dm^{-3}) solution (pH 10.1), the absorption peak of TCPP was shifted to longer wavelengths, accompanied by appearance of an isosbestic point at 416 nm. This finding indicates the formation of an inclusion complex of TM-β-CD with TCPP. The absorption spectral change for TM-β-CD is similar to that for γ-CD, except for the CD concentration range: the concentrations for TM-β-CD are two orders of magnitude lower than those for γ-CD, implying that TM-β-CD is more readily bound to TCPP than γ-CD. The strong binding of TM-β-CD to TCPP is due to the tight fit of the TM-β-CD cavity to the carboxyphenyl moiety in TCPP compared to γ-CD.

Figure 3 exhibits fluorescence spectra of TCPP (5.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of TM-β-CD. When the TM-β-CD concentration is increased, the fluorescence bands are significantly sharpened, suggesting that TCPP experiences less polar environment, i.e., the formation of an inclusion complex with TM-β-CD. Although the fluorescence spectral change in the presence of TM-β-CD is similar to that in the presence of γ-CD, the former change is more prominent than the latter change.

To determine the stoichiometry of the TM-β-CD–TCPP inclusion complex, a continuous variation method was applied using the absorbance at 417.5 nm. From the result under the conditions of $[\text{TCPP}]_0 + [\text{TM-}\beta\text{-CD}]_0 = 1.0 \times 10^{-6}$ mol dm^{-3}, it was found that the TM-β-CD–TCPP inclusion complex has a 2:1 stoichiometry.

$$2\text{TM-}\beta\text{-CD} + \text{TCPP} \overset{K_2}{\rightleftharpoons} (\text{TM-}\beta\text{-CD})_2\cdot\text{TCPP} \quad (3)$$

Here, K_2 is the equilibrium constant for the formation of the 2:1 TM-β-CD–TCPP inclusion complex ((TM-β-CD)$_2$·TCPP).

Figure 4 exhibits the TCPP fluorescence intensity at 680 nm as a function of the TM-β-CD concentration. Below a TM-β-

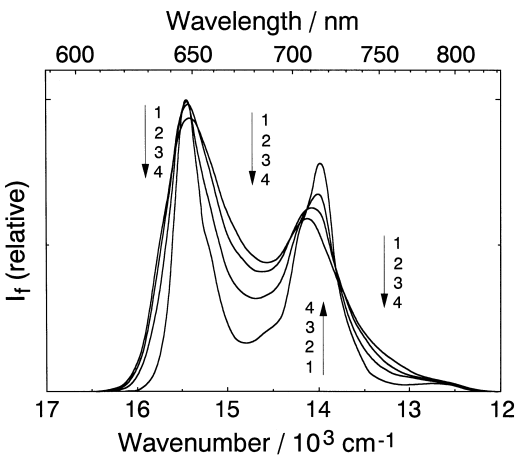

Fig. 3. Fluorescence spectra of TCPP (5.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of TM-β-CD. Concentration of TM-β-CD: (1) 0, (2) 2.0×10^{-7}, (3) 5.0×10^{-7}, and (4) 1.0×10^{-6} mol dm^{-3}. $\lambda_{ex} = 416$ nm.

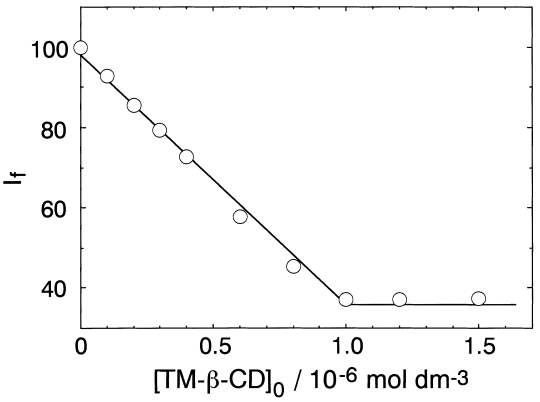

Fig. 4. Simulation of the observed fluorescence intensity data for the formation of the 2:1 TM-β-CD–TCPP inclusion complex. The best fit simulation curve for the 2:1 inclusion complex has been calculated under the assumptions of $c = 1.96 \times 10^8$ mol^{-1} dm^3, $d = 7.14 \times 10^7$ mol^{-1} dm^3, and $K_3 = 4.34 \times 10^{18}$ mol^{-2} dm^6. $[TCPP]_0 = 5.0 \times 10^{-7}$ mol dm^{-3}. $\lambda_{ex} = 416$ nm. $\lambda_{obs} = 680$ nm.

CD concentration of 1.0×10^{-6} mol dm^{-3}, the amount of the decrease in the fluorescence intensity is linear with the increase in the TM-β-CD concentration. The fluorescence intensity, however, remains constant above a TM-β-CD concentration of 1.0×10^{-6} mol dm^{-3}. This finding suggests that free TCPP is absent at TM-β-CD concentrations higher than 1.0×10^{-6} mol dm^{-3}; for TCPP of 5.0×10^{-7} mol dm^{-3}, the formation of the TM-β-CD–TCPP inclusion complex is accomplished at a TM-β-CD concentration of 1.0×10^{-6} mol dm^{-3}. The TM-β-CD concentration, where TCPP is completely converted to the inclusion complex, is just double the TCPP concentration used; this agrees with our conclusion, which has been derived from the continuous variation method, that the TM-β-CD–TCPP inclusion complex has a 2:1 host–guest stoichiometry.

Because the TCPP concentration of 5.0×10^{-7} mol dm^{-3} is in the TM-β-CD concentration range shown in Fig. 3, one cannot apply a plotting of $1/(I_f - I_f^0)$ against $1/[TM\text{-}\beta\text{-}CD]_0^2$ to the evaluation of the K_2 value. Thus, we have employed a simulation method. The equilibrium constant, K_2, is defined as

$$K_2 = [(TM\text{-}\beta\text{-}CD)_2 \cdot TCPP]/([TM\text{-}\beta\text{-}CD]^2[TCPP]) \quad (4)$$

For TCPP solution containing TM-β-CD, the observed fluorescence intensity of TCPP is given by the sum of the fluorescence intensities of free TCPP and the 2:1 TM-β-CD–TCPP inclusion complex.

Figure 4 shows the least-squares best fit simulation curve for the observed fluorescence intensities. From this simulation, a K_2 value is estimated to be 4.34×10^{18} mol^{-2} dm^6. A similar analysis was made using the absorbance change. In this case, an inflection point was seen at a TM-β-CD concentration of 2.0×10^{-6} mol dm^{-3}, which was also just twice the TCPP concentration (1.0×10^{-6} mol dm^{-3}) used in the experiment for the absorbance change. This provides additional evidence for the formation of the 2:1 TM-β-CD–TCPP inclusion complex. From the simulation for the absorbance change, a K_2 value was evaluated to be 4.87×10^{17} mol^{-2} dm^6. This K_2 value is one order of magnitude less than that obtained from the fluorescence change. The K_2 value evaluated from the fluorescence intensity change is more reliable than that from the absorbance change, because the fluorescence intensity change is greater than the absorbance change. As already noted, the K_2 value of TCPP in pH 7.0 buffer has been reported to be 1.4×10^{16} mol^{-2} dm^6.[13] Our estimated K_2 value in pH 10.1 buffer is two orders of magnitude greater than the reported one. For TSPP, the formation of the 2:1 TM-β-CD–TSPP inclusion complex has been confirmed.[11] However, the K_2 value (1.9×10^{13} mol^{-2} dm^6) of TSPP is five orders of magnitude less than that of TCPP. As mentioned previously, a similar trend is seen for the binding of γ-CD to TCPP and TSPP. The differences in the K values of TCPP and TSPP are most likely due to the fact that a carboxyphenyl moiety is more hydrophobic than a sulfonatophenyl moiety is.

Complex Formation of TCPP with HB in Basic Aqueous Solution. Figure 5 depicts absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of HB. As the HB concentration is increased below about 3.0×10^{-5} mol dm^{-3} of HB, the absorption peak is slightly shifted to longer wavelengths, accompanied by the reduction of the maximum absorbance and the appearance of an isosbestic point at 419.5 nm. Above around 1×10^{-4} mol dm^{-3} of HB, the isosbestic point disappears. The isosbestic point at low concentrations of HB suggests the 1:1 organic cation–organic anion complex formation between HB and TCPP.

$$TCPP + HB \underset{}{\overset{K_3}{\rightleftharpoons}} TCPP \cdot HB \quad (5)$$

Here, K_3 is the equilibrium constant for the formation of the 1:1 TCPP–HB complex (TCPP·HB). At high HB concentrations, a 1:2 TCPP–HB complex is most likely formed besides the 1:1 TCPP–HB complex, leading to the disappearance of the isosbestic point. In the low HB concentration range, where

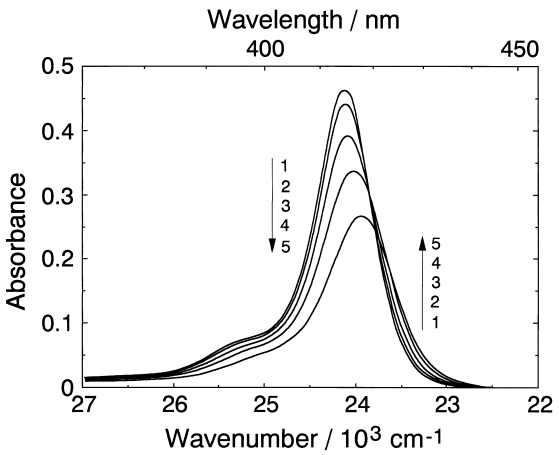

Fig. 5. Absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of HB. Concentration of HB: (1) 0, (2) 3.0×10^{-6}, (3) 1.0×10^{-5}, (4) 3.0×10^{-5}, and (5) 1.0×10^{-4} mol dm^{-3}.

only the 1:1 TCPP–HB complex is formed, K_3 can be evaluated according to the Benesi–Hildebrand type equation:

$$1/(A - A_0) = 1/b + 1/(bK_3[\mathrm{HB}]_0) \quad (6)$$

where A and A_0 are the absorbances in the presence and absence of HB, respectively, and b is a constant, and $[\mathrm{HB}]_0$ is the initial concentration of HB. From the plot based on Eq. 6, the K_3 value has been evaluated to be 43000 ± 3000 $\mathrm{mol^{-1}\ dm^3}$.

Upon the addition of HB to aqueous solution of TCPP, the TCPP fluorescence was significantly quenched, indicating the formation of the complex between TCPP and HB. On the basis of an equation similar to Eq. 2, a K_3 value has been evaluated to be 45000 ± 3000 $\mathrm{mol^{-1}\ dm^3}$. This K_3 value obtained from the fluorescence intensity change is nearly the same as that from the absorbance change, confirming that the 1:1 TCPP–HB complex alone is formed at a low HB concentration.

As in the case of HB, the addition of MB to TCPP solution resulted in the absorption spectral change of TCPP, indicating the formation of a TCPP–MB complex. From the absorbance change upon the addition of MB, a K_3 value of TCPP for MB has been evaluated to be 3800 ± 500 $\mathrm{mol^{-1}\ dm^3}$, which is one order of magnitude less than that for HB. This finding suggests that the hexyl groups of HB contribute to the complexation with TCPP. The K_3 value of Zn TSPP for MB has been reported to be 2.2×10^4 $\mathrm{mol^{-1}\ dm^3}$, which is one order of magnitude greater than that of TCPP.[23]

Interactions of TCPP with CDs and HB. Upon the addition of γ-CD, the absorption spectrum of HB has not been varied, suggesting that the interactions between γ-CD and HB are negligible. Figure 6 illustrates absorption spectra of TCPP (1.0×10^{-6} mol $\mathrm{dm^{-3}}$) in γ-CD (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) solutions containing various concentrations of HB. With the increase in the HB concentration, the absorption peak is shifted to longer wavelengths, accompanied by a reduction of the maximum absorbance.

To examine whether the absorption spectral change caused by the addition of HB is due to the formation of a γ-CD–TCPP–HB inclusion complex, induced circular dichroism (icd) spectra have been measured for TCPP in γ-CD (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) solutions in the absence and presence of HB (5.0×10^{-5} mol $\mathrm{dm^{-3}}$) (Fig. 7). In the absence of HB, a positive icd signal, which is due to the γ-CD–TCPP inclusion complex, is seen with a peak at 418 nm. A positive signal has similarly been observed for the γ-CD–TSPP inclusion complex.[11] Upon the addition of HB to TCPP solution containing γ-CD, the positive signal is reduced in intensity, accompanied by an appearance of a negative signal at around 430 nm. This finding suggests the formation of a ternary inclusion complex among γ-CD, TCPP, and HB, at the expense of the γ-CD–TCPP inclusion complex. In the γ-CD–TCPP–HB inclusion complex, the orientation of the TCPP molecule relative to the γ-CD cavity is different from that in the γ-CD–TCPP inclusion complex, leading to the negative icd signal.

For comparison, the icd spectrum of TCPP has been examined in a solution containing TM-β-CD and HB. In this case, the positive signal of TCPP in solution containing TM-β-CD has only been reduced by the addition of HB, suggesting the dissociation of the TM-β-CD–TCPP inclusion complex and no formation of a ternary inclusion complex among TM-β-CD, TCPP, and HB (not shown). The narrow cavity of TM-β-CD compared to γ-CD is responsible for no formation of the ternary inclusion complex of TM-β-CD with TCPP and HB.

The γ-CD–TCPP–HB inclusion complex most likely has a stoichiometry of 1:1:1 from a viewpoint of the steric factor.

$$\gamma\text{-CD}\cdot\mathrm{TCPP} + \mathrm{HB} \overset{K_4}{\rightleftharpoons} \gamma\text{-CD}\cdot\mathrm{TCPP}\cdot\mathrm{HB} \quad (7)$$

Here, K_4 is the equilibrium constant for the formation of the 1:1:1 γ-CD–TCPP–HB inclusion complex (γ-CD·TCPP·HB). Taking into account Eqs. 1, 5, and 7, one can derive a quadratic

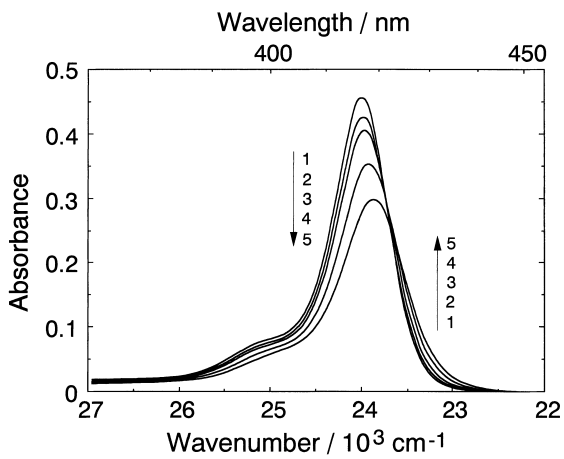

Fig. 6. Absorption spectra of TCPP (1.0×10^{-6} mol $\mathrm{dm^{-3}}$) in pH 10.1 buffers containing γ-CD (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) and various concentrations of HB. Concentration of HB: (1) 0, (2) 1.0×10^{-5}, (3) 3.0×10^{-5}, (4) 1.0×10^{-4}, and (5) 3.0×10^{-4} mol $\mathrm{dm^{-3}}$.

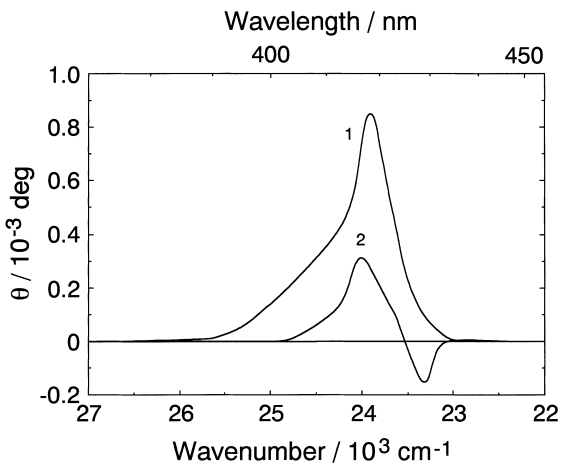

Fig. 7. Induced circular dichroism spectra of TCPP (2.0×10^{-6} mol $\mathrm{dm^{-3}}$) in pH 10.1 buffers containing γ-CD (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) (curve 1) and both γ-CD (3.0×10^{-4} mol $\mathrm{dm^{-3}}$) and HB (5.0×10^{-5} mol $\mathrm{dm^{-3}}$) (curve 2).

equation for [TCPP]:

$$(K_3 + K_1K_4[\gamma\text{-CD}]_0 + K_1K_3[\gamma\text{-CD}]_0 + K_1^2K_4[\gamma\text{-CD}]_0^2)[\text{TCPP}]^2 + (1 + K_3[\text{HB}]_0 - K_3[\text{TCPP}]_0 + K_1[\gamma\text{-CD}]_0 + K_1K_4[\gamma\text{-CD}]_0[\text{HB}]_0 - K_1K_4[\gamma\text{-CD}]_0[\text{TCPP}]_0)[\text{TCPP}] - [\text{TCPP}]_0 = 0 \quad (8)$$

When a K_4 value is assumed, the TCPP concentration is calculated from Eq. 8, since the K_1 and K_3 values are already known. With respect to the concentration of HB, the following equation holds:

$$[\text{HB}] = [\text{HB}]_0/(1 + K_3[\text{TCPP}] + K_1K_4[\gamma\text{-CD}]_0[\text{TCPP}]) \quad (9)$$

Consequently, the HB concentration can be evaluated from Eq. 9, using the TCPP concentration. The absorbance of TCPP in solution containing γ-CD and HB is represented as

$$A = (\varepsilon_0 + \varepsilon_1K_1[\gamma\text{-CD}]_0 + \varepsilon_2K_3[\text{HB}] + \varepsilon_3K_1K_4[\gamma\text{-CD}]_0[\text{HB}])[\text{TCPP}]d \quad (10)$$

where ε_0, ε_1, ε_2, and ε_3 are the molar absorption coefficients of free TCPP, the γ-CD–TCPP inclusion complex, the TCPP–HB complex, and the γ-CD–TCPP–HB inclusion complex, respectively, and d is the path length of a cell. Consequently, the absorbance of TCPP can be calculated from Eq. 10, since the TCPP and HB concentrations are evaluated for the assumed K_4 value. From the simulation based on Eq. 10, a K_4 value has been estimated to be 5000 mol^{-1} dm^3 (not shown). The excellent fit of the simulation curve to the observed data supports the formation of the 1:1:1 γ-CD–TCPP–HB inclusion complex.

As in the case of the absorbance change, we have simulated the fluorescence intensity change for TCPP solutions containing both γ-CD and HB. When HB is added to TCPP solution containing γ-CD, the fluorescence intensity of TCPP is represented by

$$I_f = (e_0 + e_1K_1[\gamma\text{-CD}] + e_2K_3[\text{HB}] + e_3K_1K_4[\gamma\text{-CD}][\text{HB}])[\text{TCPP}] \quad (11)$$

Here, e_0, e_1, e_2, and e_3 are the experimental constants that include the fluorescence quantum yields of free TCPP, the γ-CD–TCPP inclusion complex, the TCPP–HB complex, and the γ-CD–TCPP–HB inclusion complex, respectively.

The best-fit simulation curve according to Eq. 11, which has been calculated in a manner similar to the procedure for the absorbance change, is exhibited along with the observed fluorescence intensity data (Fig. 8). The excellent fit of the simulation curve to the observed data confirms the existence of the 1:1:1 γ-CD–TCPP–HB inclusion complex. From this simulation, a K_4 value is evaluated to be 7270 mol^{-1} dm^3, with $e_0 = 1.07 \times 10^8$, $e_1 = 9.66 \times 10^7$, $e_2 = 0$, and $e_3 = 0$ mol^{-1} dm^3. The K_4 value evaluated from the fluorescence intensity is 1.45 times greater than that evaluated from the absorbance. The former K_4 value is, however, more reliable, because the fluorescence intensity change is greater than the absorbance change. The finding that the e_2 and e_3 values are zero is consistent with the fact that, irrespective of the presence of γ-CD, the

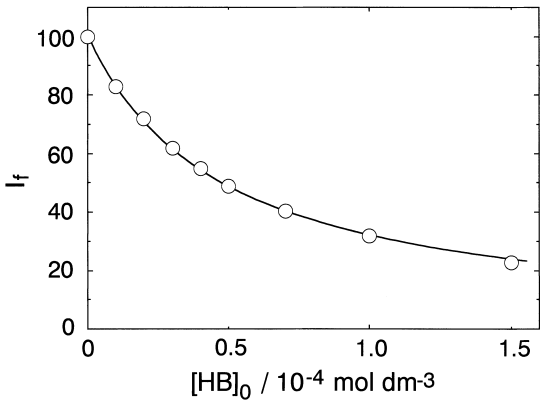

Fig. 8. Comparison of the observed fluorescence intensity data with the fluorescence intensity curve simulated for the formation of the 1:1:1 TM-β-CD–TCPP–HB inclusion complex. The best fit simulation curve for the 1:1:1 inclusion complex has been calculated under the assumption of $e_0 = 1.07 \times 10^8$, $e_1 = 9.66 \times 10^7$, $e_2 = 0$, $e_3 = 0$, and $K_4 = 7.27 \times 10^3$ mol^{-1} dm^3. $[\text{TCPP}]_0 = 1.0 \times 10^{-6}$ mol dm^{-3}. $[\gamma\text{-CD}]_0 = 3.0 \times 10^{-4}$ mol dm^{-3}. $\lambda_{ex} = 421$ nm. $\lambda_{obs} = 650$ nm.

TCPP fluorescence is quenched by HB. This implies that the TCPP–HB complex and the γ-CD–TCPP–HB inclusion complex do not emit the fluorescence.

The equilibrium constant for the formation of the γ-CD–TCPP–HB inclusion complex from γ-CD and the TCPP–HB complex, represented by K_5, is defined as

$$K_5 = [\gamma\text{-CD}\cdot\text{TCPP}\cdot\text{HB}]/([\gamma\text{-CD}][\text{TCPP}\cdot\text{HB}]) \quad (12)$$

Consequently, a relationship holds for K_1, K_3, K_4, and K_5:

$$K_1K_4 = K_3K_5 \quad (13)$$

Since the values of K_1, K_3, and K_4 are already known, the K_5 value is estimated to be 905 mol^{-1} dm^3.

The K_5 values, which are the equilibrium constants for the formation of the 1:1:1 inclusion complex from CD and an organic cation–organic anion complex, are in the range of 33 – 900 mol^{-1} dm^3 for the 1:1:1 γ-CD–2,6-bis(1-pyridiniomethyl)naphthalene dibromide–naphthalenedicarboxylate inclusion complexes.[24] The K_5 value for the γ-CD–TCPP–HB inclusion complex is nearly the same as that (900 mol^{-1} dm^3) for the γ-CD–2,6-bis(1-pyridiniomethyl)naphthalene–2,6-naphthalenedicarboxylate inclusion complex. On the other hand, the K_5 value is 21500 mol^{-1} dm^3 for the 1:1:1 γ-CD–thionine–2-naphthalenesulfonate inclusion complex.[14]

When MB was added to TCPP (2.0×10^{-6} mol dm^{-3}) solution containing γ-CD (3.0×10^{-4} mol dm^{-3}), a new signal was not observed, although the positive signal due to the γ-CD–TCPP inclusion complex was reduced in intensity. This finding indicates no formation of a ternary inclusion complex among γ-CD, TCPP, and MB. In the γ-CD–TCPP–HB inclusion complex, therefore, a carboxyphenyl moiety in TCPP and a heptyl group in HB are most likely encapsulated simultaneously into the γ-CD cavity.

Upon the addition of MB (1.0×10^{-3} mol dm^{-3}) to TSPP (2.0×10^{-6} mol dm^{-3}) solution containing γ-CD (3.0×10^{-3} mol dm^{-3}), a positive icd signal due to the γ-CD–TSPP inclusion complex disappeared. This finding suggests that MB induces the dissociation of the γ-CD–TSPP inclusion complex rather than the formation of a ternary inclusion complex among γ-CD, TSPP, and MB.

Conclusions

TCPP forms inclusion complexes with γ-CD and TM-β-CD in pH 10.1 buffers. The stoichiometries of the γ-CD–TCPP and TM-β-CD–TCPP inclusion complexes are 1:1 and 2:1, respectively. The 2:1 TM-β-CD–TCPP inclusion complex is quantitatively formed, because the K_2 value is exceedingly large (4.34×10^{18} mol^{-2} dm^6).

TCPP also forms an organic cation–organic anion complex with HB in pH 10.1 buffer, with $K_3 = 45000 \pm 3000$ mol^{-1} dm^3. When HB is added to TCPP solution containing γ-CD, the 1:1:1 γ-CD–TCPP–HB inclusion complex is formed. The K_4 value for the γ-CD–TCPP–HB inclusion complex has been evaluated to be 7270 mol^{-1} dm^3 from a simulation method using the fluorescence intensity. On the other hand, the addition of TM-β-CD instead of γ-CD does not result in the formation of the ternary inclusion complex. This finding suggests that the cavity size critically controls the formation of the ternary inclusion complex.

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