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Complex Formation of Hematoporphyrin with Cyclodextrins and 1,1′-Diheptyl-4,4′-bipyridinium Dibromide in Aqueous Solutions

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At around 5×10^{-6} mol dm^{-3} of hematoporphyrin (HP), an HP dimer exists as well as an HP monomer. The equilibrium constant for the dimerization of HP in pH 10.0 buffer has been evaluated to be 1.70×10^5 mol^{-1} dm^3 from the HP concentration dependence of the absorption spectrum. In aqueous solution, HP forms 1:1 inclusion complexes with β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD). The fluorescence of HP is significantly enhanced by the addition of CDs. From simulations of the fluorescence intensity changes, the equilibrium constants for the formation of the CD–HP inclusion complexes have been estimated to be 200, 95.7, and 938 mol^{-1} dm^3 for β-CD, γ-CD, and TM-β-CD, respectively. HP forms a 1:1 complex with 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHB) in aqueous solution. In contrast to the addition of CDs, the HP fluorescence is significantly quenched by the addition of DHB. The equilibrium constant for the formation of the HP–DHB complex has been evaluated to be 1.98×10^5 mol^{-1} dm^3 from the fluorescence intensity change of HP. The addition of DHB to an HP solution containing β-CD induces a circular dichroism signal of negative sign, indicating the formation of a ternary inclusion complex involving β-CD, HP, and DHB. In contrast, there is no evidence for the formation of a ternary inclusion complex of HP with DHB and TM-β-CD.

Keywords: Hematoporphyrin; 1,1′-Diheptyl-4,4′-bipyridinium dibromide; Cyclodextrins; Absorption spectra; Fluorescence spectra

INTRODUCTION

Cyclodextrins (CDs), which are cyclic oligomers composed of D-(+)-glucopyranose residues, are shaped like a truncated cone with a relatively hydrophobic cavity. CDs having six, seven, and eight D-(+)-glucopyranose residues are named α-CD, β-CD, and γ-CD, respectively [1]. Although the exterior of the CD cavity is hydrophilic due to the many hydroxy groups attached to the narrow and wide CD rims, a variety of organic compounds are encapsulated by the CD cavity, because of its hydrophobicity. The incorporation of the organic compounds into the CD cavity leads to variations in their physicochemical properties. Due to such useful characteristics of CDs, they are used in the food, pharmaceuticals and cosmetics industries.

Porphyrin derivatives are requisite materials in life. Consequently, the behavior of porphyrin derivatives receives a great deal of attention. Several porphyrin derivatives have been found to form inclusion complexes with CDs in aqueous solutions [2–21]. Hirai *et al.* have found that hematoporphyrin (hematoporphyrin IX), etc., form inclusion complexes with α- and γ-CD [2]. They have evaluated the equilibrium constants for the formation of such inclusion complexes, as well as the equilibrium constant for the dimerization of hematoporphyrin. Manka and Lawrence have indicated that heptakis(2,6-di-*O*-methyl)-β-CD forms a 2:1 inclusion complex with a protonated tetraamino porphyrin derivative [3]. Ribo *et al.* have described the inclusion complexation of tetrakis(4-sulfonatophenyl)porphyrin (TSPP) with α-, β-, and γ-CD by means of electronic absorption and ^{1}H NMR spectroscopy [6]. Kano *et al.* have investigated the inclusion complexation of cationic and anionic porophyrins including TSPP with β-CD, heptakis(2,6-di-*O*-methyl)-β-CD, and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin

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(TM-β-CD) on the basis of analyses of absorption and NMR data [8,20]. They have demonstrated the formation of a 2:1 TM-β-CD–TSPP inclusion complex. Carofiglio *et al.* have reported that TM-β-CD forms a 2:1 host–guest inclusion complex with tetrakis(4-carboxyphenyl)porphyrin (TCPP) [9].

Recently, we have examined the interactions of CDs with TSPP, Fe(III) tetrakis(4-sulfonatophenyl)-porphyrin (FeTSPP), and TCPP in aqueous solution [11,17,19]. γ-CD forms 1:1 inclusion complexes with TSPP, FeTSPP, and TCPP, whereas TM-β-CD forms 2:1 host–guest inclusion complexes with TSPP and TCPP, although a 1:1 TM-β-CD–FeTSPP inclusion complex has been observed. Furthermore, upon the addition of 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHB) to TCPP solution containing γ-CD, a ternary inclusion complex is formed involving γ-CD, TCPP, and DHB. In the γ-CD–TCPP–DHB inclusion complex, a heptyl group of DHB is most likely involved in the inclusion complexation [19]. In TCPP solution containing DHB, DHB forms an organic cation–organic anion complex with TCPP. Methylene Blue similarly forms an organic cation–organic anion complex with TSPP [18]. However, a ternary inclusion complex is not formed between γ-CD, TSPP, and Methylene Blue.

Ribo *et al.*, Kano *et al.*, and Carofiglio *et al.* have shown that the binding sites of TSPP and TCPP towards CD contain a sulfonatophenyl moiety and a carboxylatophenyl moiety, respectively [6,8,9]. In hematoporphyrin, a 1-hydroxyethyl moiety and/or a 2-carboxylatoethyl moiety are expected to be the binding sites for CD. Because these moieties are less bulky, there may be the possibility that a ternary inclusion complex is formed between CD, HP, and a molecule of an appropriate size, although the binding site(s) of HP is less hydrophobic than those of TSPP and TCPP. We have observed complexation between HP and DHB in aqueous solution. Thus, we examined the complexation of HP with CDs and determined whether or not a ternary inclusion complex including CD and HP is formed in the presence of DHB as the third component.

RESULTS AND DISCUSSION

Dependence of the Absorption Spectrum of HP on the HP Concentration

Figure 1 shows the HP concentration effects on the Soret band of HP in aqueous solution (pH 10.0). When the HP concentration is increased, the absorption peak at 390 nm disappears, accompanied by an increase in the absorption intensity at 300–370 nm. This finding suggests an equilibrium between a monomer and a dimer of HP. The absorption band at 390 nm is assigned to the HP monomer band, whereas the absorption band at around 375 nm is assigned to the HP dimer band. The molar absorption coefficient, ε, of HP is represented by the sum of the contributions from the HP monomer and the HP dimer [Eq. (1) in the Experimental section]. In accordance with Eq. (1), a simulation was made to estimate the value of the equilibrium constant (K_D) for the formation of the HP dimer. Figure 2 depicts the HP concentration dependence of the ε value observed at 400 nm, together with the least-squares best-fit simulation curve, which has been calculated with variables $\varepsilon_0 = 8.40 \times 10^4\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$, $\varepsilon_1 = 9.72 \times 10^4\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$, and $K_D = 1.70 \times 10^5\ \mathrm{mol^{-1}\,dm^3}$. The good fit of the simulation curve to the observed data confirms the existence of the HP dimer. Although a simulation for the monomer–trimer equilibrium was performed, the fit of the best-fit simulation curve to the observed data was not as good as that for the monomer–dimer equilibrium. This supports the existence of the monomer–dimer equilibrium of HP. The K_D value determined in this study is comparable to a reported value of

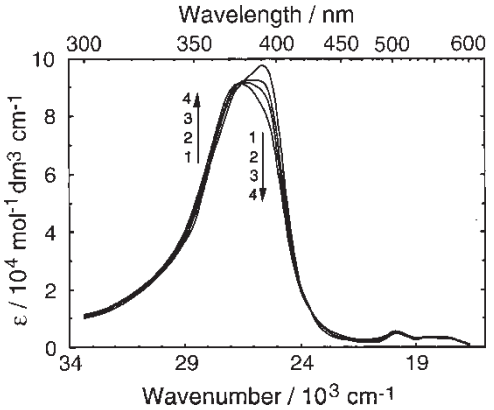


FIGURE 1 Dependence of the absorption spectrum of HP in aqueous solution (pH 10.0) on the HP concentration. Concentration of HP: (1) 5.0×10^{-7}, (2) 2.0×10^{-6}, (3) 5.0×10^{-6}, and (4) $1.0 \times 10^{-5}\ \mathrm{mol\,dm^{-3}}$.

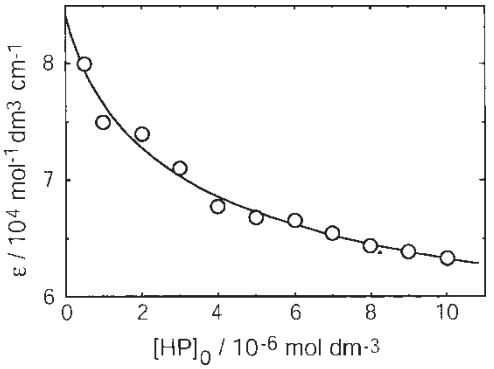


FIGURE 2 Comparison of the ε value observed at 400 nm with the least-squares best-fit simulation curve calculated with $\varepsilon_0 = 8.40 \times 10^4\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$, $\varepsilon_1 = 9.72 \times 10^4\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$, and $K_D = 1.70 \times 10^5\ \mathrm{mol^{-1}\,dm^3}$.

$4(\pm 0.4) \times 10^5\,\mathrm{mol^{-1}\,dm^3}$ (pH 7.2 Tris-HCl buffer) [22], but is about 3.5 times greater than a reported value of $4.9 \times 10^4\,\mathrm{mol^{-1}\,dm^3}$ ($0.002\,\mathrm{mol\,dm^{-3}}$ NaOH solution) [2]. The dimerization of HP is caused by the association between anionic species. Consequently, the magnitude of the K_D value of HP may be affected by the experimental conditions, such as the buffer.

Formation of Inclusion Complexes of HP with γ-CD, β-CD, and TM-β-CD

Figure 3 shows the absorption spectra of HP ($5.0 \times 10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions (pH 10.0) containing various concentrations of γ-CD. As the γ-CD concentration is increased, an absorption peak of HP at around 377 nm is reduced in intensity, accompanied by an isosbestic point at 395 nm. At around 377 nm, the molar absorption coefficient of the HP dimer is greater than that of the HP monomer. Consequently, this spectral change is explained in terms of the dissociation of the HP dimer to the HP monomers, which is induced by the formation of an inclusion complex between γ-CD and HP. Figure 4 illustrates fluorescence spectra of HP ($5.0 \times 10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions containing various concentrations of γ-CD. Upon the addition of γ-CD, the fluorescence maxima of HP are slightly shifted to longer wavelengths, with an enhancement of the fluorescence intensity. This finding indicates the formation of an inclusion complex between γ-CD and HP. The finding that the HP dimer is dissociated to HP monomers by the addition of γ-CD, indicates that the γ-CD–HP inclusion complex has most likely a 1:1 stoichiometry.

In HP solutions containing γ-CD, there exist the HP monomer, the HP dimer, and the 1:1 γ-CD–HP inclusion complex. Consequently, the HP fluorescence intensity, I_f, is given by the sum of the fluorescence intensities of the three species [Eq. (3) in the Experimental section]. Figure 5 shows the observed fluorescence intensities of HP as a function of the γ-CD concentration, together with the least-squares best-fit simulation curve calculated with $a = 7.51 \times 10^5$, $b = 5.69 \times 10^7$, $c = 3.41 \times 10^7$, and $K_1 = 95.7\,\mathrm{mol^{-1}\,dm^3}$, where K_1 is the equilibrium constant for the formation of the γ-CD–HP inclusion complex (see the Experimental section). As shown in Fig. 5, the good fit of the simulation curve to the observed data supports the 1:1 stoichiometry of the γ-CD–HP inclusion complex. The K_1 value obtained in this study is close to a reported K_1 value of $73\,\mathrm{mol^{-1}\,dm^3}$ for a $0.002\,\mathrm{mol\,dm^{-3}}$ NaOH solution of HP [2]. The K_1 value ($95.7\,\mathrm{mol^{-1}\,dm^3}$) for HP is about 17 and 59 times less than those for TSPP and TCPP, respectively, indicating the considerably weaker interactions of γ-CD with HP compared to TSPP and TCPP [11,19]. γ-CD is most likely bound to a 1-hydroxyethyl moiety and/or a 2-carboxylatoethyl moiety of HP. On the other hand, the binding sites of TSPP and TCPP are a sulfonatophenyl moiety and

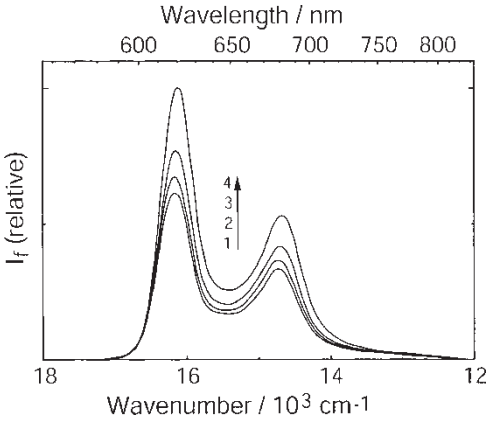


FIGURE 4 Corrected fluorescence spectra of HP ($5.0 \times 10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions (pH 10.0) containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, and (4) $1.0 \times 10^{-2}\,\mathrm{mol\,dm^{-3}}$. $\lambda_{ex} = 420$ nm.

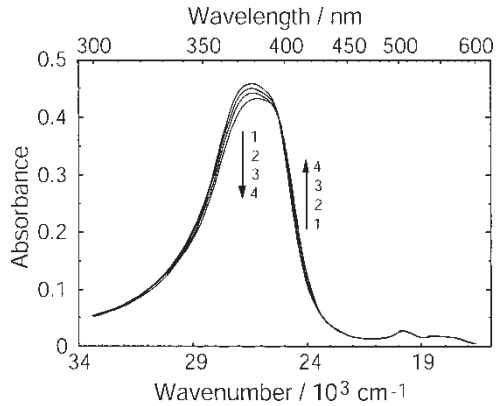


FIGURE 3 Absorption spectra of HP ($5.0 \times 10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions (pH 10.0) containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, and (4) $1.0 \times 10^{-2}\,\mathrm{mol\,dm^{-3}}$.

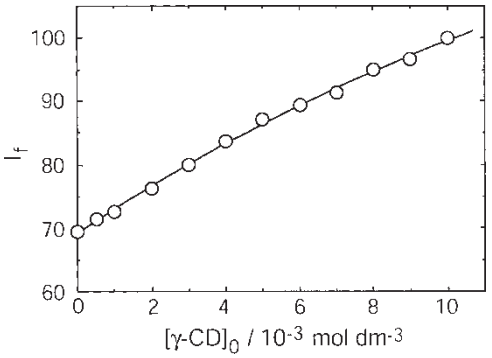


FIGURE 5 Comparison of the fluorescence intensity of HP in aqueous solution (pH 10.0) with the least-squares best fit simulation curve calculated with $a = 7.51 \times 10^5$, $b = 5.69 \times 10^7$, $c = 3.41 \times 10^7$, and $K_1 = 95.7\,\mathrm{mol^{-1}\,dm^3}$. $[\mathrm{HP}]_0 = 5.0 \times 10^{-6}\,\mathrm{mol\,dm^{-3}}$. $\lambda_{ex} = 420$ nm. $\lambda_{obs} = 617$ nm.

a carboxylatophenyl moiety, respectively, which are bulkier and more hydrophobic than the 1-hydroxyethyl and 2-carboxylatoethyl moieties. Consequently, it is reasonable that the K_1 value for HP is significantly less than those for TSPP and TCPP. If an additional γ-CD molecule is bound to the 1:1 γ-CD–HP inclusion complex, its equilibrium constant is expected to be similar to the K_1 value. To form a 2:1 γ-CD–HP inclusion complex, therefore, a significantly high concentration of the γ-CD-HP inclusion complex is required for the small K_1 value. This is not the case. Consequently, such a small K_1 value of HP is consistent with a 1:1 stoichiometry for the γ-CD–HP inclusion complex.

When β-CD was added to an HP solution, the absorption spectrum of HP exhibited a spectral change similar to that upon addition of γ-CD. This finding suggests the formation of an inclusion complex of β-CD with the HP monomer, followed by the dissociation of the HP dimer to HP monomers. As the β-CD concentration was increased, the HP fluorescence was enhanced, indicating the formation of a β-CD–HP inclusion complex. From a simulation of the change in fluorescence intensity upon addition of β-CD, a K_1 value for β-CD was estimated to be $200\,\mathrm{mol^{-1}\,dm^3}$ (not shown). The β-CD–HP inclusion complex most likely has a 1:1 stoichiometry, because the simulation curve excellently fitted the observed data, which was calculated on the basis of a 1:1 stoichiometry. The K_1 value obtained for β-CD is about twice that for γ-CD, indicating that the binding site of HP fits the β-CD cavity more snugly compared with γ-CD. This confirms that a 1-hydroxyethyl moiety and/or a 2-carboxylatoethyl moiety are/is the binding sites/site of HP.

Upon addition of TM-β-CD to HP solution, an absorption spectral change similar to that for γ-CD was observed, suggesting the formation of an inclusion complex of TM-β-CD with HP. The HP fluorescence was enhanced by an increase in the TM-β-CD concentration, confirming the formation of the TM-β-CD–HP inclusion complex. As in the case of γ-CD, the analysis based on Eq. (3) was performed; a K_1 value of $938\,\mathrm{mol^{-1}\,dm^3}$ was found for TM-β-CD (not shown). An excellent fit of the simulation curve to the data suggests the formation of the 1:1 TM-β-CD–HP inclusion complex, in sharp contrast to the 2:1 TM-β-CD–TSPP and 2:1 TM-β-CD–TCPP inclusion complexes [8,9,11,19].

The equilibrium constants for the formation of the 2:1 TM-β-CD–TSPP and 2:1 TM-β-CD–TCPP inclusion complexes were evaluated to be 1.92×10^{13} and $4.34\times10^{18}\,\mathrm{mol^{-2}\,dm^6}$, respectively, from the fluorescence intensity changes [11,19,23]. Under the assumption that the incorporation of TSPP (TCPP) into a first and a second TM-β-CD cavity has the same binding constant, the equilibrium constants (K') for the stepwise (first or second) binding of TM-β-CD to TSPP and TCPP are calculated to be 4.38×10^6 and $2.08\times10^9\,\mathrm{mol^{-1}\,dm^3}$, respectively. The K_1 value of HP for TM-β-CD is significantly less than the K' values estimated for TSPP and TCPP. Consequently, it is reasonable that the second binding of HP to TM-β-CD does not occur to form a 2:1 TM-β-CD–HP inclusion complex. The inspection of the magnitudes of the K_1 and K' values supports the 1:1 stoichiometry for the TM-β-CD–HP inclusion complex.

Complex Formation between HP and DHB

Figure 6 shows absorption spectra of HP ($5.0\times10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions containing various concentrations of DHB. As the DHB concentration is increased, the absorption maximum of HP is reduced in intensity, accompanied by isosbestic points at 406 and 493 nm. Upon addition of DHB ($1.0\times10^{-4}\,\mathrm{mol\,dm^{-3}}$), the absorption maximum of HP is shifted from 375 nm to around 390 nm. These findings indicate the complex formation between HP and DHB. The absorption maximum shift implies that the HP dimer is converted into HP monomers by the addition of DHB. Consequently, the HP–DHB complex has a 1:1 stoichiometry. In accordance with Eq. (5) (Experimental section), a K_2 value of $1.85\times10^5\,\mathrm{mol^{-1}\,dm^3}$ has been determined from the simulation of the absorbance at 370 nm (not shown), where K_2 is the equilibrium constant for the formation of the HP–DHB complex. Values of ε_0, ε_1, and ε_2 have been estimated to be 1.08×10^5, 1.32×10^5, and $6.07\times10^4\,\mathrm{mol^{-1}\,dm^3\,cm^{-1}}$, respectively.

Figure 7 depicts the fluorescence spectra of HP ($5.0\times10^{-6}\,\mathrm{mol\,dm^{-3}}$) in aqueous solutions containing various concentrations of DHB. In the presence of DHB, the HP fluorescence is quenched. At the same time, the fluorescence maxima are very slightly blue-shifted. The formation of the HP–DHB complex causes an increase in the concentration ratio of HP

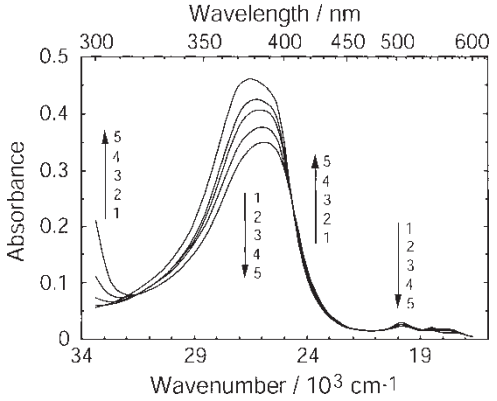


FIGURE 6 Absorption spectra of HP (5.0×10^{-6} M) in aqueous solutions (pH 10.0) containing various concentrations of DHB. Concentration of DHB: (1) 0, (2) 3.0×10^{-6}, (3) 1.0×10^{-5}, (4) 3.0×10^{-5}, and (5) $1.0\times10^{-4}\,\mathrm{mol\,dm^{-3}}$.

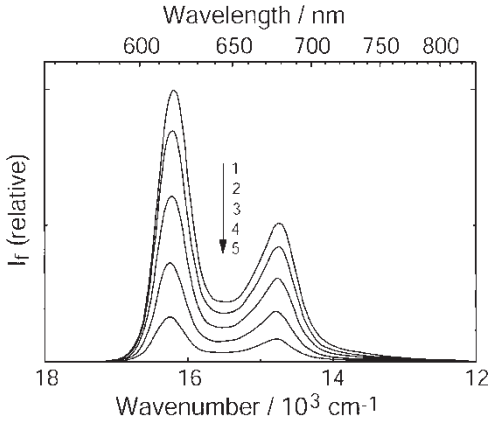


FIGURE 7 Corrected fluorescence spectra of HP (5.0×10^{-6} mol dm^{-3}) in aqueous solutions (pH 10.0) containing various concentrations of DHB. Concentration of DHB: (1) 0, (2) 3.0×10^{-6}, (3) 1.0×10^{-5}, (4) 3.0×10^{-5}, and (5) 1.0×10^{-4} mol dm^{-3}. $\lambda_{ex}=406$ nm.

monomer to HP dimer, because the sum of the concentrations of the HP monomer and dimer is decreased due to the formation of the HP–DHB complex. Consequently, the HP monomer is responsible for the very slight blue-shifts of the HP fluorescence maxima.

As in the case of the absorbance change, a K_2 value can be estimated from the fluorescence intensity change by the addition of DHB. On the basis of Eq. (8) (Experimental section), we have simulated the fluorescence intensities observed as a function of the DHB concentration. Figure 8 shows the observed fluorescence intensities, along with the least-squares best-fit simulation curve calculated with $d=3.73\times10^7$, $e=1.07\times10^6$, $f=1.86\times10^6$, and $K_2=1.98\times10^5$ mol^{-1} dm^3. The K_2 value obtained from the fluorescence intensity change is in good agreement with that obtained from the absorbance change.

For TCPP, K_2 values of 45000 ± 3000 and 43000 ± 3000 mol^{-1} dm^3 have been determined from the fluorescence intensity and absorbance changes, respectively [19]. These K_2 values of TCPP are about one-fourth that of HP, implying that the interactions between HP and DHB are stronger than those between TCPP and DHB. In TCPP, the benzene rings of the carboxylatophenyl moieties are rotated to some degree relative to the porphyrin ring. Consequently, carboxylatophenyl moieties may somewhat obstruct the interactions between TCPP and DHB, leading to a lesser K_2 value than that for HP.

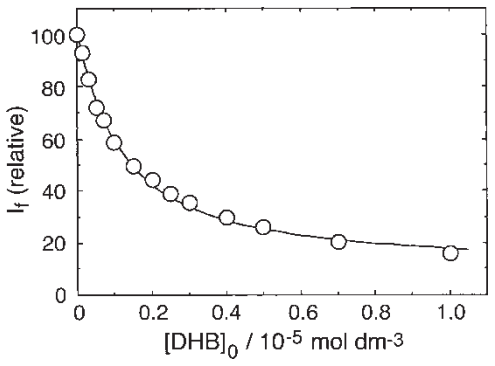


FIGURE 8 Comparison of the HP fluorescence intensity observed at 617 nm with the least-squares best-fit simulation curve calculated with $d=3.73\times10^7$, $e=1.07\times10^6$, $f=1.86\times10^6$, and $K_2=1.98\times10^5$ mol^{-1} dm^3. $[HP]_0=5.0\times10^{-6}$ mol dm^{-3}. $\lambda_{ex}=406$ nm.

The Interactions of the HP–DHB Complex with CDs

A ternary CD inclusion complex including a porphyrin derivative has been found for the system of γ-CD–TCPP–DHB [19]. The addition of β- or γ-CD induces the dissociation of a TSPP–Methylene Blue complex to its components [18]. Figure 9 depicts the absorption spectrum of HP (5.0×10^{-6} mol dm^{-3}) in aqueous solution containing DHB (3.0×10^{-5} mol dm^{-3}) and that containing both DHB (3.0×10^{-5} mol dm^{-3}) and γ-CD (1.0×10^{-2} mol dm^{-3}). The addition of γ-CD to an HP solution containing DHB results in a red-shift of about 3 nm in the absorption maximum, accompanied by a slight enhancement of the absorption intensity. As shown in Fig. 3, the addition of γ-CD shifts the monomer–dimer equilibrium in favor of the monomer. DHB and γ-CD affect the absorption spectrum of HP through complexation with HP, i.e., the formation of the HP–DHB complex and the γ-CD–HP inclusion complex, respectively. At the concentrations shown in Fig. 9, values of $K_1[\gamma\text{-CD}]_0$ and $K_2[DHB]_0$ are calculated to be 0.957 and 5.94, respectively. Consequently, the magnitude of the addition effects of γ-CD is at most one sixth of that of DHB. Nonetheless, a 3 nm red-shift upon addition of γ-CD is observed for the absorption maximum of HP in DHB solution. Therefore, this suggests that γ-CD causes the formation of not only

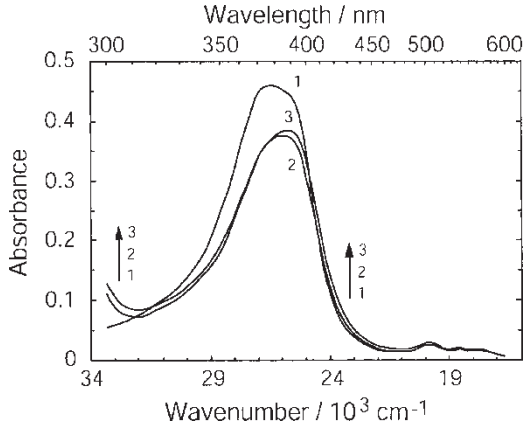


FIGURE 9 Absorption spectrum of HP in pH 10.0 buffer (spectrum 1) and those in pH 10.0 buffers containing DHB (3.0×10^{-5} mol dm^{-3}) (spectrum 2) and both DHB (3.0×10^{-5} mol dm^{-3}) and γ-CD (1.0×10^{-2} mol dm^{-3}) (spectrum 3). $[HP]_0=5.0\times10^{-6}$ mol dm^{-3}.

the γ-CD–HP inclusion complex but also the γ-CD–HP–DHB inclusion complex. To confirm the formation of the γ-CD–HP–DHB inclusion complex, we attempted to measure an induced circular dichroism (ICD) spectrum of HP (1.0×10^{-5} mol dm^{-3}) in aqueous solutions containing γ-CD (1.0×10^{-2} mol dm^{-3}) and one containing both γ-CD (1.0×10^{-2} mol dm^{-3}) and DHB (3.0×10^{-5} mol dm^{-3}). However, the ICD signals due to HP could not be observed, probably because the concentrations of the γ-CD–HP and γ-CD–HP–DHB inclusion complexes were too low to be detected by means of ICD spectroscopy.

To further examine the formation of a ternary inclusion complex of HP and DHB, we employed β-CD instead of γ-CD. The absorption spectral change for HP solution containing DHB (3.0×10^{-5} mol dm^{-3}) upon addition of β-CD (1.0×10^{-2} mol dm^{-3}) was similar to that upon the addition of γ-CD; in the presence of β-CD the absorption maximum was shifted to longer wavelengths. This finding suggests the formation of a ternary inclusion complex between β-CD, HP, and DHB. Fig. 10 illustrates the ICD spectrum of HP (1.0×10^{-5} mol dm^{-3}) solution containing β-CD (1.0×10^{-2} mol dm^{-3}) (spectrum 1) and that containing both β-CD (1.0×10^{-2} mol dm^{-3}) and DHB (3.0×10^{-5} mol dm^{-3}) (spectrum 2). In the ICD spectrum of HP solution containing β-CD, there is little signal (less than -0.1 mdeg). At around 405 nm, on the other hand, a weak, negative band (about -0.4 mdeg) is observed for the icd spectrum of HP solution containing β-CD and DHB. This finding provides evidence for the formation of the β-CD–HP–DHB inclusion complex.

For the β-CD–TSPP and γ-CD–TCPP–DHB inclusion complexes, negative ICD signals have been detected, although a positive signal has been observed for the γ-CD–TCPP inclusion complex [11,19]. The electronic transition dipole moments

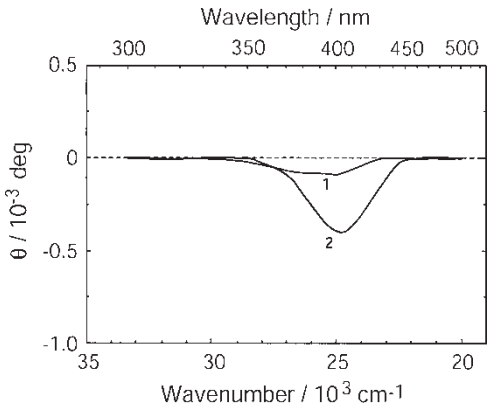


FIGURE 10 Induced circular dichroism spectra of HP (1.0×10^{-5} mol dm^{-3}) in pH 10.0 buffers containing β-CD (1.0×10^{-2} mol dm^{-3}) (spectrum 1) and both β-CD (1.0×10^{-2} mol dm^{-3}) and DHB (3.0×10^{-5} mol dm^{-3}) (spectrum 2).

responsible for the Soret band of HP are most likely directed to the N–N and NH–NH axes of the porphyrin ring [24,25]. In the inclusion complex of CD with HP, the transition dipoles of HP are located outside of the CD cavity. In this case, an ICD spectrum exhibits a negative sign under conditions where the angle between the directions of the transition dipole moment and the symmetry axis of CD is less than 54.7° [26]. Upon axial binding of CD to a 1-hydroxyethyl moiety and/or a 2-carboxylatoethyl moiety of HP, the angles between the transition dipoles and the CD symmetry axis are 36 and 54°. Consequently, the observed negative ICD signal of HP in solution containing β-CD and DHB implies that the 1-hydroxyethyl and/or 2-carboxylatoethyl moiety are axially incorporated into the β-CD cavity in spite of the co-inclusion of DHB.

When TM-β-CD (3.0×10^{-3} mol dm^{-3}) was added to HP solution containing DHB (3.0×10^{-5} mol dm^{-3}), the absorption maximum was not shifted. This finding suggests that a ternary inclusion complex is not formed between HP, DHB, and TM-β-CD. This is reasonable, probably because the TM-β-CD cavity is too narrow to simultaneously accommodate a 1-hydroxyethyl (2-carboxylatoethyl) moiety of HP and a heptyl group of DHB.

CONCLUSIONS

At an HP concentration of around 5×10^{-6} mol dm^{-3}, the HP monomer is in equilibrium with the HP dimer. The equilibrium constant for the formation of the HP dimer has been evaluated to be 1.70×10^5 mol^{-1} dm^3 from the HP concentration dependence of the absorption spectrum of HP. With a stoichiometry of 1:1, HP forms inclusion complexes with β-CD, γ-CD, and TM-β-CD. The HP fluorescence is enhanced upon addition of β-CD, γ-CD, and TM-β-CD. From simulations of fluorescence intensity changes, the equilibrium constants for β-CD, γ-CD, and TM-β-CD have been evaluated to be 200, 95.7, and 938 mol^{-1} dm^3, respectively. HP also forms a complex with DHB. For the equilibrium constant for the formation of the 1:1 HP–DHB complex, a value of 1.85×10^5 mol^{-1} dm^3 has been determined from a simulation of the absorbance change. The addition of DHB results in the quenching of the HP fluorescence. From the decrease in the fluorescence intensity upon the addition of DHB, a value of the equilibrium constant for the formation of the HP-DHB complex has been estimated to be 1.98×10^5 mol^{-1} dm^3, which is nearly the same as that evaluated from the absorbance change. In DHB solution containing β- or γ-CD, HP forms a ternary inclusion complex with CD and DHB. On the other hand, a ternary inclusion complex is not formed between TM-β-CD, HP, and DHB.

EXPERIMENTAL

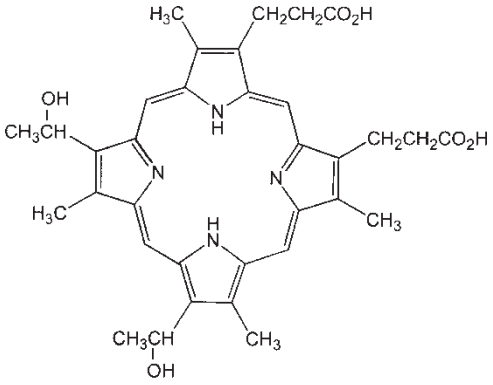
Reagents

Hematoporphyrin (HP) and 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHB), which were obtained from Tokyo Kasei Kogyo, were used as received (Scheme 1). β-Cyclodextrin (β-CD) purchased from Nakalai Tesque was recrystallized twice from water. γ-CD and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD), purchased from Wako Pure Chemicals and Nakalai Tesque, respectively, were used without further purification.

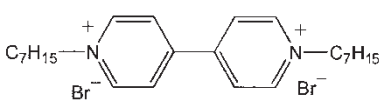
Aqueous buffers (pH 10.0) of $NaHCO_3$ (2.5×10^{-3} mol dm^{-3})–NaOH (1.1×10^{-3} mol dm^{-3}) were employed throughout this work. The concentration of HP was 5.0×10^{-6} mol dm^{-3}, except for the experiments of the HP concentration dependence of the absorption spectrum and the measurements of circular dichroism spectra of HP (1.0×10^{-5} mol dm^{-3}).

Apparatus

Absorption and fluorescence spectra were recorded on a Shimadzu UV-260 spectrophotometer and a Shimadzu RF-501 spectrofluorimeter equipped with a cooled Hamamatsu R-943 photomultiplier, respectively. The fluorescence spectra were corrected for the spectral response of the fluorimeter. 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°C.



Hematoporphyrin (HP)



1,1'-Diheptyl-4,4'-bipyridinium dibromide (DHB)

SCHEME 1

Data Analyses

Monomer–Dimer Equilibrium

In a monomer–dimer equilibrium, the molar absorption coefficient, ε, of HP is represented by the sum of the contributions from a monomer and a dimer,

$$\varepsilon = (\varepsilon_0 + \varepsilon_1 K_D[HP])[HP]/[HP]_0 \quad (1)$$

where ε_0 and ε_1 are the molar absorption coefficients of the HP monomer and dimer, respectively, K_D is the equilibrium constant for the formation of the HP dimer, and [HP] and $[HP]_0$ are the concentration of the HP monomer and the initial concentration of HP, respectively. Assuming a K_D value, the HP monomer concentration can be calculated by solving Eq. (2).

$$2K_D[HP]^2 + [HP] - [HP]_0 = 0 \quad (2)$$

In accordance with Eq. (1), therefore, a simulation was made, using ε_0 and ε_1 as parameters.

Equilibrium between Free HP and the γ-CD–HP Inclusion Complex

The HP fluorescence intensity, I_f, is given by the sum of the fluorescence intensities of the HP monomer, the HP dimer, and the γ-CD–HP inclusion complex.

$$I_f = (a + bK_D[HP] + cK_1[\gamma\text{-CD}])[HP] \quad (3)$$

Here, a, b, and c are experimental constants, including the fluorescence quantum yield of the relevant species, namely, the HP monomer, the HP dimer, and the γ-CD–HP inclusion complex, respectively, and K_1 is the equilibrium constant for the formation of the γ-CD–HP inclusion complex. Since there is the monomer–dimer equilibrium in addition to the equilibrium concerning the formation of the γ-CD–HP inclusion complex, the HP monomer concentration can be calculated from the equation, assuming a K_1 value [Eq. (4)].

$$2K_D[HP]^2 + (1 + K_1[\gamma\text{-CD}])[HP] - [HP]_0 = 0 \quad (4)$$

Consequently, the fluorescence intensity was simulated using a, b, and c as parameters, according to Eq. (3).

Equilibrium for the Formation of the HP–DHB Complex

For an HP solution containing DHB, the absorbance, A, is represented by Eq. (5).

$$A = (\varepsilon_0 + \varepsilon_1 K_D[HP] + \varepsilon_2 K_2[DHB])[HP]d \quad (5)$$

Here, ε_2, K_2, and d are the molar absorption coefficient of the HP–DHB complex, the equilibrium

constant for the formation of the HP–DHB complex, and the pathlength (1.0 cm) of the cell, respectively. For the HP monomer concentration, Eq. (6) holds.

$$2K_D[HP]^2 + (1 + K_2[DHB])[HP] - [HP]_0 = 0 \quad (6)$$

In the HP–DHB system, the initial DHB concentration is comparable to the HP concentration. Consequently, the HP monomer concentration was calculated from Eq. (6), firstly using the initial concentration of DHB as the free DHB concentration. Next, using the calculated HP monomer concentration, the free DHB concentration was evaluated from a relationship given in Eq. (7).

$$[DHB] = [DHB]_0/(1 + K_2[HP]) \quad (7)$$

In accordance with Eq. (6), the HP monomer concentration was recalculated using the DHB concentration evaluated from Eq. (7). Such an iterative procedure was carried out until the concentrations of the HP monomer and free DHB converged to constant values.

The procedure for estimating K_2 from the HP fluorescence intensity change is analogous to that from the absorbance change. The HP fluorescence intensity is represented as given in Eq. (8),

$$I_f = (d + eK_D[HP] + fK_2[DHB])[HP] \quad (8)$$

where d, e, and f are experimental constants, including the fluorescence quantum yields of the relevant species, namely, the HP monomer, the HP dimer, and the HP–DHB complex, respectively. To estimate the concentrations of the HP monomer and free DHB, the iterative procedure was performed according to Eqs. (6) and (7).

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