Journal of Inclusion Phenomena and Macrocyclic Chemistry **50**: 209–217, 2004.

Inclusion Complexes of Cyclodextrins with Tetrakis(4-carboxyphenyl)porphyrin and Tetrakis(4-sulfonatophenyl)porphyrin in Aqueous Solutions

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(Received: 12 December 2003; in final form: 7 May 2004)

Key words: cyclodextrins, electronic absorption, fluorescence, inclusion complexes, tetrakis(4-carboxyphenyl)porphyrin, tetrakis(4-sulfonatophenyl)porphyrin

Abstract

In neutral and alkaline solutions, tetrakis(4-carboxyphenyl)porphyrin (TCPP) and tetrakis(4-sulfonatophenyl)porphyrin (TSPP) form 1:1 and 2:1 host–guest inclusion complexes with α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and 6-deoxy-6-diethylamino-β-CD (DEA-β-CD), except for DEA-β-CD in alkaline solution. On the other hand, TCPP and TSPP form only 1:1 inclusion complexes with 6-deoxy-6-dihexylamino-β-CD (DHA-β-CD). The limited solubilities of DEA-β-CD in alkaline solution and DHA-β-CD are likely responsible for no observation of the 2:1 inclusion complex containing DEA-β-CD in alkaline solution and that containing DHA-β-CD. The equilibrium constants (*K*s) of TCPP and TSPP for the formation of the inclusion complexes have been estimated from the absorption and/or fluorescence intensity changes in neutral and alkaline solutions. The K_2 values, which are the equilibrium constants for the formation of the 2:1 host–guest inclusion complex from the 1:1 inclusion complex, are about one tenth the corresponding K_1 values, except for the α-CD–TSPP system in alkaline solution. In neutral solution, where DEA-β-CD and DHA-β-CD are in protonated forms, the electrostatic force operates between DEA-β-CD (DHA-β-CD) and TCPP (TSPP), leading to the greater *K* values than those in alkaline solution, where DEA-β-CD and DHA-β-CD exist as neutral species.

Introduction

Commercially available cyclodextrins (CDs) are water-soluble cyclic oligosaccharides with six, seven, and eight D-glucopyranose residues, which are called α-, β-, and γ-CD, respectively [1]. CDs, which are shaped like a truncated cone with a relatively hydrophobic cavity, incorporate a wide variety of organic compounds to form inclusion complexes.

The formation of inclusion complexes between CDs and porphyrin derivatives has been examined by many researchers [2–9]. Manka and Lawrence have reported that a cationic porphyrin possessing four ammonium groups forms a 2:1 host–guest inclusion complex with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin [4]. Kano *et al.* have demonstrated that cationic porphyrins such as tetrakis(1-methylpyridinium-4-yl)porphyrin hardly interact with CDs [8]. In contrast to cationic porphyrins, anionic porphyrins such as tetrakis(4-sulfonatophenyl)porphyrin (TSPP) forms inclusion complexes with CDs [5–11]. Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) tends to form 2:1 inclusion complexes with anionic porphyrins in aqueous solutions [7–12]. It has been reported that TM-β-CD forms 2:1 host–guest inclusion complexes with TSPP and tetrakis(4-carboxyphenyl)porphyrin (TCPP), respectively [8, 10]. For CDs other than TM-β-CD, 2:1 CD–porphyrin derivative inclusion complexes have been observed, although only a 1:1 stoichiometry has been reported for the γ-CD–TSPP system [11]. Recently, we have investigated the interactions between CDs and TSPP or TCPP in aqueous solution, and have estimated several equilibrium constants for the formation of the inclusion complexes of TSPP and TCPP [11–14].

The interactions between anionic guests and cationic CDs have so far been examined [15–19]. Protonated heptakis(6-amino-6-deoxy)-β-cyclodextrin associates with *p*-methylbenzoate and *N*-acetyltryptophan anions more strongly than a monoamino-β-CD cation does [17]. The equilibrium constants for the formation of inclusion complexes of the anionic guests with cationic CDs are greater than those of the anionic guests with neutral CDs, because the electrostatic interaction operates between cations and anions. A porphyrin nucleus is too bulky to be accommodated into the CD cavity, so that a binding site of a porphyrin derivative towards CD

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is a moiety substituted on a porphyrin nucleus. CDs possessing a dialkyl amino group exist either as neutral or cationic species, depending on the pH value of solution. When inclusion complexes are formed between porphyrin derivatives and charged CDs, charged substituents of porphyrin derivatives are expected to interact with charged CDs.

Thus, we have examined the formation of inclusion complexes of TCPP and TSPP with α-CD, β-CD, 6-deoxy-6-diethylamino-β-CD, and 6-deoxy-6-dihexylamino-β-CD in neutral and alkaline solutions. In addition, we have examined whether or not the length of an alkyl chain of a dialkylamino moiety substituted on β-CD affects its binding properties of TCPP and TSPP.

Experimental

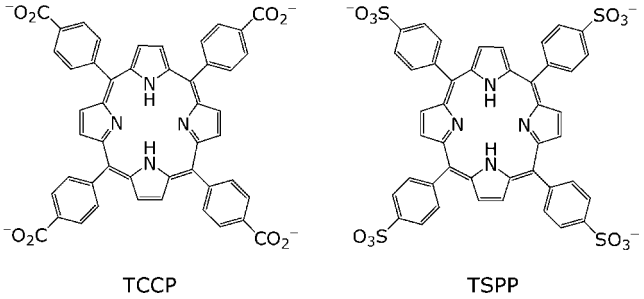
Absorption, fluorescence, and induced circular dichroism spectra were recorded on a Shimadzu UV-260 spectrophotometer, a Shimadzu RF-540 spectrofluorimeter, and a JASCO J-400X spectropolarimeter interfaced to a JASCO DP-500 data processor, respectively. The fluorescence spectra were corrected for the spectral response of the fluorimeter. However, the fluorescence spectra in the longer-wavelength region could not fully be corrected, because the sensitivity of the fluorimeter in this region was very low. The spectroscopic measurements were made at 25 ± 0.1 °C, except for the induced circular dichroism measurements (25 ± 2 °C).

Tetrakis(4-carboxyphenyl)porphyrin (TCPP) and tetrakis(4-sulfonatophenyl)porphyrin (TSPP), which were obtained from Tokyo Kasei Kogyo, were used as received.

β-Cyclodextrin (β-CD) purchased from Nacalai Tesque was twice recrystallized from water. α-CD and γ-CD, obtained from Nacalai Tesque and Wako Pure Chemical, respectively, were used without further purification. 6-Deoxy-6-diethylamino-β-CD (DEA-β-CD) was synthesized from 6-deoxy-6-tosyl-β-CD and diethylamine, and was purified twice through a Sephadex C-50 column [19]. Analysis: calculated for $C_{46}H_{72}O_{34}N \cdot 8H_2O$: C, 41.41; H, 5.44; N, 1.05; found: C, 40.83; H, 5.71; N, 1.11. 6-Deoxy-6-dihexylamino-β-CD (DHA-β-CD) was similarly synthesized using dihexylamine, and was purified twice through a Sephadex C-50 column. Analysis: calculated for $C_{54}H_{95}O_{34}N \cdot 8H_2O$: C, 44.83; H, 6.62; N, 0.97; found: C, 44.36; H, 7.02; N, 0.98.

Solutions of KH_2PO_4 (1.7×10^{-3} M)–Na_2HPO_4 (1.7×10^{-3} M), KH_2PO_4 (6.7×10^{-4} M)–Na_2HPO_4 (2.7×10^{-3} M), and $NaHCO_3$ (2.6×10^{-3} M)–NaOH (3.7×10^{-3} M) were employed as buffers of pH 6.7, 7.3, and 10.5, respectively.

Concentrations of TCPP and TSPP were 1.0×10^{-6} mol dm^{-3} for the spectroscopic measurements, except for the measurements of induced circular dichroism spectra (2.0×10^{-6} or 4.0×10^{-6} mol dm^{-3}).



Scheme 1. Molecular structures of TCPP and TSPP.

Results and discussion

Inclusion complexes of DEA-β-CD with TCPP in alkaline and neutral solutions

Because TCPP has a pK_a value of 5.8, it exclusively exists as an anion in alkaline solution [20]. At pH 10.5, on the other hand, DEA-β-CD is exclusively in an unprotonated form, because it has a pK_a value of 8.7 [19]. Figure 1 shows the Soret band in the absorption of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.5 buffers containing various concentrations of DEA-β-CD. The absorption peak is shifted to longer wavelengths with an increase in the DEA-β-CD concentration, accompanied by an isosbestic point at 417 nm. This finding indicates the formation of an inclusion complex of DEA-β-CD with TCPP. As previously stated, DEA-β-CD in pH 10.5 buffer exclusively exists as a neutral form. Consequently, the DEA-β-CD–TCPP inclusion complex at pH 10.5 is comprised of neutral DEA-β-CD and anionic TCPP. A double reciprocal plot for the absorbance of a TCPP solution containing various

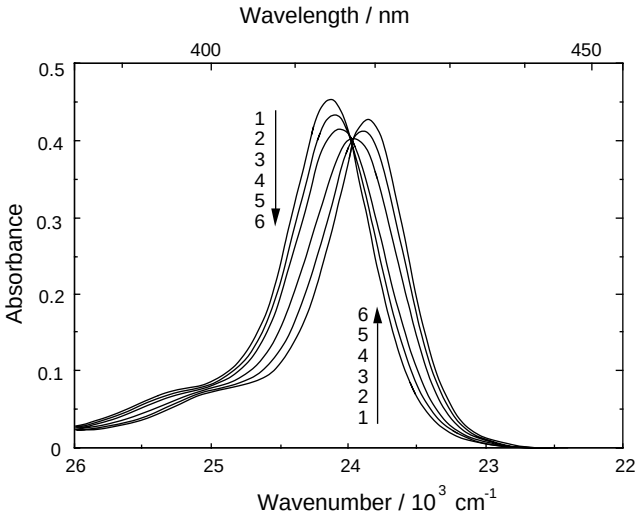


Figure 1. Absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.5 buffers containing various concentrations of DEA-β-CD. Concentration of DEA-β-CD: (1) 0, (2) 1.0×10^{-5}, (3) 2.0×10^{-5}, (4) 5.0×10^{-5}, (5) 1.0×10^{-4}, and (6) 3.0×10^{-4} mol dm^{-3}.

concentrations of DEA-β-CD has afforded a good straight line (not shown), suggesting that the DEA-β-CD–TCPP inclusion complex in pH 10.5 buffer has a 1:1 stoichiometry [21, 22].

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

Here, K_1 is the equilibrium constant for the formation of the 1:1 DEA-β-CD–TCPP inclusion complex (DEA-β-CD $\cdot$ TCPP). From the double reciprocal plot regarding the absorbance, a K_1 (K_1^{abs}) value has been evaluated to be 3300 $\pm$ 1100 mol^{-1} dm^3 (Table 1).

Figure 2 exhibits fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.5 buffers containing various concentrations of DEA-β-CD. When DEA-β-CD is added, the fluorescence intensity at a peak of around 645 nm is enhanced with a peak shift to longer wavelengths, while the fluorescence intensity at a shoulder of around 700 nm is reduced. This spectral change also indicates the formation of the DEA-β-CD–TCPP inclusion complex. At pH 10.5, DEA-β-CD is in a neutral form. Amines efficiently quench the excited state of aromatic hydrocarbons such as anthracene [23]. In pH 10.3 buffer, the fluorescence emissions of 2-naphthalenesulfonate and 2-anthracenesulfonate are quenched by DEA-β-CD [19]. As seen in Figure 2, however, the fluorescence of TCPP is not quenched by neutral DEA-β-CD. In the quenching by an amino group, the unshared electrons on a nitrogen atom flow to the excited fluorophore. In the case of TCPP, it may be difficult for the electrons on a nitrogen atom to flow to TCPP possessing largely negative charges. Consequently, DEA-β-CD would not quench the TCPP fluorescence, even when DEA-β-CD is bound to TCPP.

From a double reciprocal plot for the fluorescence intensity change, a K_1 (K_1^{flu}) value of 3100 $\pm$ 2300 mol^{-1} dm^3 was obtained for the DEA-β-CD–TCPP inclusion complex (not shown). The relatively large error of the K_1 value seems to be due to the

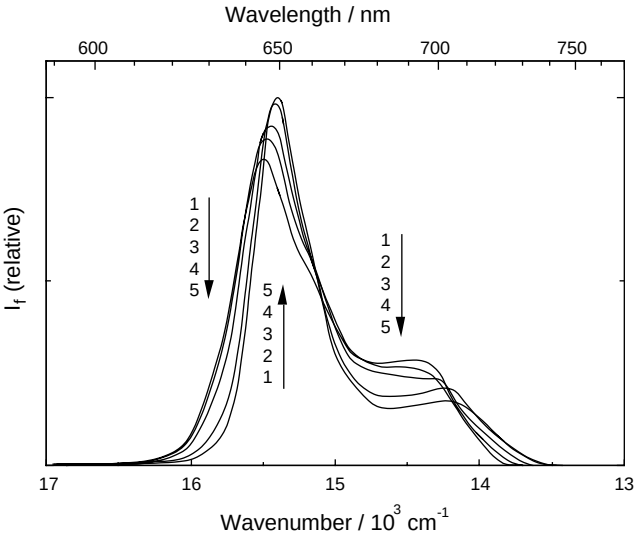


Figure 2. Fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 10.5 buffers containing various concentrations of DEA-β-CD. Concentration of DEA-β-CD: (1) 0, (2) 1.0×10^{-5}, (3) 3.0×10^{-5}, (4) 1.0×10^{-4}, and (5) 3.0×10^{-4} mol dm^{-3}. $\lambda_{ex} = 417$ nm.

experimental conditions of the low concentrations (below 1.0×10^{-4} mol dm^{-3}) of DEA-β-CD. This K_1 value obtained from the fluorescence intensity change is similar to that from the absorbance change. The agreement between the K_1 values from both procedures supports the formation of only the 1:1 DEA-β-CD–TCPP inclusion complex.

Because TCPP and DEA-β-CD have pK_a values of 5.8 and 8.7, respectively, the experiments have been made at pH 7.3 to examine the interactions between anionic TCPP and cationic DEA-β-CD. Figure 3 depicts absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DEA-β-CD. As the DEA-β-CD concentration is increased, the absorption peak is shifted to longer wavelengths. In contrast to the absorption spectral change at pH 10.5, there is no

Table 1. The K_1 and K_2 values for TCPP in neutral and alkaline solutions at 25 $\pm$ 0.1 °C

Host	pH	K_1^{abs} /mol^{-1} dm^3	K_2^{abs} /mol^{-1} dm^3	K_1^{flu} /mol^{-1} dm^3	K_2^{flu} /mol^{-1} dm^3
α-CD	7.3	300 $\pm$ 80	–[a]	240 $\pm$ 20	–[a]
	10.5	210 $\pm$ 30	–[a]	310 $\pm$ 100	–[a]
β-CD	7.3	15,000 $\pm$ 7000	1300 $\pm$ 300	11,900[b]	840[b]
	10.5	23,000 $\pm$ 15000	640 $\pm$ 310	23,100[b]	4410[b]
DEA-β-CD	7.3	36,000 $\pm$ 13000	–[a]	19,200[b]	1000[b]
	10.5	3300 $\pm$ 1100	–[c]	3100 $\pm$ 2300	–[c]
DHA-β-CD	7.3	6600 $\pm$ 200	–[c]	5700 $\pm$ 1500	–[c]
	10.5	2700 $\pm$ 700	–[c]	4700 $\pm$ 1500	–[c]
γ-CD	10.1	10,000 $\pm$ 400[d]	–[c,d]	5600 $\pm$ 300[d]	–[c,d]

[a] A K_2 value could not be estimated.
[b] A K value estimated from a simulation procedure. The errors of the K_1 and K_2 values evaluated from the simulation procedure are estimated to be less than 15% and 30%, respectively.
[c] The formation of a 2:1 inclusion complex was not observed.
[d] Ref. [14].

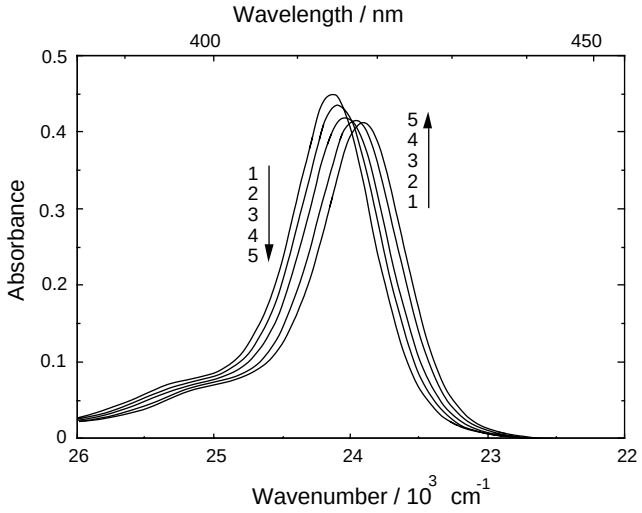


Figure 3. Absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DEA-β-CD. Concentration of DEA-β-CD: (1) 0, (2) 1.0×10^{-5}, (3) 3.0×10^{-5}, (4) 1.0×10^{-4}, and (5) 3.0×10^{-4} mol dm^{-3}.

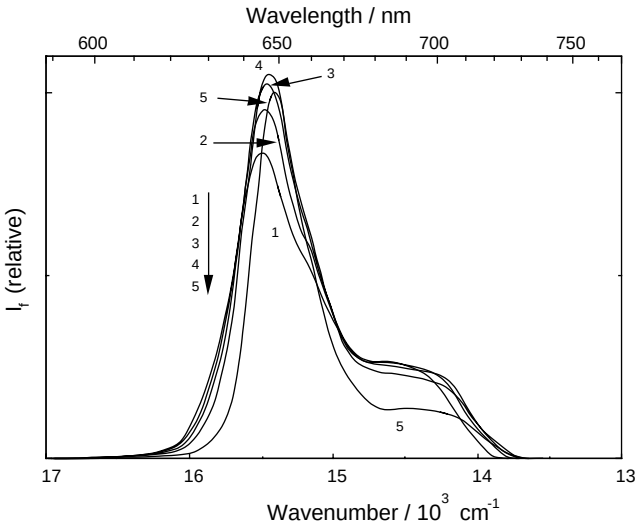


Figure 4. Fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DEA-β-CD. Concentration of DEA-β-CD: (1) 0, (2) 3.0×10^{-5}, (3) 1.0×10^{-4}, (4) 3.0×10^{-4}, and (5) 1.0×10^{-3} mol dm^{-3}. $\lambda_{ex} = 417$ nm.

isosbestic point in the absorption spectra at pH 7.3, indicating the existence of at least two kinds of inclusion complexes. The highest DEA-β-CD concentrations used at pHs 7.3 and 10.5 were 1.0×10^{-3} and 3.0×10^{-4} mol dm^{-3}, respectively. The limited solubility at pH 10.5 is likely responsible for the observation of only the 1:1 inclusion complex, in contrast to the observation of the two kinds of the inclusion complexes at pH 7.3. At low DEA-β-CD concentrations in pH 7.3 buffer, the 1:1 DEA-β-CD–TCPP inclusion complex is formed, while at high DEA-β-CD concentrations, a 2:1 DEA-β-CD–TCPP inclusion complex is formed besides the 1:1 inclusion complex.

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

Here, K_2 is the equilibrium constant for the formation of the 2:1 DEA-β-CD–TCPP inclusion complex ((DEA-β-CD)$_2$ · TCPP). At pH 7.3, the inclusion complexes are attributed to the complex between protonated DEA-β-CD and anionic TCPP.

Figure 4 illustrates fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DEA-β-CD. As the DEA-β-CD concentration is raised, the fluorescence peak at 643.5 nm is initially enhanced, accompanied by a shift to the longer wavelengths. In the high concentration range of DEA-β-CD, however, the fluorescence peak intensity is reduced with an increase in the DEA-β-CD concentration. These findings indicate that, at high DEA-β-CD concentration, the 1:1 DEA-β-CD–TCPP inclusion complex further associates with DEA-β-CD to form the 2:1 DEA-β-CD–TCPP inclusion complex. At pH 7.3, the result obtained from the TCPP fluorescence is consistent with that from the absorption spectral change of TCPP. In the presence of DEA-β-CD, the fluorescence intensity (I_f) is represented by

$$I_f = a[\text{TCPP}] + b[\text{DEA-}\beta\text{-CD} \cdot \text{TCPP}] + c[(\text{DEA} - \beta\text{-CD})_2 \cdot \text{TCPP}]. \quad (3)$$

Here, a, b, and c are experimental constants including the fluorescence quantum yields for free TCPP, the 1:1 DEA-β-CD–TCPP inclusion complex, and the 2:1 DEA-β-CD–TCPP inclusion complex, respectively. The concentration of free TCPP is given by

$$[\text{TCPP}] = [\text{TCPP}]_0/(1 + K_1[\text{DEA-}\beta\text{-CD}] + K_1K_2[\text{DEA} - \beta\text{-CD}]^2). \quad (4)$$

Consequently, Equation (3) is rewritten as

$$I_f = (a + bK_1[\text{DEA-}\beta\text{-CD}] + cK_1K_2[\text{DEA} - \beta\text{-CD}]^2)[\text{TCPP}]_0/(1 + K_1[\text{DEA-}\beta\text{-CD}] + K_1K_2[\text{DEA-}\beta\text{-CD}]^2). \quad (5)$$

As a function of the DEA-β-CD concentration, Figure 5 illustrates the fluorescence intensity observed at 644 nm and the best-fit simulation curve, which has been calculated with $a = 8.24 \times 10^7$, $b = 1.14 \times 10^8$, $c = 5.30 \times 10^7$, $K_1^{flu} = 19200$, and $K_2^{flu} = 1000$ mol^{-1} dm^3 (Table 1). A K_1^{abs} value was estimated to be $36{,}000 \pm 13{,}000$ mol^{-1} dm^3 from a double reciprocal plot, for which the absorbance data in the low concentration range of DEA-β-CD were used. This K_1^{abs} value is comparable with the K_1^{flu} value obtained from the fluorescence intensity change. The K_1^{flu} value at pH 7.3 is about six times greater than that at pH 10.5, suggesting that the electrostatic force due to the protonated diethyl-

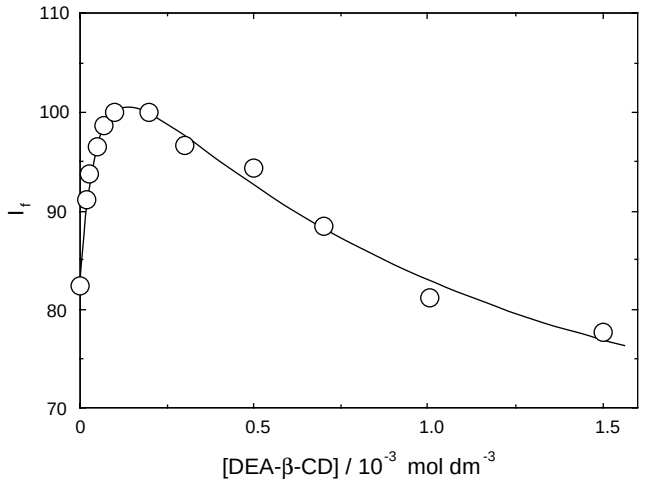


Figure 5. Observed fluorescence intensity of TCPP (4.0×10^{-7} mol dm^{-3}) in pH 7.3 buffers as a function of the DEA-β-CD concentration and the best fit simulation curve for the fluorescence intensity of TCPP calculated with $K_1^{flu} = 19200$ mol^{-1} dm^3, $K_2^{flu} = 1000$ mol^{-1} dm^3, $a = 8.24 \times 10^7$, $b = 1.14 \times 10^8$, and $c = 5.30 \times 10^7$. $\lambda_{ex} = 417$ nm. $\lambda_{obs} = 644$ nm.

amino moiety facilitates the incorporation of TCPP into DEA-β-CD. The large K_1^{flu} value at pH 7.3 causes the high concentration of the 1:1 DEA-β-CD–TCPP inclusion complex. Consequently, the large K_1^{flu} value most likely promotes the additional binding of DEA-β-CD towards the 1:1 DEA-β-CD–TCPP inclusion complex to form the 2:1 DEA-β-CD–TCPP inclusion complex. At pH 7.3, the K_2^{flu} value evaluated is an order of magnitude smaller than the K_1^{flu} value. This indicates that the first binding of DEA-β-CD towards TCPP weakens the second binding-ability of TCPP. If the second binding of DEA-β-CD was independent of the first binding, the K_2 value would be similar to the K_1 value. This is not the case. Although the thermodynamic parameters for the K values were not evaluated, the entropy changes for the K values might explain the difference in the K values. The first binding of DEA-β-CD seems to destroy the solvation shell around a TCPP molecule to a large extent. This affords the entropic gain for the first binding of DEA-β-CD to TCPP. In the second-binding process of DEA-β-CD, the entropic gain would not stem from the destruction of the solvation shell, because the solvation shell only partly encompasses the TCPP molecule located within the 1:1 inclusion complex. Consequently, the K_2 value is less than the corresponding K_1 value.

Kano *et al*. have reported that, in ethylene glycol and aqueous ethylene glycol, the K_2 values for the TM-β-CD–charged porphyrins inclusion complexes are similar to or an order of magnitude greater than the corresponding K_1 values [9]. For the β-CD–TSPP system in aqueous solution, on the other hand, the K_1 value ($17{,}000 \pm 3000$ mol^{-1} dm^3) is an order of magnitude greater than the K_2 value (2300 ± 400 mol^{-1} dm^3) [9]. At pH 7.3, the relationship in the DEA-β-CD–TCPP system between the magnitudes of K_1 and K_2 is analogous to that reported in the β-CD–TSPP system. In organic solvents, the solvation around a charged porphyrin does not seem to be strong, compared to in aqueous solution. Consequently, the first and second binding are not strongly affected by the breakage of the solvation shell, resulting in similar K_1 and K_2 values in organic solvents.

Inclusion complexes of DHA-β-CD with TCPP in neutral and alkaline solutions

Because the pK_a value of DHA-β-CD is most likely similar to that of DEA-β-CD, DHA-β-CD exists as a protonated and a neutral form at pHs 7.3 and 10.5, respectively. The absorption spectral change of TCPP in pH 7.3 buffers containing DHA-β-CD was similar to that in pH 10.5 buffers containing DEA-β-CD (Figure 1). This finding indicates the formation of an inclusion complex of DHA-β-CD with TCPP. From the absorbance change by the addition of DHA-β-CD, a K_1^{abs} value at pH 7.3 has been evaluated to be 6600 ± 200 mol^{-1} dm^3 (not shown). A good straight line in the double reciprocal plot for the absorbance change suggests the formation of the 1:1 DHA-β-CD–TCPP inclusion complex.

Figure 6 depicts fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DHA-β-CD. When the DHA-β-CD concentration is increased, the fluorescence intensity is enhanced with a slight shift of the fluorescence peak to longer wavelengths. As in the case of DEA-β-CD, the TCPP fluorescence at pH 7.3 is not quenched by the addition of DHA-β-CD.

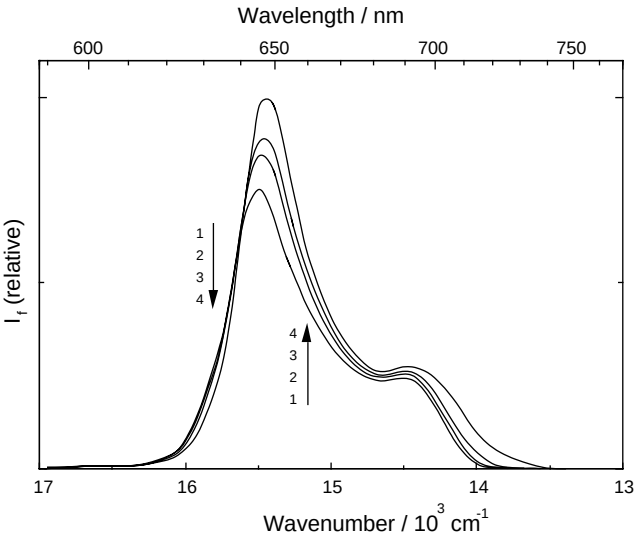
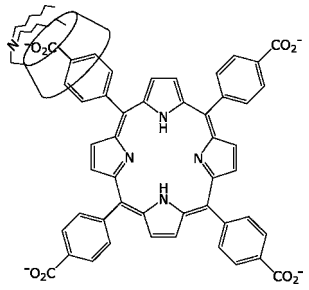


Figure 6. Fluorescence spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of DHA-β-CD. Concentration of DHA-β-CD: (1) 0, (2) 1.0×10^{-5}, (3) 1.0×10^{-4}, and (4) 3.0×10^{-4} mol dm^{-3}. $\lambda_{ex} = 418$ nm.



Scheme 2. Probable structure of the DHA-β-CD–TCPP inclusion complex.

From a double reciprocal plot using the fluorescence intensity at pH 7.3, a K_1^{flu} value of 5700 ± 1500 mol^{-1} dm^3 has been evaluated for DHA-β-CD (not shown). This K_1^{flu} value is similar to the K_1^{abs} value, supporting the 1:1 stoichiometry of the DHA-β-CD–TCPP inclusion complex. The K_1 values for DHA-β-CD at pH 7.3 are several times less than those for DEA-β-CD at pH 7.3. In the case of DHA-β-CD, a hydrophobic hexyl group in a dihexylamino moiety most likely intrudes intramolecularly into the DHA-β-CD cavity to some extent, although an ethyl group in a diethylamino moiety of DEA-β-CD may intrude slightly into the DEA-β-CD cavity.

The invasion of a hexyl group(s) obstructs the incorporation of TCPP into the DHA-β-CD cavity. This is responsible for the K_1 values of DHA-β-CD less than those of DEA-β-CD.

Upon the addition of DHA-β-CD at pH 10.5, the absorption and fluorescence spectra of TCPP exhibited changes similar to those at pH 7.3. K_1 values of 2700 ± 700 and 4700 ± 1500 mol^{-1} dm^3 were evaluated at pH 10.5 from the double reciprocal plots for the absorbance and fluorescence intensity changes, respectively. The K_1 value for DEA-β-CD at pH 7.3 is greater than that at pH10.5, whereas the K_1 value for DHA-β-CD at pH 7.3 is similar to that at pH 10.5. At pH 7.3, the protonation reduces the hydrophobicity of a diethylamino group of DEA-β-CD, leading to the extrusion of the diethylamino group from the CD cavity. In addition to the electrostatic force between DEA-β-CD and TCPP, therefore, the extrusion causes the increase in the K_1 value for DEA-β-CD at pH 7.3. On the other hand, the protonation of a dihexylamino group of DHA-β-CD does not reduce the hydrophobicity of two hexyl chains of a dihexylamino group, because of the hydrophobic, long alkyl chains. Consequently, the intrusion behavior of the hexyl chains of a dihexylamino group is not affected by the protonation, resulting in similar K_1 values for DHA-β-CD at pHs 7.3 and 10.5.

In contrast to DEA-β-CD, a 2:1 inclusion complex of DHA-β-CD with TCPP was not observed. The highest concentration (3.0×10^{-4} mol dm^{-3} at pH 7.3) of DHA-β-CD was lower than that of DEA-β-CD, because of the low solubility of DHA-β-CD. Consequently, the absence of the 2:1 DHA-β-CD–TCPP inclusion complex seems to be due to the low DHA-β-CD concentration used.

Inclusion complexes of α-CD with TCPP in neutral and alkaline solutions

When α-CD was added to TCPP solution buffered at pH 7.3 or 10.5, the absorption peak of TCPP was shifted to longer wavelengths, accompanied by a reduction of the absorption intensity. Although an isosbestic point appeared at 416 nm below about 5×10^{-3} mol dm^{-3} of α-CD, no isosbestic point was observed at higher α-CD concentrations. This finding indicates the formation of a 2:1 α-CD–TCPP inclusion complex as well as a 1:1 inclusion complex at both pHs. From the double reciprocal plots in the α-CD concentration range in which the isosbestic point was observed, K_1^{abs} values at pHs 7.3 and 10.5 were evaluated to be 300 ± 80 and 210 ± 30 mol^{-1} dm^3, respectively. Within the experimental errors, the K_1^{abs} values at the both pHs are the same, indicating that the pH value does not affect the binding ability of α-CD. This is reasonable, because α-CD is in a neutral form at pHs 7.3 and 10.5. These K_1 values for α-CD are significantly less than those for DEA-β-CD and DHA-β-CD, indicating that the α-CD cavity is too small to snugly encapsulate a carboxylatophenyl moiety of TCPP.

As α-CD was added to TCPP solution of pH 7.3 or 10.5, the fluorescence intensity was enhanced, accompanied by a slight shift of the fluorescence peak to longer wavelengths. From the double reciprocal plots for the fluorescence intensity at low α-CD concentrations where the isosbestic point was observed, K_1^{flu} values of 240 ± 20 and 310 ± 100 mol^{-1} dm^3 were obtained at pHs 7.3 and 10.5, respectively. As in the case of K_1^{abs}, the K_1^{flu} values at the both pHs are the same within the experimental errors. In addition, the K_1^{flu} values are similar to the K_1^{abs} values.

In spite of the small K_1 values for α-CD, a 2:1 α-CD–TCPP inclusion complex is formed. This is probably because a higher α-CD concentration has been used, compared to DEA-β-CD in pH 10.5 buffer and DHA-β-CD. For α-CD, K_2 values could not be estimated, because the absorbance and fluorescence intensity changes were small at high α-CD concentrations.

Inclusion complexes of β-CD with TCPP in neutral and alkaline solutions

Figure 7 illustrates absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of β-CD. As the β-CD concentration is increased, the absorption peak is shifted to longer wavelengths, accompanied by the decrease in the absorbance. Below 4.0×10^{-5} mol dm^{-3} of β-CD, an isosbestic point is observed at 415 nm. Over the β-CD concentration range examined, however, there is no

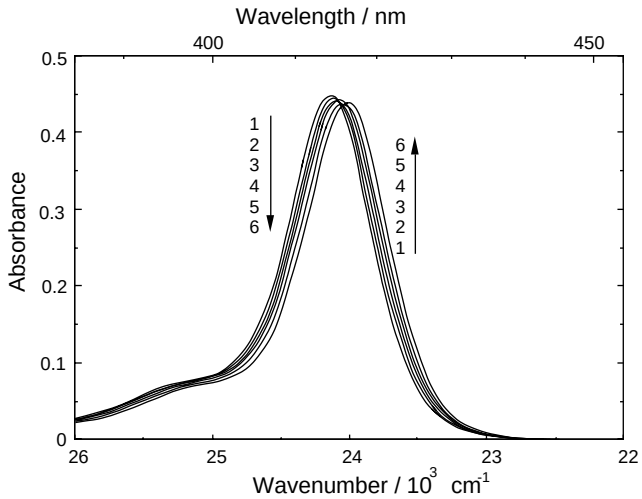


Figure 7. Absorption spectra of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers containing various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 2.0×10^{-5}, (3) 4.0×10^{-5}, (4) 7.0×10^{-5}, (5) 1.3×10^{-4}, and (6) 3.0×10^{-4} mol dm^{-3}.

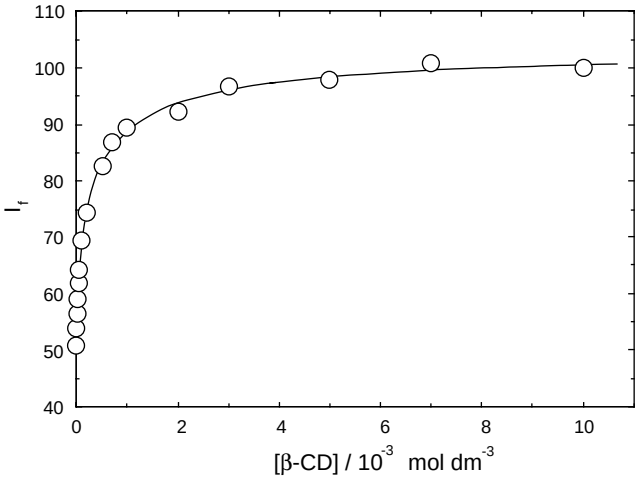


Figure 8. Observed fluorescence intensity of TCPP (1.0×10^{-6} mol dm^{-3}) in pH 7.3 buffers as a function of the β-CD concentration and the best fit simulation curve for the fluorescence intensity of TCPP calculated with $K_1^{flu} = 11{,}900$ mol^{-1} dm^3, $K_2^{flu} = 840$ mol^{-1} dm^3, $a = 5.07 \times 10^7$, $b = 7.99 \times 10^7$, and $c = 1.03 \times 10^8$. $\lambda_{ex} = 425$ nm. $\lambda_{obs} = 643$ nm.

isosbestic point, suggesting that a 1:1 inclusion complex further associates with an additional β-CD molecule to form a 2:1 β-CD–TCPP inclusion complex. In the concentration range below 4.0×10^{-5} mol dm^{-3}, therefore, we applied a double reciprocal procedure to the absorbance change, and obtained $15{,}000 \pm 7000$ mol^{-1} dm^3 as a K_1^{abs} value. Above 4.0×10^{-5} mol dm^{-3}, another isosbestic point appears at 416 nm, suggesting that the 2:1 β-CD–TCPP inclusion complex is generated from the 1:1 inclusion complex and an additional β-CD molecule. Under the assumption that the 2:1 inclusion complex is alone formed above 4.0×10^{-5} mol dm^{-3}, a K_2^{abs} value has been evaluated to be 1300 ± 300 mol^{-1} dm^3 from the double reciprocal plot. As in the case of DEA-β-CD at pH 7.3, the K_2 value is an order of magnitude less than the K_1 value, suggesting that the first binding of β-CD reduces the binding ability of TCPP towards an additional β-CD molecule.

The fluorescence intensity of TCPP in pH 7.3 buffer was enhanced by the addition of β-CD. The fluorescence spectral change in the presence of β-CD was similar to that in the presence of DHA-β-CD in pH 7.3 buffers (Figure 6). Using a simulation method similar to that applied to the DEA-β-CD–TCPP system, K_1^{flu} and K_2^{flu} values have been evaluated to be 11900 and 840 mol^{-1} dm^3, respectively (Figure 8). These K_1^{flu} and K_2^{flu} values are similar to the K_1^{abs} and K_2^{abs} values, respectively. At pH 10.5, values of K_1^{abs}, K_2^{abs}, K_1^{flu}, and K_2^{flu} have been evaluated to be $23{,}000 \pm 15{,}000$, 640 ± 310, 23,100, and 4410 mol^{-1} dm^3, respectively, from analyses similar to those performed at pH 7.3. The K_1 values for β-CD at pH 10.5 are 50–90% greater than the K_1 values at pH 7.3, although the K_1 values for α-CD are nearly the same at both pHs. At present, it is not clear why the K_1 values for β-CD at pH 10.5 are greater than those at pH 7.3.

The magnitude of the K_1 value for TCPP increases in the order, α-CD < γ-CD < β-CD (Table 1). This trend reflects the degree of the fit in size between the CD cavity and a carboxylatophenyl moiety of TCPP. The carboxylatophenyl moiety most snugly fits the β-CD cavity, resulting in the largest K_1 value. As seen for DHA-β-CD, the substitution of a dihexylamino group on the C6 atom of β-CD reduces the K_1 value. This may be explained in terms of the intrusion of the hexyl chain(s) into the DHA-β-CD cavity. On the other hand, the K_1 value of DEA-β-CD at pH 7.3 is about 1.6 times greater than that of β-CD at pH 7.3. This finding implies that electrostatic forces take place between a protonated diethylamino group of DEA-β-CD and anionic TCPP at pH 7.3.4

Inclusion complexes of α-CD, β-CD, DEA-β-CD, and DHA-β-CD with TSPP in neutral and alkaline solutions

The pK_a value of TSPP has been reported to be 4.65 ± 0.05, 4.8 ± 0.1, or 4.95 [24–26]. Solutions of pH 6.7 have been used as buffers, in which diethylamino and dihexylamino moieties of DEA-β-CD and DHA-β-CD are predominantly protonated. As in the case of TCPP, K_1 and K_2 values have been evaluated using double reciprocal plots or simulation methods similar to those employed for TCPP. These K_1 and K_2 values evaluated for TSPP are listed in Table 2. The order in the magnitudes of the K_1 values of TSPP is the same as that of TCPP; α-CD < γ-CD < β-CD. This is reasonable, because the bulkiness of a sulfonatophenyl moiety is nearly the same as that of a carboxylatophenyl moiety.

Table 2. The K_1 and K_2 values for TSPP in neutral and alkaline solutions at 25 ± 0.1 °C

Host	pH	K_1^{abs} /mol^{-1} dm^3	K_2^{abs} /mol^{-1} dm^3	K_1^{flu} /mol^{-1} dm^3	K_2^{flu} /mol^{-1} dm^3
α-CD	6.7	–[a]	–[a]	–[b]	–[b]
	10.1	720 ± 180[c]	–[a,c]	295[d]	0.0458[d]
β-CD	6.7	–[b]	–[b]	17,200[d]	1930[d]
	10.5	–[b]	–[b]	31,900[d]	4500[d]
	in water	17,000 ± 3000[e]	2300 ± 400[e]		
DEA-β-CD	6.7	25,000 ± 9000	–[a]	53,700[d]	5070[d]
	10.5	1000 ± 3100	–[f]	2900 ± 2400	–[f]
DHA-β-CD	6.7	3200 ± 290	–[f]	7400 ± 1000	–[f]
	10.5	2000 ± 230	–[f]	3800 ± 370	–[f]
γ-CD	10.1	1800 ± 100[c]	–[c,f]	1600 ± 200[c]	–[c,f]

[a] A K value could not be estimated.
[b] A K value was not estimated.
[c] Ref. [11].
[d] A K value estimated from a simulation procedure. The errors of the K_1 and K_2 values evaluated from the simulation procedure are estimated to be less than 15% and 20%, respectively, except for the K_2 value for α-CD at pH 10.1, whose error is estimated to be less than 50%.
[e] Ref. [9].
[f] The formation of a 2:1 inclusion complex was not observed.

As in the case of TCPP, the substitution of a diethylamino or a dihexylamino group on the C6 atom of β-CD decreases the K_1 value of TSPP, except for DEA-β-CD at pH 6.7. The K_1^{flu} value for DEA-β-CD at pH 6.7 is about three times greater than that for β-CD at pH 6.7, indicating the electrostatic attraction between the protonated diethylamino group and the negatively charged sulfonatophenyl moiety of TSPP. The electrostatic effect is also confirmed from the finding that the K_1^{abs} and K_1^{flu} values for DEA-β-CD at pH 6.7 are about 20 times greater than the K_1^{abs} and K_1^{flu} values for DEA-β-CD at pH 10.5, respectively. For DHA-β-CD, the K_1^{flu} value at pH 6.7 is about two times greater than that at pH 10.5. This finding is attributed to the electrostatic force between the protonated dihexylamino group and anionic TSPP at pH 6.7, although the ratio of the K_1 values at pHs 6.7 and 10.5 is not so large as that for DEA-β-CD.

As in the case of TCPP, the K_2 value of TSPP is about one tenth the corresponding K_1 value, except for α-CD, indicating that the second encapsulation of TSPP by β-CD or DEA-β-CD is decelerated by the first CD binding. For TSPP in α-CD solution of pH 10.5, the K_2^{flu} value is four orders of magnitude less than the corresponding K_1^{flu} value.

The K_1 and K_2 values for the 1:1 and 2:1 β-CD–TSPP inclusion complexes in water have been reported to be 17,000 ± 3000 and 2300 ± 400 mol^{-1} dm^3, respectively [9]. The K_1^{flu} and K_2^{flu} of TSPP for β-CD at pH 6.7, which have been evaluated in this study, are nearly the same as the reported K_1 and K_2 values, respectively. The K_1^{flu} value for β-CD at pH 10.5 is about two times greater than that at pH 6.7, although the reason is not clear at present.

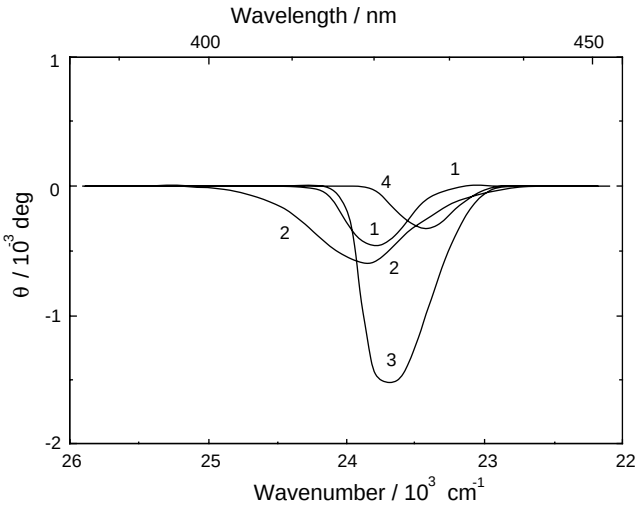


Figure 9. Induced circular dichroism spectra of TCPP in pH 7.3 buffers containing (1) α-CD (5.0×10^{-3} mol dm^{-3}), (2) β-CD (3.0×10^{-3} mol dm^{-3}), (3) DEA-β-CD (3.0×10^{-4} mol dm^{-3}), and (4) DHA-β-CD (3.0×10^{-4} mol dm^{-3}). Concentration of TCPP: (1) 2.0×10^{-6}, (2) 2.0×10^{-6}, (3) 4.0×10^{-6}, and (4) 4.0×10^{-6} mol dm^{-3}.

Induced circular dichroism spectra of TCPP and TSPP in neutral and alkaline solutions containing CD

Figure 9 illustrates induced circular dichroism (icd) spectra of TCPP in pH 7.3 buffers containing α-CD (5.0×10^{-3} mol dm^{-3}), β-CD (3.0×10^{-3} mol dm^{-3}), DEA-β-CD (3.0×10^{-4} mol dm^{-3}), and DHA-β-CD (3.0×10^{-4} mol dm^{-3}). Negative icd signals are observed for all CDs under study. Similar negative icd spectra have been obtained for TCPP in alkaline solution containing CD. The observation of the icd

signals definitely indicates the formation of inclusion complexes of TCPP with the CDs. The electronic transition moments of TCPP responsible for the Soret band in the electronic absorption are most likely directed to the N—N and NH—NH axes [27, 28]. The binding site of TCPP towards CD is a carboxylatophenyl moiety. Consequently, the transition moment of TCPP is located outside the CD cavity in the CD–TCPP inclusion complexes. In this case, the icd signal exhibits a negative sign, under the conditions that an angle between the symmetry axis of CD and the transition moment of TCPP is less than 54.7° [29, 30]. In the inclusion complexes of TCPP examined, therefore, the angle between the CD symmetry axis and the transition moment of TCPP is below 54.7°. In the case of γ-CD, a positive signal has been reported for TCPP [14]. This implies that the orientation of TCPP within the γ-CD cavity is different from those within the α-CD, β-CD, DEA-β-CD, and DHA-β-CD cavity.

Furthermore, we measured icd spectra of TSPP (4.0×10^{-6} mol dm^{-3}) in neutral and alkaline solutions containing DEA-β-CD (3.0×10^{-4} mol dm^{-3}) and DHA-β-CD (3.0×10^{-4} mol dm^{-3}). As in the case of TCPP, these icd spectra of TSPP exhibited negative signals. For TSPP in alkaline solutions containing α-CD and β-CD, the icd spectra have exhibited a negative sign [11], indicating that the binding mode of TSPP towards α-CD and β-CD is similar to that of TCPP. On the other hand, it has been reported that the icd spectra have exhibited a positive sign for alkaline solutions containing γ-CD and heptakis(2,3,6-tri-*O*-methyl)-β-CD [11]. The inclusion mode of γ-CD for TSPP is similar to that for TCPP.

Conclusions

In neutral and alkaline solutions, α- and β-CDs form 1:1 and 2:1 host–guest inclusion complexes with TCPP and TSPP. DEA-β-CD also forms 1:1 and 2:1 inclusion complexes with TCPP and TSPP in neutral solutions. The K_1 values for TCPP and TSPP increase in the order, α-CD < γ-CD < β-CD. This finding can be interpreted in terms of the fitness of the carboxylatophenyl and sulfonatophenyl moieties to the CD cavity, because these moieties have similar bulkiness. The greater the K_1 value is, the greater the K_2 value is. The K_2 values are about one tenth of the corresponding K_1 values, except for α-CD, for which the K_2^{flu} value is four orders of magnitude less than the K_1^{flu} value. Consequently, the second binding of CD towards TCPP (TSPP) is weakened by the first binding of CD towards TCPP (TSPP).

In pH 10.5 buffers, a dihexylamino group of DHA-β-CD intramolecularly intrudes into the DHA-β-CD cavity, resulting in the K_1 value of DHA-β-CD less than the K_1 value for β-CD. The same is true for DEA-β-CD in alkaline solution. In neutral solutions, where a dialkylamino group is protonated, the electrostatic attraction operates between a positively-charged dialkylamino group and a negatively-charged carboxylatophenyl (sulfonatophenyl) moiety. Consequently, the K_1 values of TCPP and TSPP for DEA-β-CD in neutral solutions are significantly greater than those in alkaline solutions, respectively. Furthermore, the K_1 values of TCPP and TSPP for DEA-β-CD in neutral solutions are greater than those for β-CD, respectively. This finding is due to the electrostatic attraction between protonated DEA-β-CD and anionic TCPP (TSPP) in neutral solutions. In neutral solution, the K_1 values for DHA-β-CD are remarkably less than those for DEA-β-CD, because of the self-intrusion of the hexyl chain(s) into the DHA-β-CD cavity.

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