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Electronic absorption, fluorescence, and circular dichroism spectroscopic studies on the inclusion complexes of tetrakis(4-sulfonatophenyl)porphyrin with cyclodextrins in basic aqueous solutions

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Abstract

In aqueous solutions of pH 10.1, inclusional complexation of tetrakis(4-sulfonatophenyl)porphyrin (TSPP) with α-cyclodextrin (α-CD), β-CD, heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), and γ-CD has been examined by means of absorption, fluorescence, and induced circular dichroism spectra. Upon the addition of CD to a basic solution of TSPP, its absorption peak is shifted to longer wavelengths and the fluorescence peak is slightly shifted to longer wavelengths, with a band sharpening. α-CD and β-CD most likely form 1 : 1 and 2 : 1 host–guest inclusion complexes with TSPP. TM-β-CD and γ-CD form 2 : 1 and 1 : 1 inclusion complexes with TSPP, respectively. An equilibrium constant for the formation of the 2 : 1 TM-β-CD–TSPP inclusion complex has been estimated to be 2.92×10^{13} mol^{-2} dm^6 from a simulation for the absorbance change of TSPP with the TM-β-CD concentration. An equilibrium constant (K_1) for the formation of the 1 : 1 γ-CD–TSPP inclusion complex has been evaluated to be 1800 ± 100 mol^{-1} dm^3 from the absorbance change. This K_1 value is nearly the same as that (1600 ± 200 mol^{-1} dm^3) obtained from the fluorescence intensity change, supporting the formation of the 1 : 1 γ-CD–TSPP inclusion complex. An induced circular dichroism spectrum of TSPP in the presence of α-CD or β-CD exhibits a negative sign, whereas that in the presence of TM-β-CD or γ-CD exhibits a positive sign, indicating different inclusion modes.

Keywords: Cyclodextrins; Tetrakis(4-sulfonatophenyl)porphyrin; Inclusion complexes; Absorption spectra; Fluorescence spectra; Induced circular dichroism spectra

1. Introduction

Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides composed of six, seven, and eight D-glucopyranose residues, which are called α-CD, β-CD, and γ-CD, respectively. Because CDs are soluble in water but have a relatively hydrophobic cavity in molecular center, many kinds of organic compounds can be incorporated into their cavities to form inclusion complexes in aqueous solutions.

Porphyrin derivatives are requisite substances in most living systems. In photosynthesis, chlorophylls, which are Mg complexes of porphyrin derivatives, act critical roles. 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 inclusion complex as a photosensitizer [1,2]. The formation of inclusion complexes of CDs with porphyrin derivatives modifies the photochemical and photophysical properties of porphyrin derivatives. Consequently, it is very important to examine the formation of inclusion complexes of CDs with porphyrin derivatives.

There are several studies on the complexation of CDs with porphyrin derivatives. From spectrophotometric studies, 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‴-(21H,23H-porphine-5,10,15,20-tetrayl)tetrakis (benzoic acid), a semi-closed complex with hydroxypropyl-β-CD has been reported [4]. A very strong binding of meso-tetrakis(4-carboxyphenyl)porphyrin with heptakis (2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) has been observed [5]. The inclusion complex has a 2 : 1 host–guest stoichiometry. The binding constants for the 2 : 1 inclusion complexes of free and zinc porphyrins have been evaluated to be

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1.4×10^{16} and $1.9 \times 10^{16}\,\mathrm{mol^{-2}\,dm^6}$, respectively. 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 [6]. In the case of 5,15-diphenylporphyrin, a 2 : 1 TM-β-CD–guest inclusion complex is formed in dimethyl sulfoxide [7].

From NMR and spectrophotometric measurements, the formation of a 4 : 1 β-CD–zinc or iron (III) tetrakis(4- sulfonatophenyl)porphyrin inclusion complex (zinc TSPP or iron (III) TSPP) has been suggested [8,9]. Venema et al. have estimated an equilibrium constant for the formation of the 1 : 1 inclusion complex between β-CD and TSPP in pH 7.0 solutions to be $1400\,\mathrm{mol^{-1}\,dm^3}$ [10]. 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 [11]. 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. In their absorption spectral study, however, the concentration of TSPP has been as high as $8 \times 10^{-5}\,\mathrm{mol\,dm^{-3}}$. Consequently, they have observed a shift in the equilibrium between TSPP monomers and its dimer, through the absorption spectra, by the addition of CDs.

In these studies, there seems to be some discrepancies concerning the stoichiometries of the CD–TSPP inclusion complexes. For porphyrins such as TSPP, which have four substituents capable of binding to CDs, it is very important to clarify the stoichiometries and formation constants of inclusion complexes with CDs. Thus, using dilute TSPP solutions where TSPP exclusively exists as a monomer, we examined the inclusional complexation of TSPP with α-CD, β-CD, TM-β-CD, and γ-CD in basic aqueous solutions, by means of absorption, fluorescence, and induced circular dichroism spectra.

2. Experimental details

2.1. Materials

Tetrakis(4-sulfonatophenyl)porphyrin (TSPP) purchased from Tokyo Kasei Kogyo was used as received. α-Cyclodextrin (α-CD), β-CD, heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD), and γ-CD obtained from Nakalai Tesque were used without further purification, except for β-CD which was twice recrystallized from water.

2.2. Apparatus

Absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were taken with a Shimadzu RF-540 spectrofluorometer. Although the fluorescence spectra were corrected for the spectral response of the fluorometer, the spectra in the longer-wavelength region were not fully corrected because the sensitivity in the longer-wavelength region was very low. Consequently, the vibrational band at around 700 nm in the fluorescence spectra of TSPP seems to be lowered to some degree. 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 $25 \pm 0.1^\circ$C, except for the induced circular dichroism spectra which were measured at $25 \pm 2^\circ$C.

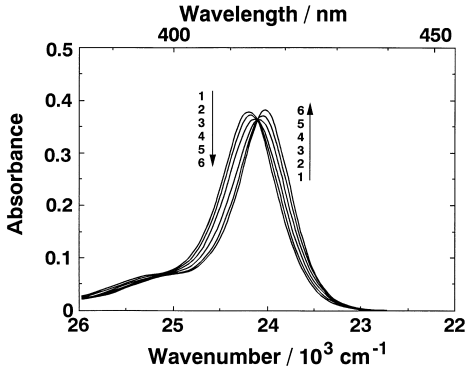
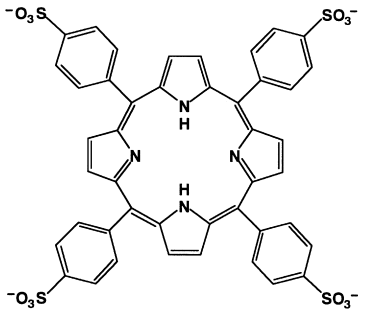


Fig. 1. Absorption spectra of TSPP ($9.0 \times 10^{-7}\,\mathrm{mol\,dm^{-3}}$) in pH 10.1 buffers containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-4}, (3) 3.0×10^{-4}, (4) 1.0×10^{-3}, (5) 3.0×10^{-3}, and (6) $1.0 \times 10^{-2}\,\mathrm{mol\,dm^{-3}}$.



Tetrakis(4-sulfonatophenyl)porphyrin (TSPP)

3. Results and discussion

3.1. Inclusion complex between γ-CD and TSPP in basic aqueous solution

From a titration curve for the absorbance of TSPP at 413 nm, a pK_a value of TSPP was evaluated to be 5.2, which is nearly the same as a reported pK_a value of 5.4 [12]. Furthermore, we evaluated an apparent pK_a^* value to be 5.6 from a titration curve for the fluorescence intensity of TSPP. Because an equilibrium for the protonation of TSPP may not be established during the lifetime of the excited singlet state of TSPP, the apparent pK_a^* value may be different from

an intrinsic pK_a^* value. In acidic solutions where TSPP is protonated, the absorption of TSPP has been found to be slightly reduced with time, indicating that protonated TSPP is relatively unstable. On the other hand, TSPP is stable in basic aqueous solution where TSPP is not protonated. In this study, therefore, we have focused on the behavior of TSPP towards CDs in basic aqueous solution.

Fig. 1 shows absorption spectra of TSPP (9.0×10^{-7} mol dm^{-3}) in buffers (pH 10.1) containing various concentrations of γ-CD. When γ-CD is added to TSPP solutions, the absorption maximum of TSPP is shifted to longer wavelengths, accompanied by an isosbestic point at 415 nm. The absorption spectral changes indicate the formation of an inclusion complex of γ-CD with TSPP. As previously stated, the formation of a 2 : 1 γ-CD–TSPP inclusion complex has been reported [11]. Assuming the formation of the 2 : 1 γ-CD–TSPP inclusion complex, therefore, we first analyzed the complexation reaction using the equation [13]:

$$\frac{1}{(A - A_0)} = \frac{1}{a} + \frac{1}{\left(aK_2[\gamma\text{-CD}]_0{}^2\right)} \quad (1)$$

Here, A, A_0, a, K_2, and $[\gamma\text{-CD}]_0$ are the absorbance in the presence of γ-CD, that in the absence of γ-CD, a constant, the equilibrium constant for the formation of the 2 : 1 γ-CD–TSPP inclusion complex, and the initial concentration of γ-CD, respectively. Eq. (1) holds under the experimental conditions of much higher concentrations of γ-CD than that of TSPP. A plot of $1/(A - A_0)$ against $1/[\gamma\text{-CD}]_0{}^2$ did not afford a straight line (not shown), indicating that the 2 : 1 inclusion complex is not formed. When a 1 : 1 γ-CD–TSPP inclusion complex is formed, an equilibrium constant (K_1) for the formation of the 1 : 1 inclusion complex is estimated from the equation [14,15]:

$$\frac{1}{(A - A_0)} = \frac{1}{a'} + \frac{1}{(a'K_1[\gamma\text{-CD}]_0)} \quad (2)$$

Here, a' is a constant. Eq. (2) also holds under the conditions that the concentration of TSPP is negligible relative to those of γ-CD. In the γ-CD concentration range of up to 1.0×10^{-2} mol dm^{-3}, a plot (double reciprocal plot) of $1/(A - A_0)$ against $1/[\gamma\text{-CD}]_0$ gives a straight line (Fig. 2); from this plot, 1800 ± 100 mol^{-1} dm^3 is evaluated as a K_1 value. This finding suggests the formation of the 1 : 1 γ-CD–TSPP inclusion complex (γ-CD·TSPP):

$$\gamma\text{-CD} + \text{TSPP} \overset{K_1}{\rightleftharpoons} \gamma\text{-CD} \cdot \text{TSPP} \quad (3)$$

The formation of the 1 : 1 γ-CD–TSPP inclusion complex is in disagreement with the result of Ribo et al. [11]. For the γ-CD–TSPP inclusion complex, they have concluded the 2 : 1 stoichiometry on the basis of a continuous variation method using NMR spectroscopy. At a high TSPP concentration (1×10^{-2} mol dm^{-3}) used in their NMR study, TSPP associates to form a dimer. Consequently, the equilibrium between monomeric and dimeric TSPPs may affect the result of the continuous variation method. In our study, the continuous variation method could not be applied to the γ-CD–TSPP system, because the concentration of TSPP was significantly low compared to those of γ-CD.

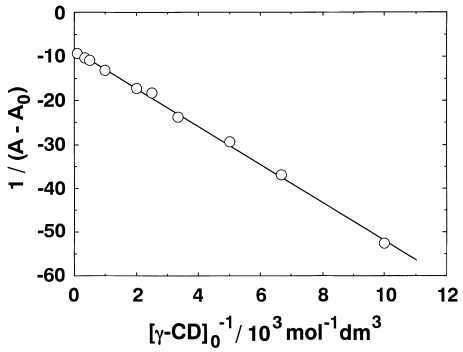


Fig. 2. Double reciprocal plot for the absorbance of TSPP (9.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. $\lambda_{obs} = 410$ nm.

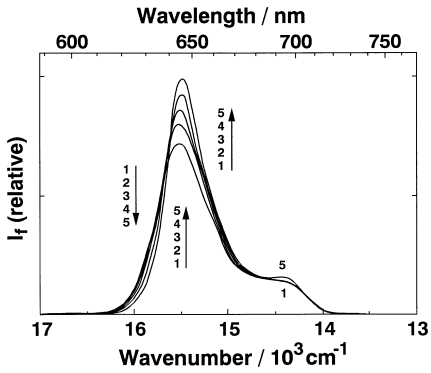


Fig. 3. Fluorescence spectra of TSPP (9.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. Concentration of γ-CD: (1) 0, (2) 1.0×10^{-4}, (3) 3.0×10^{-4}, (4) 1.0×10^{-3}, and (5) 3.0×10^{-3} mol dm^{-3}. $\lambda_{ex} = 415$ nm.

Fig. 3 exhibits fluorescence spectra of TSPP (9.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD. Upon the addition of γ-CD, the fluorescence maximum is very slightly shifted to longer wavelengths. At the same time, the fluorescence intensity is enhanced with a sharpening of the fluorescence band. These findings also indicate the formation of the inclusion complex of γ-CD with TSPP. For the formation of the 1 : 1 γ–CD-TSPP inclusion complex, one can evaluate a K_1 value according to the equation [15]:

$$\frac{1}{(I_f - I_f^0)} = \frac{1}{b} + \frac{1}{(bK_1[\gamma\text{-CD}]_0)} \quad (4)$$

Here, I_f, I_f^0, and b are the fluorescence intensity in the presence of γ-CD, that in the absence of γ-CD, and a constant, respectively. As in the analyses of the absorbance change, Eq. (4) holds when the concentrations of γ-CD are much higher than that of TSPP. From a plot of $1/(I_f - I_f^0)$ against $1/[\gamma\text{-CD}]_0$, a K_1 value of 1600 ± 200 mol^{-1} dm^3 is evaluated (Fig. 4). The results that this K_1 value is nearly the same as

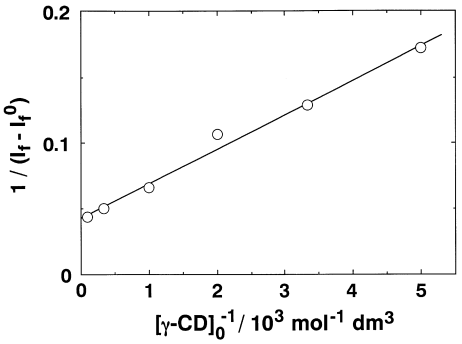


Fig. 4. Double reciprocal plot for the fluorescence intensity of TSPP (9.0×10^{-7} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of γ-CD, λ_{ex} = 415 nm, λ_{obs} = 646 nm.

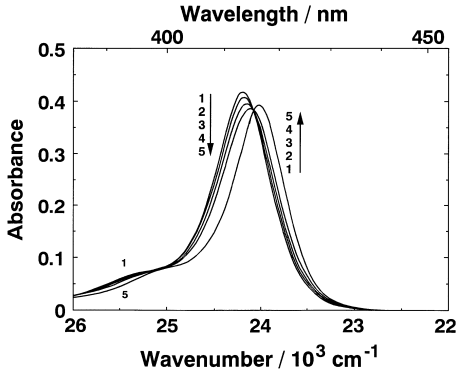


Fig. 5. Absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of TM-β-CD. Concentration of TM-β-CD: (1) 0, (2) 2.0×10^{-7}, (3) 5.0×10^{-7}, (4) 1.0×10^{-6}, and (5) 3.0×10^{-6} mol dm^{-3}.

that (1800 ± 100 mol^{-1} dm^3) obtained from the absorbance change and the plot of $1/(I_f - I_f^0)$ against $1/[\gamma\text{-CD}]_0$ shows a straight line provide additional evidence that not a 2 : 1 but a 1 : 1 γ-CD–TSPP inclusion complex is formed.

3.2. Inclusion complex between TM-β-CD and TSPP in basic aqueous solution

Fig. 5 exhibits absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of TM-β-CD. As the TM-β-CD concentration is increased, the absorption peak is shifted to longer wavelengths, accompanied by an isosbestic point at 415 nm. This finding indicates the formation of an inclusion complex of TM-β-CD with TSPP. In contrast to γ-CD, the absorption spectral changes are seen at concentrations of TM-β-CD similar to that (1.0×10^{-6} mol dm^{-3}) of TSPP, indicating that the complexation between TM-β-CD and TSPP is significantly favorable compared to γ-CD.

To determine a stoichiometry of the TM-β-CD–TSPP inclusion complex, a continuous variation method for the absorbance was applied keeping the sum of the initial concentrations of TSPP and TM-β-CD constant; $[\text{TSPP}]_0 + [\text{TM-β-CD}]_0 = 2.0 \times 10^{-6}$ mol dm^{-3}. The result

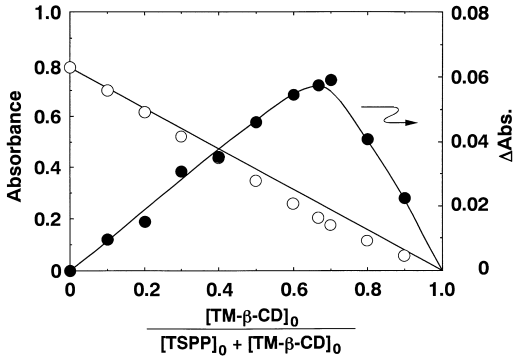


Fig. 6. Continuous variation plot for the absorbance. Open and closed circles represent the observed absorbances and the absorbances corrected for the contribution of free TSPP, respectively. $[\text{TSPP}]_0 + [\text{TM-β-CD}]_0 = 2.0 \times 10^{-6}$ mol dm^{-3}. λ_{obs} = 412.5 nm.

is depicted in Fig. 6. The open circles represent the absorbances observed at 412.5 nm and the closed circles represent the corrected absorbances (ΔAbs) which have been given by the subtraction of the raw, observed absorbance values from the absorbance values on a straight line drawn in Fig. 6. This correction means that a contribution of free TSPP to the absorbance is removed from the observed absorbance. Consequently, the ΔAbs value is proportional to the concentration of complexed TSPP. In Fig. 6, the ΔAbs value goes through a maximum at a molar fraction of 0.66, indicating a 2 : 1 stoichiometry of the TM-β-CD–TSPP inclusion complex. Our conclusion of the formation of the 2 : 1 TM-β-CD–TSPP inclusion complex is in agreement with the results of the NMR spectroscopic study reported by Kano et al. [12].

As seen in Fig. 5, the concentrations of TM-β-CD are comparable to that of TSPP. Consequently, Eq. (1) cannot be applied to estimate an equilibrium constant (K_2) for the formation of the 2 : 1 TM-β-CD–TSPP inclusion complex. For the 2 : 1 TM-β-CD–TSPP inclusion complex ((TM-β-CD)$_2$·TSPP), K_2 is expressed as

$$K_2 = \frac{[(\text{TM-β-CD})_2 \cdot \text{TSPP}]}{([\text{TM-β-CD}]^2[\text{TSPP}])} \tag{5}$$

The initial concentrations of TSPP and TM-β-CD are respectively represented by the following equations:

$$[\text{TSPP}]_0 = \left(1 + K_2[\text{TM-β-CD}]^2\right)[\text{TSPP}] \tag{6}$$

$$[\text{TM-β-CD}]_0 = (1 + 2K_2[\text{TM-β-CD}][\text{TSPP}])\,[\text{TM-β-CD}] \tag{7}$$

Using Eqs. (5)–(7), a cubic equation for the concentration of free TM-β-CD is derived as

$$K_2[\text{TM-β-CD}]^3 + 2K_2(2[\text{TSPP}]_0 - [\text{TM-β-CD}]_0)[\text{TM-β-CD}]^2 + [\text{TM-β-CD}] - [\text{TM-β-CD}]_0 = 0 \tag{8}$$

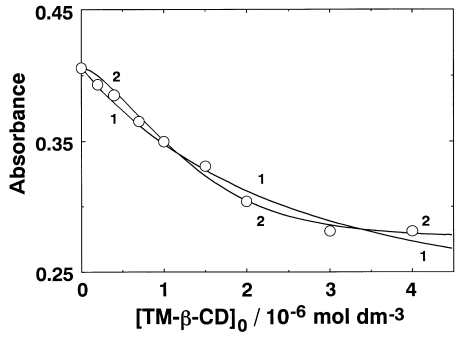


Fig. 7. Comparison of the observed absorbance data with the absorbance curves simulated for the 1 : 1 (curve 1) and 2 : 1 (curve 2) TM-β-CD–TSPP inclusion complexes. Using the known value of $\varepsilon_0 = 4.06 \times 10^5\ \mathrm{mol^{-1}\,dm^3}$ at 412.5 nm, the best fit simulation curves for the 1 : 1 and 2 : 1 inclusion complexes have been calculated under the assumptions of $\varepsilon_1 = 2.04 \times 10^5\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$ and $K_1 = 5.70 \times 10^5\ \mathrm{mol^{-1}\,dm^3}$ for the 1 : 1 inclusion complex and $\varepsilon_2 = 2.79 \times 10^5\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$ and $K_2 = 2.92 \times 10^{13}\ \mathrm{mol^{-2}\,dm^6}$ for the 2 : 1 inclusion complex, respectively. $[\mathrm{TSPP}]_0 = 1.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$.

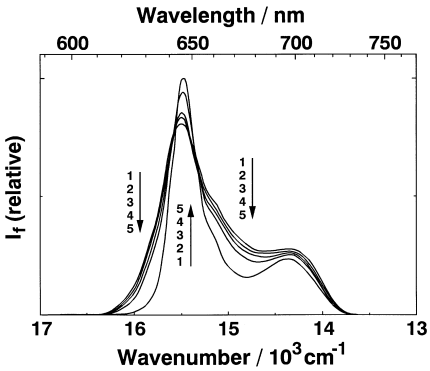


Fig. 8. Fluorescence spectra of TSPP ($1.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$) in pH 10.1 buffers containing various concentrations of TM-β-CD. Concentration of TM-β-CD: (1) 0, (2) 2.0×10^{-7}, (3) 6.0×10^{-7}, (4) 1.0×10^{-6}, and (5) $3.0 \times 10^{-6}\ \mathrm{mol\ dm^{-3}}$. $\lambda_{ex} = 415$ nm.

When a K_2 value is assumed, the solution of Eq. (8) gives the concentration of free TM-β-CD. Then, the concentration of free TSPP is evaluated from Eq. (6). After the concentrations of free TM-β-CD and free TSPP are known, the concentration of the 2 : 1 inclusion complex can be calculated from Eq. (5). Assuming the molar absorption coefficient (ε_2) of the 2 : 1 inclusion complex, the absorbance of TSPP can be calculated by

$$A = \varepsilon_0[\mathrm{TSPP}]d + \varepsilon_2\,[(\mathrm{TM\text{-}\beta\text{-}CD})_2 \cdot \mathrm{TSPP}]\,d$$
$$= \left(\varepsilon_0 + \varepsilon_2 K_2[\mathrm{TM\text{-}}\beta\mathrm{\text{-}CD}]^2\right)[\mathrm{TSPP}]d \qquad (9)$$

where ε_0 and d are the molar absorption coefficient of free TSPP and the path length (1.0 cm) of a cell, respectively. An ε_0 value at 412.5 nm has experimentally been determined to be $4.06 \times 10^5\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$. Fig. 7 displays the least-squares best fit simulation curve for the absorbances observed at 412.5 nm, which has been calculated with $\varepsilon_2 = 2.79 \times 10^6\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$ and

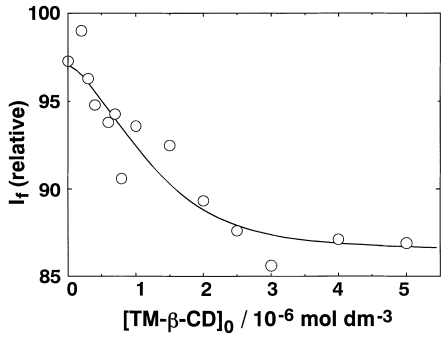


Fig. 9. Comparison of the observed fluorescence intensity data with the fluorescence intensity curve simulated for the 2 : 1 TM-β-CD–TSPP inclusion complex. The best fit simulation curve for the 2 : 1 inclusion complex has been calculated under the assumption of $K_2 = 1.92 \times 10^{13}\ \mathrm{mol^{-2}\,dm^6}$. $[\mathrm{TSPP}]_0 = 1.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$.

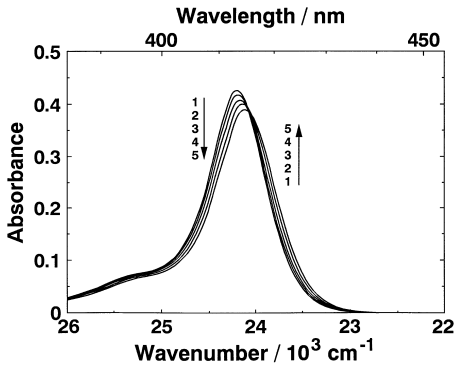


Fig. 10. Absorption spectra of TSPP ($1.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$) in pH 10.1 buffers containing various concentrations of α-CD. Concentration of α-CD: (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, (4) 6.0×10^{-3}, and (5) $1.0 \times 10^{-2}\ \mathrm{mol\,dm^{-3}}$.

$K_2 = 2.92 \times 10^{13}\ \mathrm{mol^{-2}\,dm^6}$, together with the observed absorbance data.

To further ascertain the existence of the 2 : 1 inclusion complex, we tried to similarly simulate the concentration curve for the 1 : 1 TM-β-CD–TSPP inclusion complex. Under the assumption of $\varepsilon_1 = 2.04 \times 10^6\ \mathrm{mol^{-1}\,dm^3\,cm^{-1}}$ and $K_1 = 5.70 \times 10^5\ \mathrm{mol^{-1}\,dm^3}$, the least-squares best fit curve of the TSPP absorbance for the 1 : 1 inclusion complex was obtained. This best fit curve is also shown in Fig. 7. The simulation curve for the 1 : 1 inclusion complex does not well fit the observed data above $2 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$ of TM-β-CD, as compared to the simulation curve for the 2 : 1 inclusion complex. This result provides additional evidence for the existence of the 2 : 1 inclusion complex. As the TM-β-CD concentration was raised, the fluorescence intensity of TSPP ($1.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$) in pH 10.1 buffer was enhanced, accompanied by a sharpening of the fluorescence band (Fig. 8). A continuous variation method using the fluorescence intensity was also applied to further corroborate the stoichiometry of the TM-β-CD–TSPP inclusion complex. The result also indicated the 2 : 1 stoichiometry (not shown). For the TSPP fluorescence intensity change, a simulation procedure similar to that applied to the absorbance change has been

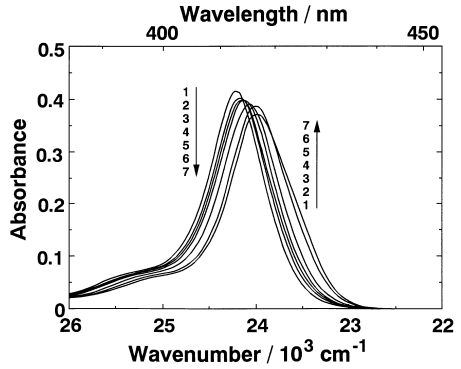


Fig. 11. Absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of β-CD. Concentration of β-CD: (1) 0, (2) 3.0×10^{-5}, (3) 5.0×10^{-5}, (4) 1.0×10^{-4}, (5) 3.0×10^{-4}, (6) 3.0×10^{-3}, and (7) 1.0×10^{-2} mol dm^{-3}.

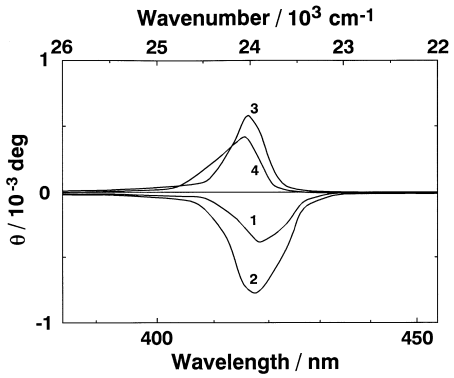


Fig. 12. Induced circular dichroism spectra of TSPP (2.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing α-CD (1.0×10^{-2} mol dm^{-3}) (curve 1), β-CD (1.0×10^{-2} mol dm^{-3}) (curve 2), TM-β-CD (4.0×10^{-6} mol dm^{-3}) (curve 3), and γ-CD (1.0×10^{-2} mol dm^{-3}) (curve 4).

performed. The least-squares best fit curve for the fluorescence intensities of the 2 : 1 inclusion complex is exhibited in Fig. 9. From this simulation, a K_2 value is estimated to be 1.92×10^{13} mol^{-2} dm^6, which is similar to the K_2 value obtained from the simulation using the absorbance.

3.3. Inclusion complexes of TSPP with α-CD and β-CD in basic aqueous solutions

Fig. 10 shows absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of α-CD. Upon the addition of α-CD of less than about 1.0×10^{-2} mol dm^{-3}, the absorption peak is shifted to longer wavelengths, accompanied by an isosbestic point at 415 nm, indicating the formation of an α-CD–TSPP inclusion complex. At an α-CD concentration of 1.0×10^{-2} mol dm^{-3}, however, the isosbestic point is not observed, suggesting the formation of two kinds of inclusion complexes. At high concentrations of α-CD, 1 : 1 and 2 : 1 α-CD-TSPP inclusion complexes may coexist. The formation of the α-CD–TSPP inclusion complexes in basic aqueous solutions is not agreement with the result reported by Ribo et al. [11]. In the α-CD concentration range of less than 1.0×10^{-2} mol dm^{-3}, a K_1 value was evaluated to be 720 ± 180 mol^{-1} dm^3 from the absorbance change, which is less than half of the K_1 value for γ-CD. The smaller K_1 value for α-CD suggests that the inclusion process of α-CD is unfavorable compared to γ-CD because of the narrower cavity of α-CD than that of γ-CD. The fluorescence maximum of TSPP in pH 10.1 buffer was very slightly shifted to longer wavelengths by the addition of α-CD.

Fig. 11 shows absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing various concentrations of β-CD. When β-CD of less than about 3.0×10^{-4} mol dm^{-3} is added to TSPP solutions, the absorption peak is shifted to longer wavelengths, accompanied by an isosbestic point at 414.5 nm, indicating the formation of a β-CD–TSPP inclusion complex. As the β-CD concentration is further increased, the absorption peak is further shifted to longer wavelengths without the isosbestic point. These findings suggest that, in addition to a 1 : 1 inclusion complex, a 2 : 1 β-CD–TSPP inclusion complex is formed in the high concentration range of β-CD. As in the case of α-CD, the fluorescence spectrum of TSPP is very slightly red-shifted upon the addition of β-CD.

In the CD concentration ranges examined, the 2 : 1 CD–TSPP inclusion complexes are formed except for γ-CD, although the concentrations where the 2 : 1 inclusion complexes appear are different form one another. The appearance of the 2 : 1 inclusion complex at higher α-CD concentrations compared to β-CD implies that the encapsulation of a sulfonatophenyl moiety by α-CD is less favorable than that by β-CD because of the narrow α-CD cavity. This seems to reflect the ability of the individual CD to form the 2 : 1 inclusion complex from the 1 : 1 inclusion.

3.4. Induced circular dichroism spectra of TSPP in basic aqueous solutions containing CDs

Fig. 12 illustrates induced circular dichroism (icd) spectra of TSPP (2.0×10^{-6} mol dm^{-3}) in pH 10.1 buffers containing α-CD (1.0×10^{-2} mol dm^{-3}), β-CD (1.0×10^{-2} mol dm^{-3}), TM-β-CD (4.0×10^{-6} mol dm^{-3}), and γ-CD (1.0×10^{-2} mol dm^{-3}). The icd spectra correspond to the 417 nm absorption band of TSPP. The sign of the icd spectrum of TSPP in the presence of α-CD or β-CD is negative, whereas that in the presence of TM-β-CD or γ-CD is positive. The transition dipole moments responsible for the 417 nm band of TSPP, which are most likely degenerate, are directed to the N–N and NH–NH axes, respectively [16,17]. In the inclusion complexes of TSPP, the binding site of CD is a sulfonatophenyl group [11,12]. Consequently, the transition dipoles of TSPP are most likely located outside the CD cavity. When a transition dipole is located outside the CD cavity and when an angle between the transition dipole moment and the symmetry axis of CD is less than 54.7°, an icd spectrum shows a

negative sign [18–21]. Conversely, a positive sign of an icd spectrum is observed under the conditions that the angle is greater than 54.7°. When the symmetry axes of CD and a sulfonatophenyl group are nearly parallel to one another, an icd spectrum of TSPP in CD solution is expected to be negative in sign. This seems to be the cases for α-CD and β-CD. As shown in Fig. 12, however, the positive signs are observed for TM-β-CD and γ-CD. These CDs may encapsulate a sulfonatophenyl group, with some angles between the symmetry axes of CD and a sulfonatophenyl group. In this situation, the transition dipoles do not lie on but are located off the symmetry axis of CD. The tilting of the two symmetry axes seems to cause the positive signs of the icd spectra for TM-β-CD and γ-CD [22].

4. Concluding remarks

Absorption and fluorescence spectra of TSPP in pH 10.1 buffers in the absence and presence of α-CD, β-CD, TM-β-CD, and γ-CD were measured to investigate the inclusional complexation of TSPP with these CDs. In the case of γ-CD, the 1 : 1 inclusion complex of TSPP is formed in pH 10.1 buffer, with the K_1 value of $1800 \pm 100\,\mathrm{mol^{-1}\,dm^3}$ evaluated from the absorbance change, which is nearly the same as that ($1600 \pm 200\,\mathrm{mol^{-1}\,dm^3}$) evaluated from the fluorescence intensity change. Nearly the same K_1 values support the existence of the 1 : 1 γ-CD–TSPP inclusion complex. The formation of the 2 : 1 TM-β-CD–TSPP inclusion complex has been confirmed on the basis of the continuous variation method using the absorbance and fluorescence intensity changes. The K_2 value has been estimated to be $2.92 \times 10^{13}\,\mathrm{mol^{-2}\,dm^6}$ from the simulation concerning the absorbance change, which is similar to the K_2 value ($1.92 \times 10^{13}\,\mathrm{mol^{-2}\,dm^6}$) obtained from the simulation concerning the fluorescence intensity change. In the cases of α-CD and β-CD, the 1 : 1 and 2 : 1 host–guest inclusion complexes are most likely formed, although the 2 : 1 inclusion complex of α-CD appears at remarkably high concentrations of α-CD compared to β-CD. There is the difference in stoichiometry of the inclusion complexes of TSPP with CDs, in spite of the same binding site (sulfonatophenyl group). In addition to the difference in stoichiometry, the binding mode of TM-β-CD and γ-CD is different from that of α-CD and β-CD, because the signs of the icd spectra for TM-β-CD and γ-CD are positive in contrast to those for α-CD and β-CD. The tilting of the symmetry axes of CD and a sulfonatophenyl group seems to cause the positive signs for the icd spectra of TM-β-CD and γ-CD, whereas the symmetry axes of α-CD and β-CD are nearly parallel to that of a sulfonatophenyl group, leading to the negative icd signs.

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