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ORIGINAL ARTICLE

Complex formation of tetrakis(4-sulfonatophenyl)porphyrin with γ-cyclodextrin, phenylalanine, and tryptophan in aqueous solution

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Abstract In pH 10.3 buffer, in which phenylalanine and tryptophan are in an anionic form, the interactions of tetrakis(4-sulfonatophenyl)porphyrin (TSPP) with γ-cyclodextrin (γ-CD) and phenylalanine (or tryptophan) have been examined by means of absorption and fluorescence spectroscopy. A 1:1 inclusion complex is formed between TSPP and γ-CD. TSPP forms 1:2 complexes with L- and D-phenylalanine (LPhe and DPhe), while TSPP forms 1:1 complexes with L- and D-tryptophan (LTrp and DTrp). In TSPP solution containing γ-CD and LPhe (or DPhe), the 1:1:2 γ-CD–TSPP–LPhe (or –DPhe) inclusion complex is formed, while the 1:1:1 γ-CD–TSPP–LTrp (or –DTrp) inclusion complex is formed for LTrp (or DTrp). The equilibrium constants for the formation of the complexes have been evaluated from the fluorescence intensity change of TSPP. The equilibrium constants are nearly the same for the optical isomers of phenylalanine and tryptophan, respectively, indicating that the optical isomers are not discriminated through the complexation. For the LPhe complexes, the equilibrium constants have also been evaluated at a fixed ionic strength of 0.2 mol dm^{-3} using NaCl. 3-Phenyl-1-propanol and L-phenylalaninol, which are analogous to phenylalanine in molecular structure, have been examined for the complexation with TSPP and γ-CD. In contrast to phenylalanine, the stoichiometries of their binary complexes with TSPP and their ternary inclusion complexes with γ-CD and TSPP are 1:1 and 1:1:1, respectively.

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Introduction

It has been reported that an organic cation forms a complex with an organic anion [1, 2]. The hydrophobic interactions as well as the electrostatic interactions likely induce the formation of an organic cation–organic anion complex as a driving force.

Porphyrins, which are a series of organic compounds capable of coordinating to a metal ion, belong to a very important group in the biological and biochemical fields. For example, chlorophyll, which is a chlorin derivative derived from porphyrin complexing with Mg, is indispensable to photosynthesis. In blood, hemoglobin, which includes porphyrin derivatives, carries oxygen to peripheral tissues. Because there are porphyrin derivatives as a key compound in living bodies, it is expected that porphyrin derivatives interact with a variety of compounds existing in the living bodies. The interactions of porphyrin derivatives with proteins or nucleic acids have been investigated [3–5]. A porphyrin ring may interact with an aromatic group in an organic compound. In human serum albumin, the complexation between tetrakis(4-sulfonatophenyl)porphyrin (TSPP) and an amino-acid residue has not been observed [3]. However, it has been suggested that the tryptophan fluorescence in human serum albumin is quenched by TSPP, and that the long distance Förster resonance energy transfer to TSPP may be responsible for the tryptophan fluorescence quenching [3]. Consequently, it is important to examine the interactions between porphyrin derivatives and amino acids in a

relatively simple system, because proteins are made of amino acids.

As a water-soluble porphyrin derivative, we selected TSPP, which is an anion. We examined the interactions of TSPP with amino acids having an aromatic moiety. It has been found that anionic TSPP forms a complex with L-phenylalanine (LPhe) in pH 10.3 buffer, in which LPhe is mainly in an anionic form; an organic anion–organic anion complex is formed. For the L- and D-optical isomers of phenylalanine, the effects of chirality have also been examined through the evaluation of the equilibrium constant for the complexation.

Cyclodextrins (CDs), which are shaped like a truncated cone, are cyclic oligosaccharides [6]. Commercially available CDs, which are composed of six, seven, and eight D-glucopyranose residues, are called α-CD, β-CD, and γ-CD, respectively. Because CD has a relatively hydrophobic cavity, CD accommodates an organic molecule of appropriate dimensions to form an inclusion complex.

The interactions of CD with TSPP have been studied by many researchers [7–14]. In these studies, binary CD–TSPP inclusion complexes have been investigated. There are a few studies on the formation of an inclusion complex of CD with an organic cation–organic anion complex [1, 2]. However, the interactions between CD with an organic anion–organic anion complex (or organic cation–organic cation complex) have not been examined. Because γ-CD forms an inclusion complex with TSPP, the interactions of γ-CD with a complex between TSPP and LPhe (or D-phenylalanine (DPhe)), which is an organic anion–organic anion complex, have been investigated in this study. In the presence of γ-CD, it has been found that TSPP forms a ternary inclusion complex with γ-CD and LPhe (or DPhe). In addition to phenylalanine, we have examined whether or not tryptophan and several compounds analogous to phenylalanine form binary complexes with TSPP and ternary inclusion complexes with γ-CD and TSPP.

Experimental

Tetrakis(4-sulfonatophenyl)porphyrin (TSPP) was purchased from Tokyo Chemical Industry Co., Ltd. and used as received (Chart 1).

γ-Cyclodextrin (γ-CD), which was obtained from Wako Pure Chemical Industries, Ltd., was used without further purification. D- and L-phenylalanine (DPhe and LPhe), D- and L-tryptophan (DTrp and LTrp), L-phenylalaninol (PA), and 3-phenyl-1-propanol (PP) purchased from Tokyo Chemical Industry Co., Ltd. were used as received. Buffers of pH 10.3 were made of 0.025 mol dm^{-3} $NaHCO_3$ and 0.0138 mol dm^{-3} NaOH.

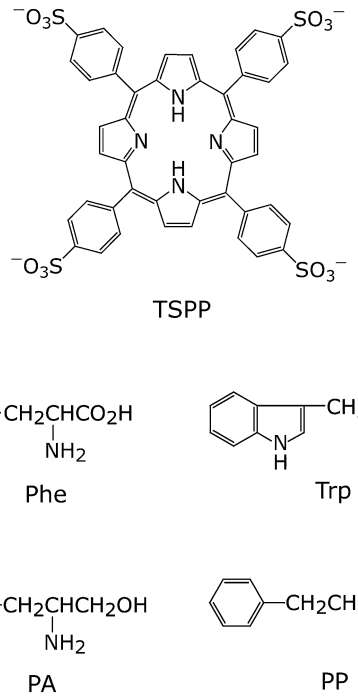

Chart 1 Chemical structures of TSPP, Phe, Trp, PA, and PP

Absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. Fluorescence spectra were taken with a Shimadzu RF-501 spectrofluorometer equipped with a cooled Hamamatsu R-943 photomultiplier. The fluorescence spectra were corrected for the spectral response of the fluorometer. Spectroscopic measurements were made at 25 ± 0.1 °C.

Results and discussion

Complexation of γ-CD with TSPP

γ-CD forms a 1:1 inclusion complex with TSPP in pH 10.1 buffer [12].

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

Here, K_1 is the equilibrium constant for the formation of the 1:1 inclusion complex (γ-CD·TSPP) of γ-CD with TSPP. From the fluorescence intensity change of TSPP, the K_1 value of TSPP in pH 10.3 buffer has been evaluated to be 1200 ± 30 mol^{-1} dm^3, which is slightly less than that (1600 ± 200 mol^{-1} dm^3) obtained for TSPP in pH 10.1 buffer [12].

Complexation of TSPP with L- and D-phenylalanine (LPhe and DPhe)

At 1.0×10^{-6} mol dm^{-3} of TSPP, its absorption bands at wavelengths longer than the 413-nm band (Soret band)

have been very weak; the absorbance of TSPP has been 0.013 at a peak wavelength of the 516-nm band. Consequently, we have focused on the Soret band of TSPP. At pH 10.3, LPhe is mainly in an anionic form, because the pKa_2 value of LPhe is 9.18. Figure 1 shows absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing various concentrations of LPhe. When LPhe is added to TSPP solution, the absorption peak of TSPP shifts to longer wavelengths with a reduction in the absorption-peak intensity, and an isosbestic point appears at 416 nm. These findings suggest the formation of a complex of TSPP with LPhe. At pH 11.2, LPhe predominantly exists as an anion. When LPhe was added to TSPP solution of pH 11.2, TSPP exhibited absorption spectral change similar to that in pH 10.3 buffer, indicating that anionic LPhe forms a complex with TSPP at pH 10.3 as well as pH 11.2. Figure 2 exhibits fluorescence spectra, excited at 424 nm, of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing various concentrations of LPhe. Upon the addition of LPhe, the fluorescence peak is shifted to longer wavelengths, accompanied by an increase in the fluorescence intensity. This finding indicates the formation of the complex between TSPP and LPhe. Because anionic TSPP forms a complex with anionic LPhe, the hydrophobic and π–π interactions of a phenyl group of LPhe most likely contribute to the stability of the TSPP–LPhe complex.

When the TSPP–LPhe complex has a 1:1 stoichiometry, the Benesi-Hildebrand equation holds for the fluorescence intensity of TSPP [15, 16].

$$1/\left(I_f - I_f^0\right) = 1/a + 1/(aK[\mathrm{LPhe}]) \quad (2)$$

where a and K are an experimental constant and the equilibrium constant for the formation of the 1:1 TSPP–LPhe complex, respectively. Figure 3a shows a plot of $1/(I_f - I_f^0)$ against 1/[LPhe] for TSPP solutions containing LPhe. The data in Fig. 3a exhibits not a straight line but a slightly convex curve. In addition, the K value estimated from the straight line in Fig. 3a has a negative K value of -11 ± 1 mol^{-1} dm^3. The negative K value evidently indicates that TSPP–LPhe complex does not have a 1:1 stoichiometry. When the TSPP–LPhe complex has a 1:2 stoichiometry, a plot of $1/(I_f - I_f^0)$ against $1/[\mathrm{LPhe}]^2$ should give a straight line:

$$1/\left(I_f - I_f^0\right) = 1/b + 1/\left(bK_2[\mathrm{LPhe}]^2\right) \quad (3)$$

where b and K_2 are an experimental constant and the equilibrium constant for the formation of the 1:2 TSPP–LPhe complex, respectively. Figure 3b depicts a plot of $1/(I_f - I_f^0)$ against $1/[\mathrm{LPhe}]^2$ for TSPP solutions containing LPhe. This plot exhibits a good straight line, suggesting the formation of the 1:2 TSPP–LPhe complex (TSPP·$(\mathrm{LPhe})_2$).

$$\mathrm{TSPP} + 2\mathrm{LPhe} \overset{K_2}{\rightleftharpoons} \mathrm{TSPP} \cdot (\mathrm{LPhe})_2 \quad (4)$$

From the straight line in Fig. 3b, the K_2 value is estimated to be 750 ± 40 mol^{-2} dm^6 (Table 1). In separate experiments, similar results for the 1/[LPhe] and $1/[\mathrm{LPhe}]^2$ dependence of the TSPP fluorescence intensity have been obtained; slightly convex curves for the 1/[LPhe] dependence, negative K values, and straight lines for the $1/[\mathrm{LPhe}]^2$ dependence. At present, it is not clear why not the 1:1 TSPP–LPhe complex but the 1:2 TSPP–LPhe complex is formed. The equilibrium constant for the formation of a 1:1 complex of TSPP with Methylene Blue, which is an organic cation–organic anion complex, has been reported to be 2.35×10^5 mol^{-1} dm^3 [17]. Compared to the equilibrium constant for Methylene Blue, the K_2 value for LPhe seems to be small, although the TSPP–Methylene Blue complex has a 1:1 stoichiometry. The relatively small K_2 value may be due to no contribution of the electrostatic attraction between TSPP and LPhe. When L-leucine was added to TSPP solution, little or no absorption spectral

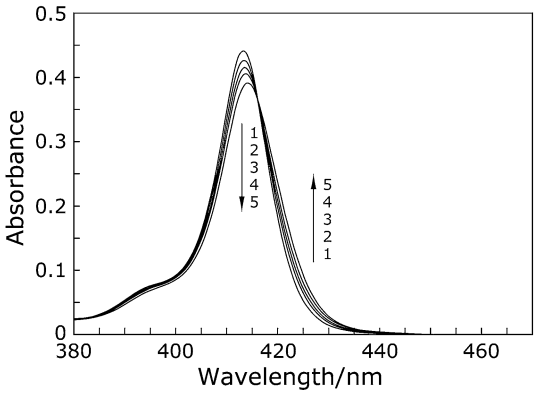

Fig. 1 Absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing various concentrations of LPhe. Concentration of LPhe: (*1*) 0, (*2*) 1.5×10^{-2}, (*3*) 2.25×10^{-2}, (*4*) 3.0×10^{-2}, and (*5*) 4.5×10^{-2} mol dm^{-3}

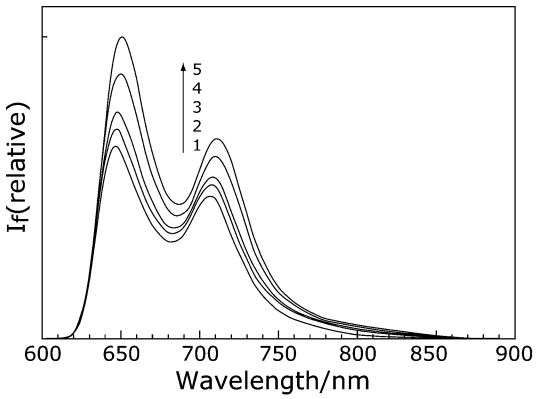

Fig. 2 Fluorescence spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing various concentrations of LPhe. Concentration of LPhe: (*1*) 0, (*2*) 7.5×10^{-3}, (*3*) 1.5×10^{-2}, (*4*) 3.0×10^{-2}, and (*5*) 4.5×10^{-2} mol dm^{-3}. $\lambda_{ex} = 424$ nm

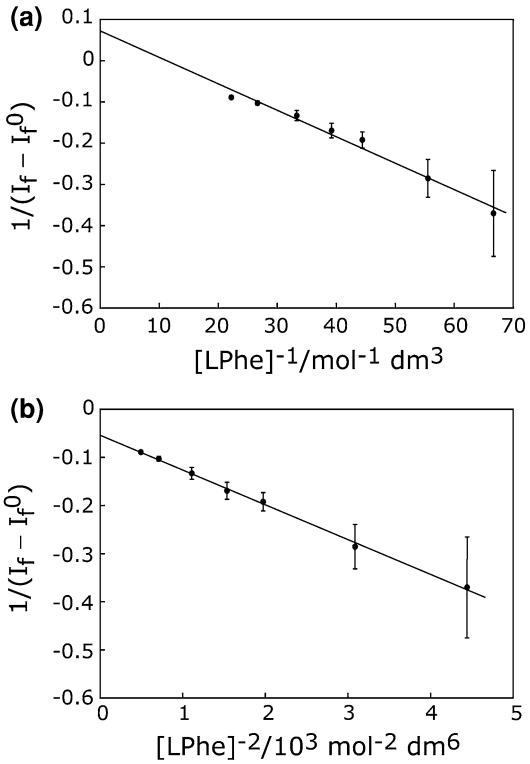

Fig. 3 **a** Plot of $1/(I_f - I_f^0)$ against 1/[LPhe] for TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing LPhe. **b** Plot of $1/(I_f - I_f^0)$ against $1/[\text{LPhe}]^2$ for TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing LPhe. $\lambda_{ex} = 406$ nm, $\lambda_{obs} = 650$ nm

Table 1 The values of K_2, K_3, and K_4 for LPhe and DPhe

	K_2 (mol^{-2} dm^6)	K_3 (mol^{-2} dm^6)	K_4 (mol^{-1} dm^3)
LPhe	750 ± 40	714	1140
	430 ± 70[a]	1220[a]	4820[a]
DPhe	800 ± 100	844	1270

[a] The K value was evaluated at an ionic strength of 0.2 mol dm^{-3} using NaCl

change of TSPP was observed. This finding suggests that a phenyl group of LPhe plays a critical role in the formation of the TSPP–LPhe complex.

Absorption and fluorescence spectral changes of TSPP in the presence of D-phenylalanine (DPhe) were similar to those in the presence of LPhe, respectively. As in the case of LPhe, a plot of $1/(I_f - I_f^0)$ against 1/[DPhe] for DPhe was curved downward. A straight line drawn for the plot gave a negative K value of -10 ± 1 mol^{-1} dm^3, while a plot of $1/(I_f - I_f^0)$ against $1/[\text{DPhe}]^2$ for TSPP solutions containing DPhe exhibited a good straight line, giving a K_2 value of 800 ± 100 mol^{-2} dm^6. Consequently, the TSPP–DPhe complex as well as the TSPP–LPhe complex has a 1:2 stoichiometry. The K_2 value (800 ± 100 mol^{-2} dm^6) for DPhe is nearly the same as the K_2 value (750 ± 40 mol^{-2} dm^6) for LPhe, suggesting that the complexation abilities of TSPP toward LPhe and DPhe are the same or almost the same; TSPP does not discriminate the optical isomers of phenylalanine in forming the complex with phenylalanine.

Ternary inclusion complex of γ-CD with TSPP and LPhe

Little or no absorption spectral change of LPhe was observed upon the addition of γ-CD to LPhe solution. Consequently, the complex formation would be neglected between γ-CD and LPhe. Figure 4 illustrates an absorption spectrum (dotted curve) of TSPP (1.0×10^{-6} mol dm^{-3}) alone in pH 10.3 buffer and absorption spectra of TSPP solutions containing γ-CD (3.0×10^{-3} mol dm^{-3}) and several concentrations of LPhe. When γ-CD is added to TSPP solution without LPhe, an absorption peak is shifted to longer wavelengths, accompanied by a slight reduction in the absorption-peak intensity. This spectral change indicates the formation of the γ-CD–TSPP inclusion complex. Further addition of LPhe to TSPP solution containing γ-CD (3.0×10^{-3} mol dm^{-3}) results in a slight peak-shit to longer wavelengths, with a slight decrease in the absorption-peak intensity. This absorption spectral change may be due to the formation of the TSPP–LPhe complex, because similar spectral changes are observed in the absence of γ-CD (Fig. 1). In Fig. 1, the wavelength shift of the TSPP absorption peak in the absence and presence of LPhe (4.5×10^{-2} mol dm^{-3}) is 0.9 nm. In Fig. 4, on the other hand, the wavelength shift of the absorption peak in the absence and presence of γ-CD (3.0×10^{-3} mol dm^{-3}) amounts to 2.5 nm, which is greater than that in the absence and presence of LPhe. When LPhe (4.5×10^{-2} mol dm^{-3}) is added to TSPP solution containing γ-CD of 3.0×10^{-6} mol dm^{-3} (Fig. 4), the absorption peak is further shifted to longer wavelengths (0.8 nm). If only the

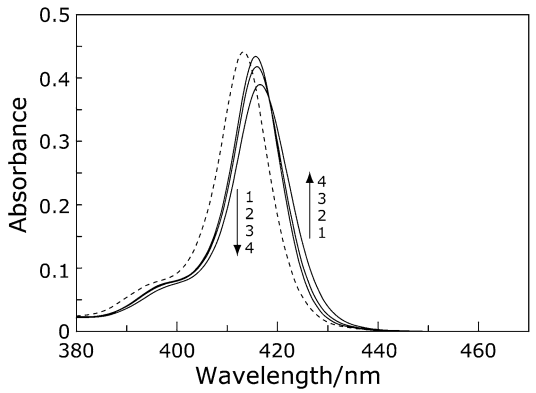

Fig. 4 Absorption spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing γ-CD and LPhe. *Spectrum 1* (*dotted curve*): [γ-CD] = 0 and [LPhe] = 0 mol dm^{-3}, *spectrum 2*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 0 mol dm^{-3}, *spectrum 3*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 1.5×10^{-2} mol dm^{-3}, and *spectrum 4*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 4.5×10^{-2} mol dm^{-3}

TSPP–LPhe complex is formed by the addition of LPhe to TSPP solution containing γ-CD, the concentration of the TSPP–LPhe complex is increased at the expense of the concentration of the γ-CD–TSPP inclusion complex. In this case, the absorption peak is expected to be rather shifted to shorter wavelengths upon the addition of LPhe to TSPP solution containing γ-CD, because the peak shift caused by the formation of the TSPP–LPhe complex is less than that caused by the formation of the γ-CD–TSPP inclusion complex under our experimental conditions. The above deduction that only the TSPP–LPhe complex is formed is not in agreement with the observed peak shift. Therefore, a ternary inclusion complex among γ-CD, TSPP, and LPhe is most likely formed in TSPP solution containing γ-CD and LPhe. Taking into account the formation of the 1:1 γ-CD–TSPP inclusion complex and the 1:2 TSPP–LPhe complex, the γ-CD–TSPP–LPhe inclusion complex likely has a stoichiometry of 1:1:2 concerning γ-CD, TAPP, and LPhe.

$$\gamma\text{–CD} \cdot \text{TSPP} + 2\text{LPhe} \overset{K_3}{\rightleftharpoons} \gamma\text{–CD} \cdot \text{TSPP} \cdot (\text{LPhe})_2 \qquad (5)$$

where K_3 is the equilibrium constant for the formation of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex (γ-CD·TSPP·$(\text{LPhe})_2$).

Figure 5 exhibits fluorescence spectra, excited at 424 nm, of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 solution containing γ-CD (3.0×10^{-3} mol dm^{-3}) and several concentrations of LPhe. In Fig. 5, is also shown the fluorescence spectrum (dotted curve) of TSPP in pH 10.3 solution without γ-CD and LPhe. When γ-CD is added to TSPP solution without LPhe, the fluorescence peaks of TSPP are shifted to longer wavelengths, accompanied by an enhancement of the peak intensity. In addition, the fluorescence band is sharpened in the presence of γ-CD. These spectral changes

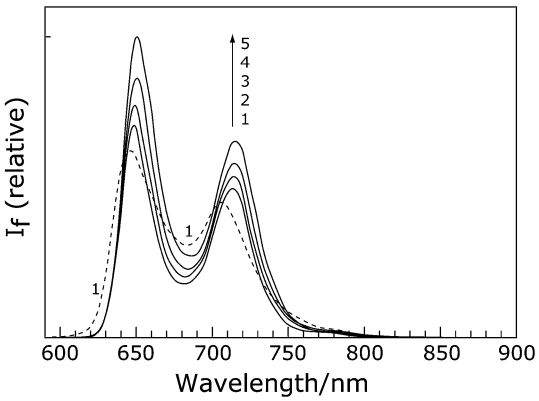

Fig. 5 Fluorescence spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing γ-CD and LPhe. *Spectrum 1* (*dotted curve*): [γ-CD] = 0 and [LPhe] = 0 mol dm^{-3}, *spectrum 2*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 0 mol dm^{-3}, *spectrum 3*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 1.5×10^{-2} mol dm^{-3}, *spectrum 4*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 3.0×10^{-2} mol dm^{-3}, and *spectrum 5*: [γ-CD] = 3.0×10^{-3} and [LPhe] = 6.0×10^{-2} mol dm^{-3}. λ_{ex} = 424 nm

indicate the formation of the γ-CD–TSPP inclusion complex. Further addition of LPhe to TSPP solution containing γ-CD results in a longer-wavelength shift of the fluorescence peaks and an increase in the fluorescence intensity. Upon the addition of γ-CD (3.0×10^{-3} mol dm^{-3}) to TSPP solution without LPhe, the longer-wavelength peak (second-band peak) at about 708 nm is shifted to longer wavelengths by about 7 nm. In Fig. 2, on the other hand, the second-band peak is shifted to longer wavelengths by about 4 nm upon the addition of LPhe (3.0×10^{-2} mol dm^{-3}). The wavelength shift in the presence of LPhe (3.0×10^{-2} mol dm^{-3}) is less than that in the presence of γ-CD (3.0×10^{-3} mol dm^{-3}). If, as complexes, only the complexes of γ-CD–TSPP and TSPP–LPhe are formed in TSPP solution containing γ-CD and LPhe (if the ternary inclusion complex among γ-CD, TSPP, and LPhe is not formed), the TSPP–LPhe complex is formed at the expense of the γ-CD–TSPP inclusion complex by the addition of LPhe to TSPP solution containing γ-CD. This would lead to a shit of the second-band peak to shorter wavelengths upon the addition of LPhe. However, this is not the case. Consequently, the fluorescence spectral change as well as the absorption spectral change suggests the formation of the ternary inclusion complex of γ-CD with TSPP and LPhe.

Evaluation of the equilibrium constants for the formation of the ternary inclusion complex of γ-CD with TSPP and LPhe

Under our experimental conditions, the absorbances of samples at an excited wavelength have been very small, so that the fluorescence intensity is proportional to the concentration of a fluorescent species. Consequently, the fluorescence intensity, I_f, for TSPP solution containing γ-CD and LPhe is represented as the sum of the fluorescence intensity from each fluorescent species:

$$I_f = c[\text{TSPP}] + d[\gamma\text{–CD} \cdot \text{TSPP}] + e\left[\text{TSPP} \cdot (\text{LPhe})_2\right] + f\left[\gamma\text{–CD} \cdot \text{TSPP} \cdot (\text{LPhe})_2\right] \qquad (6)$$

where c, d, e, and f are experimental constants that include the fluorescence quantum yields of free TSPP, the 1:1 γ-CD–TSPP inclusion complex, the 1:2 TSPP–LPhe complex, and the 1:1:2 γ-CD–TSPP–LPhe inclusion complex, respectively. Using the equilibrium constants, Eq. 6 is rewritten as

$$I_f = \left(c + dK_1[\gamma\text{-CD}] + eK_2[\text{LPhe}]^2 + fK_1K_3 \times [\gamma\text{-CD}][\text{LPhe}]^2\right)[\text{TSPP}]_0 \Big/ \left(1 + K_1[\gamma\text{-CD}] + K_2[\text{LPhe}]^2 + K_1K_3[\gamma\text{-CD}][\text{LPhe}]^2\right) \qquad (7)$$

Here, $[\text{TSPP}]_0$ is the initial concentration of TSPP. The ratios of d and e to c can be estimated from simulations of

the fluorescence intensity as a function of the concentrations of γ-CD and LPhe, respectively. For TSPP solution containing LPhe alone, the fluorescence intensity is given as the sum of the contribution from free TSPP and the 1:2 TSPP–LPhe complex:

$$I_f = c[\mathrm{TSPP}] + e\left[\mathrm{TSPP}\cdot(\mathrm{LPhe})_2\right] = \left(c + eK_2[\mathrm{LPhe}]^2\right)[\mathrm{TSPP}]_0 \Big/ \left(1 + K_2[\mathrm{LPhe}]^2\right) \quad (8)$$

Figure 6 shows a simulation of the fluorescence intensity of TSPP solution containing LPhe at an excitation wavelength of 424 nm, in which the already evaluated K_2 value (750 mol^{-2} dm^6) has been used. From the simulation, the ratio of e to c is estimated to be 1.98. The ratio, d/c, has been evaluated to be 2.29 from a similar simulation for TSPP solution containing γ-CD alone, in which the K_1 value has been fixed as 1200 mol^{-1} dm^3.

In the simulation, based on Eq. 7, for TSPP solution containing γ-CD and LPhe, the values of K_1, K_2, d/c, and e/c have already been evaluated. Consequently, c, f, and K_3 are variables. Figure 7 depicts the observed fluorescence-intensity data and a best fit simulation curve (solid curve) for the fluorescence intensity of TSPP solution containing γ-CD and LPhe as a function of the LPhe concentration. From the simulation, the values of K_3, c, and f are evaluated to be 714 mol^{-2} dm^6 (Table 1), 3.58×10^7 mol^{-1} dm^3, and 1.21×10^8 mol^{-1} dm^3, respectively.

If the ternary γ-CD–TSPP–LPhe inclusion complex is not formed (only the γ-CD–TSPP inclusion complex and the TSPP–LPhe complex are formed), the fluorescence intensity is represented by

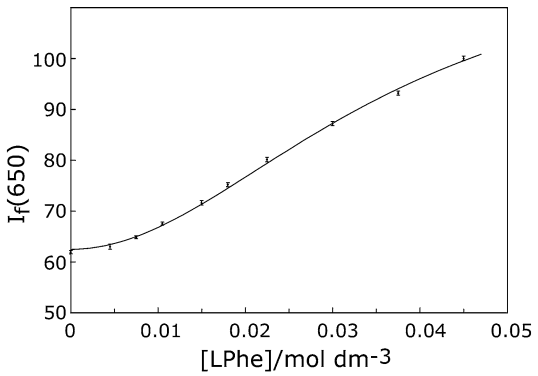

Fig. 6 Simulation for the observed fluorescence intensities of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing various concentrations of LPhe. The fluorescence intensity at an LPhe concentration of 4.5×10^{-2} mol dm^{-3} has been normalized to 100. A best fit simulation curve has been calculated on the basis of the scheme involving the formation of the 1:2 TSPP–LPhe complex. For the simulation, the evaluated K_2 value has been used, and values of c and e have been assumed to be 6.25×10^7 and 1.24×10^8 mol^{-1} dm^3, respectively. $\lambda_{ex} = 424$ nm, $\lambda_{obs} = 650$ nm

$$I_f = \left(c' + d'K_1[\gamma\text{-CD}] + e'K_2[\mathrm{LPhe}]^2\right)[\mathrm{TSPP}]_0 \Big/ \left(1 + K_1[\gamma\text{-CD}] + K_2[\mathrm{LPhe}]^2\right) \quad (9)$$

where c', d', and e' are experimental constants that include the fluorescence quantum yields of free TSPP, the 1:1 γ-CD–TSPP inclusion complex, and the 1:2 TSPP–LPhe complex, respectively. The values of d'/c' and e'/c' are equal to those of d/c and e/c, respectively. A best fit simulation curve (dotted curve) based on the scheme, in which no ternary inclusion complex is formed, is also shown in Fig. 7. This simulation curve does not fit the observed fluorescence intensity data, supporting the formation of the ternary inclusion complex. If the stoichiometry of the ternary γ-CD–TSPP–LPhe inclusion complex is not 1:1:2 but 1:1:1, the fluorescence intensity is given by

$$I_f = \left(c'' + d''K_1[\gamma\text{-CD}] + e''K_2[\mathrm{LPhe}]^2 + f''K_1K_3''[\gamma\text{-CD}][\mathrm{LPhe}]\right)[\mathrm{TSPP}]_0 \Big/ \left(1 + K_1[\gamma\text{-CD}] + K_2[\mathrm{LPhe}]^2 + K_1K_3''[\gamma\text{-CD}][\mathrm{LPhe}]\right) \quad (10)$$

where c'', d'', e'', and f'' are experimental constants including the fluorescence quantum yields of free TSPP,

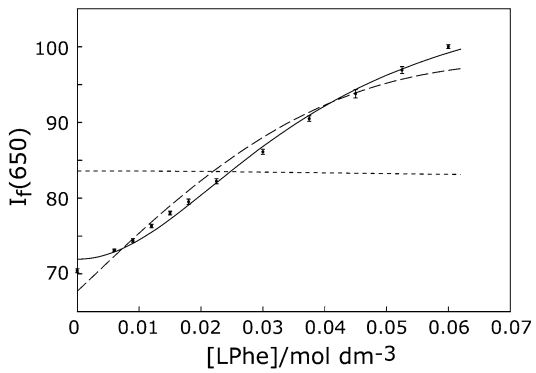

Fig. 7 Simulation for the observed fluorescence intensities of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing γ-CD (3.0×10^{-3} mol dm^{-3}) and LPhe. The fluorescence intensity has been normalized to 100 at the maximum intensity. A best fit simulation curve (*solid curve*) has been calculated on the basis of the scheme involving the formation of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex. The evaluated values of K_1, K_2, d/c, and e/c have been used, and values of K_3, c, and f have been assumed to be 714 mol^{-2} dm^6, 3.58×10^7 mol^{-1} dm^3, and 1.21×10^8 mol^{-1} dm^3, respectively. A best fit simulation curve (*dotted curve*), which has been based on the scheme not involving the formation of the ternary γ-CD–TSPP–LPhe inclusion complex, has been calculated with the evaluated values of K_1, K_2, d'/c' (= d/c) and e'/c' (= e/c), and an assumed c' value of 4.16×10^7 mol^{-1} dm^3. A best fit simulation curve (*dashed curve*) has been calculated on the basis of the scheme involving the formation of the 1:1:1 γ-CD–TSPP–LPhe inclusion complex. The evaluated values of K_1, K_2, d''/c'' (= d/c), and e''/c'' (= e/c) have been used, and values of K_3, c'', and f'' have been assumed to be 6.53×10^{-3}, 3.37×10^7, and 1.53×10^{11} mol^{-1} dm^3, respectively. $\lambda_{ex} = 424$ nm, $\lambda_{obs} = 650$ nm

the 1:1 γ-CD–TSPP inclusion complex, the 1:2 TSPP–LPhe complex, and the 1:1:1 γ-CD–TSPP–LPhe inclusion complex, respectively, and K_3'' is the equilibrium constant for the formation of the 1:1:1 γ-CD–TSPP–LPhe inclusion complex. As in the case of d'/c' and e'/c', the values of d''/c'' and e''/c'', which are equal to those of d/c and e/c, respectively, have already been known. A best fit simulation curve (dashed curve), which is based on the scheme for the formation of the 1:1:1 γ-CD–TSPP–LPhe inclusion complex, is exhibited in Fig. 7. The simulation curve does not reproduce the observed fluorescence intensity data. The bad fit of the two simulation curves to the observed data, which are based on the schemes of no formation of the ternary inclusion complex and the formation of the 1:1:1 γ-CD–TSPP–LPhe inclusion complex, respectively, provides evidence for the existence of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex.

The magnitude of the K_3 value, which represents the association between the γ-CD–TSPP complex and LPhe (Eq. 5), is similar to the magnitude of the K_2 value, which represents the association between free TSPP and LPhe (Eq. 4). This finding suggests that the binding ability of TSPP towards LPhe is not too different regardless of whether TSPP is bound to the γ-CD cavity or not. Because TSPP is a large molecule, γ-CD encapsulates only a part of a TSPP molecule; a single sulfonatophenyl group of TSPP is incorporated into the γ-CD cavity, so that the other part of a TSPP molecule is not hindered by γ-CD. Consequently, LPhe seems to be bound to the other portion of the TSPP molecule; in the γ-CD–TSPP–LPhe inclusion complex, LPhe does not enter the γ-CD cavity. Ribo et al. have suggested that the γ-CD–TSPP inclusion complex has the γ-CD primary face close to a porphyrin ring, although they have suggested the formation of a 2:1 γ-CD–TSPP inclusion complex [9]. In the 1:1:2 γ-CD–TSPP–LPhe inclusion complex, LPhe molecules are located outside of the γ-CD cavity, interacting with TSPP. A probable structure of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex, together with that of the 1:1 γ-CD–TSPP inclusion complex, is schematically drawn in Fig. 8.

In TSPP solution containing γ-CD and trimethyloctylammonium bromide (TMOA) or hexyltrimethylammonium bromide (HTMA), a ternary inclusion complex is formed among γ-CD, TSPP, and TMOA (or HTMA) [18]. In this case, an alkyl chain of TMOA or HTMA is likely bound to the γ-CD cavity together with TSPP. Because an alkyl chain is long but not too bulky in shape, the alkyl chain and TSPP may be simultaneously incorporated into the γ-CD cavity. When TSPP forms a complex with LPhe, a phenyl group of LPhe most likely interacts with TSPP. In this case, it is unlikely that γ-CD simultaneously includes a sulfonatophenyl group of TSPP and a phenyl group of LPhe, because they are too bulky for the γ-CD cavity to accommodate them together. In the 1:1:1 γ-CD–tetrakis(4-*N*-methylpyridyl)porphyrin–phthalate inclusion complex, it has been suggested that a phthalate anion is not incorporated into the γ-CD cavity.[19]

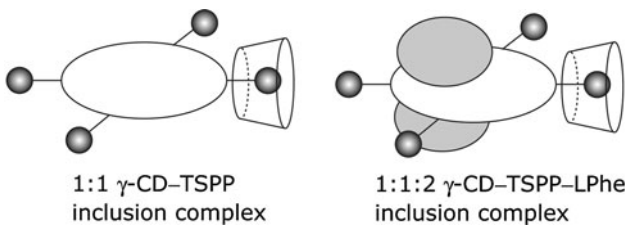

Fig. 8 Schematic drawings for probable structures of the 1:1 γ-CD–TSPP inclusion complex and the 1:1:2 γ-CD–TSPP–LPhe inclusion complex

The equilibrium constant, K_3, represents the formation of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex from the γ-CD–TSPP inclusion complex and LPhe. In TSPP solution containing γ-CD and LPhe, there is an additional equilibrium, in which the 1:1:2 γ-CD–TSPP–LPhe inclusion complex is formed from γ-CD and the 1:2 TSPP–LPhe complex.

$$\gamma\text{–CD} + \text{TSPP}\cdot(\text{LPhe})_2 \overset{K_4}{\rightleftharpoons} \gamma\text{–CD}\cdot\text{TSPP}\cdot(\text{LPhe})_2 \qquad (11)$$

Here, K_4 is the equilibrium constant for the formation of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex from γ-CD and the 1:2 TSPP–LPhe complex. From the definition of K_1, K_2, K_3, and K_4, a relationship holds among these equilibrium constants:

$$K_1K_3 = K_2K_4 \qquad (12)$$

From Eq. 12, the K_4 value for LPhe is evaluated to be 1140 mol^{-1} dm^3, which is very close to the K_1 value (1200 ± 30 mol^{-1} dm^3). This finding suggests that γ-CD encapsulates TSPP in a similar fashion irrespective of whether or not TSPP associates with LPhe. This implies that the LPhe molecules associating with TSPP are remote from a binding site between TSPP and γ-CD, and that the LPhe molecules do not obstruct the binding of γ-CD to TSPP. This is consistent with our conclusion that, within the γ-CD–TSPP–LPhe inclusion complex, γ-CD does not simultaneously encapsulate TSPP and LPhe.

Ternary inclusion complex of γ-CD with TSPP and DPhe

As previously noted, the K_2 value (800 ± 100 mol^{-2} dm^6) for DPhe is nearly the same as the K_2 value (750 ± 40 mol^{-2} dm^6) for LPhe. Absorption and fluorescence spectral changes for TSPP solution containing both γ-CD and DPhe were similar to those for TSPP solution containing both γ-CD and LPhe. Consequently, a ternary inclusion complex is most likely formed among γ-CD,

TSPP, and DPhe. A simulation similar to that for the formation of the 1:1:2 γ-CD–TSPP–LPhe inclusion complex was performed for the 1:1:2 γ-CD–TSPP–DPhe inclusion complex. From the simulation, a K_3 value of 844 mol^{-2} dm^6 was estimated for DPhe. This K_3 value is analogous to the K_3 value (714 mol^{-2} dm^6) for LPhe. In addition to the similarity between the K_2 and K_3 values and that between the K_1 and K_4 values for LPhe, the similarity between the K_3 values for DPhe and LPhe may suggest that γ-CD does not encapsulate phenylalanine in forming the ternary inclusion complex. From Eq. 12, the K_4 value of DPhe is evaluated to be 1270 mol^{-1} dm^3, which is similar to the K_4 value of LPhe. This finding also suggests that, in the ternary inclusion complex, LPhe or DPhe is not incorporated into the γ-CD cavity but associates with TSPP outside of the γ-CD cavity.

Ionic strength effects on the complexation of TSPP with γ-CD and LPhe

The ionic strength of solution may affect the complexation abilities of TSPP, LPhe, and γ-CD, because TSPP and LPhe are in an anionic form in pH 10.3 solution and because the inclusion complexation of CD is often affected by the ionic strength of solution [20–23]. Thus, we tried to estimate the values of K_2, K_3, and K_4 for LPhe and the K_1 value for TSPP at a fixed ionic strength of 0.2 mol dm^{-3} using NaCl. From the fluorescence intensity change of TSPP, the K_1 value at an ionic strength of 0.2 mol dm^{-3} has been evaluated to be 1700 ± 20 mol^{-1} dm^3, which is slightly greater than that without addition of NaCl. The slightly greater K_1 value at an ionic strength of 0.2 mol dm^{-3} is likely due to the salt effect. For solution of an ionic strength of 0.2 mol dm^{-3}, the K_2 value has been estimated to be 430 ± 70 mol^{-2} dm^6 (Table 1), which is less than that (750 ± 40 mol^{-2} dm^6) for solution without NaCl. At present, it is not clear why the K_2 value at an ionic strength of 0.2 mol dm^{-3} is less than that in the absence of NaCl. At an ionic strength of 0.2 mol dm^{-3}, the K_3 and K_4 values have been evaluated to be 1220 mol^{-2} dm^6 and 4820 mol^{-1} dm^3, respectively. The K_3 value at an ionic strength of 0.2 mol dm^{-3} is greater than that for solution in the absence of NaCl. In an ionic environment, the electrostatic interactions between organic ions having the same or different charges are expected to be weakened. Consequently, the large K_3 value at an ionic strength of 0.2 mol dm^{-3} is due to the high concentrations of ions in solution. The K_4 value for solution of the fixed ionic strength is greater than that for solution without NaCl. The K_4 value represents a measure of the inclusion of a TSPP molecule associating with LPhe by γ-CD. At an ionic strength of 0.2 mol dm^{-3}, therefore, the large K_4 value is due likely to the salt effect, which is also observed for the K_1 value.

Interactions between TSPP and tryptophan (Trp)

Tryptophan mainly exists as an anion in pH 10.3 buffer, because the pKa_2 value of tryptophan is 9.39. For LTrp and DTrp, absorption spectral changes similar to that shown in Fig. 2 for LPhe were observed, although an isosbestic point was observed at 418.5 nm. Figure 9 illustrates fluorescence spectra, excited at 406 nm, of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing various concentrations of LTrp. Upon the addition of LTrp, the fluorescence intensity is reduced, indicating the formation of a complex between TSPP and LTrp. Figure 10 depicts a plot of $1/(I_f - I_f^0)$ against 1/[LTrp] for TSPP solutions containing LTrp. This plot exhibits a good straight line, indicating that the TSPP–LTrp complex has a 1:1 stoichiometry.

$$\mathrm{TSPP} + \mathrm{LTrp} \overset{K_2'}{\rightleftharpoons} \mathrm{TSPP \cdot LTrp} \tag{13}$$

where K_2' is the equilibrium constant for the formation of the 1:1 TSPP–LTrp complex (TSPP·LTrp). From the plot in Fig. 10, the K_2' value is evaluated to be 34 ± 3 mol^{-1} dm^3 (Table 2), which is relatively small. The 1:1 stoichiometry of the TSPP–LTrp complex is in contrast to the 1:2 stoichiometry of the TSPP–LPhe (or –DPhe) complex. An aromatic moiety in an LTrp molecule is bulkier than that in an LPhe (or DPhe) molecule. The interactions between TSPP and LTrp may be weak due to the steric hindrance between TSPP and bulky LTrp. A second LTrp molecule cannot bind to TSPP, probably because the concentration of the 1:1 TSPP–LTrp complex is low due to the relatively small K_2' value. This seems to lead to the formation of only the 1:1 TSPP–LTrp complex.

When TSPP is bound to human serum albumin, the energy transfer from tryptophan residue(s) to TSPP occurs at pH 2, although the complexation between TSPP and tryptophan has not been observed [3]. However, the direct interactions between TSPP and amino-acid residues such as

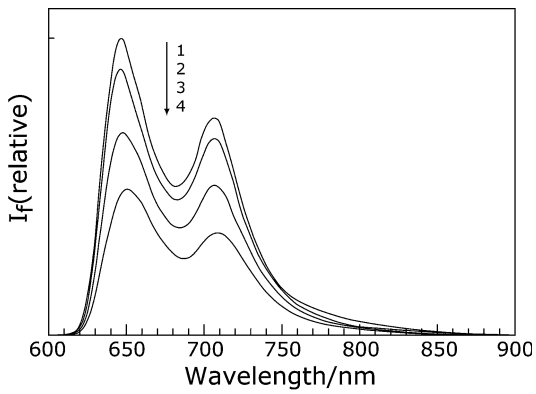

Fig. 9 Fluorescence spectra of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing various concentrations of LTrp. Concentration of LTrp: (*1*) 0, (*2*) 3.0×10^{-3}, (*3*) 9.0×10^{-3}, and (*4*) 1.8×10^{-2} mol dm^{-3}. $\lambda_{ex} = 406$ nm

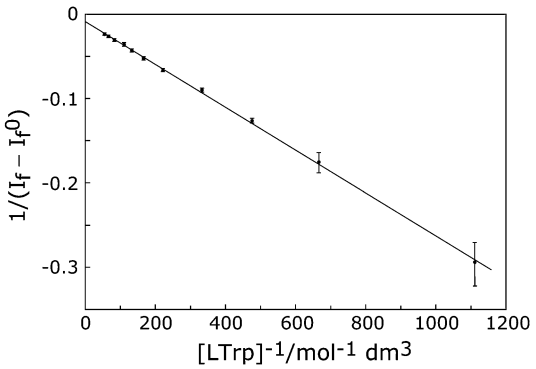

Fig. 10 Plot of $1/(I_f - I_f^0)$ against 1/[LTrp] for TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffer containing LTrp. $\lambda_{ex} = 406$ nm, $\lambda_{obs} = 650$ nm

Table 2 The values of K_2', K_3', and K_4' for LTrp, DTrp, 3-phenyl-1-propanol (PP) and L-phenylalaninol (PA)

	K_2' (mol^{-1} dm^3)	K_3' (mol^{-1} dm^3)	K_4' (mol^{-1} dm^3)
LTrp	34 ± 3	25.4	896
DTrp	29 ± 1	28.1	1160
PP	19 ± 2	37.3	2360
PA	53 ± 7	49.2	1110

phenylalanine and tryptophan may be possible in proteins, taking into account the complexation ability of TSPP toward phenylalanine (or tryptophan).

Ternary inclusion complex of γ-CD with TSPP and LTrp

Upon the addition of γ-CD to LTrp solution, little or no absorption spectral change of LTrp was observed. Consequently, the complexation would be neglected between γ-CD and LTrp. When LTrp was added to TSPP (1.0×10^{-6} mol dm^{-3}) solution containing γ-CD (3.0×10^{-3} mol dm^{-3}), the fluorescence intensity of TSPP was decreased at an excitation wavelength of 406 nm. This finding is due likely to the formation of a ternary inclusion complex of γ-CD, TSPP, and LTrp. Unlike the 1:1:2 γ-CD–TSPP–LPhe inclusion complex, the γ-CD–TSPP–LTrp inclusion complex likely has a 1:1:1 stoichiometry, because the TSPP–LTrp complex has a 1:1 stoichiometry.

$$\gamma\text{–CD} \cdot \text{TSPP} + \text{LTrp} \overset{K_3'}{\rightleftharpoons} \gamma\text{–CD} \cdot \text{TSPP} \cdot \text{LTrp} \qquad (14)$$

where K_3' is the equilibrium constant for the formation of the 1:1:1 γ-CD–TSPP–LTrp inclusion complex (γ-CD·TSPP·LTrp). For this scheme, the fluorescence intensity of TSPP solution containing γ-CD and LTrp is represented by

$$I_f = \left(g + hK_1[\gamma\text{-CD}] + iK_2'[\text{LTrp}] + jK_1K_3'[\gamma\text{-CD}][\text{LTrp}]\right) \times [\text{TSPP}]_0 / \left(1 + K_1[\gamma\text{-CD}] + K_2'[\text{LTrp}] + K_1K_3'[\gamma\text{-CD}][\text{LTrp}]\right) \qquad (15)$$

Here, g, h, i, and j are experimental constants including the fluorescence quantum yields of free TSPP, the 1:1 γ-CD–TSPP inclusion complex, the 1:1 TSPP–LTrp complex, and the 1:1:1 γ-CD–TSPP–LTrp inclusion complex, respectively. The K_1 and K_2' values have already been obtained. As in the case of the determination of the d/c and e/c values, simulations for the fluorescence intensity of TSPP solutions containing γ-CD and LTrp have been performed to evaluate the values of h/g (= 0.852) and i/g (= 0), respectively.

In the simulation based on Eq. 15, which includes the fluorescence intensity from the 1:1:1 γ-CD–TSPP–LTrp inclusion complex, K_3', g, and j are parameters. Figure 11 depicts the observed fluorescence intensity data for TSPP solution containing both γ-CD (3.0×10^{-3} mol dm^{-3}) and LTrp, and a best fit simulation curve (solid curve), which has been calculated assuming the values of K_3', g, and j to be 25.4, 1.14×10^8, and 3.06×10^5 mo^{-1} dm^3, respectively. The best fit simulation curve well reproduces the observed fluorescence intensities, providing evidence for the existence of the 1:1:1 γ-CD–TSPP–LTrp inclusion complex.

If the ternary inclusion complex is not formed, the fluorescence arises from free TSPP, the γ-CD–TSPP inclusion complex, and the TSPP–LTrp complex. Consequently, the fluorescence intensity is represented by

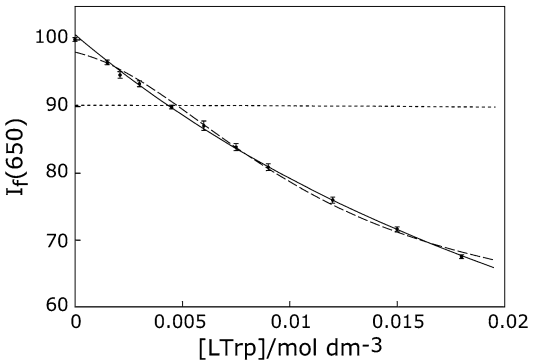

Fig. 11 Simulation for the observed fluorescence intensities of TSPP (1.0×10^{-6} mol dm^{-3}) in pH 10.3 buffers containing γ-CD (3.0×10^{-3} mol dm^{-3}) and LTrp. The fluorescence intensity has been normalized to 100 at the maximum intensity. A best fit simulation curve (*solid curve*) has been calculated on the basis of the scheme involving the formation of the 1:1:1 γ-CD–TSPP–LTrp inclusion complex. The evaluated values of K_1, K_2', h/g, and i/g have been used, and values of K_3', g, and j have been assumed to be 25.4, 1.14×10^8, and 3.06×10^5 mol^{-1} dm^3, respectively. A best fit simulation curve (*dotted curve*), which has been based on the scheme not involving the formation of the ternary γ-CD–TSPP–LTrp inclusion complex, has been calculated with the evaluated values of K_1, K_2', h'/g' (= h/g) and i'/g' (= i/g), and an assumed g' value of 1.03×10^8 mol^{-1} dm^3. A best fit simulation curve (*dashed curve*) has been calculated on the basis of the scheme involving the formation of the 1:1:2 γ-CD–TSPP–LTrp inclusion complex. The evaluated values of K_1, K_2', h''/g'' (= h/g), and i''/g'' (= i/g) have been used, and values of K_3'', g'', and j'' have been assumed to be 8.72×10^3 mol^{-2} dm^6, 1.11×10^8 mol^{-1} dm^3, and 5.88×10^7 mol^{-1} dm^3, respectively. $\lambda_{ex} = 406$ nm, $\lambda_{obs} = 650$ nm

$$I_f = \left(g' + h'K_1[\gamma\text{-CD}] + i'K_2'[\text{LTrp}]\right)[\text{TSPP}]_0 \Big/ \left(1 + K_1[\gamma\text{-CD}] + K_2'[\text{LTrp}]\right) \quad (16)$$

where g', h', and i' are experimental constants including the fluorescence quantum yields of free TSPP, the γ-CD–TSPP inclusion complex, and the 1:1 TSPP–LTrp complex, respectively. The K_1 and K_2' values have already been determined. The h'/g' and i'/g' values are equal to the h/g and i/g values, respectively. Figure 11 also shows a best fit simulation curve (dotted curve), which has been calculated on the basis of the scheme not involving the ternary inclusion complex. The best fit simulation curve does not fit the observed fluorescence intensities, supporting the formation of the ternary inclusion complex of γ-CD with TSPP and LTrp.

If the stoichiometry of the γ-CD–TSPP–LTrp inclusion complex is 1:1:2, which is identical to the stoichiometry of the γ-CD–TSPP–LPhe inclusion complex, the fluorescence intensity is represented as

$$I_f = \left(g'' + h''K_1[\gamma\text{-CD}] + i''K_2'[\text{LTrp}] + j''K_1K_3''[\gamma\text{-CD}][\text{LTrp}]^2\right)[\text{TSPP}]_0 \Big/ \left(1 + K_1[\gamma\text{-CD}] + K_2'[\text{LTrp}] + K_1K_3''[\gamma\text{-CD}][\text{LTrp}]^2\right) \quad (17)$$

Here, g'', h'', i'', and j'' are experimental constants including the fluorescence quantum yields of free TSPP, the 1:1 γ-CD–TSPP inclusion complex, the 1:1 TSPP–LTrp complex, and the 1:1:2 γ-CD–TSPP–LTrp inclusion complex (γ-CD·TSPP·$(\text{LTrp})_2$), respectively, and K_3'' is the equilibrium constant for the formation of the 1:1:2 γ-CD–TSPP–LTrp inclusion complex.

$$\gamma\text{-CD} \cdot \text{TSPP} + 2\text{LTrp} \overset{K_3''}{\rightleftharpoons} \gamma\text{-CD} \cdot \text{TSPP} \cdot (\text{LTrp})_2 \quad (18)$$

A best fit simulation curve (dashed curve), for which a K_3'' value of 8720 mol^{-2} dm^3 is assumed, is also shown in Fig. 11. The best fit simulation curve, which is based on the scheme involving the 1:1:2 γ-CD–TSPP–LTrp inclusion complex, cannot reproduce the observed fluorescence intensities, indicating that the 1:1:1 γ-CD–TSPP–LTrp inclusion complex is formed. In addition to the process shown in Eq. 14, the 1:1:1 γ-CD–TSPP–LTrp inclusion complex can be produced from γ-CD and the 1:1 TSPP–LTrp complex.

$$\gamma\text{-CD} + \text{TSPP} \cdot \text{LTrp} \overset{K_4'}{\rightleftharpoons} \gamma\text{-CD} \cdot \text{TSPP} \cdot \text{LTrp} \quad (19)$$

where K_4' is the equilibrium constant for the formation of the 1:1:1 γ-CD–TSPP–LTrp inclusion complex from γ-CD and the 1:1 TSPP–LTrp complex. A relationship among K_1, K_2', K_3', and K_4' is derived, using the definition of these equilibrium constants.

$$K_1K_3' = K_2'K_4' \quad (20)$$

For LTrp, a K_4' value of 896 mol^{-1} dm^3 is obtained from Eq. 20 (Table 2). The absorption and fluorescence spectral changes for DTrp were similar to those for LTrp, respectively. For DTrp, the K_2', K_3', and K_4' values have been evaluated to be 29 ± 1, 28.1, and 1160 mol^{-1} dm^3, respectively, which are respectively similar to those for LTrp, suggesting that the optical isomers of tryptophan as well as phenylalanine cannot be discriminated through the formation of the complexes with γ-CD and/or TSPP.

Interactions of TSPP with γ-CD, 3-phenyl-1-propanol (PP), and L-phenylalaninol (PA)

We further examined the complexation of TSPP with γ-CD and PP (or PA), which is analogous to phenylalanine in molecular structure but neutral in charge. Absorption and fluorescence spectral changes of TSPP solution in the presence of PP (or PA) were similar to those in the presence of LPhe, respectively. This finding suggests that an aromatic moiety, a phenyl group, plays a critical role in the complexation with TSPP. The Benesi-Hildebrand plot for the fluorescence intensity of TSPP solution containing PP or PA exhibited a straight line, indicating that the TSPP–PP and TSPP–PA complexes have a 1:1 stoichiometry. From the plots, the K_2' values for PP and PA were evaluated to be 19 ± 2 and 53 ± 7 mol^{-1} dm^3, respectively (Table 2), which are similar to those of LTrp and DTrp. Although there is no electrostatic repulsion between TSPP and PP (or PA), the K_2' values for PP and PA are relatively small. This may suggest that the hydrophobic and π–π interactions work in the formation of the TSPP–phenylalanine (or tryptophan) complexes as well as the TSPP–PP (or –PA) complex. From simulations similar to that for LTrp, the K_3' values for PP and PA were estimated to be 37.3 and 49.2 mol^{-1} dm^3, respectively. For PP and PA, K_4' values of 2360 and 1110 mol^{-1} dm^3 were respectively obtained from Eq. 20. The K_4' value for PA is nearly the same as that for DTrp, although the K_4' value for PP is about twice of that for DTrp. Further study is necessary to clarify the difference in the stoichiometries of the TSPP–LPhe, TSPP–PP, and TSPP–PA complexes etc.

Conclusions

In pH 10.3 buffer, TSPP forms complexes with phenylalanine and tryptophan, which are mainly in an anionic form. For phenylalanine, the 1:2 TSPP–phenylalanine complex is formed, while the 1:1 complex is formed for tryptophan. Because the equilibrium constants for the optical isomers of phenylalanine and tryptophan are nearly the same, respectively, the optical isomers of phenylalanine and

tryptophan are not discriminated through the complexation with TSPP.

γ-CD forms a 1:1 inclusion complex with TSPP. In TSPP solution containing γ-CD and LPhe (or DPhe), the 1:1:2 γ-CD–TSPP–LPhe (or –Dphe) inclusion complex is formed. From simulations of the observed fluorescence intensity of TSPP, the equilibrium constants, K_3, for the formation of the ternary inclusion complexes have been evaluated for LPhe and DPhe. The K_3 values for LPhe and DPhe are nearly the same, suggesting that phenylalanine is not bound to the γ-CD cavity within the ternary inclusion complex. This conclusion is confirmed by the results that the K_1 and K_2 values are similar to the K_4 and K_3 values, respectively. At an ionic strength of 0.2 mol dm^{-3} using NaCl, the equilibrium constants of LPhe were evaluated. The K_1 and K_4 values at an ionic strength of 0.2 mol dm^{-3} are greater than those without NaCl, respectively.

For LTrp and DTrp, the 1:1:1 γ-CD–TSPP–LTrp and –DTrp inclusion complexes are formed, respectively. The equilibrium constant for the formation of the ternary inclusion complex of LTrp is similar to that of DTrp. As in the case of phenylalanine, the optical isomers of tryptophan are not discriminated through the complexation. Although PP and PA are analogous to phenylalanine in molecular structure, the 1:1 complexes with TSPP and the 1:1:1 inclusion complexes with γ-CD and TSPP are formed.

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