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ORIGINAL PAPER

Supramolecular Interaction of Two Tryptophans with *p*-sulfonated calix[*4,6,8*]arene

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Abstract The complex characteristics of *p*-sulfonated calix[*n*]arene (SC*n*A) and two tryptophans *N*-[(*tert*-butoxy) carbonyl]-tryptophan (trp-A) and *N*-carbobenzoxy-tryptophane (trp-B) were examined through various techniques. Spectrofluorimetry was performed at different temperatures to determine the stability constants and evaluate the thermodynamic parameters of the two complexes. The effect of pH on complex formation was estimated. According to the fluorescence data, the assumption about the steric hindrance of the *tert*-butyl group of trp-A and the phenyl group of trp-B was put forward. ^{1}H NMR was also performed to determine the binding interaction mechanism. Results showed that the indole benzene rings of the two tryptophans partly penetrated into the cavity of *p*-sulfonated calix[*n*]arene. The shift in Ha, Hb and Hc, Hd positions became more significant as the number of phenolic units of the calixarene ring increased. Molecular modeling of the complexes elucidated the assumption about the steric hindrance of the *tert*-butyl group of trp-A and the phenyl group of trp-B. These observations of molecular modeling computation are consistent with previous fluorescence data and ^{1}H NMR results.

Keywords Spectrofluorimetry · *p*-sulfonated calix[*n*]arene · *N*-[(*tert*-butoxy) carbonyl-tryptophan · *N*-carbobenzoxy-tryptophane · Molecular modeling computation

Abbreviations

SC*n*A	*p*-sulfonated calix[*n*]arene
trp-A	*N*-[(*tert*-butoxy)carbonyl]-tryptophan
trp-B	*N*-carbobenzoxy-tryptophane

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Introduction

Calixarenes, as the third generation of host molecules [1], have attracted considerable attention in host-guest chemistry because of their excellent recognition ability [2, 3]. Calixarenes with specific functions have been studied extensively as a platform for constructing novel host compounds. These host molecules have a cavity-shaped structure that can hold a guest molecule and create specific affinity to a target molecule by introducing various functional groups [4]. Studies have investigated *p*-sulfonated calix[*n*]arenas, which have flexible and often poorly defined cavities that bind positively charged species. *p*-sulfonated calix[*n*]arenas are regarded as promising water-soluble hosts [5] for quaternary ammonium ions [6, 7], trimethylammonium cations [8–10], dyes [11, 12], native amino acids [13, 14] and small neutral organic molecules [15]. Recently, the possible biological and pharmaceutical applications of *p*-sulfonated calix[*n*] arenas have stimulated particular interest [14, 16, 17].

Tryptophan is an essential amino acid that affects the growth, development, and metabolism of humans and animals. Thus tryptophan is considered as the second most essential amino acid. Pigment, alkaloids, coenzyme, plant hormones, and other physiologically active substances are synthesized from tryptophan. Tryptophan, which is widely used in medicine, food and food additives can prevent and treat pellagra, eliminate mental tension, and improve sleep. For example, the D-amino-acids in human brain are used as an indicator of Alzheimer's disease [18]. Thus, chemists continuously examine the host-guest binding of amino acids and water-soluble calixarenes in many areas. For instance, water-soluble calix[*4*]arene can change their conformation to fit the size of the aromatic tryptophan, and allow its benzene ring to penetrated the hydrophobic cavity of calix[*4*]arene [19].

^{1}H NMR spectroscopy and microcalorimetry showed that the interaction between an amino-acid and *p*-sulphonated calix[*4*]arene forms 1:1 complexes in water [20, 21]. The complexation of *p*-sulphonated calix[*4*]arene with some amino

acids by reverse-phase high-performance liquid chromatography and ^{1}H NMR experiments has been widely studied [22]. Various interactions, such as hydrophobic, ion-pairing, aromatic -aromatic, and electrostatic interaction may occur between tryptophan and SC*n*A.

Given its instrumental simplicity, high selectivity and good sensitivity, fluorimetry is a useful and suitable method for studying the inclusion process of two tryptophans and SC*n*A (Fig. 1). In this study, we investigated the complexes of *N*-[(*tert*-butoxy)carbonyl]-tryptophan (trp-A) and *N*-carbobenzoxy- tryptophane (trp-B) with *p*-sulphonated calix[*4,6,8*] arenas (see Fig. 1) in aqueous solutions by spectrofluorimetry. The effect of pH was determined. Temperature-dependent inclusion constants were also obtained to calculate ΔH and ΔS. ^{1}H NMR and molecular modeling analyses were performed to investigate the possible mechanism of the binding reaction.

Materials and Methods

Instrumentation

Fluorescence spectra and intensity measurements were obtained with a Agilent Technologies Cary Eclipse. Fluorescence spectrofluorometer equipped with a pulsed lamp. The slit width of both the excitation and emission monochromators was set at 5 nm. The fluorescence spectra were recorded at a scan rate of 600 nm min^{-1}. All measurements were performed in a standard 10 mm path-length quartz cell set to a temperature of 25.0±0.5 °C. All pH values were measured with a pHS-3TC digital precision pH meter (Shanghai, China). ^{1}H NMR spectra were recorded using a Bruker DRX-600 MHz spectrometer (Switzerland) in D_2O. Molecular modeling calculations were optimized at the B3LYP/6-31G(d) level of density functional theory with the Gaussian 03 program.

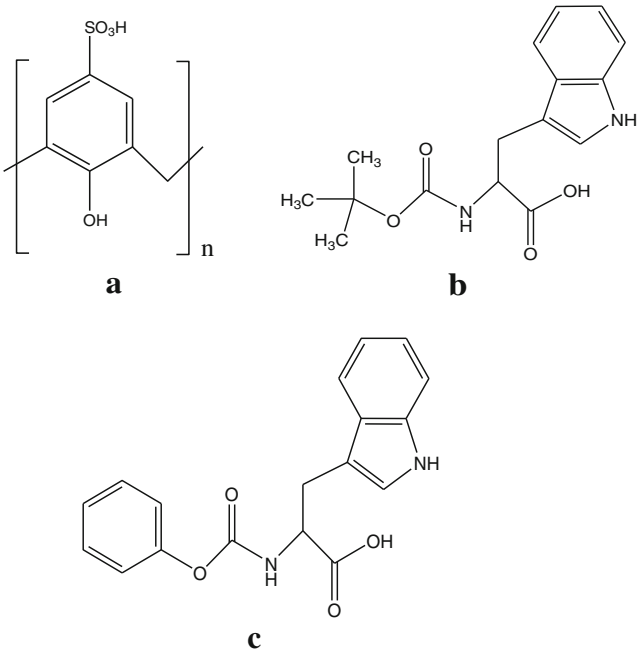

Fig. 1 The structure of ring closure of *p*-sulfonated calix[*n*]arenas (*n= 4, 6, 8*) (**a**) and *N*-[(*tert*-butoxy) carbonyl] -tryptophan (**b**) and *N*-carbobenzoxy-tryptophane (**c**)

Chemicals and Reagents

All chemicals were of analytical reagent grade, and double-distilled water was used throughout the procedures. The *N*-[(*tert*-butoxy)carbonyl]-tryptophan (trp-A, content > 98.0 %) and *N*-carbobenzoxy- tryptophane (trp-B, content > 98.0 %) used in the experiment were obtained from Great Brtian and Tokyo Chemical industry Co., Ltd, Tokyo, Japan, respectively. The stock solution of 1.0×10^{-4} M was prepared by directly dissolving in double-distilled water. SC*4*A was prepared according to literature [23] and identified by IR, ^{1}H NMR and element analysis. SC*6*A and SC*8*A were respectively purchased from Great Britain and Shanghai Chemical Industry Co, Ltd. SC*n*A(*n= 4, 6, 8*) stock solution of 1.0×10^{-4} M was prepared respectively in a 100 ml volumetric flask. Working solutions were obtained by dilution of the stock solution. All the stock standard solutions were stable for several weeks at room temperature. A Britton-Robinson buffer solution was prepared using 0.04 M boric acid, acetic acid and phosphoric acid, and then was adjusted to accurate values by using 0.2 M sodium hydroxide.

Experimental

A 1 ml aliquot of the stock solution of trp-A was transferred into a 10 ml volumetric flask and an appropriate amount of 1.0×10^{-4} M SC*n*A was respectively added, followed by 1.0 ml of Britton- Robinson buffer solution (pH 7.4). The mixed solution was diluted to final volume with double-distilled water, stirred thoroughly, and equilibrated at room temperature for 15 min. The fluorescence intensity values of the experimental and blank solution were measured at 365 nm using an excitation wavelength of 280 nm. The same experiment was conducted for trp-B as above.

Results and Discussion

Formation of Inclusion Complexes of the Two Tryptophans and SC*n*A

The fluorescence spectrum of the two tryptophans changed with addition of different amount of SC*n*A at pH 7.4 (Figs. 2 and 3). The maximum fluorescence excitation and emission wavelengths of the tryptophan were approximately at 280 and 365 nm, respectively. However, Fig. 3 showed that a new small

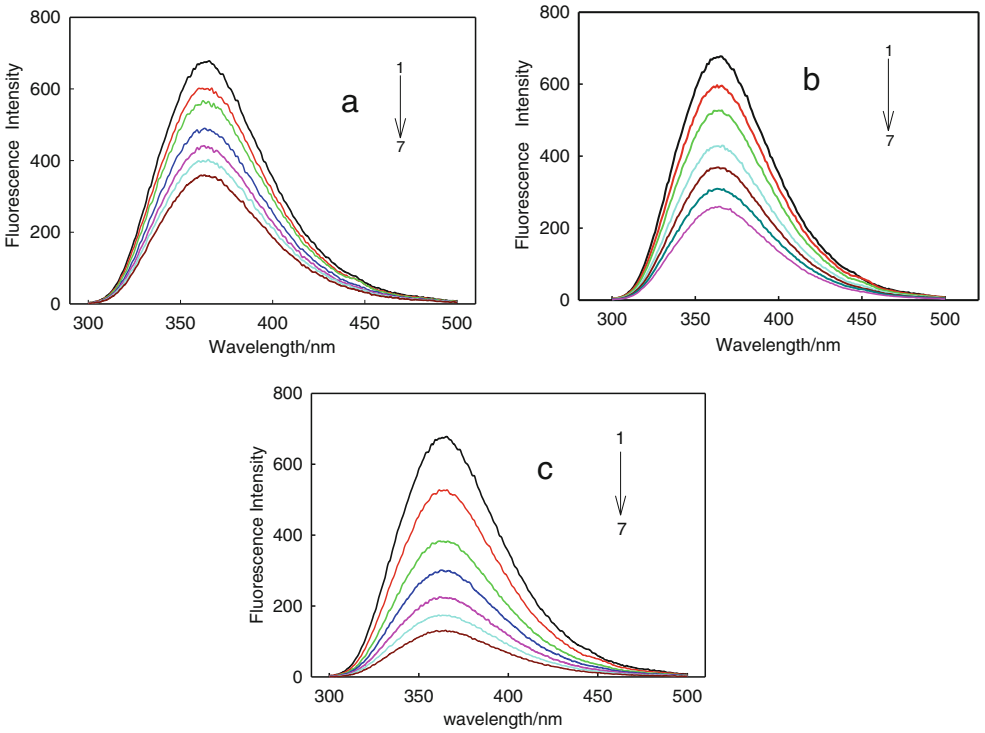

Fig. 2 The fluorescence spectra of *N*-[(*tert*-butoxy) carbonyl]-tryptophan in different concentrations of SC*4*A (**a**), SC*6*A (**b**), SC*8*A (**c**) in Britton-Robinson buffer solutions at 298 K and pH=7.4. The concentrations of SC*n*A ($\times 10^{-4}$ M): (1) 0; (2) 0.3; (3) 0.6; (4) 1.0; (5) 1.3; (6) 1.6; (7) 2.0; $C_{trp-A}=1.0\times10^{-4}$ M

benzene emission wavelength of trp-B was approximately at 455 nm. As different amounts of SC*n*A were added, the two tryptophane fluorescence emission wavelengths intensity significantly decreased. The maximum emission wavelength generated a small blue shift from 365 nm to 360 nm, 455 nm to 450 nm. Significant fluorescence quenching and the blue shift indicated that the complexes were formed.

The formation of inclusion complexes between trp-A and SC*n*A was confirmed by fluorescence spectra when the initial trp-A concentration was 1.0×10^{-4} M. Figures 2 and 3 showed

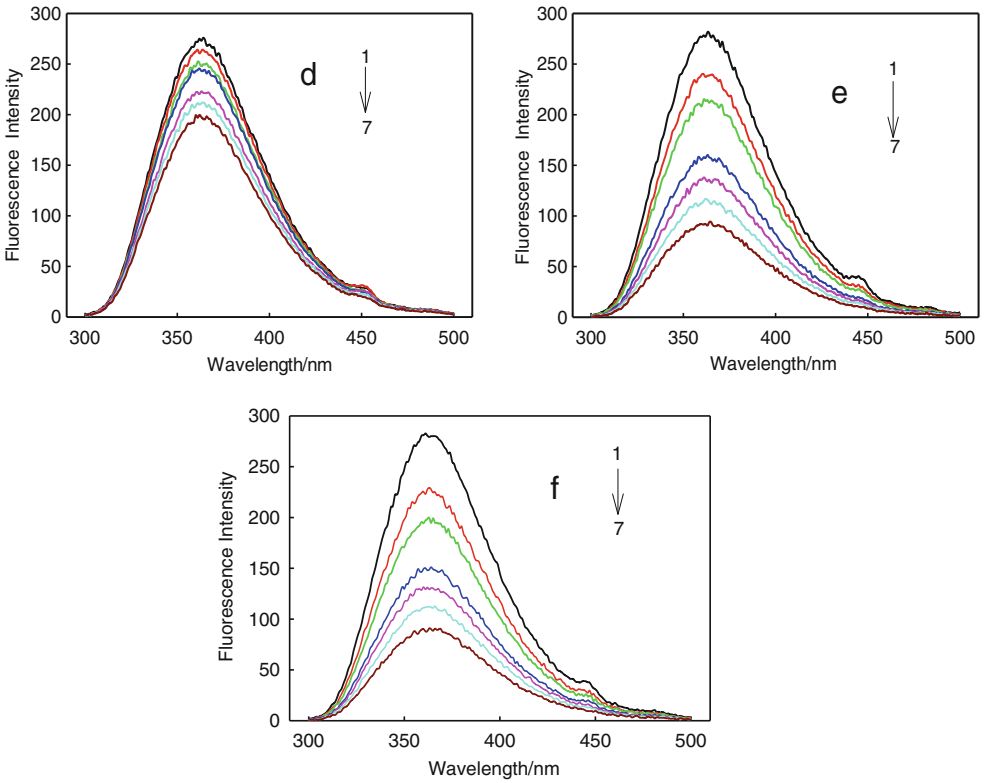

Fig. 3 The fluorescence spectra of *N*-carbobenzoxy-tryptophane in different concentrations of SC*4*A (**d**), SC*6*A (**e**), SC*8*A (**f**) in Britton-Robinson buffer solutions at 298 K and pH=7.4. The concentrations of SC*n*A ($\times 10^{-4}$ M): (1) 0; (2) 0.3; (3) 0.6; (4) 1.0; (5) 1.3; (6) 1.6; (7) 2.0; $C_{trp-B}=1.0\times10^{-4}$ M

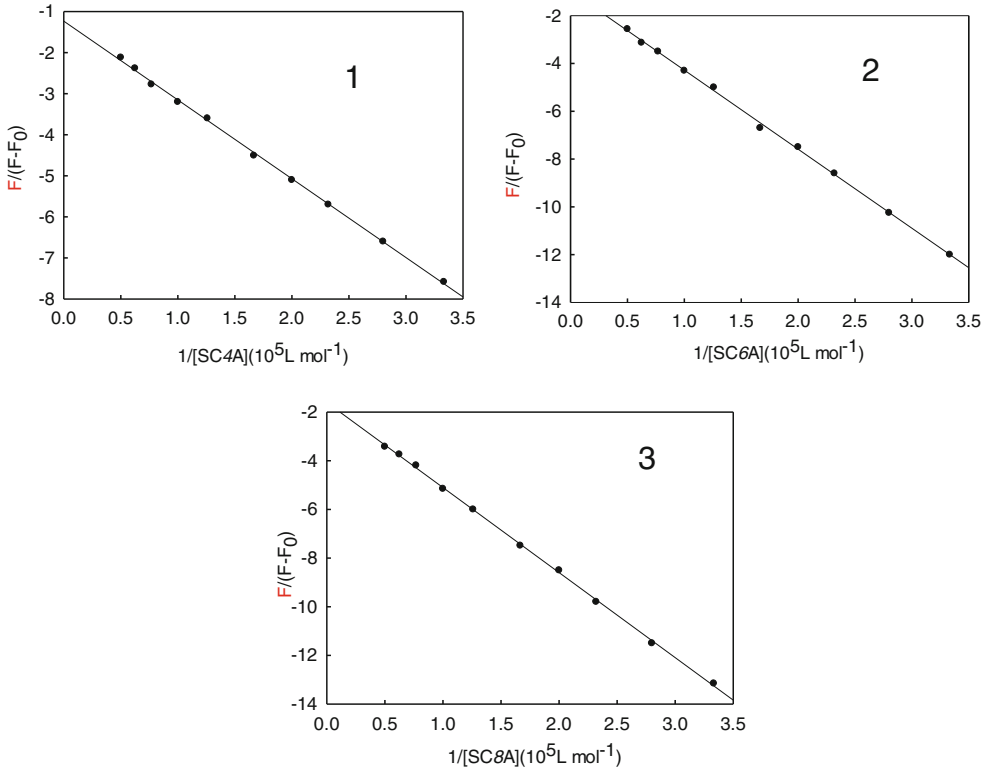

Fig. 4 The relationship of $(F-F_0)^{-1}$with SC*n*A^{-1}, *T*=298 K, C(trp-A)=10^{-4} M, pH=7.4, (1) SC*4*A, (2) SC*6*A, (3) SC*8*A

the fluorescence spectra of the two tryptophans in the absence and presence of SC*4*A, SC*6*A, and SC*8*A. The fluorescence intensity of the two tryptophans significantly decreased upon the addition of SC*n*A. Given the formation of inclusion complexes between SC*n*A and the two tryptophans, the concentration of naked tryptophans in aqueous solution decreased as SC*n*A was added, thereby decreasing of the fluorescence intensity.

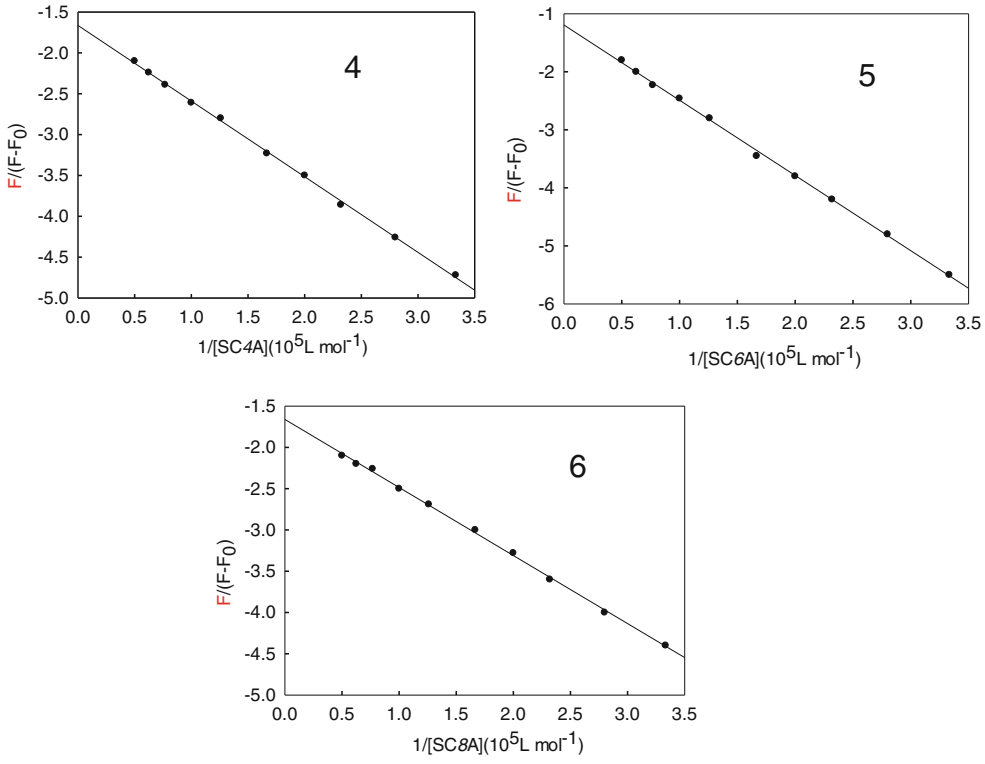

Fig. 5 The relationship of $(F-F_0)^{-1}$with SC*n*A^{-1}, *T*=298 K, C(trp-B)=10^{-4} M, pH=7.4, (4) SC*4*A, (5) SC*6*A, (6) SC*8*A

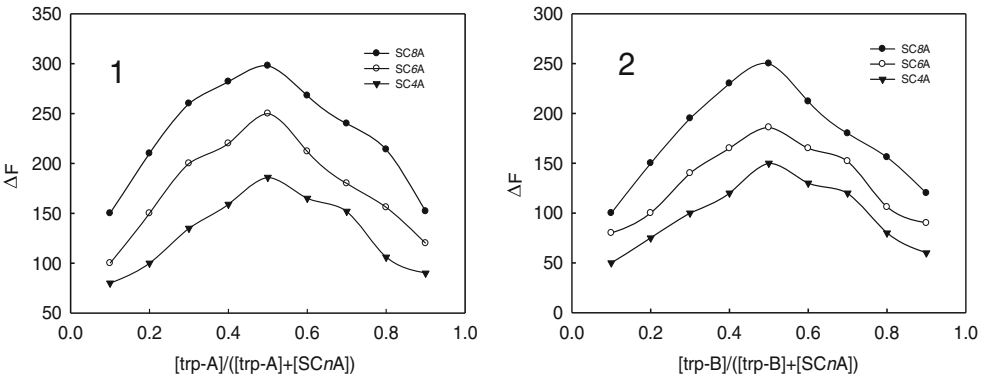

Fig. 6 Job's plot for the complex of trp-A (1), trp-B (2) with SC*n*A in Britton-Robinson buffer solution (pH 7.4) at 25 °C, respectively. (1) [trp-A] + [SC*n*A])=1.0×10^{-5} M; (2) [trp-B] + [SC*n*A])=1.0×10^{-5} M

However, the fluorescence intensity of the trp-A-SC*n*A complex was higher than that of the trp-B-SC*n*A complex at the same concentration and pH (see Figs. 2 and 3). The fluorescence quenching of trp-A was more significant than that of trp-B at the same SC*n*A concentration possibly because of the steric hindrance of the functional group. In general, the steric hindrance of the *tert*-butyl group of trp-A is greater than that of the phenyl group of trp-B. Thus, the fluorescence quenching of trp-A should be less than that of trp-B but the fluorescence quenching was high probably because the *tert*-butyl group and the indole benzene ring of the trp-A were on opposite sides after the indole benzene ring of trp-A penetrated the SC*n*A cavity. By contrast, the steric hindrance of the *tert*- butyl group of trp-A was less than that of the phenyl group of trp-B when the phenyl group and the indole benzene ring of trp-B were located on the same side. Thus, the part of the indole benzene ring of trp-A, which penetrated the SC*n*A cavity, and the degree of fluorescence quenching of trp-A was relatively larger than those of trp-B. However, further studies are needed to verify this observation.

Stoichiometry and Association Constant of the Inclusion Complexes

Assuming that SC*n*A and the two tryptophans forms a 1:1 ratio complex, the following expression can be written as

$$H + G \rightleftharpoons H{-}G \tag{1}$$

The formation constant of the complex (K) is given by

$$K = \frac{C_{H-G}}{C_H \times C_G} \tag{2}$$

An equation of inclusion constant K of the complex with guest-host was used to calculate the inclusion constant [24]:

$$\frac{1}{F-F_0} = \frac{1}{(F_\infty - F_0)KC_{SCnA}} + \frac{1}{F_\infty - F_0} \tag{3}$$

In which, C_G is the original concentration of the tryptophan, C_H is the original concentration of the SC*n*A, F_0 is the fluorescence intensity of the tryptophan in the absence of SC*n*A, F_∞ is the fluorescence intensity when all of the tryptophan molecules are essentially complexed with SC*n*A, and F is the observed fluorescence intensity at each SC*n*A concentration tested. K is the association constant of the complex.

The excellent linear relationship was obtained when $F/(F-F_0)$ was plotted against $1/C_{SCnA}$ (Figs. 4 and 5), which indicated the formation of a 1:1 complex. The binding constants of the trp-A and SC*n*A complexes at pH 7.4 were determined to be 6.84×10^4, 7.5×10^4, and 8.72×10^4 in presence of SC*4*A, SC*6*A and SC*8*A. The binding constants of the trp-B and SC*n*A complexes at pH 7.4 were 3.56×10^4, 4.89×10^4, and 6.28×10^4 in presence of SC*4*A, SC*6*A and SC*8*A, respectively. These values were determined by dividing the intercept by the slope of the corresponding lines. The complex stability constant

Table 1 Complex stability constants (K) and thermodynamic parameters for 1:1 intermolecular complex of trp-A with SC*n*A in a phosphate buffer solution (pH 7.4)

Host	T(K)	K(10^4 L mol^{-1})	H(kJ mol^{-1})	G(kJ mol^{-1})	S(J/(mol K)$^{-1}$)
SC*4*A	278	16.27	−29.9	−27.74	−7.77
	288	10.41		−27.66	
	298	6.84		−27.58	
	308	4.62		−27.51	
	318	3.20		−27.43	
SC*6*A	278	20.48	−34.56	−28.26	−22.65
	288	12.17		−28.04	
	298	7.50		−27.81	
	308	4.77		−27.58	
	318	3.12		−27.36	
SC*8*A	278	29.83	−42.38	−29.14	−47.64
	288	15.78		−28.66	
	298	8.72		−28.18	
	308	5.00		−27.71	
	318	2.97		−27.23	

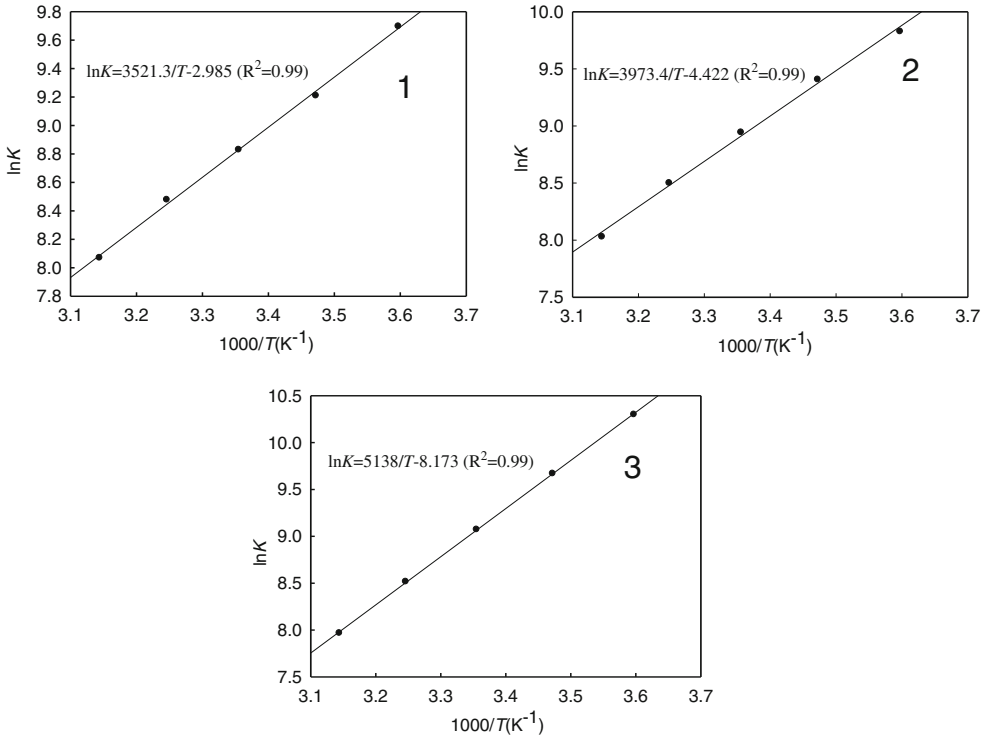

Fig. 7 Van't Hoff plot, pH 7.4, C(trp-A)=10^{-4} M,(1) SC*4*A, (2)SC*6*A, (3) SC*8*A

monotonically increased with the number of phenolic units in the calixarene ring.

Generally, the Job's plot was used to calculate the association ratio. A typical Job's plots for the inclusion complexes of SC*n*A with trp-A and trp-B were shown in Fig. 6. The maximum of the relative fluorescence intensity was at a mol fraction of 0.5, which confirmed that the 1:1 ratio complex assembly formed [25].

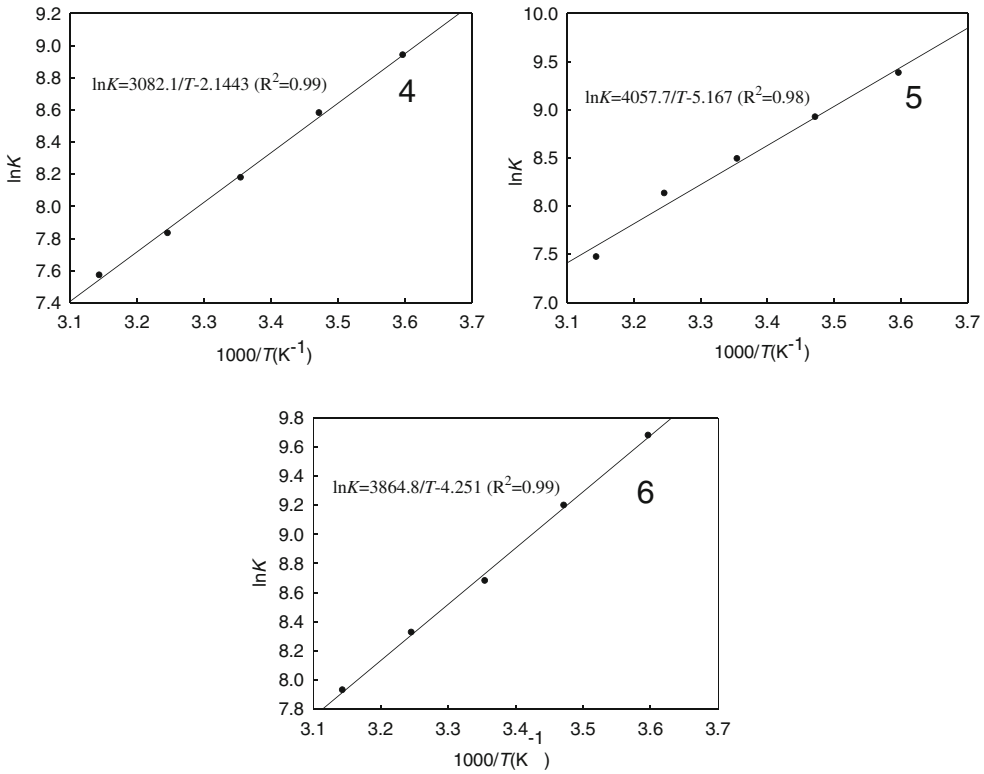

Fig. 8 Van't Hoff plot, pH 7.4, C(trp-B)=10^{-4} M,(4) SC*4*A, (5)SC*6*A, (6) SC*8*A

Table 2 Complex stability constants (K) and thermodynamic parameters for 1:1 intermolecular complex of trp-B with SC*n*A in a phosphate buffer solution (pH 7.4)

Host	T(K)	K(10^4 L mol^{-1})	H(kJ mol^{-1})	G(kJ mol^{-1})	S(J/(mol K)$^{-1}$)
SC*4*A	278	7.63	−26.34	−25.99	−1.26
	288	5.15		−25.98	
	298	3.56		−25.97	
	308	2.52		−25.95	
	318	1.82		−25.94	
SC*6*A	278	11.84	−30.48	−27.00	−12.52
	288	7.49		−26.87	
	298	4.89		−26.75	
	308	3.27		−26.62	
	318	2.25		−26.50	
SC*8*A	278	15.95	−32.13	−27.69	−15.97
	288	9.87		−27.53	
	298	6.28		−27.37	
	308	4.13		−27.21	
	318	2.78		−27.05	

Thermodynamic Parameters of Inclusion Complexes

To explain the thermodynamic origins of the formation of inclusion complex, the stability constants at various temperatures ranging from 278 K to 318 K were obtained by fluorescence spectra method as shown in Table 1. The relationship of stability constant (K) with temperature T can be described by Van't Hoff equation [26] shown as follows:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{4}$$

where R was the gas constant, The enthalpy change (ΔH) and the entropy change (ΔS) associated with complex formation. Supposing ΔH and ΔS were constant in the range of experiment temperature.

Table 3 The change of the stability constants (K) for 1:1 inclusion complexes of trp-A with SC*4*A, SC*6*A, and SC*8*A in different pH at T=298 K

pH	K/10^4 (L mol^{-1})				
	4.0	6.0	7.0	9.0	11.0
SC*4*A	3.64	4.62	6.84	3.6	1.2
SC*6*A	4.21	5	7.5	4.32	2.43
SC*8*A	5.08	6.78	8.72	6.65	4.15

Table 4 The change of the stability constants (K) for 1:1 inclusion complexes of trp-B with SC*4*A, SC*6*A, and SC*8*A in different pH at T=298 K

pH	K/10^4 (L mol^{-1})				
	4.0	6.0	7.0	9.0	11.0
SC*4*A	0.36	1.22	3.56	1.42	0.86
SC*6*A	1.35	2.25	4.89	2.78	1.05
SC*8*A	2.56	3.46	6.28	3.47	1.29

A plot of ln K versus 1/T was linear within experimental error, which was shown in Figs. 7 and 8. The value of the

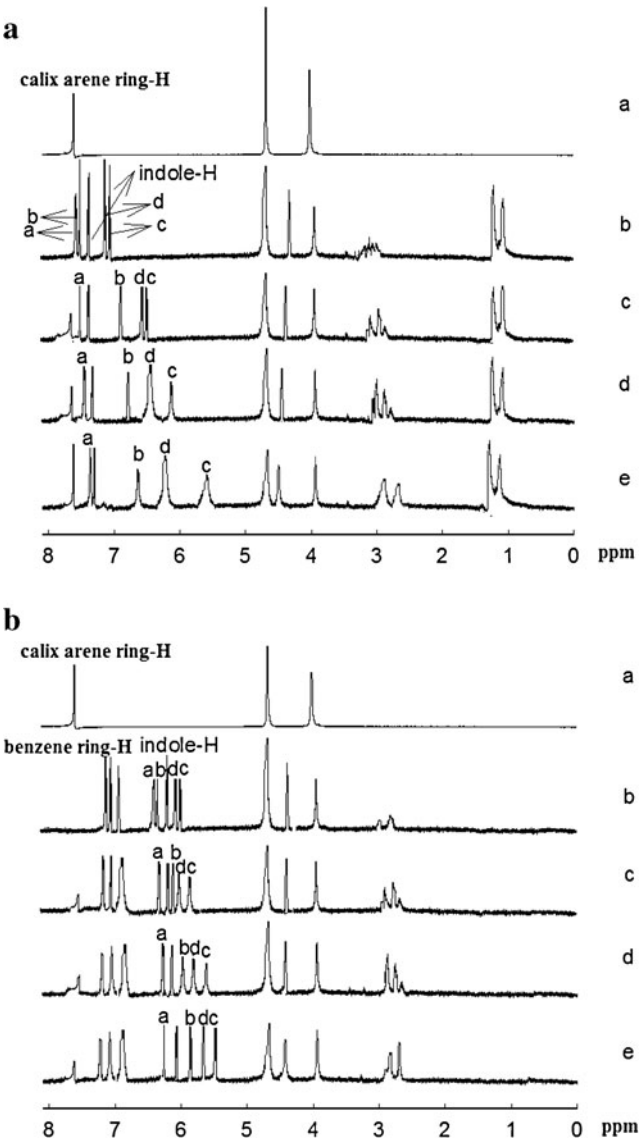

Fig. 9 **a** ^{1}H NMR spectra (600 MHz) of SC*n*A (a), trp-A (b), SC*4*A-trp-A complex (c), SC*6*A-trp-A complex (d), and SC*8*A-trp-A complex (e) in D_2O **b** ^{1}H NMR spectra (600 MHz) of SC*n*A (a), trp-B (b), SC*4*A-trp-B complex (c), SC*6*A-trp-B complex (d), and SC*8*A-trp-B complex (e) in D_2O

changes in enthalpy and entropy could be obtained from the slopes and the intercepts, respectively. The free energy change (ΔG) was estimated from the following relationship [19]:

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

From Tables 1 and 2, the negative sign for the enthalpy change and free energy mean that the interaction process was exothermal and spontaneous, but accompanied a small entropic loss. Higher temperature was unfavorable to the formation of inclusion complexes. The negative value of molar enthalpy change proved that the process of complex was exothermic reaction. The negative value of entropy change was probably due to the increased order of guest molecule after the formation of inclusion complexes and the decrease of the freedom of guest molecule relative to the host. Therefore the present experiments were conducted at room temperature. The thermodynamic parameters indicated that the complexes of SC*n*A (*n=4, 6, 8*) with the two tryptophans were all mainly driven by the enthalpy change which mainly to the electrostatic interaction.

Influence of pH

The associations of the two tryptophans with SC*n*A were also conducted in acidic, neutral and basic media. The pH dependence of association constants of was examined at a pH range of 4.0 to 11.0. Tables 3 and 4 showed the large affinity of the two tryptophans to SC*n*A in the fluorescence spectra were found irrelevant of pH. The binding constants were less sensitive to pH in spite of the increasing negative charge of SC*n*A, suggesting that the Coulomb force was not the dominant host-guest complex stabilizing factor [27]. The binding constants became more sensitive to pH with increasing the size of SC*n*A cavity, indicating that the electrostatic interaction and the structural matching effect were the dominant stabilizing factors for the host-guest complexes of the two tryptophans and SC*n*A.

Deprotonation of the phenolic OH groups of SC*n*A can be further strengthened by increasing the pH to 10.5. Thus, repulsive interaction of the two phenolic O^- of the lower rim of SC*n*A results in a small size of the cavity. The electrostatic interaction of the amino group of the two tryptophans and the

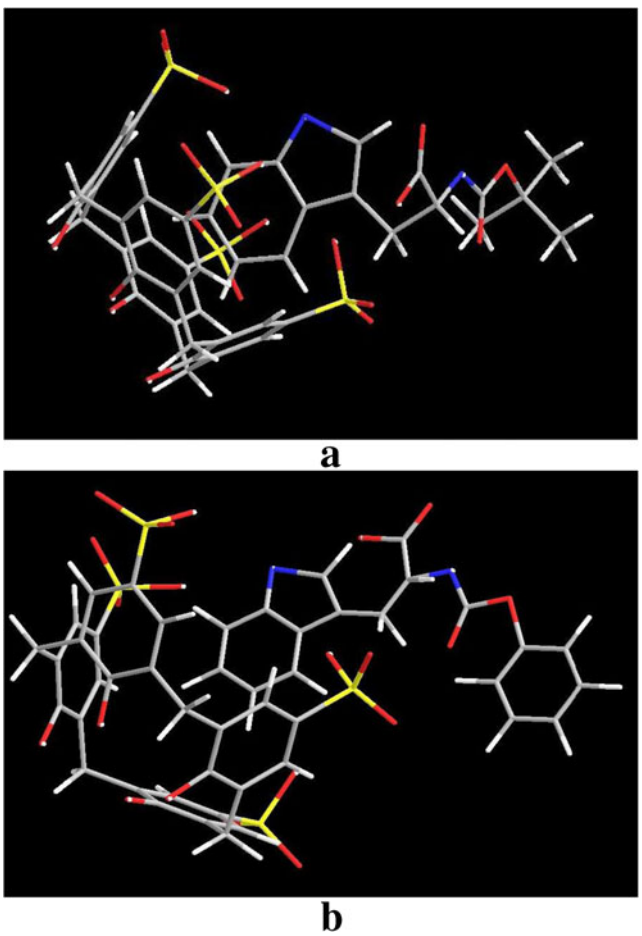

Fig. 10 Lowest energy structure of complex using ball and stick model determined by molecular dynamics simulation with direct minimization. **a** trp-A and SC*4*A complex **b** trp-B and SC*4*A complex

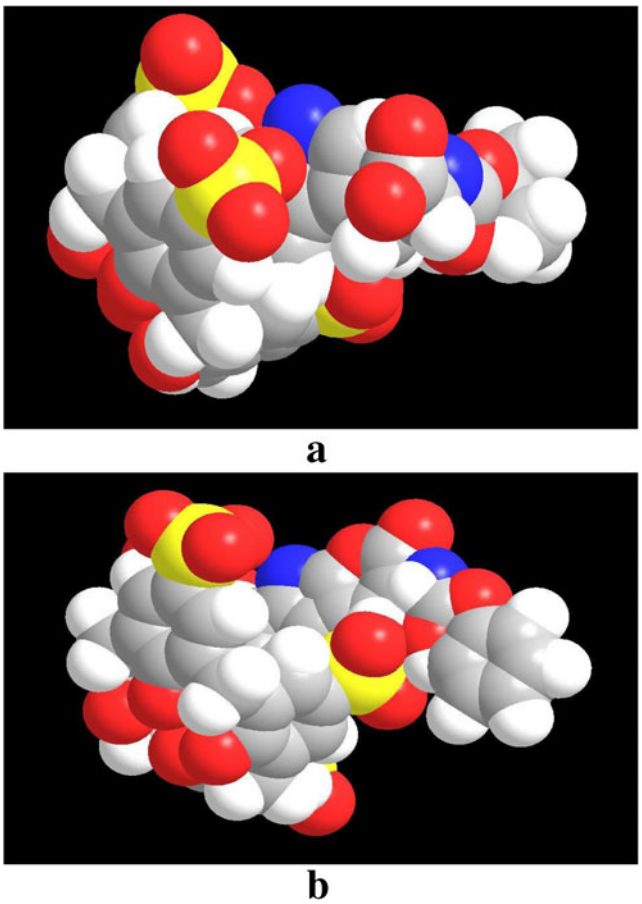

Fig. 11 Lowest energy structure of complex using Space Filling model determined by molecular dynamics simulation with direct minimization. **a** trp-A and SC*4*A complex **b** trp-B and SC*4*A complex

sulphonate anions of SC*n*A occurred at pH 7.4. As the pH from 7.4 to 9.0 or more basic condition, the electrostatic interaction between the tryptophan and SC*n*A became much weaker. Based on these results, the binding constant at pH 7.4 was higher than that at pH 9.0 or 11.0.

^{1}H NMR Study and Molecular Modeling Calculation

The ^{1}H NMR spectra of tryptophan, SC*n*A and their complexation were shown in Fig. 9. The indole proton slightly shifted, but Ha, Hb, Hc, and Hd protons of the benzene ring significantly shifted. At approximately 4.3 ppm, the protons exhibited a downfield shift upon complexation which may be attributed to the interaction between the $-NH_2^+$ of tryptophan and the $-SO_3^-$ of calixarene. This finding may also be attributed to the ring current effect of the aromatic nuclei of the host [28]. Tryptophan entered the SC*n*A cavity from the Ha, Hb, Hc, and Hd positions of the benzene ring.

The shift in Ha, Hb, Hc, and Hd positions was more significant with the increase of the number of phenolic units of the calixarene ring (Fig. 9). Thus, the interaction between SC*n*A and tryptophan was stronger, Tryptophan penetrated deeper into the SC*n*A cavity. Moreover, the ^{1}H NMR spectra showed that the shift in trp-A and the SC*n*A complex was higher than that of trp-B and the SC*n*A complex under the same experimental conditions. Thus, the part of the indole benzene ring of trp-A that penetrated into the SC*n*A cavity was relatively larger than that of trp-B. These results are consistent with the previous fluorescence data.

Molecular modeling calculation was optimized at the B3LYP/6–31G(d) [29] level of the density functional theory [30] by using Gaussian 03 program [31]. Molecular mechanics was simulated to obtain the optimized conformation of the host-guest complex (Figs. 10 and 11). The energy -minimized structAure revealed that the interaction between the indole benzene ring of trp-A or trp-B and the negative charge of the sulphonyl groups of SC*4*A dispersed the electron cloud density, and that SCX4 could partially accommodate the indolent benzene ring of the two tryptophans with a tilted conformation.

Trp-A is located above SC*4*A in a tilted orientation (Figs. 10a and 11a), which allows the indole benzene ring of tryptophan to approach the $-SO_3^-$ group of SC*4*A. The distance was observed at 2.964 Å. However, the estimated distance in the trp-B-SC*4*A complex was found to be only 2.426 Å (Figs. 10b and 11b). The smaller distance in the latter was attributed to the weaker interaction between trp-B and SC*4*A. These findings indicated that the interaction in the trp-A-SC*4*A complex was more stabilized than that of the trp-B-SC*4*A complex. The indole benzene ring of the two tryptophans entered into the SC*4*A cavity, however the *tert*-butyl group and the indole benzene ring of trp-A were located on opposite sides, but the phenyl group and the indole benzene ring of trp-B were on the same side. This finding is consistent with the trend observed in ^{1}H NMR and spectrofluorimetric results.

Conclusions

The findings of the paper are summarized accordingly: (1) The fluorescence intensity of tryptophan significantly decreased when SC*n*As were added. The changes in fluorescence intensity were also consistent with the size of the calixarene ring (i.e., SC*4*A < SC*6*A < SC*8*A). The fluorescence intensity of the trp-A-SC*n*A complex was higher than that of the trp-B-SC*n*A complex at the same concentration and pH. The stability constants, binding ratio, enthalpy, and entropy of complexation were also examined. (2) The ^{1}H NMR spectra showed that the indole benzene group of the two tryptophans partially penetrated into the hydrophobic cavity of the water-soluble SC*n*A. According to the molecular mechanics simulation, the *tert*-butyl group and the indole benzene ring of trp-A were on opposite sides, but the phenyl group and the indole benzene ring of trp-B were on the same side. Thus, the steric hindrance of the tert butyl group of trp-A was smaller than that of the phenyl group of trp-B. These observations are consistent with previous fluorescence data and ^{1}H NMR results.

The possible complexation mechanism for tryptophan and SC*n*A may involve hydrophobic and electrostatic interaction. This mechanism can be used as a fluorescence probe and sensor to detect non-fluorescent or weakly fluorescent substances. Related studies are currently underway.

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