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Journal of Photochemistry and Photobiology A: Chemistry 197 (2008) 389–393
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Interaction of water-soluble calix[4]arene with L-tryptophan studied by fluorescence spectroscopy

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Received 15 June 2007; received in revised form 8 January 2008; accepted 7 February 2008
Available online 14 February 2008

Abstract

The complexing ability of water-soluble calix[4]arene for L-tryptophan (L-try) was investigated by a variety of techniques. The spectrofluorometry titrations were performed at different temperatures to determine stability constants, as well as to evaluate the thermodynamic parameters of the obtained complex. The effect of pH on the complexation process was quantitatively assessed. Moreover, to obtain information about the binding mechanism of the interaction, ^{1}H NMR studies were carried out. Molecular modeling showed that water-soluble calix[4]arene accommodated part of L-amino acid in its cavity meanwhile the aliphatic chain of L-tryptophan stuck out of the cavity. Based on the experiment data, the association process of complexes was established. The water-soluble calix[4]arene was found to be able to adjust its conformation to fit the size of aromatic L-tryptophan, and the benzene ring of amino acid penetrated into the hydrophobic cavity of calix[4]arene.

Keywords: Spectrofluorometry; Aromatic L-amino acid; Water-soluble calix[4]arene

1. Introduction

The inclusion complexation and molecular recognition are of current interest in host–guest chemistry or supramolecular chemistry [1–3]. To date, calixarenes have been extensively studied as a platform to construct a novel host compound bearing a specific function. Calix[*n*]arenes have cavity-shaped architecture to include a guest molecule and can create a specific affinity toward a target molecule by introducing various functional groups either to the upper or to the lower rim [4]. Sulphonated calixarenes represent a particularly important class of the host molecules because these versatile macrocyclic phenolic compounds are highly soluble in water, less toxic than cyclodextrins [5]. Meanwhile, studies of the water-soluble sulphonated calixarenes have generated interest owing the similarities to biological function [6], mimic enzyme [7], and clay structure [8]. The complexation properties of sulphonated calixarenes towards small neutral organic molecules, drug molecules [9,10] and biomolecules such as amino acids, peptides [11] and protein [12] in aqueous solution have been extensively investigated by ^{1}H NMR spectroscopy [13], calorimetric titration [14] and MALDI-MS [15].

In particular, amino acids are the most important targets for molecular recognition by artificial host compounds. This is due to their relevance in the bioactive materials world and pharmaceutical and food industries [16]. For this reason, chemists have been studying host–guest binding of amino acids and water-soluble calixarenes in wide field. In 1999, the ^{1}H NMR studies of the complex formed between *p*-sulphonatocalix[4]arene (SCX4) with trimethylanilinium ion (TMA) and α-amino acids in D_2O showed that the guest was bound to the cone-shaped cavity [10,17]. In addition, the interaction between arginine and lysine with *p*-sulphonatocalix[4]arene by means of ^{1}H NMR spectroscopy [18] and microcalorimetry [19] showed that 1:1 complexes were formed in water. Similarly reasoning, an interesting study of the complexation of *p*-sulphonatocalix[4]arene with some amino acids by means of reversed phase high-performance liquid chromatography (RP-HPLC) and ^{1}H NMR experiments was reported [20]. The results obtained suggested that various interactions such as hydrophobic, ion-pairing,

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doi:10.1016/j.jphotochem.2008.02.002

aromatic–aromatic and electrostatic may occur between the amino acids under study and *p*-sulphonatocalix[4]arene. The fluorometric method is a powerful tool and suitable for the study on inclusion process of aromatic amino acids and sulphonated calixarenes, due to its sensitivity, selectivity and instrumental simplicity.

In this work, we investigated the complexation of L-tryptophan (L-trp) with *p*-sulphonatocalix[4]arene in aqueous solutions by means of spectrofluorometric titration. The effect of pH and thermodynamic property of the complex was studied. Molecular modeling and ^{1}H NMR studies were carried out to determine the possible mechanism for the binding reaction.

2. Experimental

2.1. Apparatus

The fluorescence measurements were performed with a Hitachi Model F-4500 spectrofluorometer (Kyoto, Japan) equipped with a 150 W xenon lamp source, a thermostat bath and a quartz cells (1 cm × 1 cm). All pH values were measured with a pHS-2 acidometre (The 2nd Instrument Factory of Shanghai, China). ^{1}H NMR spectra were recorded in D_2O on a Bruker-DKX-300 MHz spectrometer (Switzerland).

2.2. Reagents

The stock solution of 5.0×10^{-4} mol L^{-1} L-tryptophan (L-trp, Biological Identification Institute of Shanghai) and 1.0×10^{-2} mol L^{-1} *p*-sulphonatocalix[4]arene (SCX4, TCI, TOKYO KASEI Co. Ltd.) were prepared by directly dissolving its powder in water. Phosphate buffer solution was used to control the pH-value of the media. Doubly distilled water was used throughout.

2.3. Procedure

A 1-mL aliquot of the stock solution (5.0×10^{-4} mol L^{-1}) of L-trp was transferred into a 10 mL volumetric flask, and an appropriate amount of 1.0×10^{-2} mol L^{-1} SCX4 was added. The pH was controlled by 0.5 mol L^{-1} phosphate buffer. The mixed solution was diluted to the final volume with distilled water and shaken thoroughly, then equilibrated for 30 min at 20 ± 1 °C.

3. Results and discussion

3.1. Formation of inclusion complexes of L-trp and SCX4

Fig. 1 illustrated the alteration of the fluorescence spectrum of L-trp upon addition of gradually increasing amount of SCX4 at pH 7.3. The fluorescence maximum excitation and emission wavelengths of L-trp were at 298 and 360 nm, respectively. Addition of different concentrations of SCX4 caused a noticeable decrease in L-trp fluorescence intensity. The maximum emission wavelength produced a small red shift from 360 to 365 nm and the corresponding excitation wavelength was slightly red shifted

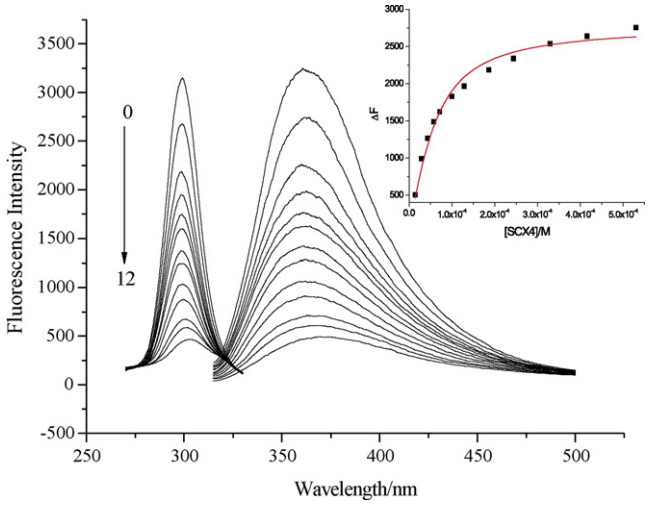

Fig. 1. Fluorescence spectral changes of L-trp (5×10^{-5} M) upon addition of various concentrations of SCX4 in phosphate buffer solution at 24 °C and the nonlinear curve fitting analysis (inset) of the differential intensity (ΔF) to calculate the complex stability constant (K).

from 298 to 302 nm. The marked fluorescence quenching and the bathochromic displacement proved complex formation.

Because significant spectral change took place at comparable concentrations of the components, the follow nonlinear curve fitting function described the relation between the fluorescence intensity at maximum emission wavelength and the total concentration of the SCX4 in the case of 1:1 complexation [21]:

$$\Delta F = \frac{1}{2}\left\{\alpha\left([H]_0 + [G]_0 + \frac{1}{K}\right) - \sqrt{\alpha\left([H]_0 + [G]_0 + \frac{1}{K}\right)^2 - 4\alpha^2[H]_0[G]_0}\right\} \quad (1)$$

where ΔF represents the change of the fluorescence intensity of L-trp with the addition of SCX4. $[H]_0$ and $[G]_0$ denote the initial concentrations of host SCX4 and guest L-trp, respectively. α is sensitive factor of the structure change of complexation composed of SCX4 and L-trp at the interactive course. K is the association constant. The fitting results all had large correlation coefficients ($R > 0.99$), which indicated that the 1:1 complex between SCX4 and L-trp was formed [22,23].

A representative Job's plot for the inclusion complexation of SCX4 with L-trp was shown in Fig. 2. The maximum of the curve was clearly at a mol fraction of 0.5, confirming the 1:1 ratio host–guest complex assembly formed in aqueous solution [24].

3.2. Thermodynamic parameters of inclusion complexation

The interaction forces between amino acids and SCX4 may include hydrophobic force, electrostatic interaction, van der Waals interaction, hydrogen bonds, etc. The stability constants (K) of the inclusion complexation of L-trp with SCX4 were determined via spectrofluorometric titration at various temperatures

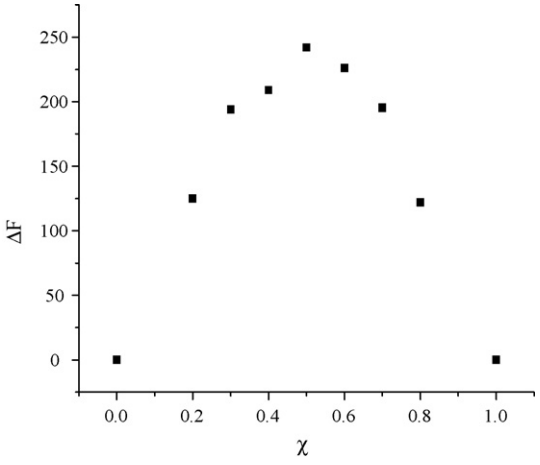

Fig. 2. Job's plot for the complexation of L-trp with SCX4 in phosphate buffer solution (pH 7.30) at 25 °C ([L-trp] + [SCX4]) = 5.0 × 10^{-5} M).

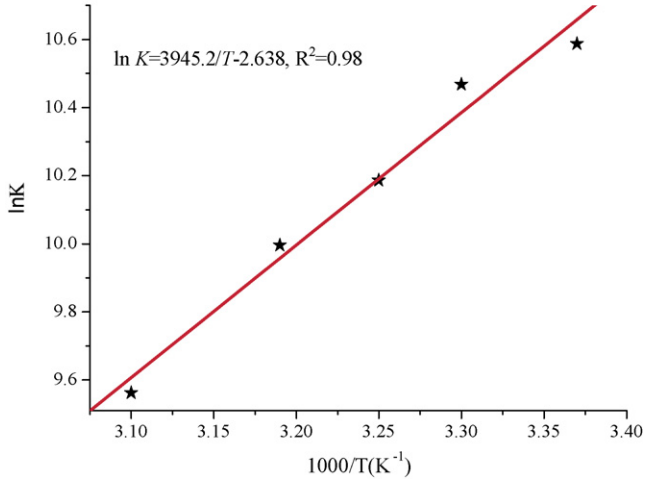

Fig. 3. Van't Hoff plot, pH 7.30, C (L-trp) = 5 × 10^{-5} M.

ranging from 24.0 to 45.0 °C. The slope of a plot of the ln K vs. 1/T (T, absolute temperature) was linear within experimental error. If the enthalpy change (ΔH) did not vary significantly over the temperature range studied, then its value and that of entropy change (ΔS) can be determined from the Van't Hoff equation [25]:

$$\ln K = \frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (2)$$

where K is analogous to the K' at the corresponding temperature [26]. The free energy change (ΔG) was estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

Fig. 3, by fitting the data of Table 1, shows that assumption of near constant ΔH was justified. Table 1 showed the values of enthalpy (ΔH) and entropy (ΔS) obtained from the slopes and ordinates at the origin of the fitted lines.

The complexation thermodynamic parameters in Table 1 indicated that the complexation of L-trp molecule with SCX4 was mainly driven by the favorable enthalpic change, but with accompanying a small entropic loss. From Table 1, it can be seen that the negative sign for free energy (ΔG) means that the interaction process was spontaneous. Although SCX4 could form effective hydrogen bonds with the hydroxyl groups in its lower rim, the hydrophobic interaction and Van Der Waals forces, as well as the electrostatic interactions with sulphonic groups, would contribute to the favorable enthalpy change. While the conformation change and the desolvation effect contributed to the entropic changes [27]. The entropy change originated from the entropic gain from the rearrangement of water molecules originally surrounding the host and guest molecules, and the entropic loss from the decrease in the motion freedom upon the complexation [28]. In this case, the L-trp molecules included into the enhanced hydrophobic cavity of SCX4, and it would be expected to balance the entropic loss due to the restricted mobility of the L-trp molecules to a much great extent.

Table 2
Complex stability constants (K_S) for 1:1 intermolecular complexation of L-trp with SCX4 at different pH

	pH				
	2	4	8	10	13
	n = 1:1	n = 1:1	n = 1:1	n = 1:1	n = 1:1
K	1201.30	4148.02	14445.79	7460.85	4051.66
R^2	0.9973	0.9874	0.9980	0.9921	0.9979

3.3. Influence of pH

The association of L-trp with SCX4 was also carried out in acidic, neutral and basic media. Large affinity of L-trp to SCX4 in the fluorescence spectra was found irrespective of pH. As seen in Table 2, the binding constants were a little sensitive to pH in spite of the growing negative charge of SCX4, which suggested that the Coulomb force was not the dominant host–guest complex stabilizing factor [29].

Further inspection of the titration data revealed that the binding constants got to maximum in neutral aqueous. SCX4 is

Table 1
Complex stability constants (K) and thermodynamic parameters for 1:1 intermolecular complexation of L-trp with SCX4 in a phosphate buffer solution (pH 7.3)

	T (K)	K (L mol^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J/(mol K)$^{-1}$)
	297	39661.68		−26.3	
	303	35168.63		−26.16	
pH 7.3	308	26538.02	−32.8	−26.05	−21.9
	313	21947.66		−25.95	
	323	14219.76		−25.73	

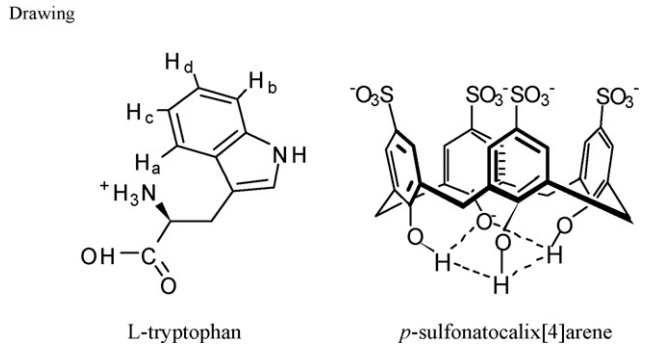

Fig. 4. Molecular structures of L-tryptophan and *p*-sulphonatocalix[4]arene.

conformationally flexible and renders its adaptable cavity to guests of various geometries. The pK_a values of the lower rim hydroxyl substituents of SCX4 were 3.34 and 11.5 [30]. Coleman et al. [31] had also reported that SCX4 exists as a penta-anion in neutral aqueous solutions because of the deprotonation of one of the phenolic OH groups, which probably reflected that the deprotonation strengthened the hydrogen bonds among the phenolic hydroxyl groups allowing conformation flexibility for the calixarene ring (as shown in Fig. 4). With increasing the pH, deprotonation of the phenolic OH groups of SCX4 could be further strengthened until the pH 11.5. So, repulsive interaction of the two phenolic O^- of the lower rim of SCX4 leads to the little size of the cavity. Another, the pK_{a1} and pK_{a2} of L-tryptophan are 2.46 (–COOH) and 9.41 ($-NH_3^+$), respectively. It is obvious that the electrostatic interaction of amino group of L-trp and sulphonate anions of SCX4 is existed in experimental condition pH 7.3. With increasing the pH from pH 7.3 to 8.0 or more basic condition, the electrostatic interaction between the L-trp and SCX4 is becoming weaker gradually. According to these, it is possible that the binding constant at pH 7.3 is larger than at pH 8.0 or pH 10.0, pH 13.0.

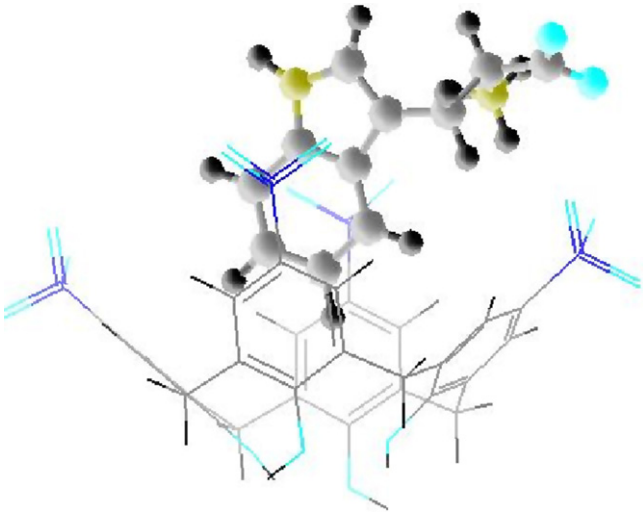

Fig. 6. Lowest energy structure of complex determined by molecular dynamics simulation with direct minimization.

3.4. Molecular binding mode and molecular recognition

^{1}H NMR titration experiments were undertaken to confirm the binding mode between water-soluble SCX4 and L-trp at room temperature at pH 7.3 in buffer solution, which excluded the possibility of leading to the change of the chemical shifts of acid–basic reaction. The spectra of 5×10^{-3} mol L^{-1} L-trp and its complex with different concentration of SCX4 were shown in Fig. 5. It is obvious that the indole-proton almost did not shift, but the Hc, Hd and Ha protons of benzene shifted remarkably. Meanwhile, the protons in the calixarene ring slightly shifted. Around 3.8–4.1 ppm, we can see the downfield of the protons upon addition SCX4. This phenomenon may attribute to the effect between the $-SO_3^-$ of calixarene and the $-NH_3^+$ of L-trp. Because of the reciprocity, the chiral hydrogen of the L-trp has been shifted obviously. It owned to the ring current effect of the aromatic nuclei of the host [13]. This indicated that the

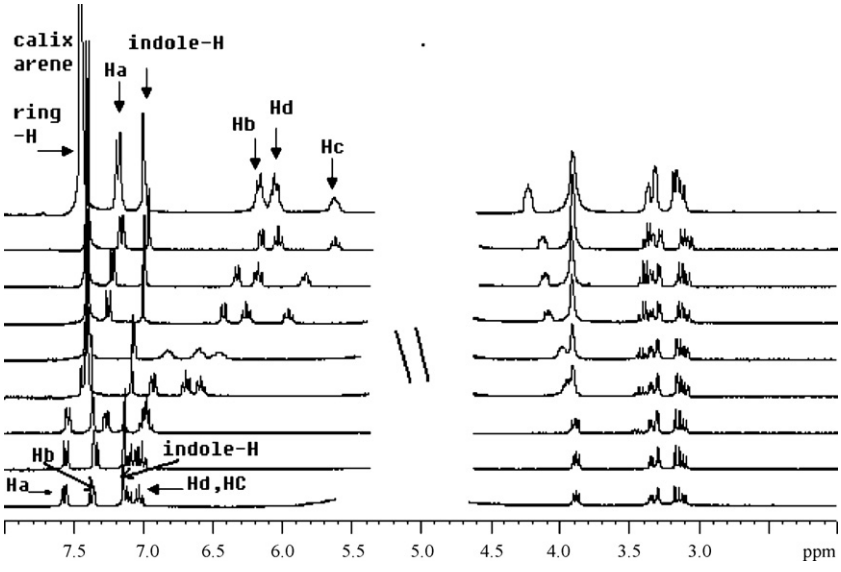

Fig. 5. Spectra of ^{1}H NMR titration of *p*-sulphocalix[4]arene with L-tryptophan at pH 7.3 buffer at 300 MHz.

L-trp penetrated into the SCX4 cavity from the Hc, Hd and Ha position of the benzene ring.

The results from molecular mechanics calculation were generally consistent with the ^{1}H NMR and spectrofluorometric experimental results. Molecular mechanics simulation with the open force field (OFF) was performed to give the optimized conformation of host–guest complex (Fig. 6). The result clearly demonstrated that SCX4 could partially accommodate benzene ring of L-trp in its hydrophobic cavity with a tilt-in conformation. In conclusion, this work showed a remarkable influence of subtle conformation changes of the cone calix[4]arene platform.

4. Conclusion

L-trp and SCX4 in aqueous solution by means of spectrofluorometric titration was studied. The stability constants, binding ratio, enthalpy and entropy of complexation were evaluated. The fluorescence intensity of the L-trp guest molecule to gradually decreased upon the addition of SCX4, accompanying with bathochromic/red shifts. From ^{1}H NMR titration, because the benzene ring protons of L-trp experienced a remarkable upfield shift, benzene apolar group of L-trp was only partially enclosed into the hydrophobic cavity of water-soluble SCX4 cavity. These observations were agreed with structures obtained by preliminary molecular mechanics calculation. This seemed to be determined by the need for the charged group of the L-trp to stick out of the apolar SCX4 cavity in order to be exposed to polar medium.

The possible mechanism involved in the complexation between L-trp and SCX4 may be a combination of hydrophobic interaction and electrostatic interaction. However, the degree to which these forces promote the formation of complex was limited by conformational changes in the SCX4 molecules. SCX4 tended to adopt different conformations upon complexation with structure-related guest and the symmetry of the guest as well as the induced-fit relationship between host and guest may be the main factors controlling these conformational adjustments.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 20575038 and 20576087) and the Foundation of Shanxi Provience (No. 2006011022).

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