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Journal of Photochemistry and Photobiology A: Chemistry 193 (2008) 178–186
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Spectral and photophysical studies on the inclusion complexation between Triton X-100 and β-cyclodextrin: A competitive method using a substituted 3*H*-indole probe

Yifeng He, Xinghai Shen*, Hongcheng Gao, Yongke He

Beijing National Laboratory for Molecular Sciences (BNLMS), Department of Applied Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

Received 29 January 2007; received in revised form 12 May 2007; accepted 15 June 2007
Available online 21 June 2007

Abstract

We report herein the interaction of the nonionic surfactant Triton X-100 with β-cyclodextrin (β-CD) using 2-(*p*-aminophenyl)-3,3-dimethyl-5-carboethyoxy-3*H*-indole (**1**) as a fluorescent probe through a competitive method. The formation of 1:1 and 1:2 (guest:host) inclusion complexes was confirmed and the association constants were estimated. Using Triton X-100 itself as a probe, the interaction pattern and the association constants obtained were in agreement with those sensed by **1**. Furthermore, the binding site of Triton X-100 with β-CD was investigated. It was found that the entire hydrophobic part of Triton X-100 was included into the cavity of β-CD. For comparison, we also employed **1** as a probe to study the interaction between Brij 56 and β-CD. The above results strongly suggest that molecule **1** is a good candidate acting as a probe to study the inclusion complex between a non-fluorescent guest and cyclodextrin.

Keywords: Inclusion complex; β-Cyclodextrin; Triton X-100; Steady-state fluorescence; Fluorescence lifetime; 2D NOESY NMR

1. Introduction

Cyclodextrins (CDs) are a series of doughnut-shaped cyclic oligosaccharides, mostly composed of 6, 7, and 8 D-(+)-glucose units named α-, β-, and γ-CDs, respectively. The cavity of cyclodextrin exhibits hydrophobic characteristic, whereas its exterior is hydrophilic. This particular structure allows various guest molecules to be included in the cavity to form what is called inclusion complexes. Noncovalent intermolecular forces are believed to play a key role on the complex formation and its stabilization. This leads to widespread applications in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis [1].

Inclusion complexes formed between surfactant molecules and CDs have received much attention [2–22], partly because they can alter chemical reactivity, act as molecular carriers, especially for drugs delivery, and mimic the effect of CDs on phospholipids. Very recently, Lu et al. used the supramolecular assembly of β-CD and the nonionic surfactant Triton X-100 as a precursor to synthesize mesoporous silica microtubules [23]. Thus, the investigation on the interaction between Triton X-100 and β-CD is of both scientific and practical importance.

The inclusion complexes between surfactants and CDs have been studied by a number of physical methods such as fluorescence spectroscopy [2,3,8,11,13,15], NMR [16], conductivity [17], surface tension [5,18], isothermal titration calorimetry [7], ultrasonic relaxation [19], UV–vis spectroscopy [20], induced circular dichroism (ICD) [21] and emf [22]. In some cases, however, the reported interaction patterns and the association constants of cyclodextrin with a same surfactant are greatly different depending on which physical method has been used. A typical example is for sodium dodecyl sulfate (SDS) interacting with β-CD [4]. While several experiments are reliable in studying the SDS/β-CD system using conductivity, sound velocity, surface tension and counterion binding measurements, their theoretical basis fails in interpreting the measurements in terms of equilibrium concentrations of different species [4]. Of all the methods, the competitive method with fluorescent and UV–vis probes and the emf method gave reasonable results since they were based on better theoretical models [4]. There-

* Corresponding author. Tel.: +86 10 62765915; fax: +86 10 62759191.
E-mail address: xshen@pku.edu.cn (X. Shen).

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doi:10.1016/j.jphotochem.2007.06.023

fore, the reliable methods, especially the competitive method using a fluorescent probe, which was first suggested by Park et al. [2], are worth studying further to obtain reasonable association constants between surfactants and CDs. The similar phenomenon also happened to the Triton X-100/β-CD system. Many groups have studied this system, but the association types and constants differed considerably [5–14]. On the basis of different methods, it was reported that Triton X-100 formed a 1:1 (guest:host) inclusion complex with β-CD [5–11]. The type of 1:2 inclusion complex between Triton X-100 and β-CD was also suggested [12,14]. However, Buschmann et al. found the coexistence of 1:1 and 1:2 inclusion complexes [13]. Besides the discrepancy of association types, the reported binding constants were also much different, for instance, the association constant of the 1:1 inclusion complex had a wide range from 145 to $(1.82 \pm 0.15) \times 10^5\ M^{-1}$. Obviously, it is necessary and interesting to get deep insight on this problem using a reliable method.

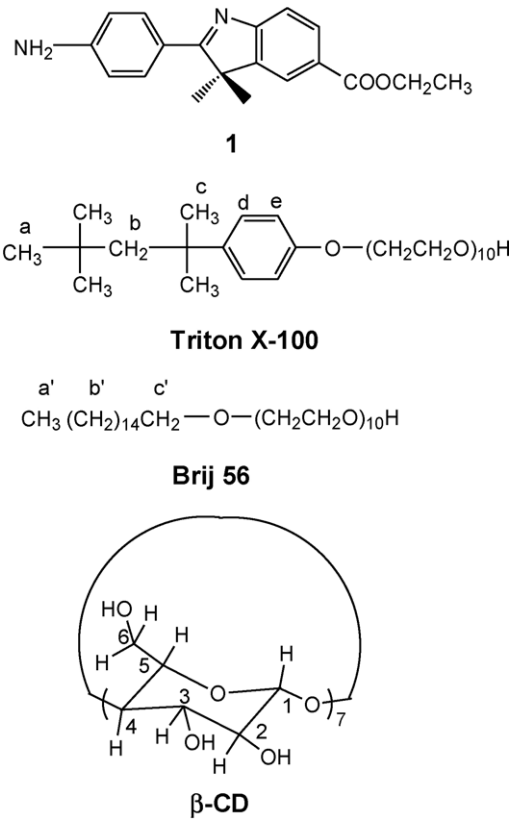
For substituted 3*H*-indoles have great sensitivity to microenvironments [24], their use as probes has been highlighted in reverse micelle [25], micelle [26,37], surfactant vesicles [27] and cyclodextrins [3,28–30,35,36]. We synthesized cationic surface-active substituted 3*H*-indole probes having long oligoethyloxyethylene chains as spacers between the cationic nitrogen and the amino nitrogen, and studied their interactions with β-CD [29]. The locations of different groups of a cationic surface-active 3*H*-indoles probe molecule in the AOT (sodium bis-(2-ethylhexyl) sulfosuccinate)-based w/o microemulsion and the physicochemical properties of Triton X-100 micelles were also investigated [31–33]. Very recently, we synthesized a substituted 3*H*-indole-β-CD, which showed novel recognition behavior and photoinduced energy transfer with naphthalene and its derivatives [34]. Other investigation has been also afforded with 3*H*-indoles, including **1** (see Scheme 1) [3,28,35–37]. **1** and β-CD in the aqueous solution can form two types of inclusion complexes, i.e., 1:1 and 1:2 types, and the interaction between SDS and β-CD has been successfully studied with a probe similar to probe **1** through a competitive method [3].

In the present work, we have used **1** and Triton X-100 itself as fluorescent probes to elucidate how Triton X-100 interacts with β-CD. For comparison, the interaction between Brij 56 and β-CD was also investigated.

2. Experimental

2.1. Materials

The synthesis and purification of probe molecule **1** were done according to the literature [38]. β-CD (AP, Fine Chemical Products of Nankai University) was recrystallized twice using tridistilled water and dried under vacuum for 24 h. Triton X-100 and Brij 56 were purchased from Acros and used without further purification. Methanol was redistilled after being dried with anhydrous sodium sulfate for about 24 h. Tridistilled water was used throughout the experiments. D_2O (99.9% isotopic purity, Beijing Chemical Reagents Company) was used as solvent for ^1H NMR.



Scheme 1. Molecular structures of **1**, Triton X-100, Brij 56 and β-CD.

2.2. Instruments

Absorption spectra were recorded on an UV-3010 (Hitachi, Japan) spectrophotometer using 1-cm path quartz cells. The slit width was 1 nm. Fluorescence spectra were measured on a FL-4500 (Hitachi, Japan) spectrophotometer. Each solution was excited near its maximum absorption wavelength using 1 cm quartz cells (356 nm for **1** and 275 nm for Triton X-100, respectively). Both the excitation and emission band passes were 5 nm throughout. The scan speed was 240 nm/min. Fluorescence lifetime measurements were made on a multiplexed time-correlated single-photon counting fluorimeter FLS920 (Edinburgh). The lifetime value was determined from data on the fluorescence transient waveform of the material to be tested and the lamp waveform data using the least-squares iterative deconvolution method. The error of the lifetime was less than 10%. The excitation and emission wavelengths were 356 and 470 nm for **1**. Three thousand counts were collected for each sample. More details on the apparatus for the fluorescence lifetime measurements were described elsewhere [39]. The ^1H NMR and 2D NOESY NMR were recorded on Bruker AV400 MHz NMR spectrometer. When 2D NOESY NMR was performed, the relaxation delay (D_1) and mixing time (D_8) were 2000 and 400 ms, respectively.

2.3. Methods

Fresh sample solutions were used in the absorption and fluorescence measurements. Stock solution of **1** was prepared in

methanol. The pH values of all the solutions with **1** as a probe in this study were adjusted to 9.5 by adding NaOH, and no buffers were used [35,36]. The concentrations of **1** for absorption and fluorescence measurements were 10^{-5} and 10^{-6} M, respectively. When Triton X-100 itself was used as a probe, its concentration was fixed at 6.0×10^{-6} M and the pH value in this case was not adjusted.

When ^{1}H NMR was applied, chemical shift was given on the δ scale (ppm) and reference to solvent residue peak of D_2O ($\delta = 4.77$ ppm, 298 K) [40]. The samples were probed under 298.0 ± 0.1 K. All other measurements were carried out at room temperature unless especial indication.

3. Results

3.1. Interaction of Triton X-100 with β-CD

3.1.1. Interaction of *1* with the Triton X-100 micelle

The absorption and fluorescence spectra of **1** in water and in the aqueous solutions of Triton X-100 are shown in Fig. 1. It can be seen that an obvious red shift accompanied by little change of absorbance in the absorption spectra and a slight blue shift accompanied by a remarkable increase of the intensity in the fluorescence spectra occur with an increase in the concentration of Triton X-100. Meanwhile, it is noted that both the absorption and fluorescence spectra change slightly at the concentrations of Triton X-100 below 0.1 mM or above 1 mM. Similar phenomena were also observed by using another substituted *3H*-indole as probe in the SDS micelle [37].

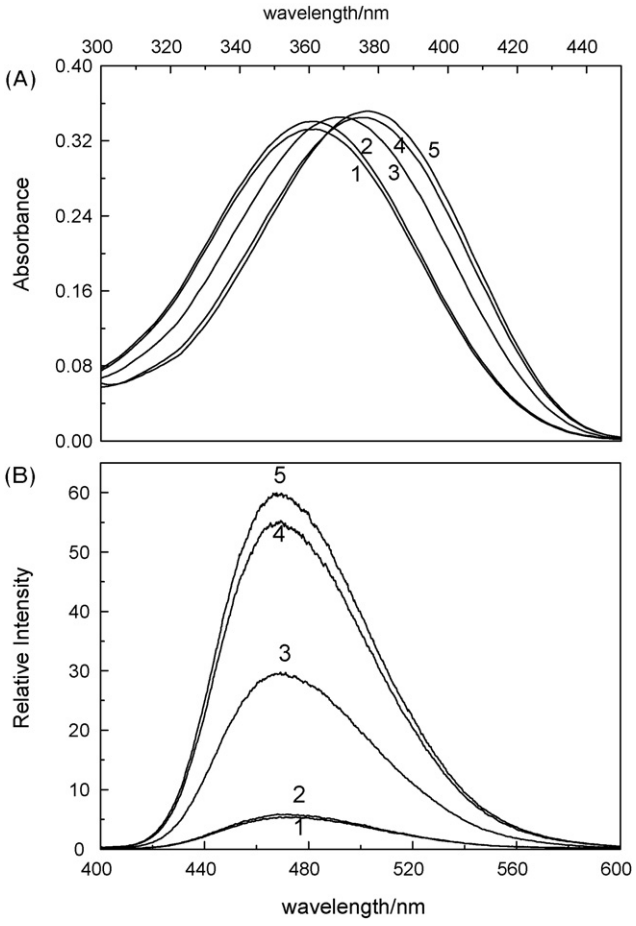


Fig. 1. Absorption (A) and fluorescence (B) spectra of **1** in aqueous solutions of Triton X-100 at various concentrations (mM): 0 (1); 0.10 (2); 0.40 (3); 1.00 (4); 3.00 (5).

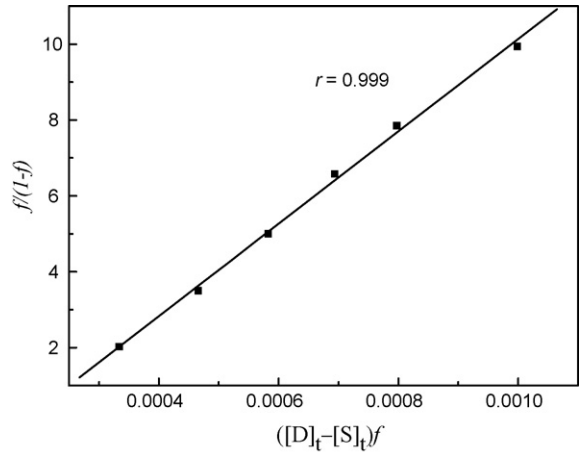


Fig. 2. $f/(1-f)$ vs. $([D]_t–[S]_t)f$ for **1** in the Triton X-100 micelle.

The relationship between the binding constant (K_s) of a substrate or a probe with a micelle and the critical micellar concentration (CMC) can be described by the following equation [28,41]:

$$\frac{f}{1-f} = K_s([D]_t - [S]_t)f - K_s\,\mathrm{CMC} \quad (1)$$

$$f = \frac{I - I_w}{I_m - I_w} \quad (2)$$

where f is the fraction of the micellar-associated probe molecule and calculated from the values of fluorescence intensities of the probe molecule in surfactant solution (I), in water (I_w), and at complete micellization (I_m). $[S]_t$ and $[D]_t$ are the total concentrations of the probe and the detergent, respectively. A plot of $f/(1-f)$ versus $([D]_t–[S]_t)f$ should give a straight line, from the slope and the intercept of which the K_s and CMC values can be obtained. Fig. 2 illustrates such kind of straight line for **1** interacting with the Triton X-100 micelle. It was estimated that the K_s and CMC values were $12\,200 \pm 300\,M^{-1}$ and $(1.7 \pm 0.2) \times 10^{-4}$ M, respectively. The CMC of Triton X-100 reported in the literature ranges from 0.14 to 0.31 mM according to different methods [6–8,10,12,44,45]. Using surface tension method, Cummings et al. [44] and Du et al. [10] gave the CMC value of Triton X-100 as 0.14 and 0.3 mM, respectively. With pyrene as a fluorescence probe, the reported CMC value of Triton X-100 was 0.24–0.26 mM [45]. It seems that the CMC value obtained in study is within an acceptable range.

3.1.2. Investigation on the Triton X-100/β-CD inclusion complex using *1* as a probe

The fluorescence spectra and the fluorescence intensity of **1** in the Triton X-100/β-CD system are shown in Fig. 3. The concen-

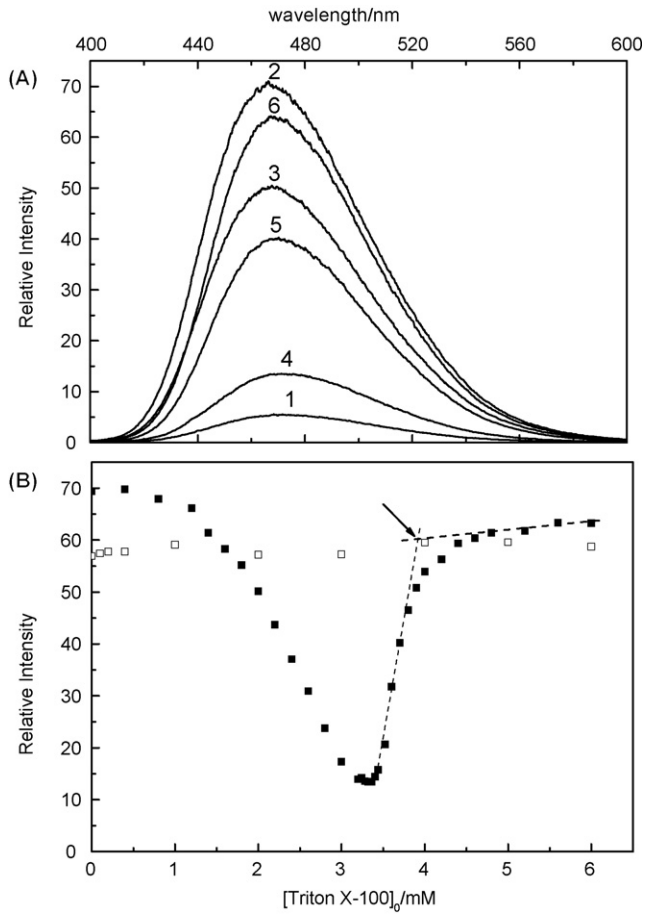


Fig. 3. (A) Fluorescence spectra of **1** in water (1), in 4.00 mM β-CD (2), and in 4.00 mM β-CD with various concentrations of Triton X-100 (mM): 2.00 (3); 3.32 (4); 3.70 (5); 6.00 (6). (B) Fluorescence intensity of **1** vs. the total concentration of Triton X-100 (■) and Brij 56 (□), respectively, in the presence of 4.00 mM β-CD.

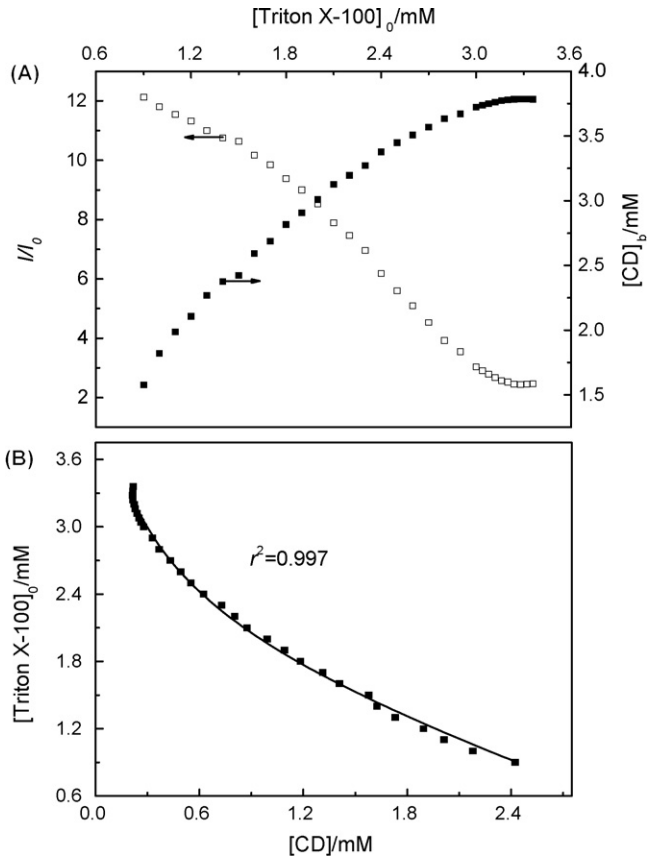


Fig. 4. (A) Relative fluorescence intensity of **1** and the concentration of Triton X-100-bound β-CD as a function of the initial concentration of Triton X-100. (B) Initial concentration of Triton X-100 vs. the equilibrium concentration of β-CD. The line is the nonlinear regression fit to the experimental data following equation (5).

trations of Triton X-100 are in the range of 0–6 mM, whereas the concentration of β-CD is fixed at 4 mM. According to the literature, the fluorescence intensity of **1** at 4 mM of β-CD reaches a plateau showing that most molecules of **1** exist in 1:2 complexes, and thus the ternary complex between **1**, Triton X-100 and β-CD can be assumed not to form [3]. The equilibrium concentrations of β-CD, i.e., [CD], at different [Triton X-100]$_0$ can be calculated, using the K_1 ($990 \pm 160\ M^{-1}$), K_2 ($2500 \pm 120\ M^{-1}$), I_1/I_0 (3.68 ± 0.40), and I_2/I_0 (14.2 ± 0.1) which we obtained according to the method reported in the literature [28]. The concentration of β-CD binding with Triton X-100 can be obtained from the relationship $[CD]_b = [CD]_0 - [CD]$. Fig. 4A shows the variation of $[CD]_b$ as a function of [Triton X-100]$_0$. At [Triton X-100]$_0$ < 3.32 mM, $[CD]_b$ is always slightly larger than [Triton X-100]$_0$ indicating the 1:2 complex between Triton X-100 and β-CD is also formed while the 1:1 complex is predominant [3]. Thus, the following stepwise equilibria exist:

$$\text{Triton X-100} + \text{CD} \overset{K_1'}{\rightleftharpoons} \text{Triton X-100–CD} \quad (3)$$

$$\text{Triton X-100–CD} + \text{CD} \overset{K_2'}{\rightleftharpoons} \text{Triton X-100–(CD)}_2 \quad (4)$$

where Triton X-100–CD and Triton X-100–$(CD)_2$ represent the 1:1 and 1:2 complexes, respectively, the association constants of which are K_1' and K_2'. Considering Eqs. (3) and (4), and the mass balances of Triton X-100 and β-CD, the following equation can be obtained [2,3]:

$$[\text{Triton X-100}]_0 = \frac{([CD]_0 - [CD])(1 + K_1'[CD] + K_1'K_2'[CD]^2)}{K_1'[CD] + 2K_1'K_2'[CD]^2} \quad (5)$$

In Fig. 3B, the inflexion shows the CMC of Triton X-100 in the presence of 4 mM of β-CD is ca. 3.95 mM. Thus, no micelles are formed in the fitting of experimental data of Fig. 4 to Eq. (5) when the concentration of Triton X-100 is below 3.32 mM. According to Eq. (5), it was estimated that the K_1' and K_2' values were $(1.14 \pm 0.32) \times 10^5$ and $(1.18 \pm 0.05) \times 10^3\ M^{-1}$, respectively. Fig. 4B shows the well fit with a correlation coefficient $r^2 = 0.997$. We have also considered the other models describing the interaction of Triton X-100 with β-CD, but no reasonable results were obtained.

3.1.3. Investigation on the Triton X-100/β-CD inclusion complex using Triton X-100 itself as a probe

Fig. 5 illustrates the fluorescence intensity of Triton X-100 at various concentrations of β-CD in the absence of **1**. The flu-

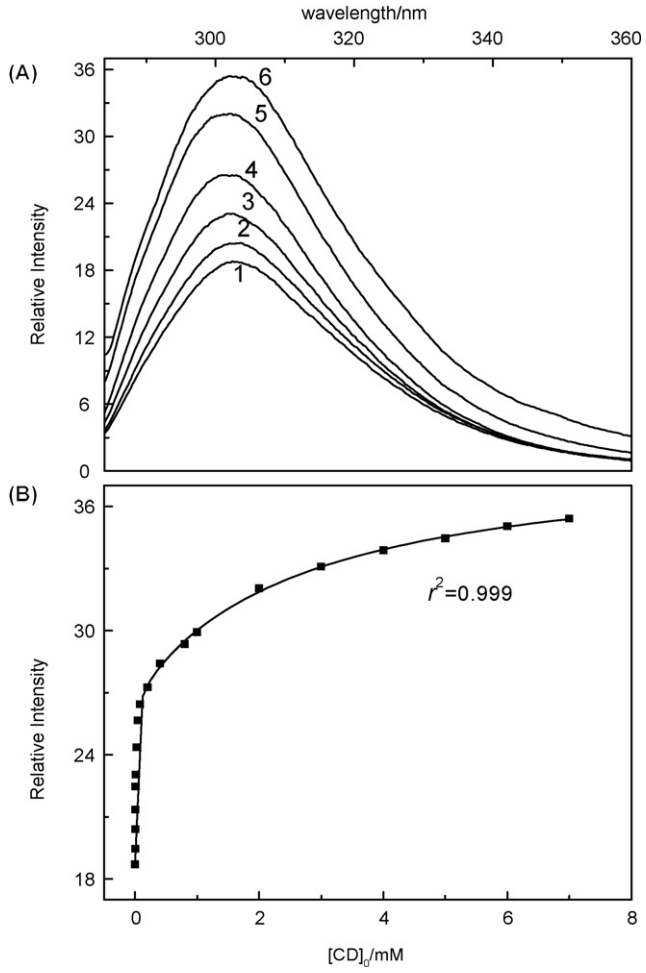


Fig. 5. (A) Fluorescence spectra of Triton X-100 in aqueous solutions of β-CD at various concentrations (mM): 0 (1); 2.00×10^{-3} (2); 1.00×10^{-2} (3); 8.00×10^{-2} (4); 2.00 (5); 7.00 (6). (B) The relative fluorescence intensity of Triton X-100 (6.0×10^{-6} M) vs. $[CD]_0$. The full line is the nonlinear regression fit to the experimental data points.

orescence intensity increases sharply at low concentrations of β-CD and then increases slowly. Triton X-100 and β-CD in the aqueous solution formed two types of inclusion complexes, i.e., 1:1 and 1:2 types by NLR analysis according to the following equation:

$$I = \frac{I_0 + I_1 K_1'[\mathrm{CD}] + I_2 K_1' K_2'[\mathrm{CD}]^2}{1 + K_1'[\mathrm{CD}] + K_1' K_2'[\mathrm{CD}]^2} \quad (6)$$

where I_0, I_1 and I_2 denote the fluorescence intensity of the substrate in pure water, in the 1:1 and 1:2 complexes, respectively, while K_1' and K_2' are the association constants for 1:1 and 1:2 complexes, respectively [35]. The association constants K_1' and K_2' were estimated to be $(1.13 \pm 0.05) \times 10^5$ and $(3.71 \pm 0.27) \times 10^2\ \mathrm{M}^{-1}$, respectively, with a correlation coefficient $r^2 = 0.999$ (see Fig. 5B). The other models describing the interaction of Triton X-100 with β-CD have been also considered, but no reasonable results were obtained.

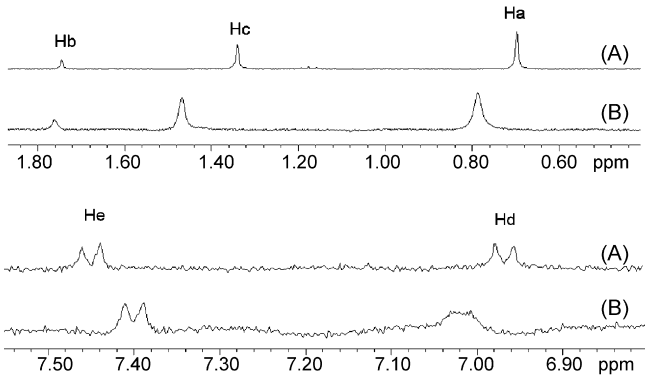


Fig. 6. Four hundred megahertz ^{1}H NMR spectra of 0.10 mM Triton X-100 in water (A) and in the aqueous solution of 0.10 mM β-CD (B), respectively.

3.2. Investigation on the Brij 56/β-CD inclusion complex using *1* as a probe

Fig. 3B shows the fluorescence intensity of **1** against the concentration of Brij 56 in the presence of 4 mM of β-CD. Unlike the fluorescence intensity of **1** in Triton X-100/β-CD system which goes through an obvious change with the addition of Triton X-100, the fluorescence intensity of **1** in Brij 56/β-CD system remains unchanged within the experimental error. The above results suggest that the interaction between Brij 56 and β-CD is weak.

3.3. NMR measurement

The influence of β-CD on Triton X-100 in D_2O was investigated using NMR (see Fig. 6). To avoid the affection of the micelle of Trtion X-100 on its NMR spectrum change originating from the addition of β-CD, the concentration of Triton X-100 is held below CMC. The ^{1}H NMR spectra of Triton X-100 consist of peaks from six kinds of proton: the terminal methyl singlet (H*a*, at δ 0.70), the internal methyl singlet (H*c*, at δ 1.34), the methylene singlet (H*b*, at δ 1.74), the strong unresolved broad peaks consisting of oxyethylene (OE) protons (at δ 3.67–3.75), and the phenyl ring protons doublets (H*d* at δ 6.98 and 6.96; H*e* at δ 7.46 and 7.44). When 0.10 mM of β-CD was added, it was found that the proton signals of H*a*, H*b*, H*c* and H*d* in 0.10 mM of Triton X-100 (see Scheme 1) were shifted downfield by 0.09, 0.02, 0.13 and 0.05, respectively, and that of H*e* upfield by 0.05. At the same time, the signals of the H-3 (triplet, at δ 3.97), H-5 (singlet, at δ 3.86) and H-6 (singlet, at δ 3.88) in β-CD exhibit upfield shifts by 0.05, 0.03 and 0.04, respectively, but the signals of H-1, H-2 and H-4 remain unaffected by Triton X-100 (see Fig. 7).

To further clarify which part of Triton X-100 was included in the β-CD cavity, the 2D NOESY NMR spectrum was measured at 6.6 mM Triton X-100 and 10 mM β-CD (see Fig. 8). According to the literature, the CMC of Triton X-100 in the presence of 10 mM β-CD is 7.25 mM [8]. Thus, no micelles were formed in our case.

The influence of β-CD on Brij 56 in D_2O was also investigated using NMR. As the CMC of Brij 56 is too low to obtain

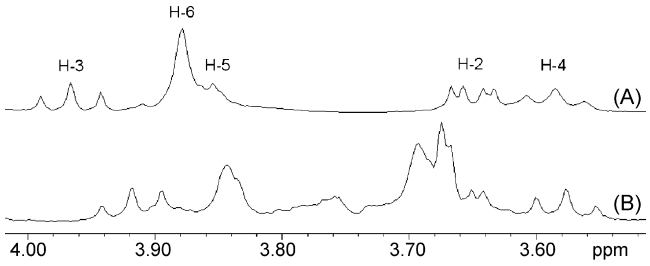


Fig. 7. Four hundred megahertz ^{1}H NMR spectra of 0.10 mM β-CD in water (A) and in the aqueous solution of 0.10 mM Triton X-100 (B), respectively.

the monomer NMR signal in this case, 1.5 mM of Brij 56 was used for NMR. Four kinds of proton peaks can be obtained in the spectra of Brij 56: the terminal methyl singlet (Ha', at δ 0.90), the internal methylene singlet (Hb', at δ 1.31), the methylene singlet which connects with oxygene of OE (Hc', at δ 1.59), and the sharp peak consisting of oxyethylene (OE) protons (at δ 3.47–3.71). Unlike Triton X-100, 1.5 mM of β-CD has negligible effect on Ha', Hb', and Hc' in 1.5 mM of Brij 56. Also, no obvious affection on all proton signals of β-CD was afforded by Brij 56.

3.4. Lifetime measurement

We measured the lifetime of **1** in the presence of 4 mM β-CD and various concentrations of Triton X-100. For comparison, the fluorescence lifetime of **1** at various concentration of Triton X-100 in the absence of β-CD was also measured (Table 1). As shown in Table 1, the lifetime of **1** in Triton X-100/β-CD system has an obvious change with adding Triton X-100. However, the fluorescence lifetime of **1** keeps unchanged at about 2.50 ± 0.05 ns with increasing the concentration of Brij 56 from 0 to 6 mM in the presence of 4 mM of β-CD.

4. Discussion

4.1. The competitive process of Triton X-100 and *1* interacting with β-CD

As can be seen from Fig. 3, the fluorescence intensity of **1** is first reduced to a minimum value, and then increases until a plateau is reached. This phenomenon can be interpreted by the fact that a competitive process exists in the Triton X-100/β-CD/**1** system. First, **1** is replaced from the hydrophobic cavity of β-CD by Triton X-100 causing the reduction in the fluorescence intensity of **1**. The fluorescence intensity of **1** reaches a minimum value at ca. 3.32 mM of Triton X-100, where the formation of the 1:1 and 1:2 inclusion complexes of Triton X-100 with β-CD reach an equilibrium and most of **1** is out of the β-CD cavity. After that, with further increasing the concentration of Triton X-100, the Triton X-100 micelle begins to form and **1** gradually enters into the hydrophobic microenvironment in the micelle. In this case, the fluorescence intensity of **1** increases again until the plateau is reached. In Fig. 3B, the inflexion shows the CMC of Triton X-100 in the presence of 4 mM of β-CD is ca. 3.95 mM. The reported values of CMC of Triton X-100 in the presence of 2 and 10 mM β-CD are ca. 2.25 and 7.25 mM, respectively

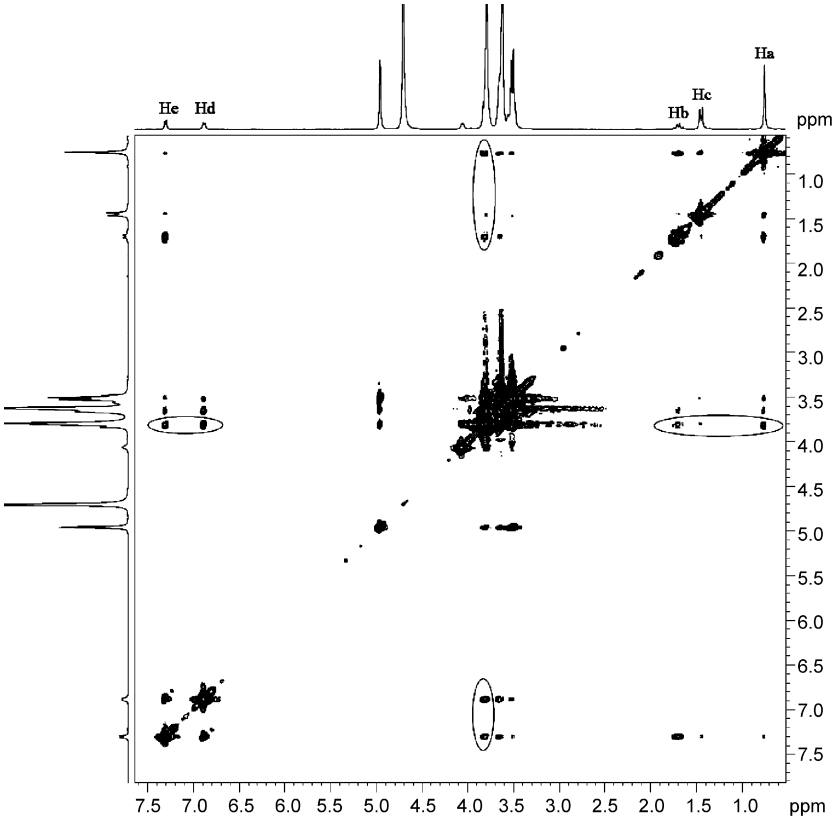


Fig. 8. 400 MHz 2D NOESY ^{1}H NMR of the interaction between 6.6 mM Triton X-100 and 10 mM β-CD.

Table 1
Lifetime of probe **1** (10^{-6} M) with different concentrations of Triton X-100 in the absence or presence of β-CD

[Triton X-100]$_0$ (mM)	[β-CD]$_0$ (mM)	τ_1 (ns)	B_1	f_1 (%)	τ_2 (ns)	B_2	f_2 (%)	χ^2
0	0	0.29	0.285	69.3	1.30	0.028	30.7	1.05
0.10	0	0.29	0.281	68.5	1.40	0.027	31.5	1.05
3.00	0	–	–	–	2.59	0.090	100	1.01
0	4.00	–	–	–	2.52	0.091	100	1.03
2.00	4.00	1.01	0.032	15.5	2.56	0.069	84.5	1.01
3.32	4.00	0.82	0.101	51.9	2.22	0.034	48.1	0.982
3.70	4.00	1.05	0.017	19.3	2.57	0.029	80.7	1.14
6.00	4.00	–	–	–	2.56	0.089	100	0.975

B_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i, $I(t) = \sum B_i \exp(-t/\tau_i)$, $f_i = (B_i\tau_i)/(\sum B_i\tau_i)$ and $\sum f_i = 1$.

[8]. Corresponding to the minimum value in Fig. 3B, a Triton X-100 molecule binds averagely with ca. 1.14 (=[CD]$_b$/[Triton X-100]$_0$ = 3.78 mM/3.32 mM, see Fig. 4A) β-CDs. That is to say the 1:1 complex is dominant and there may also exist the 1:2 inclusion complex.

The above competitive process can be confirmed by lifetime measurements. It is reported that the fluorescence lifetime of a substituted 3*H*-indole probe molecule increases when it transfers from polar to apolar solvent [34]. As can be seen from Table 1, **1** has two lifetimes in water. The similar phenomenon is also found in the literature [35]. For the short lifetime, i.e., 0.29 ns is ascribed to the formation of a nonemissive twisted intramolecular charge transfer (TICT) state in bulk water [35,42]. In the absence of β-CD, with increasing the concentration of Triton X-100, the short lifetime of **1** disappears and a longer lifetime occurs. This indicates that **1** transfers into the apolar micelle from water.

In the presence of 4 mM of β-CD but without Triton X-100, the sole long lifetime as shown in Table 1 indicates that **1** is located in the hydrophobic cavity of β-CD. However, with the addition of Triton X-100, a short lifetime appears and its proportion is increased. This process reflects that **1** is gradually driven out from the β-CD cavity by Triton X-100 and is exposed to bulk water. With further adding Triton X-100, the proportion of short lifetime is decreased. Finally, the sole long lifetime is exhibited. These phenomena imply that **1** enters an apolar environment again, i.e., the micelle of Triton X-100.

4.2. The binding type of Triton X-100 with β-CD

Many groups have studied the formation of the inclusion complex between Triton X-100 and β-CD. However, the association constants and types reported in the literature differ considerably (see Table 2) [5–14]. It can be seen from Table 2 that three types of inclusion complexes have been suggested by using different methods: (1) the 1:1 inclusion complex reported by Warner et al. [5,6], Eli and Chen [7], Bhattacharyya et al. [8], and Du et al. [9–11], respectively; (2) the 1:2 inclusion complex observed by Saito et al. [12] and Harada and Kamachi [14]; (3) the coexisting 1:1 and 1:2 inclusion complexes found by Buschmann et al. [13]. According to the literature, the Triton X-100/β-CD system was mainly studied using a probe molecule. Bhattacharyya et al. used 2,6-*p*-toluidinonaphthalene sulfonate (TNS) as a probe and assumed a 1:1 complex but not considering other binding types [8]. Du et al. employed probe phenolphthalein (PP) and reported on the 1:1 inclusion complex by absorption spectra [11]. Both Warner and Buschmann groups used Triton X-100 itself as a probe to investigate the Triton X-100/β-CD system by fluorescence spectra. However, the binding type and the association constant they obtained exhibited large discrepancy [6,13].

In this article, we report on the coexisting 1:1 and 1:2 inclusion complexes using both **1** and Triton X-100 itself as probes. Not only the binding type but also the association constants show agreement with the results obtained by **1** as a probe through a competitive method. Therefore, it is believed that **1** is a good candidate acting as a probe to investigate the interaction between cyclodextrin and a non-fluorescent guest molecule.

4.3. The binding site of Triton X-100 in the β-CD cavity

NMR is an effective method for studying the binding site of Triton X-100 in the β-CD cavity. Warner et al. have reported that the phenyl group and part of the hydrocarbon chain (*c*-CH_3 and *b*-CH_2) was included inside the CD cavity [6], whereas Saito et al. [12] and Du et al. [11] found that the entire hydrophobic moiety of Triton X-100 was included inside the cavity of β-CD. The disadvantage of above results was that the concentration of

Table 2
Association constants of inclusion complexes between Triton X-100 and β-CD

K'_1 (M^{-1})	K'_2 (M^{-1})	Method	Reference
145	–	Surface tension	[5]
3327	–	FLU	[6]
9100 ± 260	–	ITC	[7]
9400 ± 1300	–	FLU	[8]
$(1.8 \pm 0.02) \times 10^5$ [a]	–	RTP	[9]
3.05×10^4	–	Surface tension, FLU	[10]
$(1.82 \pm 0.15) \times 10^5$	–	FLU	[11]
–	5.8×10^6 [b]	Surface tension	[12]
5130	10.7	FLU	[13]
$(1.14 \pm 0.32) \times 10^5$	$(1.18 \pm 0.05) \times 10^3$	FLU[c]	Our work
$(1.13 \pm 0.05) \times 10^5$	$(3.71 \pm 0.27) \times 10^2$	FLU[d]	Our work

[a] Ternary complex Triton X-100/β-CD/1-BrN
[b] $kg^2\ mol^{-2}$.
[c] Molecule **1** as a fluorescent probe.
[d] Triton X-100 itself as a fluorescent probe.

Triton X-100 chosen for NMR was above CMC. To avoid the affection of micelles on NMR spectral change, 0.1 mM (<CMC) of Triton X-100 was employed. Our results exhibit shifts of the proton signals (H*a*–H*e*) of Triton X-100 (see Fig. 6) and upfield shifts of H-3, H-5 and H-6 in β-CD (see Fig. 7), which strongly suggest that the entire hydrophobic part including the phenyl ring of Triton X-100 is accommodated into the cavity of β-CD. This result is confirmed by 2D NOESY NMR spectra (see Fig. 8), where H*a*, H*b*, H*c*, H*d* and H*e* in Triton X-100 have strong interaction with H-3 and H-5 in β-CD (the signals are marked with ellipses).

From NMR spectra, it is difficult to get useful information on the CH_2CH_2O units in Triton X-100 interacting with β-CD because the signals of the protons in CH_2CH_2O units are broad and overlap with that in β-CD. Harada et al. reported that poly(propylene glycol) but not poly(ethylene glycol) could form polyrotaxane with β-CD [43]. In aqueous solution, the oxygen in hydrophilic ether acts as an acceptor in the hydrogen bond with the aqueous interface, whereas the apolar ethylene units are thought to reside in the interstitial cavities of the highly structured liquid water [29]. Thus, unlike the hydrophobic interaction and the hydrogen bonding effect, the solvation energy effect is unfavorable to the formation of inclusion complexes of ethylene glycol units with β-CD. In our previous study, it was found that the binding types between three substituted 3*H*-indoles including 1–3 CH_2CH_2O units and β-CD changed from 1:3 to 1:1 with increasing the number of CH_2CH_2O units [29]. The thermodynamic parameters ΔH, ΔS and ΔG estimated strongly suggested that CH_2CH_2O units were energetically unfavorable to interact with β-CD [29].

To further confirm our results, we compared the interaction between Brij 56 and β-CD with that between Triton X-100 and β-CD. The structure of Brij 56 is similar to that of Triton X-100 and the difference exhibits in the hydrophobic part, that is, Brij 56 has only a long alkyl chain, whereas Triton X-100 has a phenyl ring besides the alkyl chain.

As can be seen from Fig. 3B, the fluorescent behavior of **1** in the Brij 56/β-CD system is greatly different from that in the Triton X-100/β-CD system. For both Triton X-100 and Brij 56 have the same hydrophilic part (10 units of CH_2CH_2O), the above difference should come from the different hydrophobic parts. Obviously, the hydrophobic part of Brij 56 should interact weakly with β-CD. This can be confirmed by the result of NMR, where the shift in the proton signals of Ha', Hb', and Hc' in Brij 56 is negligible with the addition of β-CD.

5. Conclusions

The interaction between Triton X-100 and β-CD was studied by absorption, steady-state fluorescence measurements, fluorescence lifetime and NMR. Using molecule **1** as a fluorescence probe through a competitive method, the coexisting 1:1 and 1:2 inclusion complexes were obtained, and the K_1' and K_2' were estimated to be $(1.14 \pm 0.32) \times 10^5$ and $(1.18 \pm 0.05) \times 10^3\ M^{-1}$, respectively. The results were further confirmed by Triton X-100 itself as a probe, and the K_1' and K_2' were estimated to be $(1.13 \pm 0.05) \times 10^5$ and $(3.71 \pm 0.27) \times 10^2\ M^{-1}$. The association types and constants estimated show agreement. Meanwhile, the obtained CMC of Triton X-100 and the association constant between **1** and Triton X-100 were $(1.7 \pm 0.2) \times 10^{-4}$ M and $12200 \pm 300\ M^{-1}$, respectively. Our work demonstrates that molecule **1** is a very sensitive fluorescence probe for studying the interaction of a non-fluorescent guest with cyclodextrin. From ^{1}H NMR and 2D NOESY NMR, it was also found that the entire hydrophobic part of Triton X-100 was located in the β-CD cavity in the inclusion complexes. Unlike the Triton X-100, the interaction between Brij 56 and β-CD was weak.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 90206020). Y. He is thankful of Dr. Aihua Wu and Miss Pei Fu for their generous help. Also, Y. He thanked Jingxin Yang and Dr. Xianrong Guo (Beijing NMR Center) for their help with NMR.

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