Studies of the Inclusion Complexation between a 3H-Indole and â-Cyclodextrin in the Presence of Urea, Sodium Dodecyl Sulfate, and 1-Propanol

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The interaction of 2-(p-(methylamino)phenyl)-3,3-dimethyl-5-carboethoxy-3H-indole (4) with â-cyclodextrin (â-CD) in the presence of urea, sodium dodecyl sulfate (SDS), and 1-propanol, respectively, were studied by absorption and steady-state fluorescence measurements. It was found that the addition of urea gives rise to a remarkable decrease in K₁, the association constant for the 1:1 (guest:host) inclusion complex, and a much more decrease in K_2 , the association constant for the 1:2 inclusion complex. The linear correlations between ln K₂ and molecular volumes of some 3H-indoles in the absence and presence of urea, respectively, indicate the hydrophobic nature of the interaction of $\mathbf{4}$ with either \hat{a} -CD or urea. This result also shows that the urea effect study is an effective way to characterize the hydrophobic nature of the interactions between cyclodextrins (CDs) and guest molecules. Using 4 as a fluorescent probe, the interaction of SDS with \hat{a} -CD was investigated through a competitive method. The formation of 1:1 and 1:2 inclusion complexes between SDS and \hat{a} -CD was confirmed. The association constants K_1 for the 1:1 complex and K_{2} for the 1:2 complex were estimated to be 26800 ± 2500 and 440 ± 90 M⁻¹, respectively. Effect of 1-propanol on the inclusion complexation between 4 and â-CD was also investigated. The mechanism that 1:1:1 but not 1:2:2 ternary complexes of **4**, 1-propanol, and \hat{a} -CD are formed was demonstrated to be operative by a nonlinear regression routine. On the addition of either urea, SDS, or 1-propanol into the $\mathbf{\hat{4}}$ - \hat{a} -CD binary system, the fluorescence quantum yields (...F) are significantly reduced suggesting that $\mathbf{4}$ transfers from the relatively nonpolar environments to polar environments.

1. Introduction

Cyclodextrins (CDs) are toroidally shaped cyclic oligosaccharides, mostly consisting of six, seven, and eight glucose units for α -CD, \hat{a} -CD, and \hat{c} -CD, respectively. Their relatively nonpolar cavities are capable of accommodating a variety of molecules to form inclusion complexes. So far, various kinds of inclusion complexes have been reported in the literature:¹ (1) binary complexes in a stoichiometry of 1:1, 1:2, 2:1, 2:2, or 1:3; (2) ternary complexes of 1:1:1, 1:1:2, and 1:2:2 (guest A:guest B:host); (3) supramolecular assemblies such as catenanes, rotaxanes, and polyrotaxanes, nanotubular structures, or threaded cyclodextrins. Noncovalent intermolecular forces are believed to play a key role in the complex formation and its stabilization. This property leads to widespread applications in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis.² Thus, great efforts have been spurred to understand the inclusion complexation between cyclodextrins and guest molecules under different conditions.

Interest in the effect of urea on the formation of inclusion complexes has grown recently.8-10 Breslow and Halfon8 suggested that the decrease of the association constant of

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guest molecules with CDs in the presence of urea results from its antihydrophobic nature. Calorimetric studies performed by Godinez and co-workers⁹ indicate that urea induces increases in ¢ G, ¢ H, and ¢ S values accompanying the formation of the complex between trimethyl(ferrocenylmethyl)ammonium cation and â-CD, which was ascribed to the destabilization effect of urea. Very recently, we performed a quantitative study of the interaction mechanism between urea and some substituted 3H-indoles in sodium dodecyl sulfate (SDS) micelles and aqueous solutions of \hat{a} -CD.¹⁰ Some experimental evidence was presented to confirm that hydrophobic interactions of urea with 3H-indoles should be responsible for the marked reduction in the association constants between 3H-indoles and the SDS micelle or â-CD.10

Inclusion complexes formed between surfactants and CDs have also received much attention,¹¹⁻¹⁷ partly because these systems can be used to mimic the effect of cyclodextrins on phospholipids, a major constituent of cell membrane.11 The hydrophobic chains of surfactants tend to reside in the hydrophobic cavity of cyclodextrins. For ordinary surfactants, 1:1 and 1:2 surfactant-cyclodextrin complexes are believed to exist.^{11–17} Very recently, we reported the 1:3 type complex formed by a fluorescent cationic surfactant and \hat{a} -CD.¹ However, there exist large

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discrepancies of association constants for some surfactant-CD systems in the literature, a typical example of which is the SDS- \hat{a} -CD system.¹² While several experiments are reliable in studies through 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.¹² By contrast, competitive binding studies using fluorescence,^{14,15} UV and visible probes,¹⁶ and emf methods¹⁷ gave reasonable results since they were based on better theoretical models.¹² Thus, we think that reliable methods, especially the fluorescence method are worth studying further to obtain reasonable association constants between surfactants and CDs.

The formation of ternary complexes between CDs and two different guest molecules has been also extensively studied.¹⁸⁻²⁶ Addition of third components such as alcohols, alkyl sulfates, and amino acids can dramatically alter the apparent association constants between CDs and guest molecules, e.g., pyrene, ^{19,23,26} naphthalene derivatives, ^{18,24} acenaphthene,²⁵ acridine,²¹ azulene,²² and xanthone.²⁰ When an appropriate third component is added into a binary system where 1:1 and 1:2 complexes coexist, it will interact with either the 1:1 complex, the 1:2 complex, or both. As a result, either the 1:1:1 complex, the 1:2:2 complex, or both are possibly formed. It is noted from the literature^{23,26} that for the pyrene–alcohol– \hat{a} -CD ternary system, different conclusions have been drawn about the stoichiometry of the ternary complex. Thus, it is very interesting to investigate an appropriate ternary system to find out which kind of ternary complex is formed.

In the past few years, our research group has been focused on the study of fluorescent substituted 3H-indoles in various environments.²⁷⁻³⁷ So far, we have probed successfully the mean structural properties of aqueous micelles, 33, 34, 36 reversed micelles, 35 and surfactant vesicles. 37 Very recently, we started a research program on the formation of inclusion complexes between cyclodextrins and some 3H-indoles, i.e., $2 \cdot (p$ -aminophenyl)-3,3-dimethyl-5-cyano-3H-indole (1),^{38,39} $2 \cdot (p \cdot (dimethylamino)phenyl)$ -3,3-dimethyl-5-cyano-3*H*-indole (2),^{38,39} 2-(*p*-aminophenyl)-

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Figure 1. Molecular structures of the substituted 3H-indoles.

3.3-dimethyl-5-carboethoxy-3H-indole (3),¹⁰ 2-(p-(dimethylamino)phenyl)-3,3-dimethyl-5-carboethoxy-3H-indole (5),10 and iodotrimethyl(2-(p-(hexylamino)phenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole)ammonium (6).¹ It was found that 1:1 and 1:2 complexes are formed between cyclodextrins and 1, 2, 3, or 5. In the case of 6, the formation of a rotaxane-like 1:3 inclusion complex was reported for the first time.¹

The aim of this paper is manifold. First, we aim to study the formation of inclusion complexes between 2-(p-(methylamino)phenyl)-3,3-dimethyl-5-carboethoxy-3H-indole (4) (see Figure 1) and â-CD in the absence and presence of urea, respectively. A spectral and photophysical study of 4 in surfactant micelles and vesicles has been done in our research group.⁴⁰ To shed more light on the urea action on inclusion complexation, we have also estimated the association constants between \hat{a} -CD and 1 and **2** in the presence of urea. Together with the previous data of 1 and 2 in the absence of urea,³⁸ and of 3 and 5 both in the absence and presence of urea,¹⁰ one can further understand the hydrophobic interactions between urea and 3*H*-indoles in terms of the possible dependence of ln Kon the molecular volume. Moreover, on the basis of this kind of study, a simple and effective method can be established to characterize the hydrophobic nature of the interaction between CDs and the guest molecules which have hydrophobic interactions with urea. The second goal of this paper is to investigate the formation of inclusion complexes between SDS and \hat{a} -CD using 4 as a fluorescence probe. Although this method has been demonstrated to be very effective in studying the interactions of surfactants with CDs as mentioned above, this kind of work is rather scarce in the literature. The third objective of this paper is to study the possible formation of the ternary complex between 4, \hat{a} -CD, and 1-propanol and further characterize its stoichiometry, that is, 1:1:1 or 1:2:2. Finally, on the basis of above work, we want to compare the mechanisms of the influence of urea, SDS, and 1-propanol on the inclusion complexation between **4** and \hat{a} -CD.

2. Experimental Section

2.1. Materials. The synthesis and purification of the substituted 3*H*-indoles (see Figure 1) were done according to the modified methods of Skrabal et al.⁴¹ and was reported by Popowycz.⁴² Analytical grade reagents 1-propanol, sodium hydroxide, methanol, urea, â-CD (Aldrich), and electrophoresis purity reagent SDS (Bio-Rad Laboratories, CA) were used as received.

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Figure 2. Absorption (right) and fluorescence (left) spectra (normalized according to the respective absorption maximum) of **4** in various environments (pH = 9.5): (A) water (solid), 0.4 mM \hat{a} -CD (dash), 4 mM \hat{a} -CD (dot); (B) 5 M urea (solid), 0.4 mM \hat{a} -CD + 5 M urea (dash), 4 mM \hat{a} -CD + 5 M urea (dot); (C) 2 mM SDS + 4 mM \hat{a} -CD (solid), 4 mM SDS + 4 mM \hat{a} -CD (dot); (D) 0.1 mM 1-propanol + 0.4 mM \hat{a} -CD (solid), 0.4 mM 1-propanol + 0.4 mM \hat{a} -CD (dot); (D) 0.1 mM 1-propanol + 0.4 mM \hat{a} -CD (solid), 0.4 mM 1-propanol + 0.4 mM \hat{a} -CD (dot).

 Table 1.
 Spectral Characteristics and Quantum Yields of 4 in Various Environments at pH = 9.5

medium	$V_{\rm A}{}^a$ (cm ⁻¹)	ϵ^{b} (M ⁻¹ cm ⁻¹)	$\mathbf{\hat{1}}_{\mathrm{F}}^{c}$ (cm ⁻¹)	Stokes shift (cm ⁻¹)	fwhm _A (cm ⁻¹)	fwhm _F (cm ⁻¹)	•••F
water	26 400	30 200	20 300	6100	5400	3100	0.014
0.4 mM â-CD	26 400	30 300	20 600	5800	5300	3400	0.18
4 mM â-CD	26 500	33 500	20 700	5800	5200	3400	0.37
5 M urea	26 200	35 100	20 500	5700	5300	3400	0.022
0.4 mM <i>â</i> -CD + 5 M urea	26 200	35 000	20 500	5700	5200	3400	0.048
$4 \text{ mM} \hat{a}$ -CD + 5 M urea	26 400	34 500	20 700	5700	5200	3500	0.25
$4 \text{ mM} \hat{a}$ -CD + 2 mM SDS	26 500	34 100	20 700	5800	5200	3400	0.31
$4 \text{ mM} \hat{a}$ -CD + 4 mM SDS	26 400	33 500	20 500	5900	5400	3300	0.068
$4 \text{ mM} \hat{a}$ -CD + 6 mM SDS	26 300	35 400	20 300	6000	5400	3200	0.029
6 mM SDS	26 200	31 300	20 300	5900	5200	3100	0.024
$0.4 \text{ mM} \hat{a}$ -CD + 0.1 mM	26 400	35 400	20 600	5800	5300	3400	0.082
1-propanol							
$0.4 \text{ mM} \hat{a}$ -CD + 0.4 mM	26 400	37 200	20 400	6000	5300	3300	0.036
1-propanol							
$0.4 \text{ mM} \hat{a}$ -CD + 1 mM	26 400	37 600	20 300	6100	5300	3100	0.021
1-propanol							
1 mM 1-propanol	26 300	37 500	20 200	6100	5400	3100	0.025

^{*a*} Absorption wavenumber taken at the center of mass of the absorption band. ^{*b*} Molar absorption coefficient at the peak intensity maximum. ^{*c*} Fluorescence wavenumber taken at the center of mass of the fluorescence band.

2.2. Instruments. Absorption spectra were recorded on a Cary 1 Bio UV-vis spectrophotometer using 1 cm quartz cells. Fluorescence spectra corrected for the emission detection were measured on a Spex Fluorolog-2 spectrofluorometer. The excitation and emission bandpasses used were 2.6 and 1.9 nm, respectively. Each solution was excited near the absorption wavelength maximum using 1 cm path quartz cells. All corrected fluorescence excitation spectra were found to be equivalent to their respective absorption spectra.

2.3. Methods. Fresh sample solutions were used in the absorption and fluorescence measurements. The pH values of all the solutions in this study were adjusted to 9.5 by adding NaOH, and no buffers were used. For **1**, **2**, and **4**, the concentrations of absorption and fluorescence measurements were 10^{-5} and 10^{-6} M, respectively. Stock solutions of **1**, **2**, and **4** were prepared in methanol. The fluorescence quantum yields of the various species were measured using 2-(p-dimethylamino)-phenyl-3,3-dimethyl-3*H*-indole²⁷ as a standard in methanol (...F = 0.24). All measurements were carried out at room temperature.

3. Results and Discussion

3.1. Spectral Characteristics. The absorption and fluorescence spectra of **4** in various environments are

shown in Figure 2, and the spectral characteristics of **4** are summarized in Table 1.

It is noted that in all cases the change of the absorption wavenumber is very small if any. The $\dots_{\rm F}$ value greatly increases going from water to aqueous solutions of \hat{a} -CD in both the absence and presence of urea suggesting that 4 moves from water to a less aqueous site where its TICT state^{30,31} is no longer stabilized. Conversely, ...F values remarkably decrease with increasing SDS and 1-propanol concentrations in the presence of 4 and 0.4 mM \hat{a} -CD, respectively, showing that 4 transfers to a more aqueous environment. When ... F values in the presence of urea are compared with those in the absence of urea at same â-CD concentrations, one also observes an obvious decrease. This phenomenon can be rationalized by the hydrophobic interaction between urea and 4.10 Small blueshifts and small increases in the fwhm_F values of the fluorescence bands going from water to aqueous solutions of \hat{a} -CD are consistent with the changes of ..._F values. On the addition of SDS and 1-propanol, small red-shifts and

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substrate spectively, s for 1:1 and ues of the asy the use of a according to the

 $(L_{1})_{0}^{2})/(1 + K_{1}[CD]_{0} + K_{1}K_{2}[CD]_{0}^{2})$ (3)

c initial concentration of \hat{a} -CD, that of the fluorescent substrate for the total fluorescence intensity CD solutions while I_0 , I_1 , and I_2 stand e intensities of the substrate in pure 1 complex and in the 1:2 complex,

shows the relative fluorescence intensity as a non-of-the initial concentration of \hat{a} -CD. The number regression fit illustrated by the full line converges well with a correlation coefficient $r^2 = 0.9997$. The estimated values of I_1/I_0 and I_2/I_0 are 9.5 ± 3.8 and 29.3 ± 0.3 , respectively. The values of K_1 and K_2 are listed in Table 2.

3.3. Urea Effect on Association Constants (K_1, K_2). We have measured the association constants of **4** at [urea] = 3 and 5 M, respectively. It was found that the fits according to eq 3 give satisfactory results (values of the variables, standard errors, 95% confidence intervals, correlation coefficient, and absolute sum of square) in the presence of urea (figures not shown). The obtained K_1 and K_2 values are also listed in Table 2. To gain a better

Figure 3. (A) Plot of the relative fluorescence intensity versus the initial concentration of \hat{a} -CD for **4** complexed to \hat{a} -CD. The full line is the nonlinear regression fit to the experimental data points following eq 3. (B) Plot of ln K_2 as a function of the molecular volume in the absence and presence of 3 M urea, respectively, for **1** to **5** complexed to \hat{a} -CD.

understanding of the mechanism of urea action on inclusion complexation, we have also measured K_1 and K_2 values of **1** and **2** at [urea] = 3 and 5 M, respectively. In the case of **2** with 3 M urea, reasonable results were given by the fit according to eq 3. However, for **1** at [urea] = 3 and 5 M, and for **2** at [urea] = 5 M, the fits according to eq 3 do not work. Similar to the situation of **3** at 5 M urea,¹⁰ we assume that only 1:1 complexes are formed in these cases, i.e., $K_2 = 0$. Thus, eq 3 becomes

$$I = (I_0 + I_1 K_1 [CD]_0) / (1 + K_1 [CD]_0)$$
(4)

If the above assumption is true, the fits according to eq 4 should give reasonable results. Also, the Benesi–Hildebrand type double reciprocal plot⁴⁴ should be obtained, i.e., $1/(I - I_0)$ vs $[CD]_0^{-1}$ should exhibit a straight line.

The results indicate that the fits according to eq 4 indeed give reasonable results for **1** at 3 and 5 M urea, respectively, and for **2** at 5 M urea (figures not shown). In addition, the straight lines of $1/(I - I_0)$ against $[CD]_0^{-1}$ can be obtained in these situations (figures not shown), which supports that only the 1:1 complex is formed.

All the data for **1** to **5** at 0, 3, and 5 M urea are included in Table 2. It can be seen that the K_1 value is substantially reduced by the presence of urea and the K_2 value is reduced to a greater extent. A similar phenomenon was observed before and the reason has been given.¹⁰

Figure 3B illustrates the plots of $\ln K_2$ as a function of V_m , the molecular volume, in the absence and presence of

⁽⁴³⁾ Graph Pad Prism, Version 1.0, Graph Pad Software Inc.

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Table 2. Molecular volumes $(V_m)^a$ of 1 to 5 and the Association Constants (K_1, K_2) for Their Neutral Forms Complexed to \hat{a} -CD

		$K_1/M^{-1}, K_2/M^{-1}$				
molecule	$V_{ m m}/{ m \AA}^3$	[urea] = 0 M	[urea] = 3 M	[urea] = 5 M		
1 2 3 4	332 382 388 414	$\begin{array}{c} 750\pm120,^{b}760\pm60^{b}\\ 1430\pm220,^{b}1880\pm150^{b}\\ 840\pm130,^{c}2660\pm210^{c}\\ 1380\pm280,3230\pm260 \end{array}$	$\begin{array}{c} 110 \pm 10 \\ 1160 \pm 550, 500 \pm 50 \\ 810 \pm 370, ^c 660 \pm 80^c \\ 590 \pm 210, 1000 \pm 130 \end{array}$	$110 \pm 10 \\ 220 \pm 10 \\ 170 \pm 20^c \\ 430 \pm 290 510 \pm 130$		
5	441	$1000 \pm 350, c 5070 \pm 460^{c}$	780 ± 130 , ^c 1260 ± 60^{c}	$540 \pm 160, c 720 \pm 50^{\circ}$		

^a Taken from ref 34. ^b Taken from ref 38. ^c Taken from ref 10.

3 M urea, respectively. The plots exhibit straight lines in both cases. This kind of dependence of $\ln K$ (or standard transfer free energy) on molecular volume is taken as strong evidence of a hydrophobic interaction.³⁴ In the CTAB micelle, a very good linear correlation has been also obtained between the standard transfer free energy of seven 3*H*-indoles including **1** to **5** as a function of their molecular volumes.³⁴ It is also noted from Figure 3B that the slope of the straight line in the absence of urea (0.017 ${\rm \AA}^{-3}$) is approximately equal to that in the presence of urea (0.015 Å^{-3}) . Thus, it is quite safe to say that the interactions of 1 to 5 with either â-CD or urea are hydrophobic in nature. More importantly, the above results show that the urea effect study is an effective way to confirm the hydrophobic nature of the interaction between CDs and the guest molecules interacting with urea by hydrophobicity,¹ which is a necessary condition since in some situations the guest molecules lie outside the cavity to form lidlike association compounds other than inclusion complexes with cyclodextrins.7,45

It should be pointed out here that the plots of ln K_1 as a function of V_m do not exhibit straight lines (figures not shown). This shows that the solvent effect exists to a certain extent in the 1:1 complex while the hydrophobic effect is also playing the key role in the stabilization of this complex.¹⁰

3.4. Study of SDS- \hat{a} -CD Complexes Using 4 as a **Probe.** Figure 4A shows the relative fluorescence intensity (I/I_0) at varying initial concentrations of SDS ([SDS]₀) ranging from 0 to 6 mM. The \hat{a} -CD concentration was fixed to be 4 mM at which the fluorescence intensity reaches a plateau (see Figure 3A) showing that most molecules of 4 exist in 1:2 complexes, and thus the ternary complex between 4, SDS, and \hat{a} -CD can be assumed not to form. Using the K_1 , K_2 , I_1/I_0 , and I_2/I_0 , the values of the equilibrium concentrations of \hat{a} -CD, i.e., [CD], at different [SDS]₀ can be evaluated according to eq 3, from which the concentrations of \hat{a} -CD binding with SDS ([CD]_b) can be also estimated from the relationship [CD]_b = [CD]₀ – [CD]. The results of [CD]_b as a function of [SDS]₀ are also shown in Figure 4A.

It can be seen from Figure 4A that at $[SDS]_0 < 4$ mM, $[CD]_b$ is always slightly larger than $[SDS]_0$. This is an indication that the 1:2 complex between SDS and \hat{a} -CD is also formed while the 1:1 complex is predominant.^{14,15} Thus, one can consider the following stepwise equilibria

$$SDS + CD \stackrel{K_1'}{\longleftrightarrow} SDS - CD$$
 (5)

$$SDS-CD + CD \stackrel{K_2'}{\longleftrightarrow} SDS-(CD)_2$$
 (6)

where SDS-CD and SDS-(CD)₂ represent the 1:1 and 1:2 complexes, respectively, the association constants of which are K_1' and K_2' . When (5) and (6) are combined and



Figure 4. (A) Relative fluorescence intensity and the concentration of surfactant-bound \hat{a} -CD as a function of the initial concentration of SDS. (B) Plot of the initial concentration of SDS against the equilibrium concentration of \hat{a} -CD. The full line is the nonlinear regression fit to the experimental data points following eq 7.

the mass balances of SDS and \hat{a} -CD are used, [SDS]₀ is related to [CD] by the following equation^{14,15}

$$[SDS]_{0} = (1 + K_{1}'[CD] + K_{1}'K_{2}'[CD]^{2})([CD]_{0} - [CD])/(K_{1}'[CD] + 2K_{1}'K_{2}'[CD]^{2})$$
(7)

On the basis of eq 7, the parameters K_1' , and K_2' are estimated to be 26800 \pm 2500, and 440 \pm 90 M⁻¹, respectively, by NLR analysis. Figure 4B shows the satisfactory fit with a correlation coefficient $r^2 = 0.98$. Moreover, the obtained values of K_1' and K_2' are very close to those reported by Park and Song,¹⁴ i.e., 25 600 and 200 M⁻¹, which are regarded as some of the most reliable values in the literature.¹² This suggests that **4** is a good candidate to study the association constants between cyclodextrins and surfactants and other nonfluorescent molecules. It is believed that **1**, **2**, **3**, and **5** are also good candidates for the same purpose due to their similar behaviors to that of **4** in aqueous solutions of \hat{a} -CD.^{10,38,39}

3.5. Formation of the Ternary Complex of 4, \hat{a} -CD, and 1-Propanol. Provided that the 1:1:1 ternary complex between 4, \hat{a} -CD and 1-propanol is formed, one should consider the following equilibria^{25,26} besides those

where Pr, Pr–CD, and Pr–SCD represent 1-propanol, the 1:1 complex between 1-propanol and \hat{a} -CD, and the ternary complex of **4**, 1-propanol, and \hat{a} -CD, respectively. K_3 , K_4 , and K_5 denote the association constants for the formation of Pr–SCD from Pr and SCD, of Pr–CD, and of Pr–SCD from S and Pr–CD, respectively. Note that there are only four independent equilibria among (1), (2), and (8) to (10) since (1) + (8) = (9) + (10). As far as the fluorescence intensity is concerned, only (1), (2), and (8) should be considered. Thus, the following equations are obtained



where I_3 is the fluorescence intensity in the ternary complex Pr–SCD. Since the average value of K_4 taken from the literature^{46–48} is only about 4 M⁻¹, it is reasonable to replace [CD] and [Pr] in (11) to (14) with [CD]₀ and [Pr]₀ (initial concentration of 1-propanol), respectively.

Figure 5A illustrates I/I_0 as a function of $[Pr]_0$, the full line of which is the result of NLR analysis using the known values of K_1 , K_2 , I_1/I_0 , and I_2/I_0 . The fit converged well with a correlation coefficient $r^2 = 0.98$. The values of K_3 and I_3/I_0 are estimated to be 40 300 ± 4500 M⁻¹ and 1.7 ± 0.3, respectively. According to the relationship $K_1K_3 =$ K_4K_5 , one obtains $K_5 = (1.4 \pm 0.4) \times 10^7 \text{ M}^{-1}$. A similar method of obtaining association constants for ternary complexes has been used before.^{25,26}

Figure 5B shows the plot of 1/[(A/B) - I] as a function of $[Pr]_0^{-1}$ according to eq 12. A good straight line (r = 0.997) is obtained further supporting the formation of the 1:1:1 ternary complex.

To ascertain whether the 1:2:2 complex exists, we have also considered the following two cases: (1) 1:1:1 and 1:2:2 complexes coexist; (2) only the 1:2:2 complex exists. In the former case, the NLR analysis does not give a reasonable result. In the latter case, the result of the NLR analysis is improved with respect to that in the former case, but is much worse than that according to eq 11. To further confirm that the latter case does not occur, one obtains the following equation: where K_6 denotes the association constant for the 1:2:2 complex and I_4 is the fluorescence intensity of the substrate in this complex. Figure 5B shows the plot of 1/[(A/B) - I] against $[Pr]_0^{-2}$ based on eq 15, which does not exhibit a straight line. In addition, the decrease of the fluorescence intensity and quantum yield induced by 1-propanol seems not consistent with the possible structural feature of the 1:2:2 complex. Thus, the possibility of the formation of the 1:2:2 complex can be ruled out.

On the basis of the association constants obtained, the molar fraction of **4** in the 1:1:1 ternary complex at $[Pr]_0$ = 1 mM is evaluated to be about 0.91 while those in water, in the 1:1 complex, and in the 1:2 complex are 0.04, 0.02, and 0.03, respectively. This is in good agreement with the above anticipation according to the spectral characteristics and the ... F value. It should be mentioned that the very low value of I_3/I_0 with respect to the values of I_1/I_0 and I_2/I_0 is also in agreement with the low ..._F value of **4** in the ternary complex at $[Pr]_0 = 1$ mM. These results seem to suggest that in the ternary complex, the anilino moiety of **4** is no longer incorporated into the \hat{a} -CD cavity so that the TICT state of 4 is stabilized. In our previous studies, 10,33,34,40 it was found that the $\ldots_{\rm F}$ values of $\boldsymbol{1}$ and 5 in SDS or CTAB micelles are very high, which could be interpreted in terms of the fact that the ends of the anilino moieties of these molecules are partly incorporated into the interface of the micelles.¹⁰ However, further studies should be carried out to obtain a clear picture of the structure of the 1:1:1 ternary complex.

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4. Concluding Remarks

The following conclusions can been drawn: (1) The interactions of **4** with either \hat{a} -CD or urea are hydrophobic and the urea effect study is an effective method of characterizing the hydrophobic nature of the interaction between CDs and guest molecules. (2) Both 1:1 and 1:2 complexes between SDS and \hat{a} -CD are formed, and **4** is qualified to be a good fluorescent probe to study the formation of the complexes between \hat{a} -CD and surfactants and other nonfluorescent guest molecules. (3) The 1:1:1 but not the 1:2:2 ternary complex between **4**, 1-propanol, and \hat{a} -CD is formed. (4) The presence of urea, SDS, and 1-propanol causes **4** to escape from the cavity of \hat{a} -CD with different mechanisms; i.e., urea interacts with **4** through hydrophobic binding, SDS competes with **4**

through the formation of inclusion complexes with \hat{a} -CD, and 1-propanol is breaking down the 1:2 complex of **4** and \hat{a} -CD to form the 1:1:1 ternary complex.

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