Spectral and Photophysical Studies of the 1:3 (Guest/Host) Rotaxane-like Inclusion Complex Formed by a 3*H*-Indole and *β*-Cyclodextrin

Xinghai Shen,† Michel Belletête, and Gilles Durocher*

Département de Chimie, Université de Montréal, C.P. 6128, Succ. A, Montréal, Québec, H3C 3J7, Canada

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In the present paper, we report a new type of rotaxane-like inclusion complex, i.e., the 1:3 (guest/host) type formed by iodotrimethyl 2-(*p*-hexylaminophenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole ammonium (**1**), a fluorescent cationic surfactant, and *β*-cyclodextrin (*β*-CD). The time-resolved fluorescence measurement reveals that only two species of **1** exist within the whole range of *β*-CD concentrations. Both the steady-state and the time-resolved fluorescence results show that the stoichiometry of the inclusion complex is 1:3. The urea effect on the formation of the inclusion complex has been investigated. It was found that the 1:3 inclusion complex is still formed in the presence of 3 M urea, while the association constant is markedly reduced. This suggests the hydrophobic nature of the interaction of **1** with either *β*-CD or urea.

1. Introduction

Cyclodextrins (CDs) are toroidally shaped cyclic oligosaccharides, mostly consisting of six, seven, and eight glucose units for α-CD, *β*-CD, and *γ*-CD, respectively. Their hydrophobic cavities enable them to accommodate various kinds of molecules to form inclusion complexes, which leads to widespread applications in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis.[1−6] It is because of this reason that the investigations on the formation of the inclusion complexes have been the focus of great efforts in cyclodextrin chemistry. The 1:1 and 1:2 (guest/host) inclusion complexes are the most common types. Complexes in a stoichiometry of 2:1[6] and 2:2[6,7] can be also formed. Interestingly, ternary complexes of 1:1:1,[8] 1:1:2,[9] and 1:2:2[10] (guest A/guest B/host) have been reported. Under appropriate conditions, supramolecular assemblies such as catenanes,[11] rotaxanes,[12] and polyrotaxanes,[13] nanotubular structures,[14,15] or threaded cyclodextrins[16] that do not involve any covalent bonding between the cyclodextrin and the other molecule can be obtained. However, to the best of our knowledge, 1:3 inclusion complexes have not been reported in the literature.

In the past few years, our research group has been focused on the study of some fluorescent substituted 3*H*-indoles in various environments.[17−27] So far, we have probed successfully the mean structural properties of aqueous micelles,[23,24,26] reversed micelles,[25] and surfactant vesicles.[27] Very recently, we started a research program on the formation of inclusion complexes between cyclodextrins and some 3*H*-indoles, i.e., 2-(*p*-aminophenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole (**2**),[28] 2-(*p*-methylaminophenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole (**3**),[29] 2-(*p*-dimethylaminophenyl)-3,3-dimethyl-5-carboethoxy-3*H*-indole (**4**),[28] 2-(*p*-aminophenyl)-3,3-dimethyl-5-cyano-3*H*-indole (**5**),[30,31] and 2-(*p*-dimethylaminophenyl)-3,3-dimethyl-5-cyano-3*H*-indole (**6**).[30,31] The measurements through excited-state dynamics and steady-state absorption and fluorescence spectroscopy show that two types of complexes, i.e., 1:1 and 1:2 types, are formed. In the 1:2 complex, the 3*H*-indole is totally entrapped in a hydrophobic environment except for the junction of the two cyclodextrins, where the indolic nitrogen is close to the secondary rim of the macrocycles.[30]

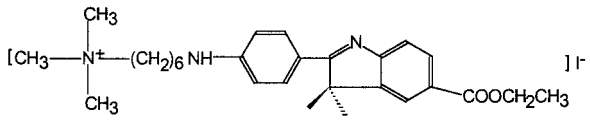

Figure 1. Molecular structure of **1**.

In this paper, a new member in the family of 3*H*-indoles; that is molecule **1** (Figure 1) is used to study the formation of the inclusion complex with *β*-CD. While the anilino moiety and the indolic moiety of **1** can fit into the cavities of cyclodextrins, respectively, the hydrophobic hexyl group is another possible site entrapped in a cyclodextrin cavity since its length is close to that of cyclodextrin (7.9 Å), thus forming a rotaxane structure. The prime objective of the present paper is to confirm the formation of the 1:3 inclusion complex between **1** and *β*-CD.

For the purpose of understanding the mechanism of urea as a strong denaturant of proteins,[32] there has been growing interest in studying the effect of urea on organized assemblies, such as micelles,[33] reversed micelles,[34] vesicles,[33a] monolayers,[35] polymers,[36] and cyclodextrins.[37,38] We have extensively studied the effect of urea on the interactions between some 3*H*-indoles and *β*-CD.[28,29] It was found that, on the addition of urea, the association constant of the 1:1 complex decreases remarkably and that of the successive 1:2 complex decreases much more. The formation of 1:2 complexes is inhibited completely for **2** and **6** at [urea] ≥ 5 M and for **5** at [urea] ≥ 3 M, respectively.[28,29] This phenomenon has been attributed to the hydrophobic interactions between urea and 3*H*-indoles.[28,29] Hydrophobic interactions between urea and aromatic hydrocarbons, alcohols, alkanes, amino acids, and surfactant SDS monomers are also believed to exist.[39] Thus, the studies of the urea effect on the formation of the 1:3 complex of **1** with *β*-CD have also been carried out to assist in further characterizing the nature of the complex. This is necessary since in some situations the guest molecules lie outside the cavity to form

* To whom correspondence should be addressed.
† Permanent address: Department of Technical Physics, Peking University, Beijing 100871, P. R. China.

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TABLE 1. Spectral Characteristics of 1 Complexed to β-CD

medium	$\bar{\nu}_A$[a] (cm^{-1})	ϵ[b] (M^{-1} cm^{-1})	$\bar{\nu}_F$[c] (cm^{-1})	Stokes shift (cm^{-1})	$fwhm_A$ (cm^{-1})	$fwhm_F$ (cm^{-1})	Φ_F
water (pH = 9.5)	25 400	14 000	20 200	5200	4800	2800	0.017
3 mM β-CD (pH = 9.5)	25 400	12 300	20 400	5000	4800	3200	0.022
6 mM β-CD (pH = 9.5)	25 400	12 400	20 500	4900	5000	3300	0.041
10 mM β-CD (pH = 9.5)	25 400	11 900[d]	20 700	4700	5200[d]	3400	0.10
14 mM β-CD (pH = 9.5)	25 500	13 800[d]	20 700	4600	5200[d]	3500	0.15
3 mM CTAB (pH = 9.5)	25 400	19 700	20 200	5200	4400	3100	0.31

[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. [d] These values are with some errors due to the scattering in the absorption spectra resulting from the large size of the 1:3 complex.[55]

lidlike association compounds other than inclusion complexes with cyclodextrins.[6,40]

2. Experimental Section

2.1. Materials. The synthesis and purification of **1** were done according to the modified methods of Skrabal et al.[41] and were reported by Popowycz.[42] Analytical grade reagent sodium hydroxide, methanol, urea, and β-CD (Aldrich) were used as received.

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 spectrofluorimeter with a F2T11 special configuration. 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. Fluorescence lifetime measurements were made on a multiplexed time-correlated single-photon-counting fluorometer (Edinburgh Instruments, model 299T). Details are described elsewhere.[43]

2.3. Methods. Fresh sample solutions were used in all measurements. The study of pH effect shows that no new species other than the neutral form of **1** are formed at pH ≥ 2.5, but the absorption spectral characteristics change to a certain extent at different pH values. Thus, for accuracy, the pH values of all solutions in this study were adjusted to about 9.5 by adding NaOH and no buffers were used. The concentrations of **1** for absorption and fluorescence spectra were 10^{-5} and 2×10^{-6} M, respectively, and that for fluorescence lifetime measurements was 7×10^{-6} M. A stock solution of **1** was prepared in methanol. The fluorescence quantum yields were measured using the DM3H molecule[17] as a standard in methanol (Φ_F = 0.24). All measurements were carried out at room temperature. To analyze the lifetime data at different concentrations, a global iterative reweighted reconvolution program based on a nonlinear least-squares method was used based on the Marquardt algorithm.[30,44] The entire decay profiles were analyzed at different concentrations of cyclodextrin solutions. Lifetime data were both individually and globally analyzed by using single, double, and triple exponentials.

3. Results and Discussion

3.1. Structural Features and Spectral Characteristics. The structure of **1** is quite similar to those of the ester para-substituted 3*H*-indoles, i.e., **2**−**4**. It was shown that the substituted 3*H*-indole molecules are not rigid and that the phenyl ring can librate within the *kT* energy barrier.[17−19,25] This torsional movement is responsible for the geometric changes taking place in the ground and excited states and provides an important deactivation pathway for the S_1 state. For **2**−**6** in water, the main nonradiative decay pathway has been ascribed to the formation of a nonemissive twisted intramolecular charge transfer (TICT) state originating in the amino group.[20−22] The very low quantum yield (see Table 1) and the short lifetime (see Table 2) of **1** in water suggest that a nonemissive TICT state is also formed for that molecule.

TABLE 2. Lifetimes, Normalized Preexponential Factors, and Fraction *f*[a] Associated with the Decay at Various Concentrations of β-CD Using the Global Analysis Method

[β-CD]/M	τ_1/ns	B_1	f_1	τ_2/ns	B_2	f_2	individual χ^2	χ_g^2
0	0.36	0.99	0.96	2.4	0.01	0.04	1.667	1.148
0.003		0.94	0.71		0.06	0.29	1.041	
0.006		0.88	0.54		0.12	0.46	1.067	
0.008		0.67	0.24		0.33	0.76	1.052	
0.010		0.41	0.10		0.59	0.90	1.017	
0.012		0.25	0.05		0.75	0.95	1.256	
0.014		0.19	0.03		0.81	0.97	1.180	
0.015		0.17	0.03		0.83	0.97	1.044	

[a] f is the fractional contribution from one species at one particular wavelength to the total fluorescence intensity defined as $f_i = (B_i\tau_i)/(\sum_i B_i\tau_i)$, where B is the preexponential factor and τ is the associated lifetime where $\sum f_i = 1$.

One can also see that **1** has the structural feature of a typical cationic surfactant, i.e., a polar headgroup and a hydrophobic chain. The concentration of **1** used in this study is not more than 10^{-5} M, which is too low for it to form micelles. Thus, as far as the energy balance is concerned, the hydrophobic chain of **1** will seek a hydrophobic environment to enter. We are performing studies on the interaction of **1** with micelles, and some preliminary results in the CTAB micelle are available now. It is found that the quantum yield of **1** first increases steadily with increasing the CTAB concentration and then reaches a plateau above the cmc, i.e., around 0.001 M (figure not shown). Table 1 shows that the fluorescence quantum yield of **1** in CTAB micelles reaches a plateau of 0.31. This phenomenon can be interpreted such that **1** first associates with the premicelles of CTAB and then its hydrophobic chain is partly encapsulated into the CTAB micelle in such a way that the amino nitrogen atom resides at the micellar interface, where it is no longer protonated by water.

We also note from the literature that the inclusion complexes between surfactants and cyclodextrins have recently received much attention,[29,45−49] partly because these systems can be used to model the effect of cyclodextrins on phospholipids, a major constituent of cell membranes.[45] It is now generally regarded that both 1:1 and 1:2 surfactant−cyclodextrin complexes can be formed.[29,45−49] On the basis of above analyses, one can imagine that in aqueous solutions of β-CD the hydrophobic chain of **1** will probably enter the cavity of β-CD.

The absorption and fluorescence spectra of **1** in aqueous solutions of β-CD and CTAB micelles are shown in Figure 2, and the spectral characteristics of **1** are compiled in Table 1.

It can be seen from Table 1 that the absorption wavenumber does not change going from water to β-CD solutions or CTAB

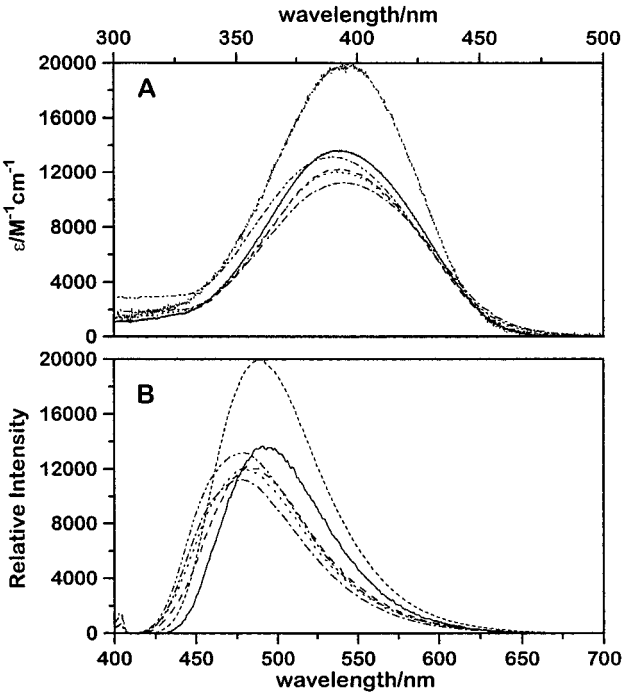

Figure 2. Absorption (A) and fluorescence (B) spectra (normalized according to the respective absorption maximum) of **1** in water (solid), 3 mM β-CD (dashed), 6 mM β-CD (dotted), 10 mM β-CD (dash−dot), 14 mM β-CD (2 dot−2 dash), and 3 mM CTAB (short dash), respectively.

micelles. The fwhm value of the fluorescence bands increases going from water to β-CD solutions, which is consistent with the observed blue-shift of the fluorescence spectra. This indicates that **1** transfers from a polar environment to a less polar environment, which is further supported by the reduction in the Stokes shift.

It is also noted from Table 1 that the quantum yield of **1** increases with increasing the β-CD concentration. This strongly suggests that **1** moves from water to less aqueous sites that avoid the intramolecular twisting responsible for the stabilization of the TICT state and the quenching of the normal fluorescence.[20−22] Comparing the quantum yield value of **1** in 14 mM β-CD solution with that in 3 mM CTAB micellar solution, one can infer that in 14 mM β-CD solution there is possibly some amount of **1** remaining in water.

3.2. Association Constants. First, we consider the following stepwise equilibria:

$$S + CD \overset{K_1}{\rightleftharpoons} SCD \quad (1)$$

$$SCD + CD \overset{K_2}{\rightleftharpoons} S(CD)_2 \quad (2)$$

$$S(CD)_2 + CD \overset{K_3}{\rightleftharpoons} S(CD)_3 \quad (3)$$

where S represents the fluorescence substrate and K_1, K_2, and K_3 denote the stepwise association constants for the 1:1, 1:2, and 1:3 complexes, respectively.

At low concentration of fluorescence probe, the total fluorescence intensity (I) is the weighted average from different species. Thus, one obtains,

$$I = I_0(1 - [SCD]/[S]_0 - [S(CD)_2]/[S]_0 - [S(CD)_3]/[S]_0) + I_1([SCD]/[S]_0) + I_2([S(CD)_2]/[S]_0) + I_3([S(CD)_3]/[S]_0) \quad (4)$$

where I_0 denotes the fluorescence intensity of the substrate in pure water and I_1, I_2, and I_3 denote the fluorescence intensity in 1:1, 1:2, and 1:3 complexes, respectively.

From eqs 1−3, the following equations can be obtained,

$$[SCD] = K_1[S][CD] \quad (5)$$

$$[S(CD)_2] = K_1K_2[S][CD]^2 \quad (6)$$

$$[S(CD)_3] = K_1K_2K_3[S][CD]^3 \quad (7)$$

The initial concentration of S ($[S]_0$) is then expressed by

$$[S]_0 = [S](1 + K_1[CD] + K_1K_2[CD]^2 + K_1K_2K_3[CD]^3) \quad (8)$$

Combining eqs 4−8, one obtains

$$I = (I_0 + I_1K_1[CD] + I_2K_1K_2[CD]^2 + I_3K_1K_2K_3[CD]^3)/(1 + K_1[CD] + K_1K_2[CD]^2 + K_1K_2K_3[CD]^3) \quad (9)$$

In our study, the concentration of CD is much larger than those of the complexes, so [CD] in eqs 5−9 can be replaced by $[CD]_0$, the initial concentration of CD.

Since only one new species is formed in the presence of β-CD according to the lifetime measurement (see section 3.4), we consider the following cases:

Case 1. Only the 1:1 complex is formed, i.e., $K_2 = 0$, $K_3 = 0$. The following relationships can be obtained from eq 9:

$$I = (I_0 + I_1K_1[CD]_0)/(1 + K_1[CD]_0) \quad (10)$$

In this case, $1/(I - I_0)$ versus $[CD]_0^{-1}$ should exhibit a straight line and the analysis by a nonlinear regression program (NLR)[28−31,50] according to eq 10 should give reasonable results.

Case 2. Only the 1:2 complex is formed, i.e., $K_3 = 0$, $[S(CD)_2] \gg [SCD]$. Thus, one obtains

$$I = (I_0 + I_2K[CD]_0^2)/(1 + K[CD]_0^2) \quad (11)$$

where $K = K_1K_2$. In this case, $1/(I - I_0)$ versus $[CD]_0^{-2}$ should exhibit a straight line, and the NLR analysis according to eq 11 should give reasonable results.

Case 3. Only the 1:3 complex is formed, i.e., $[S(CD)_3] \gg [S(CD)_2] \gg [SCD]$. Thus, eq 9 becomes

$$I = (I_0 + I_3K'[CD]_0^3)/(1 + K'[CD]_0^3) \quad (12)$$

where $K' = K_1K_2K_3$. In this case, $1/(I - I_0)$ versus $[CD]_0^{-3}$ should exhibit a straight line, and the NLR analysis according to eq 12 should give reasonable results.

It should be pointed out that there exists only one equilibrium in cases 2 and 3.

Figure 3 illustrates the plots of $1/(I - I_0)$ against $[CD]_0^{-1}$, $[CD]_0^{-2}$, and $[CD]_0^{-3}$, respectively. The plot of $1/(I - I_0)$ against $[CD]_0^{-4}$ is also shown in Figure 3, although the 1:4 complex seems impossible to form on the basis of the molecular length (see section 3.5). It can be seen from Figure 3 that only the plot of $1/(I - I_0)$ as a function of $[CD]_0^{-3}$ indeed exhibits a straight line with a good correlation coefficient ($r = 0.997$). From the slope and intercept of this straight line, one can obtain the values of K' and I_3. But we found that the standard error of the intercept obtained is 30%, while that of the slope is 3%. Thus, the K' value obtained by this method is with great error.

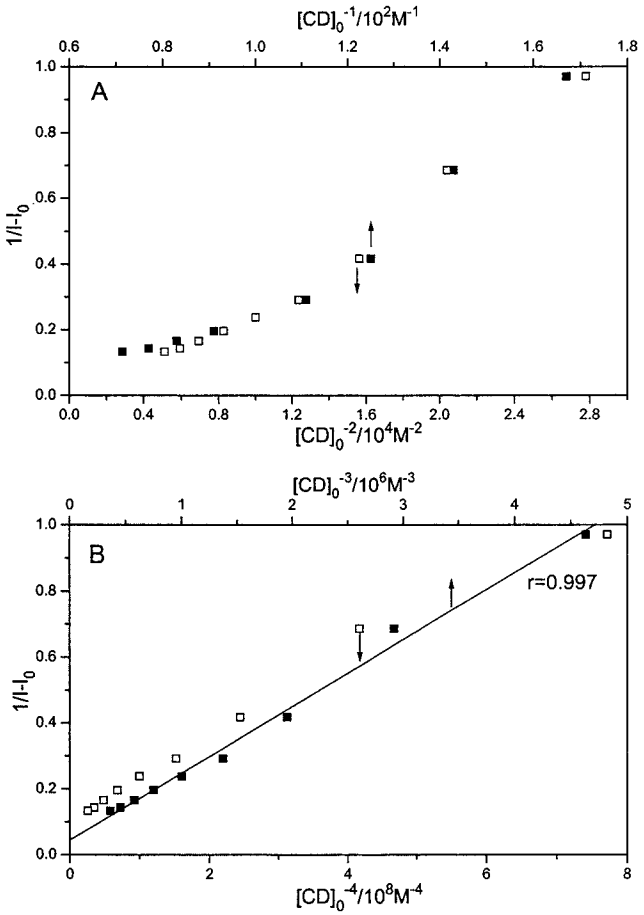

Figure 3. $1/(I - I_0)$ as a function of $[CD]_0^{-1}$, $[CD]_0^{-2}$, $[CD]_0^{-3}$, and $[CD]_0^{-4}$, respectively.

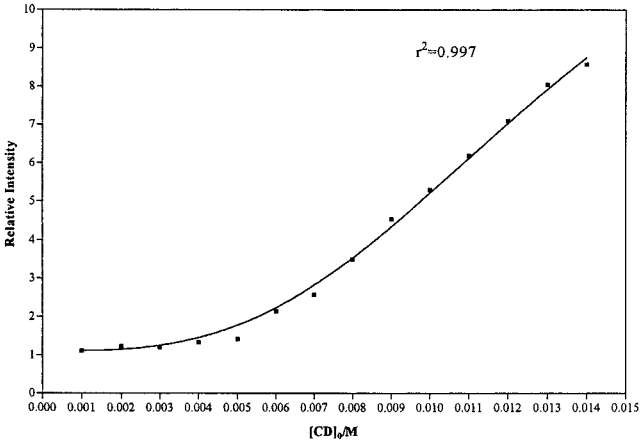

Figure 4. Plot of the relative fluorescence intensity versus $[CD]_0$ for **1** complexed to β-CD. The full line is the nonlinear regression fit to the experimental data points following eq 12.

Much more precision is gained from the NLR analysis. It indicates that reasonable results (values of the variables, standard errors, 95% confidence intervals, correlation coefficient, and absolute sum of squares) can be obtained only when case 3 applies. The fit based on eq 12 converged well with a correlation coefficient $r^2 = 0.997$ (Figure 4). The values of K' and I_3 are estimated to be $(3.7 \pm 0.4) \times 10^5$ M^{-3} and 16 ± 1, respectively. For safety, we have also considered some other cases: (1) 1:1 and 1:2 complexes coexisting in the solution;[28,30] (2) 1:2 and 1:3 complexes coexisting, and (3) only the 1:4 complex. In the first and second cases, results of NLR analyses are not reasonable, while in the third case, the obtained variable

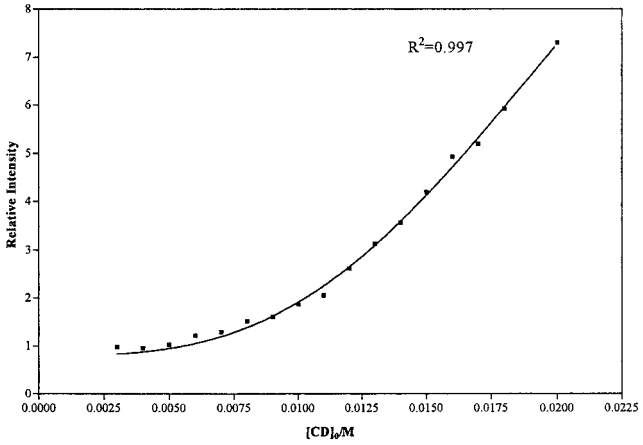

Figure 5. Plot of the relative fluorescence intensity versus $[CD]_0$ for **1** complexed to β-CD in the presence of 3 M urea. The full line is the nonlinear regression fit to the experimental data points following eq 12.

values change with the entered initial values, which means that the data set cannot fit the equation based on the 1:4 complex model.[50]

Figure 4 also shows that at the highest concentration of β-CD studied, which is very close to its maximum solubility, i.e., 16 mM,[4] the fluorescence intensity ($I = 8.6$) is increasing at only about half of the value of I_3. This means that free molecules of **1** have not completely transferred into the cavities of β-CD. This is in agreement with the results of the lifetime measurements listed in Table 2 (see section 3.4) and the fact that the total fluorescence quantum yield in 14 mM β-CD only reaches 0.15, as discussed in section 3.1.

3.3. Urea Effect on the Formation of the 1:3 Complex. Figure 5 depicts the relative fluorescence intensity as a function of the β-CD concentration in the presence of 3 M urea. The increased solubility of β-CD in the presence of urea[51] enables us to measure the fluorescence intensity at higher concentrations of β-CD compared to those in the absence of urea. Again, the fit based on eq 12 converged well with a correlation coefficient $r^2 = 0.997$, while the fits based on the other models do not work. The values of K' and I_3 were estimated to be $(5.6 \pm 0.8) \times 10^4$ M^{-3} and 22 ± 2, respectively.

Figure 6 also shows the plots of $1/(I - I_0)$ against $[CD]_0^{-1}$, $[CD]_0^{-2}$, $[CD]_0^{-3}$, and $[CD]_0^{-4}$, respectively, in the presence of 3 M urea. It is clear that only the plot of $1/(I - I_0)$ versus $[CD]_0^{-3}$ exhibits a straight line ($r = 0.997$).

Both Figures 5 and 6 suggest that the interaction pattern between **1** and β-CD in the presence of 3 M urea is the same as that in the absence of urea. It is well-known that the interactions between urea and β-cyclodextrin are not important, as discussed elsewhere.[37,52] The greatly decreased K' value indicates that a considerable amount of **1** transfers from the β-CD cavity into the bulk phase owing to the interaction of urea with free molecules of **1**. Since urea has only hydrophobic interaction with 3*H*-indoles,[28,29] the above results lead us to conclude that the nature of the interaction between **1** and β-CD is also hydrophobic.

3.4. Lifetime Measurements. The lifetime of **1** was measured at different concentrations of β-CD ranging from 0 to 0.015 M. The excitation and emission wavelengths were 395 and 485 nm, respectively. A total of 5000 counts were collected for each sample. A global analysis of the fluorescence decay was carried out. The lifetimes of various samples were linked together, and the result was judged by the statistical fitting parameters χ^2 for the individual single-curve analysis and for

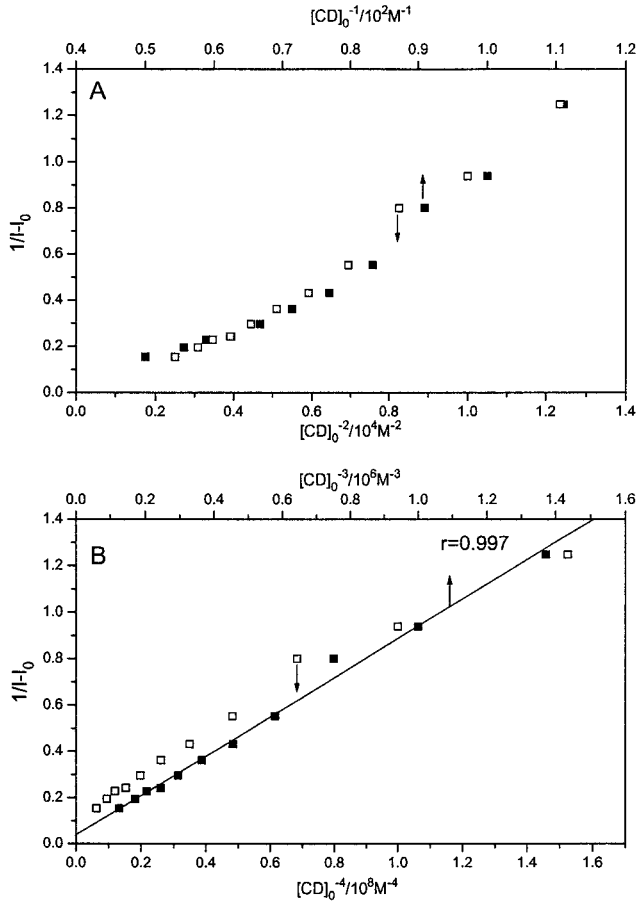

Figure 6. $1/(I - I_0)$ as a function of $[CD]_0^{-1}$, $[CD]_0^{-2}$, $[CD]_0^{-3}$, and $[CD]_0^{-4}$, respectively, in the presence of 3 M urea.

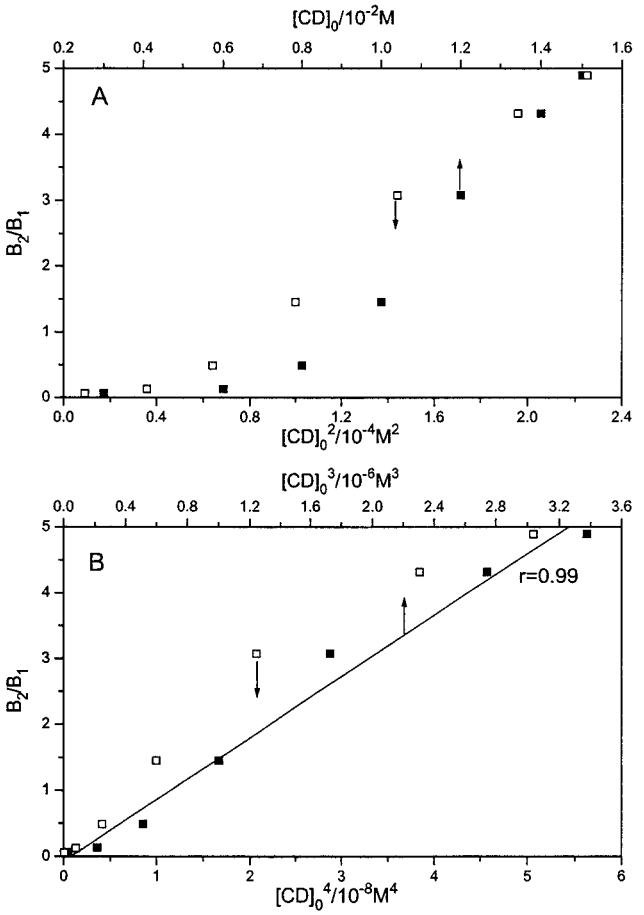

Figure 7. B_2/B_1 as a function of $[CD]_0$, $[CD]_0^2$, $[CD]_0^3$, and $[CD]_0^4$, respectively.

the global analysis (χ_g^2). Results are shown in Table 2. The statistical criteria to judge the quality of the fit include both graphical and numerical tests. The global reduced χ_g^2 statistics have been tabulated and used as a numerical test. The normal deviate $Z\chi_g^2$ corresponding to χ_g^2 was obtained from[53]

$$Z\chi_g^{\,2} = (\nu/2)^{1/2}(\chi_g^{\,2} - 1) \qquad (13)$$

where ν is the number of degrees of freedom. $Z\chi_g^2$ is always less than 1.2 for these results. It should be normally less than 1.96 for a 95% confidence level in the fit.[53]

We attempted a global double-exponential analysis, linking the lifetimes together. Two lifetimes were obtained with a very satisfactory χ_g^2 value (Table 2). The triple-exponential analysis was also attempted on the same set of conditions. However, it did not bring about any improvement of χ_g^2. It has to be pointed out here that the fluorescence decay of **1** in pure water not analyzed globally is not totally explained by a single exponential. A short lifetime of 0.30 ns with a normalized preexponential factor of 0.96 is obtained together with a longer lifetime of 1.13 ns with a preexponential factor of 0.04. This is the reason when the decay in pure water is included in the global analysis at all concentrations of β-CD, the individual χ^2 in pure water is not as good (see Table 2). On the other hand, performing the global analysis at all concentrations of β-CD excluding the decay in pure water, one obtains results for B_1, τ_1, B_2, and τ_2 similar to those reported in Table 2. Therefore, we believe that only two species exist in all the samples except that in pure water. The smaller component is close to the lifetimes of **5** and **6** in bulk water and the larger one to their lifetimes in the 1:2 inclusion complexes with β-CD obtained by global analysis.[30,31] Thus, the smaller component should correspond to the free molecules of **1** in water and the larger one to an inclusion complex.

Using the values of B_1 and B_2 at various concentrations of β-CD in Table 2,one can also estimate the association constant[31] on the basis of the following equations:

$$S + 3CD \overset{K'}{\rightleftharpoons} S(CD)_3 \qquad (14)$$

$$K' = [S(CD)_3]/([S][CD]^3) = B_2/(B_1[CD]_0^{\,3}) \qquad (15)$$

The plot of B_2/B_1 as a function of $K'[CD]_0^3$ should exhibit a straight line through the zero point, the slope of which is equal to the value of K'. Figure 7 illustrates this kind of straight line with a correlation coefficient $r = 0.99$. The value of K' is estimated to be $(1.5 \pm 0.1) \times 10^6$ M^{-3}. This value is about 4 times higher than that obtained by the NLR analysis. The reason for this discrepancy has not been ascertained yet. Further work will have to be done. Nervertheless, the fact that the plot of B_2/B_1 versus $[CD]_0^3$ indeed exhibits a straight line through the zero point strongly supports that only the 1:3 complex is formed. Again, we found that the plots of B_2/B_1 against $[CD]_0$, $[CD]_0^2$, and $[CD]_0^4$, respectively, do not exhibit straight lines as shown in Figure 7.

3.5. Location of 1 and Orientation of β-CD in the 1:3 Inclusion Complex. According to calculations from the molecular structure optimized using the AM1 semiempirical method,[22] the total linear length of **1** is about 26.2 Å. The length from the left end side to the amino nitrogen atom, from the amino nitrogen atom to the indolic nitrogen atom, and from the indolic nitrogen atom to the right end side are 9.6, 6.9, and

9.7 Å, respectively. Since the length of **1** is only slightly longer than 3 times of the length of β-CD, i.e., 23.7 Å, we believe that **1** exists in a linear form in a 1:3 complex. The amino and indolic nitrogen atoms should locate in the junctions of two cyclodextrins, respectively, where they probably interact to some extent with the −OH groups of β-CD. The cationic nitrogen is believed to be at the outside of the β-CD cavity due to its high polarity and the large water solvation layer around it.

Presently, we have no direct evidences showing the relative orientation of the three β-CDs accommodating one molecule of **1**. It was suggested in our previous studies that the indolic nitrogen atom is close to the secondary rims of two β-CD macrocycles in the 1:2 inclusion complexes of **2**−**6** with β-CD.[28−31] This seems also possible in the 1:3 complex. However, it is not easy to determine the orientation of the β-CD molecule residing between the cationic nitrogen and the amino nitrogen. In the case of decamethylenebis(trimethylammonium), the end groups are so large that the inclusion in α-CD takes several minutes.[6] It seems that the ionic end groups can provide significant steric hindrance to the inclusion and dissociation processes.[6] Thus, the cationic nitrogen of **1** sterically prevents β-CD from accommodating it. With this idea, we believe that the β-CD molecule locating between the cationic and amino nitrogens would preferably accommodate **1** from its right end side passing through the smaller water solvation layers around the indolic and the amino nitrogens.

Finally, it should be pointed out that the 1:3 inclusion complex formed by **1** and β-CD belongs to a novel kind of rotaxane. Rotaxanes are referred to as molecules in which a cyclic structure is threaded by a chain or other linear subunit having bulky ends that prevent the dissociation (or unthreading) of the cyclic and linear components.[12] In the synthesized rotaxanes, the presence of two similar[12a] or different[12b] terminal groups is utilized to cap the cyclic component. In our case, the β-CD molecule acting as the cyclic component is capped by the cationic nitrogen and the other two β-CD molecules. It was found that the steric hindrance toward the unthreading depends on the orientation of the cyclodextrin.[6] Thus, we suggest that in our case the β-CD molecule acting as the cyclic component has such an orientation that its primary rim is near the cationic nitrogen. This should correspond to a stable structural form. Similar phenomena were observed before.[6,54] The rotaxane-like structure of the 1:3 inclusion complex also sheds some light about why equilibria (1) and (2) are so weak. Indeed the rotaxane-like structure of the 1:3 inclusion complex should be very stable as compared with the 1:1 and 1:2 complexes. The large solvation layer around the cationic nitrogen acts as a "stopper". The "stoppers" are necessary to stabilize the rotaxane structure in order to prevent the end cyclodextrin molecule from dissociating. It should also be difficult for the cyclodextrin molecule in the middle to escape from the inclusion complex. For molecules **2**−**6**, it was found that both the 1:1 and 1:2 complexes exist. Absorption measurements indicate that the 1:1 complex fitted by the aniline moiety exists, but there is no evidence showing the existence of the 1:1 complex fitted by the indolic moiety.[28] But here, it seems that the interaction between the indolic moiety and β-cyclodextrin is strong. This might explain why the cyclodextrin molecule does not dissociate from the right end so that the 1:3 complex is stable.

4. Concluding Remarks

Absorption and fluorescence spectral characteristics, the change in the fluorescence quantum yield, and its structural features as a cationic surfactant indicate that **1** can interact with β-CD through its hydrophobic chain fitting the cavity of β-CD. The TICT state of **1** in aqueous solutions is inhibited by the formation of the inclusion complex.

The nonlinear least-squares approach was used to analyze the steady-state fluorescence intensities, which strongly suggest that only the model based on the formation of the 1:3 (guest/host) inclusion complex is operative. The data of the lifetimes obtained by the global analysis reveal that there are only two discrete environments, i.e., bulk water and an inclusion complex. The analysis of the preexponential factors is in good agreement with the model of the 1:3 inclusion complex.

The study of the urea effect further confirms the hydrophobic nature of the interaction between **1** and β-CD. The structural features of the 1:3 inclusion complex show that it belongs to a novel kind of rotaxane.

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