19 February 1999

ELSEVIER

Chemical Physics Letters 301 (1999) 193–199

CHEMICAL PHYSICS LETTERS

Interactions between a surface-active cationic 3H-indole molecular probe and β-cyclodextrin. Design of a novel type of rotaxane

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Received 14 September 1998; in final form 20 November 1998

Abstract

We report herein the interactions of a cationic surface-active molecular probe having long aliphatic chains, i.e., iodo-methyldioctadecyl 2-(*p*-hexylaminophenyl)-3,3-dimethyl-5-carboethoxy-3H-indole ammonium, with β-CD investigated by spectral and photophysical characterizations. It is found through lifetime measurements that only two species exist within the whole range of β-CD concentrations. Both the steady-state and the time-resolved fluorescence results further show that the stoichiometry of the inclusion complex is 1:3. It is also suggested that an interaction of the aliphatic chains of the cationic 3H-indole with β-CD takes place. Finally it is shown that a new rotaxane forms spontaneously in solution.

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]. Therefore the investigations of the inclusion complexes have been the focus of great efforts in organic chemistry. Among the various possibilities of intermolecular interactions between CDs and organic molecules, the 1:1 and 1:2 (guest:host) inclusion complexes are the most common types. However, under appropriate conditions, supramolecular assemblies such as catenanes [7], rotaxanes [8,9], polyrotaxanes [10], nanotubular structures [11,12], or threaded cyclodextrins [13] that do not involve any covalent bonding between the cyclodextrin and the other molecule, can be obtained.

In the past few years, our research group has been focused on the study of some substituted 3H-indoles in microenvironments [14–17]. Very recently, our research group started a program of studying the complexation between substituted 3H-indoles and CDs [18–22]. It was found that 1:1 and 1:2 complexes are usually formed between 3H-indoles and

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0009-2614/99/$ - see front matter
PII: S0009-2614(98)01420-1

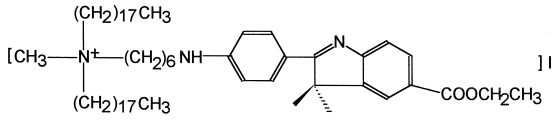

Fig. 1. Molecular structure of **2**.

CDs [18,19,21,22]. However, for a cationic 3H-indole (**1**), we have reported for the first time the formation of a 1:3 inclusion complex, which belongs to a novel kind of rotaxane [20].

In this Letter, we aim to study the interaction of a cationic 3H-indole having long aliphatic chains (**2**, see Fig. 1), with β-CD. Like **1**, quantitative analyses on the results of fluorescence intensity and lifetime measurements show that three β-CD molecules will simultaneously accommodate the aromatic chain of **2** (1:3 rotaxane-like inclusion complex). But the results strongly suggest that each aliphatic chain of **2** will also be incorporated into one or two β-CD cavities, giving rise to a new kind of inclusion complex. Inclusion complexes between surfactants and cyclodextrins have recently received much attention [19,23–27], partly because these systems can be used to model the effect of cyclodextrins on phospholipids, a major constituent of cell membranes [23]. Moreover, to the best of our knowledge, the spontaneous formation of rotaxanes in solution has not been reported yet in the literature. Thus we consider that this work is important not only because **2** forms a new kind of 1:3 inclusion complex, but also because it forms a new kind of rotaxane.

2. Experimental

2.1. Materials

The synthesis and purification of **2** were done according to the modified methods of Skrabal et al. [28] and was reported by Popowycz [29]. Analytical grade reagent 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 [30].

2.3. Methods

Fresh sample solutions were used in all measurements. The concentration of **2** for absorption spectra was $(2–3)\times 10^{-6}$ M except stated otherwise, while those for steady-state and time-resolved fluorescence measurements were 10^{-6} and 3×10^{-6} M, respectively. A stock solution of **2** was prepared in methanol, and 0.1 ml aliquots of this solution were added to aqueous solutions of β-CD. The fluorescence quantum yields were measured using the 2-(*p*-dimethylaminophenyl)-3,3-dimethyl-3H-indole molecule as a standard in methanol ($\Phi_F = 0.24$) [31]. The error in the determination of the Φ_F values is ~10%. To analyze the lifetime data, a global iterative reweighted reconvolution program based on a nonlinear least-squares method was used based on the Marquardt algorithm [21,32].

The entire decay profiles were analyzed at different concentrations of β-CD solutions. Lifetime data were globally analyzed by using single, double and triple exponentials. The excitation and emission wavelengths were 395 and 485 nm, respectively. Since what is important for global analysis is the number of total counts under an entire surface and not in any single curve [32], 5000 counts were collected for each sample. All measurements were carried out at room temperature.

3. Results and discussion

3.1. Spectral characteristics

The absorption and fluorescence spectra of **2** in water and in β-CD solutions of various concentra-

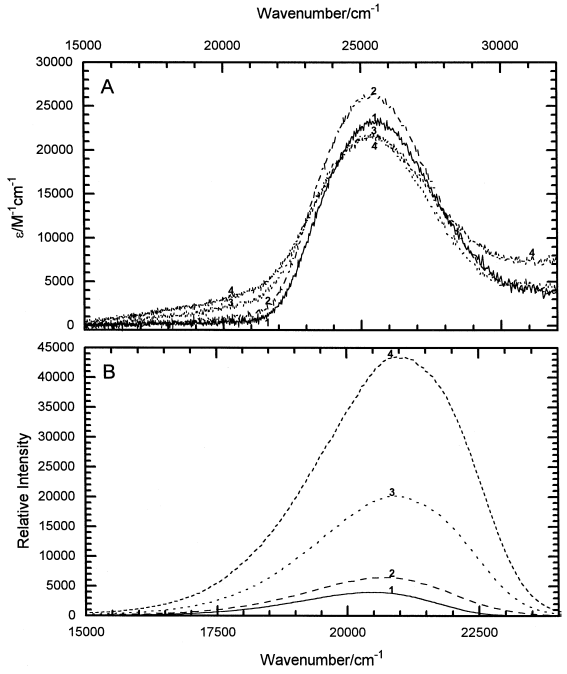

Fig. 2. Absorption (A) and fluorescence (B) spectra (normalized according to the respective fluorescence quantum yield) of **2** in: (1) water (solid); (2) 0.004 M β-CD (dash); (3) 0.008 M β-CD (dot); and (4) 0.015 M β-CD (short dash).

tions are shown in Fig. 2. The optical characteristics of these spectra are compiled in Table 1.

Fig. 2 and Table 1 show that the absorption wavenumber does not change going from water to β-CD solutions. This might suggest that both the amino and indolic nitrogen are probably at the junctions of adjacent cyclodextrins and thus experience close contacts with water molecules. However, the FWHM values of the absorption and fluorescence bands increase going from water to β-CD solutions, which is consistent with the blue shift observed in the fluorescence spectra. This indicates that **2** transfers from a polar to a less polar environment.

It is also noted from Table 1 that the fluorescence quantum yield of **2** increases with increasing the β-CD concentration. This strongly suggests that **2** 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 [31,33,34]. But by comparing the fluorescence quantum yield value of **2** in 0.015 M β-CD solution with those of 2-(*p*-aminophenyl)-3,3-dimethyl-5-carboethoxy-3H-indole and 2-(*p*-dimethylaminophenyl)-3,3-dimethyl-5-carboethoxy-3H-indole in 0.004 M β-CD solution, which are 0.37 and 0.39, respectively [18], one can infer that in 0.015 M β-CD solution there are possibly some amount of **2** remaining in water.

3.2. Association constants

Since only one new species is formed in the presence of β-CD according to the lifetime measurements (see Section 3.3), the data have been analyzed according to our most recent paper [20].

The plot of I/I_0 vs. $[CD]_0$ is shown in Fig. 3A. The NLR analysis 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 a 1:3 complex is formed. The fit converged well with a correlation coefficient $r^2 = 0.992$ (see Fig. 3A). The value of the association constant (K') estimated is

Table 1
Spectral characteristics of **2** in various environments

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)	25400	24200	20200	5200	4800	3000	0.013
0.004 M β-CD (pH = 9.5)	25300	(26600)[d]	20400	4900	(4800)[d]	3200	0.021
0.008 M β-CD (pH = 9.5)	25300	(22500)[d]	20600	4700	(5000)[d]	3300	0.065
0.015 M β-CD (pH = 9.5)	25400	(22000)[d]	20700	4700	(5100)[d]	3400	0.14

[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, especially those at [β-CD] = 8 and 15 mM, are with errors due to the scattering in the absorption spectra resulting from the large size of the 1:3 complex [20].

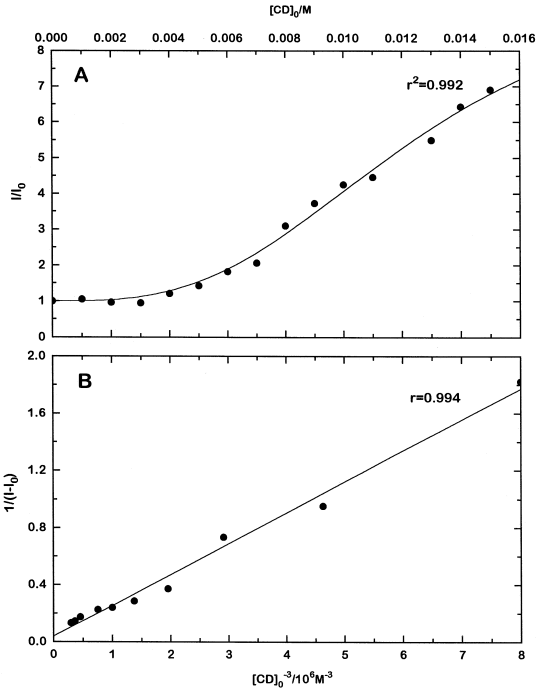

Fig. 3. (A) Plot of the relative fluorescence intensity vs. $[CD]_0$ for **2** complexed to β-CD. The full line is the nonlinear regression fit to the experimental data points following eq. (12) of Ref. [20]. (B) $1/(I-I_0)$ as a function of $[CD]_0^{-3}$.

$(5.0 \pm 0.6) \times 10^5$ M^{-3}. It should be pointed out that any reasonable results of NLR analyses are not obtained using other models, such as 1:1 and 1:2 complexes coexisting, 1:2 and 1:3 complexes coexisting and only the 1:4 complex existing. The 1:3 complexation model is further supported by the straight line of $1/(I-I_0)$ plotted against $[CD]_0^{-3}$ ($r = 0.994$; Fig. 3B). It should also be pointed out here that, when $1/(I-I_0)$ is plotted against $[CD]_0^{-1}$, $[CD]_0^{-2}$ and $[CD]_0^{-4}$, respectively (figures not shown), straight lines cannot be obtained.

Fig. 3A also shows that at the highest concentration of β-CD studied, which is very close to its maximum solubility [33], i.e., 0.016 M, the fluorescence intensity is still increasing. This means that free molecules of **2** 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 3.3) and the fact that the total fluorescence quantum yield in 0.015 M β-CD only reaches 0.14 as discussed in Section 3.1.

A recent study of urea effect has shown to be an effective way of confirming the hydrophobic nature of complexes formed between cyclodextrins and 3H-indoles [18–20]. Moreover, the hydrophobic interaction mechanism between urea and 3H-indoles has also been established [18]. For this reason, the urea effect study has been performed on the interaction between **2** and β-CD. It was found that the interaction pattern of **2** with β-CD in the presence of 3 M urea is similar to that in the absence of urea, that is, reasonable results of the NLR analysis can be obtained only when the 1:3 complexation model is employed and the plot of $1/(I-I_0)$ vs. $[CD]_0^{-3}$ exhibits a straight line (figures not shown). But, the association constant K' is decreased to $(2.2 \pm 0.4) \times 10^5$ M^{-3}. These results lead us to conclude that the nature of the interaction between **2** and either β-CD or urea is hydrophobic [18–20].

All the results presented above seem to show that each molecule **2** is encapsulated simultaneously by three β-CD molecules. However, it should be noted that the change in the fluorescence intensity reflects directly the change in the microenvironment of the aromatic chain, but not of the two long aliphatic chains of **2**. This is the reason why the interaction pattern between **2** and β-CD is similar to that between **1** and β-CD. But it is well known that the aliphatic chains of the surfactants such as SDS and

Table 2
Lifetimes, normalized pre-exponential 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	0.32	0.99	0.94	2.33	0.01	0.06	1.50	1.21
0.004		0.90	0.54		0.10	0.46	1.19	
0.006		0.82	0.38		0.18	0.62	1.28	
0.008		0.66	0.21		0.34	0.79	1.24	
0.010		0.41	0.09		0.59	0.91	1.05	
0.011		0.33	0.06		0.67	0.94	1.26	
0.012		0.27	0.05		0.73	0.95	1.11	
0.013		0.27	0.05		0.73	0.95	1.32	
0.014		0.22	0.04		0.78	0.96	1.06	
0.015		0.18	0.03		0.82	0.97	1.11	

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

CTAB form 1:1 and 1:2 complexes with β-CD (Ref. [19] and refs. cited therein). Thus, a similar phenomenon could occur for **2**, the surface active molecular probe. If the interaction between the aliphatic chains of **2** and β-CD does exist, then $[S(CD)_3]$ becomes an apparent concentration that does include the contribution from higher-order complexes (1:4 . . . 1:7) and the equilibrium involving the formation of the 1:3 complex will be moved toward the right. This is opposite to the effect of urea, which makes the equilibrium move toward the left. In the latter case, we have observed that the *apparent association constant* is reduced by the hydrophobic interaction between urea and 3H-indoles [18–20]. Obviously, in the former case, the *apparent association constant* of the 1:3 complex will increase to some extent. To shed some light on this problem, one can compare the association constants of **2** with those of **1** in the absence and presence of urea, respectively. The values for **1** without and with urea are $(3.7 \pm 0.4) \times 10^5$ and $(5.6 \pm 0.8) \times 10^4$ M^{-3}, respectively [20]. The fact that the association constant of **2** is larger than that of **1** in the absence of urea is an indication that the aliphatic chains might fit into the β-CD cavity. On the other hand, in the presence of 3 M urea, the association constant of **2** is about four times higher than that of **1**. It is worth mentioning that the urea concentration used is very high such that the hydrophobic interaction of urea and the aliphatic chains is considerable. Moreover it was reported that the hydrophobic interaction of urea on the aliphatic chain of SDS exists and thus the CMC of SDS is markedly increased [18,35]. On the basis of these facts, we believe that the complexation of the aliphatic chains of **2** with β-CD takes place. Otherwise, the association constant value between **2** and β-CD would be obviously lower than that between **1** and β-CD.

It is important to point out here that the three β-CD molecules complexed with the aromatic chain are not undergoing dynamic exchange with free β-CD because of the rotaxane-like structural feature [20]. However, the β-CD molecules complexed with the aliphatic chains should have exchange with free β-CD. Since one or two β-CD molecules might accommodate each aliphatic chain, many types of higher-order complexes between **2** and β-CD such as 1:4, 1:5, 1:6 and 1:7 complexes could possibly exist.

3.3. Lifetime measurements

The fluorescence decay curve of **2** was measured in β-CD solutions of various concentrations. A global analysis was carried out by linking the fluorescence decay curves together. The results were judged by the statistical fitting parameters χ^2 for the individual single curve analysis and for the global analysis (χ_g^2). The statistical criteria to judge the quality of the fit include both graphical and numerical tests [20,21].

We attempted a global double exponential analysis, linking the decay curves together. Two lifetimes were obtained with a 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 **2** in pure water analyzed individually is not well reproduced by a single exponential. A short lifetime of 0.28 ns with a normalized preexponential factor of 0.97 is obtained together with a longer lifetime of 1.13 ns with a preexponential factor of 0.03. This is the reason why 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 poor (see Table 2). A similar phenomenon was observed for **1** [20]. 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 and B_2, τ_2 similar as those reported in Table 2. Therefore, we believe that only two species exist in all the samples except that in pure water. The component with the smaller lifetime should correspond to the free molecules of **2** in water whereas the component with the larger lifetime involves the inclusion complex. As discussed in Section 3.1, **2** is less in contact with water in the inclusion complex. Thus the quenching of the fluorescence observed in aqueous solutions is avoided [31,33,34], giving rise to higher values of the fluorescence quantum yield and lifetime for the 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 [20,21]. The plot of B_2/B_1 as a function of $[CD]_0^3$ should exhibit a straight line through the zero point [20], the slope of which is equal to the K' value. Fig. 4 illustrates this kind of

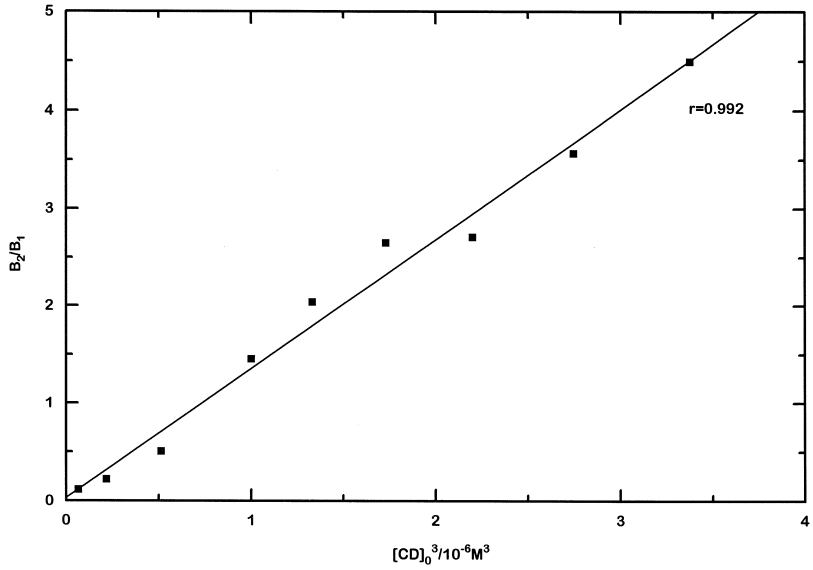

Fig. 4. Plot of the ratio of the preexponential factors (B_2/B_1) as a function of $[CD]_0^3$.

straight line with a correlation coefficient $r = 0.992$. The K' value is estimated to be $(1.3 \pm 0.1) \times 10^6$ M^{-3}, which is similar to that of **1** obtained through the similar method [20]. It is noted that this value is 2.6 times higher than that obtained from the NLR analysis on the fluorescence intensity. This kind of discrepancy also appears in the literature [20,36]. The possible reason is that the inclusion complex in the excited state is not exactly the same as that in the ground state. Nevertheless, the fact that the plot of B_2/B_1 vs. $[CD]_0^3$ indeed exhibits a straight line through the zero point strongly supports the formation of the 1:3 complex. 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 (figures not shown). Finally, it should be noted that, due to the discrepancy of the K' value, no information on the interactions between the aliphatic chains of **2** and β-CD can be obtained through the comparison of K' values of **1** and **2**.

4. Concluding remarks

The NLR analysis on the steady-state fluorescence intensity of **2** in β-CD suggests that only the model based on the formation of the 1:3 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. A comparison of the association constants of **2** and **1** in the absence and presence of urea, respectively, also suggests that the two long aliphatic chains are also incorporated into β-CD cavities. It is also shown that the inclusion complex between **2** and β-CD forms a novel kind of rotaxane. To our knowledge, the spontaneous formation of rotaxanes in solution is reported for the first time in the literature.

Acknowledgements

We gratefully acknowledge the financial assistance of the National Sciences and Engineering Research Council of Canada and the ''Fonds FCAR'' (Québec) in the form of grants. We also thank Mr. Adrian Popowycz for the synthesis and purification of the substituted 3H-indole studied here. XS thanks Peking University, PR China, and University of Montreal, Canada, for the financial assistance provided by the exchange program.

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