Fluorescence Study of Inclusion Complexes between Star-Shaped Cholic Acid Derivatives and Polycyclic Aromatic Fluorescent Probes and the Size Effects of Host and Guest Molecules

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Star-shaped host molecules containing two, three, and four cholic acid moieties have been used to form inclusion complexes with polycyclic aromatic hydrocarbon probes (guests) varying in size from four (pyrene) to five (benzo(e)pyrene) and seven aromatic rings (coronene) and investigated by steady-state fluorescence measurements and fluorescence lifetime techniques. The results indicated that these hydrophobic guest probes prefer to locate in the hydrophobic cavities formed by the host molecules in an aqueous solution. Further studies showed that the stoichiometric ratios of the complexes depended on the relative size of both the host and the guest. The complexes of 1:1 ratio (guest:host) were formed between pyrene and the host molecules of different sizes, while the complexes of 1:2 ratio (guest:host) were found for coronene in all cases. For benzo(e)pyrene with an intermediate size, the complexes with 1:1 and 1:2 ratios (guest:host) were formed depending on the relative sizes of the host molecules. The stability of the inclusion complexes was observed to change with the solvent polarity, indicative of an adaptation of the hydrophobicity of the host pockets to the polarity of the solvent. The formation of the complexes was driven by the solvophobic interactions.

1. Introduction

The past decades have witnessed much research progress in various aspects of supramolecular chemistry.[1,2] A large number of molecular receptors of varying sizes, shapes, and functionalities have been synthesized, and their interaction with guest molecules has been investigated.[3−19] Bile salts, a class of amphiphilic biomolecules, aggregate in aqueous solutions since the concave side of these molecules is hydrophilic whereas the convex side is hydrophobic.[20] Early studies on structural aspects have shown that bile salt aggregates progressively form micelles,[21−23] leading to larger structures as the concentration of bile salt monomers are raised. The primary/secondary aggregation model proposed the association of the hydrophobic faces of a small number of monomers leading to the primary aggregation at a low concentration.[21,22] At high monomer concentrations, the primary aggregation agglomerated into larger structures called secondary aggregates.[21,22,24] Evidence for stepwise aggregation of bile salts has been observed by the use of fluorescent probes,[21−24] such as polycyclic aromatic hydrocarbon (PAH) probes, ranging from pyrene with 4 aromatic rings to ovalene with 10 aromatic rings.[23] The sizes of the PAH probes did not appear to significantly affect the critical micelle concentration, although they may cause distortion in the shape of the aggregates.[23] Molecular modeling studies showed that two bile salt molecules could stabilize pyrene, while four to eight bile salt molecules were required to contain and stabilize the ovalene probes from the bulk solution.[25]

Efficient synthetic receptors capable of selective substrate binding in aqueous solution are important for the understanding of molecular recognition and self-assembly in chemical and biological systems.[1−3] Star-shaped cholic acid derivatives, called molecular umbrella,[8−10,18] molecular tweezers,[5,12,13] tripodal cholamide,[17] adaptive dendron,[11] or molecular baskets,[6,16] have been studied in the applications of environmental responsive switches,[16] molecular hydrogel,[17] transbilayers,[9,10,18] guest carriers,[17] and supramolecular ion channels.[7] We have made various star-shaped cholic acid derivatives through highly efficient synthesis and found that they can form inclusion complexes with fluorescent molecules such as pyrene.[26] The study of the physicochemical properties of host−guest complexes of these molecules is important for their application in drug delivery and transportation across membranes. The relative sizes of the host and guest are expected to be important for the interaction and the application of such host molecules. In this study, we have studied the complexation of the dimer, trimer, and tetramer derivatives of cholic acid with PAH probes of different sizes. The binding constants and stoichiometric ratios between the guest and host, which depended on their relative sizes, have been estimated by the use of fluorescence techniques. Finally, the solvent effect on the host−guest complex formation is studied to understand the major driving force for the formation of the host−guest complexes in aqueous solutions.

2. Experimental Section

Pyrene (Py), benzo(e)pyrene (BeP), and coronene (Co) (Sigma-Aldrich, 99%) were used without further purification. Anhydrous *n*-hexanol, *n*-pentanol, *n*-butanol, *n*-propanol, ethanol, and methanol (Sigma-Aldrich, 99.9%) were used as received. Phosphate buffer solution (PBS, 0.1 M, pH 10) in Milli-Q water was used throughout the experiments.

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Fresh sample solutions were used in the fluorescence measurements. Stock solutions of the dimer, trimer, tetramers **1** and **2** were prepared in methanol and diluted with PBS (0.1 M, pH 10) to obtain the desired concentrations. Aliquots of 100-μL stock solutions of Py, BeP, and Co in methanol were added, respectively, to 10-mL solutions of dimer, trimer, tetramers **1** and **2** to maintain a final concentration of 2×10^{-7} M of the probes for the steady-state fluorescence and lifetime measurements. Steady-state fluorescence spectra were recorded on an FLS-900 (Edinburgh Instruments, UK) spectrofluorimeter equipped with Xe-900 lamp with excitation wavelength of 336, 325, and 325 nm for Py, BeP, and Co, respectively. The slit widths of excitation and emission were 5 and 0.2 nm, respectively. Lifetime measurements were recorded with the same instrument equipped with a nF-900 lamp and excited at 336, 325, and 325 nm with a 10-nm slit width and detected at 384, 389, and 445 nm for Py, BeP, and Co, respectively, with a 10-nm slit width. Fluorescence decay data were analyzed by using mono-, bi-, and triexponential functions. All steady-state fluorescence and lifetime measurements were carried out at room temperature.

The preparation of trimer, tetramer **1**, and tetramer **2** of cholic acid (Figure 1) has been described previously.[26] The dimer of cholic acid was prepared with a similar procedure[26] in the presence of a small amount of water. A pressure-resistant vessel equipped with a Teflon screw cap was used as the reactor. To a solution of choloyl azide (0.23 mmol, 100 mg) in 20 mL of THF was added 100 μL of water. The solution was tightly sealed and stirred at 140 °C overnight. THF was removed under vacuum, and the residue was purified by column on silica gel using ethyl acetate−methanol (4:1, v/v) as eluent yielding 50 mg (50%) of white solid product. ^{1}H NMR (400 MHz, CD_3-OD) 3.98 (2H, s), 3.82 (2H, s), 3.39 (2H, m), 3.21 (2H, m), 3.10 (2H, m), 2.18−2.41 (4H, m), 1.12−2.41 (42H, m), 1.06 (6H, d, J=6.6 Hz), 0.94 (6H, s), 0.74 (6H, s); ^{13}C NMR (100 MHz, CD_3OD) 12.0, 17.1, 22.2, 23.3, 26.9, 27.9, 28.6, 30.2, 34.0, 34.8, 34.9, 35.5, 36.5, 37.6, 39.5, 40.0, 42.0, 42.2, 46.6, 47.4, 68.1, 71.9, 73.0, 160.4; HR-MS *m/z* calcd. for $C_{47}H_{81}N_2O_7$ $[M+H]^+$ 785.60383, found $[M+H]^+$ 785.60536.

3. Results and Discussion

3.1. Fluorescence Spectra. Hydrophobic PAH probes, such as Py, BeP, and Co, prefer to locate in a hydrophobic microenvironment. They have been widely used to study the conformation and aggregation of bile acids,[22,23] cyclodextrins,[25,27,28] and micelles.[29] The notably long lifetime of the monomeric PAH probes[23] and the efficient formation of excimers[23,30] are well-known interesting photophysical properties, which make them suitable as effective probes in the study of micellar aggregations.[29]

Fluorescence spectra of Py, BeP, and Co in aqueous buffer solutions in the absence and presence of the dimer host molecule are shown in Figure 2. At a concentration of 2×10^{-7} M, Py does not show any obvious excimer peak at 480 nm corresponding to the aggregation in aqueous solution (Figure 2A). The spectral intensities of the peaks (I_1, I_2, I_3, I_4, I_5)[31,32] in the spectra clearly show that the fine vibrational structure undergoes significant perturbations upon moving from a polar to a nonpolar environment.[25−29] As the environment surrounding pyrene is altered, a concomitant change in the ratio of I_3/I_1 vibronic bands can be observed. It has been demonstrated that the complexation equilibria between pyrene and cyclodextrins can be easily monitored by following the changes in the ratio of I_3/I_1.[25,27,28] The fluorescence spectra of Py recorded at different concentrations of the dimer (Figure 2A) show that the fluorescence intensity increases with increasing concentration of the dimer, followed by a red shift of the spectra. Particularly, the ratio of I_3/I_1 increases with increasing concentration of the dimer, indicating that the microenvironment surrounding pyrene changes from a polar bulk solution to a nonpolar environment.

In parts B and C of Figure 2, the excimers of BeP and Co at 425 and 500 nm were observed in the absence or in the presence of added small amounts of host molecules.[23,33−35] The spectra of excimers disappeared upon the addition of more host molecules, while new emission peaks of monomers appeared and were enhanced with increasing concentration of the host molecules. The phenomenon observed indicated that hydrophobic probe molecules (BeP or Co) aggregated to form excimers in aqueous solutions, whereas the addition of host molecules isolated them from each other and help them to stabilize separately in the solution.

Similar fluorescence spectra were observed for the trimer and tetramers **1** and **2** as host molecules. All the results suggested that the hydrophobic probes of Py, BeP, and Co moved from the bulk solution to the hydrophobic pockets formed by the amphiphilic star-shaped cholic acid derivatives (dimer, trimer, and tetramers). Moreover, the disappearance of the excimers indicated that the guest molecules were separated from each other by entering the hydrophobic pockets of the host molecules, where $\pi-\pi$ conjugation of the guest molecules was not detected.

3.2. Stoichiometry and Binding Constants. In the literature, different types of inclusion complexes between cyclodextrins and various guest molecules have been investigated by the fluorescence techniques.[4,25,27,28] The following equilibria, which are widely used in the study of the inclusion complexes between cyclodextrins and guest molecules,[4,25,27,28] are applied in this study

$$\text{Guest} + 0.5\ \text{Host} \overset{K_{0.5}}{\rightleftharpoons} (\text{Guest})(\text{Host})_{0.5} \quad (1)$$

$$\text{Guest} + \text{Host} \overset{K_1}{\rightleftharpoons} (\text{Guest})(\text{Host}) \quad (2)$$

$$\text{Guest} + 2\ \text{Host} \overset{K_2}{\rightleftharpoons} (\text{Guest})(\text{Host})_2 \quad (3)$$

$$\text{Guest} + 3\ \text{Host} \overset{K_3}{\rightleftharpoons} (\text{Guest})(\text{Host})_3 \quad (4)$$

where $K_{0.5}$, K_1, K_2, and K_3 denote the binding constants of the various inclusion complexes.

At low concentration of fluorescent probes, the following equation on the fluorescence intensity (or the ratio of I_3/I_1) can be obtained[4,25,27,28]

$$P = \frac{P_0 + P_n K_n [\text{Host}]^n}{1 + K_n [\text{Host}]^n} \quad (5)$$

where P_0 and P_n are the fluorescence intensity (or the ratio of I_3/I_1 for Py) in an aqueous solution and in 1:n (guest:host) inclusion complexes and K_n represents the binding constants of the 1:n (guest:host) inclusion complexes. The Benesi−Hildebrand type double reciprocal equations[36] should give a straight line when a proper value of n is applied in the following equation

$$\frac{1}{P - P_0} = \frac{1}{K_n (P_n - P_0)} \frac{1}{[\text{Host}]^n} + \frac{1}{P_n - P_0} \quad (6)$$

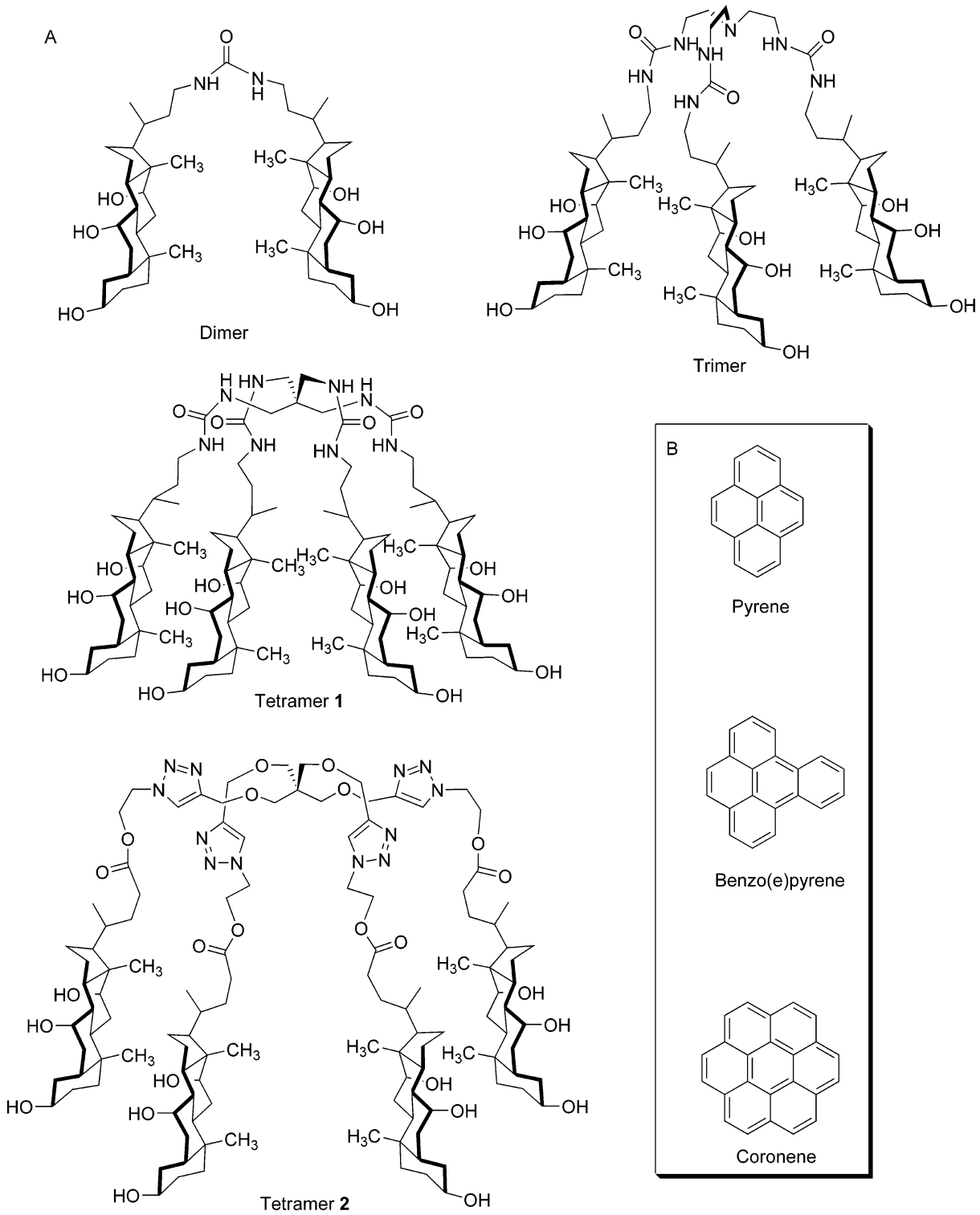

Figure 1. Molecular structures of (A) cholic acid derivatives (dimer, trimer, tetramers **1** and **2**) and (B) PAH fluorescent probes Py, BeP, and Co.

The fluorescence intensities of BeP and Co or the ratio of I_3/I_1 of Py in the absence and presence of dimer, trimer, and tetramers **1** and **2** vs the concentration of the host molecules are shown in Figure 3. All the experimental data were analyzed by eq 5 with different n values. Reasonable results of the values of parameters, standard errors, and correlation coefficient can only be obtained when a correct value of n is used in eq 5.

For the inclusion complexes between Py and the host molecules (dimer, trimer, and tetramers **1** and **2**), the experimental data are analyzed by nonlinear fitting method and yield good fits only when $n = 1$ in eq 5 (correlation coefficients $R^2 \approx 0.99$). In addition, $1/(P - P_0)$ vs $[\text{Host}]_0^{-1}$ also give straight lines ($R > 0.99$). This implies that the 1:1 inclusion complexes are formed between Py and the host molecules (dimer, trimer, and tetramers **1** and **2**). Data obtained for the inclusion complexes between Co and the host molecules are tested by nonlinear fitting analysis according to 1:2 inclusion complex model ($n = 2$) and by linear plots of $1/(P - P_0)$ vs $[\text{Host}]_0^{-2}$, which yield good correlation coefficients ($R^2 \approx 0.99$ and $R > 0.998$, respectively). BeP is of medium size; the experimental

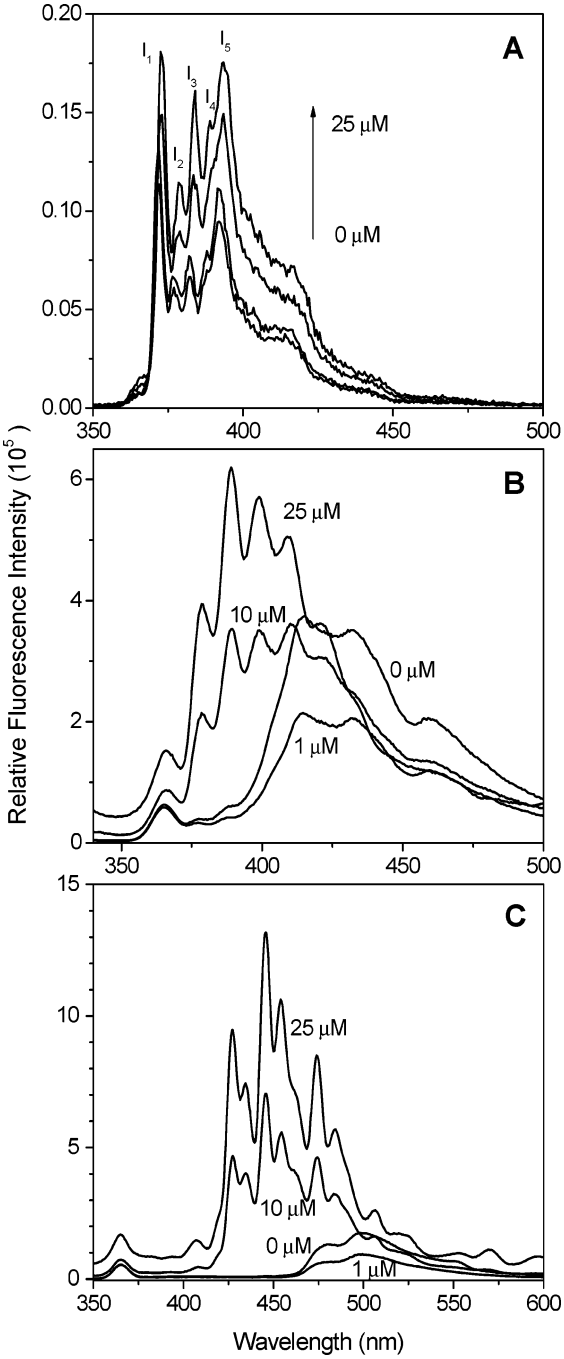

Figure 2. Fluorescence spectra of Py (A), BeP (B), and Co (C) all at 2×10^{-7} M in the presence of 0, 1, 10, and 25 μM of the host molecules (dimer) in aqueous PBS solutions (0.1 M, pH 10).

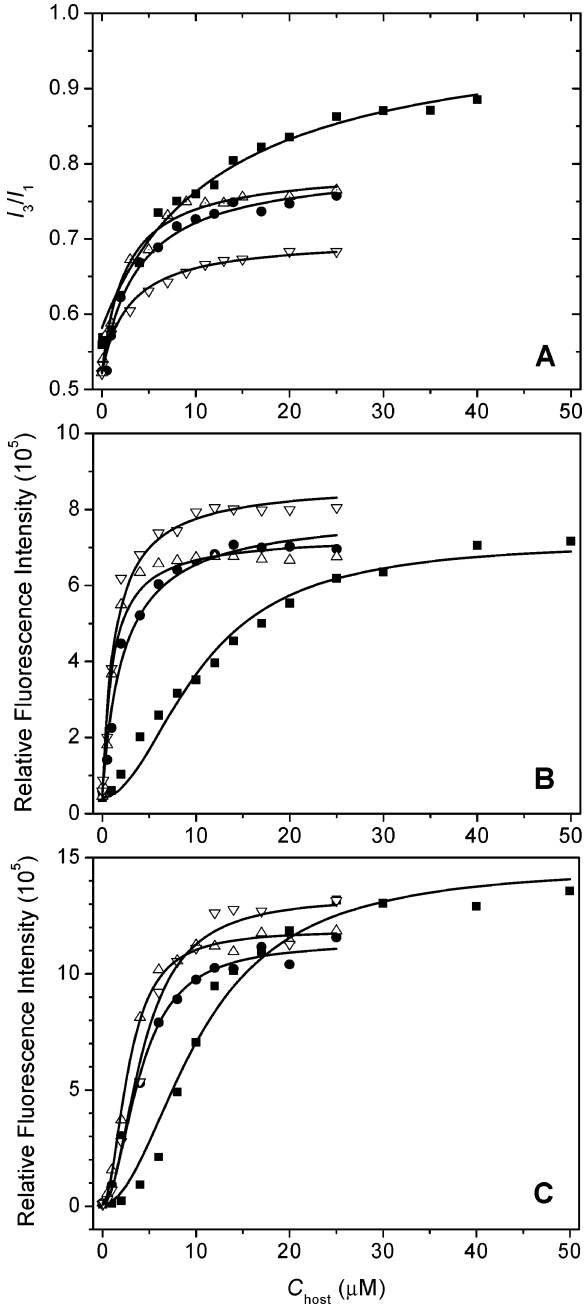

Figure 3. The ratio of I_3 to I_1 of Py (A), the fluorescence intensities of BeP (B) and Co (C) as a function of the concentration of the host dimer (■), trimer (●), tetramer **1** (△), and tetramer **2** (▽). The fluorescent probe concentration is at 2×10^{-7} M. The data are fits to eq 5.

data of trimer and tetramers **1** and **2** can be fitted well to a 1:1 inclusion complex model with good correlation coefficients ($R^2 \approx 0.98$ and $R \geq 0.997$, respectively). For the inclusion complexes between BeP and the dimer, only when $n = 2$ good correlation coefficients are obtained in both nonlinear ($R^2 = 0.98$) and linear ($R = 0.999$) fitting analyses. Double reciprocal plots are shown in Figure 4 and in the Supporting Information.

The coexisting complex models of 2:1 and 1:1, 1:1 and 1:2, and 1:2 and 1:3 were also examined. However, no reasonable result (including the values of parameters, standard errors and correlation coefficient) was obtained. The following facts are observed. For a small guest molecule such as Py, all of the four hosts form 1:1 inclusion complexes; for a large guest molecule such as Co (the largest guest molecules), all these hosts form 1:2-type complexes; for a guest molecule of intermediate size such as BeP, 1:2 inclusion complexes are formed with the smallest host molecule (dimer), while 1:1 complexes are formed with the larger host molecules (trimer and tetramers **1** and **2**). The stoichiometric ratios of all the inclusion complexes formed between the guests (Py, BeP, and Co) and the hosts (dimer, trimer, and tetramers **1** and **2**) are shown in Table 1.

The binding constants between the guest and the host are listed in Table 2. The results obtained from nonlinear fitting analysis with eq 5 are similar to those by linear fitting analysis with eq 6. In Table 2, the binding constants with a certain guest of the inclusion complexes of the same stoichiometric ratio increase from the dimer to the tetramers, indicating that the number of the cholic acid units of the star-shaped cholic acid derivatives can significantly affect the stability of the inclusion complexes. In addition, the binding constants of the same host

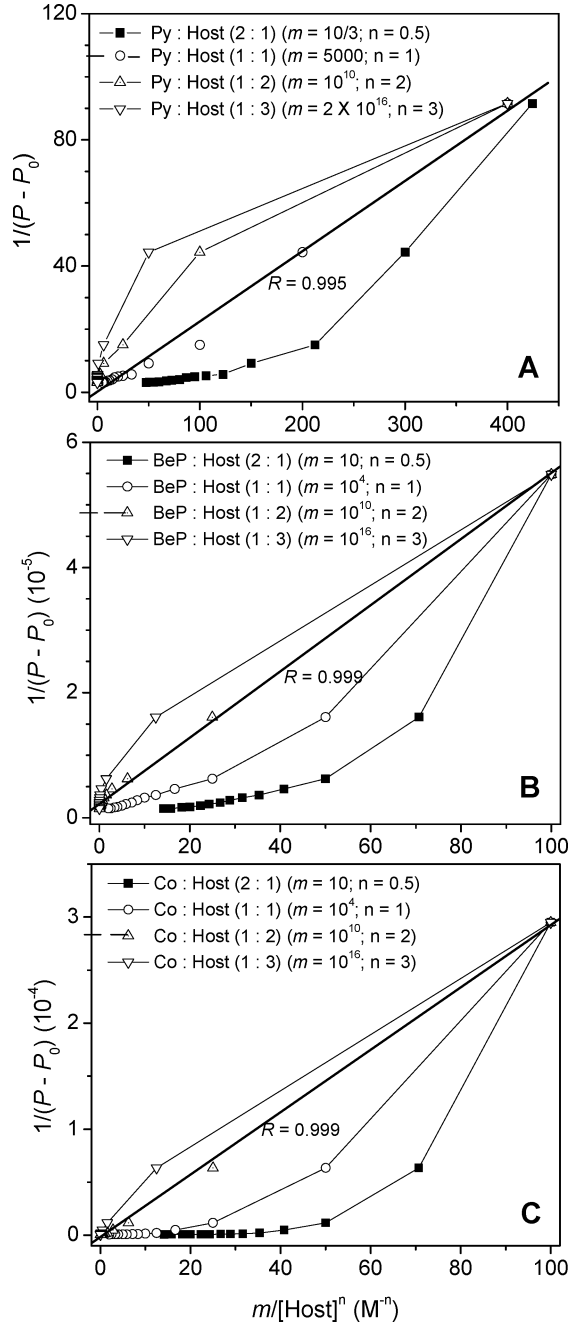

Figure 4. Plot of $1/(P - P_0)$ vs $m/[\text{Host}]^n$ for Py (A), BeP (B), and Co (C) in the presence of the host molecules (dimer). The straight lines are fits to eq 6.

TABLE 1: Stoichiometric Ratios of the Guest:Host in the Inclusion Complexes

	dimer	trimer	tetramer **1**	tetramer **2**
Py	1:1	1:1	1:1	1:1
BeP	1:2	1:1	1:1	1:1
Co	1:2	1:2	1:2	1:2

with the same stoichiometric ratio increase from Py to Co because of the more hydrophobic nature of the larger aromatic structures. The standard binding free energy changes calculated from $\Delta G^\circ = -RT \ln K$ are about -30 and -60 kJ/mol for the 1:1 and 1:2 complexes, respectively, at room temperature. The negative ΔG° values indicate that, in the standard states, the inclusion processes can proceed spontaneously. The values of

TABLE 2: Binding Constants of the Host−Guest Complexes Obtained from Linear and Nonlinear Analyses

		nonlinear fits			linear fits		
guest	host	P_n	K_n	R^2	P_n	K_n	R
Py[c]	dimer[a]	0.99	8.0×10^4	0.984	1.02	4.9×10^4	0.995
	trimer[a]	0.80	2.6×10^5	0.987	0.84	1.9×10^5	0.992
	tetramer **1**[a]	0.79	3.9×10^5	0.991	0.82	2.9×10^5	0.996
	tetramer **2**[a]	0.70	3.5×10^5	0.987	0.71	2.6×10^5	0.999
BeP[d]	dimer[b]	0.72	9.2×10^9	0.980	0.49	4.2×10^{10}	0.999
	trimer[a]	0.80	4.2×10^5	0.987	0.91	2.0×10^5	0.997
	tetramer **1**[a]	0.73	9.3×10^5	0.978	1.0	2.9×10^5	0.998
	tetramer **2**[a]	0.87	7.3×10^5	0.985	1.3	2.3×10^5	0.999
Co[d]	dimer[b]	1.5	9.1×10^9	0.983	0.63	5.5×10^9	0.999
	trimer[b]	1.1	6.1×10^{10}	0.995	1.6	4.3×10^{10}	0.999
	tetramer **1**[b]	1.2	1.3×10^{11}	0.998	1.1	1.6×10^{11}	1
	tetramer **2**[b]	1.3	5.4×10^{10}	0.989	1.0	7.7×10^{10}	0.998

[a] Data fitted with a 1:1 model. K_n is binding constant of the 1:1 complexes with a unit of M^{-1}. [b] Data fitted with a 1:2 model, K_n is binding constant of the 1:2 complexes with a unit of M^{-2}. [c] P_n represents the ratio of I_3 to I_1, when pyrene is fully bound to the host molecules. [d] P_n represents the fluorescence intensity (with a unit of 10^6) for BeP or Co at a concentration of 2×10^{-7} M, when BeP or Co are fully bound to the host molecules.

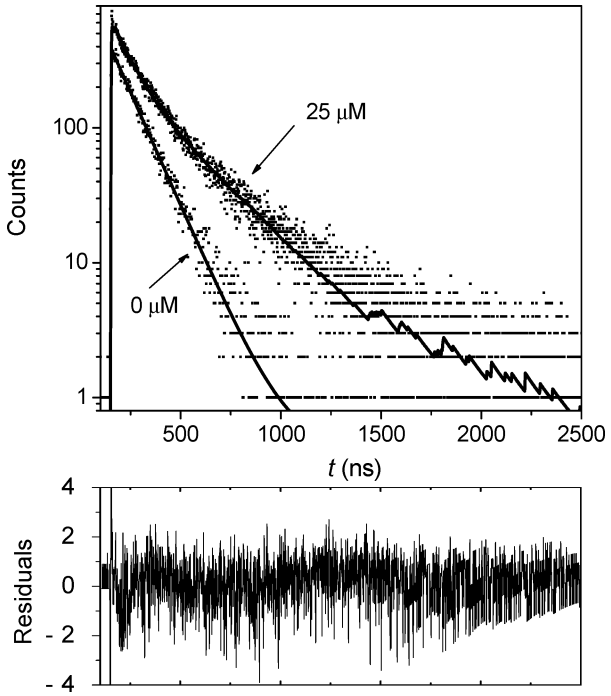

Figure 5. Fluorescence lifetime measurements of pyrene (2×10^{-7} M) in the absence and presence of the host molecules (dimer).

ΔG° are comparable with those of inclusion complexes between cyclodextrins and various guest molecules[4] through hydrophobic interactions.

3.3. Fluorescence Lifetime. Fluorescence lifetimes of the PAH probes, a useful indication of the microenvironment experienced by the probes,[22−35] were measured as a function of the concentration of the host molecules (dimer, trimer, and tetramers **1** and **2**). The lifetime decays of Py in the absence and presence of host molecules were analyzed by using mono-, bi-, and triexponential functions. In Figure 5, Py shows a monoexponential decay with lifetime around 130 ns in the absence of host dimer molecules, while biexponential decays with lifetimes around 110 and 300 ns are obtained in the presence of the host molecules (Table 3). The residuals of the fitting analysis in Figure 5 and the values of χ^2 all indicate that the mono- and biexponential analyses are acceptable. For BeP

TABLE 3: Fluorescence Lifetimes of Pyrene, BeP, and Co at Different Host Concentrations at pH 10

guest	host	C_{host} (μM)	τ_1 (ns)	Φ_1	τ_2 (ns)	Φ_2	χ^2
Py	dimer	0	129.0	1			1
		1	129.5	1			1
		10	100.2	0.3449	290.1	0.6551	1
		25	92.3	0.2995	279.5	0.7005	1
		40	112.7	0.1046	290.7	0.8954	1
	trimer	0.1	127.9	1			0.963
		1	131.6	0.9099	332.6	0.0901	0.752
		6	122.3	0.4438	321.4	0.5562	0.985
		17	100.3	0.2088	333.9	0.7912	1
		25	99.9	0.1460	330.1	0.854	1
	tetramer **1**	0.1	134.3	1			1
		1	124.6	0.5243	334.1	0.4757	1
		6	122.2	0.2489	325.4	0.7511	1
		17	123.4	0.1848	349.4	0.8152	1
		25	111.3	0.1334	360.1	0.8666	1
	tetramer **2**	0.1	129.0	1			1
		1	129.6	0.7938	316.8	0.2062	0.975
		6	119.8	0.3722	305.8	0.6278	1
		17	113.2	0.2115	332.4	0.7885	1
		25	100.0	0.1663	309.5	0.8337	1
BeP[a]	dimer	10	4.0	0.0518	49.3	0.9482	1
		25			50.9	1	0.993
		40			47.4	1	1
	trimer	10	2.2	0.0381	51.0	0.962	1
		25			49.4	1	1
Co[a]	dimer	10	1.2	0.0428	223.1	0.9572	1.04
		25			214.3	1	1
		40			222.7	1	1
	trimer	10	1.3	0.0151	218.2	0.9849	1
		25			230.1	1	1

[a] Lifetime data are not available because of low fluorescence intensity when $C_{host} < 10$ μM.

and Co (Table 3), two decay constants are obtained ($\sim$ 3 and 50 ns for BeP, and 1.2 and 220 ns for Co) at low concentrations of the host molecules, while at high concentrations of the host only a long lifetime of 50 and 220 ns were observed for BeP and Co, respectively. According to the literature,[23−28,31−35] the short lifetimes are attributed to the species in the bulky solution and the longer ones to the single guest molecules individually solubilized in the hydrophobic pockets of the hosts. The PAH probes in an excited state are easily quenched by water molecules, while they can be protected in the hydrophobic pockets of the hosts to avoid quenching so that their lifetimes are prolonged. A comparison of the relative concentration of the species (Φ) shows that the concentration of probes in bulky solution decreased with increasing concentration of host molecules, at the same time the relative concentration of probes in the hydrophobic pockets increased (Table 3). Moreover, the relative concentration of probes in the hydrophobic pockets increased from dimer to tetramer at similar concentrations of host molecules, indicating that the binding constants between tetramer and guest probes are larger than that between dimer and guest probes. The fluorescence intensities of BeP and Co in bulky solution are too weak to be detected when $C_{host} < 10$ μM and the τ_1 were observed to be much smaller than τ_2. Therefore, the probes in solution contributed little to the overall fluorescence decay. The BeP and Co are more hydrophobic than Py and thus form complexes more easily. Therefore, only one (longer) fluorescence lifetime τ_2 of BeP and Co was observed in the presence of host molecules at high concentrations (Table 3).

All the fluorescence lifetime results suggest that short-lived and long-lived species of the probes existed in the host−guest systems. It is easy to understand that only two species exist at equilibrium in the complexes of 1:1 stoichiometric ratio. For the complexes in 1:2 stoichiometric ratio, only two species were found in the analysis, a short-lived species (corresponding to the free PAH probes) and a long-lived one (PAH in the complexes). It is possible that three species, free guest, (Guest)(Host), and (Guest)$(Host)_2$, coexist in the solution at the same time. Either the concentration of (Guest)(Host) is too low to be detected or the lifetimes for (Guest)(Host) and (Guest)$(Host)_2$ are similar, resulting in the fact that only two lifetimes were observed in the analysis.

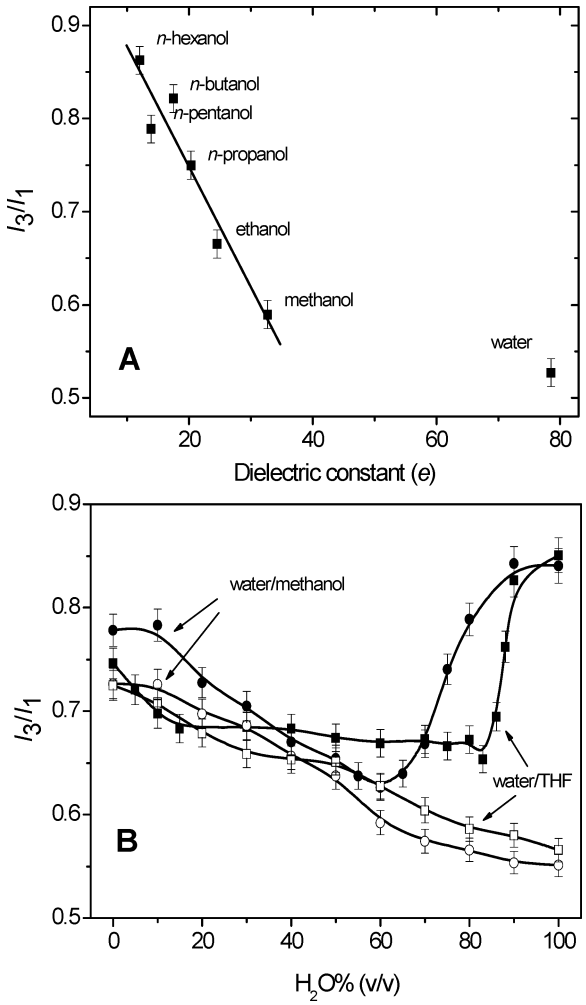

Figure 6. (A) The ratio of I_3 to I_1 of Py (2×10^{-7} M) in pure water and in different alcohols with different alkyl groups vs the dielectric constant of the solvents; (B) the ratio of I_3 to I_1 of Py (2×10^{-6} M) in the absence (open symbols) and presence (closed symbols) of host molecules (trimer, 2×10^{-5} M) as a function of water content (vol %) in THF (squares) and in methanol (circles).

3.4. Solvent Effects. The intensities for various fine vibronic structures of monomeric pyrene in the fluorescence spectrum have been reported to show strong solvent dependence.[29,31,32] For example, the intensity of I_1 may be enhanced in the presence of polar solvents at the expense of other peaks. This strong perturbation in the vibronic band intensities depends on both the dipole and the dielectric constant of the solvent.

To estimate the polarity and the nature of the binding sites of Py in the above-mentioned pockets formed by star-shaped cholic acid derivatives according to the method in the literature,[29] the following correlation, between the ratios of I_3/I_1 for Py in alcohols and dielectric constant (ϵ) of the alcohols (from methanol to *n*-hexanol)[29] and water (Figure 6A), can be obtained

$$\frac{I_3}{I_1} = 1.01 - 0.013\epsilon \quad (7)$$

As compared with the ratios of I_3/I_1 in Figure 3A, the dielectric constant of the pocket environment surrounding Py were estimated to be around 12, 20, 22, and 30 in the inclusion complexes of dimer, trimer, and tetramers **1** and **2**, respectively. The increase in micropolarity from dimer to tetramers may indicate that the structures of the inclusion complexes between the dimer and Py is more compact and that the complexes with the trimer and tetramers are less tight and more accessible to water molecules due to the steric hindrances between the cholic acid units, even though the binding constant for the trimer and tetramers are larger than that for the dimer.

It has been reported that the conformations of the star-shaped cholic acid derivatives can respond to the environment of surrounding solvent.[6,11,14,16,19] The fluorescence spectra of Py in the absence and presence of trimer were recorded in the mixture solvents of water/THF and water/methanol with varying component ratios. The ratios of I_3/I_1 are plotted vs the concentration of water (vol %) in Figure 6B. It showed that the ratio of I_3/I_1 continuously decreases with increasing water contents in the absence of trimer. While in the presence of trimer, the ratio of I_3/I_1 decreases to a certain value of 60 vol % for water/methanol and 80 vol % for water/THF, respectively, and then it increases with further increase in water content. It means that the polarity of the solvent mixture allows the formation of the hydrophobic pockets by the host molecules in which Py can be sheltered and that the pockets become tighter with increasing polarity of the solvent mixture. Since THF is more hydrophobic than methanol, higher water content (80 vol %) is needed to increase the polarity of the environment for the formation of pockets of similar characteristics (water/methanol mixture at 60 vol % water content).

3.5. The Structural Characteristics of the Inclusion Complexes. Bile acids are natural amphiphilic molecules that can form complexes with hydrophobic guest molecules such as naphthalene, pyrene, and their derivatives via stepwise formation of primary and secondary aggregates or through hydrogen bonding.[21−23,37−39] Sandwich structures have been suggested[21−23] during the primary aggregation, while a more complex structure is formed by the primary aggregates into secondary aggregates. It also has been reported that two cholic acid molecules are necessary to solubilize one Py molecule, while four, six, or eight cholic acid molecules are needed to complex with larger guest probes of BeP, Co, and ovalene.[23] On the basis of the results of the stoichiometric analysis in this study, the star-shaped cholic acid oligomers can fold in one direction and form a hydrophobic cavity, a process driven by solvophobic interactions. For 1:1 complexes of Py and dimer, a pyrene molecule is sandwiched between two cholic acid units of the dimer; its aromatic ring surface was just covered by the two steroid units. For 1:1 complexes between Py/BeP and trimer/tetramers **1** and **2**, cholic acid monomers formed an umbrella-shaped structure with the Py/BeP guest probes in the middle. Water molecules may have access to the guest inside since such a structure is not very compact. This is consistent with the evaluated dielectric constants of the pocket interior for the Py complexes with different host molecules discussed previously. The relative size and shape play important role in the formation of inclusion complexes. A single dimer is clearly too small to effectively solubilize the larger BeP and Co. Therefore, 1:2 host−guest complexes would be formed.

Molecular modeling results in the literature[23] indicate that four cholic acid units match the size of the two larger probes (BeP and Co). The fluorescence study in this work indicates the formation of 1:2 complexes. To maximize the contact with guest probes, two optimal conformations can be proposed for such complexes with the four cholane surfaces of two dimers assembling into a cage with the guest in the middle. The energy difference between the two conformations is within thermal energy ($\sim kT$).[23] The relatively large probe molecule located in the center of the cavity helps to minimize the energy of the complexes.

4. Concluding Remarks

Inclusion complexes are formed between the star-shaped cholic acid derivatives and the PAH probes driven by the solvophobic effect as evidenced by fluorescence studies. The binding constants, the stoichiometric ratio and the fluorescence lifetime of the free and bound PAH probes can be estimated. The stoichiometric ratio of such complexes depends on the relative shape and size of the host and guest molecules. The formation of the complex also depends on the relative polarity of the environment in the solution. It is important to note that no micellar aggregation of the host and guest molecules was observed in the concentration range used in this study because of the solubility limit of these compounds. The application in regard to the flipping of such star-shaped cholic acid derivatives will be further explored.

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Supporting Information Available: Benesi−Hildebrand-type double reciprocal analysis (Figure S1, S2, and S3), $1/(P - P_0)$ vs $m/[\text{Host}]^n$, for Py, BeP, and Co in the presence of host molecules (trimer, tetramer **1**, tetramer **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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