Probing the Interactions of Calix[4]arene-Based Amphiphiles and Cyclodextrins in Water

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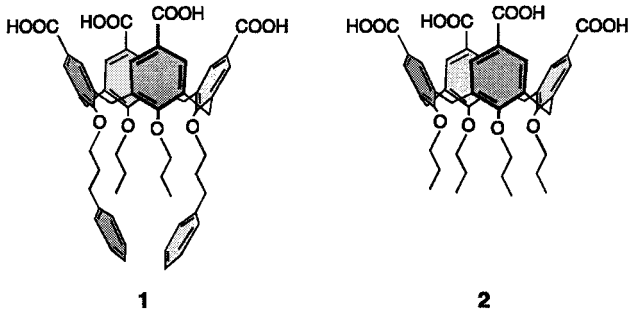
The surfactant behavior of the water-soluble calix[4]arene **1**, which has carboxylate groups at the upper rim and two phenyl groups at the lower rim, and its complexation to γ-cyclodextrin were studied. The critical micelle concentration (cmc) of **1** and of tetrapropoxycalix[4]arenetetracarboxylate **2** are 35 and 650 μM, respectively. The stabilities of the 1:1 inclusion complexes of γ-cyclodextrin with **1** and **2** are 1.3×10^4 and 1.5×10^3 M^{-1}, respectively. The stability of the former complex was derived from a calorimetric titration under conditions where **1** is largely micellized. A minimization routine is described for determining the heat contributions of demicellization and complexation. The association of **1** to γ-cyclodextrin is enthalpy-driven, which, together with the high K value and the 1:1 binding stoichiometry, indicates that both pendant phenyl rings of **1** are included in the γ-cyclodextrin cavity.

Introduction

Previously, in our group receptor molecules were synthesized by both covalent and noncovalent combinations of supramolecular building modules such as calix[4]arenes, cavitands, cyclodextrins, and porphyrins.[1,2] Covalent 1:1 couples of calix[4]arene and β-cyclodextrin containing a fluorescent group have been prepared as sensors for organic analytes in water.[3] These compounds show strong amphiphilic behavior and form vesicles in solution. The sensing properties depend heavily on this aggregation behavior, which is caused mainly by the calixarene moiety. This prompted us to study noncovalent combinations of cyclodextrins and calix[4]arenes in a more systematic manner. To this purpose, we designed calix[4]-arenes with distinct amphiphilic character and which could also act as guest molecules for complexation in cyclodextrins. The synthetic versatility of calix[4]arenes renders them attractive as platforms to which several functionalities or guests for cyclodextrins can be attached.

Cyclodextrins are widely known for their ability to complex hydrophobic guests in aqueous solution.[4] The stability of cyclodextrin complexes is known to depend on the fit of the guest in the cyclodextrin host. The size-fit concept[5] predicts the highest complex stabilities for guests that match the cyclodextrin cavity in size and shape. These complexations involve a large negative enthalpy change. Due to the fact that this favorable enthalpy change is partly compensated for by a large, negative complexation entropy (the so-called enthalpy−entropy compensation effect[5]), such complexations are enthalpy-driven. In contrast, complexation of loosely bound guest molecules, which retain some of their rotational degrees of freedom in the complexed state, is entropy-driven.

Chart 1



1 **2**

In this study, we describe the micellar behavior and γ-cyclodextrin inclusion of two calix[4]arene derivatives. Modeling studies pointed out that even the γ-cyclodextrin cavity is too small to actually encapsulate the aromatic framework of calix[4]arenes. However, substituents converging to one large hydrophobic "trunk" may be introduced at the lower rim, which then provides a binding site for γ-cyclodextrin. Since two aromatic moieties fit very well in γ-cyclodextrin,[6] a calix[4]arene with two lower rim-attached propoxyphenyl groups was synthesized (**1**, Chart 1). Solubility in water was achieved by introduction of carboxylate groups at the calix[4]arene upper rim. The tetrapropoxycalix[4]arene tetraacid **2** was synthesized as a reference compound to investigate the influence of the aromatic groups of **1** on the aggregation behavior and the γ-cyclodextrin association process.

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Experimental Section

General Information. Tetrapropoxycalix[4]arenetetracarboxylic acid (**2**) was synthesized according to literature procedures, by oxidation of the tetraformyl species,[7] which was obtained via lithium exchange of the tetrabrominated upper rim of tetrapropoxycalix[4]arene.[8] Chemicals were obtained from commercial sources and used as such. γ-Cyclodextrin was dried in vacuo at 80 °C in the presence of P_2O_5 for at least 5 h before use. Solvents were dried using standard laboratory procedures.

25,27-Bis(3-phenylpropoxy)calix[4]arene (4). This reaction was carried out in a fashion analogous to a literature procedure for the selective functionalization of the calix[4]arene lower rim.[9] Calix[4]arene (**3**) (2.5 g, 5.9 mmol) was added to a suspension of K_2CO_3 (1.9 g, 14.5 mmol) and NaI (0.3 g, 1.8 mmol) in acetonitrile (100 mL). 1-Phenyl-3-bromopropane (2.7 g, 14.5 mmol) was added, and the mixture was refluxed for 24 h. The product was purified by column chromatography and isolated as colorless crystals (2.7 g, 70%). Mp: 206 °C. ^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm): 8.28 (s, 2H, OH), 7.29−7.14 (m, 10H, $CH_2CH_2CH_2Ph$), 7.07, 6.91 (2d, 8H, $^3J_{m-p}$ = 7.3 Hz, Ar *m*-H), 6.73, 6.65 (2t, 4H, Ar *p*-H), 4.33 (d, 4H, $^2J_{gem}$ = −13.2 Hz, ArCH_2Ar), 4.00 (t, 4H, ^3J = 6.4 Hz, OCH_2), 3.37 (d, 4H, ArCH_2Ar), 3.01 (t, 4H, ^3J = 7.7 Hz, CH_2Ph), 2.35 (m, 4H, OCH_2CH_2). ^{13}C NMR (66 MHz, $CDCl_3$), δ (ppm): 153.27, 151.88, 141.40, 133.28, 128.88, 128.52, 128.45, 128.36, 128.08, 125.88, 125.31, 119.99 (Ar C), 75.79 (OCH_2), 32.05 (ArCH_2Ar), 31.59, 31.43 (CH_2CH_2Ph). FAB-MS: calcd for $C_{46}H_{44}O_4$, *m*/*z* = 661.3 [M + H]; found, *m*/*z* = 660.2 [M + H]. Anal. Calcd for $C_{46}H_{44}O_4 \cdot 0.5H_2O$: C, 82.48; H, 6.77, Found: C, 82.72; H, 6.42.

25,27-Bis(3-phenylpropoxy)-26,28-dipropoxycalix[4]arene (5). This reaction was carried out in a fashion analogous to a literature procedure for the selective functionalization of the calix[4]arene lower rim.[10] NaH (0.26 g of a 60% dispersion in mineral oil, 6.7 mmol) was washed with hexane under an argon atmosphere. DMF (50 mL) was added, followed by **4** (2.0 g, 3.0 mmol). The mixture was heated at 60 °C for 1 h. 1-Iodopropane (0.65 mL, 6.7 mmol) was added, and the mixture was stirred overnight at 60 °C. The product was purified by column chromatography and isolated as a colorless oil (1.6 g, 72%). ^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm): 7.33−7.20 (m, 10H, CH_2CH_2Ph), 6.70−6.55 (m, 12H, calix Ar H), 4.46 (d, 4H, $^2J_{gem}$ = −13.4 Hz, ArCH_2Ar), 3.99 (t, 4H, ^3J = 7.2 Hz, OCH_2-CH_2CH_2Ph), 3.84 (t, 4H, ^3J = 7.5 Hz, OCH_2CH_2Me), 3.17 (d, 4H, ArCH_2Ar), 2.73 (t, 4H, ^3J = 8.1 Hz, CH_2CH_2Ph), 2.23 (m, 4H, CH_2CH_2Ph), 1.91 (sx, 4H, CH_2Me), 0.99 (t, 6H, ^3J = 7.3 Hz, CH_2CH_3). ^{13}C NMR (66 MHz, $CDCl_3$), δ (ppm): 156.54, 156.29, 141.97, 135.27, 134.76, 128.30, 128.29, 128.20, 128.02, 125.76, 121.98, 121.91 (Ar C), 76.71, 74.40 (OCH_2), 32.63, 31.97, 30.97, 26.87, 23.35 (CH_2), 10.49 (CH_3). FAB-MS: calcd for $C_{52}H_{56}O_4$, *m*/*z* = 745.4 [M + H]; found, *m*/*z* = 745.2 [M + H].

5,11,17,23-Tetrabromo-25,27-bis(3-phenylpropoxy)-26,28-dipropoxycalix[4]arene (6). This reaction was carried out in a fashion analogous to a literature procedure for the direct introduction of functionalities at the para position of the calixarene ring system.[11] *N*-Bromosuccinimide (3.1 g, 1.7 mmol) was added to a solution of **5** (1.6 g, 2.2 mmol) in DMF (50 mL). The reaction mixture was stirred at room temperature for 40 h. The product was purified by recrystallization from $CHCl_3$/MeOH and isolated as colorless crystals (1.6 g, 71%). Mp: 174−175 °C. ^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm): 7.33−7.14 (m, 10H, CH_2Ph), 6.87, 6.73 (2s, 8H, calix Ar H), 4.33 (d, 4H, $^2J_{gem}$ = −13.4 Hz, ArCH_2Ar), 3.93 (t, 4H, ^3J = 7.5 Hz, $OCH_2CH_2CH_2$Ph), 3.76 (t, 4H, ^3J = 7.5 Hz, OCH_2CH_2Me), 3.08 (d, 4H, ArCH_2Ar), 2.65 (t, 4H, ^3J = 7.9 Hz, CH_2CH_2Ph), 2.15 (m, 4H, CH_2CH_2Ph), 1.83 (sx, 4H, CH_2Me), 0.95 (t, 6H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C NMR (66 MHz, $CDCl_3$), δ (ppm): 155.50, 155.20, 141.41, 136.66, 136.00, 131.08, 130.86, 128.41, 128.18, 125.97, 115.22 (Ar C), 77.06, 74.70 (OCH_2), 32.42, 31.62, 30.69, 23.81 (CH_2), 10.39 (CH_3). FAB-MS: calcd for $C_{52}H_{52}Br_4O_4$, *m*/*z* = 1057.1 [M + H]; found, *m*/*z* = 1060.6 [M + H]. Anal. Calcd for $C_{52}H_{52}O_4Br_4$: C, 58.88; H, 4.95; Br, 30.13. Found: C, 58.70; H, 5.02; Br, 29.79.

25,27-Bis(3-phenylpropoxy)-26,28-dipropoxycalix[4]arene-5,11,17,23-tetracarboxylic acid (1). This reaction was carried out in a fashion analogous to a literature procedure for the selective upper rim functionalization via di- or tetralithiated calix[4]arenes.[8] A solution of **6** (1.2 g, 1.1 mmol) in THF (50 mL) was cooled to −78 °C under argon. *tert*-Butyllithium (7.0 mL of a 1.7 M solution in pentane, 11.9 mmol) was added, and the reaction mixture was stirred for 30 min at −78 °C. An excess of dry gaseous CO_2 was bubbled through the mixture while being warmed slowly to room temperature. A 6 M HCl solution (15 mL) was added to precipitate the crude product. The product was purified by recrystallization from 1-butanol and isolated as colorless crystals (0.63 g, 62%). Mp: >290 °C. ^{1}H NMR (250 MHz, DMSO-d_6), δ (ppm): 12.37 (br, COOH), 7.48, 7.17 (2s, 8H, calix Ar H), 7.33−7.21 (m, 10H, CH_2Ph), 4.34 (d, 4H, $^2J_{gem}$ = −13.1 Hz, ArCH_2Ar), 4.02 (t, 4H, ^3J = 7.4 Hz, $OCH_2CH_2CH_2$Ph), 3.79 (t, 4H, ^3J = 7.2 Hz, OCH_2CH_2Me), 3.40 (d, 4H, ArCH_2Ar), 2.65 (t, 4H, ^3J = 7.8 Hz, CH_2CH_2Ph), 2.16 (m, 4H, CH_2CH_2Ph), 1.83 (m, 4H, CH_2Me), 0.95 (t, 6H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C NMR (66 MHz, DMSO-d_6), δ (ppm): 166.83, 166.54 (COOH), 160.02, 159.36, 141.36, 134.76, 133.68, 129.80, 129.39, 128.28, 128.02, 125.81, 124.66, 124.49 (Ar C), 76.70, 74.29 (OCH_2), 31.65, 31.46, 29.97, 22.84 (CH_2), 10.21 (CH_3). FAB-MS: calcd for $C_{56}H_{56}O_{12}$, *m*/*z* = 919.4 [M − H]; found, *m*/*z* = 919.3 [M − H]. Anal. Calcd for $C_{56}H_{56}O_{12}$: C, 73.03; H, 6.13. Found: C, 72.73; H, 6.01.

UV Spectroscopy. UV measurements were carried out at 25 °C using a Hewlett-Packard 8452A diode array spectrophotometer. The calixarene tetraacids were dissolved in a $NaHCO_3$/NaOH buffer at pH = 10; ionic strength I = 0.123 M. Aliquots of 5 or 10 μL of stock solutions of **1** (4.6 mM) or **2** (7.2 mM) were titrated into a solution of Reichardt's dye (5.2 μM) or Methylene Blue (4.0 μM) in the cuvette (d = 1.0 cm), monitoring the wavelength corresponding with the solvatochromic charge-transfer band or the absorbance of the 670 nm band, respectively. The stock solutions of the calix[4]arenes also contained the same concentrations of the corresponding dyes to keep their concentrations constant throughout the titration.

Calorimetry. Calorimetric measurements were carried out at 30 °C using a Microcal VP-ITC microcalorimeter with a cell volume of 1.4115 mL. In all measurements, the same buffer solution was employed, as mentioned above for the UV titrations. For studying demicellization, 5 μL aliquots (50 injections, 10 s per injection, 7 min between injections) of a 0.49 mM stock solution of the tetrasodium salt of **1** were titrated into the pure buffer solution in the calorimetric cell. The experiment was carried out two times and appeared to be fully reproducible. In another demicellization experiment, a 2.49 mM stock solution of the tetrasodium salt of **1** was titrated into the buffer solution initially using 2 μL aliquots (10 injections) followed by 10 μL injections. For studying the complexation in γ-cyclodextrin, 10 μL aliquots (29 injections, 20 s per injection) of a 2.49 mM stock solution of the tetrasodium salt of **1** were titrated into a 0.49 mM solution of γ-cyclodextrin in the calorimetric cell.

NMR. NMR titrations were carried out at 25 °C using a Bruker AC 250 and a Varian Inova 300 NMR spectrometer. ^{1}H NMR chemical shifts (250 or 300 MHz) are given relative to residual $CHCl_3$ (7.25 ppm), DMSO (2.50 ppm), or HDO (4.65 ppm). ^{13}C chemical shifts (66 or 75 MHz) are given relative to $CDCl_3$ (77.00 ppm). ^{1}H NMR titrations were performed in a 0.01 M Na_2CO_3 buffer in D_2O. The guest concentrations were kept constant at 0.1 mM for **1** and 0.2 mM for **2**. The Job's plot analysis was carried out by mixing 0.1 mM stock solutions of **1** and γ-cyclodextrin.

Mass Spectrometry and Elemental Analysis. Mass spectra were recorded with a Finnigan MAT 90 spectrometer using *m*-nitrobenzyl alcohol (NBA) as a matrix. Elemental analyses were carried out with a model 1106 Carlo-Erba Strumentazione element analyzer.

Results and Discussion

For the synthesis of **1**, the lower rim of calix[4]arene **3** was first alkylated at the 25- and 27-positions with

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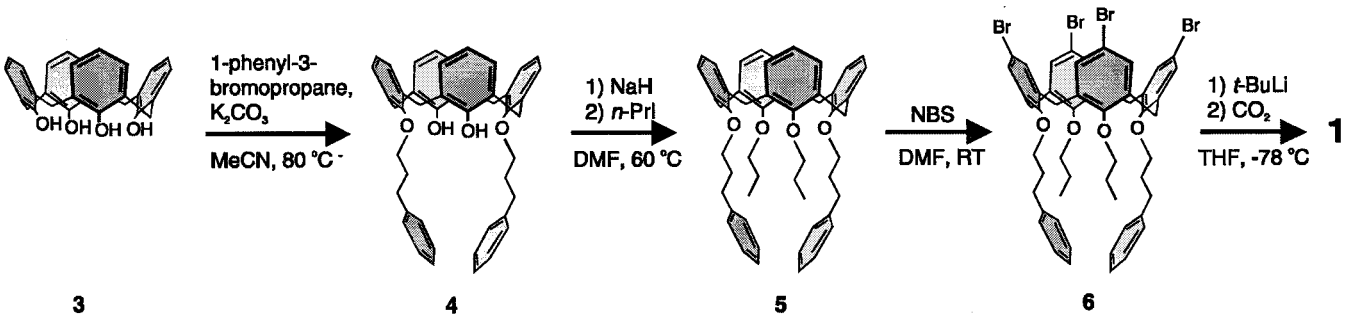
Scheme 1



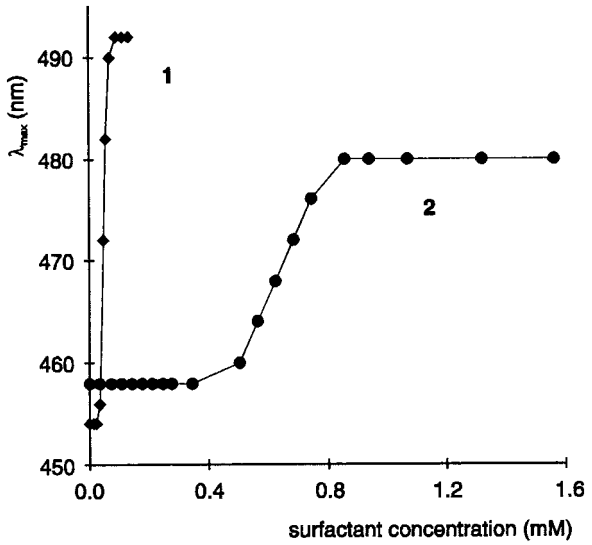


Figure 1. Absorption maxima of Reichardt's dye as a function of the concentrations of surfactants **1** and **2** ($NaHCO_3$ buffer, pH = 10, I = 0.123 M, [dye] = 5.0 μM, T = 25 °C).

3-bromo-1-phenylpropane and then alkylated at the 26- and 28-positions with 1-iodopropane (**5**, Scheme 1). Solubility in water was introduced by carboxylic acid groups at the upper rim, which were introduced by quenching the tetralithiated species with CO_2.[8] Calixarene **2** was prepared as described before.[8]

Calix[4]arenes **1** and **2** are water soluble when all four carboxylic acid groups are deprotonated (pH > 7). Since the hydrophilic upper rim is well separated from the hydrophobic bulk consisting of the calix[4]arene framework and the lower rim substituents, both species are amphiphilic. The critical micelle concentrations (cmc's) of calix[4]arene-based surfactants **1** and **2** were determined by UV spectroscopy, using two different dye probes.

The first dye used was the solvatochromic Reichardt's dye.[12] The wavelength corresponding to the intramolecular charge transfer transition is strongly dependent on the polarity of the environment of the dye. In Figure 1, the wavelength of this band is depicted versus the calix[4]arene concentration. A strong increase of λ_{max} is observed upon reaching the cmc, which is 45 μM for **1** and 650 μM for **2** under the conditions employed here.

The rather hydrophobic Reichardt's dye is believed to interact with the hydrophobic parts of the micelles, as soon as they are formed, resulting in a more apolar environment, which leads to destabilization of the *polar* ground state and stabilization of the *apolar* excited state of the dye. This gives rise to the observed red shift of the charge transfer absorption band.

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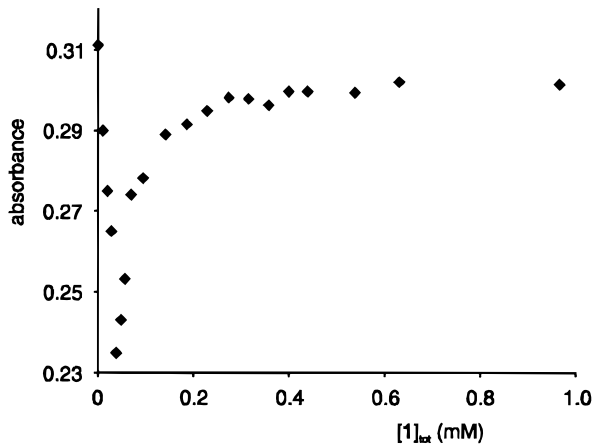

Figure 2. Absorbance of Methylene Blue at 670 nm as a function of the concentration of **1** ($NaHCO_3$ buffer, pH = 10, I = 0.123 M, $[MB^+]$ = 5.0 mM, T = 25 °C).

In an independent UV experiment, the concentration of **1** was increased in the presence of Methylene Blue. The 670 nm absorbance of this dye is strongly dependent on the polarity of the environment.[13] In Figure 2, the absorbance at 670 nm is depicted versus the concentration of **1**. Around the cmc a marked drop in the absorbance is observed, followed by a steep increase at higher concentrations until a limiting value is reached. The concentration of **1** at the minimum of the curve is the cmc. This method gives a cmc of 35 μM for **1**, which agrees well with the value obtained by the Reichardt's dye method. The decrease in absorbance at concentrations below the cmc was attributed to the interaction between the surfactant monomers and the Methylene Blue (MB^+) ions and to stacking of the flat MB^+ ions.[13,14] This stacking of the MB^+ ions might induce the formation of aggregates of the surfactant molecules *below* the cmc.

Cmc's of some tetraalkylammonium calix[4]arene- and calix[6]arene-based surfactants have previously been determined by Shinkai et al. using surface tension measurements, fluorescence spectroscopy, and conductivity measurements.[15] They found low cmc's (5×10^{-7} to 5×10^{-5} M) for cone calix[4]arene amphiphiles, in which the cationic charges at the upper rim ($Me_3N^+-(CH_2)_n-$ calix groups) are well separated from the hydrophobic bulk. Substantially higher cmc's (3×10^{-5} to 3×10^{-3} M) were found for the isomeric surfactants in the 1,3-alternate conformation due to equal charge distribution at both sides

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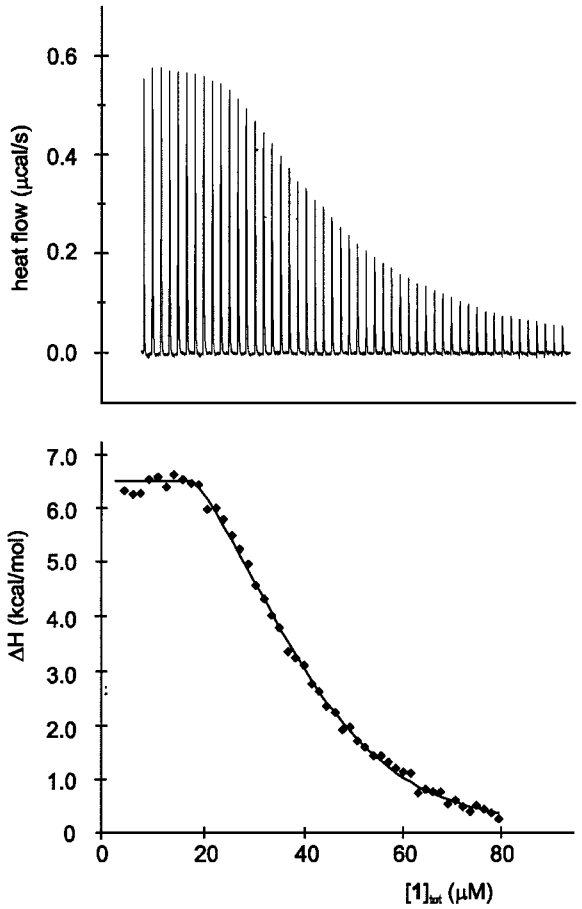

Figure 3. Heat flow after each injection of 0.49 mM **1** into the buffer as a function of time (top) and the resulting enthalpy as a function of the total surfactant concentration $[\mathbf{1}]_{tot}$ (bottom) ($NaHCO_3$ buffer, pH = 10, I = 0.123 M, T = 30 °C).

of the molecule. Interestingly, the cmc's for **1** and **2** fall in the latter range although they both are in the cone conformation. It shows that calix[4]arene amphiphiles with a wide range of aggregation strengths can be made.

The strength of the association of **1** and γ-cyclodextrin was determined using titration microcalorimetry. The assessment of the stability constant is not trivial since the aggregation behavior of **1** interferes strongly with the association process and we must dissect the contributions of the (de)aggregation processes of **1** and of the 1:1 association with γ-cyclodextrin. At first, we performed a demicellization experiment at the lowest possible concentration (0.49 mM) where the observed heat effects were still reliable and enough data points could be obtained before reaching the cmc. This allows an accurate determination of the enthalpy of demicellization and of the cmc. Figure 3 shows the isothermal titration calorimetric (ITC) data as well as the enthalpogram obtained from this titration in which a micellized (0.49 mM) solution of **1** was titrated into the buffer. As long as the concentration of **1** in the calorimetric cell remained below the cmc, equally large endothermic heat pulses were obtained as a result of complete micellar breakup. With increasing concentration, the effect diminished due to incomplete breakup of the micelles at concentrations approaching the cmc and eventually reduced to zero. The calorimetrically determined cmc (at the inflection point of the curve) of **1** is about 30 μM and agrees well with the values obtained by the UV methods discussed above.

The enthalpogram (Figure 3, bottom) depicts the resulting enthalpy (i.e., the evolved heat per added mole

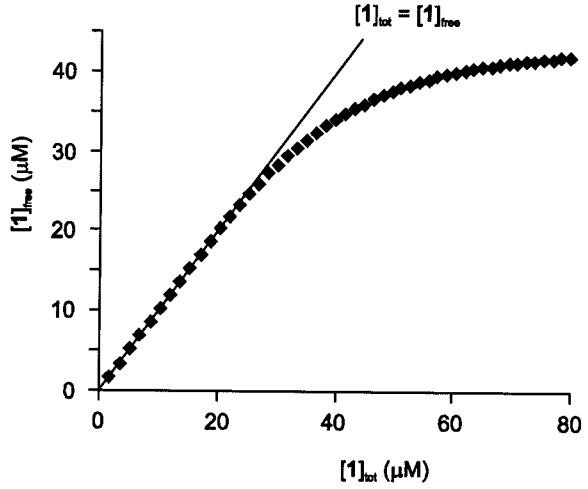

Figure 4. Free surfactant concentration $[\mathbf{1}]_{free}$ as a function of the total concentration $[\mathbf{1}]_{tot}$.

of surfactant) as a function of the total surfactant concentration $[\mathbf{1}]_{tot}$, which is the sum of the concentrations of micellized surfactant $[\mathbf{1}]_{mic}$ and the free surfactant $[\mathbf{1}]_{free}$ in the cell. In the ideal case, the latter equals the cmc at $[\mathbf{1}]_{tot}$ > cmc.[16] From the average value of the data points in the horizontal part of the enthalpogram, the enthalpy of demicellization ΔH_{dem} (7.1 kcal mol^{-1}) was derived after correcting for the fraction of nonmicellized surfactant (42 μM, 8.6%; see below).

In the ideal case, the transition from the monomeric surfactant in solution to the micellized species occurs at a well-defined concentration (the cmc). This would correspond to a titration in which the heat effects reduce to zero once the cmc is reached. In the present case, however, the endothermic heat effect already starts to decrease at a concentration of about 20 μM and then slowly diminishes to zero. For every injection of the titration (Figure 3), the evolved heat was divided by ΔH_{dem}, which gave the free surfactant concentration $[\mathbf{1}]_{free}$ as a function of $[\mathbf{1}]_{tot}$ (Figure 4). It is obvious that demicellization is incomplete at $[\mathbf{1}]_{tot}$ > 20 μM and that $[\mathbf{1}]_{free}$ levels off to about 42 μM.

For an accurate assessment of the stability constant of the inclusion complex of **1** and γ-cyclodextrin, a higher titrant concentration (2.49 mM) of **1** was needed (see below). We also performed a demicellization experiment using the same titrant concentration to check whether the demicellization heat, which is dependent on the micellar state of the surfactant, was the same at this concentration. Although only two data points could be taken at a concentration below the cmc, the general trend of the enthalpogram was similar to the one shown in Figure 3. The enthalpy of demicellization was 7.9 kcal mol^{-1}, which was slightly higher than the value (7.1 kcal mol^{-1}) measured at the lower titrant concentration.

The association of **1** with γ-cyclodextrin was studied by a titration of a 2.49 mM stock solution of **1** into a solution of 0.5 mM γ-cyclodextrin (Figure 5). The process occurring during this titration is depicted by the equilibrium given in Scheme 2. It is assumed that there is no direct interaction between the micelles and γ-cyclodextrin and that association occurs in a 1:1 fashion (see NMR results, shown below). Micellized **1** was titrated into the γ-cyclodextrin solution, and demicellization occurred at low cell concentrations of **1** until the cmc was reached. Owing to association of **1** with γ-cyclodextrin, the cmc was reached

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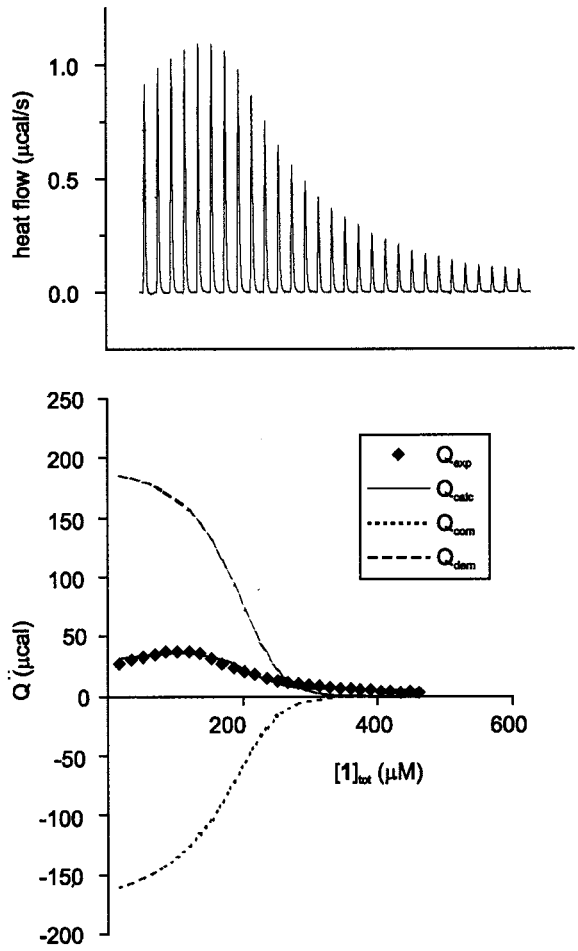

Figure 5. Heat flow after each injection as a function of time (top) and net heat developed after each injection of 2.49 mM **1** into 0.49 mM γ-cyclodextrin as a function of the total guest concentration $[\mathbf{1}]_{tot}$ (bottom) ($NaHCO_3$ buffer, pH = 10, $I = 0.123$ M, $T = 30$ °C).

Scheme 2

$$\mathbf{1}_{mic} \underset{\Delta H_{dem}}{\rightleftharpoons} \mathbf{1}_{free} \overset{+\gamma\text{-CD}}{\underset{\Delta H_{com}}{\rightleftharpoons}} \mathbf{1}\cdot\gamma\text{-CD}$$

at a higher total concentration of **1**. Therefore, the inflection point was found at 160 μM. A similar inflection point shift was observed previously by Cooper et al.[16] The observed heat effects were smaller because part of the endothermic demicellization heat was canceled by the exothermic association process. In the initial part of the curve, at which $[\mathbf{1}]_{free}$ remained below the cmc, complete demicellization occurred. Association, however, occurred only partly and was dependent on the free γ-cyclodextrin concentration. Therefore, the exothermic contributions to the observed heat effects diminished, leading to the observed increase of the endothermic heat effects in the initial part of the curve in Figure 5. The decrease of the observed heat effects in the remainder of the curve arose from incomplete demicellization analogous to the behavior discussed above.

As explained above, the inflection point of the curve (160 μM) occurred at higher $[\mathbf{1}]_{tot}$ than in the demicellization experiment (30 μM). As an approximation, this inflection point shift is attributed to uptake of **1** into the associate **1**·γ-CD ($[\mathbf{1}\cdot\gamma\text{-CD}] = 130$ μM). With $[\gamma\text{-CD}]_{tot} = 500$ μM and $[\mathbf{1}]_{free} = 30$ μM (cmc), this leads to a stability constant of $K = 1.2 \times 10^4$ M^{-1}.

K was determined more accurately by taking into account the complete enthalpogram depicted in Figure 5. The observed heat effects (Q_{exp}) contain contributions from demicellization of **1** (endothermic), inclusion into γ-cyclodextrin (exothermic), and dilution of the titrant. In our model, the calculated heats (Q_{calc}) therefore consisted of three terms for each of these contributions, respectively (eq 1). Both Q_{dem} (demicellization) and Q_{com} (complexation)

$$Q_{calc} = Q_{dem} + Q_{com} + Q_{dil} \quad (1)$$

required the calculation of the concentrations of free ($[\mathbf{1}]_{free}$) and micellized **1** ($[\mathbf{1}]_{mic}$) and of free ($[\gamma\text{-CD}]_{free}$) and complexed γ-cyclodextrin ($[\mathbf{1}\cdot\gamma\text{-CD}]$), both in equilibrium and directly after the titrant addition. This was achieved using the mass balance equations for **1** and γ-cyclodextrin, the equilibrium constant K for **1**·γ-CD (as an initial estimate), and the relationship between the free and micellized concentrations as observed in Figure 4. The demicellization heat is given by eq 2, where ΔH_{dem} is the

$$Q_{dem} = -\Delta H_{dem}(\Delta[\mathbf{1}]_{mic})V_{cell} \quad (2)$$

demicellization enthalpy (7.9 kcal mol^{-1}), $\Delta[\mathbf{1}]_{mic}$ is the change of the concentration of micellized **1** upon equilibration after the titrant addition, and V_{cell} is the cell volume. Analogously, Q_{com} was obtained from eq 3, where

$$Q_{com} = \Delta H_{com}(\Delta[1\cdot\gamma\text{-CD}])V_{cell} \quad (3)$$

ΔH_{com} is the complexation enthalpy and $\Delta[1\cdot\gamma\text{-CD}]$ is the increase of the inclusion complex concentration upon equilibration after the titrant addition. The differences between Q_{calc} and Q_{exp} were minimized in a least-squares optimization using K, ΔH_{com}, and Q_{dil} as the fitting parameters. Introduction of the dilution heat Q_{dil}, which is a constant heat effect, as a fitting parameter did not affect the value for K but significantly improved the overall fit. The optimization was carried out using a spreadsheet methodology described before.[17] Figure 5 shows the above-defined heat effects as a function of $[\mathbf{1}]_{tot}$.

From two separately performed calorimetric titrations, an association constant of $K = (1.28 \pm 0.03) \times 10^4$ M^{-1} was obtained, as well as $\Delta H_{com} = -7.9 \pm 0.2$ kcal mol^{-1}. This completes the thermodynamic picture of the association of **1** with γ-cyclodextrin; $\Delta G_{com} = -5.7$ kcal mol^{-1} and $T\Delta S_{com} = -2.2$ kcal mol^{-1}. It clearly shows that the association is enthalpy driven, which suggests a tight fit of **1** in the cavity[5] (see below). Despite the equal magnitude of ΔH_{com} and ΔH_{dem}, an overall endothermic heat effect was observed when micellized **1** was added to the γ-cyclodextrin solution. This is explained by the fact that less of **1** is complexed to γ-cyclodextrin than is demicellized upon addition.

Previously, titration microcalorimetry was applied to study cyclodextrin inclusion phenomena with a large number of organic analytes.[5,18] Also surfactant (de)micellization processes were effectively studied using this technique.[19] The complexation of surfactants by α-, β-, and γ-cyclodextrins has been investigated using various techniques, including NMR spectroscopy,[20] competitive binding using UV, visible, and fluorescent probes,[21,22]

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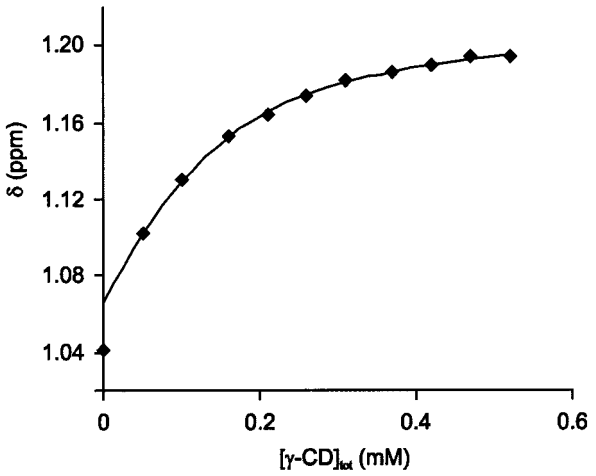

Figure 6. ^{1}H NMR titration of **1** with γ-cyclodextrin (Na_2CO_3 solution, pH = 10, I = 0.03 M, $[\mathbf{1}]_0$ = 0.12 mM, T = 25 °C).

surface tension,[23] sound velocity,[24,25] conductivity,[26] electrochemical methods,[25,27] and titration microcalorimetry.[16,28] These studies involve classical ionic surfactants such as *n*-alkylammonium derivatives and *n*-alkanesulfonates. In a comparative study of literature data, it was emphasized that large discrepancies exist in the values found for the complexation constants of surfactants and cyclodextrins, determined by direct versus indirect methods.[28] It was concluded that techniques which yield direct measurements of the free and bound surfactant gave consistent and reliable data. The consistency of microcalorimetric data with data obtained using these techniques showed that microcalorimetry, though being an indirect method, is a reliable technique for the determination of CD−surfactant complexation constants. To our knowledge, accurate calorimetrically determined K values have only been assessed using surfactant concentrations below the cmc, since demicellization processes are then avoided.[28] Because of the low cmc of **1**, however, we were forced to measure at concentrations of **1** higher than the cmc in order to obtain considerable heat contributions owing to complexation of **1** with γ-cyclodextrin. The rigorous mathematical treatment applied here allowed us to determine the stability constant even under these conditions.

To support the calorimetric data, the association of **1** and **2** with γ-cyclodextrin was also studied using NMR spectroscopy, which yields in a direct manner concentration ratios for free and bound guest. Titrations of **1** and **2** with γ-cyclodextrin were carried out at constant total calixarene concentrations ($[\mathbf{1}]_{tot}$ = 0.12 mM, $[\mathbf{2}]_{tot}$ = 0.20 mM) in a 0.01 M Na_2CO_3 solution in D_2O. The curve corresponding to the titration of **1** with γ-cyclodextrin is shown in Figure 6. A separate performed dilution experiment showed no line broadening at $[\mathbf{1}]_{tot} < 0.1$ mM (**2** showed no line broadening). The binding stoichiometry was verified by a separate Job's plot analysis (Figure 7), which clearly showed the formation of a 1:1 inclusion complex.

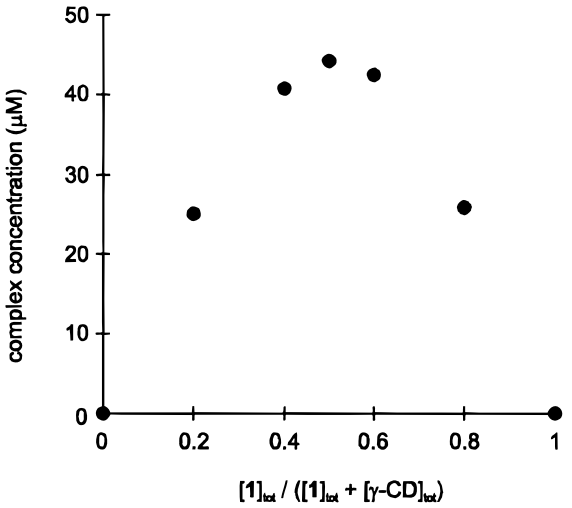

Figure 7. Job's plot for the complexation of **1** with γ-cyclodextrin.

The first data point in the 1:1 fitting procedure of the titration was omitted because there the guest was partly micellized. At all other data points, the guest appeared to be completely demicellized, probably due to the fact that the cmc is somewhat higher at this lower ionic strength. The association constant was found to be $(1.3 \pm 0.2) \times 10^4$ M^{-1}, in good agreement with the value obtained by calorimetry. The binding of **2** in γ-cyclodextrin is weaker ($K = 1.5 \times 10^3$ M^{-1}), which confirms that **1** fits better inside the cavity than **2** owing to the presence of the aromatic substituents in the former.

For calix[4]arene-based surfactants **1** and **2**, the largest complexation-induced shifts (CIS's)[29] upon addition of γ-cyclodextrin were observed for the ^{1}H signals corresponding to the terminal methyl groups of the propoxy chains (supposed to be included in the γ-cyclodextrin cavity) and to one of the two aromatic proton pairs of the calix[4]arene upper rim (supposed to be protruding from the γ-cyclodextrin cavity). The CIS of the methyl signal is probably caused by a change in the microenvironment due to the inclusion, whereas the CIS of the aromatic signal is attributed to an electronic effect or a conformational change of the still rather flexible calix[4]arene framework upon association. The CIS's of the other upper rim ^{1}H signal was not significant. The more complex signal set corresponding to the protons of the lower rim pendant phenyl rings of **1** also showed clear CIS's, but their overlap rendered them useless for monitoring association.

The cmc values of **1** as determined by the two UV spectroscopic dye methods are in good agreement (45 μM using Reichardt's dye and 35 μM using Methylene Blue), and they also agree with the value obtained from calorimetry (30 μM). These experiments reveal that micelles of **1** start to form at 20 μM. No line broadening was observed in the ^{1}H NMR spectrum of **1** at concentrations in the range 20−200 μM. At concentrations above 200 μM, gradual broadening of the signals was observed in the NMR spectrum upon increasing the concentration of **1**. This may be attributed to changes in micellar shape or to NMR exchange phenomena. The only other indication

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(29) The cyclodextrin cavity normally causes small but distinct shifts of the signals corresponding to the guest protons that are actually included due to weak shielding by the alicyclic cyclodextrin skeleton. Though small, these CISs are usually large enough for performing NMR titrations in an accurate manner. For NMR studies on cyclodextrin inclusion phenomena, see: Schneider, H.-J.; Hacket, F.; Rudiger, V. *Chem. Rev.* **1998**, *98*, 1755 and references therein.

for the possible existence of different micellar types in solutions of **1** are the different demicellization heats found at different titrant concentrations (see above). Such an expression of micellar states into microcalorimetric enthalpy data has been studied previously.[30] Since it is outside the scope of this study, we did not further investigate this behavior.

The association constants for the association of **1** with γ-cyclodextrin obtained using calorimetry (1.2×10^4 M^{-1} using the inflection point shift and 1.28×10^4 M^{-1} using the fitting procedure) are in excellent agreement with the value obtained by NMR titration (1.3×10^4 M^{-1}). This supports the accepted notion that microcalorimetry is as valuable as direct methods for reliably determining association constants.[28] It also confirms our rigorous mathematical treatment allowing a reliable association constant determination even under micellized conditions.

Furthermore, the association of **1** with γ-cyclodextrin is enthalpy driven. This means that the negative (unfavorable) value for $T\Delta S_{com}$ is canceled by the larger negative (favorable) value for ΔH_{com}. This is commonly observed for complexes whose association constants are relatively high and whose fits of guests inside the cyclodextrin cavity are rather tight, depriving the guests of their rotational degrees of freedom.[5] A tight fit leads to a contribution to the complex stability by van der Waals forces between the guest and the inner wall of the cyclodextrin cavity. These additional forces are not present in the case of a smaller guest, which is too far away from the cavity wall to experience van der Waals attraction. The high K value and the fact that the complexation is enthalpy driven strongly indicate that both pendant phenyl rings of **1** are included in the γ-cyclodextrin cavity. For comparison, inclusion of a single aromatic ring is orders of magnitude weaker; e.g., *n*-propylbenzene binds in γ-cyclodextrin with an association constant of $K = 52$ M^{-1}.[31] This complexation mode with both phenyl rings is supported by the observed 1:1 binding stoichiometry of the complexation.

Conclusions

An effective method is described to calorimetrically determine the association constant for the association between a cyclodextrin and a micellizing guest. The net enthalpogram resulting from the demicellization and complexation processes can be used to determine K using a least-squares fitting procedure. The value obtained is in good agreement with the ^{1}H NMR data. This means that the determination of the strength of association of a surfactant with a cyclodextrin does not necessarily need to be done at guest concentrations below the cmc to avoid "disturbing" aggregation processes. The complexation between **1** and γ-cyclodextrin is clearly enthalpy driven. It is therefore concluded that both phenyl rings are included, experiencing van der Waals interactions and thus providing stronger complexes.

This study expands our efforts toward the extension of well-defined supramolecular systems to aqueous environments. We envisage that strong cyclodextrin−guest interactions may be employed for the aqueous assembly of different units and functionalities.

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