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Controlling the Conformation of Oligocholate Foldamers by Surfactant Micelles

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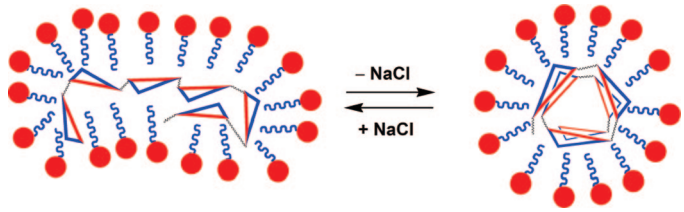
Received April 1, 2008



Fluorescence resonance energy transfer (FRET) occurred readily in a cholate hexamer labeled with a naphthyl donor and a dansyl acceptor at the chain ends when the hexamer was solubilized by sodium dodecyl sulfate (SDS) micelles in water. Independence of the energy transfer efficiency over 1−70 mM SDS suggested that the energy transfer resulted from the folding of the hexamer instead of its intermolecular aggregation within the micelle. Upon addition of sodium chloride to the solution, energy transfer became less efficient, indicating unfolding of the oligocholate. In contrast, the oligocholate stayed folded in the micelle of nonionic Brij 30, in the presence or absence of NaCl. These results suggested that the oligocholate preferred to fold within the small spherical SDS micelles but unfold when the preference for spherical over rodlike micelles was not strong enough to overcome the tendency for the oligocholate to unfold.

Introduction

Foldamers have attracted a great deal of interest in recent years as the synthetic analogues of biomolecules that adopt compact, ordered conformations.[1–3] On the fundamental level, learning to control the conformation of a chain-like molecule allows chemists to gain insight into the folding of biological polymers. Also, the conformation of a molecule dictates its size, shape, and the distribution of functional groups, all of which directly affect its chemical and physical properties. Therefore, on the practical level, conformational control can help chemists design materials that respond to environmental stimuli in predictable manners.

We recently reported amphiphilic foldamers[4] derived from cholic acid (Scheme 1).[5] These so-called oligocholates fold into helical structures in nonpolar solvents (e.g., hexane/ethyl acetate or CCl_4) containing a small amount of a polar solvent (e.g., DMSO or small alcohol). Intrastrand NH/OH hydrogen bonds are shown to be *not* important to the folding.[4d] Instead, a nanometer-sized hydrophilic cavity is created as the polar

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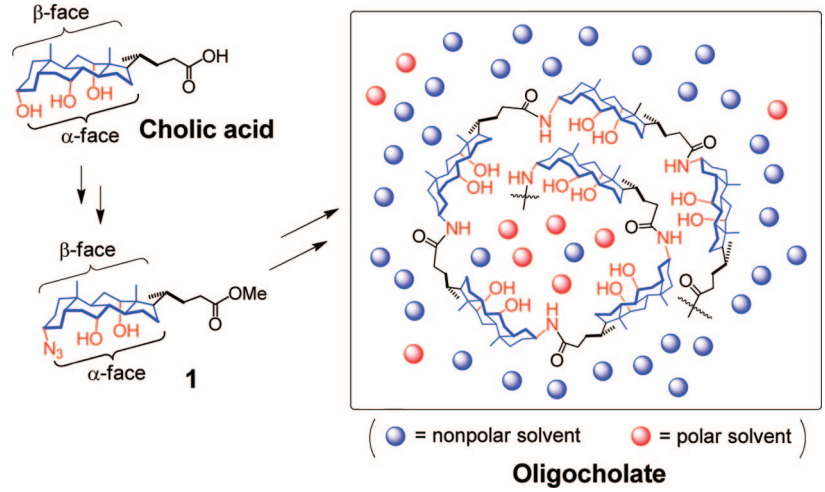
10.1021/jo800724j CCC: $40.75
Published on Web 06/24/2008

SCHEME 1. Cholic Acid and Oligocholate



hydroxyl groups of an oligocholate point inward and is solvated by the polar solvent preferentially.[4] This preferential solvation allows the folded oligocholate to minimize the unfavorable solvophobic contact between its polar faces and the nonpolar solvent.[4] Moreover, some of the polar solvent molecules now "happily" reside within a hydrophilic microenvironment instead of in the bulk, mostly nonpolar medium. Because the oligocholate relies on this preferential solvation to fold, unless stabilized by other interactions such as metal−ligand complexation,[4b,e] its folding requires the above mentioned special solvent mixtures.

In this paper, we report the folding of the oligocholates within surfactant micelles. An interesting discovery is that surfactant micelles, as nanosized hydrophobic microenvironments dispersed in aqueous solution, can control the conformation of the oligocholate. A small, spherical micelle forces the oligocholate into the folded conformation, whereas an elongated micelle easily accommodates an unfolded oligocholate. Nearly all foldamers reported in recent years fold in homogeneous solutions and/or in the solid state.[1–3] Although foldamers have been used to interact with lipid membranes,[6] their folding within surfactants assemblies (micelles or membranes) has not been studied in detail.[7] Importantly, surfactant micelles represent unique environments and are frequently used by biochemists to study how membrane-associated peptides/proteins behave in a membrane-like environment. [8] As the foldamer chemistry undergoes rapid development, studying the conformation of synthetic foldamers in surfactant assemblies should provide valuable insight into the folding of membrane proteins and enable new applications of foldamers.

Results and Discussion

Folding Models for the Oligocholates within SDS Micelles. According to our previous work, three units make up one turn in the helical conformer of the oligocholate.[4a] Interestingly, more than ten years ago, Sanders and co-workers reported that, when cyclic oligocholate esters were subjected to transesterification, the trimer was always the most thermodynamically favorable species.[9] Although their cyclic oligocholates are connected by ester bonds and our linear cholate foldamers by amide, both Sanders' work and ours suggest that the cholate backbone prefers trimeric periodicity. Because of the large size of the repeat unit (ca. 1.4 nm from head to tail) and the trimeric periodicity, the molecule changes its dimension rather dramatically during folding/unfolding. As shown by the molecular models of a cholate hexamer, the molecule can extend over several nanometers in length in the unfolded state but shrinks to <2 nm upon folding (Scheme 2).

Another noticeable feature of the oligocholate is the dominance of hydrophobic groups in the structure. Apart from the hydroxyl groups in the α faces and the amide linkages, the majority of the molecule consists of hydrocarbon (see the

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SCHEME 2. Molecular Models of an Unfolded and Folded Cholate Hexamer (Reprinted with permission from ref 4d. Copyright 2007, American Chemical Society, Washington, DC)

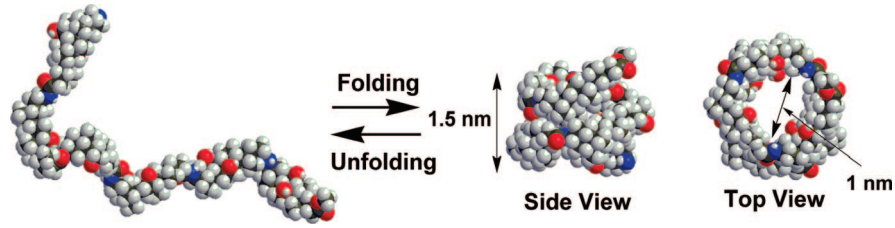
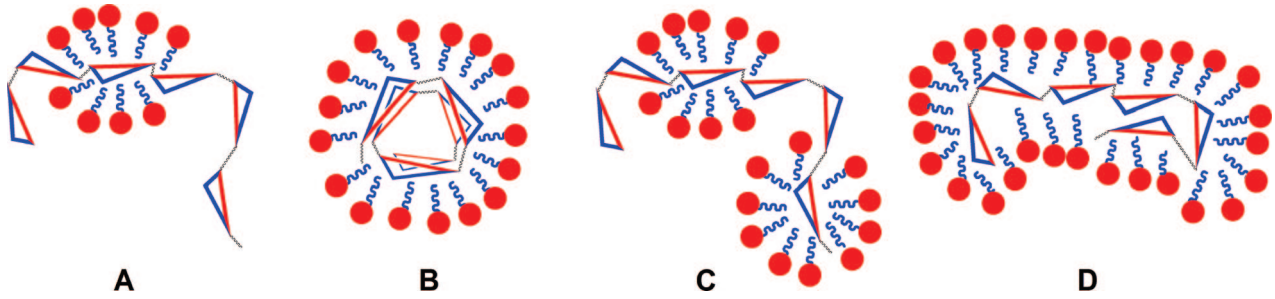


CHART 1. Schematic Representations of Potential Co-assemblies of an Oligocholate and SDS Surfactants



molecular models in Scheme 2). As a result, the cholate oligomers are suited mostly for nonpolar solvent mixtures, such as 1−5% DMSO in hexane/ethyl acetate (2/1).[4a] Apparently also as a result of the hydrophobicity, the oligocholates can be solubilized by surfactant micelles in aqueous solution. In a previous work of ours, a chloate-methionine hybrid foldamer was solubilized by micelles in water and used as a sensor for mercury ions. [4c] Its folding within the micelle is facilitated by Hg^{2+}. It was unclear, however, in that work whether an oligocholate within a micelle would fold or unfold *without* any metal−ligand complexation. This is the question that we set out to answer in the current investigation.

When an oligocholate is solubilized by surfactant micelles in water, only one solvent (i.e., water) is present. Hence, the preferential solvation (Scheme 1) responsible for the folding in the mixed solvents is no longer possible. Without any particular driving force for folding, the oligocholate should prefer unfolded, random conformations in order to maximize its conformational entropy. The unfolded, random conformer tends to dominate unless certain interactions favor the ordered conformer. This is clearly the case if the oligocholate is dissolved in a single solvent.[4] However, the interior of a micelle is a very different environment in comparison to a homogeneous solution. For example, many micelles have rather limited sizes. SDS micelles are quite small, ~4 nanometers in diameter.[10] (This is the hydrodynamic diameter. The hydrophobic core is even smaller.) Even though the micelle might swell upon the incorporation of an oligocholate, the oligocholate within the micelle is still constrained and does not enjoy as much freedom as it does in a homogeneous solution. Will unusual conformational behavior result under such a cirsumstance? This is a question important not only to the oligocholates but also to foldamers in general, especially because surfactant micelles are frequently used by biochemists as media to study the folding of membrane-associaed peptides and proteins.[8]

The dodecyl chain, even in the all-trans conformation, is about 1.5 nm in length. Intuitively, an SDS micelle, with a hydrophobic core about 3 nm or so in diameter, would have difficulty containing an unfolded oligocholate. Being largely hydrophobic, the oligocholate clearly does not want to stretch outside the micelle and immerse itself in the surrounding water (Chart 1, **A**). From this perspective, the folded oligocholate seems to be more favorable, as it can be more easily accommodated by the micelle (Chart 1, **B**). Another possibility is a "pearl-necklace-like" structure (Chart 1, **C**), in which two or more SDS micelles solubilize segments of the oligocholate, much as how micelles interact with a hydrophobic polymer.[11] Such a structure, however, also seems to be problematic for the oligocholate because large areas of hydrophobic surface are exposed to water. Additionally, due to the much shorter chain length of the cholate oligomer compared to a polymer, the neighboring SDS micelles would have strong electrostatic repulsion with one another. A fourth possibility is shown in scenario **D**. In this case, an elongated, rodlike micelle is formed to accommodate the unfolded oligocholate. Of course, it is also possible that multiple cholates reside wihin a single SDS micelle. This last situation can be minimized if the oligocholate is kept at a sufficiently low concentration.

It is difficult to predict a priori whether the oligocholate prefers to fold or unfold in a surfactant micelle. In the case of folding in a solvent mixture, the entire system including the oligocholate and the solvents must be considered. In the current system, the oligocholate, the surfactants, and the water molecules must all be taken into account. For charged surfactants, electrostatic repulsion is weaker in a small spherical micelle (**B**) than in a rodlike micelle (**D**).[12] Spherical micelles are typically formed for surfactants with a single hydrocarbon tail unless other additives (salt, polyelectrolyte, or oppositely charged surfactants, etc.) are added.[12–14] With the oligocholate included in the picture, an interesting competition takes place. The oligocholate prefers to unfold in order to maximize its conformational entropy, but the surfactants, to miminze electrostatic interactions, favor small, spherical micelles better suited

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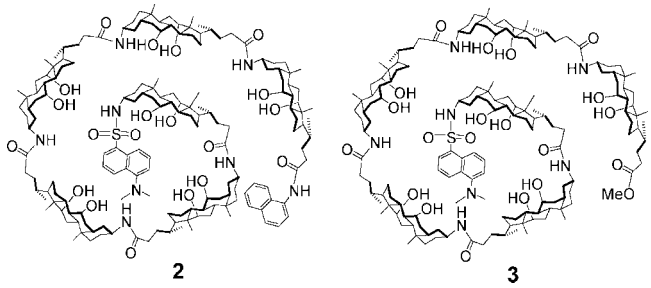
to accommodate the folded oligocholate. The question is "Who will win in this tug of war?"

It should be noted that unfavorable hydrophobic/hydrophilic contact (shown in blue/red in Chart 1) exists in both **B** and **D**. In either case, the hydrophilic faces of the oligocholate are incorporated into the hydrophobic interior of a micelle. This is certainly unfavorable, but the alternative (**A** or **C**) appears even worse because large areas of hydrophobic surfaces are exposed to water. As discussed previously, the oligocholate is overall a hydrophobic molecule, and thus, the unfavorable hydrophobic/hydrophilic contact is more severe in **A**/**C**. In **B** and **D**, at least only a limited number of polar groups scattering along the hydrophobic backbone (see Scheme 2) are in contact with the hydrophobic tails of the surfactants. Because water can penetrate the interior of a micelle appreciably,[15] it is easy to imagine that some water molecules enter the micelle, providing solvation to the polar groups in either **B** or **D**.[16]

Conformations of the Oligocholates in the SDS Micelles in Water. In order to understand the conformational behavior of an oligocholate in the SDS micelle, we synthesized two cholate oligomers, **2** and **3**, by standard amide coupling reactions.[4a] Both have six cholate units and, thus, are expected to form two turns upon folding, resulting in a relatively short end-to-end distance. Foldamer **2** is labeled with a naphthyl group on one end and a dansyl group on the other. These fluorophores are the donor (D) and the acceptor (A) for fluorescence resonance energy transfer (FRET), respectively.



2 **3**

In FRET, the energy-transfer efficiency (E) is related to the D−A distance (r) by equation $E = R_o^6/(R_o^6 + r^6)$, in which R_o is the Förster distance for a specific D−A pair and corresponds to the distance at which the energy-transfer efficiency is 50%.[17] Because R_o typically ranges from 1 to 10 nm and such a distance is comparable to most biofoldamers, FRET is widely used to study the conformations of proteins and DNAs. FRET is especially suited for the oligocholates because of their nanometer-sized dimension and highly dynamic conformations.[18]

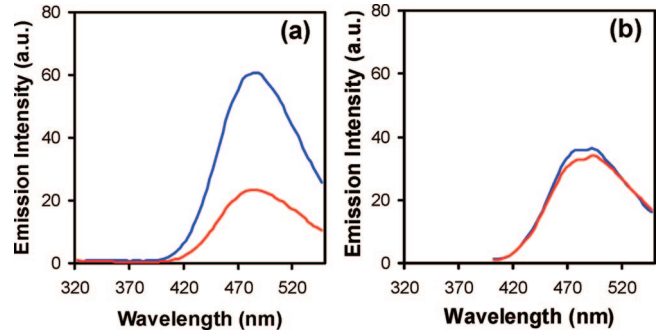


FIGURE 1. Fluorescence spectra of foldamer **2** (blue) and foldamer **3** (red) in 2 mM SDS in water, with the excitation wavelength being (a) 287 nm and (b) 350 nm, respectively. The emission at ca. 490 nm is that of the dansyl acceptor. [**2**] = [**3**] = 2.0×10^{-6} M.

With the R_o value equal to 2.2 nm,[17,19] the naphthyl−dansyl pair can easily detect distance between 1.5 nm (transfer efficienty $E = 0.9$) and 3.2 nm ($E = 0.1$). FRET can be measured either by an increase in the acceptor emission or the decrease of the donor emission. Our previous work indicates that the naphthyl emission is weak under most conditions and FRET is bettter detected by the (increase of the) dansyl emission.[4] For this reason, control foldamer **3** was synthesized, labeled only with the dansyl group. Its size, hydrophobicity, and conformational behavior should be very similar to those of foldamer **2**, except that it cannot undergo FRET without the naphthyl donor.

We first recorded the fluorescence spectra of compounds **2** and **3** in water in the presence of 2 mM SDS. This concentration is below the CMC of SDS (ca. 8 mM)[13] but was shown to solublize oligocholates effectively in our previous work.[4c] When the excitation wavelength is 350 nm, at which only the dansyl acceptor absorbs light, both compounds have similar emission intensity (Figure 1b). The nearly identical emission indicates that both the concentration and the local environment of the dansyl acceptor are the same for **2** and **3**. On the other hand, when the samples are excited at 287 nm, at which the donor absorbs more strongly than the acceptor, the *acceptor* emission around 490 nm is much stronger in the donor−acceptor-labeled **2** than in the acceptor-only **3** (Figure 1a). Clearly, after the naphthyl donor is excited, some of its excited-state energy is transferred to the dansyl, resulting in a higher emission in **2**.

(12) SDS forms monodispersed, spherical micelles in water and polydispersed, rodlike micelles in high salt solutions. (a) Turro, N. J.; Yekta, A. *J. Am. Chem. Soc.* **1978**, *100*, 5951–5952. (b) Lianos, P.; Zana, R. *J. Phys. Chem.* **1980**, *84*, 3339–3341. (c) Coll, H. *J. Phys. Chem.* **1970**, *74*, 520–528. (d) Emerson, M. F.; Holtzer, A. *J. Phys. Chem.* **1967**, *71*, 1898–1907. (e) Anaker, E. W. In *Solution Chemistry of Surfactants*; Mittel, K. L., Ed.; Plenum: New York, 1979; Vol. 1. (f) Ikeda, S.; Hayashi, S.; Imae, T. *J. Phys. Chem.* **1981**, *85*, 106–112. (g) Mazer, N. A.; Benedek, G. B.; Carey, M. C. *J. Phys. Chem.* **1976**, *80*, 1075–1085. (h) Missel, P. J.; Mazer, N. A.; Benedek, G. B.; Young, C. Y.; Carey, M. C. *J. Phys. Chem.* **1980**, *84*, 1044–1057. (i) Missel, P. J.; Mazer, N. A.,; Benedek, G. B.; Carey, M. C. *J. Phys. Chem.* **1983**, *87*, 1264–1277. (j) Corti, M.; Degiorgia, V. *J. Phys. Chem.* **1981**, *85*, 711–717. (k) Flamberg, A.; Pecora, R. *J. Phys. Chem.* **1984**, *88*, 3026–3033. (l) Lianos, P.; Zana, R. *J. Phys. Chem.* **1980**, *84*, 3339–3341. (m) Kratohvil, J. P. *J. Colloid Interface Sci.* **1980**, *75*, 271. (n) Lindman, B.; Wennerstrom, H. *Top. Curr. Chem.* **1980**, *87*, 1. (o) Chen, J.-M.; Su, T.-M.; Mou, C. Y. *J. Phys. Chem.* **1986**, *90*, 2418–2421. (p) Almgren, M.; Swarup, S. *J. Phys. Chem.* **1982**, *86*, 4212–4216.

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(16) Undoubtedly, water molecules would rather join other water molecules in the bulk. Just like the water molecules within a conventional micelle, the water molecules in B or D are probably highly dynamic, coming in and out of the micelle on a fast time scale.

(17) In general, FRET is better used for measuring relative instead of absolute distances; see: (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819–846. (b) Selvin, P. R. *Methods Enzymol.* **1995**, *246*, 300–334. (c) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd Ed.; Kluwer: New York, 1999; Chapter 13.

(18) NOE-based NMR techniques are useful for short-range distances (<5 Å). Although standard in protein characterization, they are unsuitable for the oligocholates. In addition to severe signal overlapping in the ^{1}H NMR spectra, the folded oligocholates are stabilized by solvent effects only. Without specific hydrogen bonds to fix the folded conformer, many degenerate folded states are expected to interconvert on a fast time scale. For a detailed discussion on solvophobic foldamers, see: Zhao, Y.;Moore, J. S. Foldamers Based on Solvophobic Effects. In *Foldamers: Structure, Properties, and Applications*; Hedt, S.; Huc, I., Eds.; Wiley-VCH: Weinheim, 2007.

(19) (a) Stryer, L.; Haugland, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 719–726. (b) Haas, E.; Wilchek, M.; Katchalski-Katzir, E.; Steinberg, I. Z. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1807–1811.

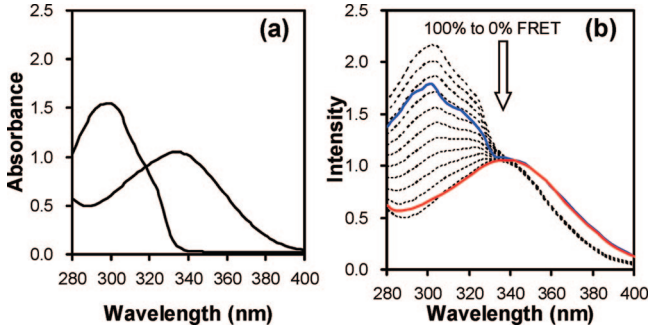
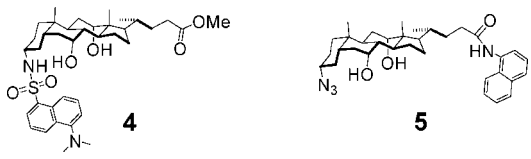


FIGURE 2. (a) UV spectra of compounds **4** (λ_{max} ~300 nm) and **5** (λ_{max} ~340 nm) in THF. [**4**] = [**5**] = 2.0×10^{-4} M. (b) Normalized excitation spectra of compounds **2** (blue) and **3** (red) in 2 mM SDS in water. The acceptor emission at 492 nm was monitored. The dotted spectra correspond to excitation spectra calculated from the UV spectra of **4** and **5** with 100, 90, 80, 70...0.0% energy transfer from top to bottom. [**2**] = [**3**] = 2.0×10^{-6} M.

Foldamer **3** is incapable of FRET. It fluorescence is due to direct excitation at a weakly absorbed wavelength (287 nm).

To determine the energy-transfer efficiency quantitatively, we recorded the UV spectra of the monomer acceptor **4** and donor **5** (Figure 2a). The spectra shown in dotted lines in Figure 2b are obtained by adding 100, 90, 80, 70...0.0% of the donor's UV absorbance to that of the acceptor from top to bottom. In this way, the dotted spectra serve as references for calibrating the energy-transfer efficiency.[17] If FRET occurs in foldamer **2**, its excitation spectrum, monitored at the *acceptor* emission, should have contribution from both the donor and the acceptor. Indeed, when the excitation spectra of foldamers **2** and **3** are normalized so that the emission intensity at 342 nm (the λ_{max} of the acceptor) was the same as the UV absorbance of **4** at the λ_{max}, the excitation spectrum of **3** is nearly identical to the UV spectrum of **4**, whereas that of the donor−acceptor-labeled hexamer **2** has significant contribution (nearly 80%) from the donor (Figure 2b). This contribution from the *donor* absorption is not related to the direct excitation of the acceptor and can only result from FRET. Because **2** and **3** are nearly identical in hydrophobicity and folding, it is reasonable to assume that they are in the same microenvironment when solubilized by SDS. As long as the donor−acceptor-labeled and the acceptor-only compounds are in the same microenvironment, the above method for calculating FRET efficiency can be correctly applied.[19a] Note that some spectral shifts between the UV and the excitation spectra are observed but are fully anticipated, as the UV spectra are recorded in THF whereas the excitation spectra are for oligocholates solubilized in SDS/water. Different environments are known to affect the absorption and the emission of polarity-sensitive fluorophores such as dansyl.[20]



With R_o = 2.2 nm for the naphthyl−dansyl D−A pair,[17,19] the energy-transfer efficiency ($E \approx 0.8$ according to Figure 2b) gives an average calculated D−A distance of 1.7 nm. Of course, a short D−A distance does not necessarily come from the folding of **2**. If two or more oligocholates reside within the same micelle, intermolecular aggregation can also give rise to FRET. To understand whether intermolecular aggregation contributes to the energy transfer in **2**, we performed similar fluorescence experiments with the concentration of SDS varied between 1 and 70 mM. Figure 3 compares the emission intensities of the dansyl acceptor in **2** and **3** at different concentrations of SDS. When the dansyl acceptor is selectively excited (at 342 nm), both **2** (□) and **3** (△) have similar emission intensity (Figure 3b). On the other hand, when the excitation wavelength is 287 nm, at which the naphthyl donor absorbs strongly, the acceptor emission is consistently stronger in **2** (□) than in **3** (△) over 1−70 mM of SDS (Figure 3a). Although some scattering of the data is observed, the energy-transfer efficiency is largely independent of the SDS concentration.

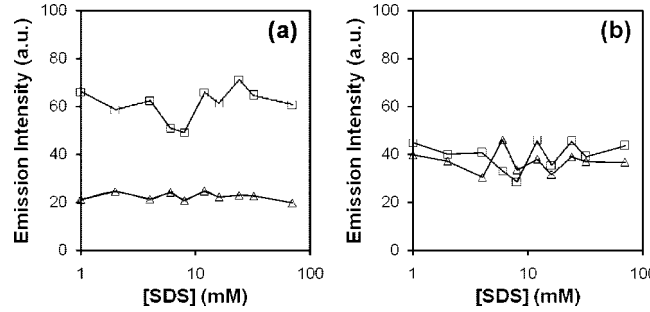


FIGURE 3. Emission intensity at 492 nm of foldamer **2** (□) and foldamer **3** (△) as a function of the concentration of SDS in water. The excitation wavelength was (a) 287 nm and (b) 342 nm, respectively. [**2**] = [**3**] = 2.0×10^{-6} M. The data points are connected to guide the eye.

The CMC of SDS is about 8 mM in water.[13] Below this concentration, the surfactants do not form micelles by themselves. Hydrophobic polymers are known to induce micellization as a result of enhanced hydrophobic interactions.[14] Being largely hydrophobic, the oligocholate probably plays a similar role. Hence, below 8 mM, micelles are only formed around the oligocholates. SDS micelles begin to form without the help of the oligocholates at 8 mM and progressively increase in numbers at higher concentrations. The fact that the emission properties of both **2** and **3** are by and large independent of the SDS concentration (Figure 3a,b) suggests that the fluorophores are more or less in similar microenvironments over the entire concentration range. Because SDS (and also its micelle) is much higher in concentration than the oligocholates, only a tiny fraction of the micelles actually contain a foldamer. The data strongly suggests that the energy transfer results from the folding of the oligocholates instead of from their intermolecular aggregation within the same micelle.

Conformation of the Oligocholates in SDS Micelles in NaCl Solution. According to the results presented so far, the oligocholates prefer to fold within SDS micelles in water (Chart 1, **B**). Without any additives, SDS molecules are known to favor small, spherical micelles.[12] Micellization results from a tradeoff between favorable hydrophobic interactions and unfavorable electrostatic repulsion. Spherical micelles are best at minimizing electrostatic interactions.[12] When a neutral, hydrophobic guest enters a micelle, a spherical micelle is still better at minimizing electrostatic interactions than a rodlike one—this is simply the result of lower surface charge density in the former and is frequently seen when hydrocarbon is included in a micelle.[12p] A similar situation probably happens when the neutral, hydrophobic oligocholate gets inside the micelle. It seems that, even

(20) Li, Y.-H.; Chan, L.-M.; Tyer, L.; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118–3126.

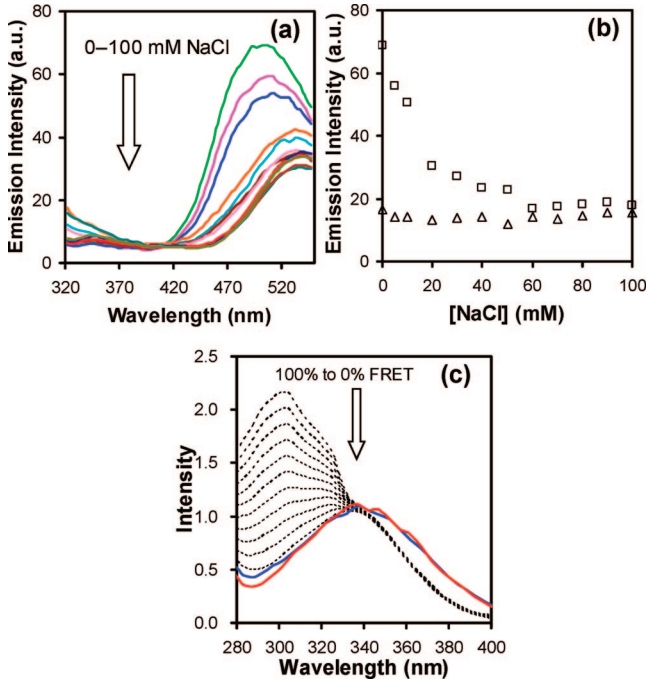


FIGURE 4. (a) Fluorescence spectra of foldamer **2** in 70 mM SDS with various concentrations of NaCl. (b) Emission intensity at 492 nm of foldamer **2** (□) and foldamer **3** (△) as a function of the concentration of NaCl in 70 mM SDS. The excitation wavelength was 287 nm. (c) Normalized excitation spectra of compounds **2** (blue) and **3** (red) in 70 mM SDS in 200 mM NaCl. The acceptor emission at 492 nm was monitored. The dotted spectra correspond to excitation spectra calculated from the UV spectra of **4** and **5** with 100, 90, 80, 70...0.0% energy transfer from top to bottom. [**2**] = [**3**] = 2.0×10^{-6} M.

with an oligocholate in it, the SDS micelle has a strong tendency to maintain its spherical shape in water. When this preference is stronger than that for the oligocholate to unfold, the oligocholate is forced to fold, as small spherical micelle cannot accommodate the unfolded conformer easily. This "confinement effect" of the spherical micelle is apparently quite strong. The energy transfer efficiency observed in SDS micelles is actually higher that what we observed ($E < 0.7$) for **2** in even the most "folding-friendly" solvent mixture, 1% DMSO in hexane/ethyl acetate (2/1).[4a]

Whereas small, spherical micelles are preferred in water, addition of a salt, such as NaCl, reduces the electrostatic repulsion among the head groups of SDS, inducing the formation of elongated, rodlike micelles.[12] For example, fluorescent quenching experiments suggested that the mean aggregation number of the SDS micelle increased by 3−7-fold with the addition of 0.6 M NaCl, depending on different probes used.[12a,b] This estimation was similar to the results obtained with membrane osmometry.[12c] Static[12d–f] and dynamic light-scattering studies,[12g–k] on the other hand, revealed more dramatic growth, showing rodlike micelles >90 nm in length in 0.6 NaCl.[12g] It was pointed out later that the fluorescence experiments underestimated the micelle size due to the lifetime of the probe and the different way of averaging in a polydispersed system.[12l–o] These studies together established the formation of rodlike SDS micelles in NaCl. If the "confinement effect" is indeed the main reason for the folding of the oligocholate in SDS micelles in water, additional of NaCl should make it unfold.

Figure 4a shows the fluorescence spectra of foldamer **2** in 70 mM SDS at various concentrations of NaCl when the donor is preferentially excited at 287 nm. As more salt is added, the acceptor emission near 490 nm gradually decreases. The donor emission around 350 nm, even though not as obvious, increases overall with the higher salt concentration. Thus, as expected, the addition of NaCl lowers the energy-transfer efficiency and unfolds the oligocholate. Figure 4b compares the acceptor emission in foldamers **2** (□) and **3** (△) over different concentrations of NaCl. It seems that the oligocholate begins to unfold as soon as NaCl (even as low as 5 mM) is added to the solution. By the time the salt concentration reaches 60 mM, most of **2** is unfolded, as its acceptor emission is nearly identical to that of **3**, indicating little (if any) FRET being present.

It should be mentioned that the literature work all indicates that rodlike micelles form in high salt solutions (e.g., 0.3−0.8 M).[12] Low concentrations of NaCl (5−20 mM) are not expected to give rise to rodlike micelles. Yet our FRET data clearly shows the unfolding of the oligocholate. Most likely, even though the oligocholate is forced to fold within the SDS micelle in water, the entire system (**B** in Chart 1) is "spring-loaded" due to the "unhappily" folded oligocholate. Its folding is caused by the small, spherical micelle strongly favored by the ionic headgroups to minimize electrostatic repulsion. As soon as salt is added to reduce the repulsion, the confinement effect is weakened. The oligocholate seizes the opportunity, springs open, and pushes the micelle into a rod shape (**D** in Chart 1). It would be highly desirable if we can monitor the size changes of the oligocholate-containing micelles during salt addition. However, at the concentration ratio of SDS/oligocholate = 35000/1 and with the mean aggregation number of the SDS micelles being ca. 60,[12a] less than 0.2% of the micelles contain oligocholates. It is impossible for typical methods such as dynamic light scattering to detect the size change of the micelles under such a circumstance. Although the FRET data does not give direct information about the oligocholate-containing micelle, it *is* direct evidence for the unfolding. Unfolding starts as soon as NaCl is added; the energy-transfer efficiency (E), nonetheless, decreases gradually and reaches about 0.05 or less in 200 mM NaCl (Figure 4c). Although exact quantification is difficult in these FRET measurements, a change of D−A distance from ca. 1.7 nm ($E = 0.80$) to >3.6 nm ($E < 0.05$) leaves no doubt for the unfolding of the oligocholate upon NaCl addition. Considering the dimension of the unfolded oligocholate and its hydrophobicity, it is reasonable to assume that rodlike micelles are formed around the unfolded foldamer.

Another way to increase the micelle size (length) is to keep the salt concentration constant while increasing the SDS concentration.[12] Indeed, when the concentration of NaCl is maintained at 100 mM and that of SDS is increased from 1 to 70 mM, oligomer **2** is observed to unfold also, as judged by the decrease of the acceptor emission (Figure 5a). This result is fully consistent with the "confinement effect". Note that the acceptor emission of foldamer **2** is much stronger in the NaCl micellar solution than in water, particularly at low concentrations of SDS. For example, the emission intensity of dansyl in **2** is >200 in 1 mM SDS in 100 mM NaCl (Figure 5a, green trace) but is <70 under the same conditions without NaCl (Figure 3a, □, the first data point). Dansyl is highly sensitive to its local environment, being essentially nonfluorescent in water and highly fluorescent in nonpolar environments.[20] Thus, the difference in the fluorescence intensity suggests that the interior of SDS micelle is more hydrophobic in NaCl than that in water—the "salting out" effect.[14]

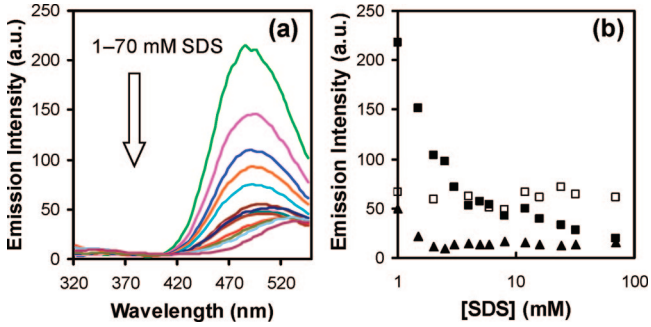


FIGURE 5. (a) Fluorescence spectra of foldamer **2** in 100 mM NaCl solution at various concentrations of SDS. (b) Emission intensity at 492 nm of foldamer **2** (■) and foldamer **3** (▲) as a function of the concentration of SDS in 100 mM NaCl. The data for foldamer **2** in water (□) is shown for comparison. The excitation wavelength was 287 nm. [**2**] = [**3**] = 2.0×10^{-6} M.

The comparison in Figure 5b is interesting. Here, the dansyl emission in foldamers **2** (■) and **3** (▲) in 100 mM NaCl, as well as that of **2** in water (□, taken from Figure 3a) are plotted against the SDS concentration. At low concentrations of SDS, foldamer **2** fluoresces more strongly in 100 mM NaCl (■) than in water (□), probably due to the more hydrophobic micellar interior in the salt solution.[14] Oligomer **2** is folded within the micelle, with and without salt at this point. As more SDS is added, the preference for spherical micelle is weakened in the salt solution and **2** starts to unfold in the salt solution but stays folded in water. Above 10 mM SDS, **2** fluoresces more strongly in the SDS micelles in water than in NaCl solutions because FRET still contributes to the (acceptor) emission in water but no longer does so for the unfolded **2** in the SDS micelles in NaCl solution.

Conformation of the Oligocholates in Brij 30 Micelles. Ionic surfactants are known to be highly sensitive to electrolyte, in terms of both their CMC and the size/shape of the resulting micelles.[12–14] Nonionic ones, on the other hand, are less affected by salt during micellization.[13,14] If unfolding of the oligocholates in the SDS micelles upon the addition of NaCl is indeed caused by the reduction of the electrostatic interactions among the headgroups and the transition from spherical to rodlike micelles, the same salt-induced unfolding should be absent in nonionic micelles.

We thus studied the folding of **2** and **3** in Brij 30, a nonionic surfactant containing the same dodecyl hydrophobic tail as SDS. Its headgroup is oligo(ethylene glycol), containing four units of ethylene glycol per molecule on average. The CMC of Brij 30 is extremely low (~0.06 M in water) without any electrostatic repulsion among the headgroups.[13,14] Figure 6 shows the data obtained from the excitation spectra, with the acceptor emission at 492 nm being monitored. The concentration of Brij 30 is varied between 0.05 and 4 mM. When the foldamers are excited at the donor absorption (287 nm), the acceptor emission of **2** (Figure 6a, □) is consistently stronger than that of **3** (Figure 6a, Δ). When the acceptor is selectively irradiated (at 342 nm), however, both foldamers have similar fluorescence (Figure 6b).

The concentration independence of the FRET over 80-fold dilution once again suggests that folding instead of aggregation is responsible for the energy transfer. This is similar to the situation in the SDS micelles in water (Figure 3a,b). The difference between the two micelles is the emission intensity of **2** or **3**, which is much higher in the Brij micelles than in SDS (compare Figure 6 with Figure 3). Micelles of nonionic

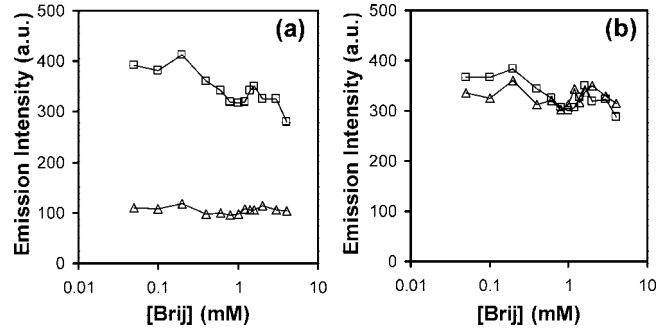


FIGURE 6. Emission intensity at 492 nm of foldamer **2** (□) and foldamer **3** (△) as a function of the concentration of Brij 30 in water. The excitation wavelength was (a) 287 nm and (b) 342 nm, respectively. [**2**] = [**3**] = 2.0×10^{-6} M. The data points are connected to guide the eye.

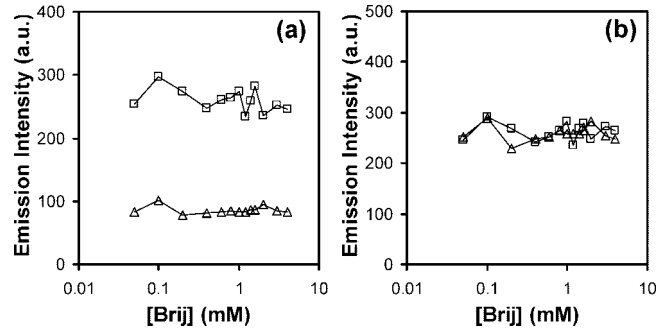


FIGURE 7. Emission intensity at 492 nm of foldamer **2** (□) and foldamer **3** (△) as a function of the concentration of Brij 30 in 100 mM NaCl. The excitation wavelength was (a) 287 nm and (b) 342 nm, respectively. [**2**] = [**3**] = 2.0×10^{-6} M. The data points are connected to guide the eye.

surfactants are well-known to be more hydrophobic in the interior than ionic micelles.[21] Since dansyl fluoresces more strongly in a less polar environment,[20] this result is not surprising at all. Similar observations were made with the cholate-methionine hybrid foldamers in our previous work.[4c]

Another difference of the nonionic micelles is their insensitivity to electrolytes.[13,14] Indeed, when 100 mM NaCl instead of water is used to make the Brij micelles, efficient FRET (shown by the higher acceptor emission in **2** than in **3**, Figure 7a) is still observed, in contrast to what happens in SDS micelles (Figure 5b). Thus, when solubilized by a nonionic surfactant, the oligocholate stays folded regardless of the concentration of the surfactant and the presence or absence of NaCl.

Micellization of alkyl polyoxyethylene ethers depends on the number of oxyethylene units in the headgroup. For the surfactants with six or less oxyethylene units (e.g., Brij 30), the primary micelles may aggregate further to form very large aggregates, partly because the oxyethylene groups are not as hydrophilic and repulsive as the ionic groups.[22] The stronger emission of **2** and **3** in the Brij 30 than in SDS micelles suggests that the foldamers are most likely located in the hydrophobic domain of the micelle. Although the Brij 30 micelles are complicated by secondary aggregation, it is clear that salt has very little effect on the coassembly of the oligocholates and the Brij 30 micelles.

(21) Kano, K.; Ueno, Y.; Hashimoto, S. *J. Phys. Chem.* **1985**, *89*, 3161–3166.

(22) (a) Tanford, C.; Nozaki, Y.; Rohde, M. F. *J. Phys. Chem.* **1977**, *81*, 1555–1560. (b) Ottewill, R. H.; Storer, C. C.; Walker, T. *Trans. Farady Soc.* **1967**, *63*, 2796–2802.

Conclusions

Most synthetic foldamers in the literature fold in the solid state or in homogeneous solutions.[1–3] Folded proteins are found both in solution (i.e., in water) and in the lipid membrane. Lipid molecules have a profound influence on the folding of membrane-associated peptides and proteins.[23] As foldamer chemistry continues to evolve, folding of synthetic foldamers in other media such as micelles or membranes may shed light on how biopolymers fold and function in these media.

Solvophobic effects typically are used to describe direct association of poorly solvated molecular surfaces. Folding of the oligocholates is mediated by the entrapped polar solvents in a solvent mixture (Scheme 1). This is a manifestation of the solvophobic effects because folding is driven by the avoidance of the hydrophilic faces of the cholates from the bulk solvent, a mostly nonpolar mixture. This paper describes another way of utilizing the solvophobic/hydrophobic effects to control the conformation of the oligocholates. Oligomers **2** and **3** fold within SDS micelles, not so much because folding can shield the polar NH/OH groups from the hydrophobic tails of the surfactants, but because the unfolded conformer cannot be accommodated within a spherical micelle ~3−4 nanometers in diameter. Extending the chain outside the micelle into the water (**A** or **C** in Chart 1) is unfavorable because large areas of hydrophobic surface would be exposed to water. The folded oligocholate (**B** in Chart 1), however, is not totally tamed by the SDS micelle. As soon as NaCl is added to reduce the electrostatic repulsion, the oligocholate springs open, pushing the micelle into a rod shape (**D** in Chart 1).

(23) Tamm, L. K., Ed.; *Protein−lipid interactions: from membrane domains to cellular networks*; Wiley-VCH: Weinheim, 2005.

Experimental Section

General Procedures. The syntheses of compounds **2**−**5** were reported previously.[4a] Cholic acid was crystallized from 95% ethanol and dried under vacuum at 60 °C for several hours. SDS (≥99.0%) and Birj 30 were purchased from commercial suppliers and used as received. All other reagents and solvents were of ACS certified grade or higher and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use. All aqueous solutions for the CMC measurements were prepared using Millipore water. Fluorescence spectra were recorded at ambient temperature on a Varian Cary Eclipse Fluorescence spectrophotometer.

Fluorescence. A typical procedure is as follows. SDS solutions (0, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 32.0, and 70.0 mM) were prepared by weighing 0, 1.5, 2.9, 5.8, 8.7, 11.5, 17.3, 23.0, 34.6, 46.1, and 100.8 mg of SDS into 11 separate vials. The samples were dissolved in NaCl aqueous solution (100 mM), transferred to 11 separate volumetric flasks, and diluted with 100 mM NaCl solution to a total volume of 5.00 mL. An aliquot (20.0 μL) of the stock solution of compound **2** or **3** (2.0×10^{-4} M in THF) was added to 2.00 mL of a SDS solution prepared above. The sample was allowed to sit at room temperature for 2 h and was transferred to a quartz cuvette. The fluorescence and the excitation spectra were recorded. The excitation wavelength was 287 nm for the fluorescence spectra. For the excitation spectrum, the dansyl emission at 492 nm was monitored as the excitation wavelength was scanned.

Acknowledgment. is made to the Roy J. Carver Charitable Trust, NSF (CHE-0748616), and the Iowa State University Research Foundation for the support of this research.

JO800724J