JOC The Journal of Organic Chemistry

Article

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Effects of Micelle Properties on the Conformation of Oligocholates and Importance of Rigidity of Foldamers

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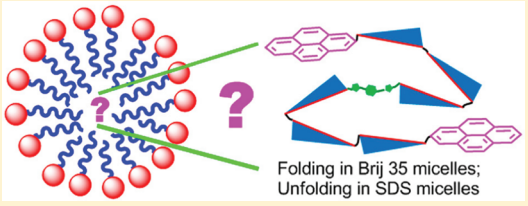
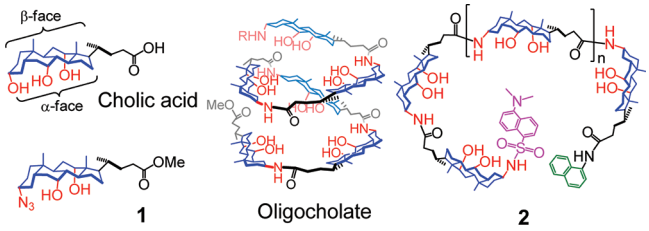
ABSTRACT: The conformation of a cholate hexamer with a clicked tether in between two tricholate units and pyrene groups at the chain ends was studied by fluorescence spectroscopy. In contrast to the parent cholate hexamer that folded in all micelles investigated, the folding of the clicked hexamer was highly dependent on the type of surfactant used to solubilize the compound. The clicked oligocholate folded in the Brij 35 micelle, possibly due to the latter's small size and strong internal hydrophobicity. The oligocholate formed intermolecular aggregates in SDS solutions below the CMC of the surfactant. The aggregates were dissociated by the SDS micelles but the individual oligocholate stayed unfolded. In Triton X-100 and sodium cholate solutions, the aggregated, unfolded, and folded oligocholates coexisted and gradual unfolding occurred with an increasing concentration of the surfactant. The conformation of the clicked oligocholate was sensitive to the nonideal mixing of ionic/nonionic micelles and to the unconventional aggregation of sodium cholate.



INTRODUCTION

Foldamers, synthetic analogues of proteins and nucleic acids, have attracted much interest from researchers in various disciplines.[1] The research is driven by two primary motivations. First, with a fundamental knowledge in the conformational control of relatively simple chain molecules, scientists should be able to better understand the conformation of complex biomolecules. Until now, it remains impossible to predict the conformation of a protein from its primary amino acid sequence. Second, conformational control is frequently used by nature to regulate the function of biomolecules including their response to environments. As chemists master the skills in the conformational control of abiotic molecules, they will not only be able to develop better ways to intervene with biological processes, but also create biomolecule-like, environmentally responsive materials.

Numerous foldamers have been synthesized in the last two decades.[1] Some are reminiscent of natural peptides and made of *β*- and *γ*-amino acids.[2] Others, including the *meta*-phenyleneethynylene (*m*PE) oligomers[3] and the aromatic electron donor−acceptor foldamers (aedamers),[4] deviate significantly from what can be found in nature. Our group synthesized foldamers from facially amphiphilic building block **1**, a derivative of cholic acid.[5] Because the cholate group curves toward the hydrophilic face, the resulting oligocholate could fold into a helix with introverted hydrophilic groups in appropriate nonpolar environments such as a nonpolar solvent containing a small amount of a polar solvent. The polar solvent is needed not only to solubilize the foldamer but also to create the solvophobic driving force for the folding. The folded helix, with the hydrophilic groups pointing inward resembling a unimolecular reversed micelle, has its interior filled disproportionally with the polar solvent. These polar solvent molecules can solvate the introverted hydrophilic groups efficiently in the overall nonpolar medium.



We also discovered that cholate-based foldamers,[6] baskets,[7] and macrocycles[8] could mimic membrane proteins in transporting hydrophilic molecules across lipid bilayers. In the literature, surfactant micelles are frequently used to mimic the bilayer environments to study the conformation of membrane-associated peptides and proteins.[9] In our hands, the parent oligocholates such as **2** indeed fold well in SDS micelles, although the folding mechanism is completely different from that in homogeneous solution.[10] The hydrophobic core of an SDS micelle is about 3 nm in diameter. A fully folded cholate hexamer is less than 2 nm in dimension and an unfolded conformer can stretch to several nanometers in length. Because the SDS micelle has strong preference to maintain its spherical shape in water (to minimize the charge density on the micellar surface),[11] the micelle can accommodate the folded conformer much better than the unfolded one. Metaphorically, solubilizing the oligocholate in a small micelle is similar to pushing a snake into a small cage—the snake (oligocholate) has no choice but to coil up (fold).

In this paper, we report the conformational study of a semirigid oligocholate in different surfactant micelles. Unlike rigid parent oligocholates that tend to fold in surfactant micelles and flexible ones that always unfold, the oligocholate was highly

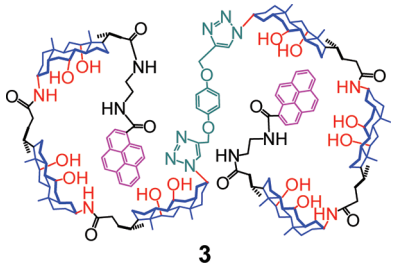
Received: October 18, 2011
Published: December 8, 2011

dx.doi.org/10.1021/jo202156d | *J. Org. Chem.* 2012, 77, 556–562

sensitive to the type of surfactant used. The size and internal hydrophobicity of surfactant micelles seem to be the most important factors controlling the conformation of the semirigid oligocholate. Most interestingly, the oligocholate was able to sense the nonideal mixing of ionic/nonionic micelles and the unconventional aggregation of sodium cholate.

RESULTS AND DISCUSSION

We recently synthesized semirigid oligocholate **3** by the highly efficient click reaction.[12] The parent oligocholate (**2**) is quite rigid, with only short tethers in between the fused aliphatic steroid rings. The clicked **3** has two tricholate fragments joined by a triazole−1,4-phenylenoxy−triazole spacer. The majority of the foldamer backbone is thus similar to the parent oligocholate in structure. The head-to-head arrangement of the cholate groups in the middle of the foldamer causes no problem in mixed organic solvents, due to the strong tolerance of the solvophobic folding to structural perturbation.[5b] Because the clicked tether contains six rotatable bonds around the 1,4-phenylene unit, **3** overall should be more flexible than the parent oligocholate.



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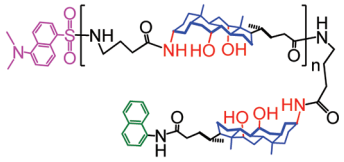

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It is known that the rigidity of the oligocholates is important to their folding in both mixed organic solvents and surfactant micelles although the effects are opposite in the two media.[10] In organic solvents, flexible spacers enable the compound to achieve the "reverse micelle-like" conformation with minimal strain and thus beneficial to the folding (Figure 1, left). 4-Aminobutyroyl-spaced oligocholate **4**, for example, folds cooperatively with three or four cholates[13] but **2** needs at least five cholates to do the same.[14] In surfactant micelles, the effect is completely opposite. Whereas **2** folds well both below and above the CMC of SDS due to the "cage" or confinement effect (Figure 1, right),[10a] **4** aggregates intermolecularly in SDS below the surfactant's CMC. Above CMC, the aggregates are dissociated by the micelles but **4** remains unfolded. The unfolded conformer is favored because the flexible spacers allow the unfolded **4** to adjust easily within the SDS micelle to expose its hydrophilic faces to water while keeping its hydrophobic faces in contact with the surfactants' hydrophobic tails. Essentially, when the unfolded conformer can meet its hydrophobic/hydrophilic needs within the confines of the small spherical micelle (which is preferred by its low surface charge density), it is more favorable than the folded form by its higher conformational entropy.[10b] For these reasons, the intermediate flexibility of **3** makes it a particularly interesting system to study. We reasoned that, unlike **2** that tends to fold in all types of micelles[6a,10a] and **4** that always unfolds,[10b] the semirigid oligocholate may be sensitive to its microenvironment and useful as a probe for the micelle properties.

Our study began with the characterization of the conformation of **3** in mixed organic solvents, taking advantage of the fluorescent pyrene labels at the chain ends. Because the oligocholates prefer three cholate units per turn in the folded helix,[14,15] the folded **3**, with two helical turns, will have the pyrene labels in close proximity. Folding, therefore, should be accompanied by the formation of pyrene excimer that emits at ~470 nm.[16]

As shown in Figure 2, in a folding-friendly solvent mixture such as 1% methanol in 2:1 hexane/ethyl acetate,[17] strong excimer emission was indeed observed. The addition of methanol initially caused little change but then diminished the excimer emission. Beyond 10% methanol, the excimer peak disappeared almost completely and the fluorescence spectrum became nearly constant. The solvent response is similar to what is observed in other oligocholates and corresponds to a methanol-induced folding−unfolding transition.[5,14] The low concentration of **3** in the solution (0.14 μM) excludes intermolecular aggregation as a possible cause of the excimer.[5] The weakening of the excimer by methanol also suggests that the $\pi-\pi$ interactions between the two pyrene groups do not contribute significantly to the folding.[18]

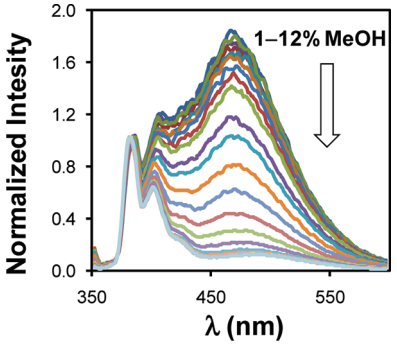


Figure 2. Normalized fluorescence spectra of **3** in 2:1 hexane/ethyl acetate with different amounts of methanol. A spectrum was recorded after each addition of 0.5 vol % methanol. [**3**] = 1.4 × 10^{-7} M. λ_{ex} = 336 nm.

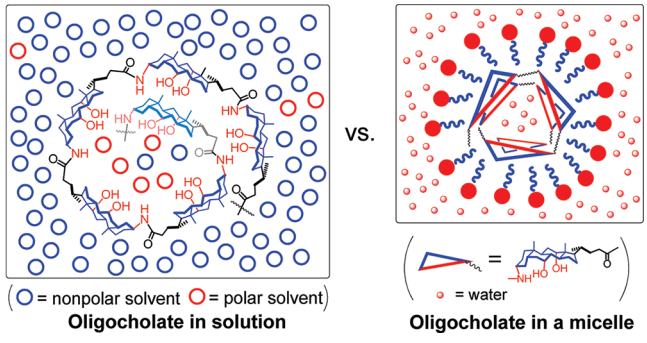


Figure 1. Schematic representation of the folding of an oligocholate in mixed organic solvents and a surfactant micelle.

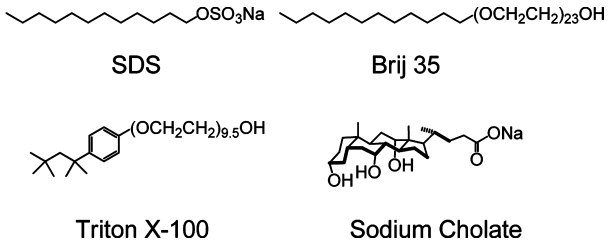
After we obtained the spectroscopic signatures for the fully folded and fully unfolded conformers in solution, we studied the conformation of **3** in micelles. Solvent titration is no longer possible in micellar solutions. Also, since pyrene excimer forms

dx.doi.org/10.1021/jo202156d | J. Org. Chem. 2012, 77, 556−562

as the result of the proximity of the probes, any process that brings the probes together would give positive results, whether of intramolecular (i.e., folding) or intermolecular (i.e., aggregation) origin. To distinguish between the intra- and intermolecular process, we monitored the fluorescence of **3** at different concentrations of the surfactant. Since the oligocholate is essentially insoluble in water, changing the surfactant concentration changes the amount of solubilizing agent and is equivalent to varying the effective concentration of **3** in the surfactant assembly. Folding, as an intramolecular process, is expected to be independent of the surfactant concentration but aggregation should occur more easily in low surfactant solutions. As the amount of surfactant increases, therefore, folding-derived excimer emission is expected to be more or less constant but that from aggregation should decrease.

The first two surfactants studied were SDS, an anionic surfactant with a sulfate headgroup, and Brij 35, a nonionic surfactant with 23 units of ethylene glycol on average (Chart 1).

Chart 1. Structures of Surfactants Used in the Study



Both surfactants have a C12 carbon chain and their CMCs in water are 8 and ~0.1 mM, respectively.[19] Figure 3 shows the excimer/monomer ratio (F_{ex}/F_{mon}) as a function of the surfactant concentration. The dashed lines in the figure represent the excimer/monomer ratio for the fully folded and unfolded conformers in mixed organic solvents, respectively.

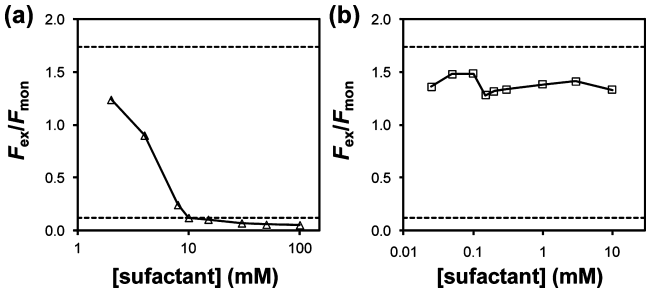
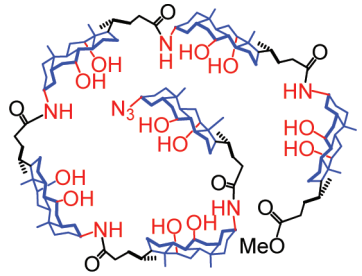


Figure 3. Excimer/monomer ratio for **3** as a function of surfactant concentration for (a) SDS and (b) Brij 35. [**3**] = 3.1×10^{-7} M. The dashed lines at 1.74 and 0.12 correspond to F_{ex}/F_{mon} for the fully folded and fully unfolded conformer of **3** in MeOH/(ethyl acetate/hexane = 2/1) mixture.

The oligocholate clearly behaved differently in the two surfactant solutions. In SDS, **3** gave strong excimer emission at 470 nm below the CMC of the surfactant. An increase in the SDS concentration caused a precipitous drop of the emission near the CMC of the surfactant (Figure 3a). The excimer disappeared completely upon a further increase in the surfactant concentration. In Brij 35 solutions, the excimer was nearly constant over a 400-fold increase of the surfactant concentration, below and above the CMC of the surfactant (Figure 3b).

Although the excimer emission of **3** in Brij 35 was not as strong as that of the fully folded conformer in solution, the average $F_{ex}/F_{mon} \approx 1.4$ was much closer to that of the fully folded ($F_{ex}/F_{mon} = 1.7$) than that of the unfolded ($F_{ex}/F_{mon} = 0.1$) conformer. The pyrene labels thus must be in close proximity when the oligocholate was solubilized in the nonionic micelle. Note that the folded conformers in the two media do not have to be exactly the same, as the folding mechanisms are quite different. The concentration-independent excimer formation was unlikely to come from intermolecular aggregation of the oligocholate. Above the CMC of the surfactant, the solubilizing power of the surfactant for hydrophobic agents increases sharply.[19] The aggregation of oligocholate in water is driven by hydrophobic interactions and such aggregates are known to dissociate above the CMC of a surfactant.[10b] Compound **3**, thus, most likely is folded in the nonionic surfactant assembly, similar to the parent oligocholate **2**.[10a]

The sharp decrease in the excimer emission of **3** at the CMC of SDS in Figure 3a can be explained by either an aggregation−deaggregation or a folding−unfolding transition. Unfolding of (ionically functionalized) oligocholates has been observed previously upon micellization.[6a] To distinguish between the two possibilities, we studied the fluorescence of **3** in the presence of unlabeled hexamer **5** in 2 mM SDS, a concentration lower than its CMC. Because rigid parent oligocholates similar to **5** are known to fold in SDS solutions,[10a] **3** and **5** should fold independently if **3** also folds under the same condition. Addition of **5**, in this case, would have little effect on the pyrene excimer.



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If the excimer of **3** below the CMC of SDS comes from intermolecular aggregation, the addition of **5** would bring a different effect. When any hydrophobic molecules (including **5**) are placed in water, they need to avoid unfavorable exposure to the solvent. An oligocholate may do so in two ways—intermolecular aggregation or folding into the reversed micelle-like conformation with the external hydrophobic surface protected by the hydrophobic tails of the surfactant.[20] Rigid parent oligocholates cannot pack tightly because of the awkwardly shaped steroidal structure, the constraint imposed by the facial amphiphilicity of the building blocks, and the short linkages in between the fused aliphatic rings.[21] Our previous research shows that, folding of the parents oligocholates in micelles was NOT due to their intrinsic strong foldability but originates from the disfavored intermolecular aggregation, the alternative process to folding.[10b] One, however, has to realize that, although **5** does not want to aggregate (due to its difficulty in close packing), there is no reason for **3** and **5** not to coaggregate. If **3** could form stable intermolecular aggregates, the same flexibility that enables **3** to aggregate would help **3** coaggregate with **5**. Entropy in general favors disordered

dx.doi.org/10.1021/jo202156d | J. Org. Chem. 2012, 77, 556−562

mixtures such as randomly mixed aggregates of **3** and **5**. Indeed, from the entropic point of view, it is difficult to imagine "pure" aggregates of **3** formed separately from individually folded **5**, given the similarity of the two molecules.

The above analysis, therefore, suggests that the addition of **5** would diminish the pyrene excimer if intermolecular aggregation was responsible for the excimer emission of **3** in 2 mM SDS, as the coaggregation of **3** and **5** would dilute the concentration of pyrene. This was indeed what was observed in our experiments. By the time 60% of **5** was added, the pyrene excimer disappeared almost completely, with F_{ex}/F_{mon} identical to that of the fully unfolded **3** in solution (Figure 4, △).

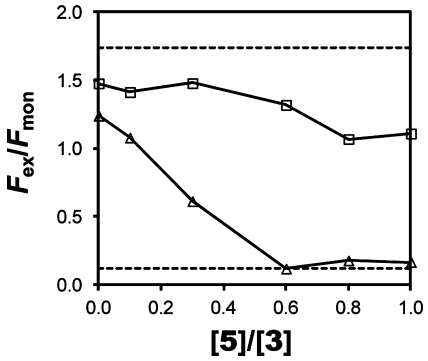

Figure 4. Excimer/monomer ratio for **3** as a function of the [**5**]/[**3**] ratio in 2 mM SDS (△) and 1 mM Brij 35 (□). [**3**] = 3.1×10^{-7} M. The dashed lines at 1.74 and 0.12 correspond to F_{ex}/F_{mon} for the fully folded and fully unfolded conformer of **3** in MeOH/(ethyl acetate/hexane = 2/1) mixture.

The same mixing experiment was used to confirm the folding of **3** in Brij 35. This time, the mixing was performed above the CMC of the surfactant because a higher surfactant concentration presents a more challenging environment to the folding. As mentioned earlier, some (ionically functionalized) folded oligocholates were observed to unfold during micellization of the surfactant.[6a] According to Figure 4 (□), although there was a slight drop in F_{ex}/F_{mon} upon the addition of unlabeled **5**, the change was rather small in comparison to what occurred in SDS solutions (△). This result thus corroborates with the concentration-independent excimer formation in Figure 3b and strongly supports the folding of **3** in Brij 35 solutions.

Why does **3** prefer to fold in Brij 35 but unfold in SDS micelles? Although the two micelles have different ionic characteristics, it is difficult to rationalize the different conformations by ionic interactions since **3** is neutral and the similar **2** (n = 3) folds well in both surfactants.[6a,10a] The hydrophobic core size of SDS micelle is about 3 nm, as determined by the chain length of the dodecyl chain.[22] The micellar aggregation number for SDS and Brij 35 are 64 and 40 in water, respectively.[19] Thus, the hydrophobic core of Brij 35 micelle is somewhat smaller than that of SDS, if only the number of dodecyl chains is considered. Our previous work shows that the size of the micelle is a major reason for the parent oligocholates to fold.[10a] The folded form is <2 nm in diameter but the unfolded form can extend to several nanometers in length according to CPK molecular models. A small micelle, hence, can accommodate the folded form better than the unfolded, as the oligocholate is dominated by hydrophobic groups and needs to stay within the hydrophobic core of the micelle. For the same reasons, solubilizing the oligocholate in a smaller micelle means that the foldamer is in a more "compressed" state. The "cage" effect is thus stronger and should facilitate the folding of even more difficult oligocholates such as **3**. Another difference between the two micelles is their internal hydrophobicity, higher for the nonionic surfactant.[23] The folded conformer has a hydrophobic exterior and prefers nonpolar environments.[5] As long as the micelle contains enough water to solvate the hydrophilic groups of the oligocholate,[24] a nonpolar micelle does seem to be a better environment for the folded structure.

If **3** was able to sense the difference of the two micelles, how about mixed micelles formed by the two? When SDS was added to **3** solubilized in 1 mM of Brij 35, strong excimer emission was observed for the folded oligocholate. As the mixture was titrated with the ionic surfactant, the excimer was found to be quite resistant, tolerating up to 10 mM or 10× SDS (Figure 5).

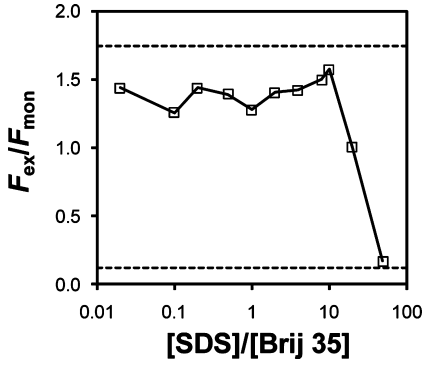

Figure 5. Excimer/monomer ratio for **3** in SDS/Brij 35 mixed micelles. [Brij 35] = 1 mM. [**3**] = 3.1×10^{-7} M. The dashed lines at 1.74 and 0.12 correspond to F_{ex}/F_{mon} for the fully folded and fully unfolded conformer of **3** in MeOH/(ethyl acetate/hexane = 2/1) mixture.

Although a further increase of SDS eventually brought down the excimer emission, the complete unfolding required 50× SDS. At first sight, it might be surprising to see the oligocholate stay folded with only 10% of Brij 35 in the solution. The result, nonetheless, is fully consistent with the nonideal mixing of the two surfactants. When an ionic and nonionic surfactant form mixed micelles, the micelle composition is frequently different from the bulk composition. Because of the strong electrostatic repulsion of the sulfate headgroups, it is more favorable to transfer SDS molecules into Brij 35 micelles than vice versa. Even at very high ratio of SDS (>90%), the mixed micelles mainly consist of the nonionic Brij 35.[25] It is interesting that the conformation of **3** reflected the phase separation of the surfactants and displayed the folding most favorable in the nonionic micelle. Also, because both mixed micelles and SDS micelles were present in the aqueous solution containing 1 mM Brij 35 and 10 mM SDS and **3** displayed the folding in Brij 35 micelles, the oligocholate must prefer to stay inside the nonionic micelle.

It is encouraging to see that the conformation of **3** was sensitive to the type of surfactant used. Although **3** is a very large probe in comparison to the size of a typical surfactant micelle, its folding seemed to reflect well the micelle property. Possibly, although the **3**–surfacant coassembly was different from the surfactant micelle itself, similar hydrophobic, ionic, and steric interactions are involved in both systems, making the conformation of **3** able to report the micelle's property.

dx.doi.org/10.1021/jo202156d | J. Org. Chem. 2012, 77, 556–562

To explore the scope of this "conformational probe", we studied the folding of **3** in aqueous solutions of two other surfactants, Triton X-100 and sodium cholate. Similar to Brij 35, Triton X-100 has a poly(ethylene glycol) headgroup, although the chain length is shorter (Chart 1). The nonionic surfactant forms oblate ellipsoid micelles and the micelle aggregation number (=140) is significantly larger than that of SDS and Brij 35.[19] Unlike SDS micelles, Triton X-100 micelles are morphologically flexible, reconstructing their structures when guests are incorporated.[26] Sodium cholate is similar to SDS as far as ionic characteristic is concerned. The facially amphiphilic bile salt, however, is known to aggregate differently from conventional head–tail surfactants. Typical head–tail surfactants form micelles abruptly at the CMC with tens of surfactants in the assembly. Bile salt surfactants, on the other hand, form oligomeric, primary aggregates at the early stage of aggregation followed by larger aggregates at higher concentrations.[27] Unlike the cooperative micellization of head–tail surfactants, the aggregation of bile salts could occur in a progressive manner over a broad range of concentration.

The conformation of **3** indeed reflected the changes in the micelle property. In Triton X-100, the excimer/monomer ratio started at ca. 1.1 at 0.05 mM of the surfactant and stayed nearly constant until the CMC (0.22–0.24 mM), where a sudden drop occurred (Figure 6a, □). Above the CMC of the nonionic

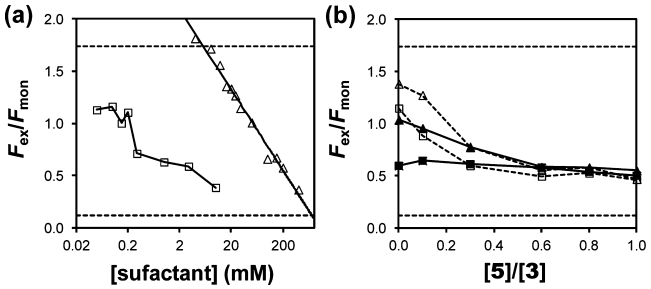

Figure 6. (a) Excimer/monomer ratio for **3** as a function of [Triton X-100] (□) and [sodium cholate] (△). (b) Excimer/monomer ratio for **3** as a function of [**5**]/[**3**] in 0.1 mM (□) and 1 mM (■) Triton X-100 (△), and in 12 mM (△) and 50 mM (▲) sodium cholate. [**3**] = 3.1×10^{-7} M. The dashed lines at 1.74 and 0.12 correspond to F_{ex}/F_{mon} for the fully folded and fully unfolded conformer of **3** in MeOH/(ethyl acetate/hexane = 2/1) mixture.

surfactant, the excimer emission continued to decrease, albeit more gradually. To understand the origin of the excimer formation, we performed the mixing experiment with unlabeled **5**. As shown by Figure 6b, in 0.1 mM of Triton X-100, the initial addition of **5** weakened the excimer emission but the excimer stabilized above 30% of the unlabeled oligocholate (□). The final F_{ex}/F_{mon} of 0.55 was significantly higher than the 0.12 for the unfolded conformer in the mixed organic solvents. The behavior was very different from that observed in SDS below its CMC (Figure 4, △). The results revealed that, in 0.1 mM Triton X-100, not all the excimer emission of **3** came from its aggregation (which would be eliminated by the addition of **5**). In other words, a fraction of **3** must have stayed folded under this condition and it was the folding-derived excimer that remained at high ratio of the unlabeled **5**.

The above explanation is consistent with the drop of F_{ex}/F_{mon} at the CMC of Triton X-100 (Figure 6a, □). As mentioned earlier, the oligocholate aggregates typically are dissociated by surfactant micelles,[10b] which have strong abilities to solubilize hydrophobic agents. In essence, once there are micelles in the solution, the oligocholates no longer have to hide their hydrophobic surfaces by self-association. The oligocholates can migrate into the surfactant micelles and satisfy their hydrophobic needs in the folded or unfolded conformation, depending on the nature of the foldamer.

Further support for the presence of the folded conformer comes from the mixing experiment above the CMC of Triton X-100. In 1 mM of this surfactant, although F_{ex}/F_{mon} (~0.55) was lower than that of the fully folded **3** (1.74), the excimer/monomer ratio was completely unaffected by the addition of unlabeled **5** (Figure 6b, ■)—a result only possible for folded **3**. Assuming that only the fully folded and fully unfolded conformers are present in 1 mM Triton X-100, we calculated that the percentage of the folded conformer to be 30% based on the excimer/monomer ratio of 0.55. The partial folding of the clicked oligocholate in Triton X-100 agrees with our early finding that the most folding-friendly micelles are those small and nonpolar in the interior. Although a Triton X-100 micelle is also quite nonpolar,[26] its somewhat larger size must make it less ideal than the Brij 35 micelle to the folded **3**.

The behavior of **3** in sodium cholate solutions was in line with the bile salt's noncooperative aggregation. As shown by Figure 6a, the excimer/monomer ratio displayed a continuous, nearly linear decrease against log[surfactant]. No step change in the excimer emission was observed, unlike what happened in SDS or Triton X-100 solutions. Upon the addition of unlabeled **5**, F_{ex}/F_{mon} decreased gradually as the pyrene labels were diluted in both 12 mM (Figure 6b, △) and 50 mM (▲) sodium cholate but stabilized beyond 60% of **5**. The CMC of sodium cholate is 16–20 mM as determined by the dye-solubilization method.[28] According to Figure 6b, part of the excimer emission came from aggregated **3** below the CMC of sodium cholate, as the addition of **5** lowered F_{ex}/F_{mon}. Some of the oligocholates must be folded, as the final excimer/monomer ratio remained significantly higher than of the unfolded form.

The excimer/monomer ratio was still affected by the mixing of **5** in 50 mM of sodium cholate, above the CMC of the surfactant (Figure 6b, ▲). Thus, cholate micelles could not fully dissociate the aggregated **3**, unlike those of SDS or Triton X-100. This was a reasonable result. Dissociation of the oligocholate aggregates requires the surfactant to satisfy the hydrophobic needs of the oligocholate. A linear head/tail surfactant can adjust its hydrophobe and should be able to dissociate the oligocholate aggregates more easily than the rigid sodium cholate.

The parent cholate hexamer **2** (n = 3) was previously found to fold in SDS[10a] and Brij 35[6a] but was never studied in Triton X-100 and sodium cholate. Since the clicked **3** was able to distinguish between these micelles, we thought it would be interesting to understand the conformation of **2** (n = 3) in these two surfactants.

The best way to study the aggregation and/or folding of dansyl–naphthyl-labeled oligocholates is by examining the Förster energy transfer from the naphthyl donor to the dansyl acceptor.[10] Similar to the pyrene excimer, energy transfer from naphthyl to dansyl only indicates the proximity of the probes, which could come from either folding or aggregation of the oligocholates. In our experience, information about the energy transfer is best extracted from the excitation spectrum of the acceptor, collected by measuring the dansyl emission at 492 nm while the excitation wavelength is scanned.[6a,10b] In the absence of energy transfer, the spectrum resembles the absorption

dx.doi.org/10.1021/jo202156d | J. Org. Chem. 2012, 77, 556–562

spectrum of the acceptor. Any appearance of the donor peak (λ_{max} = 300 nm) in the excitation spectrum indicates energy transfer from the naphthyl.

As shown in Figure 7a, as the concentration of Triton X-100 was varied from 0.05 to 10 mM, the donor peak at 300 nm was

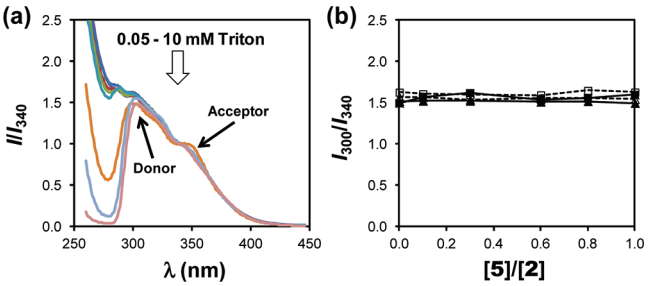

Figure 7. (a) Excitation spectra of **2** (n = 3) in different concentrations of Triton X-100. (b) I_{300}/I_{340} ratio for **2** (n = 3) as a function of [**5**]/[**2**] in 0.1 mM (□) and 1 mM (■) Triton X-100 (△), and in 12 mM (△) and 50 mM (▲) sodium cholate. [**2**] = 3.1 × 10^{-7} M.

clearly visible and essentially constant. Although there were some differences in the excitation spectra below 300 nm, the change was probably due to the difference in the environmental polarity over the CMC of the surfactant and has been observed before.[10b] Further support for the folding of **2** (n = 3) was obtained from the mixing experiment. In both Triton X-100 and sodium cholate, below and above the CMC, the intensity ratio of the acceptor emission at 300 and 340 nm, an indicator of the energy-transfer efficiency,[6a,10b] was completely unaffected by the addition of unlabeled **5** (Figure 7b). Clearly, the energy transfer in **2** in both surfactant solutions was from an intramolecular process (i.e., folding).

CONCLUSIONS

The conformational stability of the oligocholates can be tuned judiciously by the introduction of spacer groups in between the cholate building blocks. The parent, rigid oligocholate **2** (n = 2) stays folded in every micelle studied so far, both below and above the CMC of the surfactant. The flexible oligocholate **4** with 4-aminobutyroyl spacers in between every cholate group was found previously to prefer to aggregate below the CMC of a surfactant and unfold above the CMC.[10b] With intermediate rigidity, clicked hexacholate **3** displays conformation highly sensitive to its microenvironment. Despite its large size in comparison to a typical surfactant micelle, its folding reflected well the properties of micelles, particularly the size and internal hydrophobicity of the surfactant assembly. Most interestingly, **3** could be used as conformational probe, revealing the nonideal mixing of ionic/nonionic mixed micelles and the noncooperative aggregation of sodium cholate.

The more general implication of the research is related to how hydrophobic foldamers as mimics of membrane proteins might interact with surfactants of different kinds. Sodium cholate and Triton X-100 are commonly used to solubilize membrane proteins. These surfactants indeed are more "folding-friendly" toward the semirigid oligocholate than SDS, which tends to denature proteins. Our work also demonstrated that sodium cholate is less able to dissociate the intermolecular aggregate of **2** than flexible, linear surfactants. Quite likely, the rigidity of an amphiphile is very important to its ability to solubilize membrane proteins without denaturation.[29]

EXPERIMENTAL SECTION

General. The syntheses of **2**,[14] **3**,[12] and **5**[14] were reported previously. All reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Millipore water was used to prepare all aqueous solutions.

Fluorescence Titration in Homogeneous Solution. A stock solution (2.8 × 10^{-4} M) of **3** in 20:1 THF/MeOH was prepared. An aliquot (1.0 μL) of the stock solution was added to 2.00 mL of hexane/EA (v/v = 2/1) with 1 vol % methanol in a quartz cuvette. Aliquots of MeOH (22 × 10.0 μL) were added to the sample. The sample was gently vortexed for 30 s after each addition before the fluorescence spectrum was recorded.

Typical Fluorescence Titration in Micellar Solution. A stock solution (2.5 × 10^{-4} M) of **3** in 20:1 THF/MeOH was prepared. Aliquots (5.0 μL) of the stock solution were added to 8 separate vials containing 4.0 mL of aqueous SDS solutions. The concentrations of the SDS were 2, 4, 8, 10, 15, 30, 50, 100 mM, respectively. The samples were allowed to stand at room temperature for 3 h before the fluorescence spectra were recorded.

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ACKNOWLEDGMENTS

We thank U.S. Department of Energy-Office of Basic Energy Sciences (grant DE-SC0002142) for supporting the research.

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