Effects of Amphiphile Topology on Aggregation Properties: Distinctive Behavior of Contrafacial Amphiphiles

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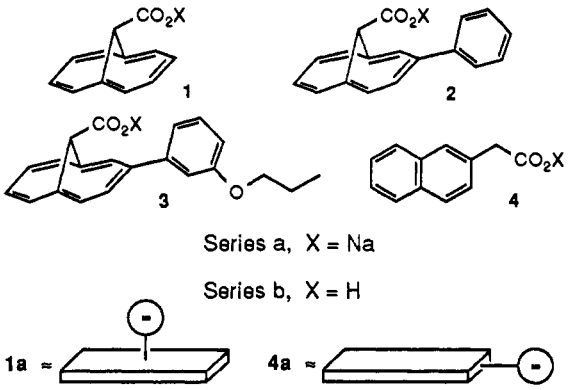
Amphiphilic molecules play important roles in many settings.[1] Biological systems, for example, use lipid-based bilayers for structural and functional definition of living cells and subcellular compartments. Synthetic surfactants find applications in many personal care products and industrial processes. The most important properties of amphiphiles, which stem from their aggregation and interaction with other molecules, are determined to a significant extent by the topological relationship between hydrophilic and lipophilic surfaces.[1,2] Amphiphiles with the most common topology, involving a compact polar "headgroup" and a flexible nonpolar "tail", aggregate cooperatively in aqueous solution to form micelles or vesicles. Alternative amphiphile topologies can lead to distinctive and useful properties. For example, the cholic acids (steroids with rigid polar and nonpolar surfaces) are physiologically important in the uptake and excretion of insoluble lipids[3] and have proven to be unique tools for the manipulation and purification of proteins.[4,5] Cholate itself aggregates less cooperatively and with greater size polydispersity than typical compact headgroup/flexible tail amphiphiles;[6] this nonmicellar mode of association is undoubtedly related to the cholic acids' functional merits.

The exploration of new amphiphilic architectures is a matter of continuing interest and importance.[7] We have been examining synthetic amphiphiles that have rigid planar structures with one polar face and one nonpolar face,[8] species that we designate "contrafacial amphiphiles".[9] (Cholate and derivatives do not completely fit this description, because the carboxylate is attached at the end of a flexible alkyl appendage that can extent approximately laterally from the steroid nucleus.) Here we report the aggregation properties of two synthetic contrafacial amphiphiles, **1a** and **2a**, and of a related structure bearing a short flexible tail (**3a**).[10] The behavior of **1a** is compared to that of isomer **4a**, which is planar and rigid but has its polar group peripherally rather than facially disposed. The topologies of contrafacial amphiphile **1a** and isomer **4a** are indicated schematically below.



Aggregation of **1a**–**4a** in D_2O could be detected by monitoring ^1H NMR signals as a function of amphiphile concentration. In each case, ^1H chemical shifts were independent of concentration in dilute solutions (<5 mM), but most or all resonances of each amphiphile began to move upfield at higher concentrations, signaling the onset of intermolecular interactions.[11] For each amphiphile, the changes in chemical shift with increasing concentration were gradual, indicating that the associations were not highly cooperative, i.e., that these aggregation processes do not correspond to the classical models of micelle formation.[1] This noncooperative behavior is consistent with observations reported for rigid amphiphiles of more conventional topology, including benzoate and benzenesulfonate derivatives.[12] According to the ^1H NMR data, the onset of association occurs in the range 20–40 mM for isomers **1a** and **4a** and in the range 5–10 mM for **2a** and **3a**.[11]

Aggregation of **1a**–**4a** was probed also by monitoring uptake of the water-insoluble azo dye orange OT as a function of amphiphile concentration (Figure 1).[13] Aggregates of **1a** did not solubilize dye even at the limit of this contrafacial amphiphile's solubility (0.54 M). In contrast, aggregates of **4a** began to solubilize dye at ca. 0.33 M. The onset of dye uptake for **3a** was ca. 0.06 M, but no dye solubilization was detected for **2a** up to 0.16 M, which is near this amphiphile's solubility limit.[14] The difference in behavior between isomers **1a** and **4a** demonstrates that the contrafacial amphiphile topology does indeed confer unique properties: the structure of the aggregates formed by **1a** must differ fundamentally from the structure of the aggregates formed by **4a**, with only the latter capable of providing a microenvironment that can solubilize the dye. Comparison of **2a** and **3a** shows that the attachment of a short flexible segment to a rigid contrafacial amphiphile unit can exert a significant effect on aggregation and solubilization properties.

For each of the amphiphiles we have studied, aggregation is detected by ^1H NMR at concentrations at which dye solubilization

(1) For leading references, see: Myers, D. *Surfactant Science and Technology*; 2nd ed.; VCH Publishers, Inc.: New York, 1992. (b) Fendler, J. H. *Membrane Mimetic Chemistry*; John Wiley & Sons: New York, 1982.

(2) (a) Mukerjee, P. *J. Pharm. Sci.* **1974**, *63*, 972. (b) Israelachvili, J. N.; Marcelja, S.; Horn, R. G. *Q. Rev. Biophys.* **1980**, *13*, 121.

(3) Carey, M. C.; Small, D. M. *Arch. Intern. Med.* **1972**, *130*, 506.

(4) See, for example: Wu, S.-H.; Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990.

(5) The synthesis of glycosylated derivatives of cholic acid and allocholic acid, with enhanced hydrophilicity on one side, has been reported: (a) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7319.

(6) (a) Roda, A.; Hofmann, A.F.; Mysels, K. J. *J. Biol. Chem.* **1983**, *258*, 6362. (b) Mukerjee, P.; Moroi, Y.; Murata, M.; Yang, A. Y. S. *Hepatology* **1984**, *4*, 61S.

(7) (a) Regen et al. have developed an imaginative membrane-directed drug design strategy based upon the "molecular harpoon" concept; for leading references, see: Naka, K.; Sadownik, A.; Regen, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2278. (b) Careful choice of polar group allows redox-switched vesicle formation: Muñoz, S.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 4899 and references therein. (c) "Gemini surfactants": Menger, F. M.; Littau, C. A. *J. Am. Chem. Soc.* **1991**, *113*, 1451. (d) "Bolaamphiphiles": Fuhrhop, J.-H.; Fritsch, D. *Acc. Chem. Res.* **1986**, *19*, 130.

(8) Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943.

(9) Kahne et al. (ref 5b) have used the term "facial amphiphile" to describe cholic acids and their derivatives. For molecules like **1a** and **2a**, we prefer "contrafacial amphiphile", a term that emphasizes the fact that all polar functionality is facially disposed.

(10) Compound **1b** was prepared as previously described: Barrett, D. G.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 6915. (For the original synthesis of **1b**, see: Vogel, E. *Spec. Publ.—Chem. Soc.* **1967**, *21*, 113.) The syntheses of acids **2b** and **3b** were similar to the previous route, except that intermediate allyl rather than methyl esters were used.

(11) Data may be found in the supplementary material.

(12) For leading references on the association of benzene carboxylates and sulfonates, see: (a) Saleh, A. M.; El-Khordagui, L. K. *Int. J. Pharm.* **1985**, *24*, 231. (b) Balasubramanian, D.; Srinivas, V.; Gaikar, V. G.; Sharma, M. M. *J. Phys. Chem.* **1989**, *93*, 3865. (c) Pyrene sulfonate in aqueous solution: Menger, F. M.; Whitesell, L. G. *J. Org. Chem.* **1987**, *52*, 3793.

(13) Schott, H. *J. Phys. Chem.* **1966**, *70*, 2966 and references therein.

(14) For aqueous solutions of **3a**, the following dye:amphiphile ratios were observed: 80 mM **3a**, 1:7700; 166 mM **3a**, 1:520. For aqueous solutions of **4a**, the ratios were the following: 425 mM **4a**, 1:24 000; 525 mM **4a**, 1:6200.

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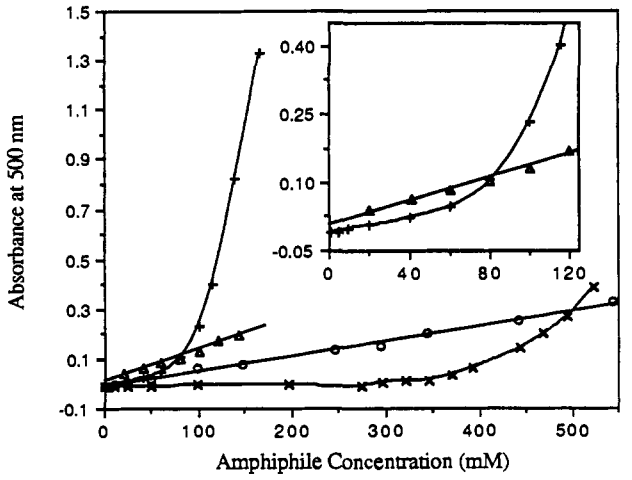

Figure 1. Orange OT solubilization as a function of amphiphile concentration in aqueous solution: **1a** (○), **2a** (△), **3a** (+) and **4a** (×). The lines for **1a** and **2a** are results of linear regression; the lines for **3a** and **4a** are arbitrary. For each amphiphile, aqueous solutions with varying amphiphile concentrations and excess suspended orange OT were agitated gently at room temperature for 2–3 days. The solutions were then filtered through cotton to remove undissolved dye, aliquots of each filtrate were diluted with 4 vol of absolute EtOH, and the absorbance was measured at 500 nm. Orange OT has an absorbance maximum near this wavelength, but amphiphiles **1a–4a** do not. The electronic absorption bands of bridged annulenes **1a–3a** (but not naphthalene derivative **4a**) "tail" weakly out to 500 nm, which causes the small amount of absorbance seen for concentrated solutions of **1a** and **2a**. The inset shows an expanded plot at low concentrations for **2a** and **3a**.

does not occur. This behavior differs from that of typical micelle-forming amphiphiles, for which critical micelle concentration (CMC) values determined by these two methods are usually comparable.[1] Our observations suggest that aggregates of **3a** and **4a** must grow to a minimum size before a dye-solubilizing microenvironment is created, behavior that is consistent with the noncritical nature of the aggregation processes for these amphiphiles.

The crystal structures of acid forms **1b–4b** were determined to allow comparison of the molecular packing of the methano-bridged annulene and naphthalene skeletons. Neighboring naphthyl units in **4b** display a "herringbone" arrangement, as is observed for naphthalene itself[15] (interplanar angle for **4b** = 48°; for naphthalene = 51°). Figure 2 shows that such a "herringbone" juxtaposition occurs as well in crystalline **1b**. Similar aromatic–

(15) (a) Abrahams, S. C.; Monteath Robertson, J.; White, J. B. *Acta Crystallogr.* **1949**, *2*, 238. (b) Brock, C. P.; Dunitz, J. D. *Acta Crystallogr.* **1982**, *B38*, 2218 and references therein. (c) For a general discussion of aromatic hydrocarbon crystal packing, see: Desiraju, G. R.; Gavezzotti, A. *Acta Crystallogr.* **1989**, *B45*, 473.

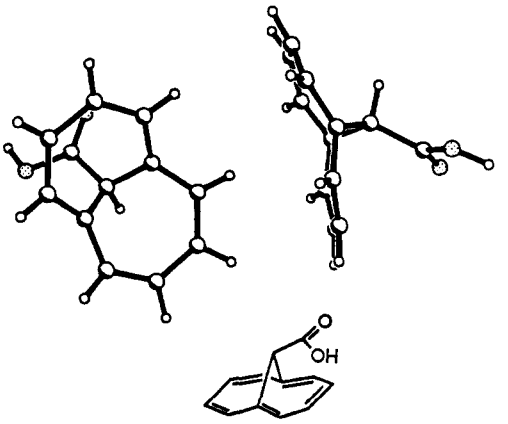

Figure 2. Ball-and-stick representation of a neighboring pair of molecules in the crystal lattice of **1b**. The angle between the mean planes of the annulene rings is 67°.

aromatic juxtapositions are observed in crystalline **2b** and **3b**.[16] In light of the qualitative difference in solution behavior between **4a** and bridged annulene-based contrafacial amphiphiles **1a** and **2a**, it is interesting that the faces and edges of bridged annulenes behave similarly to the analogous surfaces of more conventional aromatic species in the solid state. The nature of the intermolecular juxtapositions in solution aggregates of **1b–4b** is unclear. It has been assumed that aromatic sulfonates and carboxylates engage in parallel "stacking" interactions in aqueous aggregates,[12a,b] but there appear to be no structural data to support this assumption.

We have shown that the contrafacial amphiphile topology confers unique solution properties relative to more traditional amphiphile topologies. The fact that **1a** and **2a** present substantial expanses of nonpolar surface to their aqueous surroundings but, in their aggregated states, do not create a microenvironment that can solubilize a hydrophobic moiety suggests that these and related molecules may display interesting nondisruptive behavior toward proteins and biological membranes.

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Supplementary Material Available: Crystallographic details for **1b–4b**, and plots of ^{1}H NMR chemical shifts vs concentration for aqueous solutions of **1a–4a** (34 pages); listing of observed and calculated structure factors for **1b–4b** (20 pages). Ordering information is given on any current masthead page.

(16) Barrett, D. G.; Desper, J. M.; Hayashi, R. K., unpublished results.