Hydrogen Bonding in Micelle Formation

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Aggregation in water is thought to be driven primarily by the hydrophobic effect.[1] Indeed, for most amphiphiles in a given structural class, hydrophobicity is inversely correlated with the aqueous critical micelle concentration (CMC).[1] However, certain amphiphiles, because of their distribution of hydrophobic and hydrophilic domains, cannot aggregate without burying at least some polar groups. For these amphiphiles, the energetics of aggregate formation are not well understood. We recently reported the synthesis and crystallographic characterization of some novel amphiphiles generated by glycosylating the hydroxyls at C-7 and C-12 of cholic acid and allocholic acid.[2,3] We called these compounds *facial amphiphiles* because they have a polar surface and a nonpolar surface. We now report the results of studies on the synthetic facial amphiphiles and their non-glycosylated counterparts which imply that hydrogen bonding can play a dominant role in micelle formation in water.

One of the most common and rapid methods to measure CMCs involves monitoring the solubilization of a hydrophobic dye such as Orange OT in the presence of increasing concentrations of an amphiphile.[4] Table 1 shows the CMCs of facial amphiphiles **1**, **2**, and **3** and four different bile acid salts as measured by the solubilization of Orange OT.[4b] As others have also reported, the CMCs of the hydrophobic bile acid salts chenodeoxycholate **4** and deoxycholate **5** are signficantly lower than the CMCs of the more hydrophilic bile acid salts cholate **6** and allocholate **7**.[5] Since hydrophobicity and CMC are thought to be correlated for the bile acid amphiphiles,[6] one might expect the CMCs of the bis-glycosylated bile acid salts **1** and **2** (Figure 1) to be higher than those of their non-glycosylated counterparts. However, even though the glycosylated bile salts **1** and **2** have six more hydroxyls than their non-glycosylated counterparts, they actually have *lower* CMCs (Figure 2). In fact, **1** has a lower CMC than deoxy- and chenodeoxycholate (**4** and **5**). Interestingly, the permethylated glycosteroid **3**, which has no free hydroxyls on the sugars, has a higher CMC than its polyhydroxylated counterpart **1**.

To assure ourselves that the additional hydroxyls do, in fact, influence the hydrophilicity of the molecules in the expected manner, we measured the retention times of all the compounds in Table 1 using an HPLC assay specifically developed to rank the hydrophobicities of the bile acids.[7a] In this assay, the retention time reflects the partitioning of an amphiphile between a stationary hydrocarbon phase and a mobile polar phase. The shorter the retention time, the more hydrophilic the compound is considered to be. Retention times are usually expressed as retention factors normalized to a standard, typically deoxycholic acid.[7b,c] As shown in the last column of Table 1, the normalized retention factors of amphiphiles **1**–**7** followed the expected order, and the glycosylated compounds **1** and **2** were by far the most hydrophilic (*i.e.*, had the smallest retention factors).

The results reported shed light on a controversy of long standing. It is known that bile salts aggregate in a stepwise manner, forming

Table 1

compd	CMC[a] (mM)	rk'[b]
1	1–2	0.188
2	3–4	0.244
3	3–4	1.364
4	2–3	0.914
5	2–3	1.000
6	7–8	0.421
7	11–12	0.475

[a] CMC of the salt in 0.15 M NaCl, pH 7.0. [b] rk', normalized HPLC retention factor (Ultrasphere ODS column, 75% MeOH/25% 0.005 M KH_2PO_4, pH 5.0 buffer).

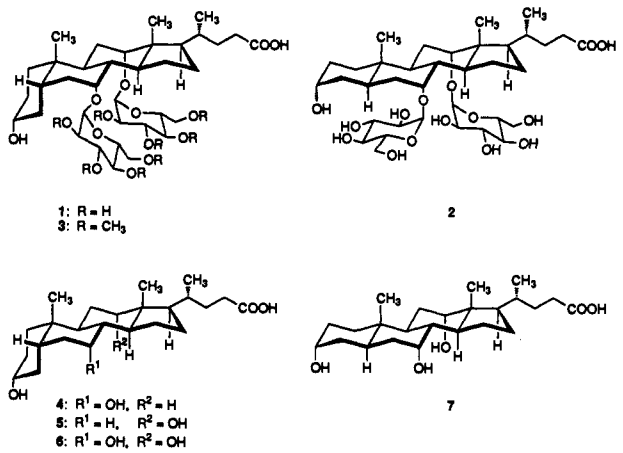

Figure 1. Structures of glycosylated and non-glycosylated bile acids **1**–**7**.

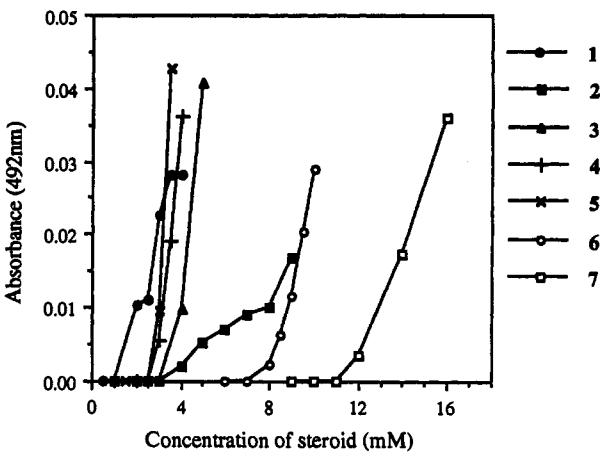

Figure 2. Determination of the CMCs of glycosylated steroids (**1**–**3**) and bile salts (**4**–**7**) in 0.15 M NaCl (pH 7.0) at 25 °C. Solutions of **1**–**7** containing excess crystalline Orange OT were shaken for 48–50 h, unsolubilized dye was removed by filtration (0.22 μm Millex GV), and the absorbance of the solution was measured at 492 nm. The CMC was taken as the concentration at the first point at which there was a non-zero absorbance.

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(3) For recent reports of other unusual amphiphiles, see: (a) Barrett, D. G.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9343. (b) Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943. (c) Menger, F. M.; Littau, C. A. *J. Am. Chem. Soc.* **1993**, *115*, 10083 and references therein. (d) Menger, F. M.; Mounier, C. E. *J. Am. Chem. Soc.* **1993**, *115*, 12222. (e) Moss, R. A.; Li, G.; Li, J.-M. *J. Am. Chem. Soc.* **1994**, *116*, 805 and references therein. (f) Nicolis, I.; Coleman, A. W.; Charpin, P.; Villain, F.; Zhang, P.; Ling, C. C.; Rango, C. *J. Am. Chem. Soc.* **1993**, *115*, 11596.

(4) (a) Schott, H. *J. Phys. Chem.* **1966**, *70*, 2966. (b) For a listing and evaluation of the different methods for CMC determination, see: Mukerjee, P.; Mysels, K. J. *Natl. Stand. Ref. Data Ser. (U.S., Natl. Bur. Stand.)* **1971**, *36*, 1 and references therein. Although solubilization methods can themselves induce micelle formation, Orange OT is known to have an extremely low mole fraction at saturation (0.01) and is considered to give reliable CMCs.

(5) The Orange OT dye solubilization method has been used to measure the CMCs of bile acids under conditions identical to ours, and the same results were obtained. (a) Roda, A.; Hofmann, A. F.; Mysels, K. J. *J. Biol. Chem.* **1983**, *258*, 6362. (b) Small, D. M. In *The Bile Acids: Chemistry, Physiology, and Metabolism*; Nair, P. P., Kritchevsky, D., Eds.; Plenum: New York, 1971; Vol. 1, p 249 and references therein.

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small primary micelles first and then larger secondary micelles, and it is generally agreed that the hydrophilic surfaces associate at some point in the aggregation process.[8-10] However, there is a debate about whether hydrogen bonds play a stabilizing role in the formation of the micelles. Mazer *et al.* have argued that hydrogen bonds are not worth much in water and that hydrophobic interactions control the association process.[9b] They have supported their arguments with the observation that deoxy- and chenodeoxycholate aggregate at lower concentrations than cholate. However, others data suggest that hydrogen bonds do in fact play a role in the energetics of bile salt micelle formation.[11]

The CMC results on the compounds in Table 1 provide strong evidence that hydrogen bonding can play a dominant role in micelle formation. Since the hydrophobic surfaces of the facial amphiphiles in Table 1 are identical within a series (cholic acid *vs* allocholic acid nucleus), the differences in the CMCs must be related to the hydrophilic surfaces. The CMCs of the glycosylated bile acid salts are lower than those of the non-glycosylated bile acid salts, implying that the hydrophilic surfaces of the former must self-associate more readily than those of the latter. Therefore, the drive to minimize hydrophobic surface area cannot play the determining role in the self-association process.[9b] Hydrogen bonds must facilitate aggregation of **1** and **2**. The fact that **3**, which has no potential hydrogen bond donors on the sugars, has a higher CMC than **1** bolsters this argument. It should be noted that there is growing evidence that *polyfunctional* hydrogen bonds can play a significant stabilizing role even in water.[11,12]

A comparison of the data in Table 1 shows, however, that the absolute number of potential hydrogen bonding groups does not correlate directly with the CMC of a facial amphiphile any more than the overall hydrophobicity does. Evidently, the hydrophilic surfaces must contain complementary arrays of hydrogen bonds to facilitate association. In this regard, it should be noted that previously reported crystal structures of the potassium salt of **1** and the methyl ester derivative of **2** show that the glycosylated steroids organize in pairs held together by multiple hydrogen bonds between opposing sugars.[2,13] However, the non-glycosylated bile acids do not crystallize in pairs but form part of an infinite hydrogen-bonded network.[14] Thus, the solid-state evidence suggests that there is better complementarity between hydrogen bonding partners in the glycosylated bile acids than in their non-glycosylated counterparts. We believe this accounts for the lower CMCs of the more hydrophilic glycosteroids. (Ironically, the crystal structures of the glycosteroids are similar in key respects to the models for bile acid micelles, in which the molecules are shown as associating in pairs with their hydrophilic surfaces in contact.)[9b]

Our findings provide new information about how facial amphiphiles associate to form micelles that should be useful in the design of other amphiphiles. Moreover, since amphiphilic peptides are also facially amphiphilic, these findings further suggest that oriented hydrogen bonds may be used in peptide design to help control the structure at the interface of peptide aggregates.[15]

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Supplementary Material Available: HPLC and UV data for compounds **1–7** are provided (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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