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New Lamellar Structure Formed by an Adamantyl Derivative of Cholic Acid

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The self-aggregation of the sodium salt of a new adamantyl amide of the 3β-amino derivative of cholic acid (Na-AdC) in aqueous solution has been investigated by surface tension, dynamic light scattering, fluorescence, small-angle X-ray scattering (SAXS), and transmission electron microscopy (TEM) measurements. These last two techniques suggest that a lamellar phase, consisting of charged bilayers of Na-AdC separated by solvent and periodically stacked, is formed in aqueous solution. The structure of the bilayer is inferred from the resolution of the crystal of the compound in its acid form. The adamantyl moieties, which are mutually interlocked, reside in the central region of the bilayer, and the carboxylic groups are directed toward the hydrophilic region. The structure is open enough to allow water molecules to interact with a fluorescence probe located at the central hydrophobic region.

Some of the most important physiological properties of bile salts are derived from their amphipathic nature, since they have a hydrophobic β side and a hydrophilic α side.[1−3] Bile salts self-aggregate in aqueous solution, forming micelles,[4] gels,[5] fibers,[6] and nanotubes.[7] The resolution of the crystal structure is very useful to infer the structure of such aggregates in aqueous solution.[8] It is expected that modifications of the hydrophilic−hydrophobic balance of bile salts will induce changes on the physicochemical properties of the native bile salt, as well as on their biological effects. Here, we present the effect of adding a bulky hydrophobic (adamantyl) group to the 3-position of cholic acid. The new derivative (either in its acid, H-AdC, or salt, Na-AdC, forms; Figure 1) is obtained by reacting the 1-adamantoic acid with the 3β-amino derivative of cholic acid.[9]

Surface tension measurements (Wilhelmy plate method) show that the plot of γ versus log[Na-AdC] is linear until 0.5 mM, reaching a plateau above this concentration. This is the typical behavior for a surfactant in aqueous solution, suggesting that Na-AdC self-aggregates in this solvent. That critical concentration value is 30 times lower than the critical micelle concentration (cmc) of sodium cholate,[10] NaC, indicating that the new compound acts as a better surfactant than the natural bile salt. From the Gibbs adsorption equation, the area A_s per surfactant molecule at the interface can be calculated from the slope of the linear part, with the resulting value being 251 Å^2. A comparison with the A_s values for other bile salts is not straightforward, since they are highly dependent on experimental conditions.[11,12] For instance, for NaC, Swanson-Vethamuthu et al.[12] have published A_s values of 207 Å^2 (in 50 mM NaCl) and 288 Å^2 (in 45 mM NaCl and 5 mM NaOH). For sodium deoxycholate (NaDC), these authors obtained values of 149 and 204 Å^2. Lower values than the previous ones have been published elsewhere.[2] Even so, it can be accepted that the obtained value for Na-AdC supports the idea of an enlargement of the hydrophobic area due to the bulky adamantyl group.

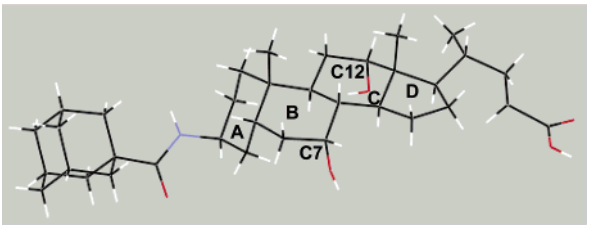

Figure 1. Structure of H-AdC.

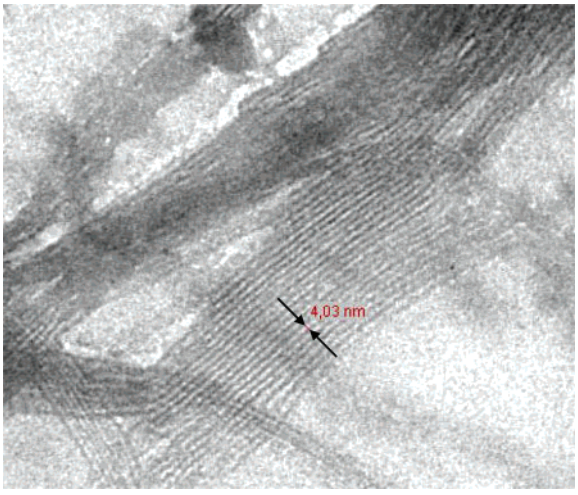

Figure 2. TEM image of lamellae formed by Na-AdC.

To characterize the structure of the aggregates in solution, dynamic light scattering experiments were carried out. The value obtained for the hydrodynamic radius at a concentration of 1.2

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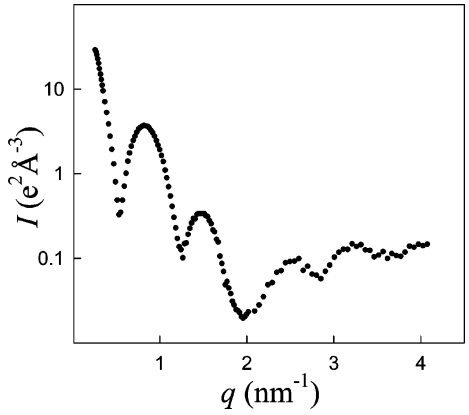

Figure 3. SAXS spectrum of a Na-AdC aqueous solution (8 wt %). The scattering intensities are expressed in electron units (electrons^2 Å^{-3}) per cm of primary beam length.

mM is 100 nm at zero time, with very low polydispersity (0.17), but the aggregates grow continuously. After 24 h, the hydrodynamic radius is 1.4 μm (polydispersity, 0.5), and after 48 h, it is 3.8 μm (polydispersity, 1). This observation is in agreement with Nagarajan equations,[13] since the polydispersity of aggregates will increase with the aggregation number.

To further investigate the structure of the aggregates, transmission electron microscopy (TEM) (in solid state) and small-angle X-ray scattering (SAXS) (in aqueous solution) measurements were performed. The TEM image (Figure 2) contains a regular organization of dark elongated objects in parallel arrays. They show very little bending over several hundred nanometers, suggesting a large bending modulus typically associated with a lamellar phase. The SAXS spectrum (Figure 3) shows four peaks which space approximately in the ratio 1:$^1/_2$:$^1/_3$:$^1/_4$, characteristic of a lamellar organization. They are consistent with an average interlamellar distance of 7.4 ± 0.3 nm. This value is similar to those observed for lamellae formed by cholesteryl derivatives.[14]

To understand the origin of this unusual lamellar organization of a bile salt derivative, it is necessary to analyze the Na-AdC structure. Here, the oxygen atom of the amide group lies toward the α side (Figure 4a, adapted from the crystal), maintaining the number of hydrophilic groups of this side, but the hydrophobic area has been enormously enlarged. Along its longitudinal axis, the molecule exhibits three regions: a charged carboxylate group at the end of the flexible side chain, a rigid steroid nucleus, and the bulky hydrophobic tail. This structure resembles the amphipathic nature of classical alkyl chain surfactants, suggesting that the lamellar phase could consist of infinite charged bilayers of Na-AdC separated by solvent and periodically stacked. The repulsive electrostatic forces between lamellae would lead to rigid and highly ordered membranes which seem flat on large scales. These structural features are in agreement with TEM observations and with the classical description of electrostatically stabilized lamellar phases.[15]

Further information on the bilayer structure can be inferred from the H-AdC crystal. [The cif data of the crystal can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif (CCDC 600368)]. Figure 4b shows a view of the crystal structure along the *b* axis. The H-AdC crystal evidences the existence of a lamellar structure, with hydrophobic and hydrophilic regions clearly distinguished. The adamantyl groups lay in the same region, facilitating hydrophobic interactions, while the carboxylic acid groups form hydrophilic channels where water molecules, involved in hydrogen bonds with these groups, are located (Figure 4b). The

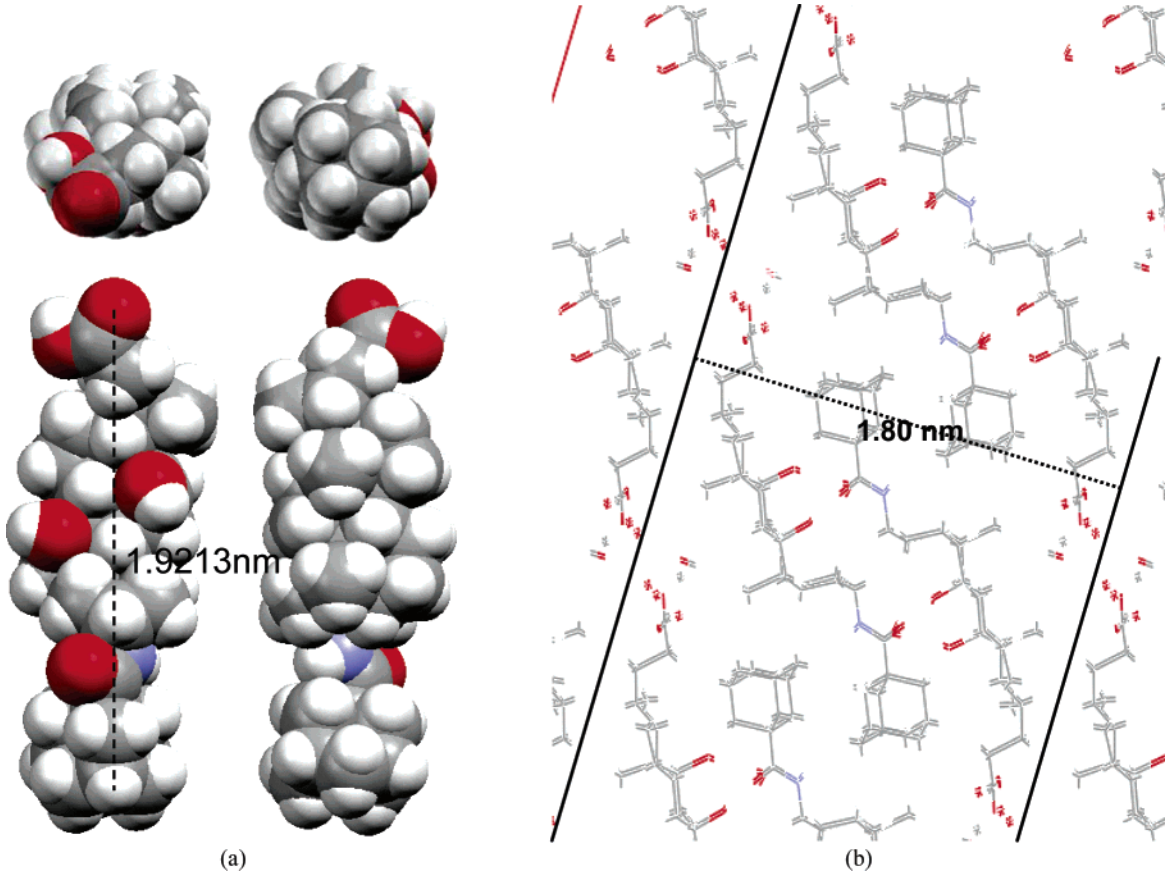

Figure 4. (a) Structure of H-AdC. (b) Structure of the crystal along the *b* axis. The lines indicate the hydrophilic channels.

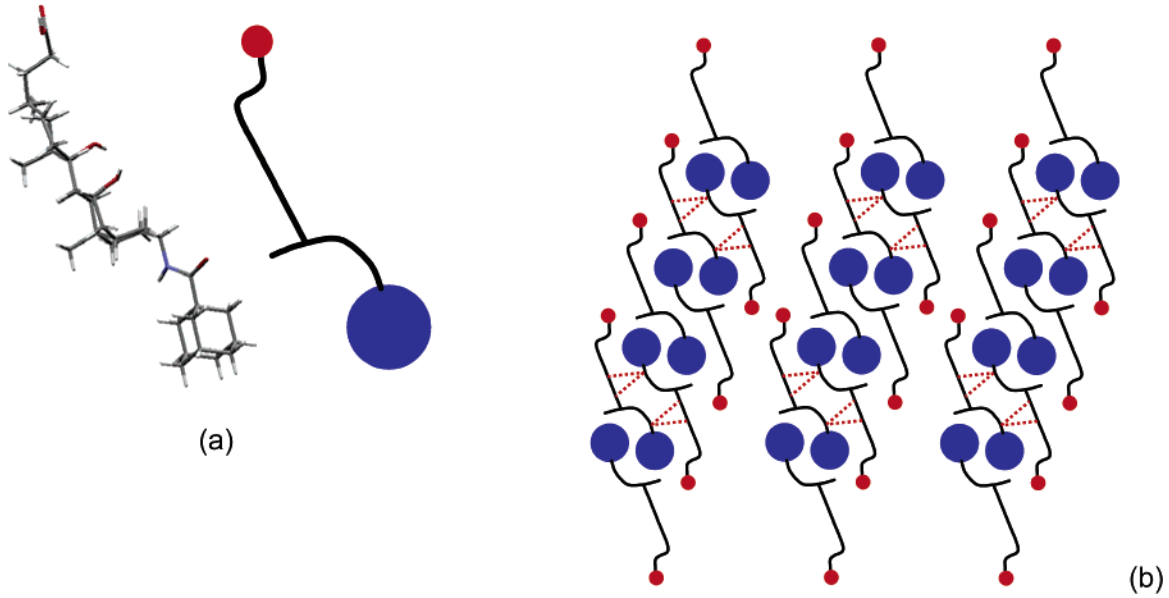

Figure 5. Schematic representations of (a) the structure of the H-AdC molecule and (b) the structure of the crystal (compare with Figure 4b). It illustrates how the adamantane residues are mutually interlocked.

width of the bilayer in the crystal, determined as the distance between hydrophilic layers, is 1.80 nm (Figure 4b) which perfectly agrees with the one (approximately 1.8 nm) obtained from TEM images (black lines of Figure 2).

The A ring, the hydrogen atom of the amide group, and the adamantyl residue form a hydrophobic semicage. The other part of the cage is formed by the methylene group (attached to the carboxylic acid group) of the side chain, the methyl group attached to this side chain, and protons of the D ring. Considered along the *b* axis, such a cage originates hydrophobic cylinder channels where other adamantyl residues are located (in alternate layers), being oriented in opposite directions and mutually interlocked (see schemes in Figure 5).

The hydrophobic interactions are reinforced by the formation of O−H···O hydrogen bonds, implying the oxygen atom of the amide group and the two hydroxyl groups of the steroid body, the hydrogen bond pattern being as follows: the amide group of one H-AdC molecule forms a hydrogen bond with the hydroxyl group at C7 of a second H-AdC molecule, while the amide group of this second molecule forms a hydrogen bond with the hydroxyl group at C12 of the first one. Therefore, there is a self-recognition process leading to the self-assembly in bilayers. The hydrogen bond network has a zigzag structure.

The aggregation behavior of amphiphiles is often explained using the molecule-specific packing parameter.[16] Conical molecules pack in spherical micelles, while cylindrical ones do it in flat lamellae. Spacefill models (Figure 4a) indicate that Na-AdC has almost a cylindrical shape, and therefore, the observed lamellae structure is in agreement with that prediction.

The proposed structure for the bilayer suggests that water could deeply enter inside the assembly. To test this hypothesis, the I_1/I_3 ratio of pyrene fluorescence has been measured. This probe has been frequently used to determine the polarity of the environment in micellar solutions, including bile salt aggregates.[5] At [Na-AdC] > 4 mM, the I_1/I_3 ratio reaches a plateau with a value of 1.19. This value is close to the observed one for SDS micelles (equal to 1.16)[17] but far from the values for NaDC (=0.70) and NaC (=0.75).[5] The fraction of pyrene in contact with water in the bile salt aggregates of these two bile salts is very low (4 and 0%, respectively),[18] but in classical surfactant micelles, pyrene is located in the palisade layer,[19] where water molecules reside (5−10 water molecules per surfactant molecule),[20] facilitating their contact with the probe. These results suggest that the packing in the Na-AdC lamellae is open enough to allow pyrene−water contact.

It may be concluded that Na-AdC is a building block which, after a self-recognition process, self-assembles in bilayers both in aqueous solution and in the solid state. This novel interlocking could be used for designing new biomaterials.

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