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Review Article:

From Crystal to Micelle: a New Approach to the Micellar Structure

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Abstract. The structure of sodium and rubidium deoxycholate micellar aggregates in aqueous solutions was found to be helical and to be stabilized mainly by polar interactions. Astonishingly, the lateral surface of the helix is covered by nonpolar groups and the interior part is filled with cations surrounded by water molecules, as in the case of an inverted micelle. This helical model was inferred from the crystal structures of sodium and rubidium deoxycholates and proved by spectroscopic and diffractometric experimental data. The strategy of the approach to the determination of the micellar structure and the comparison with another model, previously proposed for the bile salt micelles, are reported. On the basis of some results obtained for sodium tauro- and glyco-deoxycholates, micellar models are suggested which could account for the biological function of these important conjugated bile salts.

Key words. Micellar structure determination, bile salts.

1. Introduction

The salts of bile acids play an important role in many physiological and biological systems because of their detergent-like and surface active properties. Their ability to form molecular aggregates in aqueous solutions, capable of solubilizing many water-insoluble compounds, is a crucial property for understanding their physico-chemical behaviour. The physical chemistry of bile acids and their salts has been extensively reviewed several times [1–6] on the basis of the copious literature existing in this field. However, there are several discrepancies and open questions which seem to indicate that the approximations inherent in the theory are too drastic and the limits of the experimental techniques are too unfavourable to obtain a reasonable picture of the physico-chemical characteristics of bile salts [7]. Moreover, very little is known about the structure of the micellar aggregates since the study of their size and shape often provides ambiguous answers. Hence, the basic starting point to improve this confused situation consists in trying to solve the structure of the micelles.

2. Strategy

A satisfactory choice of reasonable structural models can only be made with great difficulty owing to the nearly always dubious interpretation of the experimental data obtained from the liquid state (aqueous micellar solutions). Furthermore, even

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though a model can be hypothesized, its geometry is generally poorly defined. A natural reservoir and useful source of models is the crystalline state, since the same structural unit, or a very similar one, could be present both in the crystal and liquid state, as frequently occurs for macromolecular compounds. Thus, we tried to find compounds which form micellar aggregates in aqueous solutions, increase the size of their micelles by varying parameters such as pH or ionic strength, and subsequently give rise to the transitions aqueous micellar solution → gel → macromolecular fibre → crystal. Moreover, we verified that the X-ray diffraction patterns of all these phases show intensity maxima and minima in the same regions in order to have a good chance of finding equal or very similar structural units in the liquid and solid phases. If this is the case, the determination of a crystal structure by means of X-ray diffraction techniques provides an actual model, sufficiently precise, which can be checked and possibly confirmed in the study of the micellar solutions.

3. Sodium and Rubidium Deoxycholate

Sodium and rubidium deoxycholates (NaDC and RbDC, respectively) were found to be compounds satisfying the abovementioned requirements. We solved the RbDC crystal structure [8] and now we also know in detail that of NaDC, which will be published elsewhere. The two crystal structures are strictly related and very similar to each other [9–11]. Their structural unit turned out to be a left-handed helix with a pseudo-sixfold screw axis, depicted in Figure 1 for RbDC. The backbone of the helix comprises Rb^+ ions surrounded by water molecules in a very peculiar fashion. Ion–ion interactions between Rb^+ and carboxylate ions, ion–dipole interactions between Rb^+ ions and water molecules and a close net of

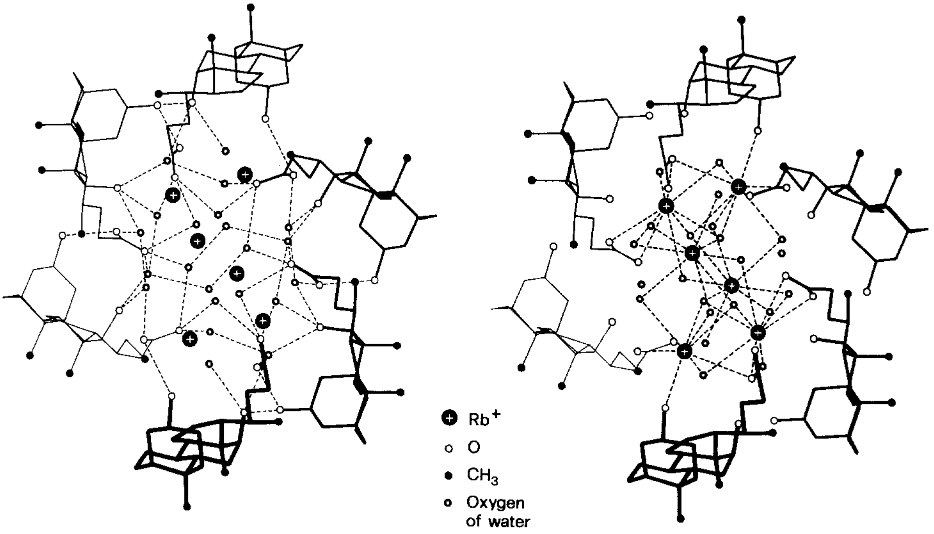

Fig. 1. View of the left-handed RbDC helix along the twofold (pseudo-sixfold) helical axis. The broken lines represent hydrogen bonds in the helix on the left and ion–dipole and ion–ion interactions within 3.4 Å in the helix on the right.

hydrogen bonds give rise to a very stable helix (see Figure 1). The outer lateral surface of the helix, which, in solution, should be in contact with the aqueous medium, is covered by nonpolar groups such as the angular methyl groups. Nevertheless, although this aggregate surprisingly resembles that of an inverted micelle (polar inside and nonpolar outside), its solubility in water can be accounted for. In fact, hydroxyl and carboxyl polar groups can be approached by solvent molecules perpendicularly both to the bases and to the lateral surface of the helix, since the separation between two adjacent deoxycholate anions is sufficiently large.

This rather unusual model was supported by previous results [12, 13], even though the helical parameters suggested were very different from those found by us [8, 11] and the structure of the helix was unknown. On the other hand, a bile salt molecule is two-faced, showing both one hydrophilic and one hydrophobic side, so that Small proposed [14, 15] that about 4–10 molecules associate in aqueous solution their hydrophobic surfaces in a back-to-back manner, in order to minimize the van der Waals energy, thus forming 'primary' micelles with hydrophilic exterior surfaces. These 'primary' micelles can polymerize under proper conditions by means of hydrogen bonds to give larger 'secondary' micelles (see Figure 2). Since the helical model and that of Small are very dissimilar and incompatible, the second stage of this research was to check the helical model in the study of the NaDC and RbDC micelles in aqueous solutions. Nuclear magnetic resonance (NMR) [11, 17], circular dichroism (CD) [16], electron spin resonance (ESR) [18], small-angle X-ray scattering (SAXS) [18], and extended X-ray absorption fine structure (EXAFS) [19] measurements verified the helical model unambiguously within the range of the thermodynamic parameters investigated.

The most significant results, easily interpretable with the helical model, can be briefly summarized as follows.

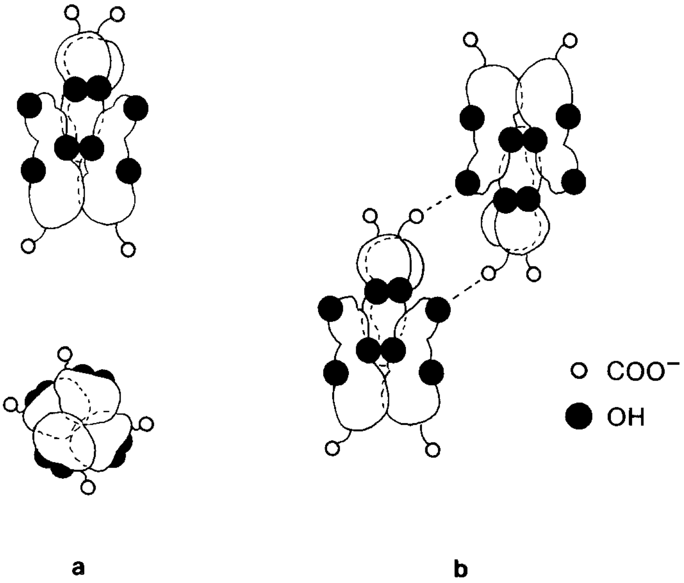

Fig. 2. Schematic model of the primary (a) and secondary (b) micelle.

(a) NMR and CD studies of NaDC micellar solutions containing some solubilizates confirm that these molecules are micelle bound and located at the micelle–water interface. One of the three methyl groups is the most protruding group of the helix (see Figure 1) and is by far the most frequent binding site of the solubilizates [11, 16, 17].

(b) SAXS and ESR measurements indicate that the NaDC micelles are growing cylindrical objects at increasing ionic strength and at lowering temperature [18]. SAXS data allowed us to obtain the electron pair distance distribution function $p(r)$, which is strongly dependent on the shape and size of the scattering particles. Plainly, $p(r)$ can be calculated and compared with the observed function if a molecular model of known geometry is assumed. Some helical parameters were changed and several models were checked, thus confirming the diagnostic power of $p(r)$, which, however, is only slightly sensitive to the short-range structure. A satisfactory agreement was achieved only with a helical model similar to that of Figure 1. The comparison of the experimental scattered intensity $I(k)$ ($k = 4\pi \sin \theta / \lambda$) and $p(r)$ of an NaDC micellar solution and the calculated ones for the helical

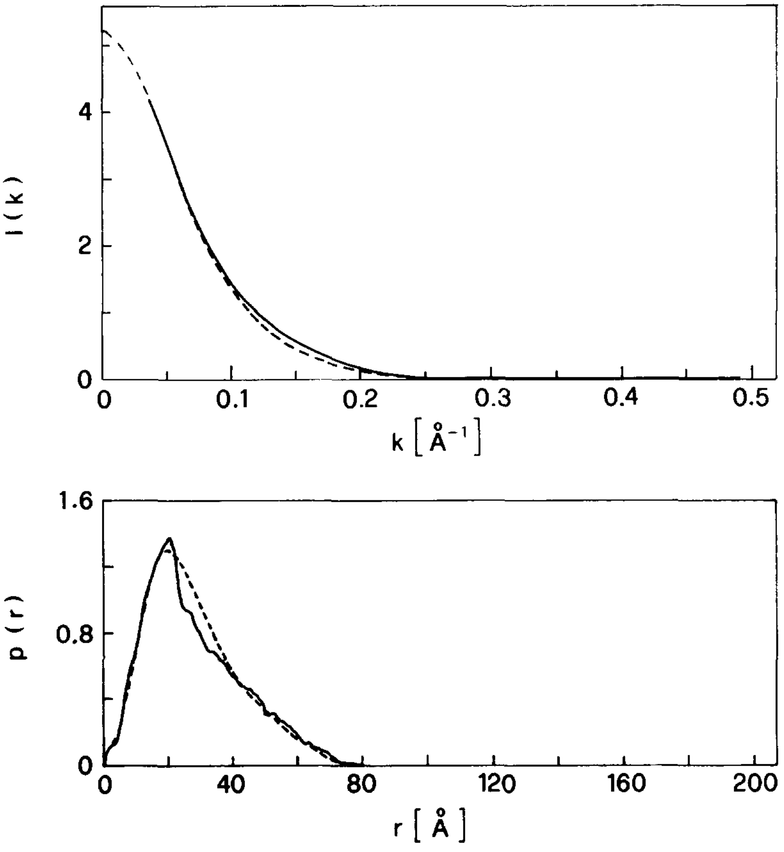

Fig. 3. Observed $I(k)$ and $p(r)$ for a 0.10 M NaDC aqueous solution at a NaCl concentration of 0.30 M (——) and calculated $I(k)$ and $p(r)$ for the helical model with 36 monomers and with an enlargement of the helical radius of 2 Å (- - - -).

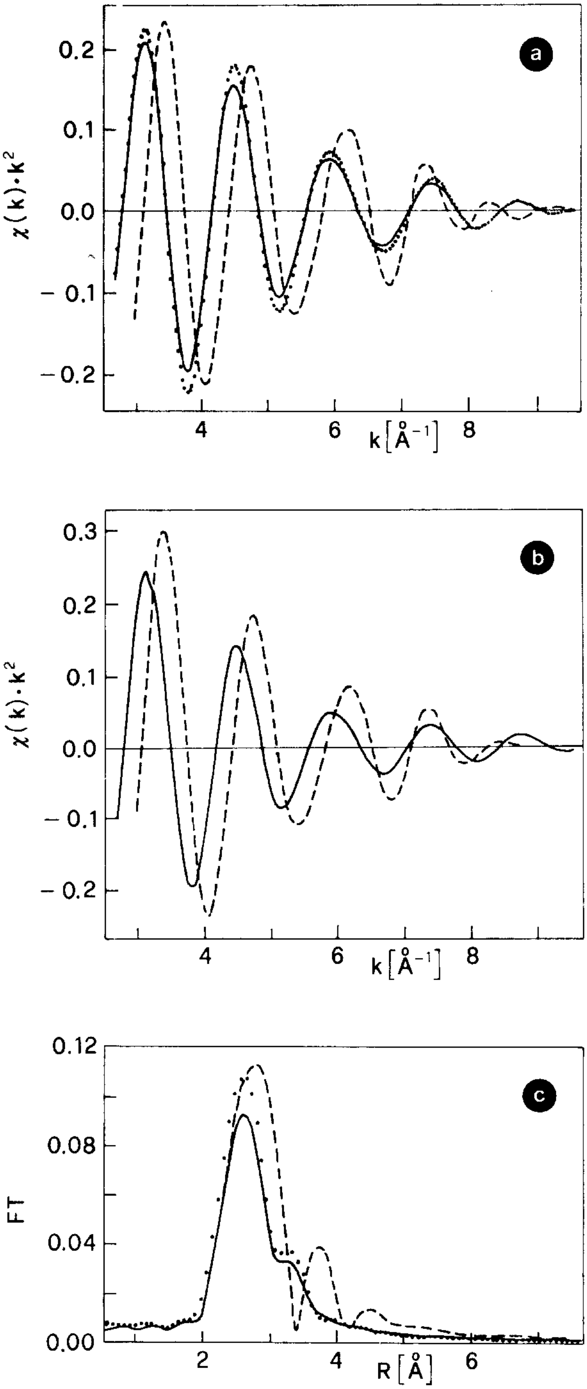

Fig. 4. Experimental filtered spectra (a), calculated spectra (b) and Fourier transforms of the experimental filtered spectra (c) of a RbDC crystal (——), an aqueous micellar solution (····) and a rubidium oxalate aqueous solution (- - - -). k is the wave number and $\chi(k)$ is the normalized oscillatory part of the X-ray absorption coefficient of Rb$^+$. The RbDC crystal and aqueous micellar solution have the same spectra in (b).

model is shown in Figure 3 (an aggregation number of 36 was obtained for this solution from SAXS data).

(c) EXAFS measurements, carried out on a RbDC crystal, a macromolecular fibre and an aqueous micellar solution, clearly indicate that the very peculiar coordination of the Rb^+ ions observed inside the helix in the crystal [8] is equal or practically equal to that in the macromolecular fibre and in the aqueous micellar solution [19]. A rubidium oxalate aqueous solution was also studied for comparison, since in this case the Rb^+ ions are surrounded by water and their coordination is similar to that of the counterions of a classical micelle. Inspection of Figure 4 shows the good agreement between the RbDC crystal and micellar solution and the disagreement between these two samples and the rubidium oxalate aqueous solution. These results definitively confirm the helical model for the micelle.

4. A Comparison Between Two Models

Small's model can hardly explain the experimental results previously cited for the following reasons (points A, B and C refer to a, b and c, respectively):

(A) It is easy to pack back-to-back 4 molecules in a primary micelle. If the number of molecules increases an increasingly larger intramicellar cavity is formed and the back-to-back van der Waals interactions are weakened. The cavity may be filled by water molecules (and this is very unlikely on the basis of energy considerations) or by solubilizates, if they are present. Since the primary micelle is a disordered aggregate and has no symmetry operators, it is very difficult for two of the three methyl groups, pointing towards the interior part of the micelle, to be only weakly engaged, especially when the size of the occluded molecule is very large as compared with the distances between methyl groups. An NMR study of the interaction between NaDC micelles and a nitroxide probe (cholestan spin label, CSL) enables us to determine approximately the distances of the unpaired electron from some protons of NaDC [17]. It appears difficult to accommodate a bulky molecule such as CSL within a primary micelle without breaking or dramatically changing the structure of the micelle itself. On the other hand, we were unable to reproduce the abovementioned distances as average values in a primary micelle–CSL adduct using stick and ball molecular models.

(B) Small's model could account for the cylindrical shape of the micelles by invoking the formation of secondary micelles of the 'string of pearls' type. However, the primary micelle has its hydroxyl and carboxyl groups protruding along several directions, so that the formation along one direction only of hydrogen bonds to get the 'string of pearls' is hardly explainable. Of course, if the primary micelles polymerize by means of a three dimensional network of hydrogen bonds, they give rise to an aggregate which reasonably does not assume a cylindrical shape.

(C) The Rb^+ counterions are outside the micelle, surrounded by water molecules and, perhaps, by some oxygen atoms of carboxylic groups, in the model of Small. We investigated by EXAFS spectroscopy an aqueous solution of rubidium oxalate to mimic this model. The results reported in point (c) allow us to reject the model of Small and any other model with the Rb^+ ions outside the micelle.

5. Concluding Remarks

The helix satisfies the very restrictive condition of the equivalence of the monomeric units along the helical axis and, hence, can have the requirements to be an energetically very stable aggregate. Therefore, it is one of the most frequent objects of cylindrical shape in nature and, probably, many rod-shaped micellar aggregates are helices. As far as the bile salts are concerned many experimental investigations were interpreted assuming that the bile salt molecules self-associate in a progressive and continuous manner, especially in water and at low ionic strengths [6]. Other studies imply a cooperative association of a large number of molecules, especially at high ionic strengths [6]. The helix is a system which fulfils both types of growing, since it can grow by a stepwise addition of molecules or by welding of helices. Moreover, the helix presents the advantage of being a one-dimensional open system, namely a system containing monomers with unsaturated interactions at the end-points if the solvent molecules are disregarded. An open system very easily explains the phenomenon of polydispersity, which entails the presence of micelles with different aggregation numbers. Reasonably, the structure of every micelle corresponds to a minimum of the potential energy, not necessarily the deepest one, since the entropic contribution must be taken into account in the evaluation of the free energy. In the case of helices all the micelles with different aggregation numbers populate the same energy minimum and the energy per monomer is always equal, if the end-monomers are neglected. Thus, the different aggregation numbers depend on kinetic factors, like for crystals of different sizes grown from the same solution. A closed system, like the sphere or an approximate spherical object, has all the monomers almost with the same saturated interactions. Now, it is hardly conceivable that spheres with different aggregation numbers, and, therefore, corresponding to different potential energy minima, have comparable free energy values since the minima are simultaneously populated.

Two last points deserve some attention. It is often assumed that the micellar behaviour of all the bile salts can be accounted for by a unique model, so far identified with that of Small. Unfortunately, a unique model is inadequate to describe the physico-chemical properties of all the bile salts. This can be supported by the study of sodium glycodeoxycholate (NaGDC) and taurodeoxycholate (NaTDC), which satisfy the requirements previously mentioned in the 'Strategy' section. NaGDC and NaTDC have been investigated owing to their paramount importance, since the bile salts of man are glycine and taurine conjugates. Although we have only preliminary results, it seems that again the micellar aggregates are helices [20, 21]. The helix of NaGDC (Figure 5) presents approximately the same helical parameters and a slightly less nonpolar lateral surface than NaDC [20], whereas NaTDC forms a helix with different symmetry axis and helical parameters and a more polar lateral surface [21] (see Figure 6). Now the NaTDC helical model has been strengthened by a recent SAXS study [22], which supplies a value of 18 Å for the radius of the micelles considered as cylindrical particles, in good agreement with the values 16–17 Å observed in the unhydrated helix [21]. Furthermore, a CD investigation of the interaction between bilirubin and NaDC [23], NaGDC or NaTDC [20] shows that the optically inactive bile pigment exhibits the same bisignate Cotton effect on binding to the micelles of the three bile salts. The

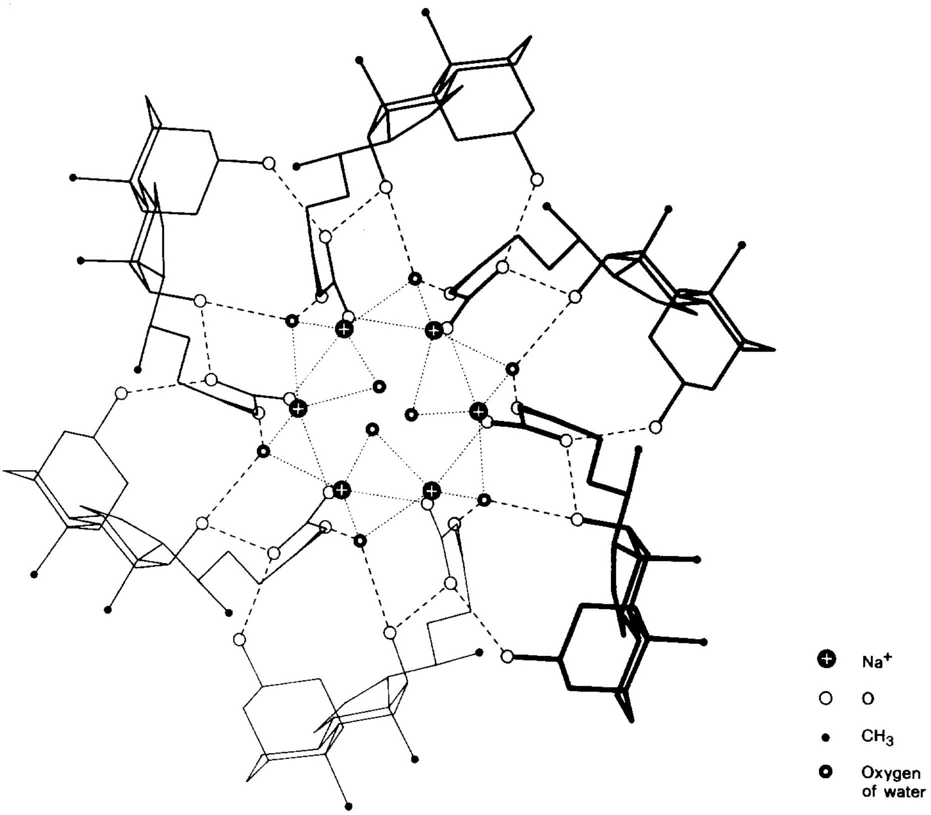

Fig. 5. View of the sodium glycodeoxycholate helix along the sixfold axis. The broken and dotted lines represent hydrogen bonds and ion–dipole or ion–ion interactions, respectively.

outcome is that one of the two enantiomeric conformers of bilirubin interacts, at least preferentially, with the micelles, which, reasonably, must be chiral objects like the helices proposed by us. Thus, NaGDC and NaTDC, and perhaps the other glycine and taurine conjugates in the bile of man, may act chiefly as hydrophobic and hydrophilic 'magnets', respectively.

The second point regards the hydrophobic–hydrophilic properties of bile salts when they form micelles. Since the micelle–solubilizate interactions occur at the micelle–water interface [11, 17] these properties depend on the micellar structure, on the size of the micelle, and, in particular, on the hydrophobic–hydrophilic balance existing in the outer surface of the micelle rather than on the chemical composition of the single molecule, even though, of course, the chemical composition influences the micellar structure. Our results show that the hydrophobicity decreases in the order NaDC > NaGDC > NaTDC, in agreement with the experimental data as, for instance, equilibrium cholesterol–solubilizing capacities [24].

In conclusion, it appears that the joint use of powerful techniques, especially SAXS and EXAFS, has allowed us unambiguously to check the helical model. However, it must be stressed that these techniques are incapable of providing the complicated helical structure in a sufficiently precise picture. For these reasons we

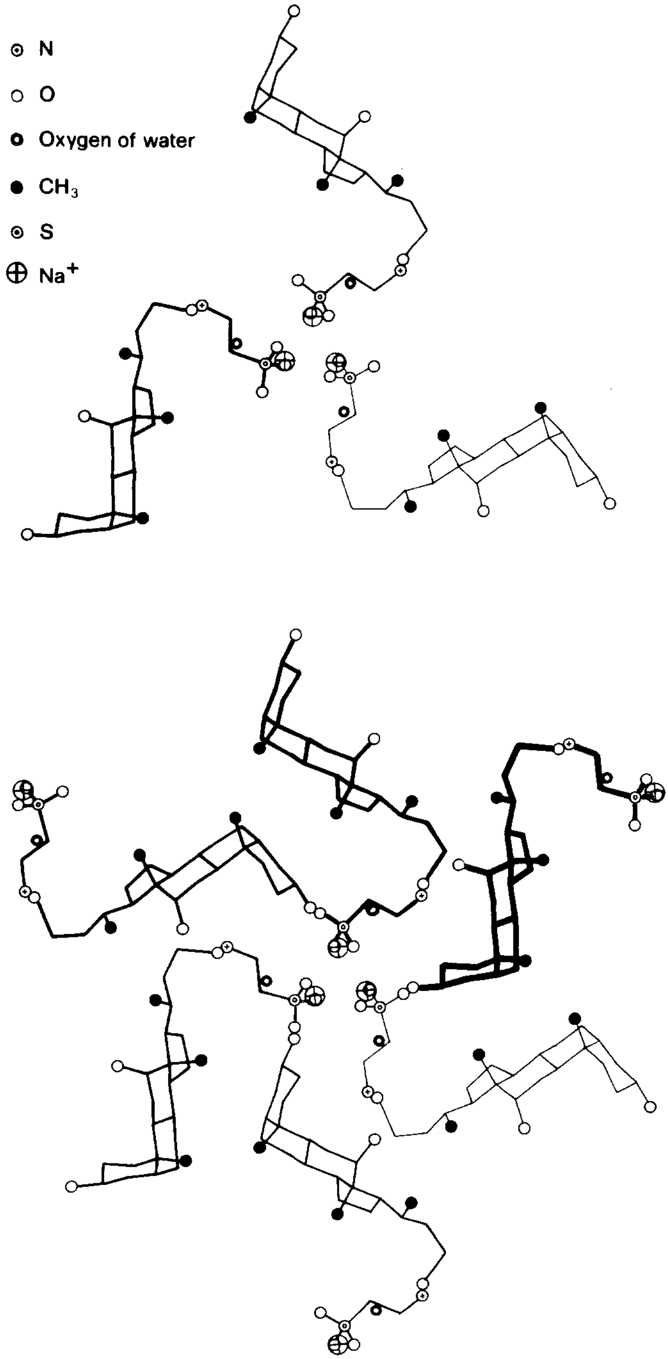

Fig. 6. View along the helical axis of the two possible helices observed in the crystal structure of sodium taurodeoxycholate. The types of interaction reported in Figure 1 are omitted for clarity. The helices at the top and at the bottom are right- and left-handed, respectively. The helix at the bottom is formed by two parallel spirals related by a translation of about 7.1 Å along the helical axis (only one spiral is shown).

are applying to other surfactants the same strategy used for NaDC and RbDC to determine their structures useful in the debate of the micellar models. Work is in progress.

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