Mixed micelles of dioctanoyl-L-alpha-lecithin and hydrocarbon amphiphiles: aspects of fluidization of the micellar interior

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Mixed Micelles of Diocanoyl-L-α-lecithin and Hydrocarbon Amphiphiles. Aspects of Fluidization of the Micellar Interior

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13C NMR measurements of diocanoyl-L-α-lecithin micellar solutions detect an almost complete visibility of the intrinsic magnetic nonequivalence behavior of the two lipid acyl chains. This is an extension of recently published observations. According to the latter, the chemical shift data are interpreted in terms of effectively different lengths of the sn-1 and sn-2 acyl chains due to a bending near the C2 carbon atom of the sn-2 chain. Mixed micellar systems of DOPC and several n-alkyltrimethylammonium bromides show a difference in effective chain lengths of the constituent detergent types. 13C NMR shieldings are observed for n-alkyl detergent fragments which are longer than both acyl chains of the lipid molecules. At an effective chain length difference of seven carbon atoms a sizeable contribution of extra gauche conformers with respect to their single micelles occur for these n-alkyl surfactants. For smaller differences decreasing van der Waals interactions (i.e., decreasing molecular packing) participate almost exclusively leading to chain separation. The deshieldings observed for the n-alkyl segments situated directly between neighboring lecithin chains indicate conformational changes toward more extended forms, as compared with their single micellar solutions, rather than increasing van der Waals interactions. The lipid molecules do not undergo measurable conformational changes upon mixed micelle formation but are only subject to increased molecular packing. This may indicate that conformational changes are of minor importance for solubilizing micelle bound hydrocarbon-like compounds.

Introduction

The importance of micelle-forming phospholipids in biological membranes has often been stated, for example, as carriers for membrane bound enzymes1 2 or trans-membrane transport-enhancing constituents within a bilayer membrane or particles stimulating cell division.3 Conformational and motional behavior of head groups and acyl tails and perhaps also intermolecularly correlated molecular ordering3 may well be of great interest for these important regulations.

Efforts have been made in the elucidation of the conformational structures of micelles of short-chain lecithins by means of different spectroscopic methods.4 By 1H NMR the intrinsic nonequivalence of the sn-1 and sn-2 acyl chains of diocanoyl-L-α-phosphatidylcholine and dipalmitoylphosphatidylcholine is visible.4 In total, four separate α protons were observed; the remaining proton signals either overlapped or were not assigned. The explanation was given in terms of different conformational behaviors of both chains, as suggested earlier by Seelig et al,5 who studied the gel phase and liquid crystalline phase of dipalmitoylphosphatidylethanolamine and dipalmitoylphosphatidylcholine.6 Again, the intrinsic nonequivalent chains resulted in partially resolved spectra. In the present study 13C NMR spectra with considerably better resolution will be presented; for all but two carbons separate signals were observed and assigned to the sn-1 and sn-2 chains. It will be shown that micelles of diocanoyl-L-α-lecithin (DOPC) resemble mixed micelles of n-alkyl detergents bearing chains of nonequivalent lengths. Increasing the effective chain length difference upon elongation of the sn-1 chain causes fluidization near the apolar middle region of the bilayer. Keshou et al7 described this fluidization in terms of intermolecular ordering. Stümpel et al8 supposed intramolecular contributions like disordering conformational changes. However, van der Waals attractive interactions either were ignored8

Acknowledgment. This investigation has been supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

(4) M. F. Roberts, A. A. Bothner-By, and E. A. Dennis, Biochemistry, 17, 935 (1978).
(5) R. A. Burns and M. F. Roberts, Biochemistry, 19, 3100 (1980).
TABLE I: 13C NMR Chemical Shifts of the Micelle Solutions (50 mM) Relative to Me$_3$Si

<table>
<thead>
<tr>
<th>carbon no.</th>
<th>sn-1</th>
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<th>C$_{2}$TAB</th>
<th>C$_{3}$TAB</th>
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<td>29.51</td>
<td>29.00</td>
<td>29.57</td>
<td>29.32</td>
<td>29.62</td>
</tr>
<tr>
<td>5</td>
<td>29.35b</td>
<td>29.41b</td>
<td>28.55</td>
<td>28.55</td>
<td>29.71b</td>
<td>29.74b</td>
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<td>31.41</td>
<td>29.71b</td>
<td>29.84b</td>
<td>29.84b</td>
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<tr>
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<td>22.94</td>
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<td>29.84b</td>
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</tr>
<tr>
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<td>29.84b</td>
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</tr>
<tr>
<td>9</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>10</td>
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<td>30.14</td>
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<td>11</td>
<td>22.84</td>
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<td>30.38</td>
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<tr>
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<tr>
<td>13</td>
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<td>30.22</td>
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</tr>
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<td>23.08</td>
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<tr>
<td>18</td>
<td>14.25</td>
<td>30.22</td>
<td></td>
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</tbody>
</table>

a C$_6$D$_6$ at 128 ppm downfield from Me$_3$Si. b Resonances could not be assigned properly because of little or no differences in $T_1$ values.

or were presumed to be constant.7 In our opinion, the latter have to contribute. On the contrary, the extent to which intermolecular van der Waals attractions on the one hand and intramolecular conformational changes on the other hand cause fluidization should be a function of the effective chain length difference.

Recently, a method has been developed for interpreting 13C NMR spectra of mixed micelles of different alkanoates.9 This was performed in terms of conformational equilibria changes with respect to the single micelles on the one hand and growing interchain distances on the other hand. It allowed us to distinguish these two factors to a certain extent. We also mentioned the possible influence of solvent effects on the spectra. Our previously developed model will be applied to mixed micelles of DOPC and several trimethylammonium bromides. It will describe the connection between van der Waals interactions, conformational changes, and the effective chain length difference (vide supra) with respect to the fluidization process. Also larger interchain distances due to van der Waals interactions9 only are once more considered.

Experimental Section

The n-alkyltrimethylammonium bromides were prepared by the reaction of trimethylamine with the n-alkyl bromides in alcoholic solution according to literature data.10 Dioctanoyl-L-a-lecithin was purchased from Supelco, Inc. A lipid stock solution was prepared by removal of the organic storage solvent under a stream of nitrogen at $-20 \, ^\circ\mathrm{C}$. Mixed micelle solutions were obtained by adding the appropriate amounts of deionized water to the solid ammonium bromide and dried samples of the lipid stock solutions. The resultant solutions were sonicated for 1 min at 25 $\, ^\circ\mathrm{C}$.

All 13C NMR spectra were run at 62.95 MHz on a Bruker WM 250 spectrometer under proton noise decoupling at 45 $\, ^\circ\mathrm{C}$. The deuterium signal from C$_6$D$_6$ was employed as an external lock signal. All chemical shifts are related to Me$_3$Si (C$_6$D$_6$ at 128 ppm downfield from Me$_3$Si). 2000-9000 transients were accumulated of spectral width 2000 Hz in 32K data points limiting the resolution to 0.005 ppm. The pulsewidth was set to a 90° flip angle.

TABLE II: (De-)shieldings upon Mixed Micelle Formation of C$_{1}$TAB and DOPC

<table>
<thead>
<tr>
<th>carbon no.</th>
<th>4:1</th>
<th>2:1</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{1}$TAB</td>
<td>+0.16</td>
<td>-0.11</td>
<td>+0.08</td>
<td>+0.06</td>
<td>+0.03</td>
</tr>
<tr>
<td>C$_{2}$TAB</td>
<td>+0.19</td>
<td>-0.14</td>
<td>+0.10</td>
<td>+0.07</td>
<td>+0.04</td>
</tr>
<tr>
<td>C$_{3}$TAB</td>
<td>+0.26</td>
<td>-0.17</td>
<td>-0.13</td>
<td>+0.08</td>
<td>+0.04</td>
</tr>
<tr>
<td>C$_{4}$TAB</td>
<td>+0.26</td>
<td>-0.17</td>
<td>-0.13</td>
<td>+0.08</td>
<td>+0.04</td>
</tr>
<tr>
<td>C$_{5}$TAB</td>
<td>+0.21</td>
<td>-0.14</td>
<td>+0.10</td>
<td>+0.07</td>
<td>+0.04</td>
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<tr>
<td>C$_{6}$TAB</td>
<td>+0.13</td>
<td>-0.11</td>
<td>+0.05</td>
<td>+0.04</td>
<td>+0.02</td>
</tr>
<tr>
<td>C$_{7}$TAB</td>
<td>+0.10</td>
<td>-0.07</td>
<td>+0.05</td>
<td>+0.04</td>
<td>+0.02</td>
</tr>
</tbody>
</table>

TABLE III: Formation of C$_{1}$TAB and DOPC

<table>
<thead>
<tr>
<th>mixing ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{1}$TAB</td>
</tr>
<tr>
<td>C$_{2}$TAB</td>
</tr>
<tr>
<td>C$_{3}$TAB</td>
</tr>
<tr>
<td>C$_{4}$TAB</td>
</tr>
</tbody>
</table>

- The total detergent concentration is 50 mM. Mixing ratios are defined as the quotient of the concentrations of the DOPC and the ammonium bromide.

Results

13C NMR chemical shifts of the micelles have been assigned by combining literature data and relative relaxation time values, assuming that $T_1$ values increase toward the apolar ends.11 Such a pattern was first suggested by Allerhand et al.11b-e and subsequently used by others.11b-e It is primarily based on increased segmental motions or rotational diffusions near the free ends of the chains. Such...
The signals of the carbon atoms could not be assigned properly.

Formation of C,

mixing ratios

carbon no. 4:1 2:1 1:1 1:2 1:4

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

C,

A pattern is quite general. Results are presented in Table I. Tables II–VI show the chemical shift changes of the mixed micelles with respect to the micelle solutions of the pure detergents.

Assignments of the different carbon pairs to the sn-1 and sn-2 chains have been performed by assuming that the spin–lattice relaxation times of the carbon atoms of the sn-1 chain are larger compared to those of the sn-2 chain.5

Discussion

Micelle Solutions of DOPC. The present measurements yield a better resolution for the dioctanoyl-l-α-phosphatidylcholine (DOPC) micelles (see Table I) with respect to recently published data.3 The visibility of the non-equivalent behavior is not restricted to carbon atoms close to the carbonyl function3 but extends over almost the complete chains.

Comparing the ω-methyl resonances of the lecithin micelles with micellar solutions of the quaternary ammonium detergents shows a decreased chain packing for the

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Former. Since significant water penetration can be ruled out,8 a possible explanation is that the lecithin head groups are much bulkier than those of the n-alkyl detergents and that the micellar shape is rather rodlike (vide infra).

The 13C NMR chemical shifts of the DOPC micelles were insensitive to the concentration of the solution from 5 to 50 mM. This indicates that no changes in molecular packing and concomittant conformational changes occur. Recently, Tausk et al.12 showed that, at the concentrations investigated here, the micelle size is larger than spherical. Apparently, this growth of the DOPC micelles does not influence the 13C NMR spectra significantly, since the line widths are also constant over the entire concentration range.

Furthermore, the results indicate a similarity between the behavior of mixed n-alkanoates of different chain lengths9 and synthetic short-chain lecithins in an aqueous medium presented here: both systems represent a magnetic nonequivalence between the different alkyl chains for comparable carbon atoms.

**Mixed Micelles of DOPC and Several n-Alkylimethylammonium Bromides.** Several aspects have to be considered upon interpreting 13C NMR chemical shifts of DOPC micelles mixed with amphiphiles of different effective chain lengths. Analogous situations in simple mixed micelles of alkanoates have been discussed in detail in a previous paper.9 It was shown that mainly two factors will influence 13C NMR chemical shifts of alkane moieties, viz. different conformational equilibria and/or different environments.8,16 Sometimes, these two factors are interconnected.15 Different environments per se (solvents, packing) will cause different medium effects on the chemical shifts. The relative magnitudes at different positions in a solute are governed by site factors.16 For convenience, relative site factors within an alkane chain are presented in Figure 2e.

So that van der Waals solvent effects on the one hand and conformational changes on the other hand could be distinguished, a schematic representation of Δ's is given (see Figure 2). In terms of 13C NMR chemical shift changes Figure 2a reproduces schematically the influence of contributions of gauche conformers for the alkyl fragments investigated here. Literature data from Mann, Bovey, Schneider, de Haan, and co-workers13,19 were combined in order to arrive at the absolute values of the Δ's upon formation of a single gauche from an anti conformer. With respect to an anti isomer carbon atoms at a relative 1,4 gauche position are shifted upfield by about 4 ppm (except ω-methyl carbons which are shielded by about 5 ppm). Intermediate carbons change by about half this magnitude. Taking into account all possible gauche conformers in different ratios results in the shielding patterns presented in Figure 2b-c. Figure 2d shows the opposite effects due to generating possible anti conformers from gauche conformers. Quite obviously, no clear-cut distinction between the two aspects is possible in all cases. However, combining the literature data of Figure 2 and experimental data of Tables II–VI, we are now able to indicate approximately to what extent conformational changes on the one hand and medium effects on the other hand will contribute to the observed Δ's.

A few consequences of the combined influences of conformational changes and alterations in environment will now be mentioned, in close analogy with mixed alkanoate micelles.9 First, shielding is expected for carbon atoms of those parts of the detergent chains which protrude from the DOPC acyl chains (see Figure 1). The average distances between those alkyl fragments are then larger than in their single micellar solutions. This is the case for the C12TAB up to and including the C16TAB detergent. (The following abbreviations have been used: C4TAB, n-octyltrimethylammonium bromide; C6TAB, n-dodecyltrimethylammonium bromide; C8TAB, n-tetradecyltrimethylammonium bromide; C10TAB, n-hexadecyltrimethylammonium bromide; C12TAB, n-octadecyltrimethylammonium bromide; DOPC, dioctanoyl-L-α-lecithin.) Larger interchain distances cause a diminution of deshielding van der Waals solvent effects. In addition, increased differences in effective chain lengths may possibly lead to conformational changes toward more kinking. This is consistent with the conclusions of Petersen and Chan14 and de Haan and van de Ven,15 who describe intramolecular alterations as a consequence of a decrease of correlated intramolecular motion of the DOPC attractive interactions going from a relatively ordered (single micellar) to a relatively disordered (mixed micellar) state, rather than the reverse. A decrease in intramolecular interactions (i.e., by "chain unpacking") will lead to shielding, as mentioned for neat and diluted alkanes.16 Similar conclusions were reached from 13C studies of polyethylene in different packing states.17 Furthermore, the above-mentioned shielding effects will be enhanced upon raising the concentration of the zwitterionic surfactant.

Second, for the carbons of the part of the n-alkyl detergent chains which are situated directly between DOPC molecules in the mixed micelles, deshieldings are anticipated, caused by increased deshielding van der Waals interactions and/or by an increase in the ratio of anti to gauche isomers. These effects are thus expected to decrease upon raising the concentration of the quaternary detergent.

Consequently, for the lecithin acyl chains incorporated in micelles of longer n-alkyl chain amphiphiles, also de-
shiftings are expected due to increasing solvent effects along with conformational changes toward more extended forms. If only solvent effects participate, deshiftings should correspond to the respective site factors, thus leading to maximal differences for the methyl carbons. These differences have to increase upon lowering the lipid concentration.

Finally, for the alkyl detergents possessing effectively shorter alkyl chains as compared with DOPC, deshielding effects are anticipated (vide supra). Thus, shiftings will occur for the carbons for those parts of the lecithin acyl chains which protrude from the n-alkyl amphiphile chains.

Regarding DOPC/C12TAB mixed micelles, deshiftings are observed for the C11-C13 alkyl chain fragment indicating effects comparable with the DOPC/C14TAB mixed micelle. However, pronounced shielding is obtained for the C13 carbon atom. Since no extra contributions of gauche conformers are feasible (Figure 2 vs. Table III), only decreasing van der Waals solvent effects are responsible. The reason is that the C12TAB surfactant possesses a greater effective length than the DOPC molecules in the mixed micelles. Incorporating C14TAB in DOPC micelles increases the effective chain length of the n-alkyltrimethylammonium detergent. This is reflected by the observed shielding effects for the C11-C14 segment of the C14TAB micelle with respect to its single micelle. It is clear that conformational changes are possible in principle. For folding around the C12-C13 bond one would expect shielding at C11 (see Figure 2a). This, however, is not observed (see Table IV). A decrease in molecular packing is able to cause the experimental Δδ's. Thus alkyl chain separation as compared with the single micelles is accomplished. Furthermore, the pattern of the observed Δδ's of the first eleven carbons resembles that of the C14TAB molecules in their mixed micelles.

The DOPC/C14TAB mixed micelles are observed for the C11-C14 part of the C14TAB amphiphile, thus showing once more an increase of the effective chain length. It is still impossible to take large contributions of gauche isomers into account (see Figure 2 and Table V), due to the discrepancy between the observed and calculated Δδ's (for all anti/gauche ratios). However, comparing observed with calculated Δδ's for the C14-C14 fragment of the C14TAB surfactant in its mixed micellar systems with DOPC indeed indicates pronounced contributions of gauche conformers, around the C14-C17 carbon bond, apart from unpacking effects (cf. the experimental shielding of the C14 methylene carbon with its calculated value based on conformational changes).


**Figure 2.** The influence (in ppm) of single gauche conformers on the 2H chemical shifts of all extended hydrocarbon chain fragments (a). (b) Possible gauche conformers of that part of the quaternary C8TAB detergent chain protruding from the DOPC molecules in their mixed micelles. Full circles at the left side of the chain fragments represent the methyl carbon atoms. The calculated shielding pattern which resembles closest the experimental data of the C12-C14 segment is given. It was obtained by weighing the gauche conformers in the ratio indicated. (c) Similar to b, for C14TAB in mixed micelles with DOPC. Again only values which are closest to the experiments are mentioned. (d) Deshielding pattern of C8TAB in DOPC micelles obtained with opposite values of α, assuming equal almost equal contributions of anti conformers around all bonds. (e) Relative site factors within an alkane chain fragment due to decreasing van der Waals interactions. **10**

In retrospect, the situation described here is similar to that of the mixed micelles of potassium dodecanoate and potassium hexanoate described in a previous paper. For this latter case, we were not in a position to rule out conformational changes of the dodecanoate chains with respect to their single micelles. A definite conclusion could not be reached due to our inability to detect the C8 resonance of the dodecanoate chain properly.

For the phospholipid in its mixed micelles the chemical shift differences of both chains are retained almost independently of chain length and concentration of the n-alkyltrimethylammonium bromide detergents. Conse-
Mixed Micelles of GM1 Ganglioside and a Nonionic Amphiphile

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Department of Biological Chemistry, The Medical School, University of Milano, Milano, Italy (Received: November 13, 1981; In Final Form: February 8, 1982)

Aqueous solutions containing a mixture of two amphiphiles, a nonionic surfactant, n-dodecyl octaoxyethylene glycol monoether (C12E8), and a biological lipid, the ganglioside GM1, are investigated by static and dynamic light scattering. It is found that mixed micelles are formed in the whole range of investigated molar ratios. The theory of light scattering from solutions of homogeneous micelles is generalized to the case of mixed micelles. The final formula is used to derive from the experimental data the aggregation number of the mixed micelle. A simple phenomenological law is proposed to describe the dependence of the aggregation number on the molar ratio between the two amphiphiles.

Introduction

The micelles formed in aqueous solutions of two amphiphiles contain usually both components and are in equilibrium with the monomeric species in the aqueous phase. Several experimental and theoretical investigations have discussed the dependence of the monomer concentrations on the molar ratio between the two components and on the total amphiphile concentration.1–3 In particular, calculations of the mixed critical micelle concentration have been performed and compared with experimental data obtained through surface tension and electrical conductivity measurements.1–3 Little information exists in the literature about the size and the aggregation number of the mixed micelles. We have recently reported experiments on mixed micelles of a biological glycolipid, the ganglioside GM1, and a commercial nonionic surfactant, Triton X-100.4 Such experiments were performed with the aim of establishing a correlation between the structural organization of lipid monomers and the activity of an enzyme which uses the GM1 as a substrate. Other authors had previously studied mixed micelles of phospholipids and Triton X-100 for similar biochemical applications.5,6

We present in this paper a light-scattering investigation of aqueous solutions containing the ganglioside GM1 and a pure nonionic surfactant, n-dodecyl octaoxyethylene glycol monoether (C12E8). From the point of view of a physicochemical study of mixed micelles, such a system is interesting because the molecular weight of the GM1 micelle is about 8 times that of the C12E8 micelle. This allows one to establish in a very direct way by the light-scattering measurement that the two amphiphiles form mixed micelles, as shown later on. Besides the fact that C12E8 is a better characterized component than Triton X-100, there is a further advantage in using C12E8 because the lower consolute temperature is considerably higher for C12E8 than that found for Triton X-100. This means that the temperature range over which the experimental results reflect the properties of the individual micelle instead of cooperative properties associated with the second-order phase transition is much larger for C12E8 and includes, in particular, the room temperature.

Acknowledgment. This investigation has been supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).