Effects of single-stranded n-alkyl amphiphiles on the conformational and dynamic behavior of lecithin sonicated bilayers and micelles studied by 13C NMR: a measure of lipid resistance against disruption of the bilayer orientation

Citation for published version (APA):

DOI:
10.1021/j150665a059

Document status and date:
Published: 01/01/1984

Document Version:
Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

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Effects of Single-Stranded \(n\)-Alkyl Amphiphiles on the Conformational and Dynamic Behavior of Lecithin Sonicated Bilayers and Micelles Studied by \(^{13}\)C NMR. A Measure of Lipid Resistance against Disruption of the Bilayer Orientation

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The triplet fine structures in the \(^{13}\)C NMR spectra of carbons in the \(\alpha\)-position to nitrogen in several \(n\)-alkyltrimethylammonium bromides (TAB's) were first published by Allerhand et al.\(^2\) in 1973 without any specific comment. In 1979 London et al.\(^3\) published \(^{13}\)C\(^{14}\)N NMR spectra of choline head groups of phospholipids. They showed that for sonicated dipalmitoyl-L-\(\alpha\)-phosphatidycholine (DPPC) vesicles in D\(_2\)O the triplet fine structure as such disappears below 60 °C but that \(^{14}\)N decoupling still causes line narrowing, e.g. for the \(-N(CH_3)_3\) signal. The disappearance of the triplet proper was tentatively assigned to a combination of decreasing quadrupolar relaxation times of \(^{14}\)N and increasing \(^{13}\)C\(^{1}\)H dipolar interactions upon lowering the temperature.

Subsequently, Murari et al.\(^4\) stated that the differences in spectral appearances were due exclusively to diminishing \(T_1\) values for the \(^{14}\)N nucleus. Moreover, they drew attention to the large quadrupolar relaxation of the lipid -\(N(CH_3)_3\) mobility around the CH\(_2\)-CH\(_2\) head-group linkage nor a decrease in acyl chain mobility. Moreover, no changes in acyl chain kinking are detected. On the other hand, the incorporated TAB molecules are forced by the lecithin molecules toward severely restricted head-group and tail mobilities. For those combinations of PC and TAB's where the TAB \(^{13}\)C NMR signals are detectable, a change in the conformational equilibrium toward more extension is found. A packing model for the incorporation of TAB's in PC vesicles is presented which probably has a rather general validity. The behavior of mixed micelles of PC's and TAB's, originating from enhancing the latter's concentration, is also described. In these systems, mobilities and chain kinking are increased with respect to the vesicular state.

Introduction

Fine structures on \(^{13}\)C NMR signals of \(-N(CH_3)_3\) groups and of \(\alpha-CH_2\) groups of \(n\)-alkyltrimethylammonium bromides (TAB's) were first published by Allerhand et al.\(^2\) in 1973 without any specific comment. In 1979 London et al.\(^3\) published \(^{13}\)C\(^{14}\)N NMR spectra of choline head groups of phospholipids. They showed that for sonicated dipalmitoyl-L-\(\alpha\)-phosphatidycholine (DPPC) vesicles in D\(_2\)O the triplet fine structure as such disappears below 60 °C but that \(^{14}\)N decoupling still causes line narrowing, e.g. for the \(-N(CH_3)_3\) signal. The disappearance of the triplet proper was tentatively assigned to a combination of decreasing quadrupolar relaxation times of \(^{14}\)N and increasing \(^{13}\)C\(^{1}\)H dipolar interactions upon lowering the temperature.

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Materials and Methods

Dioctanoyl-L-\(\alpha\)-lecithin was purchased from Supelco, Inc. A lipid stock solution was prepared by removal of the organic storage solvent under a stream of nitrogen and by dissolving in chloroform. This stock solution was stored at -20 °C. Mixed micelle solutions were obtained by adding the appropriate amounts of deionized water to the solid ammonium bromides and dried samples of the lipid stock solutions. The resultant solutions were sonicated for 1 min at 25 °C.

The alkyltrimethylammonium bromides were prepared by the reaction of trimethylamine and the \(n\)-alkyl bromides in alcoholic solution according to literature procedures.\(^1\) Dimyristoyl-L-\(\alpha\)-
phosphatidylcholine (DMPC) and dipalmitoyl-L-\(\beta\)-phosphatidylcholine (DPPC) were purchased from Supelco, Inc. and Sigma, respectively. To obtain mixed micelle/bilayer solutions of DMPC and DPPC, dry samples of the lecithin and the \(n\)-alkyl detergents were added to the appropriate amount of deionized water. Solutions were sonicated in NMR tubes within a Branson Model 50-D ultrasonic water bath. The temperature of the solutions was at all times above the main phase transition temperature of the lecithin concerned. In order to achieve this, the temperature of the water bath was kept at 0–15 °C.

Typically, solutions were clear after a period of 10 min. During this interval no thermal degradation or hydrolysis occurred, as monitored by thin-layer chromatography with CHCl3:CH3OH:H2O = 65:25:4 (w/w) as eluents. Multilayer suspensions of DMPC and DPPC were prepared by adding the appropriate amounts of deionized water to dry lecithin and vortexing vigorously above the main phase transition temperature. Pure DMPC and DPPC vesicles were prepared by means of sonication in the above-mentioned water bath between 0 and 15 °C (operation time typically 1 h). Solutions were bufflesh and transparent, and no hydrolysis or thermal degeneration occurred according to thin-layer chromatography. Laser beat spectroscopy showed that these samples were not monodisperse: particle sizes varied between 250 and 1000 Å. The distribution function showed a large fraction of small particles (around 250 Å) and a small fraction of large particles (exceeding 1000 Å). Clearly, the small particles are observed by our NMR measurements.

The total amphiphile concentration was 50 mM for all pure and mixed lipid samples.

All \(^{13}\)C NMR spectra were run at 62.93 MHz on a Bruker WM 250 spectrometer under proton noise decoupling at 50 °C, unless indicated otherwise. The deuterium signal of C\(_d\)\(_z\) was employed as an external lock signal. All chemical shifts are related to Si(CH\(_3\))\(_4\). 10,000–100,000 transients were accumulated in 4K data points zero filled to 32K points before Fourier transformation. Spectral width was 2 kHz. No relaxation delay was employed. Pulsewidth was set to a 90° flip angle. The decoupling circuit was carefully tuned prior to the various experiments in order to operate at levels exceeding 2 W (\(\approx 0.1 \) G).

Results and Discussion

A. Head Groups of Lecithins in Single Vesicles. In the introduction the important aspects of the influences of \(^{14}\)N quadrupolar relaxation and of \(^{13}\)C–H dipolar interactions as well as their dependences on molecular motions were already mentioned. As also the relative influences of both mechanisms on the final \(^{13}\)C NMR line shapes were briefly mentioned. In turn, as already published earlier by London et al.\(^4\) and by Murari et al.\(^4\), the molecular motions in the head groups of neighboring lecithins are strongly influenced by the way in which head groups of neighboring lecithins are forced to interact.

In Figure 1 some results are shown for DOPC micelles. At 45 and 50 °C a somewhat broadened singlet is observed (see Figure 1A). Incorporation of a few percent of C\(_{18}\)TAB at 318 K causes no change (Figure 1A), but at 323 K this small incorporation is sufficient to yield a clear triplet with a slightly narrower and higher central line (see Figure 1B). When the detergent concentration is enhanced to ca. 20%, triplets are observed at 318 K (see Figure 1C). Further concentration rises of the detergent do not make much difference (results not shown).

The above results show that relatively very small changes in intermolecular interactions (packing and lateral expansion) within the micelles are sufficient to "tip the balance". Enough room is obviously created for the head groups to resume sufficiently fast rotations around the central CH\(_2\)–CH\(_2\) bond for the \(^{13}\)C–H dipolar interactions and the \(^{14}\)N relaxation rate to become as small as in monomers. This is, in our view, a rather important and useful result. It allows the study of rather subtle changes in head group–head group interactions by careful observation of the

\(^{14}\)N resonances of DOPC observed by means of a decoupling power of 0.1 G. Mixed-micelle ratios are indicated.

-\(^{14}\)N(CH\(_3\))\(_3\) or \(\alpha\)-CH\(_3\) \(^{13}\)C NMR signals, given that the necessary experimental precautions are taken. In retrospect, the results presented by Murari et al.\(^4\) can be seen as extreme situations in which monomers are compared with (very) tightly packed lecithins and liposomes. A further discussion of this point is deferred to section C.

Comparing the line shapes of the \(-N^+\)Me\(_3\) signals of DOPC (Figure 1A) and of DPPC (Figure 2A) at the same temperature and decoupling level may indicate somewhat larger mobilities of the DMPC head groups in their sonicated bilayer structures. This may well be due to the difference in average surface area per lipid molecule: \(N_{\text{DMPC}} \approx 2850\) in sonicated bilayers (vesicles) and \(N_{\text{DPPC}} \approx 8100\) in rodlike micelles.\(^12\) Any explanation in terms of different monomer contributions fails because DOPC has the higher cmc value. Increased contributions of monomers should lead to a more pronounced triplet (see below). Enhancement of the decoupling power from \(B_1 \approx 0.1\) to 0.6 G causes additional splitting of the DMPC triplet (see Figure 2B). Due care was taken to ensure that the extra decoupling did not result in a mere heating of the sample (the solutions did not contain buffer electrolytes; see Materials and Methods). This was done by careful thermostating of the samples and by running test experiments over a temperature range, keeping the decoupling level constant. The results presented here show unequivocally that, contrary to Murari's interpretations,\(^4\) residual \(^{13}\)C–H dipolar interactions do contribute to the final line widths. This is in line with London's original proposals.\(^3\) As can be seen from Figure 2C,D, DPPC vesicles require even higher decoupling levels to make the triplet structures visible.

Without proper proton noise decoupling levels, one is tempted to conclude that head-group mobilities in DMPC and DPPC vesicles are rather slow, making \(\gamma^2 \approx 25J_{\text{CH}}, T_2 \approx 10^{-12}\) pointing to fast \(^{14}\)N relaxation. Although CH\(_3\)–CH\(_2\) rotations with frequencies of the order of \(10^{10}\)–\(10^{11}\) Hz are clearly influenced by the aggregation, the effect is partially masked by other processes. This is clearly visible upon application of sufficient decoupling power when part of the triplet structure is restored (see Figure

Figure 2. \(-N^+Me_3\) line shapes of DMPC and DPPC sonicated bilayers (50 mM in water) at 323 K and variable magnitudes of proton noise decoupling in 62.9-MHz \(^{13}\)C NMR spectra (DP = decoupling power).

2B. With enough decoupling power, line shapes corresponding approximately with \(\gamma^2 = 100\) can be obtained (cf. Figure 2B,D). This implies a considerably larger value for \(T_1\) of \(^{14}\)N than obtained "at first sight" from Figure 2A,C.

The fact that proton noise decoupling power has such a large influence on the DMPC- and DPPC-\(^{14}\)N(CH\(_3\))\(_2\) line shapes proves that besides fast rotations slower processes of the order of \(10^{-5}-10^{-6}\) s are also affected by vesicle formation. Remember that rotation around C\(_2\)-C\(_3\) of partially oriented lecithin molecules does not produce isotropic motions which would work optimally to completely remove \(^{13}\)C-H dipolar interactions. At this stage, we ascribe the changing slower components to a coupling motion between the head groups and the rest of the lecithin molecules, the motions of which are known to possess components with the relevant frequencies.

B. Head Groups of \(n\)-Alkyltrimethylammonium Bromide Detergents. In order to demonstrate the influence of different molecular packings on \(^{13}\)C NMR line shapes of \(-N^+(CH\(_3\))\(_2\)\) groups, several TAB's were studied in the monomeric and in the micellar states. It should be stressed that whereas in lecithin micelles or vesicles the CH\(_2\)-N\(^+\) vector will be approximately parallel to the bilayer plane, corresponding vectors in the TAB's will be approximately perpendicular to the aggregate surface. A comparison is possible with \(^{13}\)C NMR line shapes of TAB incorporated in mixed micelles or in mixed vesicular systems (see next paragraph).

The \(n\)-octyl detergent is not capable of forming aggregates at the concentration investigated here\(^{(14)}\) and shows well-resolved triplets for the \(-N^+(CH\(_3\))\(_2\)\) group (see Figure 3A). The relatively large \(n\)-octadecyl micelles show broadened singlets under these conditions (see Figure 3E). This behavior is consistent with the picture of a highly mobile CH\(_2\)-N\(^+\) vector at low aggregational densities as for monomeric C\(_18\)TAB. At high aggregation densities a "singlet" resonance is observed, as in C\(_14\)TAB. \(^{13}\)C NMR \(T_1\) values show that when the molecular packing increases, head-group mobilities (time scale \(10^{-10}-10^{-11}\) s) around the CH\(_2\)-CH\(_2\) bond decrease. This behavior is qualitatively the same in TAB's and in lecithins, in spite of the different head-group orientations with respect to the bilayer normal. It should also be noted that in these two "extreme cases" the influence of decoupling power at the levels available to use is negligible. This, in turn, means that in C\(_18\)TAB motions with time scales in the order of \(10^{-5}-10^{-6}\) s are effectively blocked for \(-N^+(CH\(_3\))\(_2\)\) and probably for a number of carbons near the head group as well.

For detergents possessing intermediate chain lengths, a trend in line shapes can be observed (see Figure 3B-D). For these detergents the appearance of triplet structures is correlated with the decoupling level and also with the temperature. The latter point underscores the importance of the quadrupolar mechanism.
as described by Murari et al.\textsuperscript{4} for lecithins; the former points to the additional importance of lower frequency movements of the chains. The most likely candidate for this motion is, in our view, a (segmental) wobbling of the chain which has been described\textsuperscript{15} as occurring at about the frequency range important for the occurrence or disappearance of \textsuperscript{13}C--H dipolar interactions as a line of broadening.

At 50 °C and with 0.8 G one can observe a pattern of head-group resonances. The reason for this might be twofold. First, the cmc decreases from \textce{C}_6\text{TAB} to \textce{C}_10\text{TAB}. As a consequence, monomeric concentrations decrease to almost zero in this series, due to a shift of the equilibrium monomer to aggregate to the right. On the NMR scale, essentially this shift is monitored, and it is expressed in increased contributions of aggregate resonances to the \textce{-N(CH}_3}_3\text{CH}_2\text{N}^+ \text{C}^1\text{NMR line shapes. Second, from geometrical considerations we learn\textsuperscript{16,17} that increasing aggregation sizes from \textce{C}_6\text{TAB} to \textce{C}_10\text{TAB} leads to more parallel head groups in the latter case. Therefore, stronger head-group--head-group interactions occur, resulting in an extra loss of motional freedom.}

\textbf{C. Head Groups of Lecithins and Longer \textce{TAB}'s in Mixed Micelles and Vesicles.} Adding increasing amounts of \textce{TAB} to a PC vesicle leads to disruption and finally results in formation of micelles which have a looser packing than vesicles.\textsuperscript{16} The surprising fact occurs that for the DMPC head groups in all cases—pure DMPC vesicles (see Figure 2B) and in vesicular and micellar mixtures with e.g. \textce{C}_6\text{TAB} (see Figure 4B)—clear triplet resonances were found for the \textce{-N(CH}_3}_3\text{CH}_2\text{N}^+ group. Apparently, head-group mobilities are not affected appreciably by micellization or vesicle formation in these systems.

In DMPC/\textce{C}_10\text{TAB} dispersions the solubilized \textce{n-octyl} surfactants are in very rapid exchange with the extravesicular monomers, because the effective concentration is far below the cmc value.\textsuperscript{14} Thus, an average state is monitored with relatively large intermolecular separations, and hence triplet structures for the \textce{-N(CH}_3}_3\text{CH}_2\text{N}^+ \text{C}^1\text{NMR line shapes. This broadening is partially caused by \textsuperscript{13}C--\textsuperscript{1}H dipolar interactions as is demonstrated by experiments at variable decoupling power and at several temperatures (now shown). This points to hindering of (segmental) motions on a time scale of ca. 10^-6 s (see above). Also responsible, although to a much lesser extent, is a rotation on the lower end of the 10^-10^-11 s time scale, causing faster relaxation of \textsuperscript{13}N and hence some broadening of the directly bonded carbons. Our results substantiate literature data\textsuperscript{14,16} that bilayer ordering is maintained up to the 1:1 mixing ratio. Lysis of the bilayer toward micellar ordering and concomitant lower aggregational densities is accomplished at lower mixing ratios. That is, the \textsuperscript{13}C--\textsuperscript{1}H dipolar interactions of the surrounding lecithin molecules force incorporated lytic compounds to reduced mobilities of the head groups with respect to pure lytic micelles. At the same time this process does not decrease notably the time scale of motions of the lipid CH\textce{2-N}^+ vectors, compared with that of the simple PC vesicles.

This packing situation may be envisaged by the difference in relative orientations of the two types of head groups (see Figure 5). It is conceivable that, in tight-packing situations, the lytic head groups are more or less "squeezed" between neighboring head groups. In the section on hydrophobic tails it will be shown that this squeezing process will also influence the alkyl chains remarkably.

For the \textce{C}_6\text{TAB} mixed dispersions with DMPC a similar change in the dynamic behavior of the \textce{(CH}_3}_3\text{N}^+--site is observed during transition from mixed bilayer to mixed micelle. Lysis of the bilayer does not provide resolved triplets for the detergent like in the case of \textce{C}_10\text{TAB} (see Figure 4). The increase in line width, also at high levels of proton noise decoupling, has to be ascribed to inhibition of the fast (10^-10^-11 s) motions. This is in line with our results for \textce{C}_6\text{TAB} in DMPC (not shown). This explanation is also in agreement with increasing \textsuperscript{13}C T_1 values of especially \textce{C}_1--\textce{C}_5 in TAB's of this length. Still, differences are observed between the head-group methyl \textsuperscript{13}C NMR resonances of \textce{C}_6\text{TAB} or \textce{C}_10\text{TAB} in the simple TAB micelle and in a 1:4 mixed micellar solution with DMPC (see Figures 3 and 4). Obviously, the presence of only small amounts of PC molecules already decreases the head-group mobilities of these particular TAB's. Although not presented here, similar patterns were observed when intercalating this series of lytic compounds in vesicle structures of DPPC, taking into account the different mixing ratios where vesicle structures are broken up into micellar aggregates.

\textbf{D. Hydrophobic Tails.} The \textsuperscript{13}C NMR spectra of the pure vesicles of DMPC and DPPC—which will serve as reference samples for the mixed lipid systems—are presented in Figure 6. As indicated, the line widths are very much smaller than previously reported.\textsuperscript{7} Besides that, it became clear that the main part of this line narrowing was a consequence of eliminated dipolar coupling. Spectra recorded at lower magnitudes of proton noise decoupling indicated this. Thus, experiments performed under elevated power decoupling conditions are a useful improvement in overcoming difficulties in the detection of broad resonances. Comparing the spectra of DMPC and DPPC sonicated bilayers shows no significant differences: line widths and chemical shifts are nearly identical. From this point of view, in combination with the results of the head groups, it is stated that on the \textsuperscript{13}C NMR time scale the DMPC and the DPPC vesicular dispersions are equivalent as far as conformational equilibria and time scales of motions are concerned. This stands in contrast to earlier reports based on vibrational spectroscopy.\textsuperscript{18}

Recently, a description of the phenomena involving fluidization of the hydrophobic core of mixed micellar systems of lipids and lysins (\textce{C}_n\text{TAB}--\textce{C}_10\text{TAB}) has been offered.\textsuperscript{16} \textsuperscript{13}C NMR chemical shift differences of the carbon resonances of the bilayer when compared to their single micelle solutions were treated in terms of an intra- and an intermolecular process: the change of conformational equilibria and the change in chain packing (i.e. van der Waals interactions), respectively. It was shown that those hydrophobic parts of the lysin located between the lipid molecules altered their conformational equilibria toward more extension and were subject to increased chain packing similar to that of the surrounding lipid molecules. However, the latter were shown not to change their conformations upon solubilization of these TAB's. Furthermore, it was discussed that the hydrophobic part of the detergent protruding from the lipid hydrophobic region was subject to conformational changes toward more kinking and/or decreased packing with respect to its single micelles. With these phenomena we are concerned here. In the present work the phenomenon was found to be associated with the effective chain length of the incorporated TAB. The lysis of the (curved) bilayer upon incorporation of TAB's is now discussed in similar terms in conjunction with time scales of motion. The information regarding the latter point was extracted from line widths.

\textit{No \textsuperscript{13}C NMR signals could be detected} for the single-stranded amphiphile upon incorporation of \textce{C}_6\text{TAB} in sonicated DMPC bilayers by using ratios where no lysis occurs. Under the same conditions, however, \textit{no detectable changes} occur for line widths and chemical shifts of the phospholipid chains with respect to its pure vesicle. The latter indicates that no changes occur in the bilayer ordering and dynamics that are directly visible in \textsuperscript{13}C NMR. The undetectability of the detergent resonances, also with enhanced decoupling levels, must point to immobilization of practically all chain movements that occur at time scales below ca. 10^-4 s. This must also include, besides kink diffusion and segmental motions ("wobbling"), rotations of the chain around the long axis. Therefore, \textsuperscript{13}C--\textsuperscript{1}H dipolar interactions remain existent to a level where strong noise decoupling cannot cope with it. (Since vesicular solutions will probably not survive MAS conditions (strong centrifugal forces), an obvious experiment would
Figure 4. (A) $\text{N}^+\text{Me}_3$ line shapes for mixed dispersions of $\text{C}_8\text{TAB}$ and DMPC at 323 K and DP = 8 W ($\zeta_t \approx 0.6$ G). Similar spectra were observed for other mixing ratios. (B) $\text{N}^+\text{Me}_3$ line shapes for mixed dispersions of $\text{C}_{12}\text{TAB}$ and DMPC at 323 K and DP = 8 W ($\zeta_t \approx 0.6$ G). Lipid/detergent mixing ratios are indicated. $J_{C-N}$ couplings were, when visible, 3.0–3.6 Hz. (C) $\text{N}^+\text{Me}_3$ line shapes for mixed dispersions of $\text{C}_{14}\text{TAB}$ and DMPC at 323 K and DP = 8 W ($\zeta_t \approx 0.6$ G). Lipid/detergent mixing ratios are indicated. $J_{C-N}$ couplings were, when visible, 3.0–3.6 Hz.
Figure 5. Relative average orientations of the lecithin and the TAB in their mixed dispersions. The hydrophobic interior is indicated by tubes.

Figure 6. $^1$H spectra of the acyl region of DMPC and DPPC vesicles. Line widths and chemical shifts are indicated. Diluting the samples from 50 to 20 mM did not affect the line widths or chemical shifts. The 40 mM samples are indicated (DP = 8 W (β, ≈ 0.6 G)).

be proton-enhanced (CP) $^1$H NMR on a stationary sample, provided the problem of heat dissipation can be solved. Up to now, only the $^1$H NMR signals of bulky substrates such as proteins and cholesterol were shown to broaden extremely upon incorporation in vesicles. It is demonstrated in the present study that relatively small lysins are immobilized in a similar way.

The apparently unrestricted mobility of the lecithin chains during the process described above means that those chains retain essentially their freedoms of motion. In all probability kink diffusional motions can be carried out partially in the form of large amplitude librations. Interchain distances between sn-1 and sn-2 chains may well, on the average, be larger than those between either of the two and a neighboring single-stranded amphiphile chain. This apparently unrestricted lipid chain motion might be correlated with the effective sn-1 – sn-2 chain length difference in the lipids. It is known that smaller effective differences in chemically modified lipids result in stronger chain packing and in concomitant restrictions. Obviously, when the membrane lipids contain amounts of lysins, these are pressed ("squeezed") between neighboring lipid molecules, the consequence being that head-group mobilities (vide supra), but particularly tail mobilities, are drastically changed.

At elevated quantities of the C$_{14}$TAB (i.e. at mixing ratios lower than 3:2), the resonances of the single-stranded detergent become visible, because the bilayer undergoes lysis and mixed micelles are formed. In addition, the decrease of DMPC acyl chain line widths is another indication for this disruption of the bilayer: line widths are typically 2–3 Hz for mixed micelles (Figure 7). Then, one is able to notice (Figure 8) that the C$_{14}$TAB shows de-shieldings as compared with the case of its single micelle solution. The pattern of deshielding indicates that the lipid molecules force the C$_{14}$TAB toward more extension upon incorporation, analogously to our detailed description regarding mixed aggregates of short-chain lipids and several TAB's. Also, the lipid resonances change position when lysis of the bilayer takes place. The observed shielding (Table I) for the C$_{17}$–C$_{14}$ lipid fragment suggests that increased chain folding and/or decreased chain packing are the result from bilayer disruption. Moreover, axial rotation and/or
Conclusions and Further Developments

It has been demonstrated in this paper that when the proper experimental precautions are taken (no buffer electrolytes and adequate decoupling power without significant heating of the sample), the observation or nonobservation of resolved fine structure of the 13C NMR signal of the -N+(CH3)3 can be used to describe the motional freedom of lipid and detergent head groups in a rather detailed manner. Moreover, the behavior of

Figure 8. Deshieldings (ppm) of C4TAB and C12TAB upon incorporation in DMPC sonicated dispersions at 323 K and DP = 8 W (B1 ~ 0.6 G). Total amphiphile concentration was 50 mM. Molar mixing ratios are indicated.

Figure 9. Deshieldings (ppm) of C4TAB upon incorporation in DPPC sonicated dispersions at 323 K and DP = 8 W (B1 ~ 0.6 G). Total amphiphile concentration was 50 mM. Molar mixing ratios are indicated.

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).

Registy No. DOPC, 19191-91-4; DMPC, 18194-24-6; DPPC, 63-89-8; C4TAB, 2083-68-3; C12TAB, 1119-94-4; C14TAB, 1119-97-7; C16TAB, 57-09-0; C18TAB, 1120-02-1.