Lipid bilayer conformational equilibria and dynamics studied by ¹³C PMAS NMR : influence of hydration and of incorporation of detergents

Citation for published version (APA):

Haan, de, J. W., Weerd, de, R. J. E. M., Ven, van de, L. J. M., Otter, den, F. A. H., & Buck, H. M. (1985). Lipid bilayer conformational equilibria and dynamics studied by ¹³C PMAS NMR : influence of hydration and of incorporation of detergents. *Journal of Physical Chemistry*, 89(25), 5518-5521.
<https://doi.org/10.1021/j100271a042>

DOI:

[10.1021/j100271a042](https://doi.org/10.1021/j100271a042)

Document status and date:

Published: 01/01/1985

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:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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radicals and those formed from FI by $\cdot\text{CO}_2^-$ and e_{aq}^- on the 20–50- μs time scale cannot be fortuitous and leads to the conclusion that equilibrated $\cdot\text{FIH}/\text{FI}^-$ is the product in each case.

While studies of the oxidation of FIH $^-$ are clearly of intrinsic value, the technique used here is relatively complex. Thus in our view reduction of FI by $\cdot\text{CO}_2^-$ remains the preferred method for spectral studies in flavin semiquinones. The spectra thus formed have not been found to exhibit any dependence on formate ion concentration in the range 10–200 mM. This, taken with our present observations in the complete absence of formate, indicates that perturbations of the spectrum by HCO_2^- must be minimal, if not negligible.

As pointed out in Results there is some evidence for a minor perturbation of the $\cdot\text{FI}^-$ spectrum by 0.1 M Br^- at pH 11. It occurs in the region 410–480 nm, the same region where perturbation by RS^- was observed to be strong. At pH 7 the spectral perturbation by RS^- is much weaker, implying a weaker complexa-

tion.¹⁸ The behavior with Br^- seems to parallel that of RS^- , since again at pH 7 there was no real evidence for a long-lived enhancement of the absorbance in the 410–480-nm range. However, it is interesting to note that the large error bars in that region in Figure 4a were required because the ΔA vs. time traces in that range approached steady values more slowly than at all other wavelengths. Detailed examination of this would require further studies on shorter time scales. However, it may be noted that polarization of $\text{Br}_2\cdot^-$ on approach would cause the atom of highest negative charge to be furthest from FIH $^-$. That atom might separate as Br^- , leaving the second neutral Br to accept the electron. Its slower departure might be responsible for the slower spectral changes in the 410–480-nm region.

Registry No. $\text{Br}_2\cdot^-$, 68565-50-4; $\text{N}_3\cdot$, 12596-60-0; RS^- , 68570-60-5; $\text{CO}_2\cdot^-$, 14485-07-5; KBr , 7758-02-3; NaN_3 , 26628-22-8; HCO_2^- , 71-47-6; dihydrolumiflavin, 23542-56-5; lumiflavin, 1088-56-8; monohydrolumiflavin radical, 64135-79-1; dihydrolumiflavin radical anion, 34533-61-4.

Lipid Bilayer Conformational Equilibria and Dynamics Studied by ^{13}C CPMAS NMR. Influence of Hydration and of Incorporation of Detergents

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High-resolution ^{13}C CPMAS NMR spectra of anhydrous and of hydrated dipalmitoylphosphatidylcholine (DPPC) in gel-phase bilayers have been obtained. Judged from ^{13}C NMR chemical shifts one can conclude that no substantial changes in the conformational equilibria of the acyl chains take place upon hydration, in contrast to conclusions drawn earlier from vibrational spectra. Incorporation of *n*-tetradecyltrimethylammonium bromide in the bilayers does not cause conformational changes in the chains. Measurements of relaxation times in the rotating frame, $T_{1\rho}$, both for ^{13}C and ^1H lead to the result that the mobilities on the 10^5 Hz time scale of the lecithin acyl chains and head groups are progressively decreased upon solubilization of more detergents. Opposite trends are found for the detergents. Those results are in agreement with previously published findings for, e.g., cholesterol solubilization in lipid bilayers, provided that one defines a cross-over region in the frequency domain at ca. 10^5 Hz. This view is supported strongly by the results of cross-polarization time (T_{CH}) measurements. The previously postulated squeezing action of phospholipids on solubilized detergents in vesicles is shown to exist also in the gel phase.

Introduction

In the past a large number of studies have been devoted to the changes brought about by addition of water to anhydrous phosphatidylcholines, e.g., dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC). Besides thermochemical methods, many of these studies involved the extensive use of vibrational spectroscopy to monitor conformational changes in the hydrophobic chains and/or differences in intermolecular interactions caused by changing interactions among head groups of neighboring molecules.¹ These changes, in turn, were described as consequences of alterations in dielectric properties in the head group region. Possibly, however, partially obsolete interpretations of IR and/or Raman spectral changes have been used in (at least) a number of cases.² Also, ^{31}P NMR studies were performed on

this subject but these were necessarily concerned with the head groups exclusively.³

Another interesting problem concerning bilayers is related to incorporation of molecules like cholesterol or proteins. The influences of, e.g., cholesterol on bilayers has been the subject of many studies.⁴ It has also been known for some time now that cholesterol, intercalated in vesicular DMPC or DPPC, is partly immobilized, at least to the extent that ^{13}C NMR signals cannot be obtained in high-resolution experiments for all but a few carbon atoms.⁵

In a previous study in these laboratories we showed that this kind of immobilization of solubilized molecules in vesicular DMPC is not confined to molecules such as cholesterol and proteins but can also be found for detergents like *n*-alkyltrimethylammonium bromides where the effective head group area is comparable to that of the lecithins.⁶ It would certainly be of interest to investigate whether this property carries over to unsonicated, lamellar

(1) (a) S. Fowler Bush, R. G. Adams, and I. W. Levin, *Biochemistry*, **19**, 4429 (1980), and references therein. For similar use of vibrational spectra for related purposes, see e.g. (b) B. P. Gaber and W. L. Peticolas, *Biochim. Biophys. Acta*, **465**, 260 (1977). (c) B. P. Gaber, P. Yager, and W. L. Peticolas, *Biophys. J.*, **21**, 161 (1978).

(2) (a) R. G. Snyder, D. G. Cameron, H. L. Casal, D. A. C. Compton, and H. Mantsch, "Proceedings of the 7th International Conference on Raman Spectroscopy", W. F. Murphy, Ed., North-Holland, New York, 1980, pp 622–623. (b) D. A. Pink, T. J. Geen, and D. Chapman, *Biochemistry*, **19**, 349 (1980). (c) R. G. Snyder, D. G. Cameron, H. L. Casal, D. A. C. Compton, and H. H. Mantsch, *Biochim. Biophys. Acta*, **684**, 111 (1982). (d) H. L. Casal, H. H. Mantsch, D. G. Cameron, and R. G. Snyder, *J. Chem. Phys.*, **77**, 2825 (1982).

(3) R. G. Griffin, *J. Am. Chem. Soc.*, **98**, 851 (1976).

(4) (a) E. Oldfield, M. Meadows, D. Rice, and R. Jacobs, *Biochemistry*, **17**, 2727 (1978). (b) P. K. Wolber and B. S. Hudson, *Biochemistry*, **20**, 2800 (1981). (c) M. R. Paddy, F. W. Dahlquist, J. H. Davis, and M. Bloom, *Biochemistry*, **20**, 3152 (1981).

(5) (a) J. R. Brainard and E. H. Cordes, *Biochemistry*, **20**, 4607 (1981). See also (b) R. L. Ong and J. H. Prestegard, *Biochim. Biophys. Acta*, **692**, 252 (1982).

(6) J. W. de Haan, R. J. E. M. de Weerd, L. J. M. van de Ven, and H. M. Buck, *J. Phys. Chem.*, **88**, 5093 (1984).

multibilayers with their much larger radii of curvature and therefore different packing arrangements.

From the point of view of spectral resolution, ^{13}C NMR seems the method of choice over, e.g., ^2H NMR. It suffers, however, from low intrinsic sensitivities as a consequence of the low natural abundance of ^{13}C and from long relaxation times. Moreover, dipolar interactions, especially with directly bonded protons, cause considerable line broadening. The former problems were largely overcome some time ago with the introduction of proton enhancement (PE) or cross polarization (CP) between carbons and protons. The combination with rapid sample spinning around the so-called magic angle also made available the intrinsically high spectral resolution (see above) by averaging the chemical shift anisotropies.

The present contribution presents a ^{13}C CPMAS NMR study of anhydrous DPPC, mono- and dihydrated DPPC (often referred to as "gel" phase although this is questionable), and to multilamellar bilayers in the gel phase with special emphasis on the above-mentioned two aspects of the addition of water to anhydrous lecithins and of the intercalation of detergents in such bilayers. In both cases the attention will be directed mainly to the hydrophobic acyl chains.

Experimental Section

DPPC was purchased from Supelco, Inc. C_{14}TAB was prepared from the corresponding bromide by reaction with trimethylamine in an alcoholic solution. Purities were checked by TLC. Multibilayer dispersions of DPPC were prepared, starting by the evaporation of a chloroform solution of the lecithin to dryness under a stream of argon. Residual traces of organic solvent were removed overnight at 60°C and ca. 5×10^{-6} atm. Each sample was dried independently (no "stock" dry DPPC was used). Anhydrous DPPC was transferred to the rotor under nitrogen. $\text{DPPC} \cdot x\text{H}_2\text{O}$ was prepared by immediate addition of the required amount of deionized water to anhydrous DPPC, after which the sample was transferred to the rotor. The rotor was then spun at ca. 2.5 kHz for ca. 1 h prior to starting the NMR experiments.

The Andrew rotor material was either Delrin or boron nitride. Teflon caps were used which provided efficient sealing of the rotor by radial expansion during rotation. TLC was used to check that no sample deterioration occurred during the NMR experiments. No loss of water could be detected by weighing the filled rotors before and after the NMR experiments. The latter two points were also checked independently by running series of spectra under identical conditions over prolonged periods, e.g. ten spectra in 10 h at 2.5-kHz frequency MAS rotation.

Mixed multibilayer dispersions of DPPC and *n*-tetradecyltrimethylammonium bromide (C_{14}TAB) were prepared similarly by drying chloroform solutions containing the appropriate amounts of both constituents. The amounts of added deionized water were calculated with respect to the total amounts of DPPC and C_{14}TAB on a molar basis.

^{13}C CPMAS NMR spectra were run at 75.476 MHz on a Bruker CXP-300 spectrometer. Chemical shifts were related to Me_4Si by using adamantane as a secondary reference compound. Field drift was less than 0.1 ppm (long term) and less than 0.02 ppm in a period comparable to the time necessary to run a typical experiment. Typically, 4000 to 60000 transients of 1K data points were accumulated and zero-filled to 32K points prior to transformation. The spectral width was 17 kHz. Single contacts were made. The pulse delays varied from 4 s for anhydrous samples to 15 s for hydrated samples. Using shorter pulse delays in the latter case resulted in too much heat generation in the sample, causing phase transition(s) and consequently rotor instability (see below). A B_1 field of ca. 20 G was employed ($\pi/2$ pulse durations of 3 μs). The acquisition times were limited to 29 ms; longer times also caused too much heat development. The contact times were 2–5 ms during the hydration studies, giving reasonably constant overall spectral intensities. During the measurements of $T_{1\rho}$ values the contact times were variable. In practice this also meant an optimization for the observation of methyl signals where the resolution is maximal.

It was checked independently with some low-melting hydrocarbons and with some other compounds as well that rotor instabilities (see above) after a smooth start were invariably caused by melting of the rotor contents. The same behavior could be "provoked" with hydrated DPPC by using shorter pulse delays and/or longer acquisition periods with dipolar decoupling. We therefore conclude that stable rotation, at least in our experimental setup, is only possible with DPPC in the gel, rather than in the liquid crystalline phase. A direct measurement of the sample temperature is not feasible. In view of the above and of the fact that $\text{DMPC} \cdot 2\text{H}_2\text{O}$ could not be measured under these conditions, we estimate that the sample temperature was $33 \pm 5^\circ\text{C}$. Furthermore, it was shown that the use of dipolar decoupling was needed⁷ in order to obtain high-resolution quality spectra, also the signal-to-noise ratio suffered severely when no high level decoupling was used.

It was demonstrated recently by Schaefer and Stejskall that in the liquid crystalline state dipolar decoupling does not bring about improvements in either the resolution or the signal-to-noise ratios, contrary to gel phases.⁷ This can be construed as supporting the statement concerning the aggregational state of the single and mixed DPPC multilamellar systems investigated here.

Discussion

Some time ago ^{13}C CPMAS NMR spectra of DMPC and of DPPC multilamellar bilayers were published by Griffin et al.⁸ Apart from an overall *apparent* shielding which may well have been caused by referencing problems (the ^{13}C NMR chemical shifts in this study are referred to Me_4Si via the secondary standard adamantane), our experimental results are in general agreement with theirs with the possible exception of C_3 of the glyceryl moiety. We find a chemical shift very close to that reported by Griffin et al. for the supposedly liquid crystalline phase of DMPC.

Moreover, we found this same signal also in spectra of DMPC, measured with long pulse delays and thus, in our opinion, in the gel phase. Finally, the use of dipolar decoupling turned out to be mandatory in our experiments, without decoupling very broad "powder-type" lines were obtained.⁷ At present, we are unable to explain this small discrepancy between our results and those of Griffin et al. definitely,⁸ unless the assignments of C_1 and C_3 , as given in the legend of Table I of Griffin, are taken as the correct ones. An alternative explanation is suggested that the DMPC and the DPPC measurements of Griffin et al. have been obtained above and below the respective *pretransition* temperatures.

The effects of adding small amounts of water to anhydrous phosphatidylcholines aroused considerable interest in the past. Pearson and Pascher performed X-ray studies on crystalline hydrates of DMPC.⁹ They stated that the first two water molecules will reside in the head group region exclusively and that one of the main functions is to "shield" the anionic PO_2^- moieties. It should be realized, however, that also the structures of the crystalline hydrates differ with water content. ^{31}P NMR measurements by Griffin et al. are in accordance with rotation of the PO_4^- moiety as a whole upon addition of 1 to 2 mol of water *per* lecithin (5–10% w/w). Neutron diffraction work by Bldt¹⁰ indicated that water does not penetrate beyond the C_3 atom of the glyceryl moiety and electrostatic interactions between head groups diminish accordingly. Some of these results were corroborated recently by Albon et al.¹¹ See, however, also the more recent Raman studies by Levin et al.^{11c}

The most comprehensive description of adding water to amorphous, anhydrous lecithins was given by Levin et al. using

(7) M. D. Sefcik, J. Schaefer, E. O. Stejskall, R. A. McKay, J. F. Ellena, S. W. Dodd, and M. F. Brown, *Biochem. Biophys. Res. Commun.*, **114**, 1048 (1983).

(8) R. A. Haberkorn, J. Herzfeld, and R. G. Griffin, *J. Am. Chem. Soc.*, **100**, 1296 (1978).

(9) R. H. Pearson and T. Pascher, *Nature (London)*, **281**, 499 (1979).

(10) G. Bldt, H. U. Gally, J. Seelig, and G. Zaccari, *J. Mol. Biol.*, **134**, 673 (1979).

(11) (a) N. Albon, *J. Chem. Phys.*, **78**, 4676 (1983). (b) N. Albon, *J. Chem. Phys.*, **79**, 469 (1984). (c) E. Mushayakarara, N. Albon, and I. W. Levin, *Biochim. Biophys. Acta*, **686**, 153 (1982).

TABLE I: Chemical Shifts of Hydrated and Nonhydrated DPPC at 2.6-kHz Sample Spinning^a

	DPPC ^d	DPPC·2H ₂ O ^d
CO	173.1	173.2
	172.2	
CHO ^b	70.8	71.2
CH ₂ N ^c	66.5	66.5
CH ₂ OP ^b	62.7	63.3
CH ₂ O ^b	64.5	64.6
CH ₂ OP ^c	60.4	60.1
N(CH ₃) ₃	54.0	54.5
C-2	35.3	35.3
	34.9	34.8
C-3	27.4	26.9
	27.0	
C-4-C-13	32.7	33.0
C-14	33.9	34.3
	33.5	33.6
C-15	24.3	24.3
	23.7	23.9
C-16	14.2	14.4

^aThe chemical shifts (ppm) are related to adamantane ($\delta = 29.23$ ppm). ^bBackbone resonances. ^cHead group resonances. ^dThe resolved resonances correspond to the *sn*-1 and *sn*-2 acyl chains of the DPPC. The reproducibility of the measurements was 0.5 ppm.

Raman spectroscopy.¹ The first water molecule was claimed to selectively hydrate the more exposed *sn*-2 carbonyl group.¹ Furthermore, comparisons were made with the earlier studies, cited above.

The addition of two, and especially of four, water molecules, however, was in their view sufficient to provoke rotations within the glyceryl backbones with a concomitant growing equivalence of the *sn*-1 and the *sn*-2 chains. This process would go hand in hand with the appearance of more *intrachain* disorder, i.e. the formation of more *gauche* conformations in the alkyl chains: "a dramatic decrease" in the ratios of *trans* and *gauche* markers was found.

Our own studies reveal that, within experimental uncertainties, the *same* ¹³C NMR chemical shifts are found for anhydrous and for hydrated DPPC with the sole exception of the carbonyl signals. With increasing amounts of water (maximum ca. 7%, see Experimental Section) the two signals merge into one single absorption, see Table I. This is additional evidence that the first two molecules of water are preferentially in the vicinity of the carbonyl groups. It underscores also the point of indistinguishability of the two chains at these positions as put forward by Levin et al.¹ No indication of changing head group or glyceryl backbone conformations can be obtained from our results, however.

If we return to the *intrachain* disorder, described by Levin et al.,¹ it is clear that changes of the magnitude claimed by Levin should have resulted in a clear shielding of the hydrocarbon parts of the acyl chains over their entire length. This should still be augmented by the "unpacking" of the chains, necessary to enable the extra kinking. The total estimated shielding is ca. 1 ppm.⁶ For comparable conclusions, see ref 12 and 13. Such an overall shielding is, however, *not* observed, see Table I. Since the study of Levin,¹ the use of IR and Raman criteria has been amended considerably.² It is a definite possibility that, instead of time-averaged situations like conformational equilibria, in fact rates of occurrence and/or time scales of certain motions change upon addition of water to amorphous lecithins. Tentative NMR investigations of the type discussed below for mixed bilayers indicate this. Of course the time scales, and thus also the nature and background of motions, influencing vibrational spectra differ considerably from those inherent in ¹³C NMR.

Upon addition of *n*-tetradecyltrimethylammonium bromide (C₁₄TAB) to DPPC bilayers no significant changes in ¹³C NMR chemical shifts were noted, neither for DPPC nor for C₁₄TAB. Altogether, six different mixed bilayers were investigated varying in C₁₄TAB concentrations from 10 to 80 mol %. In all mixed bilayers as well as in the reference single bilayer systems 2 mol of water *per* mol lecithin and/or detergent were added.

Figure 1. Cross-polarization characteristics of the chain methyls in ¹³C CPMAS NMR of the constituents of the DPPC or C₁₄TAB·2H₂O aggregates. Mixing ratios are indicated. Vertical scales are in arbitrary units: (Δ) DPPC/C₁₄TAB·2H₂O (1:4); (○) DPPC/C₁₄TAB·2H₂O (1:1); (●) DPPC·2H₂O-C₁₄TAB·2H₂O.

Obviously, no appreciable changes in conformational equilibria (and/or packing; see, however, below) take place. Presumably, all three chains are in the all-anti conformation in the single systems and remain so in the mixtures. A peculiar point is the marked deshielding by ca. +2.4 ppm of only C_ω of C₁₄TAB with respect to the *sn*-1 and *sn*-2 C_ω's of DPPC. Since no differences in head group induced average conformations are likely (see above) we surmise that this difference is due to the extra tight packing of the C₁₄TAB molecules, even with respect to DPPC chains as a consequence of the smaller head group area in C₁₄TAB. It is known that ¹³C NMR signals of methyl groups are more susceptible toward changes in packing densities than those of methylene groups.¹² The extra tight packing exists in single C₁₄TAB bilayers as well for C₁₄TAB in the mixed DPPC/C₁₄TAB systems investigated in the present study.

Besides ¹³C NMR chemical shifts, also ¹³C NMR cross-polarization characteristics and ¹³C rotating frame relaxation times (*T*_{1ρ}(¹³C)) were measured in order to help to clarify any motional changes taking place in the mixed systems with respect to the single systems. In spite of the intrinsically high resolution obtained in our CP MAS with dipolar decoupling experiments, we limited the main part of the dynamic study to the methyl regions where the spectral resolution for the head groups as well as for the chain ends is maximal. It has become clear from the recent work of Boroske and Thrahms¹⁴ that, although differences in proton dipolar order between methyl and methylene groups cause a constantly larger *T*_{1ρ}(¹³C) value for the methyl signals, the *changes* in the values are similar upon passing the various transition temperatures. We assume that the same is true for mixing phenomena. Again, mixed DPPC/C₁₄TAB systems with 2 mol of water were used. In these systems, practical problems inherent in the use of high-power decoupling levels can be overcome most easily. The results for the terminal methyl groups of DPPC show clearly that incorporation of 50 mol % C₁₄TAB into DPPC bilayers causes the *T*_{1ρ}(¹³C) value (average for *sn*-1 and *sn*-2) to rise from ca. 30 to

(12) J. W. de Haan, L. J. M. van de Ven, and A. Bučinská, *J. Phys. Chem.*, **86**, 2518 (1982).

(13) D. L. VanderHart, *J. Magn. Reson.*, **44**, 117 (1981).

(14) E. Boroske and L. Thrahms, *Biophys. J.*, **42**, 275 (1983).

TABLE II: $T_{1\rho}(\text{C})$ (in ms) for DPPC-2H₂O and for C₁₄TAB-2H₂O in Single and in 1:1 Mixed Bilayers

		DPPC	C ₁₄ TAB	DPPC/C ₁₄ TAB
N ⁺ (CH ₃) ₃	DPPC	16		24
	C ₁₄ TAB		11	41
(CH ₂) _n	DPPC	8.5		
	C ₁₄ TAB		31	14 ^a
C ω	DPPC	30		43
	C ₁₄ TAB		8	~12

^a In mixed DPPC-2H₂O/C₁₄TAB-2H₂O the (CH₂)_n signals of DPPC and C₁₄TAB overlap.

ca. 43 ms. The corresponding value for C₁₄TAB could not easily be measured but appeared to increase as well (see Table II and Figure 1).

For comparisons with similar reports in the literature one is limited to the studies of Schaefer and Stejskal⁷ and those of Cornell et al.¹⁵ Both studies concern, however, liquid crystalline phases, while in the latter study $T_{1\rho}(\text{H})$ values rather than $T_{1\rho}(\text{C})$ values were reported. If we assume that $T_{1\rho}$ values of ¹H and ¹³C react in the same way to changes in mobilities, both studies point to enhanced lecithin mobilities (smaller $T_{1\rho}$ values) upon incorporation of cholesterol in DMPC and of rhodopsin in DLPC. The two studies disagree on the point of changes in mobilities at higher frequencies monitored with T_1 values. The essentially flat molecular shape of cholesterol requires that the lecithins immediately surrounding cholesterol ("boundary lipids") adapt their conformational equilibria and/or mobilities. This situation differs considerably from that concerning C₁₄TAB. The all-stretched polyunsaturated chain of retinal in rhodopsin resembles C₁₄TAB (or lecithins) more. Furthermore, the modes of incorporation, intercalation or solubilization, might differ as well among cholesterol, rhodopsin, and C₁₄TAB. Since cross-polarization characteristics of lecithins in the gel phase containing either cholesterol or rhodopsin have, as yet, not been reported to our knowledge, all comparisons must be considered tentative.

This having been stated, we nevertheless conclude that upon incorporation of C₁₄TAB into gel-phase DPPC bilayers, the mobilities of the *sn*-1 and *sn*-2 chains on the 10⁻⁵–10⁻⁶ s time scale are diminished with respect to the single DPPC bilayers. It should be stressed that in the present work both $T_{1\rho}(\text{C})$ and $T_{1\rho}(\text{H})$ values of DPPC C ω show the same trend (see Table II and Figure 1).

It thus can be concluded that upon incorporation of C₁₄TAB into DPPC unsonicated bilayers in the gel phase, the changes in mobilities are in the *opposite* direction to those obtained earlier, see above. In view of the discussion above we conclude that this is due to the fact that our measurements have been carried out in the gel phase instead of in the liquid crystalline phase.

Furthermore, the mobilities in the DPPC chains in the low-frequency range, 10–10² Hz, change upon incorporation of C₁₄TAB. From an estimated 0.5 ms for DPPC bilayers the T_{CH} value grows to ca. 3 ms for a 1:1 (molar) mixture of DPPC and C₁₄TAB and eventually to ca. 7 ms for a mixed bilayer containing 20 mol % DPPC. This represents a clear increase in motional freedom for the DPPC chains since the CH₃ groups, by virtue of their fast rotations around the threefold axis, are less susceptible than, e.g., methylene moieties close to the head groups, where the local proton dipolar fields are considerably higher.

In relation with our previous experiments of C_nTAB's solubilized in DMPC vesicles,⁶ it is quite obvious that in unsonicated, multilamellar mixed bilayers of DPPC and C₁₄TAB also "squeezing" interactions are enforced on C₁₄TAB by DPPC. This is evident from the approximate fourfold increase in the $T_{1\rho}(\text{C})$ value of the head group methyls of C₁₄TAB upon solubilization in DPPC. The $T_{1\rho}(\text{C})$ value of C₁₄ increases also, albeit to a smaller extent.

The same differences in packing may also be responsible for the increased mobility of the C₁₄TAB chains in mixed systems with respect to the single aggregates (see $T_{1\rho}(\text{H})$ changes in Figure 1). The opposite was observed in vesicular DMPC/C₁₄TAB systems.⁶

It should be kept in mind that for the head group methyls of C₁₄TAB the local proton dipolar fields will be lower than for the centers of the chains. In contrast to the results obtained for mixed DMPC/C_nTAB vesicles, where it was demonstrated that solubilization of limited amounts of C_nTAB increases the head group mobilities of the lecithins around their CH₂–CH₂ choline bonds, it is clear that in the unsonicated mixed bilayers of DPPC–C₁₄TAB the DPPC head groups are *restricted* in their mobilities by the C₁₄TAB solubilizates. This is due to the fact that the relative positions of the lecithins in the multilamellar phase are, especially in the head group regions, different from those in vesicles where the curvature is much more pronounced.

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).

Registry No. DPPC, 2644-64-6; C₁₄TAB, 1119-97-7.

(15) B. A. Cornell, J. B. Davenport, and F. Separovic, *Biochim. Biophys. Acta*, **689**, 337 (1982).