



Slow motions in bilayers containing anionic phospholipid

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Abstract

Lung surfactant monolayer formation and maintenance requires substantial reorganization of phospholipid assemblies containing up to 10% anionic lipid. Nuclear magnetic resonance was used to study chain order and slow reorientation in bilayer mixtures of dipalmitoylphosphatidylcholine (DPPC) and an anionic phospholipid, dipalmitoylphosphatidylglycerol (DPPG), with one or both lipids chain-perdeuterated and in the presence and absence of calcium in the aqueous medium. Anionic lipid broadens the chain-melting transition in the presence of calcium but has little effect on phase behaviour or chain order in its absence. Chain spectra suggest that any calcium-induced DPPG aggregation in the liquid crystalline phase is short-lived. While bilayers containing DPPG can exist in a metastable hydration state, their stable hydration state is found to be one in which the contribution of slow collective or diffusive bilayer motions to liquid crystal phase quadrupole echo decay is substantially greater than observed for DPPC bilayers. © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

It is common to model biological membranes by self-assembled phospholipid bilayer systems which remain relatively stable after hydration. There are, however, important natural processes which involve a collective transformation from a bilayer to non-bilayer structure. For example, non-bilayer structures are believed to play an important role in membrane fusion or vesiculation events. Non-bilayer phases which may be relevant to cell fusion have been studied extensively [1,2].

Another process which depends on formation of non-bilayer lipid structures is adsorption and maintenance of the surfactant monolayer film at the air–water interface in lungs. The monolayer, which is predominantly dipalmitoylphosphatidylcholine (DPPC), a saturated chain zwitterionic phospholipid, modifies surface tension at the air–water

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interface and reduces the work associated with inflation of the lungs. Cells in the alveolar wall generate lamellar bodies consisting of zwitterionic and anionic phospholipids, other lipids such as cholesterol, and protein. By mass, the composition of pulmonary surfactant includes about 40% DPPC and about 10% protein [3]. The remainder of the surfactant mass is made up of other phospholipids and glycolipids. The aqueous medium into which the lamellar bodies are secreted contains Ca^{2+} ions. As lung volume cycles, the lamellar bodies transform to a more open bilayer structure, known as tubular myelin, which may be a precursor to monolayer formation. The monolayer seems to be refined by the preferential removal, or squeeze-out, of components other than DPPC while lung volume decreases. The physical chemistry and properties of lung surfactant are described in a number of reviews [3–5].

Interactions between protein and lipid components of pulmonary surfactant have been studied extensively in model systems containing subsets of the complete surfactant mixture. In particular, ^2H NMR has been used to study the interactions of the hydrophobic surfactant proteins SP-B and SP-C with bilayers containing DPPC and an anionic lipid having the same acyl chains, dipalmitoylphosphatidylglycerol (DPPG) [6,7]. The hydrophobic proteins have a limited effect on bilayer phase behaviour and acyl chain orientational order of deuterated lipids in these samples but do appear to affect motions with correlation times longer than 10^{-5} s, as indicated by quadrupole echo decay measurements [6,7]. The extent to which such motions are altered by the hydrophobic proteins is, in turn, sensitive to the presence of calcium ions in the aqueous medium [7].

Anionic phospholipid accounts for between 7% and 10% of surfactant mass [3]. The hydrophobic surfactant proteins carry a net positive charge at physiological pH and the presence of anionic lipid in the lamellar body bilayer material presumably influences protein-lipid interaction in this material. However, it is also important, to consider the possibility that the presence of anionic lipid may modify physical properties of the bilayer itself in a way which might be significant for surfactant function. The extensive work previously done on bilayer mixtures of phosphatidylcholine and phosphatidylglycerol lipids having a common acyl chain length makes these ideal model systems in which to address this possibility.

In the absence of calcium, phosphatidylcholine (PC) bilayers and phosphatidylglycerol (PG) bilayers containing saturated acyl chains of the same length have similar chain melting transition temperatures and the chain-melting transitions of their mixtures are sharp [8,9]. Addition of Ca^{2+} to the aqueous medium in which such mixed lipid bilayers are dispersed raises the chain-melting transition temperature [8,9] but the transition remains sharp for PG concentrations below 80 mol%. Observations from ^2H NMR studies on headgroup labeled phospholipids in the presence of Ca^{2+} suggest that any association of a Ca^{2+} ion with a particular PG headgroup in such a mixture must persist for less than the characteristic time of the NMR experiment, 10^{-6} – 10^{-5} s [10,11]. These results have been interpreted as indicating that PC and PG headgroups are miscible in the bilayer surface [8] and that Ca^{2+} ions move freely in a potential well near the negatively charged surface of the PC–PG bilayer [11]. ^2H NMR studies

of liquid crystalline bilayers consisting of 17 mol% DPPG in DPPC, have shown that addition of Ca^{2+} to the aqueous medium containing the mixed bilayer dispersion results in an increase in acyl chain orientational order [12]. The effect of aqueous Ca^{2+} on the phase behaviour and hydration state of bilayers containing only saturated diacyl PG lipids appears to be greater [13–15].

The phase behaviour of PC–PG mixed lipid bilayers and the interaction of Ca^{2+} with the charged bilayer surface of such mixtures have been well characterized. Relatively less attention has been paid to the effect of anionic phospholipid on slow motions of the bilayer surface which might provide insights into how lipid composition affects large scale reorganization of lipid assemblies. In the present study, ^2H NMR is used to examine phase behaviour, acyl chain orientational order and, particularly, quadrupole echo decay in bilayer mixtures of DPPC and DPPG with one or both components chain-perdeuterated. The effect of excess Ca^{2+} in the aqueous phase is also examined.

2. Materials and methods

Chain-perdeuterated dipalmitoylphosphatidylglycerol (DPPG- d_{62}) was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Chain-perdeuterated palmitic anhydride [16] was used in the acylation [17] of glycerophosphocholine (Sigma, St. Louis MO) to synthesize chain-perdeuterated dipalmitoylphosphatidylcholine (DPPC- d_{62}). The product of this synthesis was purified on a 1.5 m Sephadex LH-20 liquid chromatography column (Pharmacia Biotech, Baie d'Urfe, PQ) and eluted with 100% ethanol which was redistilled before use. The product was found to migrate as a single spot using thin-layer chromatography.

Samples were prepared by dissolving each bilayer component lipid in a 3 : 1 mixture of chloroform and redistilled ethanol and then mixing the resulting solutions. Solvents were removed under reduced pressure in a rotary evaporator. Samples were further dried under high vacuum overnight. Samples were hydrated in buffer containing 150 mM NaCl and 130 mM HEPES. For samples containing calcium, the buffer also included 10 mM CaCl_2 . The volume of buffer was always chosen so that the molar ratio of Ca^{2+} to anionic lipid exceeded 2. The buffer pH was adjusted to 7 to ensure DPPG ionization.

Two hydration protocols were employed. A protocol referred to as “stirring” involved scraping the dried liquid mixture from the walls of the flask from which solvent was removed. Buffer was added to the dried material following which the suspension was stirred gently with a glass rod. The sample was then warmed above the chain-melting transition before each series of ^2H NMR experiments. In the other hydration protocol, labeled “soaking”, buffer was added to the flask containing the dried lipid mixture. The flask was then rotated in a hot water bath maintained above the temperature of the chain-melting transition for the mixture. Following hydration, samples were centrifuged to obtain a pellet which was transferred to a 500 μl NMR tube. All samples contained less than 10% lipid by weight with respect to water.

^2H NMR experiments were performed on a locally constructed spectrometer using a 3.5 T superconducting solenoid (Nalorac Cryogenics, Martinez, CA). Spectra were obtained using a quadrupole echo sequence [18] with $\pi/2$ pulses of 3.6 μs . The number of transients averaged for a given experiment varied between 4000 and 7000 depending on the degree to which the sample was deuterated. Free induction decays were obtained with oversampling by a factor of two [19]. Effective digitizer dwell times were 4 μs in the liquid crystalline phase and 2 μs in the gel phase.

The weighted mean splitting of ^2H NMR spectra was obtained by calculating the first spectral moment over half of the spectrum [20] using

$$M_1 = \frac{\int_0^\infty \omega f(\omega) d\omega}{\int_0^\infty f(\omega) d\omega}, \quad (1)$$

where $f(\omega)$ is the spectrum. For chain-perdeuterated lipid in the liquid crystalline phase, M_1 is proportional to the average, over all deuterons, of the orientational order parameter,

$$S_{CD} = \frac{1}{2} \langle 3 \cos^2 \theta_{CD} - 1 \rangle, \quad (2)$$

where θ_{CD} is the instantaneous angle between a given carbon–deuterium bond and the molecular axis or rotational symmetry.

Transverse relaxation was studied by measuring the characteristic time for decay of the quadrupole echo. The quadrupole echo sequence consists of two $\pi/2$ pulses, shifted in phase by 90° and separated by an interval τ . A quadrupole echo is formed at time 2τ following the initial pulse. Motions which modulate the orientation-dependent quadrupole interaction on the timescale of the quadrupole echo experiment interfere with refocusing of the echo. The initial decay of the echo amplitude, $A(2\tau)$ then has the form

$$A(2\tau) = A(0)e^{-2\tau/T_{2e}}, \quad (3)$$

where T_{2e}^{-1} is the decay rate averaged over all deuterons.

3. Results and discussion

Fig. 1 shows ^2H NMR spectra of DPPC- d_{62} , in buffer containing no Ca^{2+} , at a series of temperatures. In the liquid crystalline phase, the spectra are superpositions of axially symmetric Pake doublets. The distribution of doublet splittings reflects the decay of orientational order from a plateau value near the outer bilayer surface to lower values in the more disordered bilayer interior. Below the sharp transition, the broader, less featured spectrum reflects the slower, less axially symmetric reorientations of the gel phase. Similar ^2H NMR spectra were obtained, in the absence of Ca^{2+} , for DPPC- d_{62} , DPPG- d_{62} , and for DPPC- d_{62} /DPPG- d_{62} mixtures containing DPPG- d_{62} concentrations of 30 and 50 mol%. Fig. 2a shows the temperature dependence of the spectral moments (M_1) for a series of DPPG- d_{62} concentrations in the absence of aqueous Ca^{2+} . These

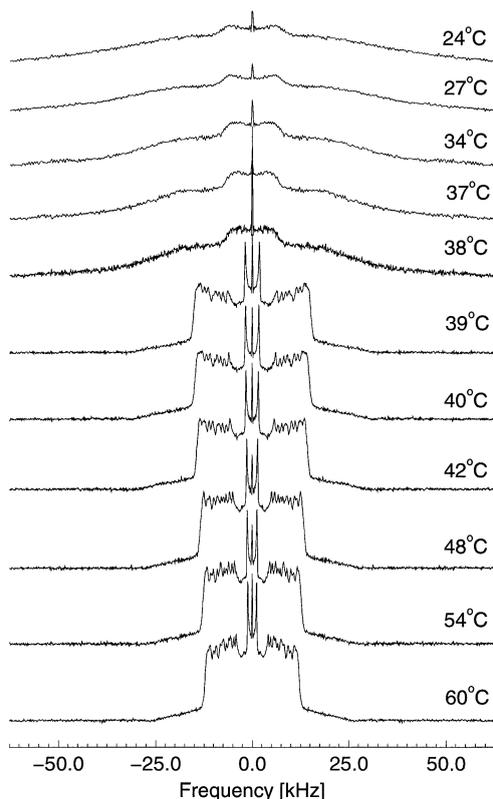


Fig. 1. Temperature dependence of ^2H NMR spectra of DPPC- d_{62} bilayers dispersed in buffer containing no Ca^{2+} .

results show that, for concentrations up to 50 mol%, DPPG- d_{62} has only a small effect on the chain melting transition and acyl chain order in the liquid crystal or gel phases. This observation is consistent with the results of earlier calorimetric studies of PC–PG mixed bilayers [8,9].

If divalent cations are present in the aqueous medium, membrane surface charge has a much greater influence on bilayer properties. ^2H NMR spectra for DPPC- d_{62} bilayers containing DPPG- d_{62} were collected over a wide range of bilayer compositions. Fig. 2b shows the temperature dependence of M_1 corresponding to these spectra. In the presence of excess calcium, the sample with 50 mol% DPPG- d_{62} displays a region of two phase coexistence spanning approximately 8° and centered 9° above the pure DPPC- d_{62} transition. These results are also consistent with calorimetric observations of the effect of bilayer composition on the phase behaviour of PC–PG mixed bilayers in the presence of Ca^{2+} [9]. Comparison of M_1 results for pure DPPC- d_{62} bilayers displayed in Fig. 2a and b shows that the presence of Ca^{2+} has no significant effect on the phase behaviour or acyl chain order of DPPC- d_{62} bilayers in the absence of DPPG.

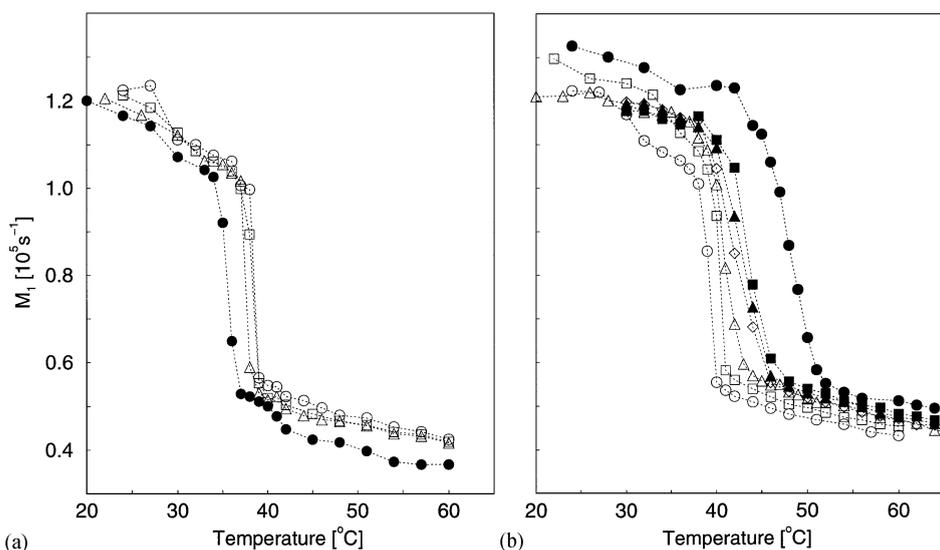


Fig. 2. (a) Temperature dependence of first spectral moments (M_1) for bilayers of DPPC- d_{62} (\circ), 30 mol% DPPG- d_{62} in DPPC- d_{62} (\square), 50 mol% DPPG- d_{62} in DPPC- d_{62} (\triangle), and DPPG- d_{62} (filled circle) dispersed in buffer containing no Ca^{2+} . (b) Temperature dependence of first spectral moments (M_1) for bilayers of DPPC- d_{62} (\circ), 7 mol% DPPG- d_{62} in DPPC- d_{62} (\square), 15 mol% DPPG- d_{62} in DPPC- d_{62} (\triangle), 25 mol% DPPG- d_{62} in DPPC- d_{62} (\diamond), 30 mol% DPPG- d_{62} in DPPC- d_{62} (\blacktriangle), 35 mol% DPPG- d_{62} (\blacksquare), and 50 mol% DPPG- d_{62} in DPPC- d_{62} (\bullet) dispersed in buffer containing excess Ca^{2+} .

The influence of Ca^{2+} on the bilayer is clearly mediated by the presence of the anionic lipid. Previous calorimetric studies and ^2H NMR studies of headgroup labeled samples have shown that PC and PG headgroups remain well-mixed even in the presence of Ca^{2+} [8,11]. A closely related question is whether average chain properties display any sensitivity to headgroup in PC–PG mixed bilayers dispersed in buffer containing Ca^{2+} . Liquid crystal phase spectra of DPPC- d_{62} /DPPG- d_{62} mixed bilayers for a series of DPPG- d_{62} concentrations were “dePaked” in order to examine whether the interaction with calcium resulted in a selective modification of chain order for the two species. DePaking of a powder spectrum yields the spectrum which would be observed if the sample were composed of oriented bilayers. It allows doublets arising from individual deuteron sites to be better distinguished. Fig. 3 shows that as DPPG- d_{62} concentration in the bilayers is increased, there is no emergence of a substantially different set of doublets. This indicates that, over the timescale of the quadrupole echo experiment, the DPPC- d_{62} and DPPG- d_{62} chains sample similar environments and are thus effectively indistinguishable. This environment is influenced by the interaction of DPPG headgroups with divalent calcium ions in the aqueous medium but lateral diffusion within the bilayer distributes the effect over all chains. In particular, these observations confirm that Ca^{2+} does not induce any long-lived (relative to the 10^{-5} s NMR timescale for these experiments) aggregation of DPPG- d_{62} in these mixtures.

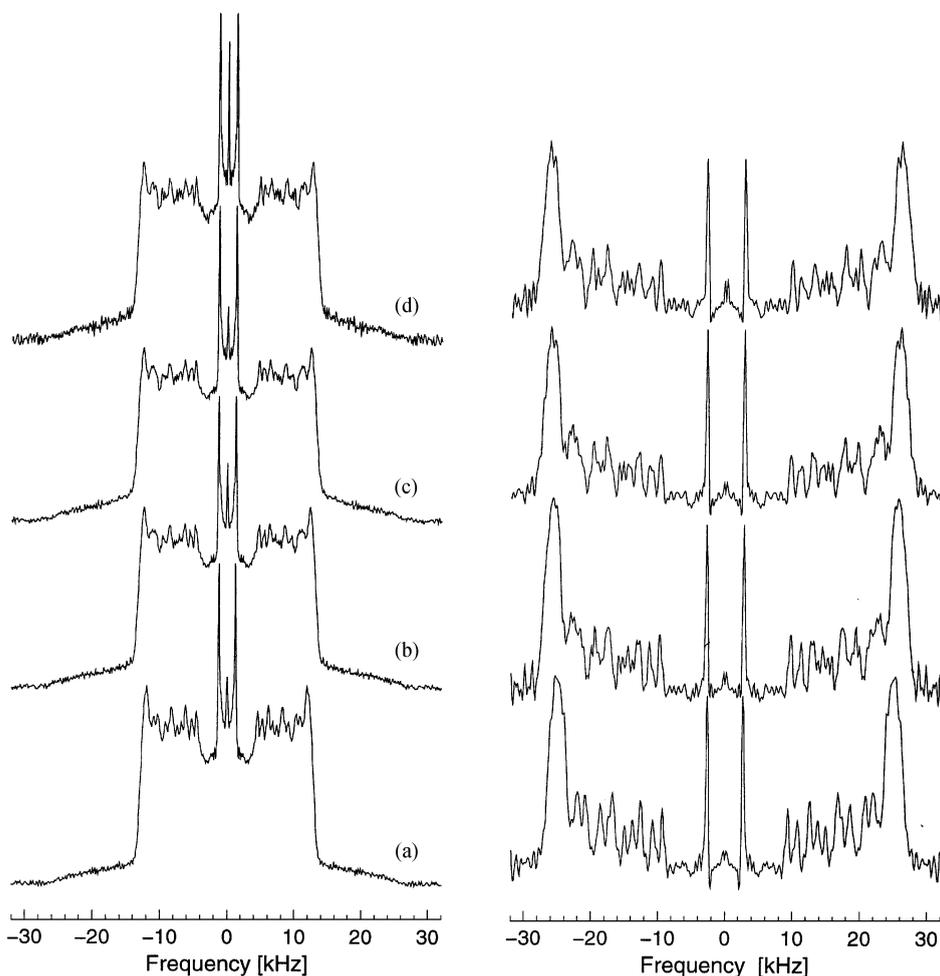


Fig. 3. ^2H NMR powder spectra (left) and corresponding oriented bilayer spectra obtained by dePakeing (right) for (a) 15 mol% DPPG- d_{62} in DPPC- d_{62} , (b) 25 mol% DPPG- d_{62} in DPPC- d_{62} , (c) 30 mol% DPPG- d_{62} in DPPC- d_{62} , and (d) 35 mol% DPPG- d_{62} in DPPC- d_{62} dispersed in buffer containing excess Ca^{2+} .

Motions which modulate the orientation dependent quadrupole echo on the timescale of the NMR experiment give rise to a decay of the echo amplitude with a characteristic time, T_{2e} . In the liquid crystalline phase, motions which contribute to quadrupole echo decay include collective modes of the bilayer surface, molecular reorientation resulting from diffusion of molecules around curved bilayer surfaces, and local reorientation (libration) of molecules [21–24]. The longest range motions may be slow enough to influence echo decay without having an effect on average orientational order as reflected by deuteron quadrupole splittings. The existence and significance of such motions, which are labeled adiabatic, were pointed out by Bloom and Sternin [21]. In the gel phase the adiabatic motions are largely frozen out but internal molecular

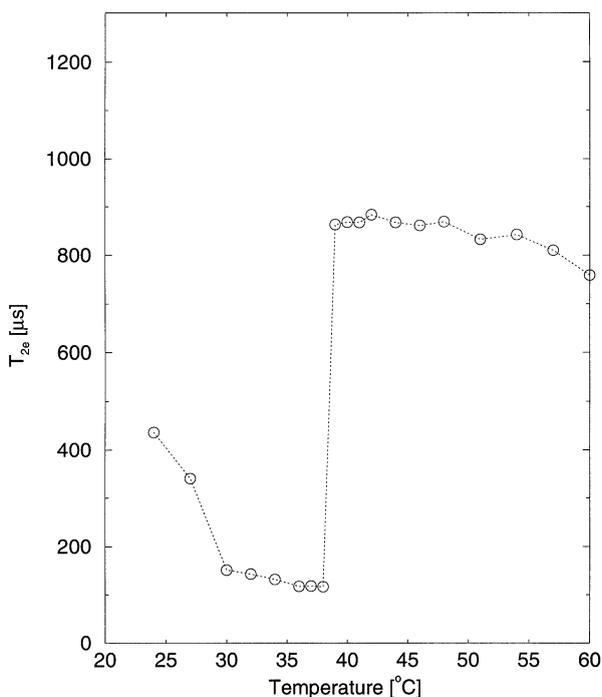


Fig. 4. Temperature dependence of the quadrupole echo decay time (T_{2e}) for DPPC- d_{62} bilayers dispersed in buffer containing no Ca^{2+} .

motions, such as trans-gauche chain isomerizations slow into the regime where they are effective contributors to echo decay. The result is a sharp drop in echo decay time at the liquid crystal to gel transition as shown in Fig. 4 for DPPC- d_{62} . As the temperature is lowered further in the gel phase, the more localized motions freeze out, thus becoming less effective contributors to echo decay, and T_{2e} increases.

A variety of motions may contribute to decay of the echo from a given deuteron. A given motion, labeled i , is characterized by a correlation time, τ_{ci} , and by the second moment, ΔM_{2i} of that portion of the quadrupole Hamiltonian modulated by the motion. An approximate expression for the amplitude of the quadrupole echo or subsequent echoes in a multiple pulse experiment, in which the quadrupole interaction is modulated by a particular motion, has been discussed by a number of authors [21,25–27]. Generalizing to the case of a carbon-deuterium bond subject to a superposition of motions gives, for the echo amplitude at 2τ ,

$$A(2\tau) = A(0) \exp \left[-2\tau \sum_{i=1}^N \Delta M_{2i} \tau_{ci} \left[1 - \frac{\tau_{ci}}{\tau} \tanh \left(\frac{\tau}{\tau_{ci}} \right) \right] \right], \quad (4)$$

where τ is the pulse separation in the quadrupole echo pulse sequence. Motions which satisfy $\tau_{ci} \ll (\Delta M_{2i})^{-1}$ contribute to motional narrowing. For such a motion, ΔM_{2i} corresponds to the reduction in spectral second moment resulting from the motion. For

an adiabatic motion, which causes no motional narrowing, ΔM_{2i} corresponds to the residual second moment not removed by motional narrowing. For fast motions which satisfy $\tau_{ci} \ll \tau$, the contribution to the echo decay rate is $\Delta M_{2i} \tau_{ci}$. For slow motions which satisfy $\tau \ll \tau_{ci}$, the contribution to the echo decay rate is proportional to τ_{ci}^{-1} . For a motion having a given ΔM_{2i} , the contribution to the echo decay rate will pass through a maximum as the correlation time for that motion increases.

The temperature dependence of the DPPC- d_{62} quadrupole echo decay time displayed in Fig. 4 is not very sensitive to details of sample preparation. Just above the transition, T_{2e} is typically found to be close to or greater than 800 μs for a variety of hydration protocols and sample histories. Bilayers containing DPPG, however, display a more complex dependence of transverse relaxation behaviour on sample history. In particular, T_{2e} values differing by as much as a factor of two have been observed for deuterium-labeled DPPC/DPPG mixtures prepared in different ways. In order to investigate this behaviour systematically, and to examine the implications of this behaviour for the way in which bilayers containing anionic lipids self-assemble, we have studied quadrupole echo decay in DPPC- d_{62} /DPPG- d_{62} bilayers prepared with two different hydration protocols as described above. In both protocols, the lipid components were mixed in solution and the solvent removed under reduced pressure in a rotary evaporator. For the “stirring” protocol, the dried lipid film was scraped from the flask and hydrated by addition of buffer followed by gentle stirring with a fine glass rod. The sample hydrated in this way was allowed to equilibrate above the bilayer chain-melting transition temperature for at least an hour before experiments were begun. For the “soaking” protocol, the samples were hydrated by adding buffer to the flask and rotating it for about an hour while holding the temperature above the chain-melting transition of the lipid mixture. Initial comparisons of the effect of hydration protocol on quadrupole echo decay were carried out in buffer containing an excess of Ca^{2+} ions.

Fig. 5 which shows the temperature dependence of T_{2e} for DPPC- d_{62} bilayers containing 30 mol% DPPG- d_{62} illustrates the effect of hydration protocol on motions responsible for transverse relaxation. In the gel phase in which the motions responsible for transverse relaxation are primarily internal or local modes, the result is insensitive to the details of the hydration protocol. In the liquid crystalline phase, on the other hand, transverse relaxation is sensitive to long range collective or diffusive motions and transverse relaxation is extremely sensitive to the hydration protocol. The temperature dependence of M_1 for these two samples is indistinguishable which suggests that the motions being affected by the hydration protocol are not those which are responsible for averaging the quadrupole interaction on the time scale of the NMR experiment. Hydration protocol apparently alters the mixed lipid bilayer state in a way which substantially affects very slow motions while having almost no effect on the local bilayer organization which influences acyl chain orientational order in both phases and quadrupole echo decay in the gel phase.

Mixed bilayer sample states obtained by the “stirring” protocol, corresponding to a longer quadrupole echo decay time in the liquid crystalline phase, were ultimately found to be metastable. By cycling the sample through the liquid crystal to gel transition, it

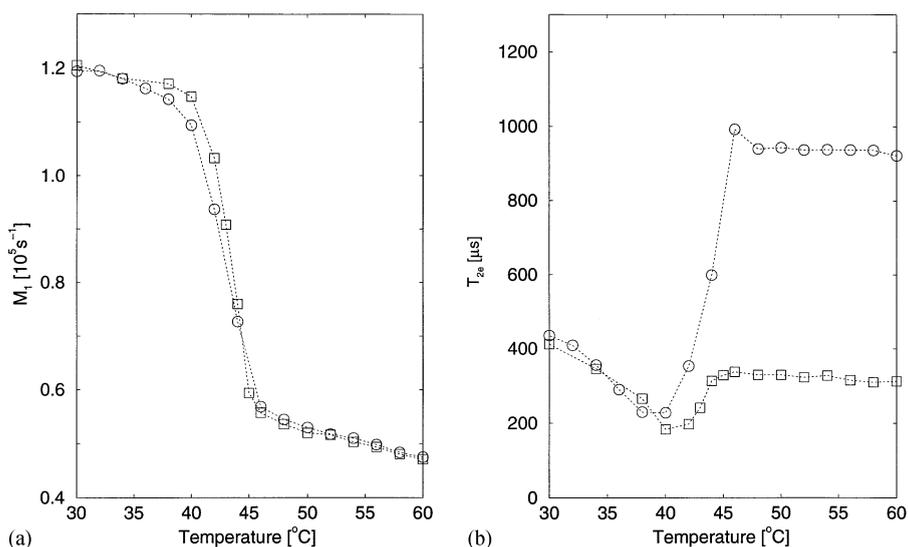


Fig. 5. (a) Temperature dependence of first spectral moments (M_1) for bilayers of 30 mol% DPPG- d_{62} in DPPC- d_{62} hydrated by stirring the dry lipid mixture in buffer (\circ) and by adding buffer to the flask containing the dried lipid mixture and rotating the flask in a water bath held above the chain-melting transition temperature for the mixture (\square). In both cases, the buffer contains excess Ca^{2+} . (b) Temperature dependence of the quadrupole echo decay time (T_{2e}) for bilayers of 30 mol% DPPG- d_{62} in DPPC- d_{62} hydrated by stirring the dry lipid mixture in buffer (\circ) and by adding buffer to the flask containing the dried lipid mixture and rotating the flask in a water bath held above the chain-melting transition temperature for the mixture (\square). In both cases, the buffer contains excess Ca^{2+} .

was possible to irreversibly transform a sample with a longer quadrupole echo decay time, obtained by “stirring”, into a state equivalent to that obtained by soaking. Fig. 6 shows the evolution of the quadrupole echo decay time in the liquid crystalline phase as a 50 mol% DPPG- d_{62} sample, prepared by “stirring”, was cycled repeatedly through the liquid crystal to gel transition. For comparison, the temperature dependence of a sample with the same composition but prepared by “soaking” is also shown. The quadrupole echo decay times in the liquid crystalline phase are comparable after the fourth cycle. Cycling of the sample through the transition was found to have little effect on mean acyl chain orientational order.

The potential for a sample to display the short quadrupole echo decay time in the liquid crystalline phase was found to reflect the presence of DPPG and was not found to be directly related to the presence or absence of Ca^{2+} in the aqueous medium. Fig. 7a shows that hydration of samples containing 30 mol% DPPG- d_{62} in DPPC- d_{62} by “soaking” resulted in short liquid crystal phase quadrupole echo decay times regardless of whether or not the buffer contained excess Ca^{2+} . Fig. 7b and c show that for samples containing little or no DPPG, both hydration protocols resulted in a state displaying longer quadrupole echo decay times in the liquid crystalline phase. Cycling of these samples through the liquid crystal to gel transition was not found

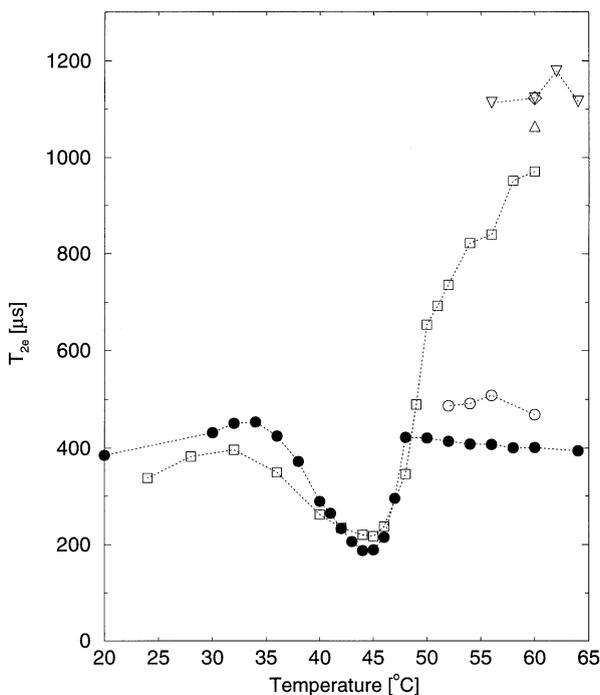


Fig. 6. Quadrupole echo decay time (T_{2e}) for 50 mol% DPPG- d_{62} in DPPC- d_{62} for a sample hydrated by soaking in buffer above the main transition temperature (filled circle) and for a sample hydrated by stirring the dried lipid mixture during the first (∇), second (\diamond), third (\triangle), fourth (\square) and fifth (\circ) cycles through the liquid crystal to gel transition.

to result in significant evolution of the sample toward a state having a substantially shorter quadrupole echo decay time.

These observations seem to indicate that the presence of anionic lipid in the bilayer has consequences for organization of the bilayer over the length scales sampled by collective or diffusive motions. Samples having the same bilayer composition but prepared so as to display different quadrupole echo decay times in the liquid crystalline phase display effectively identical chain deuteron quadrupole splittings. This suggests that the enhanced quadrupole echo decay rate observed for samples hydrated by “soaking” or by “stirring” and then cycling through the transition is associated with the adiabatic motions such as diffusion around a curved bilayer surface or collective bilayer modes. Such motions will fall on the long correlation time side of the T_{2e} minimum so that an enhancement of quadrupole echo decay presumably reflects either a reduction in correlation time for the motion or an increase in ΔM_2 for that motion. Enhanced lateral diffusion might also affect quadrupole echo decay in the liquid crystalline phase but this might be difficult to reconcile with the observation that whatever is enhancing the echo decay does not appear to affect local chain motions significantly. Most other possibilities involve enhanced bilayer curvature in some form or other. A reduction

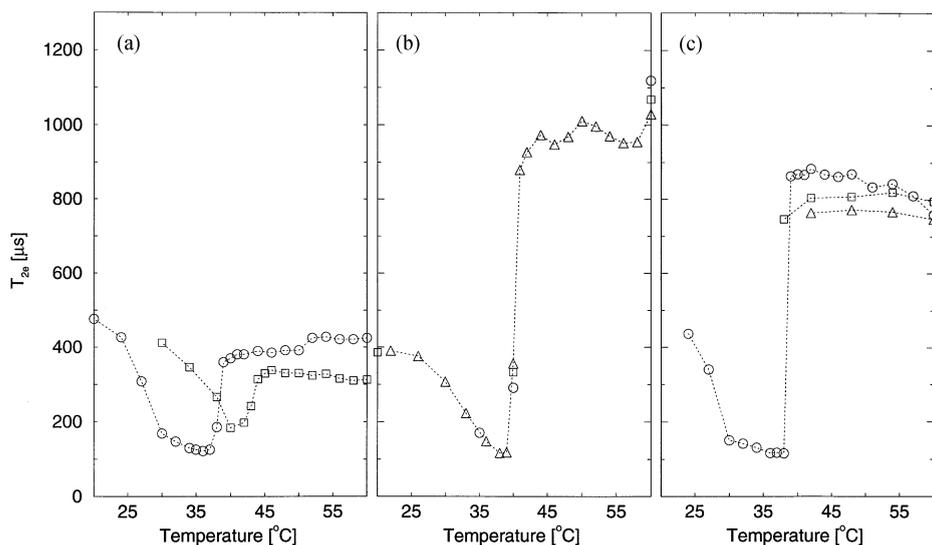


Fig. 7. (a) Temperature dependence of the quadrupole echo decay time (T_{2e}) for 30 mol% DPPG- d_{62} hydrated by “soaking” above the main transition temperature in buffer containing excess Ca^{2+} (\square) and no Ca^{2+} (\circ). (b) Temperature dependence of T_{2e} for 7 mol% DPPG- d_{62} in DPPC- d_{62} hydrated “soaking” above the transition temperature in buffer containing excess Ca^{2+} during the first (\circ), second (\square) and third (\triangle) cycles through the liquid crystal to gel transition. (c) Temperature dependence of T_{2e} for DPPC- d_{62} in the absence of Ca^{2+} obtained during the first (\circ), second (\square) and third (\triangle) cycles through the liquid crystal to gel transition.

in mean vesicle size would increase the quadrupole echo decay rate but it might be hard to reconcile this explanation with observation of two effectively distinct modes of behaviour rather than a continuum. Another possibility is enhanced local bilayer curvature or intensity of long wavelength bilayer modes. This would imply an effect on mechanical properties of the bilayer within the multilamellar environment of the vesicle. Given that tubular myelin, a highly convoluted bilayer structure, is thought to be a precursor to monolayer formation in lung surfactant, the possibility that anionic lipid might promote increased bilayer curvature in some way is interesting.

4. Conclusions

The observations described here suggest that in bilayer mixtures of the zwitterionic phospholipid, DPPC, and the anionic phospholipid, DPPG, the motions of the acyl chains which are responsible for averaging of the quadrupole interaction on the time scale of the ^2H NMR experiment are not headgroup-specific. In DPPC- d_{62} bilayers, the adiabatic motions which contribute to chain deuteron quadrupole echo decay in the liquid crystalline phase are not very sensitive to the details of the hydration protocol or to cycling of the bilayer through the chain-melting transition. Bilayers containing 30 mol% DPPG- d_{62} or more can be prepared such that they reflect adiabatic motions like those

observed in DPPC- d_{62} but this state is not stable and cycling through the chain-melting transition progressively transforms the sample to a state in which adiabatic motions appear to be much more effective contributors to quadrupole echo decay. Such a state is also obtained immediately on slow hydration of the sample in buffer above the chain-melting transition temperature. Neither chain deuteron quadrupole splittings nor gel phase quadrupole echo decay appear to be sensitive to the difference between the two DPPG/DPPC bilayer states.

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