Pulmonary Surfactant Protein SP-B Interacts Similarly with Dipalmitoylphosphatidylglycerol and Dipalmitoylphosphatidylcholine in Phosphatidylcholine/Phosphatidylglycerol Mixtures†

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ABSTRACT: Porcine pulmonary surfactant-associated protein SP-B was incorporated into bilayers of chain-perdeuterated dipalmitoylphosphatidylglycerol (DPPG-d_{62}) and into bilayers containing 70 mol % dipalmitoylphosphatidylcholine (DPPC) and 30 mol % DPPG-d_{62} or 70 mol % chain-perdeuterated DPPC (DPPC-d_{62}) and 30 mol % DPPG. The effect of SP-B on the phase behavior, lipid chain order, and dynamics in these bilayers was examined using deuterium nuclear magnetic resonance (^{2}H-NMR). In both DPPG-d_{62} and the mixed lipid system, SP-B is found to have little effect on chain order in the liquid crystalline phase. With 11% (w/w) SP-B present, both bilayer systems display a continuous change from liquid crystal to gel with no evidence of two-phase coexistence near the transition. Despite its limited effect on chain order in these bilayers, SP-B is found to strongly perturb chain deuteron transverse relaxation in the liquid crystal and gel phases of DPPG-d_{62} and the DPPC/DPPG (7:3) mixtures. The observation that SP-B associates with the bilayer in a way which substantially alters the slow motions responsible for transverse relaxation without significantly affecting chain order in either the liquid crystal or gel phases may place some constraints on possible models for that association.

Pulmonary surfactant is a mixture of lipid and protein synthesized and secreted by type II pneumocytes in the alveoli. Approximately 90% of surfactant mass is lipid, of which about 85% is phospholipid. Anionic lipids such as phosphatidylglycerol and phosphatidylinositol constitute 10−15% of the surfactant phospholipid. Saturated phosphatidylcholine, the major portion of which is dipalmitoylphosphatidylcholine (DPPC),[1] constitutes more than 40% of the total surfactant mass (Kahn et al., 1995). The composition, properties, and physical chemistry of pulmonary surfactant have been described in a number of recent reviews (Weaver & Whitsett, 1991; Keough, 1992; Johansson et al., 1994)

Pulmonary surfactant is generally thought to reduce the work associated with breathing by forming a DPPC-enriched film which modifies the surface properties of the air−water interface in the lung. Enrichment of the film in DPPC may result from a process of selective exclusion of other surfactant components. SP-B and SP-C are hydrophobic surfactant proteins which appear to facilitate the rapid spreading of surfactant material from lamellar structures into the monolayer (Oosterlaken-Dijksterhuis et al., 1991a,b; Pèrez-Gil et al., 1992, 1994). In order to gain a clearer understanding of how the composition of pulmonary surfactant contributes to an enhanced monolayer-spreading rate, it is important to learn how the components, particularly the hydrophobic proteins, interact with lamellar bilayer structures from which the surfactant monolayer, or the possible precusor structure, tubular myelin, may be derived.

SP-B is a cysteine-linked homodimer of a 79-amino acid residue monomer which is suggested to contain one or more regions of amphipathic α-helix. Recent work has shown that synthetic SP-B does not substantially affect chain order in bilayers of dipalmitoylphosphatidylcholine (Morrow et al., 1993). Both SP-B and SP-C have excess positive charge at physiological pH. In order to examine the extent to which the interaction of SP-B with bilayer lipid is sensitive to lipid charge, we have carried out ^{2}H-NMR and DSC studies of the effect of porcine SP-B on the phase behavior, chain order, and dynamics in bilayers of chain-perdeuterated diplamitoylphosphatidylglycerol (DPPG-d_{62}) and mixed bilayers consisting of either 30 mol % DPPG-d_{62} and 70 mol % dipalmitoylphosphatidylcholine (DPPC) or 30 mol % DPPG and 70 mol % DPPC-d_{62}.

MATERIALS AND METHODS

Chain-perdeuterated dipalmitoylphosphatidylglycerol (DPPG-d_{62}) and dipalmitoylphosphatidylcholine (DPPC-d_{62}) were purchased from Avanti Polar Lipids (Pelham, AL). Unlabeled dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma Chemical Co. (St. Louis, MO). The lipids ran as single spots on thin layer chromatography and were used without further purification.

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, chain-perdeuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt); DPPG-d_{62}, chain-perdeuterated 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt); SP-B, pulmonary surfactant-associated protein (M_r = 17 400); SP-C, pulmonary surfactant-associated protein (M_r = 4186); ^{2}H-NMR, deuterium nuclear magnetic resonance; DSC, differential scanning calorimetry.

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Pulmonary surfactant protein SP-B was obtained from lipid extracts of porcine lung lavage as described previously (Taneva & Keough, 1994). Isolation and purification of the surfactant proteins SP-B and SP-C from the lipid extract were performed by gel exclusion chromatography on Sephadex LH-60 (Pharmacia, Uppsala, Sweden) in 1:1 (v/v) chloroform/methanol containing 0.1 M HCl (2%). SDS−polyacrylamide gel electrophoresis (16% gels) (Laemmli, 1970; Taneva & Keough, 1994) of the SP-B under nonreducing conditions yielded a band at about 18 kDa.

Lipids were weighed and dissolved in $CHCl_3$/MeOH (1:1) to give a concentration of 2 mg/mL. Three lipid compositions were used in the samples. These were DPPG-d_{62}, DPPC/DPPG-d_{62} (7:3 mole:mole), and DPPC-d_{62}/DPPG (7:3 mole:mole). The protein concentration in the column eluent was determined by fluorescamine assay (Udenfriend et al., 1972). Samples containing SP-B were prepared with a lipid:protein ratio of 9:1 (w/w). Solvents were removed by rotary evaporation under N_2 followed by overnight evacuation. Samples were suspended by adding buffer (135 mM NaCl and 15 mM Hepes at pH 7.0) to the flask containing the dried sample film and then rotating the flask in a water bath at 45−50 °C for about 1 h. Films containing the protein were scraped from the walls of the flask to ensure complete suspension in the buffer. The amount of buffer added was chosen to yield a suspension of about 2 mg/mL which was then centrifuged at 27000*g* for 10 min. Most of the supernatent was removed. The resulting pellet was scraped into an 8 mm NMR tube with a volume of about 400 μL.

^{2}H-NMR measurements at 23.215 MHz were carried out in a superconducting solenoid (Nalorac, Martinez, CA) using the quadrupole-echo pulse sequence (Davis et al., 1976) with $\pi/2$ pulses with lengths of between 2.4 and 2.7 μs. For spectra from which first spectral moments (Davis, 1983) were obtained, the two $\pi/2$ pulses of the quadrupole-echo sequence were separated by 40 μs. For transverse relaxation time measurements, pulse separations were varied from 40 to 400 μs. The effective transverse relaxation time, T_{2e}, was taken to be the inverse of the mean transverse relaxation rate for the chain deuterons and was obtained from the initial slope of the quadrupole-echo decay as described elsewhere (Morrow et al., 1996). Typical spectra were obtained by averaging 24 000 transients, obtained with phase cycling at a repetition time of 0.5 s. The sample tube and probe coil were enclosed within a copper oven, the temperature of which was maintained by a microprocessor-based temperature controller. Experiments were carried out for a series of temperatures beginning at 55 °C and descending to 10 °C in steps of 2 °C (1 °C near the transition). Samples were allowed to equilibrate for at least 20 min after each cooling step.

Before centrifugation, 2 mL aliquots of each bilayer suspension were removed for analysis by differential scanning calorimetry (DSC). These samples and a reference buffer were degassed under vacuum and scanned using an MC-2 differential scanning calorimeter (Microcal Inc., Northhampton, MA). Scans were carried out from 20 to 60 °C at a rate of 30 °C/h using a filtering constant of 15. Each sample was scanned a minimum of three times to obtain reproducible results. Samples were analyzed for phosphate content before and after scanning using the method of Bartlett (1959). The lipid content was used to normalize thermograms using software supplied with the calorimeter.

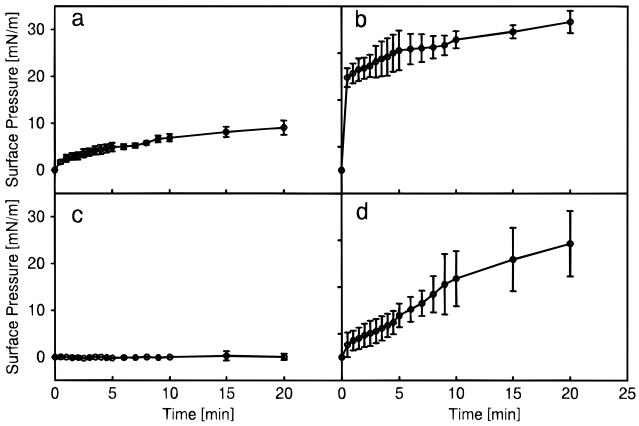

FIGURE 1: Surface pressure adsorption isotherms for (a) DPPG-d_{62}, (b) DPPG-d_{62} plus 11% (w/w) SP-B, (c) DPPC/DPPG-d_{62} (7:3), and (d) DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B. The final phospholipid concentration in each case is 50 μg/mL. The subphase was 135 mM NaCl and 15 mM Hepes at pH 7.0. The temperature was 37 ± 1 °C. Values are the means of two or three experimental runs.

Samples of uncentrifuged material were also assessed for their ability to be adsorbed into a clean air−water interface at 37 °C in an apparatus that has been described previously (King & Clements, 1972; Pèrez-Gil et al., 1992).

RESULTS

Porcine SP-B was initially incorporated into bilayers of DPPG-d_{62} and into bilayers containing 70 mol % DPPC and 30 mol % DPPG-d_{62}. Surface pressure measurements using the Wilhelmy plate (King & Clements, 1972) were used to confirm that SP-B was incorporated into the bilayers in a manner capable of affecting the spreading characteristics of each mixture. As shown in Figure 1, SP-B was found to enhance the initial adsorption rate for both DPPG-d_{62} and the DPPC/DPPG-d_{62} (7:3) mixture. Similar results (not shown) were obtained for SP-B in DPPC-d_{62}/DPPG (7:3). These results are consistent with earlier observations of the effect of SP-B on the spreading properties of lipid mixtures (Pèrez-Gil et al., 1992). The effect of SP-B on the initial spreading rate was greater for DPPG-d_{62} than for the lipid mixture, suggesting that SP-B has a greater influence on the acidic lipid than on the zwitterionic DPPC. In addition, as can be seen from a comparison of panels a and c of Figure 1, liposomes of acidic lipid alone have an intrinsically greater adsorption than do liposomes containing a high proportion of DPPC.

Figure 2 shows DSC scans for DPPG-d_{62}, DPPG-d_{62} plus 11% (w/w) SP-B, DPPC/DPPG-d_{62} (7:3), and DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B. For both the DPPG-d_{62} and the mixed lipid system, the protein broadens the transition slightly and reduces the transition enthalpy. The analyses of the system of DPPC-d_{62}/DPPG (7:3) with and without protein (data not shown) produced thermograms consistent with the ones shown. The protein induces a small increase in the transition temperature for the DPPG-d_{62} system and has little effect on the transition temperature of the mixed lipid system.

Figure 3 shows ^{2}H-NMR spectra at selected temperatures for DPPG-d_{62} and for DPPG-d_{62} plus 11% (w/w) SP-B. In the absence of SP-B (Figure 3a), the spectra above 36 °C are superpositions of sharp doublets characteristic of axially

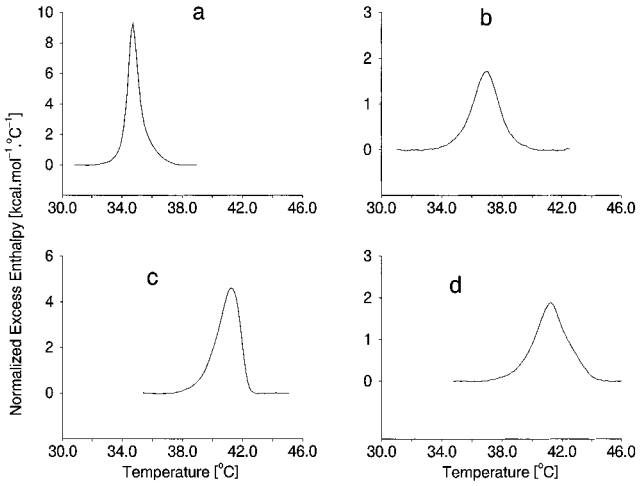

FIGURE 2: Differential scanning calorimetric scans for (a) DPPG-d_{62}, (b) DPPG-d_{62} plus 11% (w/w) SP-B, (c) DPPC/DPPG-d_{62} (7:3), and (d) DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B. The buffer was 35 mM NaCl and 15 mM Hepes at pH 7.0. The heating rate for the scans was 30 °C/h.

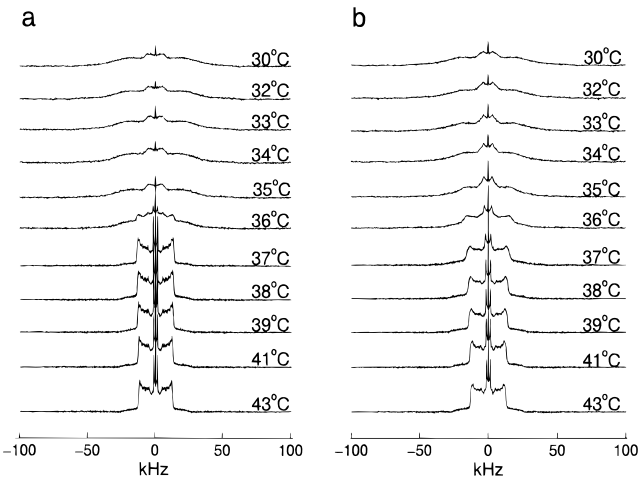

FIGURE 3: (a) ^{2}H-NMR spectra of DPPG-d_{62} for selected temperatures. (b) ^{2}H-NMR spectra of DPPG-d_{62} plus 11% (w/w) SP-B for selected temperatures.

symmetric chain reorientation in the liquid crystalline phase. Below 36 °C, spectra display the wider and more continuous intensity distribution characteristic of the gel phase in which chain motions are axially asymmetric and reorientation occurs on a time scale close to that of the inverse spectral width. A relatively sharp transition from the liquid crystal to the gel phase occurs at 36 °C where spectra characteristic of the two coexisting phases are superimposed. For the DPPG-d_{62} sample containing SP-B, the spectra display a more continuous change from liquid crystal to gel characteristics. None of the spectra in Figure 3b display a clear superposition of liquid crystal and gel phase spectra. The largest change in spectral shape occurs between 37 and 36 °C.

Using the process known as dePakeing, powder pattern spectra can be transformed to yield the spectrum which would be seen for an oriented sample (Bloom et al., 1981; Sternin et al., 1983). Figure 4 shows dePaked spectra, corresponding to 0° orientation of the bilayer normal, for DPPG-d_{62} and DPPG-d_{62} plus 11% (w/w) SP-B at 45 °C. The distribution of resolved doublet splittings is typical of the dependence of orientational order on position along the chain for a range of saturated diacylphosphatidylcholines

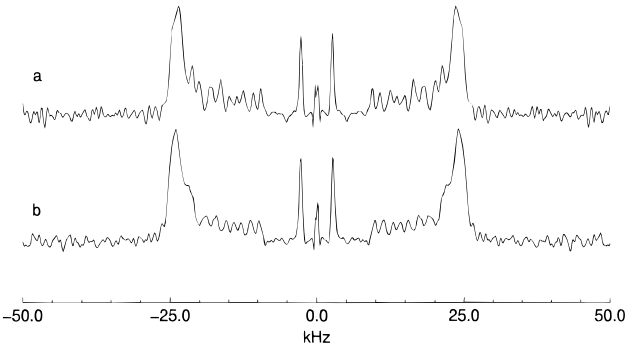

FIGURE 4: DePaked ^{2}H-NMR spectra for (a) DPPG-d_{62} and (b) DPPG-d_{62} plus 11% (w/w) SP-B at 45 °C.

(Morrow & Lu, 1991). SP-B has no appreciable effect on either the mean orientational order or the dependence of deuteron orientational order on position along the DPPG-d_{62} chains.

Figure 5 shows spectra at selected temperatures for DPPC/DPPG-d_{62} (7:3) and for this lipid mixture plus 11% (w/w) SP-B and 15% (w/w) SP-B. The sample with no protein shows a relatively sharp transition from liquid crystal to gel at 40 °C where a superposition of gel and liquid crystal phase features can be seen. The increase in the transition temperature relative to that of the DPPG-d_{62} sample is an isotope effect and reflects the reduced degree of deuteration in the mixed lipid sample. It is interesting that, despite the difference in transition temperatures of DPPC (41 °C) and DPPG-d_{62} (36 °C), the transition remains relatively narrow. The mixed lipid samples containing SP-B again show a continuous change of spectral character from liquid crystal to gel.

While SP-B promotes a more continuous transition in both DPPG-d_{62} and the mixed lipid bilayers, close inspection of the gel phase spectra suggests that the perturbation of the bilayer may not be identical in the two cases. Because of rotation about the methyl group symmetry axis, methyl group deuterons in the gel phase give rise to significantly narrower spectral components than the rest of the acyl chain deuterons. Gel phase spectra for both of the DPPC/DPPG-d_{62} samples with SP-B present display methyl features which are somewhat narrower and more prominent than those seen for DPPG-d_{62} plus 11% (w/w) SP-B. This indicates that there is a population of molecules in an environment where reorientation is less restricted than in more typical gel phases. As discussed below, however, the mean transverse relaxation rates in the gel phases for the protein-containing samples with different lipid compositions are not substantially different.

In order to consider the possibility of a specific interaction between SP-B and either DPPC or DPPG in the mixture, ^{2}H-NMR spectra were also collected for DPPC-d_{62}/DPPG (7:3) and for this lipid mixture plus 11% (w/w) SP-B. Figure 6 shows spectra for these two samples at selected temperatures. The difference in the transition temperatures observed for the two mixed lipid samples in the absence of SP-B reflects the higher degree of deuteration in the DPPC-d_{62}/DPPG (7:3) sample compared to that in the DPPC/DPPG-d_{62} mixture. When this is taken into account, the sequences of spectra observed for the mixed lipid samples in the presence of SP-B are nearly independent of which lipid is deuterated. There is no indication of a significant specific

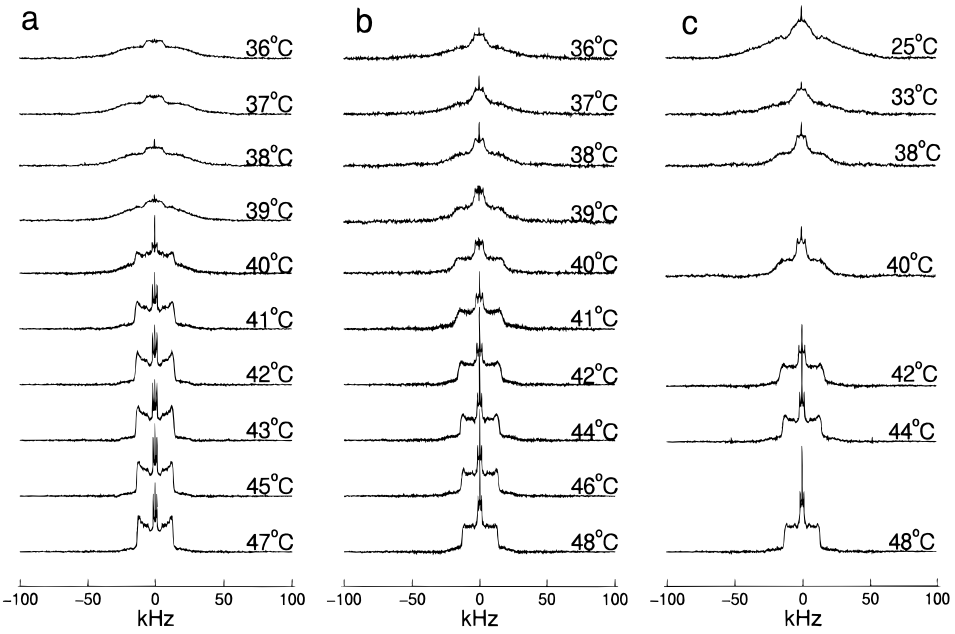

FIGURE 5: (a) ^{2}H-NMR spectra of DPPC/DPPG-d_{62} (7:3) for selected temperatures. (b) ^{2}H-NMR spectra of DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B for selected temperatures. (c) ^{2}H-NMR spectra of DPPC/DPPG-d_{62} (7:3) plus 15% (w/w) SP-B for selected temperatures.

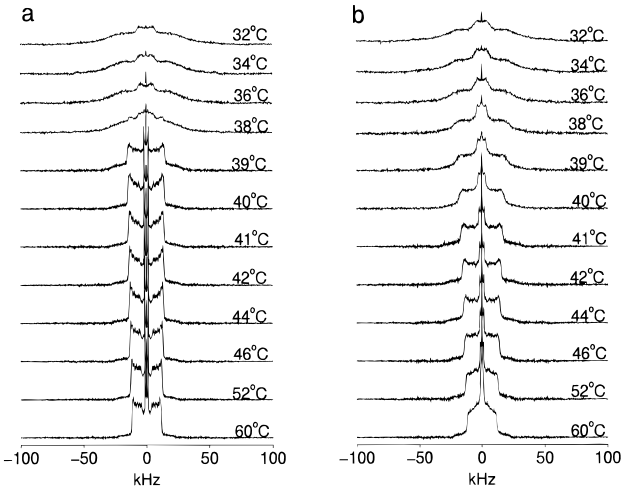

FIGURE 6: (a) ^{2}H-NMR spectra of DPPC-d_{62}/DPPG (7:3) for selected temperatures. (b) ^{2}H-NMR spectra of DPPC-d_{62}/DPPG (7:3) plus 11% (w/w) SP-B for selected temperatures.

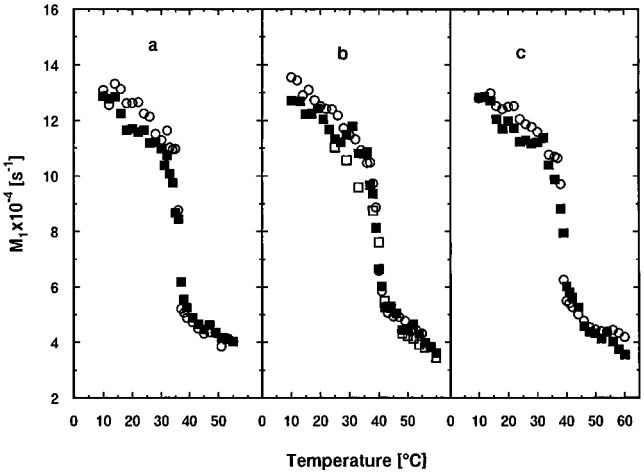

FIGURE 7: (a) Temperature dependence of ^{2}H-NMR first spectral moments for (○) DPPG-d_{62} and (■) DPPG-d_{62} plus 11% (w/w) SP-B. (b) Temperature dependence of ^{2}H-NMR first spectral moments for (○) DPPC/DPPG-d_{62} (7:3), (■) DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B, and (□) DPPC/DPPG-d_{62} (7:3) plus 15% (w/w) SP-B. (c) Temperature dependence of ^{2}H-NMR first spectral moments for (○) DPPC-d_{62}/DPPG (7:3) and (■) DPPC-d_{62}/DPPG (7:3) plus 11% (w/w) SP-B.

interaction of SP-B with one or the other lipid components of the mixture.

Figure 7 shows first spectral moments (M_1) derived from the ^{2}H-NMR spectra of DPPG-d_{62} and the mixed lipid samples with and without 11% (w/w) SP-B. While the transition in DPPG-d_{62} is slightly sharper than that of the mixed lipid samples in the absence of SP-B, all of the samples containing SP-B display a change from liquid crystal to gel phase values of M_1 over roughly the same width in temperature. As was reported for synthetic SP-B in bilayers of DPPC-d_{62} (Morrow et al., 1993), the protein has little effect on chain order in the liquid crystalline phase at temperatures away from the transition. The effect, if any, on chain order in the gel phase is small. Figure 7b also shows M_1 values for DPPC/DPPG-d_{62} plus 15% (w/w) SP-B. The increased protein concentration has some effect on the gel phase but little effect on liquid crystal phase chain order. From a comparison of panels b and c of Figure 7, there is again no indication of a specific interation of SP-B with one of the lipid components in the DPPC/DPPG bilayer.

Additional information about the lipid−protein interaction can be obtained from an examination of deuteron transverse relaxation times which can indicate how slow lipid reorientation is perturbed by the presence of the protein. The quadrupole-echo sequence consists of two $\pi/2$ radio frequency pulses separated by an interval τ. The echo is formed at time 2τ following the start of the sequence. Motions which alter the orientation-dependent quadrupole interaction during the interval 2τ diminish the extent to which transverse magnetization present after the initial pulse is refocused in the echo. The characteristic time for decay of the echo is labeled T_{2e}, the effective transverse relaxation time. Motions which are believed to affect transverse relaxation include diffusion of lipid molecules along curved bilayer surfaces (Bloom & Sternin, 1987), bilayer undulations (Bloom & Evans, 1991) and collective modes (Stohrer et al., 1991),

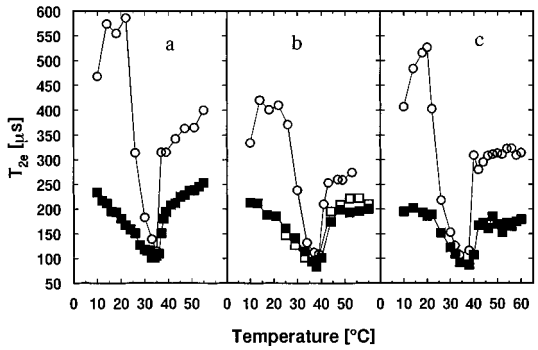

FIGURE 8: (a) Temperature dependence of T_{2e} for (○) DPPG-d_{62} and (■) DPPG-d_{62} plus 11% (w/w) SP-B. (b) Temperature dependence of T_{2e} for (○) DPPC/DPPG-d_{62} (7:3), (■) DPPC/DPPG-d_{62} (7:3) plus 11% (w/w) SP-B, and (□) DPPC/DPPG-d_{62} (7:3) plus 15% (w/w) SP-B. (c) Temperature dependence of T_{2e} for (○) DPPC-d_{62}/DPPG (7:3) and (■) DPPC-d_{62}/DPPG (7:3) plus 11% (w/w) SP-B.

and, under some conditions, slow conformational changes (Meier et al., 1986). For motions of the carbon−deuterium bond having correlation times (τ_c) which are short compared to the inverse width of the deuteron quadrupole splitting, the transverse relaxation rate is given by $T_{2e}^{-1} = \Delta M_2 \tau_c$, where ΔM_2 is that part of the second moment modulated by the motion (Pauls et al., 1985). This is generally the case for some motions in the liquid crystalline phase. If the correlation time is long compared to the inverse splitting, the transverse relaxation time is proportional to the correlation time (Pauls et al., 1985). This is normally the situation for transverse relaxation in the gel phase. As a result, the transverse relaxation time for deuterated lipid bilayers generally passes through a minimum at the main phase transition. As the temperature is lowered in the gel phase, the relevant correlation times, and thus the transverse relaxation times, increase as the slow motions freeze out.

Figure 8a shows the temperature dependence of T_{2e} for DPPG-d_{62} with and without 11% (w/w) SP-B. For DPPG-d_{62}, there is a sharp drop in T_{2e} at the transition due to an abrupt increase in correlation times for motions which determine transverse relaxation. In the gel phase of DPPG-d_{62}, T_{2e} rises more quickly with decreasing temperatures than for chain-perdeuterated saturated diacylphosphatidylcholines. This presumably indicates that the relevant slow reorientational motions freeze out more easily in bilayers containing DPPG. The T_{2e} maximum observed in the gel phase indicates that, while some slow motions are freezing out and having less effect on transverse relaxation, at least one faster, presumably intramolecular, motion is slowing into the correlation time window in which it contributes effectively to transverse relaxation. A T_{2e} maximum has also been observed in the gel phase of specifically labeled dimyristoylphosphatidylcholine (Meier et al., 1986).

The liquid crystal phase T_{2e}values for DPPG-d_{62} are lower than have been reported for chain-perdeuterated diacylphosphatidylcholines. It may be that the hydration protocol used for these samples results in a distribution of vesicle sizes which differs from that obtained when dried lipid films are scraped from a flask and then hydrated by gentle stirring. A separate investigation has shown that, for mixtures of DPPC-d_{62}/DPPG-d_{62}, hydrated in a calcium-containing buffer, the rotating flask hydration protocol employed here consistently, and reproducibly, yields liquid crystal transverse relaxation times shorter than those observed when dried lipid is scraped from the walls of a flask and hydrated by gentle stirring at a temperature above the transition (M. Kilfoil, unpublished results). Significantly, the same study shows that the gel phase behavior of T_{2e} is unaffected by hydration protocol. This may mean that the motions responsible for transverse relaxtion in the gel phase are predominantly localized reorientations which might be expected to be less sensitive to details of vesicle formation.

Figure 8a also shows that addition of SP-B to DPPG-d_{62} lowers T_{2e} in the liquid crystalline phase, reduces its sensitivity to temperature in the gel phase, and gives rise to a more continuous variation of T_{2e} through the minimum. This is typical of the effect of a number of membrane-associated proteins and polypeptides. In the liquid crystalline phase, the effect of the protein is an increase in the correlation times for those slow reorientational motions which are responsible for transverse relaxation. In the gel, the protein appears to interfere with the freezing out of the localized reorientations which affect transverse relaxation in this phase.

Figure 8b shows the temperature dependence of T_{2e} for DPPC/DPPG-d_{62} (7:3) in the absence of SP-B and with 11% (w/w) and 15% (w/w) SP-B added. Figure 8c shows the temperature dependence of T_{2e} for DPPC-d_{62}/DPPG (7:3) with and without 11% (w/w) SP-B. Aside from a shift in the transition temperature due to an altered isotope effect, the temperature dependence of T_{2e} for the mixed lipid samples with no SP-B present is qualitatively similar to that of DPPG-d_{62}. Compared to the behavior of DPPG-d_{62}, both lipids in the mixed lipid samples with no SP-B present display slightly lower T_{2e} values in the liquid crystalline phase. In light of the sensitivity of liquid crystal T_{2e} values to sample preparation, such small differences are probably not significant. With SP-B present, though, the behavior of T_{2e} is strikingly similar to that seen for DPPG-d_{62} plus 11% (w/w) SP-B. The similarity of T_{2e} results for 11% (w/w) SP-B and 15% (w/w) SP-B suggests that there may be a threshold SP-B concentration beyond which the response of T_{2e} to SP-B concentration saturates.

DISCUSSION

A recent study of synthetic SP-B in DPPC-d_{62} showed that, while the protein broadened the main phase transition, it had little effect on acyl chain order in the bilayer (Morrow et al., 1993). The present work has extended this observation to natural SP-B in bilayers of DPPG-d_{62} and in DPPC/DPPG-d_{62} or DPPC-d_{62}/DPPG mixed bilayers. These observations suggest that SP-B interacts with the bilayer in a way which has little effect on the amplitude of the fast chain conformational fluctuations which determine mean orientational order for the perdeuterated chain in the liquid crystalline phase. This would seem to be evidence showing that SP-B does not alter interactions between neighboring lipid molecules in a way which would significantly modify chain packing. In particular, it would be difficult to reconcile these observations with models in which the amphipathic helical segments intercalate substantially into the bilayer head group region. For samples prepared using the formulation employed in this work, the effect of SP-B on bilayer lipid properties is found to be largely insensitive to the relative amounts of DPPC and DPPG. Furthermore, the effect of SP-B on DPPC/DPPG mixed bilayers, as indicated by ^{2}H-

NMR observations, shows no evidence of specific interaction of SP-B with one or the other lipid components in the mixed lipid bilayer. As indicated above, these observations also argue against a model in which the interaction of SP-B with the bilayer takes place predominantly through intercalation into the head group region if the interaction is at the bilayer surface. At the same time, SP-B is observed to substantially alter deuteron transverse relaxation in both the liquid crystal and gel phases of these bilayer systems. This suggests that the interaction of SP-B with the bilayer does perturb the slower reorientations which are responsible for transverse relaxation and thus that it is strongly associated with the bilayer. One possibility which might be consistent with these observations is that the distribution of SP-B is uniform for larger length scales but displays some degree of self-association at shorter length scales. This would be the case, for example, if the SP-B associated to form boundaries around discs of relatively unperturbed lipid which then aggregate to form large sheets, an arrangement which is suggested by electron micrographs presented by Williams et al. (1991).

While SP-B has only a limited effect on mean chain order in the liquid crystal and gel phases, it does have some effect on the details of the ^{2}H-NMR spectral shape, particularly in the gel phase of the mixed lipid samples. The effect of SP-B on the width of the gel phase methyl deuteron spectral component is weaker in both DPPG-d_{62} and in DPPC-d_{62} (Morrow et al., 1993) than in the mixed lipid. The spectra close to and above the transition, for the mixed lipid bilayers with SP-B present, also show a narrow component which suggests that a small fraction of the sample may be reorienting isotropically. One intriguing possibility, suggested by the earlier observation of disklike structures in bilayers containing SP-B (Williams et al., 1991), is that this enhanced intensity close to zero quadrupole splitting might indicate a small population of more freely reorienting bilayer fragments or disks as outlined above. Alternatively, if SP-B could associate into boundaries surrounding regions of lipid, this arrangement might provide a means for the amphipathic protein to slightly intercalate into the bilayer center, thus reducing methyl group ordering.

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