# Effect of Calcium on Phospholipid Interaction with Pulmonary Surfactant Protein C

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ABSTRACT Porcine pulmonary surfactant-associated protein SP-C was incorporated into bilayers of chain-perdeuterated dipalmitoylphosphatidylglycerol (DPPG-$d_{62}$) and chain-perdeuterated dipalmitoyl-phosphatidylcholine (DPPC-$d_{62}$) and into bilayers containing 70 mol% dipalmitoyl-phosphatidylcholine (DPPC) and 30 mol% DPPG-$d_{62}$ or 70 mol% DPPC-$d_{62}$ and 30 mol% dipalmitoylphosphatidylglycerol (DPPG). The effect of SP-C on the phase behavior, lipid chain order, and dynamics in these bilayers was examined by using deuterium nuclear magnetic resonance. SP-C was found to have a similar effect on the chain order and phase behavior of DPPC-$d_{62}$ and DPPG-$d_{62}$ in bilayers with a single lipid component. In gel phase DPPC/DPPG (7:3) bilayers with one or the other lipid component chain-perdeuterated, SP-C was found to affect first spectral moment more strongly for DPPG-$d_{62}$ than for DPPC-$d_{62}$. This may indicate that SP-C induced a nonrandom lateral distribution in the mixed lipid bilayer. SP-C was also found to influence motions responsible for deuteron transverse relaxation in both the gel and liquid crystalline phases. The presence of 5 mM $Ca^{2+}$ in the aqueous phase substantially altered the effect of SP-C on transverse relaxation in the bilayer.

## INTRODUCTION

Pulmonary surfactant is a mixture of lipid and protein synthesized and secreted by type II pneumocytes in the alveoli. Its composition, properties, and physical chemistry have been described in a number of recent reviews (Weaver and Whitsett, 1991; Keough, 1992; Johansson et al., 1994). Approximately 10% of surfactant mass is protein. Saturated phosphatidylcholine, most of which is dipalmitoylphosphatidylcholine (DPPC), accounts for more than 40% of the total surfactant mass (Kahn et al., 1995). From 7% to 10% of surfactant mass is anionic phospholipids such as phosphatidylglycerol and phosphatidylinositol. About 13% of surfactant mass is lipid other than phospholipid. The remainder of the mass is composed of other phospholipids such as unsaturated phosphatidylcholines and a very small amount of glycolipid (Keough, 1992).

Pulmonary surfactant facilitates cycling of lung volume by giving rise to a molecular film that modifies the surface properties of the air-water interface in the lung. This film is believed to be highly enriched in DPPC, possibly because of a process by which other surfactant components are selectively excluded. Four proteins have been identified as significant components of pulmonary surfactant. Two of these, SP-B (pulmonary surfactant-associated protein, $M_r$ 17,400) and SP-C (pulmonary surfactant-associated protein, $M_r$ 4186), are hydrophobic proteins that appear to promote the rapid spreading of surfactant material from lamellar structures into the monolayer (Oosterlaken-Dijksterhuis et al., 1991a,b; Pérez-Gil et al., 1992, 1994). SP-B and SP-C have both been estimated to constitute ~1–2% of the surfactant mass (Keough, 1992). An understanding of how these proteins interact with lipids in lamellar structures is an important step toward a clearer picture of how pulmonary surfactant composition is related to the rapid monolayer spreading properties that are essential to effective surfactant function. The hydrophobic protein SP-C consists of 33–35 amino acid residues and is largely $\alpha$-helical (Pastrana et al., 1991; Vandenbussche et al., 1992). It is generally palmitoylated at two viscinal residues near the N-terminus of the peptide chain. SP-C can enhance the spreading of DPPC into a monolayer, although it is not as effective as SP-B (Oosterlaken-Dijksterhuis et al., 1991a; Pérez-Gil et al., 1994). There is some indication that SP-C may be involved in monolayer refinement through the removal of material from the monolayer under compression (Taneva and Keough, 1994).

An earlier $^2H$ NMR study of porcine SP-C in chain perdeuterated DMPC (DMPC-$d_{54}$) showed that 8% (w/w) SP-C had no noticeable effect on acyl chain order in the liquid crystalline phase, but did result in the phase change from liquid crystal to gel being continuous (Simatos et al., 1990). This is very similar to the effect on the bilayer of other transbilayer polypeptides for which there appears to be a threshold polypeptide concentration beyond which the transition is replaced by a continuous phase change (Morrow and Whitehead, 1988; Morrow and Davis, 1988). Natural pulmonary surfactant, though, contains a mixture of charged and neutral lipids in an aqueous environment containing $Ca^{2+}$ ions. A more complete understanding of protein-lipid interaction within pulmonary surfactant thus requires some consideration of how that interaction is

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affected by bilayer composition, lipid charge, and the ionic environment.

In a recent study, $^2$H NMR was used to examine the effect of the hydrophobic pulmonary surfactant protein, SP-B, on the properties of bilayers consisting of DPPG or DPPC/DPPG mixtures (Dico et al., 1997). SP-B was found to influence slow lipid motions in the bilayer, but did not display a preferential interaction with one or the other component in DPPC/DPPG mixtures.

In the present work, $^2$H NMR measurements of mean chain order have been used to compare the interaction of SP-C with DPPC and DPPG in bilayers containing a single lipid component and in bilayers containing both lipids. The effect of $Ca^{2+}$ on the protein-lipid interaction has been examined through measurement of the $^2$H transverse relaxation time in DPPC/DPPG-$d_{62}$ (7:3) bilayers with and without SP-C present.

## 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 obtained from Sigma Chemical (St. Louis, MO). The lipids were found to be pure by thin-layer chromatography and were used without further purification.

Pulmonary surfactant protein SP-C was obtained from lipid extracts of porcine lung lavage as described previously (Taneva and Keough, 1994). Surfactant protein SP-C from the lipid extract was isolated and purified on Sephadex LH-60 (Pharmacia, Uppsala) in chloroform/methanol, 1:1 (v/v), containing 2% by volume 0.1 M HCl. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (16% gels) (Laemmli, 1970; Taneva and Keough, 1994) of the SP-C under nonreducing conditions yielded a band at ~5 kDa.

DPPC was dissolved in chloroform and DPPG was dissolved in $CHCl_3$/MeOH (3:1); the concentrations of the solutions were determined by phosphorus assay (Bartlett, 1959; Keough and Kariel, 1987). The lipid compositions used for these samples were DPPC-$d_{62}$, DPPG-$d_{62}$, DPPC:DPPG-$d_{62}$ (7:3 mol/mol), and DPPC-$d_{62}$/DPPG (7:3 mol/mol). Protein concentration in the liposomes was determined by fluorescamine assay (Udenfriend et al., 1972). Samples containing SP-C were prepared with a protein concentration of 10% (w/w). Solvents were removed by rotary evaporation, flushed with $N_2$, and evacuated overnight. Samples were suspended by adding buffer (135 mM NaCl, 15 mM HEPES, with or without 5 mM $CaCl_2$, pH 7.0) to the flask containing the dried sample film and then rotating the flask in a water bath at 50°C for ~1 h. Films containing the protein were scraped from the walls of the flask to ensure complete suspension in the buffer. For samples containing $Ca^{2+}$, the amount of buffer added was chosen to yield a suspension of ~2 mg/ml, which was then centrifuged at 14,000 r.p.m. for 10 min. Some samples without $Ca^{2+}$ were also prepared by centrifugation. Most of the supernatant was removed. The resulting pellet was scraped into an 8-mm NMR tube and suspended in 400 μl of buffer (135 mM NaCl, 15 mM HEPES, and 5 mM $CaCl_2$ for samples containing $Ca^{2+}$).

$^2$H NMR measurements at 23.215 MHz were carried out in a superconducting solenoid (Nalorac, Martinez, CA), using quadrupole echo pulse sequences (Davis et al., 1976) with $\pi/2$ pulse lengths of 2.3–2.75 μs. For spectra from which first spectral moments were taken, the pulse separation was 40 μs. For transverse relaxation time measurements, quadrupole echo sequence pulse separations were varied from 40 μs to 400 μs. Typical spectra were obtained by averaging 24,000 transients obtained with phase cycling, using 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.

The first spectral moment, $M_1$, is given by (Davis, 1983)

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

where $f(\omega)$ is the spectral intensity as a function of frequency. The orientational order parameter for a particular acyl chain deuteron undergoing fast, axially symmetrical reorientation is given by

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

where $\theta_{CD}$ is the angle between the carbon-deuterium bond axis and the molecular axis of rotational symmetry. The average is over accessible chain conformations. In the liquid crystalline phase of bilayers containing perdeuterated acyl chains, $M_1$ is proportional to the average of the deuteron orientational order parameter over all labeled positions on the chain. The temperature dependence of $M_1$ provides information about bilayer phase behavior and mean orientational order of the chain segments.

## RESULTS AND DISCUSSION

Fig. 1 shows $^2$H NMR spectra for DPPG-$d_{62}$ and DPPG-$d_{62}$ containing 10% (w/w) porcine SP-C. Fig. 2 shows $^2$H NMR spectra for DPPC-$d_{62}$ and DPPC-$d_{62}$ containing 10% (w/w) SP-C. In the absence of $Ca^{2+}$, the DPPG-$d_{62}$ main transition is lower than the DPPC-$d_{62}$ transition by ~2°. In both cases, the presence of SP-C has little effect on chain deuteron quadrupole splittings in the liquid crystalline phase. For both systems, SP-C induces coexistence of the liquid crystal and gel phases over a narrow temperature range just below the pure lipid main transition temperature.

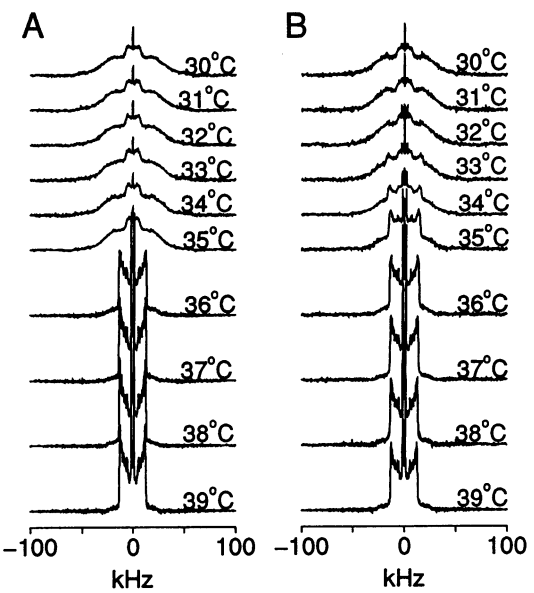


FIGURE 1 $^2$H NMR spectra at selected temperatures for (*A*) DPPG-$d_{62}$ and (*B*) DPPG-$d_{62}$ containing 10% porcine SP-C (w/w). Buffer is 135 mM NaCl, 15 mM HEPES.

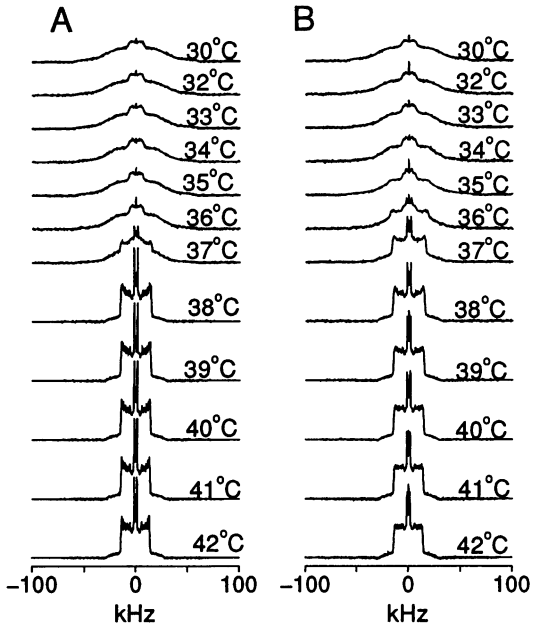


FIGURE 2 $^2$H NMR spectra at selected temperatures for (*A*) DPPC-$d_{62}$ and (*B*) DPPC-$d_{62}$ containing 10% porcine SP-C (w/w). Buffer is 135 mM NaCl, 15 mM HEPES.

Fig. 3 shows first spectral moments ($M_1$) corresponding to the spectra shown in Figs. 1 and 2. These results illustrate, more quantitatively, the small broadening of the main transition by SP-C and the lack of any substantial perturbation of liquid crystal chain order by SP-C. An earlier study also showed no perturbation of chain order in liquid crystalline DMPC-$d_{54}$ by up to 8% (w/w) SP-C (Simatos et al., 1990). In the gel phase of DMPC-$d_{54}$, however, 8% (w/w) SP-C was found to reduce $M_1$. This difference may indicate that the interaction in the gel phase is more sensitive to the relative sizes of the protein and lipid components in the mixed bilayer or to the mismatch in hydrophobic regions of the components (Mouritsen and Bloom, 1984).

The results shown in Fig. 3 suggest that differences between the interactions of SP-C with DPPC-$d_{62}$ and DPPG-$d_{62}$ are not large enough to be apparent in the chain order of bilayers containing a single lipid species. In a bilayer containing a mixture of lipid species, however, it is conceivable that a small difference in the interactions might lead to a nonrandom distribution of bilayer components. To test this possibility, bilayers containing SP-C in a mixture of DPPC and DPPG, with one or the other lipid component deuterated, were examined by $^2$H NMR. Fig. 4 shows $^2$H NMR spectra at selected temperatures for DPPC/DPPG-$d_{62}$ (7:3) without SP-C and with 10% (w/w) and 15% (w/w) SP-C present. Fig. 5 shows $^2$H NMR spectra for analogous samples containing DPPC-$d_{62}$/DPPG (7:3). For both lipid mixtures, 10% (w/w) SP-C induces two-phase coexistence over a narrow temperature range. With 15% (w/w) SP-C, superpositions of liquid crystal and gel spectra, which would be characteristic of two-phase coexistence, are not observed, and the change from liquid crystal to gel is

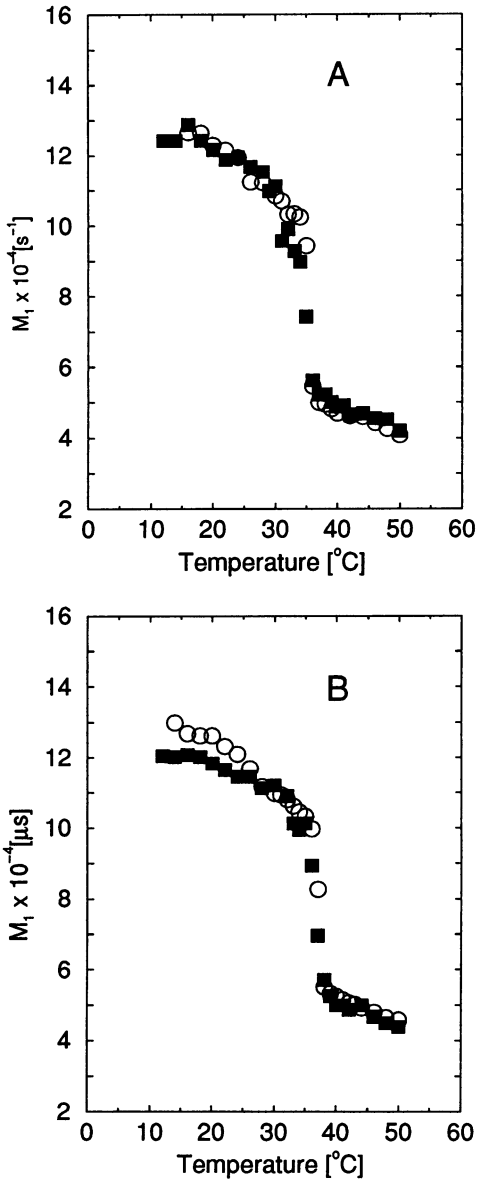


FIGURE 3 (*A*) Temperature dependence of $^2$H-NMR first spectral moments for (○) DPPG-$d_{62}$ and (■) DPPG-$d_{62}$ plus 10% (w/w) SP-C. (*B*) Temperature dependence of $^2$H-NMR first spectral moments for (○) DPPC-$d_{62}$ and (■) DPPC-$d_{62}$ plus 10% (w/w) SP-C.

presumed to proceed continuously. The occurrence of a limiting peptide concentration beyond which there is no two-phase coexistence has been discussed previously (Morrow and Davis, 1988; Morrow and Whitehead, 1988).

Fig. 6 shows first spectral moments ($M_1$) for both lipid compositions with and without 15% SP-C (w/w). In the gel phase of the lipid mixture, SP-C appears to have a larger effect on $M_1$ for the sample containing DPPG-$d_{62}$ than for the sample containing DPPC-$d_{62}$. In the liquid crystalline phase, SP-C seems to have a slight ordering effect on DPPC-$d_{62}$ but not on DPPG-$d_{62}$ in the mixture. This may indicate that, in a mixture of PC and PG, it is possible for SP-C to associate preferentially with one or the other com-

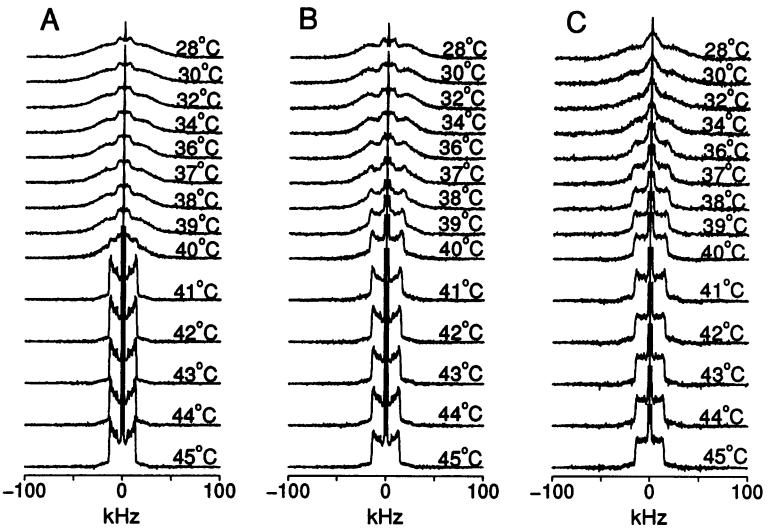


FIGURE 4 $^2$H NMR spectra at selected temperatures for (*A*) DPPC/DPPG-$d_{62}$ (7:3), (*B*) DPPC/DPPG-$d_{62}$ (7:3) with 10% (w/w) SP-C, and (*C*) DPPC/DPPG-$d_{62}$ (7:3) with 15% (w/w) SP-C present. Buffer is 135 mM NaCl, 15 mM HEPES.

ponent and thus promote a nonrandom lateral distribution in the bilayer. The behavior observed in the gel phase suggests a significantly stronger perturbation of DPPG-$d_{62}$ and thus, presumably, a higher effective concentration of SP-C in the neighborhood of DPPG molecules. The observed difference in the liquid crystalline phase is smaller and may not represent a significant preferential interaction of SP-C with liquid crystal phase DPPC. Pérez-Gil et al. (1995), using specific phospholipid spin probes in a liquid crystal DPPC matrix, also observed little selectivity of interaction between SP-C and various lipids, including PC and PG.

At a pH of 7.0, SP-C carries a net positive charge and DPPG is negatively charged. The presence of $Ca^{2+}$ in the pulmonary aqueous subphase may thus influence the way in which SP-C interacts with a mixture of PC and PG in the lamellar bodies of pulmonary surfactant. To examine this possibility, we compared the effect of SP-C on lipid dynamics in DPPC/DPPG-$d_{62}$ (7:3) bilayers in the presence and absence of 5 mM $Ca^{2+}$. Fig. 7 (*A* and *B*) shows $^2$H NMR spectra at selected temperatures for DPPC/DPPG-$d_{62}$ (7:3) in the presence of 5 mM $Ca^{2+}$ without SP-C and with 10% SP-C (w/w). Comparison with the spectra shown in Fig. 4 indicates that the presence of $Ca^{2+}$ shifts the transition temperature by ~6°C. For the same SP-C concentration, the temperature range over which the gel and liquid crystal phases coexist is not significantly altered by the presence of $Ca^{2+}$. Fig. 7 *C* shows the temperature dependence of $M_1$ corresponding to the spectra in Fig. 7, *A* and *B*. The perturbation of DPPG-$d_{62}$ by SP-C in the gel phase of DPPC/DPPG-$d_{62}$ (7:3), as seen in Fig. 6 *A*, is largely removed in the presence of $Ca^{2+}$.

The effect of protein-lipid interactions on slow motions in the bilayer can be probed by deuteron transverse relaxation. For a pulse separation $\tau$, the quadrupole echo pulse sequence gives rise to an echo at time $2\tau$ after the start of the sequence. Decay of the echo with increasing $2\tau$ is characterized by the effective transverse relaxation time, $T_{2e}$, which is the inverse of the transverse relaxation rate averaged over all deuterons in the sample. This decay is sensitive to motions that alter the quadrupole interaction during the interval $2\tau$. Contributions to the transverse relaxation rate from motions with correlation times $\tau_c$ that are short compared to the inverse width of the deuteron quadrupole splitting are given by $T_{2e}^{-1} = \Delta M_2 \tau_c$, where $\Delta M_2$ is that

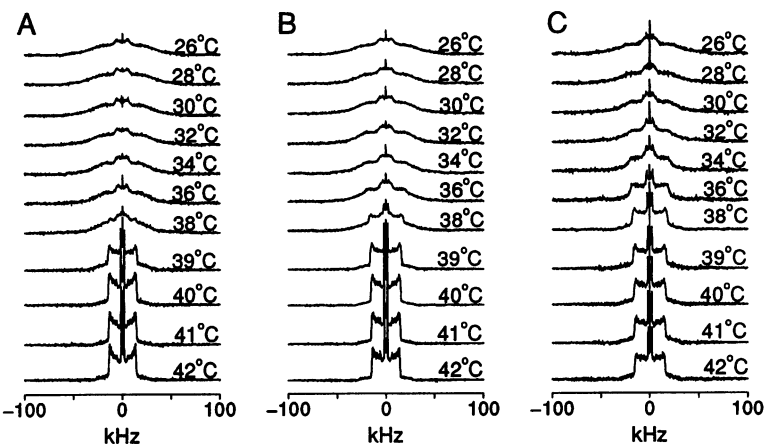


FIGURE 5 $^2$H NMR spectra at selected temperatures for (*A*) DPPC-$d_{62}$/DPPG (7:3), (*B*) DPPC-$d_{62}$/DPPG (7:3) with 10% (w/w) SP-C, and (*C*) DPPC-$d_{62}$/DPPG (7:3) with 15% (w/w) SP-C present. Buffer is 135 mM NaCl, 15 mM HEPES.

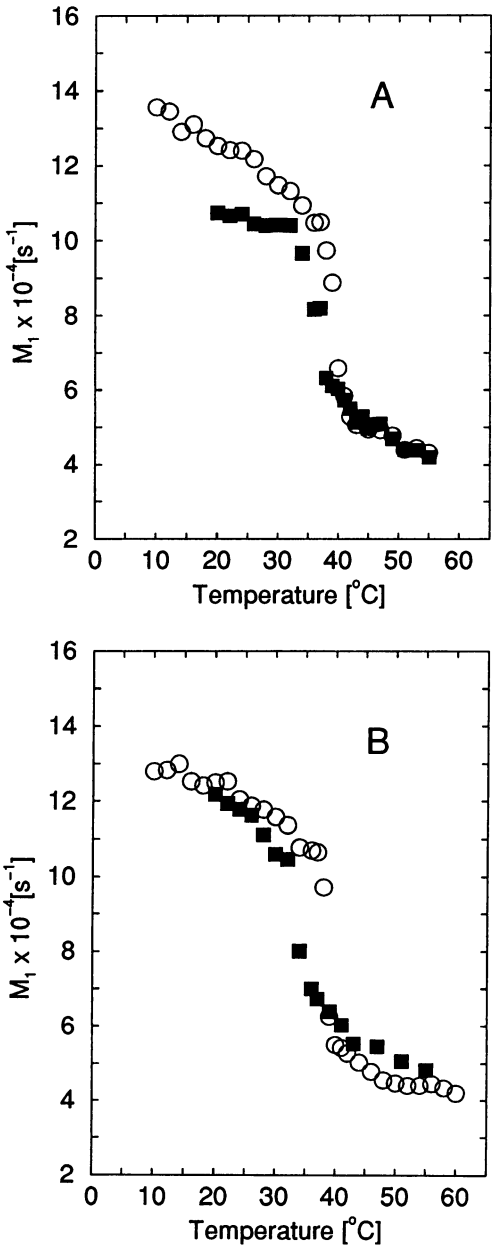


FIGURE 6 (*A*) Temperature dependence of $^2$H-NMR first spectral moments for (○) DPPC/DPPG-$d_{62}$ (7:3) and (■) DPPC/DPPG-$d_{62}$ (7:3) with 15% (w/w) SP-C. (*B*) Temperature dependence of $^2$H-NMR first spectral moments for (○) DPPC-$d_{62}$/DPPG (7:3) and (■) DPPC-$d_{62}$/DPPG (7:3) with 15% (w/w) SP-C.

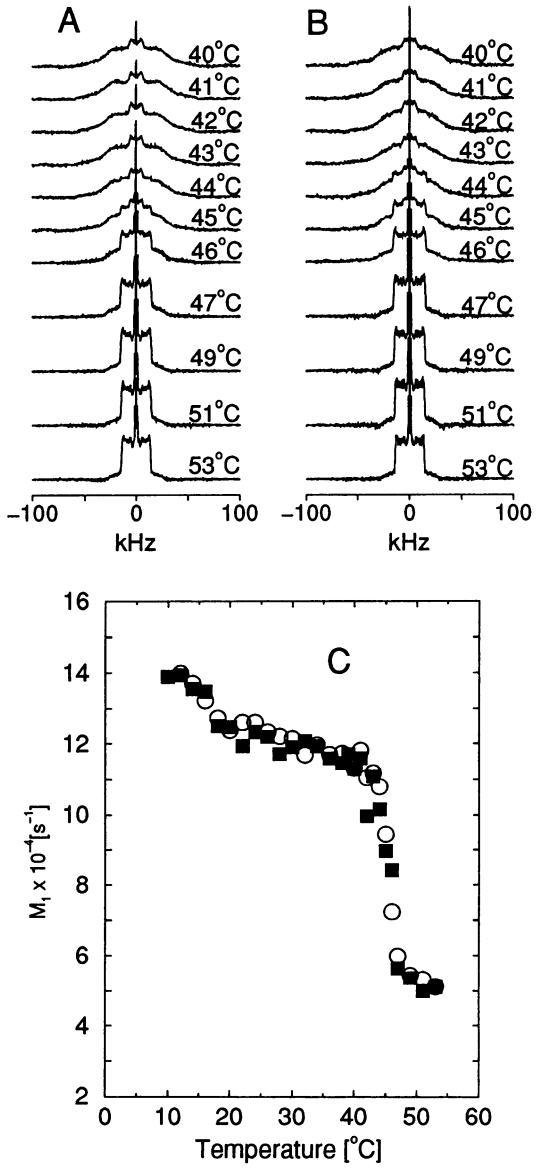


FIGURE 7 (*A*) $^2$H NMR spectra at selected temperatures for DPPC/DPPG-$d_{62}$ (7:3) in the presence of 5 mM $Ca^{2+}$. (*B*) $^2$H NMR spectra at selected temperatures for DPPC/DPPG-$d_{62}$ (7:3) with 10% (w/w) SP-C in the presence of 5 mM $Ca^{2+}$. (*C*) Temperature dependence of $^2$H-NMR first spectral moments for (○) DPPC/DPPG-$d_{62}$ (7:3) in the presence of 5 mM $Ca^{2+}$ and (■) DPPC/DPPG-$d_{62}$ (7:3) with 10% (w/w) SP-C in the presence of 5 mM $Ca^{2+}$. Buffer is 135 mM NaCl, 15 mM HEPES.

part of the second moment modulated by the motion (Pauls et al., 1985). If the correlation time is long compared to the inverse splitting, the transverse relaxation time is proportional to $\tau_c$ (Pauls et al., 1985). Transverse relaxation is found to be sensitive to the presence of proteins and polypeptides in the membrane (Morrow, 1990; Simatos et al., 1990). The precise way in which such motions are altered by interaction with proteins is not fully understood, although comprehensive modeling has provided valuable insights into specific cases, such as the interaction of a synthetic polypeptide with specifically labeled DPPC (Prosser et al., 1992).

Fig. 8 shows the temperature dependence of $T_{2e}$ for DPPC/DPPG-$d_{62}$ and DPPC/DPPG-$d_{62}$ plus 10% SP-C (w/w) in the absence of $Ca^{2+}$ (Fig. 8 *A*) and in the presence of 5 mM $Ca^{2+}$ in the aqueous subphase (Fig. 8 *B*). In the absence of SP-C, the addition of 5 mM $Ca^{2+}$ has a small effect on deuteron transverse relaxation in the mixed lipid bilayer, but the qualitative picture is not altered. In the absence of SP-C, the samples with and without 5 mM $Ca^{2+}$ both display $T_{2e}$ values that start above 400 $\mu$s in the liquid

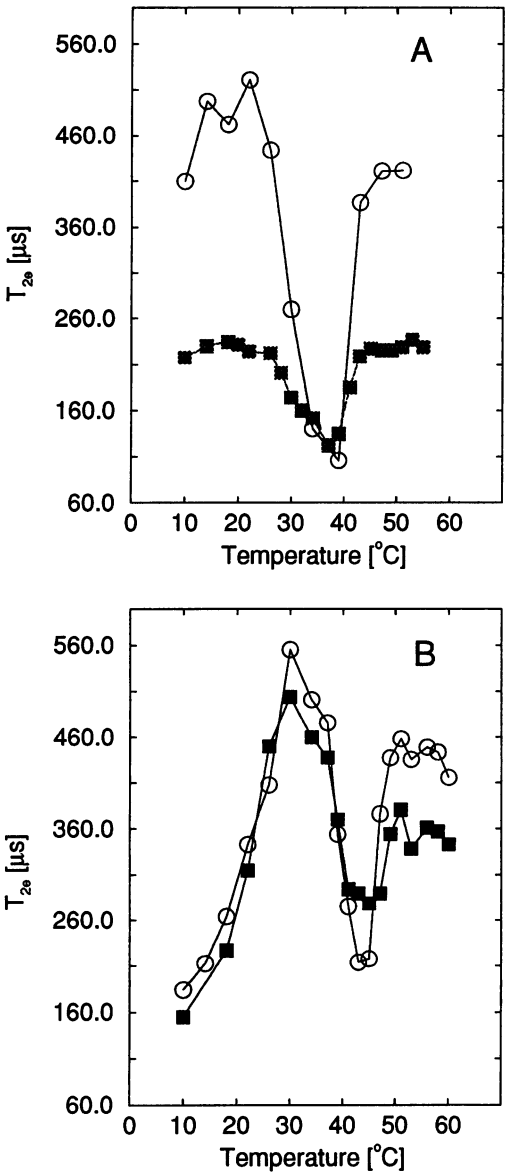


FIGURE 8 (*A*) Temperature dependence of $T_{2e}$ for (○) DPPC/DPPG-$d_{62}$ and (■) DPPC/DPPG-$d_{62}$ with 10% (w/w) SP-C. (*B*) Temperature dependence of $T_{2e}$ for (○) DPPC/DPPG-$d_{62}$ and (■) DPPC/DPPG-$d_{62}$ with 10% (w/w) SP-C in the presence of 5 mM $Ca^{2+}$ in the aqueous subphase.

crystalline phase and drop as temperature is lowered toward the transition. For both samples without SP-C, $T_{2e}$ passes through a minimum at or just below the transition and then rises toward a maximum as the temperature is lowered into the gel phase region.

In the liquid crystalline phase, transverse relaxation is sensitive to a wide range of bilayer motions. Some are slow or adiabatic motions, which may include bilayer undulation (Bloom and Evans, 1991), diffusion along curved surfaces (Bloom and Sternin, 1987), and collective bilayer modes (Stohrer et al., 1991). Such motions are expected to be in the regime where their contributions to the transverse relaxation rate are proportional to their respective correlation times. Although such slow motions contribute significantly to the transverse relaxation rate in the liquid crystalline phase, faster intramolecular motions such as rotational diffusion around the long and short molecular axes are also present (Meier et al., 1986; Prosser et al., 1992). The contribution of faster motions to the transverse relaxation rate can be enhanced in the presence of bilayer perturbations that increase the correlation times for such motions. The decrease in $T_{2e}$ as the temperature is lowered toward the transition indicates that some faster motions contribute to transverse relaxation and that this contribution is growing as their correlation times increase with decreasing temperature. Below the liquid crystal to gel transition, collective modes and diffusion presumably freeze out and the correlation times for other motions fall into the slow motion regime. Just below the transition, the motions dominating transverse relaxation are in the regime where their contributions to the transverse relaxation rate are inversely proportional to their correlation times. Accordingly, as the temperature is lowered and the correlation times for these remaining motions increase, the transverse relaxation rate drops and $T_{2e}$ increases. The resulting minimum in transverse relaxation time near the transition is a general feature of perdeuterated and specifically deuterated lipid bilayer systems (Meier et al., 1986; Yue et al., 1988; Morrow, 1990; Simatos et al., 1990). Gel phase bilayers containing DPPG are found to display a faster increase of transverse relaxation time with decreasing temperature than bilayers containing only phosphatidylcholines (Dico et al., 1997). The maximum in $T_{2e}$ below the transition indicates that, even in the gel phase, one of the motions contributing to transverse relaxation remains in the short correlation time regime and begins to contribute significantly to transverse relaxation below the temperature of this maximum.

The effect of SP-C on transverse relaxation depends strongly on whether $Ca^{2+}$ is present. In the absence of $Ca^{2+}$, SP-C influences transverse relaxation in both the liquid crystal and gel phases, as can be seen in Fig. 8 *A*. Proteins and polypeptides have been observed to reduce $T_{2e}$ in both the liquid crystal and gel phases of other model systems (Morrow, 1990; Simatos et al., 1990; Prosser et al., 1992; Dico et al., 1997). Branched-chain amphiphiles have been reported to similarly affect $T_{2e}$ in the gel phase (Yue et al., 1988).

Because collective modes and diffusive motions in the liquid crystalline phase contribute to transverse relaxation in the slow motional regime, a decrease in $T_{2e}$ in this phase could reflect an increase in the amplitude with which the quadrupole interaction is modulated by such motions. Alternatively, the observed decrease in $T_{2e}$ could indicate that the protein is increasing the correlation time, and thus the contribution to liquid crystal transverse relaxation rate, of a faster motion such as rotational diffusion or chain orientational fluctuations. In the gel phase, the reduction in $T_{2e}$ indicates that SP-C in the bilayer interferes with the freezing out of motions that contribute to transverse relaxation in this phase. Fig. 8 *B* shows that the presence of 5 mM $Ca^{2+}$ in the

aqueous phase substantially alters the way in which SP-C influences the motions responsible for transverse relaxation. In both the liquid crystal and gel phases, the presence of $Ca^{2+}$ reduces the extent to which SP-C lowers $T_{2e}$. In the gel phase, $Ca^{2+}$ appears to fully remove the ability of SP-C to influence transverse relaxation. This is particularly surprising, in light of the observation that $Ca^{2+}$ does not significantly alter the properties of the bilayer in the absence of SP-C, except for a shift in the transition temperature. One possible explanation is that the interaction with $Ca^{2+}$, which shifts the bilayer transition, may also make it more difficult for SP-C to be accommodated in the mixed bilayer gel phase. Removal of the effect of SP-C on gel phase transverse relaxation might thus reflect a $Ca^{2+}$-induced partial separation of bilayer components at the transition. It is interesting to note that the presence of $Ca^{2+}$ in the aqueous subphase has been observed to weaken the interaction between DPPG and SP-C in spread monolayers (Taneva and Keough, 1994).

The observation that there is some selectivity of interaction between SP-C and PG in the gel phase may have consequences for bilayer rearrangement and the bilayer-to-monolayer transition in surfactant in situ. The observation that calcium "removes" the influence of SP-C on lipid motions in the gel phase may also be of consequence in situ. Inside the cell, the surfactant lipid-protein complex exists in tightly packed bilayer arrays known as lamellar bodies. When these lamellar bodies are secreted into the hypophase, they become more loosely packed, transform into unusual packing arrays called tubular myelin, and form films at the air-water interface. As the surfactant transits from inside to outside the cell, the free calcium concentration changes from the micromolar to the millimolar ranges. Changes in surfactant protein-lipid interactions caused by the change in calcium concentration might play a role in initiating this extracellular transformation of surfactant.

## CONCLUSIONS

Porcine pulmonary surfactant-associated protein SP-C has been found to have a similar effect on the chain order and phase behavior of DPPC-$d_{62}$ and DPPG-$d_{62}$ in bilayers containing a single lipid component. In both cases, SP-C broadened the transition slightly, but had little effect on chain order in the liquid crystalline phase. In DPPC/DPPG (7:3) mixed bilayers with one or the other component deuterated, SP-C was found to have a stronger effect, in the gel phase, on DPPG than on DPPC. This may be due to a departure from random lateral distribution of the lipid components due to interaction with the protein. The protein was found to influence lipid motions in such a way as to increase the mean transverse relaxation rate in both the liquid crystal and gel phases. In the presence of 5 mM $Ca^{2+}$ in the buffer, SP-C was found to have no significant effect on transverse relaxation in the gel phase of DPPC/DPPG (7:3) mixed bilayers.

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