Eur Biophys J (2004) 33: 285–290
DOI 10.1007/s00249-003-0357-0

ARTICLE

**Michael R. Morrow · June Stewart · Svetla Taneva
Awel Dico · Kevin M. W. Keough**

Perturbation of DPPC bilayers by high concentrations of pulmonary surfactant protein SP-B

Received: 8 April 2003 / Accepted: 28 August 2003 / Published online: 23 September 2003

Abstract Deuterium (^{2}H) NMR has been used to observe perturbation of dipalmitoylphosphatidylcholine (DPPC) bilayers by the pulmonary surfactant protein B (SP-B) at concentrations up to 17% (w/w). Previous ^{2}H NMR studies of DPPC/dipalmitoylphosphatidylglycerol (DPPG) (7:3) bilayers containing up to 11% (w/w) SP-B and DPPC bilayers containing up to 11% (w/w) synthetic SP-B indicated a slight effect on bilayer chain order and a more substantial effect on motions that contribute to decay of quadrupole echoes obtained from bilayers of deuterated DPPC. This is consistent with the perturbation of headgroup-deuterated DPPC reported here for bilayers containing 6 and 9% (w/w) SP-B. For the higher concentrations of SP-B investigated in the present work, ^{2}H NMR spectra of DPPC deuterated in both the headgroup and chain display a prominent narrow component consistent with fast, large amplitude reorientation of some labeled lipid. Similar spectral perturbations have been reported for bilayers in the presence of the antibiotic polypeptide nisin. The observation of large amplitude lipid reorientation at high SP-B concentration could indicate that SP-B can induce regions of high bilayer curvature and thus provides some insight into local interaction of SP-B with DPPC. Such local interactions may be relevant to the formation, in vitro and in vivo, of tubular myelin, a unique structure found in extracellular pulmonary surfactant, and to the delivery of surfactant material to films at the air–water interface.

Keywords Pulmonary surfactant protein · SP-B · Deuterium NMR · Model membrane

Abbreviations DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine · DPPG: 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol · DPPC-d_{62}: 1,2-perdeutero-dipalmitoyl-*sn*-glycero-3-phosphocholine · DPPC-d_4: 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(α,β perdeutero)-choline

M. R. Morrow (✉) · A. Dico
Department of Physics and Physical Oceanography,
Memorial University of Newfoundland,
St. John's, NF, A1B 3X7, Canada
E-mail: myke@physics.mun.ca
Tel.: 709-737-4361
Fax: 709-737-8739

J. Stewart · S. Taneva
Department of Biochemistry,
Memorial University of Newfoundland,
St. John's, NF, A1B 3X9, Canada

K. M. W. Keough
Department of Biochemistry and Discipline of Pediatrics,
Memorial University of Newfoundland,
St. John's, NF, A1B 3X9, Canada

Introduction

In lungs, surface tension at the air–water interface is reduced by the presence of a surfactant layer derived from a lamellar protein–lipid complex secreted into the alveolar hypophase by type II cells. Approximately 80% of this material, by weight, is accounted for by phospholipids including DPPC, which represents ~40% of the total phospholipid, and PG, which accounts for up to 10% of the phospholipid (Yu et al. 1983; Goerke 1998; Veldhuizen et al. 1998). Proteins account for 5–10% of the total surfactant weight.

Surfactant protein B (SP-B), a 79-residue hydrophobic protein, is an essential requirement for effective surfactant function. It forms disulfide-linked homodimers with regions of amphipathic α-helix and a net charge of +12 (Curstedt et al. 1990; Hawgood et al. 1998). The resistance of SP-B to thermal denaturation is demonstrated by the similarity of its circular dichroism spectra for 90 and 20 °C (Andersson et al. 1995). The extent to which SP-B inserts into phospholipid bilayers has been reported to depend on the way in which mixtures are reconstituted (Cruz et al. 1998). SP-B has been found to promote lipid mixing and fusion of vesicles containing anionic phospholipids (Poulain et al. 1992).

Deuterium (^{2}H) NMR was previously used to observe the motions of chain-deuterated lipids in dipalmitoylphosphatidylcholine (DPPC) bilayers containing synthetic SP-B (Morrow et al. 1993a) and DPPC/dipalmitoylphosphatidylglycerol (DPPG) (7:3) bilayers containing SP-B from porcine lung extract (Dico et al. 1997). These studies showed that SP-B interacts similarly with DPPC and DPPG and that, for concentrations up to 11% (w/w), SP-B has little effect on bilayer chain order in the liquid-crystalline phase. The presence of SP-B did eliminate coexistence of liquid crystal and gel domains near the bilayer transition, and resulted in the change from liquid crystal to gel proceeding continuously as the temperature was lowered. SP-B also had a substantial effect on motions that determine the decay of quadrupole echoes from deuterons on labeled lipids in protein–lipid mixtures.

Natural surfactant is a complex mixture of proteins and lipids. If interactions between specific components can result in nonuniform distributions, then local concentrations of a particular component might deviate from the observed physiological average for that component. In this work, we report ^{2}H NMR observations of chain-deuterated and headgroup-deuterated DPPC in bilayers containing SP-B concentrations up to 17% (w/w). These observations suggest that high local concentrations of SP-B might substantially perturb bilayer organization and dynamics by inducing regions of high bilayer curvature. This could be relevant to understanding how protein–lipid interactions within the surfactant complex contribute to the transformation of material from bilayer morphology to structures with surface activity.

Materials and methods

Lipids

The chain-perdeuterated phospholipids DPPC-d_{62} and choline-labeled DPPC-d_4 were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and were used without further purification.

Protein preparation

SP-B

Surfactant protein B was prepared from porcine lung lavage fluid (Curstedt et al. 1988; Taneva and Keough 1994; Taneva and Keough 1995). The protein was isolated from lipid extracts of surfactant using gel exclusion chromatography on Sephadex LH-60 in chloroform/methanol (1:1 v/v) plus 2% by volume 0.1 M HCl.

Analytical methods and sample preparation

Concentrations of SP-B were estimated by the fluorescamine method using bovine serum albumin as standard (Udenfriend et al. 1972) and were verified by amino acid analysis (Sarin et al. 1990). Lipid concentrations were determined by phosphorus analysis (Bartlett 1959; Keough and Kariel 1987).

Samples containing lipid and SP-B were first codissolved in chloroform/methanol (1:1 v/v). Solutions were dried, evacuated overnight in the presence of P_2O_5, and then hydrated in buffer containing 135mM NaCl and 15mM HEPES at pH 7.0 as described by Dico et al. (1997).

Deuterium NMR

Wideline ^{2}H NMR observations were performed using a locally constructed spectrometer and a 3.55-T superconducting solenoid. Spectra were obtained using a quadrupole echo pulse sequence (Davis et al. 1976) with $\pi/2$ pulse lengths ranging from 3.7–4.8 μs. The number of transients averaged was typically 30,000–40,000 per spectrum, but ranged from 12,000 to 80,000 depending on the amount of deuterated material present in a given sample. For samples containing chain-deuterated lipids, the $\pi/2$ pulses of the quadrupole echo sequence were typically separated by 35 μs and the free-induction decays were oversampled by a factor of two (Prosser et al. 1991) to give effective dwell times of 4 and 2 μs for liquid-crystalline and gel-phase samples, respectively. Spectra were symmetrized by zeroing the imaginary channel before Fourier transformation. Spectra of choline-deuterated lipids were obtained with a $\pi/2$ pulse separation of 50 μs and an effective dwell time, after oversampling, of 10 μs. For echo decay experiments, the pulse separations were varied to provide the ranges implicit in each decay shown. For a given sample, the number of transients used to obtain a spectrum was typically 2–3 times larger than the number averaged in the corresponding echo decay experiment. For each series of measurements, the sample was first warmed to 60 °C. Spectra and echo decay times were then obtained for a series of decreasing temperatures. The sample was allowed to equilibrate at each temperature for at least 30 min before the start of data acquisition. The conformation of SP-B is expected to be stable over the range of temperatures used in this work (Andersson et al. 1995).

Results

Figure 1 shows ^{2}H NMR spectra for choline-labeled DPPC-d_4 bilayers containing SP-B up to 17% by weight. In the absence of protein, three doublets can be seen in the highest temperature spectra. The shapes of these doublets are characteristic of fast axially symmetric reorientation. For multilamellar vesicle samples, the axis of symmetry for reorientation is typically the bilayer normal, and the prominent edges of the doublet arise from molecules reorienting about bilayer normals that are perpendicular to the applied magnetic field. The splitting of a given doublet is proportional to the orientational order parameter, $S_{CD} = (3\cos^2\theta - 1)/2$, where θ is the angle between the CD bond and the axis about which the molecule is reorienting, and the average is over motions of the CD bond that modulate the quadrupole interaction with correlation times that are short relative to the characteristic time scale ($\sim 10^{-5}$ s) of the ^{2}H NMR measurement (Davis 1983).

The two largest splittings in the DPPC-d_4 liquid crystal spectra correspond to the α and β choline deuterons. The smallest splitting arises from slight labeling of choline methyl groups. Between 42 and 41 °C, there is an abrupt transition from fast axially-symmetric reorientation in the liquid-crystalline phase to slower reorientation in the gel phase.

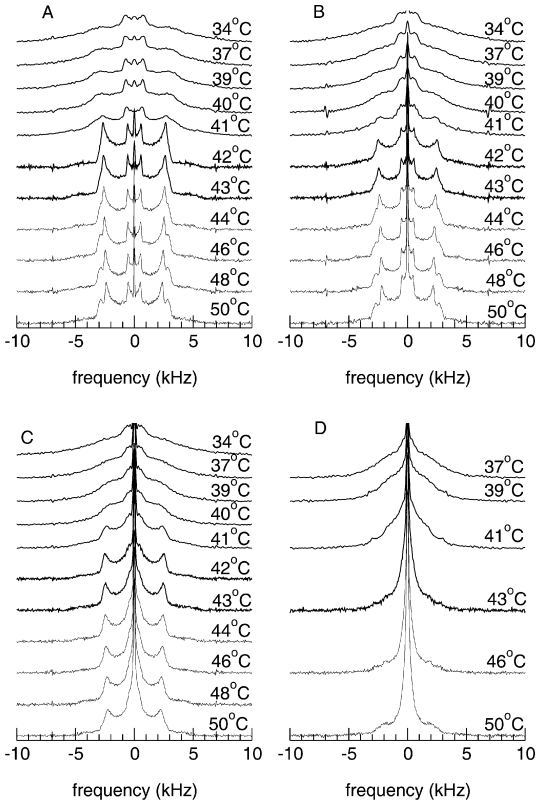

Fig. 1a–d Deuterium NMR spectra for bilayers of choline-labeled DPPC-d_4 containing **a** no SP-B, **b** 6% SP-B, **c** 9% SP-B, and **d** 17% SP-B by mass. Where they can be resolved, the largest and second largest splittings correspond to the α and β choline deuterons, respectively. The small doublet with $\sim$1 kHz splitting arises from partial deuteration of choline methyl groups. Samples were hydrated in buffer containing 135mM NaCl plus 15mM HEPES

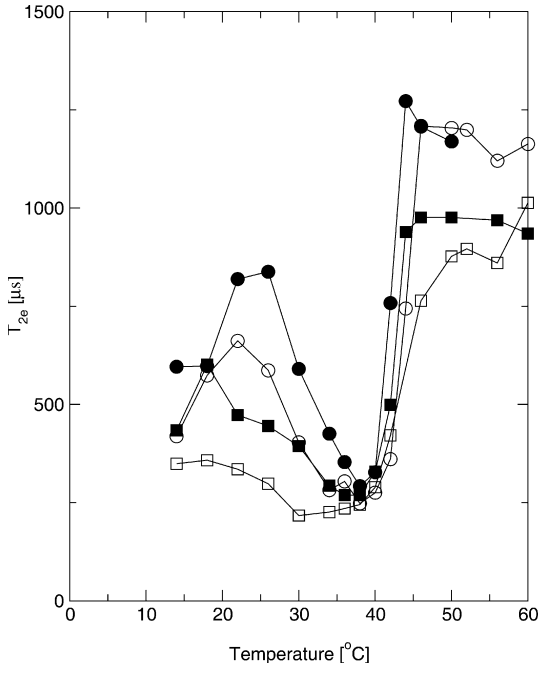

Fig. 2 Quadrupole echo decay times (T_{2e}) for DPPC-d_4 containing ● no SP-B, ○ 6% SP-B, ■ 9% SP-B, and □ 17% SP-B by mass. Samples were hydrated in buffer containing 135mM NaCl plus 15mM HEPES

The splittings of α and β choline deuterons are known to undergo counterdirectional shifts in response to changes in average headgroup orientation resulting from electrostatic or steric interactions (Seelig et al. 1987; Fiech et al. 1998). The small changes in α and β deuteron splittings upon addition of 6% SP-B likely reflect a slight change in average headgroup orientation upon binding of the protein. The more substantial departure of the lineshape from a superposition of well-defined doublets with increasing SP-B concentration suggests a significant perturbation of lipid motion at higher SP-B concentration.

As the concentration of SP-B is increased, the change from liquid crystal to gel becomes more continuous. The prominent edges of the α and β doublets broaden and become indistinguishable but the width of the corresponding feature remains typical of bilayer organization. This implies a change in the correlation time, but not the amplitude of the headgroup motions, for those molecules contributing to the residual normal bilayer feature. At the same time, the striking emergence of a very narrow spectral component with increasing SP-B concentration suggests that some molecules are undergoing more isotropic reorientation as the SP-B concentration increases.

Figure 2 shows quadrupole echo decay times for the choline-labeled samples. The general temperature and protein concentration dependences of the echo decay times are qualitatively similar to what has been observed previously for signals from chain-deuterated and headgroup-deuterated lipid in bilayers containing pulmonary surfactant protein C (SP-C) (Simatos et al. 1990; Morrow et al. 1993b). Deuterons precessing after an initial $\pi/2$ radio frequency pulse dephase due to heterogeneity of the orientation-dependent quadrupole interaction. Dephasing is reversed by a second $\pi/2$ pulse at time τ and an echo forms at time 2τ. Motions which alter the orientation-dependent quadrupole interaction during the time 2τ contribute to echo decay. For motions with correlation times (τ_c) that are short compared to the inverse spectral width, the contribution to the echo decay rate is proportional to τ_c (Abragam 1961). For slow reorientations, the contribution to the echo decay rate is proportional to τ_c^{-1} (Pauls et al. 1985). Just above the liquid crystal to gel transition, slow long-range motions, such as bilayer undulation or diffusion across bilayer curvature, and faster local lipid motions, such as reorientation about the bilayer normal or internal lipid motions, can both contribute to the echo decay rate (Bloom and Sternin 1987; Bloom and Evans 1991; Stohrer et al. 1991). On cooling through the transition, the correlation times for all motions are expected to increase. For motions that are already in the slow regime in the liquid-crystalline phase, the contribution to echo decay will decrease on cooling into the gel phase. For faster local motions, the increase in correlation time on cooling into the gel phase will result in an increased contribution to

echo decay. The transition into the gel phase may result in local motions, such as reorientation about the bilayer normal or rotation of lipid segments about bonds, slowing such that their contributions to the echo decay rate become inversely proportional to τ_c. If so, the contributions to echo decay from such motions will decrease as temperature is reduced further. The observed increase in echo decay time with decreasing temperature just below the liquid crystal to gel transition likely reflects further slowing of such local motions (Kilfoil and Morrow 1998).

Figure 2 shows that the average headgroup deuteron echo decay times in the liquid-crystalline phase range from ~1200 μs for the pure lipid to ~900 μs for the highest SP-B concentration. These average decay times are slightly longer than what is observed in the liquid-crystalline phase for dimyristoylphosphatidylcholine chain deuterons in the absence of protein, and substantially longer than what is observed for chain deuterons in the presence of protein (Simatos et al. 1990).

Figure 3 shows ^{2}H NMR spectra for chain-perdeuterated DPPC-d_{62} containing 15% SP-B (w/w). Selected spectra from DPPC-d_{62} alone are shown for comparison. In the absence of protein, the liquid-crystalline spectra of DPPC-d_{62} are superpositions of Pake doublets arising from fast, axially symmetric reorientation of CD bonds with respect to the bilayer normal. The distribution of splittings reflects the dependence of orientational order parameter on position along the acyl chain, that characterises the liquid-crystalline phase of saturated phospholipid bilayers. Below the sharp transition into gel phase, DPPC-d_{62} spectra are characteristic of reorientation that is of intermediate rate and less axially symmetric on the time scale of the measurement.

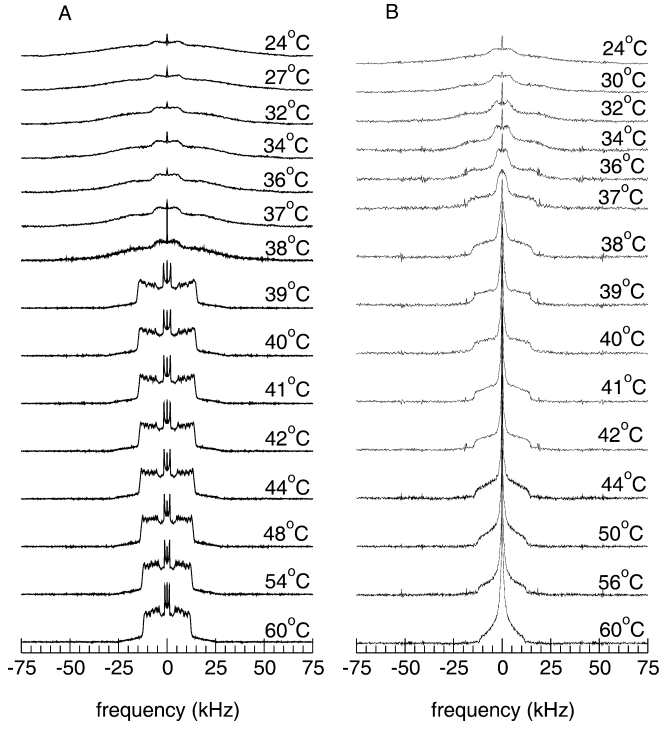

Fig. 3a, b Deuterium NMR spectra at selected temperatures for **a** DPPC-d_{62} bilayers and **b** DPPC-d_{62} bilayers containing 15% SP-B by weight. Some of the DPPC-d_{62} spectra (*panel A*) were shown, in a different form, in Kilfoil and Morrow (1998). Samples were hydrated in buffer comprising 135mM NaCl plus 15mM HEPES

The DPPC-d_{62} spectra at high SP-B concentration are very similar to the headgroup deuteron spectra under similar conditions. They are also extremely similar to spectra reported by El Jastimi et al. (1999) for DPPC-d_{62} bilayers in the presence of the antibiotic polypeptide nisin at a polypeptide to lipid ratio of 0.2. The narrow central feature is characteristic of fast, large amplitude reorientation. Intensity decreases continuously from the central feature out to a splitting comparable to that of the spectrum for protein-free DPPC-d_{62}, but the prominent edges characteristic of fast axially symmetric reorientation in liquid crystal DPPC-d_{62} bilayers are absent. Doublets arising from specific methylene deuterons along the acyl chain cannot be resolved.

Figure 4 shows the temperature dependence of DPPC-d_{62} first spectral moments and quadrupole echo decay times at high SP-B concentration. First moments for DPPC-d_{62} alone are shown for comparison. For a spectrum $f(\omega)$, the first spectral moment, $M_1 = \int_0^\infty \omega f(\omega) d\omega / \int_0^\infty f(\omega) d\omega$ is proportional to the orientational order parameter averaged over all deuterated segments. The presence of 15% (w/w) SP-B substantially reduces DPPC-d_{62} chain order in both the liquid crystal and gel phases. This differs from the effect of SP-B at lower concentrations, where it has little effect on liquid crystal chain order and induces a slight decrease in gel phase chain order (Morrow et al. 1993a; Dico et al. 1997).

The quadrupole echo decay times for DPPC-d_{62} containing 15% (w/w) SP-B are shorter than those typically observed from DPPC-d_{62} alone (Kilfoil and Morrow 1998) but do display a qualitatively similar temperature dependence. In the absence of protein, quadrupole echo decay times in the liquid-crystalline phase of DPPC-d_{62} are typically between 800 and 1,000 μs. The effect of SP-B on quadrupole echo decay time in the liquid-crystalline phase thus appears to be more pronounced for chain deuterons than for headgroup deuterons.

Discussion

Previous ^{2}H NMR observations of DPPC and DPPC/DPPG (7:3) bilayers containing SP-B showed that, for protein concentrations up to 11% (w/w), SP-B perturbs DPPC chain order only slightly in the liquid-crystalline phase (Morrow et al. 1993a; Dico et al. 1997). The observations reported here suggest that at higher concentrations, SP-B can have a substantially greater effect. If local concentrations of surfactant components can depart from average values found in natural surfactant,

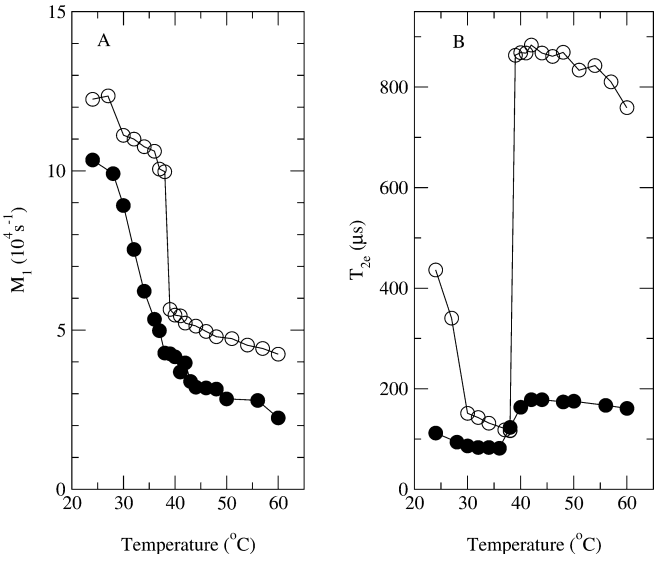

Fig. 4a, b Temperature dependence of **a** first spectral moments (M_1) and **b** quadrupole echo decay times for • DPPC-d_{62} bilayers containing 15% SP-B by weight. First spectral moments and quadrupole echo decay times for ○ DPPC-d_{62} alone, taken from Kilfoil and Morrow (1998), are shown for comparison. Samples were hydrated in buffer comprising 135mM NaCl plus 15mM HEPES

perturbation of bilayer properties by high concentrations of SP-B might be directly relevant to understanding how protein–-lipid interactions contribute to the reorganization of material implicit in the maintenance of a surfactant layer in lungs. The most pronounced perturbations observed here provide some insight into the nature of protein–lipid interactions that may be relevant to the formation of tubular myelin-like structures (see below).

The observation of narrow spectral components in the liquid-crystalline phases of different samples containing both headgroup-deuterated and chain-deuterated DPPC at high SP-B concentration suggests that entire molecules undergo large amplitude reorientations in those regions most perturbed by SP-B. The reorientation giving rise to such motional narrowing could result from fast diffusion of molecules through highly curved bilayer regions or from rapid modulation of bilayer orientation by large amplitude undulations. The gel-phase spectra of headgroup and chain deuterons at high SP-B concentration are characteristic of more usual bilayer organization and the narrow component is absent or much smaller. It is thus unlikely that the narrow component observed in the liquid-crystalline phase reflects substantial protein-induced fragmentation of the bilayer into small particles that can reorient rapidly.

In addition to the prominent narrow component, a feature with a width characteristic of the normal liquid-crystalline bilayer phase appears in the spectra of both headgroup-deuterated and chain-deuterated DPPC at high SP-B concentration. The absence of prominent doublet edges corresponding to different methylene segments in either the headgroup or the acyl chain implies that, for molecules contributing to this feature, SP-B perturbs the timescale for reorientation without substantially altering the amplitude of reorientation or local bilayer organization. This is consistent with the reduction in quadrupole echo decay time observed for both chain and headgroup deuterons in the presence of SP-B.

Decay of the quadrupole echo signal from deuterated lipids in the liquid-crystalline phase can reflect reorientation due to motions that are fast enough to contribute to motional narrowing or motions, referred to as adiabatic, that are too slow to contribute to motional narrowing (Bloom and Sternin 1987). The contribution to quadrupole echo decay rate from a fast motion is proportional to both the correlation time for that motion and the second moment of that part of the quadrupole interaction modulated by that motion. The enhanced amplitude of reorientation responsible for the emergence of a narrow spectral component likely accounts for at least part of the protein-induced reduction in quadrupole echo decay time for the liquid-crystalline phase.

These observations at high SP-B concentration are consistent with the existence of bilayer regions having varying degrees of increased local curvature. Rapid diffusion of lipid molecules across the most highly curved portions of the bilayer could result in the enhanced motional narrowing, reflected by the narrow components of the headgroup-deuteron and chain-deuteron spectra for high SP-B concentration. Slower reorientation resulting from diffusion over less highly curved regions could reduce the quadrupole echo decay time, and thus the resolution of residual bilayer spectral features, without contributing substantially to motional narrowing of the lipid spectra.

It is interesting that the spectra observed in this study for DPPC-d_{62} bilayers containing 15% SP-B (w/w) are so similar to those reported by El Jastimi et al. (1999) for DPPC-d_{62} bilayers in the presence of nisin at a polypeptide to lipid ratio of 0.2. Like the spectra reported here for high SP-B concentration, the DPPC-d_{62}/nisin spectrum for $R = 0.2$ displayed a narrow feature characteristic of isotropic molecular reorientation and the remnant of a more normal lipid bilayer spectrum with individual methylene doublets broadened to the extent of being unresolvable (El Jastimi et al. 1999). The authors of the DPPC-d_{62}/nisin study interpreted the narrow feature and the short quadrupole echo decay time as being due to lipid diffusion across highly curved bilayer planes. This interpretation was supported by the observation of thread-like aggregates and globular structures in electron micrographs of samples with the same composition as the sample from which the NMR spectrum was obtained.

The observations reported here suggest that at high concentrations, SP-B may also promote formation of highly curved bilayer regions. Such perturbation of bilayer morphology by SP-B at high concentration may provide some insight into how surfactant protein promotes the reorganization of bilayer material, initially

secreted as lamellar bodies, into surface active material at the alveolar air–water interface. The unusual structural form of extracellular surfactant called tubular myelin, when observed in vivo and in vitro, displays areas of apparently intersecting or abutting bilayers with very high curvature (Suzuki et al. 1989; Williams et al. 1991). A local increase of SP-B abundance in these areas could correlate with the types of perturbations that we have observed in this work for high SP-B concentrations.

Acknowledgements K.W.M.K. is Chief Scientist, Health Canada, Ottawa, Ontario, Canada. This research was supported by grants from the Natural Science and Engineering Research Council of Canada to M.R.M. and from the Canadian Institutes of Health Research to K.M.W.K. The authors are grateful to Dr. Maria Kilfoil for DPPC-d_{62} data used for comparison in this work.

References

Abragam A (1961) The principles of nuclear magnetism. Oxford University Press, London

Andersson M, Curstedt T, Jörnvall H, Johansson J (1995) An amphipathic helical motif common to tumorolytic polypeptide NK-lysin and pulmonary surfactant polypeptide SP-B. FEBS Lett 362:328–332

Bartlett G (1959) Phosphorus assay in column chromatography. J Biol Chem 234:466–468

Bloom M, Evans E (1991) Observation of surface undulations on the mesoscopic length scale by NMR. In: Peliti L (ed) Biologically inspired physics. Plenum, New York, pp 137–147

Bloom M, Sternin E (1987) Transverse nuclear spin relaxation in phospholipid bilayer membranes. Biochemistry 26:2101–2105

Cruz A, Casals C, Plasencia I, Marsh D, Pérez-Gil J (1998) Depth profiles of pulmonary surfactant B in phosphatidylcholine bilayers studied by fluorescence and electron spin resonance spectroscopy. Biochemistry 37:9488–9496

Curstedt T, Johansson J, Barros-Soderling J, Robertson B, Nilsson G, Westberg M, Jornvall H (1988) Low-molecular-mass surfactant protein type 1. The primary structure of a hydrophobic 8-kDa polypeptide with eight half-cystine residues. Eur J Biochem 172:521–525

Curstedt T, Johansson J, Persson P, Eklund A, Robertson B, Löwenadler B, Jörnvall H (1990) Hydrophobic surfactant-associated polypeptides: SP-C is a lipopeptide with two palmitoylated cysteine residues, whereas SP-B lacks covalently linked fatty acyl groups. Proc Natl Acad Sci USA 87:2985–2989

Davis J, Jeffrey K, Bloom M, Valic M, Higgs T (1976) Quadrupole echo deuteron magnetic resonance spectroscopy in ordered hydrocarbon chains. Chem Phys Lett 42:390–394

Davis J (1983) The description of membrane lipid conformation, order and dynamics by ^{2}H-NMR. Biochim Biophys Acta 737:117–171

Dico A, Hancock J, Morrow M, Stewart J, Harris S, Keough K (1997) Pulmonary surfactant protein SP-B interacts similarly with dipalmitoylphosphatidylglycerol and dipalmitoylphosphatidylcholine in phosphatidylcholine/phosphatidylglycerol mixture. Biochemistry 36:4172–4177

El Jastimi R, Edwards K, Lafleur M (1999) Characterization of permeability and morphological perturbations induced by nisin on phosphatidylcholine membranes. Biophys J 77:842–852

Fiech D, Bonev B, Morrow M (1998) Effect of pressure on dimyristoylphosphatidylcholine headgroup dynamics. Phys Rev E 57:3334–3343

Goerke J (1998) Pulmonary surfactant: functions and molecular composition. Biochim Biophys Acta 1408:79–89

Hawgood S, Derrick M, Poulain F (1998) Structure and properties of surfactant protein B. Biochim Biophys Acta 1408:150–160

Keough K, Kariel N (1987) Differential scanning calorimetric studies of aqueous dispersions of phosphatidylcholines containing two polyenoic chains. Biochim Biophys Acta 902:11–18

Kilfoil M, Morrow M (1998) Slow motions in bilayers containing anionic phospholipids. Physica A 261:82–94

Morrow M, Pérez-Gil J, Simatos G, Boland C, Stewart J, Absolom D, Sarin V, Keough K (1993a) Pulmonary surfactant-associated protein SP-B has little effect on acyl chains in dipalmitoylphosphatidylcholine dispersions. Biochemistry 32:4397–4402

Morrow M, Taneva S, Simatos G, Allwood L, Keough K (1993b) ^{2}H NMR studies of the effect of pulmonary surfactant SP-C on the 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine headgroup: a model for transbilayer peptides in surfactant and biological membranes. Biochemistry 32:11338–11344

Pauls K, MacKay A, Söderman O, Bloom M, Tangea A, Hodges R (1985) Dynamic properties of the backbone of an integral membrane polypeptide measured by ^{2}H-NMR. Eur Biophys J 12:1–11

Poulain F, Allen L, Williams M, Hamilton R, Hawgood S (1992) Effects of surfactant apolipoproteins on liposome structure: implications for tubular myelin formation. Am J Physiol 262 (Lung Cell Mol Physiol 6):L730–L739

Prosser R, Davis J, Dahlquist F, Lindorfer M (1991) ^{2}H nuclear magnetic resonance of the gramicidin A backbone in a phospholipid bilayer. Biochemistry 30:4687–4696

Sarin V, Gupta S, Leung T, Taylor V, Ohning B, Whitsett J, Fox J (1990) Biophysical and biological activity of a synthetic 8.7 kDa hydrophobic pulmonary surfactant protein SP-B. Proc Natl Acad Sci USA 87:2633–2637

Seelig J, Macdonald P, Scherer P (1987) Phospholipid head groups as sensors of electric charge in membranes. Biochemistry 26:7535–7541

Simatos G, Forward K, Morrow M, Keough K (1990) Interaction between perdeuterated dimyristoylphosphatidylcholine and low molecular weight pulmonary surfactant protein SP-C. Biochemistry 29:5807–5814

Stohrer J, Gröbner G, Reimer D, Weisz K, Mayer C, Kothe G (1991) Collective lipid motions in bilayer membranes studied by transverse deuteron spin relaxation. J Chem Phys 95:672–678

Suzuki Y, Fujita, Y, Kogishi K (1989) Reconstitution of tubular myelin from synthetic lipids and proteins associated with pig pulmonary surfactant. Am Rev Respir Dis 140:75–81

Taneva S, Keough K (1994) Pulmonary surfactant proteins SP-B and SP-C in spread monolayers at the air–water interface. I. Monolayers of pulmonary surfactant protein SP-B and phospholipids. Biophysical J 66:1137–1148

Taneva S, Keough K (1995) Calcium ions and interactions of pulmonary surfactant proteins SP-B and SP-C with phospholipids in spread monolayers at the air/water interface. Biochim Biophys Acta 1236:185–195

Udenfriend S, Stein S, Bohlen P, Dairman W, Loimgrukes W, Weigele M (1972) Fluorescamine: a reagent for assay of amino acids, peptides, and primary amines in the picomole range. Science 178:871–872

Veldhuizen R, Nag K, Argeig S, Possmayer F (1998) The role of lipids in pulmonary surfactant. Biochim Biophys Acta 1408:90–108

Williams M, Hawgood S, Hamilton R (1991) Changes in lipid structure produced by surfactant proteins SP-A, SP-B, and SP-C. Am J Respir Cell Mol Biol 5:41–50

Yu S, Smith N, Harding P, Possmayer F (1983) Bovine pulmonary surfactant: chemical composition and physical properties. Lipids 18:522–529