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Structure and Orientation of Lung Surfactant SP-C and L-α-Dipalmitoylphosphatidylcholine in Aqueous Monolayers

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ABSTRACT SP-C, a pulmonary surfactant-specific protein, aids the spreading of the main surfactant phospholipid L-α-dipalmitoylphosphatidylcholine (DPPC) across air/water interfaces, a process that has possible implications for in vivo function. To understand the molecular mechanism of this process, we have used external infrared reflection-absorption spectroscopy (IRRAS) to determine DPPC acyl chain conformation and orientation as well as SP-C secondary structure and helix tilt angle in mixed DPPC/SP-C monolayers in situ at the air/water interface. The SP-C helix tilt angle changed from ~24° to the interface normal in lipid bilayers to ~70° in the mixed monolayer films, whereas the acyl chain tilt angle of DPPC decreased from ~26° in pure lipid monolayers (comparable to bilayers) to ~10° in the mixed monolayer films. The protein acts as a "hydrophobic lever" by maximizing its interactions with the lipid acyl chains while simultaneously permitting the lipids to remain conformationally ordered. In addition to providing a reasonable molecular mechanism for protein-aided spreading of ordered lipids, these measurements constitute the first quantitative determination of SP-C orientation in Langmuir films, a paradigm widely used to simulate processes at the air/alveolar interface.

INTRODUCTION

Pulmonary surfactant, a lipid/protein mixture that lines the air-alveolar interface, is generally thought to exist in vivo as a monomolecular film that lowers surface tension (γ) to near-zero values, a requirement essential for normal breathing. There are two apparently mutually exclusive attributes of this material that any molecular description must reconcile. The main phospholipid component of surfactant, L-α-dipalmitoylphosphatidylcholine (DPPC), is indeed able to sustain high surface pressures (i.e., low γ) in monolayer films at the air/water (A/W) interface under conditions of film compression. However, the spreading of gel-phase (conformationally ordered) DPPC at the A/W interface occurs far too slowly to be effective at the rates required in vivo (Goerke and Clements, 1986). Two small hydrophobic surfactant-specific proteins, SP-B and SP-C, have been shown to promote spreading of the surface-active phospholipids (Wang et al., 1996b; Johansson et al., 1994a; Weaver and Whitsett, 1991; Hawgood, 1989; Oosterlaken-Dijksterhuis et al., 1991a,b), although the molecular basis for this action remains obscure. A thorough understanding of the role of these proteins in the facilitation of spreading will aid the rational design of therapeutic agents in pathological conditions such as respiratory distress syndrome.

As monomolecular films at the air/alveolar interface in situ are extremely difficult to study, Langmuir films at the A/W interface have been adopted as experimental paradigms for investigating the interaction of pulmonary surfactant components. Nevertheless, molecular-level information about surfactant protein/lipid interactions that facilitate the spreading process is sparse, because of the lack of spectroscopic approaches that provide conformational and orientational information about the film components. As a prelude to monolayer studies, bulk-phase measurements have contributed useful background structural information about SP-C/lipid interaction. The protein, a hydrophobic, S-palmitoylated 33–35-amino acid chain proteolytically generated from 21-kDa precursor, assumes a predominantly (60–70%) α-helical structure in bilayers with a helix orientation slightly tilted from the normal to the bilayer plane (i.e., transbilayer orientation; Pastrana et al., 1991; Vandenbussche et al., 1992). The more polar amino terminal is assumed to be unordered and located closer to the membrane surface because of the presence of two proline residues at positions 4 and 7 and three positively charged residues at positions 2, 11, and 12. In addition, two cysteine residues at positions 5 and 6 are covalently linked through thiol esters to two palmitoyl groups, suggesting interaction with the lipid acyl chains.

Monolayer studies with surfactant proteins have included surface pressure-area (π-A) isotherms allowing for the characterization of surface thermodynamics, whereas microscopic techniques have been employed to determine domain sizes and shapes (Lipp et al., 1996; Wang et al., 1996a; Post et al., 1995; Taneva and Keough, 1994). For example, the domain size of liquid condensed lipid monolayers was found to be significantly reduced by SP-C (Perez-Gil et al., 1992; Nag et al., 1996).

To acquire molecular-level understanding of the means by which SP-C facilitates the spreading of phospholipids in monolayer films, we have applied the relatively new technique of external infrared-reflection absorption spectroscopy (IRRAS). Developed by Dluhy and co-workers for the study of aqueous monolayers (Dluhy, 1986), IRRAS has recently been extended to provide quantitative information about molecular functional group orientation (Flach et al.,

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1997), in addition to the structural information inherent in the IR frequencies (Mendelsohn et al., 1995). The IRRAS approach is thus ideally suited to the study of SP-C/phospholipid interaction. The current report describes the orientation of both components in mixed monolayer films of DPPC with SP-C. Use of isotopically labeled *sn*-2-^{13}C=O DPPC also permits measurement of the orientation of each DPPC C=O bond. Our experiments reveal a large change in the tilt angle of the SP-C helix tilt away from the interface normal during bulk phase to monolayer transitions, and lead to a tentative explanation for SP-C facilitation of phospholipid spreading at the A/W interface.

MATERIALS AND METHODS

Materials

L-α-DPPC and acyl chain perdeuterated DPPC (DPPC-d_{62}) were purchased from Avanti Polar Lipids (Alabaster, AL). Isotopically labeled *sn*-2-^{13}C=O DPPC was generously provided by Dr. Ruthven Lewis and Prof. Ronald McElhaney (University of Alberta). Highly purified S-palmitoylated porcine SP-C (characterized by matrix-assisted laser desorption/ionization mass spectrometry) was generously provided by Prof. Kevin Keough (Memorial University of Newfoundland). Chloroform, methanol, and sodium chloride were obtained from Fisher Scientific (ACS certified; Pittsburgh, PA). High-performance liquid chromatography-grade H_2O was used (Fisher), and deuterium oxide (D_2O) with 99.9% isotopic enrichment was purchased from Isotec (Miamisburg, OH).

Preparation of samples for IRRAS measurements

DPPC and *sn*-2-^{13}C=O DPPC were dissolved in $CHCl_3$ (~2 mg/ml) for IRRAS measurements of pure DPPC monolayers. Solutions of DPPC/SP-C, *sn*-2-^{13}C=O DPPC/SP-C, or DPPC-d_{62}/SP-C (20:1 mol ratio) were prepared by mixing the appropriate amounts of $CHCl_3$:MeOH (2:1, v:v) solutions of DPPC (2 mg/ml) and SP-C (1 mg/ml). A subphase of high-performance liquid chromatography-grade H_2O was used for the pure DPPC monolayers, whereas 0.15 M NaCl in D_2O was used for DPPC/SP-C films (pD 7.0). It has been shown that the acyl chain methylene stretching bands for DPPC are not effected by the presence of salt in the subphase (Flach et al., 1993).

IRRAS measurements

The temperature of the subphase was 19.0 ± 0.5°C. Typically, 5–10 μl of solution was spread on a surface area of ~83 cm^2. Initial surface pressure values (at maximum surface area) were 2–3 mN/m for the pure DPPC monolayers and 7–10 mN/m for the mixed lipid/protein films. Our current trough design dictates that these pressures are required before the intermittent compression procedure can be used to reach a final surface pressure of ~30 mN/m. After an initial relaxation period of 45 min, the film was compressed discontinously to the desired surface pressure, which took 3–4 h. Before IR data collection the film was allowed to relax for at least 1 h. Details of the instrument design have been described elsewhere (Flach et al., 1997). The polarizer was accessible, so that spectra from perpendicular (s) and parallel (p) polarized radiation were easily acquired from the same monolayer. Typically, 2048 scans were acquired for s-polarization and 4096 scans for p-polarization. Interferograms were collected at 4 cm^{-1} resolution, apodized with a triangular function, and Fourier-transformed with one level of zero filling to yield spectra encoded at 2 cm^{-1} intervals. Experiments were repeated at least five times for each angle of incidence.

Analysis of IRRAS data

Spectra were baseline corrected before peak positions and intensities were determined. Occasionally, residual water vapor bands were subtracted, using an appropriate reference spectrum. Fourier smoothing using a Gaussian lineshape and breakpoint of no less than 0.80 was performed before the determination of carbonyl and amide I band intensities. Peak heights rather than integrated intensities were used to minimize interference from partially overlapped spectral features.

IRRAS spectra are presented as reflectance-absorbance (RA) versus wavenumber (cm^{-1}). RA is defined as $-\log(R/R^F)$, where R is the reflectivity of the film covered surface, and R^F is the reflectivity of the film-free surface. RA values can be positive or negative, depending on the polarization of the incident light, the direction of the dipole moment change for the normal mode of vibration, and the magnitude of the incident angle relative to the Brewster angle. The reflectivity of p-polarized light is minimum at the Brewster angle, resulting in maximum band intensities in the vicinity of and a change in the direction of the band at the Brewster angle. The most accurate orientational information can be obtained close to the Brewster angle. Small amounts of s-polarized light leaking through the polarizer may strongly influence the observed intensities for p-polarization in an angular range of at least ±15° around the Brewster angle. Therefore, the degree of polarization realized must be considered for all calculations of reflectance-absorbances (Flach et al., 1997).

Determination of molecular functional group orientation

The mathematical formalism used for the simulation has been detailed elsewhere (Flach et al., 1997). Briefly, the following parameters are required for the calculations. The optical setup is characterized by the angle of incidence of the incoming beam with respect to the surface normal, ϕ_1, and the overall degree of polarization, Γ. Optical features of the subphase include the real and imaginary parts of the H_2O and D_2O refractive index, n_2 and k_2. These values are known and can be interpolated to the desired stepwidth (Bertie et al., 1989). The real part of the refractive index has been obtained from IR ellipsometric measurements of thin films on solid supports (A. Röseler, unpublished results). The directional extinction coefficients for the monolayer, $k_x = k_y$ and k_z, are obtained using Fraser and MacRae's formalism (Fraser and MacRae, 1973) and are generated for a given tilt angle relative to the surface normal, θ; dipole moment direction relative to the local molecular axis, α; and the film extinction coefficient, k_{max}; for the particular vibration. The magnitude of k_{max} depends on the strength of the oscillation and the density of the film-forming molecules at the A/W interface, which varies throughout the compression of the monolayer. The k_{max} values derived for monolayers at the A/W interface are usually larger than those obtained by ellipsometric measurements of thin films on solid supports. These differences can be attributed to the following shortcomings of the optical model: the presence of the film causes structural changes in the water proximal to the film, extending the region of optical inhomogeneity beyond the thickness of the monolayer (see Blaudez et al., 1996, for a recent discussion); second, the profile of the refractive index in the region of inhomogeneity is unknown. It can be shown that the derived tilt angle is independent of the film thickness when k_{max} is adjusted accordingly, i.e., smaller thicknesses result in larger k_{max} values. The ratio of s- and p-polarized band intensities and, in particular, the curvature for p-polarized band intensities over a range of incident angles are determined by the anisotropy of the optical constants and define the tilt. Other film parameters include the vacuum wavelength of the IR radiation at the band maximum; the full width of the band at half-height; and the monolayer thickness, d, which can be obtained by taking into account the tilt angle for a molecule of known length, L. The angle between the dipole moment and the local molecular axis is known, leaving the tilt angle and k_{max} as unknowns. The tilt angle and k_{max} were determined by comparing measured and calculated RA values at the different angles of incidence for the two polarizations. The whole band contour was simulated and, as with the experimental bands, baseline corrected and offset to zero before intensity measurements.

RESULTS AND DISCUSSION

IRRAS spectra of the methylene stretching region (2800–3000 cm^{-1}) for pure DPPC monolayers in a condensed phase (28 ± 2 mN/m) on an H_2O subphase using s- and p-polarized radiation at three different incident angles are displayed in Fig. 1. The observed frequencies of the asymmetric and symmetric methylene stretching vibrations ($\nu_a(CH_2)$ and $\nu_s(CH_2)$), 2917.9 ± 0.5 and 2849.5 ± 0.3 cm^{-1}, respectively, are indicative of acyl chains in the all-*trans* conformation (Snyder et al., 1978). Thus intensity measurements are suitable for calculation of chain orientation because the acyl chains can be regarded as fully extended (all-*trans*, planar).

In Fig. 2 *A*, the results of simulated and experimental peak intensities for a pure DPPC monolayer are compared for $\nu_a(CH_2)$ on an H_2O subphase. The best fit was achieved for an average acyl chain tilt angle from the surface normal of 26 ± 2°. As a validation of our approach, these calculated tilt angles may be compared with those determined by other physical methods. A recent study using synchrotron x-ray diffraction determined a tilt angle of 25° or 30°, depending on surface pressure for the DPPC acyl chains in a condensed phase (Brezesinski et al., 1995). Previous neutron and x-ray reflection measurements yielded 33 ± 3° for the tilt angle of acyl chain perdeuterated DPPC monolayers at a surface pressure of 42 mN/m (Vaknin et al., 1991; Helm et al., 1987). These values compare favorably with the tilt angle of 30 ± 3° found for hydrated DPPC vesicles in the (bulk) $L_{\beta'}$ phase (Wiener et al., 1989), and with the 28° value determined from attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) studies (Brauner et al., 1987). The relatively slight variation among the monolayer values may arise (aside from experimental uncertainties) from differences in the amount of lipid initially spread on the surface, subphase temperature, and the rates and methods of compression, i.e., continuous versus intermittent (Gericke et al., 1993). The relatively large tilt angle of the DPPC acyl chains reflects the greater cross-sectional area of the choline headgroup compared to the ordered acyl chains.

For mixed DPPC/SP-C monolayers (data not shown) on D_2O subphases at π = 24 ± 2 mN/m, $\nu_a(CH_2)$ is observed at 2917.4 ± 0.5 cm^{-1}. As for pure DPPC monolayers, this value suggests an all-*trans* chain conformation. The chain tilt angle for DPPC in the presence of SP-C, determined from simulations of the $\nu_a(CH_2)$ band intensities for DPPC/SP-C monolayers (Fig. 2 *B*), is 10 ± 5°. To quantify possible interference from the two palmitoyl chains located at the two cysteines of SP-C, mixed monolayers of DPPC-d_{62}/SP-C on both H_2O and D_2O subphases were investigated and showed negligible intensities (in the range of the noise level, i.e., 0.0001 reflectance-absorbance unit) in the methylene stretching region. To summarize, SP-C induces a decrease in the average tilt angle of the phospholipid acyl chains from ~26° to ~10°. It should be noted that the reported tilt angles represent an average of a range of tilt angles that can be found on the surface. The exact tilt angle spread in the monolayer is unknown; however, results from Brewster angle measurements indicate that for DPPC monolayers at high surface pressures, a reasonably narrow distribution of tilt angles exist (Vollhardt, 1996). The error bars in Fig. 2, *A* and *B*, and in similar figures to follow indicate the accuracy of the measured intensities.

To determine tilt angles of the SP-C helix, spectra in the 1600–1800 cm^{-1} region (Fig. 3) were analyzed for DPPC/SP-C samples at several angles of incidence. The spectra acquired at an incident angle of 50° for p-polarized radiation show a greatly reduced signal-to-noise ratio due to the diminution of the reflectivity of p-polarized radiation in the vicinity of the Brewster angle and are therefore excluded from the quantitative analysis. The lipid carbonyl stretching vibrations and protein amide I′ band appear at ~1735 and ~1647 cm^{-1}, respectively. The shoulder at ~1720 cm^{-1} on the former arises from hydrogen-bonded carbonyl groups (Gericke and Hühnerfuss, 1995; Blume et al., 1988). The frequency observed for the protein amide I′ band, along with the absence of amide II intensity, is consistent with a hydrogen-deuterium-exchanged α-helical secondary structure for SP-C (Flach et al., 1994). A predominantly α-heli-

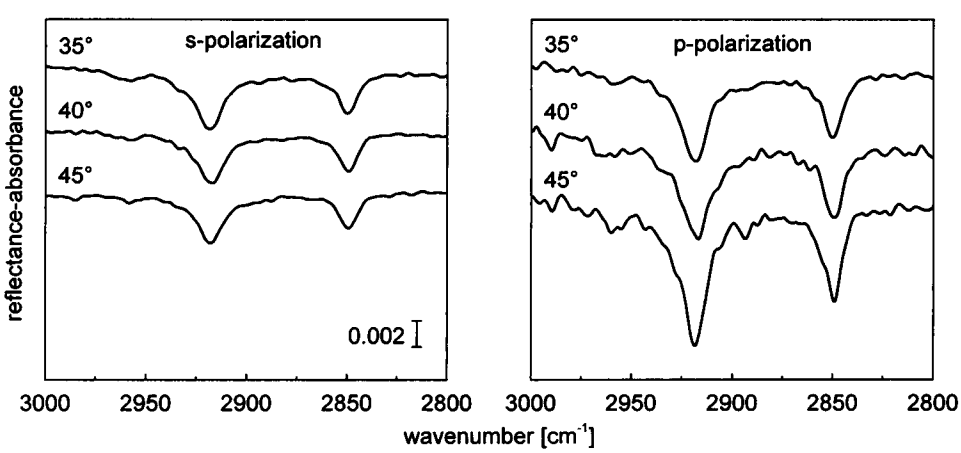

FIGURE 1 IR reflection-absorption spectra of the methylene stretching band region (2800–3000 cm^{-1}) for pure DPPC monolayers at the air/H_2O interface at different angles of incidence for perpendicular (s) and parallel (p) polarized radiation. The data were acquired at a surface pressure of 28 ± 2 mN/m and a temperature of 19.0 ± 0.5°C. The bar reflects the intensity in reflectance-absorbance units.

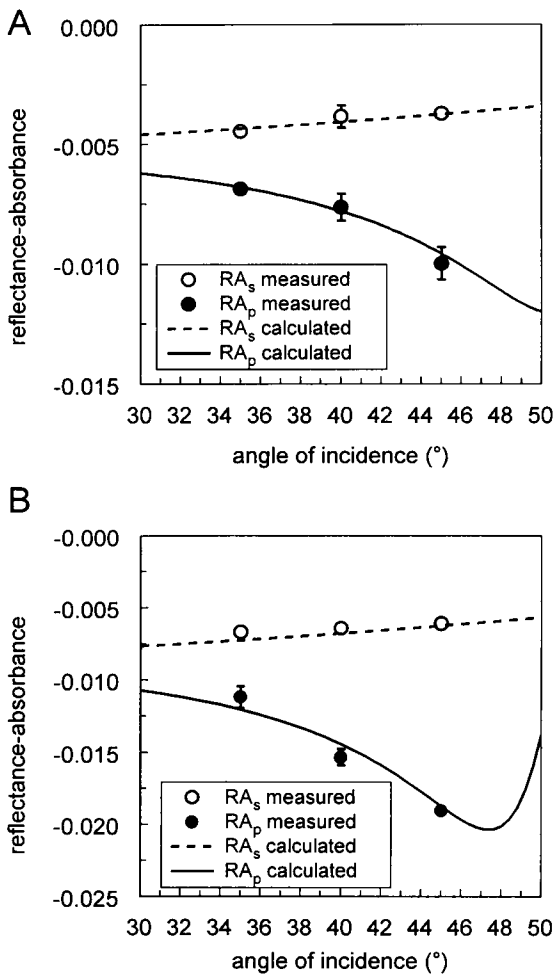

FIGURE 2 Simulated and measured (—— and ● p-polarization, - - - and ○ for s-polarization, respectively) reflectance-absorbances for the asymmetric methylene stretching vibration versus angle of incidence. The real part of the refractive index at the center of the band is $n = 1.41$, the length of the DPPC molecule is 2.66 nm, and the degree of polarization is 98.7%. (*A*) The pure DPPC monolayer (air/H_2O interface). The best fit was obtained for $k_{max} = 0.54$ and a chain tilt angle of 26°. (*B*) The DPPC/SP-C 20:1 monolayer (air/D_2O interface). The best fit was obtained for $k_{max} = 0.42$ and a chain tilt angle of 10°. We note that reflectance-absorbances for the methylene stretching vibrations are generally higher on D_2O subphases than on H_2O subphases (Flach et al., 1997).

cal secondary structure observed for SP-C is consistent with previous reported monolayer results (Flach et al., 1994; Pastrana-Rios et al., 1995) and bulk phase and ATR-FTIR measurements for DPPC/SP-C bilayers (Pastrana et al., 1991; Vandenbussche et al., 1992).

To account for the fact that the secondary structure of SP-C in nonpolar solvents is 70–80% helical (Johansson et al., 1994b), the following considerations were applied to the theoretical calculations. First, peak heights rather than the integrated intensities of the amide I′ band are used for the calculation of the helix tilt angle. Since the amide I′ band position of the SP-C helix is ~1647 cm^{-1}, whereas a random or unordered structure generally shifts the frequency down by several wavenumbers, the use of peak heights reduces possible interference at the position of the helical component. Second, and more important, is the fact that the amide I (or I′) molar extinction coefficient for random coil structures is smaller than that for helices (Venyaminov and Kalnin, 1990). In addition, for p-polarized radiation (which provides most of the useful tilt angle information) the transition moments in the z direction produce IRRAS bands opposite in sign to the reflectance-absorbances of transition moments in the x direction. The partial cancellation of intensities is important for random coil structures, because the distribution of the amide I vibrational transition moments is (by definition) isotropic, and, therefore, intensities found for random coils are considerably weaker than helical amide I intensities. Although simulation parameters for random structures are obscure, calculations (this laboratory, unpublished data) assuming that 25% of the structure is random coil suggest an amide I′ intensity at the center of the random coil band that is less than 8% the intensity of the helical band. Allowing for the fact that the frequencies for the two structures differ reduces any potential interference to less than ~5%, which is within the experimental error.

In the case of an extended and ordered structure in the amino terminal, the corresponding amide I mode will be near 1620 cm^{-1} and will produce negligible interference at 1647 cm^{-1}. It should also be noted that under the current set of experimental conditions, the spectra do not show signs of peptide aggregation (Van Stokkum et al., 1995; Pastrana et al., 1991).

A comparison of the simulated and experimental peak intensities for the amide I′ band of SP-C over the range of incident angles in mixed monolayers is shown in Fig. 4 *A*. The best fit to the experimental data was obtained for a helix tilt angle of 70 ± 5°, using a k_{max} of 0.48. The angle the transition dipole moment makes with the helical axis was taken as 28° (Rothschild and Clark, 1979). The length of the molecule used in the simulations is an estimate of the helical segment of the protein (see Materials and Methods for a discussion of film thickness). To judge the significance of this result, the experimental data are compared to the intensities calculated for a random tilt angle distribution. At the "magic" tilt angle of 54° for the helix, the film's directional extinction coefficients are equal, and therefore a tilt of 54° generally cannot be distinguished from an isotropic, random distribution of the molecules. To test whether the obtained tilt angle of 70° can be distinguished from the magic angle, k_{max} was adjusted to match the measured s-polarized intensities for a tilt of 54° (Fig. 4 *B*). In this case, the p-polarized simulated intensities do not fit the measured data, particularly for the 45° incident angle, highlighting the importance of the p-polarized measurement close to the Brewster angle. It should be emphasized that measurements at a single incident angle, removed by more than ±10° from the Brewster angle, do not provide adequate orientational sensitivity to determine tilt angles accurately. To estimate the accuracy of the derived tilt angle, simulations at varying tilt angles

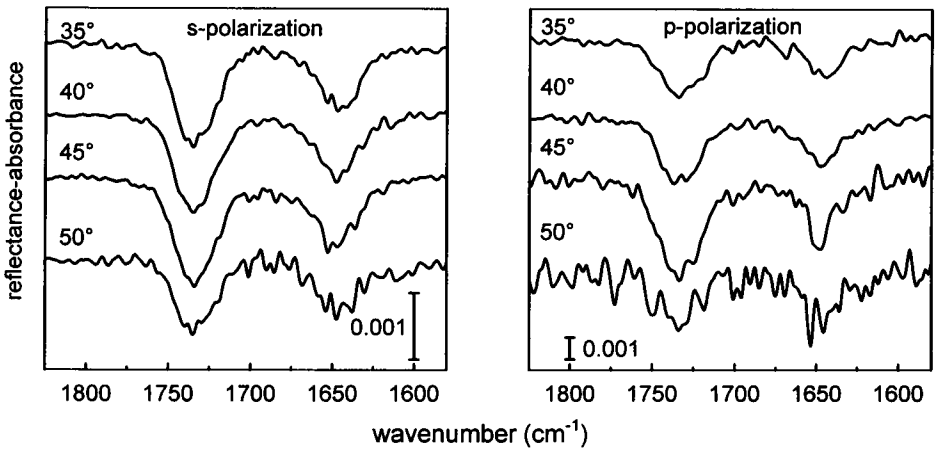

FIGURE 3 IR reflection-absorption spectra in the region 1800–1600 cm^{-1} for DPPC/SP-C monolayers (air/D_2O interface) at a surface pressure of 28 mN/m (19.0 ± 0.5°C) at different angles of incidence for s- and p-polarization. The bar reflects the intensity in reflectance-absorbance units. The original, unsmoothed spectra are shown.

around 70° were performed, and an accuracy of 5° was found.

In addition to the intensity dependence on tilt angle, bandshapes, band position, and signs, i.e., positive or negative bands, have also been shown to vary as a function of tilt angle and have been used to estimate helix orientation at the A/W interface (Cornut et al., 1996). IRRAS spectra for a pure α-helical peptide were calculated for a 45° incident angle over a range of tilt angles, using optical parameters similar to those for Fig. 4, *A* and *B*. As the tilt angle is decreased to 0°, the sign of the amide I band changes from negative to positive, with an anomolous dispersion lineshape for the tilt angle range of ~10 to 40°. Therefore, bandshape analysis shows unequivocally that the tilt of the helical segment of SP-C in lipid monolayers must be larger than 40°, in agreement with the derived tilt angle of 70°.

A 23 ± 3° angle was obtained by analyzing carbonyl band intensities for the mixed DPPC/SP-C film (Fig. 3). This angle, which represents the angle between the surface normal and the normal to the carbonyl stretching dipole, is an average for the two carbonyl groups. The diagnostic value of this result is limited because of likely differences in the orientation of the two carbonyl groups caused by the bend in the *sn*-2 chain adjacent to the carbonyl group (Pearson and Pascher, 1979). Therefore, additional IRRAS experiments were performed with *sn*-2-^{13}C=O-labeled DPPC to reveal whether SP-C interaction with DPPC differs between the *sn*-1 and *sn*-2 positions. The weakness of the C=O intensities forced us to restrict data collection to a single angle of incidence (40°) from which individual tilt angles were calculated. Spectra in Fig. 5, *A* and *B*, show the carbonyl and amide I′ regions for pure *sn*-2-^{13}C=O DPPC and *sn*-2-^{13}C=O DPPC/SP-C monolayers on D_2O, respectively. In Fig. 5 *A*, the C=O stretching band from the *sn*-1 position is observed at ~1735 cm^{-1}, whereas the *sn*-2-^{13}C-labeled C=O stretch is observed at ~1690 cm^{-1}. The isotopic shift of 45 cm^{-1} is expected from the change in reduced mass of the C=O group (Blume et al., 1988). The mixed monolayer displays (Fig. 5 *B*), in addition to the two lipid C=O stretching bands, the expected amide I′ band of SP-C at ~1645 cm^{-1}. In view of the previous comments describing the importance of measurements at several angles of incidence, as well as the weakness of the signal, the derived angles for the C=O group must be considered semiquantitative.

To verify the experimental and simulation procedures for a single incident angle, average tilt angles for the acyl chains in the labeled pure lipid and binary systems were calculated by using the same simulation parameters as in the unlabeled systems. The results are summarized in Table I. The average acyl chain tilt angle computed from the $\nu_a(CH_2)$ band for the pure labeled DPPC monolayer was 22°, and for the binary system it was 13°, in good agreement with the unlabeled monolayer values (26° and 10°, respectively). In addition, the average tilt angle for the helical components of SP-C in the mixed labeled film as acquired from analysis of the amide I′ band compares well with the unlabeled system (72° and 70°, respectively). Finally, the k_{max} values used for the simulations for the individual carbonyl bands in the labeled films were approximately half the magnitude of the k_{max} used for the combined carbonyl band in the unlabeled systems. The angles between the surface normal and the normal to the carbonyl bond for the pure DPPC film are 31° for the *sn*-1-C=O and 21° for the *sn*-2^{13}C=O stretching modes. In the presence of SP-C, the calculated angles are 29° and 42°, respectively. The value for the *sn*-1 carbonyl group is essentially unchanged from the pure phospholipid, whereas the orientation of the *sn*-2 carbonyl group changes substantially upon interaction with the protein. Considering the small average tilt angle for the lipid acyl chains in the presence of SP-C (10°), it appears as if the observed reorientation is restricted to the vicinity of the *sn*-2 carbonyl group. However, additional experiments must be conducted to probe this preferential interaction further.

The 70° tilt angle for SP-C in lipid monolayers is the first rigorous quantitative measure of protein orientation at the A/W interface. To date, reports on protein orientation at the A/W interface have been semiquantitative (Cornut et al., 1996; Blaudez et al., 1996) or based on analysis of films

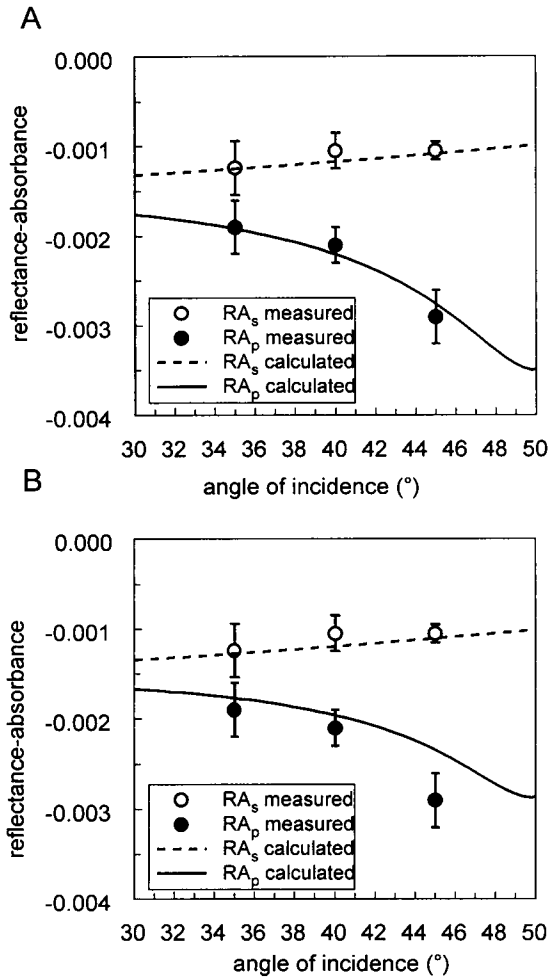

FIGURE 4 Simulated and measured (—— and ● for p-polarization, - - - and ○ for s-polarization, respectively) reflectance-absorbances for the protein amide I′ band versus angle of incidence. The real part of the refractive index at the center of the band is n = 1.41, the length of the protein is 3.41 nm, the degree of polarization is 99.0%, the full-width at half-height is 15 cm^{-1}, and the angle the transition dipole moment makes with the helix axis is taken as 28° (Rothschild and Clark, 1979). (*A*) The best fit was obtained for k_{max} = 0.480 and a tilt angle of the helix of 70°. (*B*) Simulations were performed using a k_{max} = 0.355 and a tilt angle of 54°.

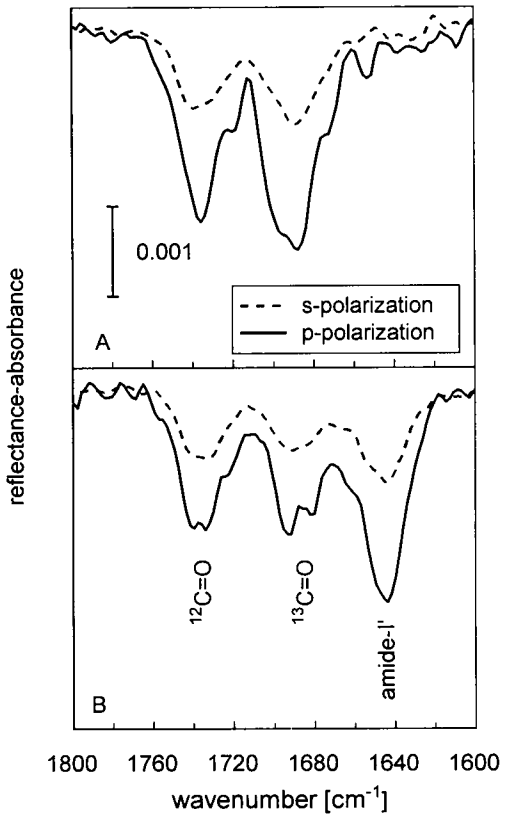

FIGURE 5 IR reflection-absorption spectra in the region 1800–1600 cm^{-1} for (*A*) *sn*-2 ^{13}C═O DPPC and (*B*) *sn*-2 ^{13}C═O DPPC/SP-C monolayers at the air/D_2O interface for a surface pressure of 28 mN/m (19.0 ± 0.5°C), respectively. The bar reflects the intensity in reflectance-absorbance units. The spectra are slightly smoothed (second-order, seven-point Savitzky-Golay smoothing).

transferred to solid supports (Briggs et al., 1986; Creuwels et al., 1993). The current IRRAS studies offer the major advantages that film transfer is not required (minimizing possible transfer artifacts) and that structural and orientational information is available with quite high accuracy from both the lipid and protein components in the film. An additional benefit of the approach is the ability to use isotopic substitution to probe issues of differential interaction. In the current study, this differential interaction is manifested in the angle changes reported (see Table 1) for the *sn*-1 compared to *sn*-2 lipid carbonyl groups. The greater effect of SP-C on the *sn*-2 carbonyl group is consistent with the presence of a bend in the *sn*-2 chain adjacent to the carbonyl group, making it more accessible for interactions.

The major difference between SP-C helix orientation in lipid bilayers and monolayers provides insight into the means by which SP-C facilitates the spreading of DPPC across the A/W interface. In oriented bilayers spread from the same solvents as used in the current work, a tilt of 24° was reported (Pastrana et al., 1991), whereas a tilt of 70° is calculated in the current study. For both monolayer and bilayer preparations, the observed tilt angles allow for maximum hydrophobic matching of the lipid acyl chains and the protein helical segment. The role of hydrophobic length matching between proteins and lipids is currently under intense investigation and has been implicated in many aspects of membrane structure, function, and biosynthesis (Mouritsen and Bloom, 1984; Killian et al., 1996; Zhang et al., 1995). The large tilt of SP-C in the monolayer allows the helical portion of the protein to act as a "hydrophobic lever" that anchors and spreads a maximum number of lipid molecules. The DPPC chains are required to adopt a more vertical orientation to maximize the interaction. The helix tilting may facilitate DPPC spreading by disrupting intermolecular acyl chain packing, while simultaneously presenting a surface to the DPPC that permits the persistence of high conformational order in the chains. By this simple

TABLE 1 Calculated tilt angles for specific molecular groups in pure lipid and lipid/protein monolayers

Monolayer	Component	Vibrational mode for simulation	Structural element	Tilt angle (°)*
DPPC	Lipid	$\nu_a(CH_2)$	Acyl chain	26 ± 2
sn-2-^{13}C=O DPPC	Lipid	$\nu_a(CH_2)$	Acyl chain	22
		sn-1-C=O	C=O#	31
		sn-2-^{13}C=O	C=O#	21
DPPC/SP-C	Lipid	$\nu_a(CH_2)$	Acyl chain	10 ± 5
		Average C=O	C=O#	23 ± 3
	Protein	amide I′	α-helix	70 ± 2
sn-2-^{13}C=O DPPC/SP-C	Lipid	$\nu_a(CH_2)$	Acyl chain	13
		sn-1-C=O	C=O#	29
		sn-2-^{13}C=O	C=O#	42
	Protein	amide I′	α-helix	72

Tilt angle of the specific molecular group with respect to the surface normal.
The quantity reported is the angle between the surface normal and the normal to the C=O dipole.

mechanism, SP-C may facilitate the rapid spread of the monolayer while ensuring its stability at high surface pressures, two essential biophysical characteristics for an effective lung surfactant.

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