Infrared spectroscopic investigations of pulmonary surfactant

Surface film transitions at the air–water interface and bulk phase thermotropism

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ABSTRACT The molecular structure of the phospholipid component of intact pulmonary surfactant isolated from bovine lung lavage has been examined by Fourier transform infrared spectroscopy. Two different physical states of the surfactant were examined by means of different infrared spectroscopic sampling techniques. Transmission infrared experiments were used to study the surfactant in the bulk phase. In these experiments, the thermotropic behavior of the bulk surfactant was monitored by temperature-induced variations in the phospholipid acyl chain CH_2 stretching frequencies. A broad phase transition (confirmed by differential scanning calorimetry) was noted with an onset temperature near 15°C and a completion temperature near 42°C. In addition to the bulk transmission experiments, external reflection infrared spectroscopy was used to examine surfactant films *in situ* at the air–water interface. As surface pressure was increased from 0 to 43 dyn/cm, a gradual and continuous decrease in the CH_2 stretching frequency was noted for the surfactant. Thus, under surface pressures which correspond to large lung volumes *in vivo*, the surfactant acyl chains exist mostly in the ordered (*trans*) configuration. The frequency shift in the CH_2 stretching mode is consistent with a continuous ordering of the acyl chains upon compression over the pressure range 0–43 dyn/cm, and implies that a weakly cooperative phase transition occurs in the hydrocarbon region of the surface film. The surface film transition is especially noted in the pressure–area curve of the surfactant and approximates in two dimensions the broad thermotropic phase transition of the bulk phase surfactant. Substantial differences were observed between the response to surface pressure changes of intact surfactant compared with the main surfactant phospholipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. The changes in response are attributed to the presence of additional surfactant components. The current work demonstrates the ability of infrared spectroscopy to obtain structural information on the surfactant in physical states that directly relate to those *in vivo*.

INTRODUCTION

Pulmonary surfactant is a complex lipid–protein mixture secreted by type II cells of the pulmonary alveolus, and functions to reduce surface tension at the air–water (A/W) interface in the lung (1, 2). Chemical analyses of surface-active substances isolated from lung lavage fluid reveal the two main constituents to be lipid (primarily phospholipid) and protein (3, 4). The main lipid is 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), with smaller amounts of phosphatidylglycerols, unsaturated phosphatidylcholines, cholesterol, and other trace components. A monomolecular film of DPPC is generally believed to be the physiologically relevant configuration of the pulmonary surfactant at the A/W interface in the lung under high levels of surface film compression.

The role of the various components in surfactant function is poorly understood. Although DPPC alone can effect a reduction in surface tension to near zero at the A/W interface, it spreads too slowly to be effective *in vivo* (5, 6). Other surfactant components, in particular phosphatidylglycerols, protein, and Ca^{2+}, have been suggested to play a role in this process (7).

Biophysical studies are expected to enhance our current understanding of the mechanism by which surfactant spreads at the A/W interface to form a stable film and to lower surface tension. The physical techniques used to date in surfactant biophysics have centered around surface balance determination of monolayer pressure-area characteristics (8, 9), as well as turbidimetric and calorimetric studies of surfactant aggregation and thermotropic behavior in bulk phases (7, 10, 11). These techniques, while useful for macroscopic thermodynamic characterization of materials, yield no detailed information on molecular structure. Molecular-level information on surfactant has been obtained using spectroscopic techniques. However, because of the problems associated with the lack of spectral sensitivity from spread monolayers at the A/W interface, previous studies have been limited to the study of bulk phases. To date, Hook et al. (12) have reported electron paramagnetic studies of surfactant lipid fluidity in bulk phases, whereas Shiffer et al. (13) investigated the interaction of low molecular mass surfactant proteins (5 through 18 kD) with phospholipids using

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fluorescence spectroscopy measurements of protein-induced alterations of liposomal fusion. Finally, a previous infrared study by Mautone et al. (14) has described the effect of Ca^{2+} and protein on the thermotropic properties of surfactant lipids.

The present work describes an in-depth infrared (IR) spectroscopic study of pulmonary lung surfactant isolated from bovine lung lavage. A comparison is made of transmission IR measurements of bulk surfactant with external reflectance IR studies of a surfactant surface film *in situ* in the A/W interface. The advantages of infrared spectroscopy for surfactant research are the following: (*a*) The technique has the potential to measure in a single experiment lipid acyl chain configuration, subphase ion interactions with phospholipid head groups, and protein secondary structure (15). (*b*) The technique requires no extrinsic probe molecules, which often perturb the properties of the system under investigation (16). (*c*) The technique requires relatively small amounts of material. (*d*) Current development in Fourier transform (FT)IR instrumentation and sampling methods have made infrared spectroscopy a sensitive analytical techinque adaptable to many areas of surface chemistry. For example, recent experiments (17–22) have shown that external reflection IR spectroscopy can be utilized as an *in situ* probe of spread monolayers at the A/W interface. This is a unique capability of IR spectroscopy just beginning to be applied to biophysical systems.

The current study sheds new insight as to the configuration of the surfactant phospholipid acyl chains both in bulk phase and at the A/W interface. In addition, alterations of molecular structure in the surfactant acyl chains are examined as induced by variations in temperature (for bulk phase surfactant) and surface pressure (for surfactant in surface films).

EXPERIMENTAL

Isolation and characterization of bovine surfactant

Bovine surfactant, collected by lung lavage, was centrifuged to remove cellular debris, further centrifuged to obtain a surface-active fraction, and used as received in this form, from Dr. Bruce Holm, Children's Hospital, Buffalo, NY. The phospholipid composition (% of phosphorous recovered) was phosphatidylcholine, 81.0%; phosphatidylglycerol, 5.0% phosphatidylethanolamine, 3.4%; phosphatidylserine/phosphatidylinositol, 4.7%; and sphingolipids, 2.5% (23, B. Holm, unpublished results).

The protein fraction of the surfactant was resolved on 10% SDS polyacrylamide gel electrophoresis under reducing conditions. Protein bands corresponding to relative molecular masses (M_r) of ~66, 55, 36-30, 29, 18, 12 and 10 kD were observed (K. E. Reilly, A. J. Mautone, and R. Mendelsohn, submitted for publication).

Differential scanning calorimetry

Endotherms were obtained on a Micro-cal MC1 unit (Micro-cal Inc., Amherst, MA). The sample size was 0.7 ml, with each sample containing ~14 mg/ml phospholipid. Scan rates were 30–35°C/h.

Surface films

Lung surfactant surface films were formed on the Teflon trough of an automated Langmuir film balance which has been described in detail elsewhere (20). The trough was cleaned before each experiment by soaking it for 1 h in a chromic acid solution (Chromerge, Fischer Scientific Co., Pittsburgh, PA). The trough was then exhaustively rinsed and filled with triply deionized water (nominal resistivity of 18 MΩ/cm, Milli-Q, Millipore Corp., Bedford, MA). Temperature control of the trough water at 22 ± 1°C was obtained by passing a thermostatted water solution through a hollow aluminum base plate on which the trough was mounted. The surfactant surface films were prepared by spreading 5.0 μl of a 15 mg/ml solution of phospholipids from raw bovine lung surfactant onto the surface of the A/W/ interface (12.75 × 23.10 cm total surface area) by aliquot addition down a glass rod. The surface film was then allowed to equilibrate for 20 min. Individual points on the pressure-area isotherm were obtained by driving the dual motorized barriers toward the center of the trough and recording the weight of a filter paper Wilhelmy plate (GVWP, 0.22 μm, Millipore Corp.) which was suspended from a microbalance (model 27, Cahn, Inc., Cerritos, CA). For a continuous compression pressure–area curve, the barrier compression rate of 0.20 cm/min or a trough area change of 5.1 cm^2/min was used. The distance between the wipers and the weight of the Wilhelmy plate was recorded every 30 s. The conversion of balance weights and barrier distances to surface pressure and molecular area (for pure DPPC films) has been reported earlier (19, 20).

Infrared spectroscopy: bulk phases

Bulk phase surfactant samples were examined in a demountable IR transmission cell (Harrick Scientific Corp., Ossining, NY) equipped with 25-μm path length and CaF_2 windows. Transmission FT-IR spectra of bulk phases were recorded on a spectrometer (Sirius 100, Mattson Instruments, Madison, WI) equipped with a HgCdTe detector. Routinely, 400 interferograms were collected, co-added, apodized with a triangular function, and Fourier transformed to give a resolution of 4 cm^{-1} with data encoded every 2 cm^{-1} (14). Temperature was controlled via a circulating water bath and monitored with a digital thermometer, whose thermocouple sensor was placed close to the IR windows in the cell. The spectrum of water (matched for temperature and path length) was subtracted from all data sets. Residual sloping baselines were removed with a linear baseline routine supplied with the instrument software.

Infrared spectroscopy: surface films

Single-reflectance FT-IR spectra of the surfactant surface film at the A/W interface were obtained using an FT-IR spectrometer (model FTS-40, Digilab, Inc., Cambridge, MA). A diagram of the optical path and a detailed description of the Langmuir film balance used in these experiments have been previously published (20). The angle of incidence of the incoming IR radiation was 30 ± 2° from the surface normal, which was in the optimum range for observing both polarizations

reflected from a H_2O substrate. The IR spectra were collected using 1,024 scans at 8 cm^{-1} resolution with triangular apodization and one level of zero filling with a narrow-band HgCdTe detector. For both polarizations, a single-beam reflectance spectrum of triply deionized water was recorded and ratioed as a background to a single-beam reflectance spectrum of the film to produce a reflection-absorption spectrum. For most surface film IR spectra, the wipers remained stationary after the film was compressed to the desired surface pressure. However, at higher surface pressures (>20 dyn/cm), the perpendicular and parallel polarized IR spectra were taken while the film was being compressed at a rate of 0.015 cm/min (0.38 cm^2/min) to maintain a constant surface pressure. Frequencies of the CH_2 stretching modes were calculated using a center-of-gravity algorithm. The data reduction procedures for surface film IR spectra have been described previously (19).

RESULTS

A typical mid-infrared transmission spectrum of bovine surfactant in the bulk phase is shown in Fig. 1. The absorbance bands seen in Fig. 1 arise primarily from the phospholipid components of the surfactant and resemble those of rabbit surfactant (14). The assignment of individual spectral features has been discussed elsewhere (14). In the current work, melting curves are constructed from the phospholipid acyl chain CH_2 symmetric and asymmetric stretching modes near 2,850 and 2,920 cm^{-1}, respectively. These bands have been widely used to monitor phospholipid conformational order in vesicle systems (15). The peak positions of these modes can be measured with a precision of better than 0.05 cm^{-1} (14, 15) and their temperature-induced variation provides a convenient method for monitoring lipid thermotropic behavior. Although frequency increases in these bands are small (1.5–5.0 cm^{-1}) during melting events, the available precision allows a good representation of the process to be achieved. The origin of the frequency increase upon melting arises from variations in interaction constants between C-H stretching coordinates on adjacent methylene groups when the lipid physical state is altered (24). The absorbance band near 2,850 cm^{-1} is better suited for construction of spectroscopic melting curves than is the 2,920 cm^{-1} mode since the spectral region around 2,850 cm^{-1} suffers from minimal interference of overlapping protein vibrations (15).

The temperature dependence of the 2,850 cm^{-1} symmetric CH_2 stretching band for intact bovine surfactant is shown in Fig. 2. A broad melting event with an onset temperature of 18–20°C and a completion temperature of ~40°C is discernible. The continuous variation in the frequency of the C–H stretching vibration below the onset temperature and above the completion temperature make more precise determination of the transition temperatures difficult. A frequency increase of ~2 cm^{-1} is noted during the melting process. The occurrence of a melting process in the bulk phase is further confirmed by differential scanning calorimetry as shown in Fig. 3. The onset and completion temperatures, 15–16 and 42–44°C, are in reasonable agreement with those observed by the transmission IR measurements. Thus, the broad thermotropic event in the bulk surfactant is accompanied by an increase in acyl chain disorder as monitored by an increase in the frequency of the symmetric CH_2 stretching vibration in the transmission IR spectra.

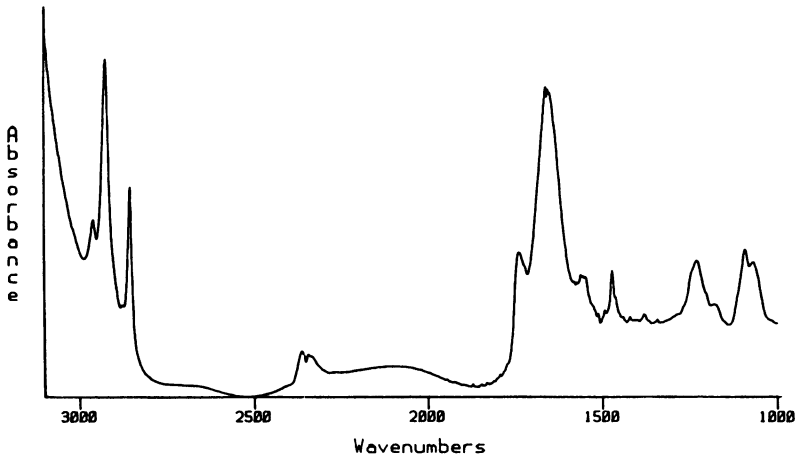

FIGURE 1 Typical transmission FT-IR spectrum for bulk bovine surfactant in aqueous media. Spectral features arise mainly from the phospholipid component. The absorbance bands in the 3,000–2,800 cm^{-1} region arise primarily from the C–H stretching vibrations of the phospholipid acyl chains. The 1,800–1,000 cm^{-1} region vibrational bands arise mostly from phospholipids with water contributing its bending mode near 1,640 cm^{-1} and protein contributing weak amide I and amide II modes near 1,650 and 1,550 cm^{-1}, respectively.

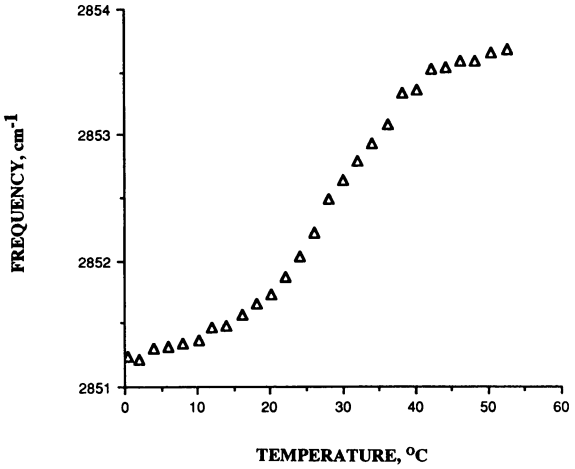

FIGURE 2 Temperature dependence of the CH_2 symmetric stretching vibrational frequency near 2,850 cm^{-1} for surfactant. Frequencies were measured from the transmission IR spectra of bulk bovine surfactant.

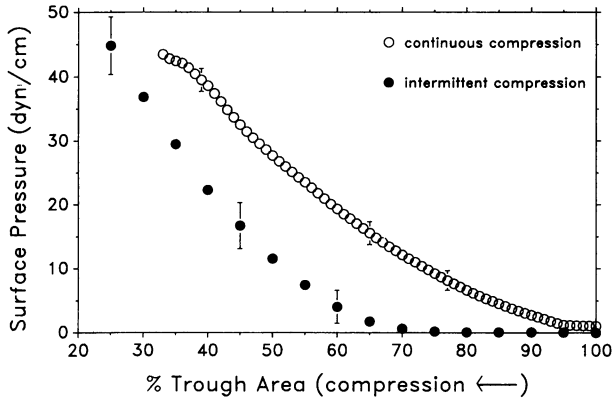

FIGURE 4 The surface pressure of a bovine pulmonary surfactant surface film is plotted as a function of the surface area of the trough. (○) Surface pressures obtained while the film underwent continuous compression. (●) Intermittently measured surface pressure data. The intermittently measured data points coincide with the acquisition of external reflection IR spectral data (see Fig. 5).

Although bulk phase studies provide valuable information regarding surfactant properties, we wish to directly address the question of the molecular structure of the surfactant in physical states thought to be most related to those *in vivo* (i.e., a surface film). Therefore, the external reflection IR technique for the study of monolayers at the A/W interface (17–22) has been applied to surface films of surfactant.

Isotherms for monolayer surface pressure vs. percent trough surface area of compression for pulmonary surfactant are shown in Fig. 4. The reproducibility of the method used to generate the films is illustrated by representative error bars in the figure. The isotherms resemble those published elsewhere (8). In the absence of accurate measurements of two-dimensional molecular area for the heterogeneous surfactant mixture at the A/W interface, only qualitative data interpretations may be suggestion. At large surface areas, the linear horizontal region (~95–100% in Fig. 4) may represent a transition between the gaseous and lipid expanded state, while between compressions of 95% and 40% the general shape of the pressure-area diagram is that of liquid-expanded film, comparable in general features to that of myristic acid between 33 and 50 $Å^2$/molecule (25). At higher surface pressures in the continuous compression mode, a transition to a condensed phase is evident. Data points for surface pressure–percent trough surface area experiments run using intermittent rather than continual film compression were obtained in parallel with the reflectance IR measurements of the surfactant surface films. For the IR reflection experiments, intermittent compression is used since the IR measurements require an ~15-min time interval in which to acquire spectra at each individual surface area data point. The difference between the surface pressure–percent trough surface area isotherms for continuous vs. intermittent compression seen in Fig. 4 has been observe previously for monolayer films. Shah and co-workers (26, 27) have found intermittent rather than continual film compression allows time for equilibration of a lecithin monolayer from the stress of the compressing force. They have obtained π–A curves for DPPC similar to our intermittent compression data for the same molecule.

Polarized external reflection IR spectra for the 3,000–2,800 cm^{-1} region of the surfactant surface film are shown in Fig. 5. The ordinate scale is plotted in absorbance units [i.e., $-\log(R/R_o)$], where R is the single-beam reflectance spectrum of the film and R_o is the single-beam reflectance spectrum of the pure H_2O substrate. The most

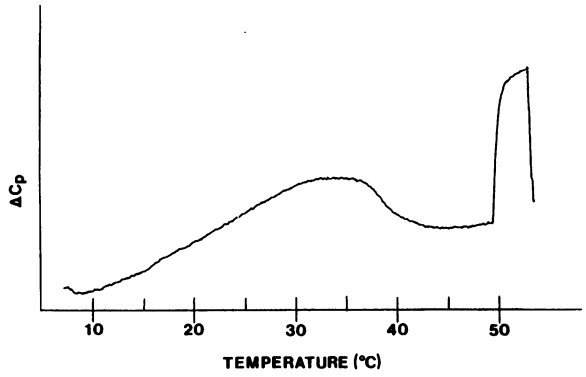

FIGURE 3 Differential scanning calorimetry endotherm for bulk bovine surfactant in aqueous medium. The feature starting at 50°C is a power pulse used for the calibration of sample heat capacity.

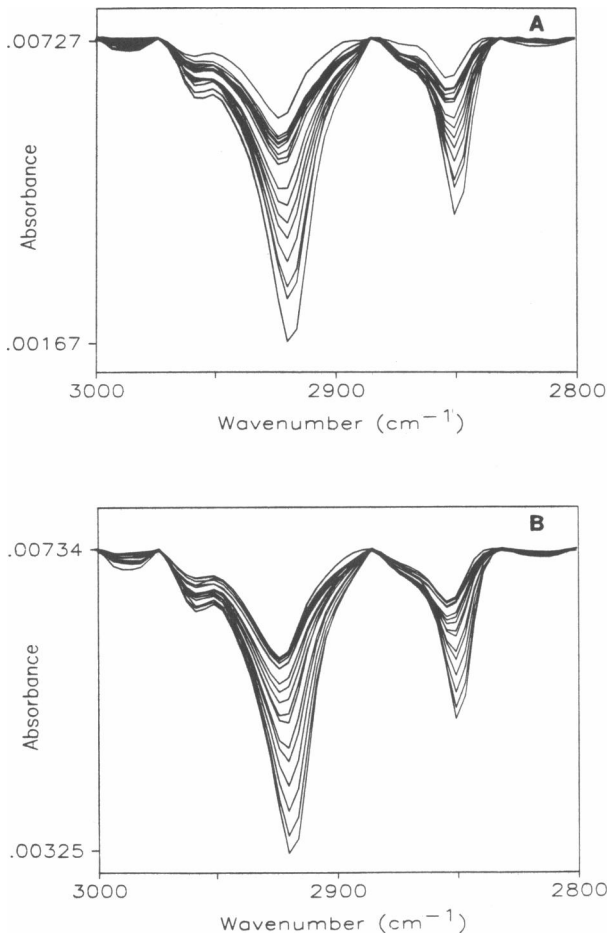

FIGURE 5 External reflection IR spectra of the raw bovine lung surfactant monolayer in the C–H stretching region. The CH_2 stretching bands are plotted for both (*A*) parallel and (*B*) perpendicular polarizations between 3,000 and 2,800 cm^{-1}. The spectra increase in intensity with increasing surface pressure. The surface pressure associated with each spectrum is given by the intermittently compressed data in Fig. 4, beginning with 0 dyn/cm and increasing to 37 dyn/cm. The band at ~2,920 cm^{-1} is the antisymmetric CH_2 stretch. The ~2,850-cm^{-1} band is the symmetric CH_2 stretch of the phospholipid hydrocarbon chains.

notable difference in the reflectance IR spectrum of Fig. 5 is compared with the transmission IR spectrum in Fig. 1 is the negative absorption bands in the former. Such bands are predicted in external reflectance IR spectroscopy when the attenuation constant (k) of the complex refractive index ($\hat{n}$) of the substrate is small (28). The current negative absorbances arise from the low k values for H_2O (k = 0.03 at 3,000 cm^{-1} [18]).

Despite the qualitative differences between transmission and external reflectance IR spectra, the observed spectral parameters (e.g., frequencies, bandwidths, intensities) still may be used as an index of phospholipid molecular conformation. For example, in addition to the surfactant film's surface pressure (Fig. 4), we may also plot the frequency of the CH_2 symmetric stretching vibration at 2,850 cm^{-1} in the spectrum of the surfactant surface film as a function of the two-dimensional trough surface area (Fig. 6). As is the case for the bulk phase surfactant spectra (Fig. 2), decreases in the measured frequency of the conformation-sensitive C–H stretching vibrations are empirically correlated with an increase in order of the surfactant lipid's hydrocarbon acyl chains (24).

The average frequencies of three sets of surfactant surface film compression experiments along with their standard deviations are plotted in Fig. 6. In addition, the frequencies of both the parallel and perpendicular polarized spectra are included. The data in Fig. 6 indicate a relatively constant frequency (~2,852.8 cm^{-1}) at minimal film compression. This frequency would indicate a high degree of acyl chain fluidity in the surfactant phospholipids at this point. Upon further compression, and at a two-dimensional trough surface area ~60% of that of the initial trough area for a constant volume of surfactant, the frequency of this absorbance band continually decreases until reaching a value of ~2,849 cm^{-1} at ~20% of the initial trough area. At this point, the lipid acyl chains are in a mostly ordered conformation. The observed value of 2,849 cm^{-1} is indicative of highly ordered acyl chains (15). It is unlikely that further compression would result in any substantial frequency decrease.

The broadened sigmoidal curve seen in Fig. 6 suggests

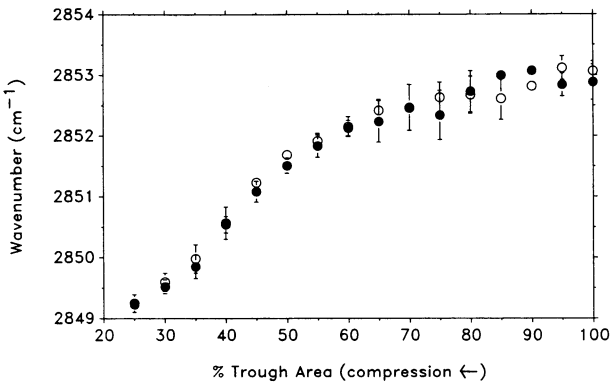

FIGURE 6 The frequency of the symmetric CH_2 stretching mode is plotted as a function of percentage of surface area for a surface film of bovine pulmonary surfactant at the A/W interface. Data was obtained for both perpendicular (●) and parallel (○) polarized spectra. The frequency–area isotherm was measured for a monolayer at 22°C by applying 5 μl of a 15-mg/ml solution of surfactant to the A/W interface of a 12.75 × 23.10-cm Langmuir trough (20). Surface area for the heterogeneous surfactant monolayer was measured as a percentage of the initial trough surface area. The frequency was measured using a center-of-gravity algorithm.

a weakly cooperative phase transition is occurring in the surfactant upon film compression. This is in contrast to the abrupt, highly cooperative transition seen in the frequency-molecular area diagrams for the first-order thermodynamic phase transition of pure DPPC monolayer films (22). The broadened transition seen in the surfactant surface film (Fig. 6) can be compared to the broadened phase transition observed in the bulk surfactant (Fig. 2). In this instance, the frequency shift in the C–H stretching band seen in the surfactant film spectra upon compression can be considered to be the two-dimensional analogue of the phase transition occurring in the bulk surfactant. The presence of this kind of weakly cooperative transition in surfactant is observed here for the first time and would not necessarily have been predicted based on the surface pressure–percent trough surface area curve for the surfactant (Fig. 4). As discussed above, this pressure-area diagram for the surfactant is indicative of a liquid-expanded monolayer film.

The difference in the surface film phase transition for the surfactant as compared with that of pure DPPC is emphasized when the symmetric C-H stretching frequencies from the surface film spectra are plotted as a function of surface *pressure,* not surface *area.* For example, the symmetric C–H stretching frequencies of the surfactant acyl chains are plotted as a function of surface pressure in Fig. 7. At low surface pressures the observed frequencies are near 2,852.5 cm^{-1} in both parallel and perpendicular polarizations, indicating (by analogy with the thermotropic behavior of surfactant) a disordered acyl chain. As the surface pressure is increased from 0 to 45 cyn/cm (Fig. 7), a continuous decrease is noted in the frequency, indicative of a gradual ordering of the hydrocarbon chains at the interface in response to increasing film pressure. As discussed above, the observed frequency of ~2,849.5 cm^{-1} at 45 dyn/cm, is suggestive of highly ordered (mostly all-*trans* C–C bonds) acyl chains. The response to increased surface pressure of the CH_2 stretching frequency in the surfactant film spectra differs markedly compared with that of the CH_2 stretching frequency in pure DPPC monolayers (Fig. 8). The broad, continual response of the surfactant is replaced by a rapid decrease in the C–H stretching frequency over a surface pressure range of 0–10 dyn/cm for DPPC, indicating the existence of ordered chains at all pressures above this. The highly cooperative, first-order thermodynamic nature of the DPPC monolayer transition vs. the weakly cooperative nature of the surfactant transition is thus highlighted in these graphs.

DISCUSSION

The CH_2 stretching modes measured in the current work provide a convenient means to elucidate and compare acyl chain conformation in bulk phases and in surface films. Increasing the film surface pressure and decreasing the temperature of a bulk phase preparation (which is presumably bilayer in nature) produce similar alterations in frequency, indicating acyl chain ordering in each instance. The underlying assumption on which this conclusion is based, namely that the response of the CH_2 frequency to changes in conformational order is the same in bulk (bilayer) phases as in surface films at the A/W interface, is supported from a spectroscopic viewpoint and is further developed below.

From a general standpoint, one must use caution when

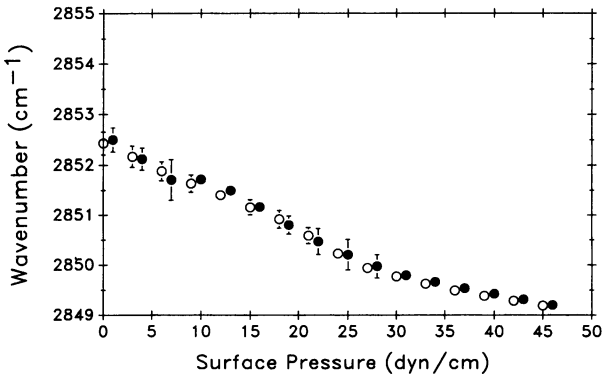

FIGURE 7 The frequency of the CH_2 stretching mode is plotted as a function of surface pressure for a surface film of bovine pulmonary surfactant at the A/W interface. Data was obtained for both perpendicular (●) and parallel (o) polarized spectra. Other experimental details as in Fig. 6.

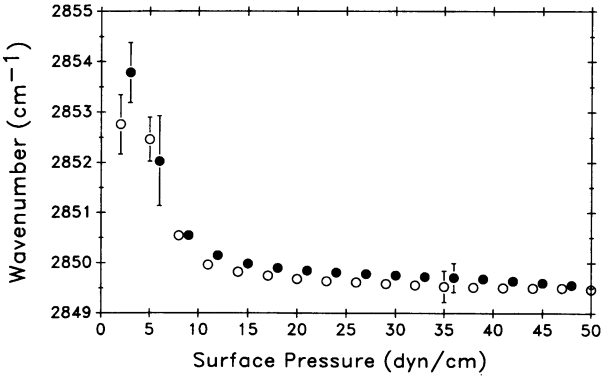

FIGURE 8 The frequency of the CH_2 stretching mode is plotted as a function of surface pressure for a monolayer of DPPC at the A/W interface. Data was obtained for both perpendicular (●) and parallel (o) polarized spectra. The frequency was measured with a center-of-gravity algorithm.

comparing spectra obtained from an external reflection IR experiment with those obtained from transmission IR spectroscopy. This is due to a fundamental difference in the nature of these spectra. Infrared transmission spectra are dominated by the attenuation constant (k) of the complex refractive index ($\hat{n}$) of the sample, and are fairly insensitive (to a first approximation) to the value of the sample's real refractive index (n). The quantities n and k are mutually dependent, and are related by the expression $\hat{n} = n + ik$ (29). For infrared reflection measurements it is n, the real part of the complex refractive index of the monolayer, that initially dominates the observed spectra. This is especially true for the external reflection spectra of monolayers on polished metal substrates, where significant band distortions (including broadening and peak shifts to higher frequency) can occur relative to transmission spectra (29, 30). These effects are primarily seen in relatively thick films (i.e., 1–2 μm) but have been noted in samples of monolayer thickness (29). The origin of these effects has been traced to the change in the refractive index of the monolayer film in the vicinity of an absorption band (an optical phenomenon known as anomalous dispersion), as well as the experimental conditions under which these spectra are acquired (29).

For monolayers at the A/W interface, however, the optical effects are substantially different when compared with monolayers at an air–metal interface. The optical constants of water are significantly different from those of metal substrates; also, the experimental conditions under which spectra are obtained differ substantially depending upon the substrate. As a result, the reflectivities, phase shifts, and electric fields present at the A/W interface are notably different when compared to an air–metal interface (18). As discussed above, the band distortions due to optical and experimental conditions that may be present in spectra of monolayers at an air–metal interface are a consequence of the nature of the metal substrate and its corresponding optical properties. These metal substrate-dependent optical distortions are of course absent from spectra of monolayers at the A/W interface any band distortions that might be seen in the spectra of monomolecular films on water would necessarily be due to the unique optical properties of the water interface. However, to this date, previous studies of monolayers at the A/W interface have not identified any band distortions attributable to the optical effects of a water substrate (17–22).

The CH_2 stretching modes that are the subject of discussion in this paper are localized, characteristic group frequencies, relatively insensitive to long range forces. Changes in the modes thus reflect alterations in the immediate environment of the CH_2 groups. Van Deenen and coworkers have shown (31) that on compression to surface pressures >10 dyn/cm, phospholipids with saturated hydrocarbon chains possessing 15 or more carbon atoms all form condensed monolayers. The current data indicate that monolayers of DPPC have a surprisingly high degree of conformational order. In constrast, surfactant at this compression level (10–20 dyn/cm) is rather disordered. The current study highlights the difficulties in inferring molecular structural information from surface measurements alone.

To extract physiologically relevant parameters from these experiments, we begin with the data of Schurch (32) and Schurch et al. (33), who have demonstrated that the surface pressure of alveoli *in situ* at both 22 and 37°C varies from ~72 dyn/cm (which corresponds to the surface pressure from ~60% of total lung capacity to minimal volume, the range in which normal resting breathing takes place) to ~42 dyn/cm (the surface pressure that corresponds to between ~80% and 100% total lung capacity, a maximal inspiratory maneuver). The current results at 22°C strongly suggest that surfactant, at physiologically relevant surface pressures, has a CH_2 frequency characteristic of an ordered acyl chain. A direct comparison at 37°C must await somewhat more technically difficult experiments. In addition, the compression experiments combined with the reflection IR measurements suggest that the surfactant at the A/W interface is capable of a weakly cooperative type of phase transition (Fig. 6).

That protein and other non-DPPC components play a vital role in surfactant organization is clearly seen by a comparison of frequency-pressure curves for surfactant with those for pure DPPC (Figs. 7 and 8). Whereas the response for surfactant is gradual and continuous over the range of pressures studied, DPPC undergoes a rapid ordering over substantially narrower pressure ranges. Similar changes in cooperativity were noted in comparing the bulk phase thermotropic response of pure DPPC to that of intact surfactant (Fig. 2). Pure DPPC undergoes its gel–liquid crystal transition over a narrow (<1°C) termperature interval centered near 41°C, whereas surfactant (Fig. 2) melts over a 20°C range of temperature. The molecular interpretation for the increased melting range in surfactant is that other surfactant components insert themselves between DPPC molecules to reduce the cooperativity of the melting process. In addition, the presence of unsaturated phosphatidylcholines and phosphatidylglycerol tend to lower the transition temperature. Analogously, we suggest that, in the surface film studies, non-DPPC components insert themselves at the surface to prevent a cooperative response to altered pressure. It has been suggested that non-DPPC components are squeezed from the A/W interface during successive compression cycles of surfactant, leaving a pure DPPC surface (9). Presumably, the non-DPPC components could then function to increase surfactant spreading rate. Those compo-

nents which could not sustain close-to-zero surface tension are thought (9, 34) to be selectively removed from the surface, by mechanisms as yet unknown, but which are suggested to be related to the saturation of the acyl chains. A direct testing of at least parts of this suggestion appears feasible with the IR external reflection spectroscopy techniques described here.

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