Fourier-Transform Infrared Spectroscopy Studies of Lipid/Protein Interaction in Pulmonary Surfactant†

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ABSTRACT: The thermotropic behavior of intact bovine lung surfactant and its hydrophobic extract has been monitored via the temperature dependence of the 2850 cm^{-1} phospholipid acyl chain CH_2 symmetric stretching frequencies in the IR spectrum. A broad, reversible, melting event was noted from about 15 to 40 °C in both the lipid extract and the native surfactant. Slight protein-induced disordering of the lipid acyl chains was evident. The melting event was confirmed by differential scanning calorimetry. The major surfactant protein, a 30–36-kDa class of glycoprotein (SP-A), has been isolated from bovine lung lavage and purified by affinity chromatography. SP-A was reconstituted into a binary lipid mixture of acyl chain perdeuterated dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol (DPPC-d_{62}/DPPG, 85:15 w/w), a ratio which approximates that in surfactant. Use of DPPC-d_{62} permitted the FT-IR determination of the effect of protein on the thermotropic behavior of individual phospholipids in the binary mixture. High levels of SP-A induced an ordering of the phospholipids, as shown by an increase in the transition temperature of DPPC-d_{62} compared to the lipid model. In contrast, a mixture of the other surfactant proteins induced a progressive disordering of the phospholipids and disruption of the cooperativity of the melting event. Transition widths of about 3°, 9°, and 27° were noted for protein:lipid ratios of 0, 1:1, and 2:1 (w/w), respectively. Possible roles for the various proteins in surfactant function are discussed in light of these data.

Pulmonary surfactant, a complex lipid/protein mixture secreted by type II cells of the pulmonary alveolus, functions by reducing the surface tension at the air/liquid interface. Chemical analyses of surface-active substances isolated from lung reveal the two main constituents to be phospholipid and protein. The main phospholipid is 1,2-dipalmitoylphosphatidylcholine (DPPC),[1] with smaller amounts of PG's, unsaturated PC's, other phospholipids, and cholesterol.

A variety of surfactant protein classes have been identified (King, 1982; Ng et al., 1983). The most plentiful class consists of a family of proteins (termed SP-A) with molecular weights ranging between approximately 28K and 36K. Ng et al. (1983) have shown that this family specifically binds to a concanavalin A column and is degraded by neuraminidase, observations which indicate that the peptides have been glycosylated posttranslationally. The canine class of protein possesses a family of M_r 28K, 32K, and 36K, with nearly identical amino acid compositions and variable glycosylation (Benson et al., 1985). The gene for the human pulmonary surfactant apoprotein has been isolated and characterized (White et al., 1985). Additional groups of hydrophobic peptides with relative molecular weights of 3.7K (occasionally reported as 6K; Yu & Possmayer, 1986; Curstedt et al., 1988), 8K, and 18K (Shiffer et al., 1988) have been reported.

The function of the various protein classes has not been elucidated, although a variety of suggestive studies have appeared. Yu and Possmayer (1986) showed that lipid extracts of bovine surfactant containing the low molecular weight protein mimicked the surface characteristics of the native material. SP-A enhanced the rate of both Ca^{2+}-induced surfactant/lipid aggregation (Hawgood et al., 1985) and adsorption of surfactant lipids to the air/water interface (Notter et al., 1983; Hawgood et al., 1985). King et al. (1983) studied the binding of SP-A to a variety of lipid mixtures. Efficient binding, suggested to be hydrophobic in nature, was observed with DPPC/DPPG (85:15) mixtures. Finally, Wright et al. (1987) have suggested that SP-A acts to direct surfactant lipids to type II cells, as shown by enhanced uptake of liposomes in these cells.

Biophysical approaches are expected to be helpful in elucidation of the molecular nature of the interaction between the proteins and phospholipids of surfactant, clearly an important determinant of surfactant function. The physical techniques used to date in surfactant biophysics have centered around surface balance determination of monolayer properties [for reviews, see Notter (1984), Goerke and Clements (1986), and Scarpelli (1988)] and (frequently) turbidimetric (Hawgood et al., 1984; King & MacBeth, 1979) or calorimetric (Keough et al., 1985) studies of surfactant aggregation or thermotropic phase properties, respectively. Surface balance or calorimetric measurements, while extremely useful for macroscopic thermodynamic characterization of materials, provide no direct molecular structural information. The techniques of spectroscopy are needed for this purpose.

To date, few biophysical molecular spectroscopic methods have been applied to studies of the molecular interaction of

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[1] Abbreviations: FT-IR, Fourier-transform infrared spectroscopy; DPPC, 1,2-dipalmitoylphosphatidylcholine; DPPC-d_{62}, acyl chain perdeuterated 1,2-dipalmitoylphosphatidylcholine; DPPG, 1,2-dipalmitoylphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; SP-A, 30–36-kDa class of pulmonary surfactant protein, as observed in the current work.

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surfactant components. Hook et al. (1984) reported electron paramagnetic studies of surfactant lipid fluidity. More recently, Shiffer et al. (1988) investigated the interaction of low molecular weight surfactant proteins (5K–18K) with phospholipids using fluorescence spectroscopy to measure protein-induced alteration of liposomal fusion. This laboratory (Mautone et al., 1987) reported FT-IR studies of the effect of Ca^{2+} and the entire protein fraction on the thermotropic properties of surfactant lipids from rabbit lung lavage.

The advantages of FT-IR for studies of surfactant are the following: (1) The technique has the potential to measure lipid acyl chain configuration, phospholipid head group/ion interactions (Dluhy et al., 1983), and protein secondary structure in a single experiment. (2) No extrinsic probe molecules which could perturb the properties of the system under investigation [e.g., see Taylor and Smith (1980)] are required. (3) Relatively small amounts of material (microgram range) may be examined in a variety of physical states, such as bilayer vesicles or monolayer films on an IR substrate or aqueous surface (Dluhy & Cornell, 1985), which may be relevant to understanding the in vivo properties of surfactant.

The current work reports the results of an FT-IR study of intact bovine surfactant and its hydrophobic extract, as well as SP-A and the remainder of the surfactant proteins (the latter taken as a group) reconstituted into lipid mixtures selected to mimic the composition of surfactant.

Materials and Methods

Separation of Surfactant Components. Lipid and protein components were separated by the method of Ng et al. (1983). Briefly, 0.5 mL of bovine surface-active material as defined in Mautone et al. (1987) (34 mg/mL phospholipid as estimated from lipid phosphorus assays) was added to a test tube containing 4.8 mL of isopropyl ether, 3.2 mL of *tert*-butyl alcohol, and 2.5 mL of water. The mixture was agitated by vortex action at room temperature every 5 min for 30 min and then centrifuged at 1000*g* for 10 min. The upper (mostly organic) phase was removed and stored at −20 °C. The aqueous phase and interfacial material were added to cold (−20 °C) 100% ethanol (ethanol:water final ratio 5:1) and stored at 4 °C overnight. Precipitates were collected by centrifugation at 18000*g* for 30 min, 4 °C, using a Beckman JA-20 rotor, resuspended in double-distilled water, and stored as a suspension.

SP-A was isolated by concanavalin A–Sepharose affinity chromatography (Ng et al., 1983); 1–1.5 mg of protein suspension was applied to the column at a flow rate of 1.5 mL/h. A fraction termed "unbound" protein was eluted with buffer I (0.2 M Tris-HCl and 0.5 M NaCl, pH 7.5) at a flow rate of 3.5 mL/h. Protein levels were monitored with an absorbance detector at 280 nm. A fraction termed "bound" protein was eluted with a freshly prepared solution of 1.0 M methyl α-D-mannoside in buffer I, at a flow rate of 8 mL/h. Bound and unbound protein fractions were separately precipitated overnight in 100% ethanol (−20 °C) (5:1 v/v). Precipitates were collected in pellet form by centrifugation as above and resuspended in double-distilled water prior to chemical and electrophoretic characterization. Bound protein was dialyzed against double-distilled water for 2 days at 4 °C to remove all methyl α-D-mannoside.

Chemical Characterization. Lipid phosphorus was determined for purified surfactant and its organic extract by the method of Chen et al. (1956). Protein concentrations were determined by the Bradford (1976) assay. In accord with King (1982), we have found that 10% by weight of the surfactant is protein. Sueishi and Benson (1981) have also shown that 30–40% of the total protein is the sought SP-A.

Protein molecular mass was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Ten percent mini slab gels were run under reducing conditions with a phosphate buffer, pH 7.2 (Weber & Osborn, 1969). Gels were developed by using a modified silver stain procedure.

Endo-β-*N*-acetylglycosaminidase H (Endo H) digestion for carbohydrate analysis of the bound fraction was performed as follows: 10 milliunits of enzyme was added to 0.1 mL of protein sample (2 mg/mL in 0.1 M sodium citrate buffer at pH 5.5), sonicated gently in a bath-type sonicator for 5 min, and incubated at 37 °C for 17 h. Samples were placed in a boiling water bath for 2–3 min to stop enzyme action. Total sugar was determined by a modified orcinol/sulfuric acid assay (White & Kennedy, 1981) using mannose as the standard.

Reconstitution of Lipid with Surfactant Proteins. The method used was a modification of a reconstitution protocol for glycophorin from erythrocyte membranes into phosphatidylcholines (MacDonald & MacDonald, 1975). The desired levels of lipid in $CHCl_3$/MeOH, and protein in water, were mixed in a ratio of 3:1.5:0.2 v/v/v ($CHCl_3$/MeOH/H_2O). Bulk solvent was evaporated under a stream of nitrogen gas, and the resultant damp sample was thoroughly dried under vacuum (0.1–1 torr). The resultant dry film was resuspended in 0.145 M NaCl/0.25 M phosphate buffer, pH 7.4, mixed by vortex action, sonicated gently in a bath-type sonicator for 15 min, and incubated overnight at 37 °C.

FT-IR Spectroscopy. Samples of lipid/protein complexes for FT-IR were centrifuged at 43000*g* for 1 h, 4 °C, using a Beckman TLA 100.3 rotor. The resulting pellet was collected and squeezed (still fully hydrated) between two 6-mm-diameter CaF_2 windows separated by a 25-μm spacer. The windows were placed in a thermostated cell constructed in this laboratory. Temperature control was achieved with a Haake (Model A80) circulating bath.

Spectra were recorded on a Mattson Instruments Sirius 100 spectrometer equipped with a Bach-Shearer microscope attachment. The microscope facilitated the acquisition of spectra from small amounts (50–500 μg) of complex. Routinely, 400 interferograms were collected, co-added, and processed as previously described (Mautone et al., 1987). Temperature calibration was provided by monitoring temperature-induced alterations in the CH_2 stretching bands of standard pure lipid components. Temperature accuracy is estimated at ±1 °C.

Spectra were taken over the temperature range 0–50 °C for normal melting curves or 1–37 °C for reversibility studies. The spectrum of water matched for temperature and cell path length was used for background subtraction. Linear base-line flattening routines were used in the C–H or C–D stretching regions to ensure uniform conditions for frequency measurements. Peak positions were calculated with a parabolic peak-picking routine supplied with the instrument.

Differential Scanning Calorimetry. Endotherms were obtained on a Micro-cal MC1 unit (Micro-cal, Inc., Amherst, MA). The sample size was 0.7 mL; the samples contained 14 mg/mL phospholipid. Scan rates were 35 °C/h.

Results

Lectin Affinity Chromatography. Typical silver-stained gels for the bound and unbound species are shown in Figure 1. Track 1 shows (from top to bottom) seven molecular weight standards of 66K, 45K, 36K, 29K, 24K, and 20.1K, and 14.2K, respectively. Tracks 2 and 3 (unbound fraction) show bands at positions corresponding to relative molecular weights of about 66K, 55K, 29K, 18K, 12K, and 10K with a very weak feature at 30K–36K. These data are consistent with those of

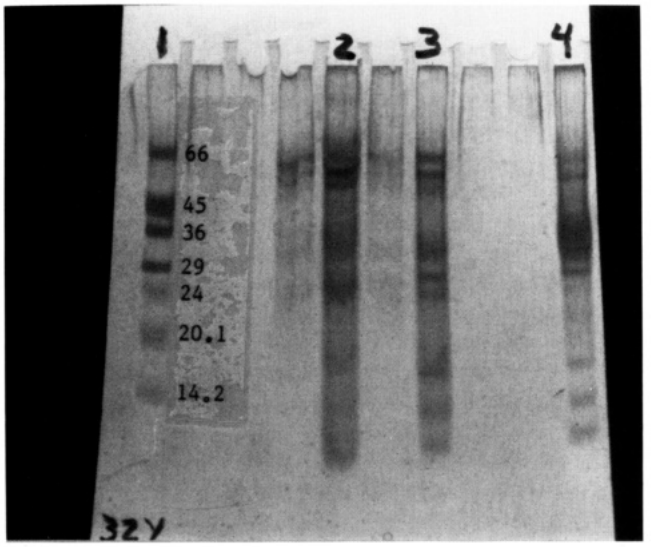

FIGURE 1: Ten percent acrylamide SDS–PAGE gels under reducing conditions. Track 1, 1.15 μg of standards: bovine albumin, egg ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor, and α-lactalbumin with molecular weights 66 000, 45 000, 36 000, 29 000, 24 000, 20 100, and 14 200, respectively, as indicated. Track 2, 3.56 μg of unbound protein fraction; track 3, 0.41 μg of unbound protein fraction; track 4, 1.15 μg of bound protein fraction. Proteins were stained with silver staining.

Ng et al. (1983) and Wright et al. (1984). Track 4 shows a gel of the bound fraction containing a main feature corresponding to M_r 29K–36K with substantially weaker features at molecular weights corresponding to those found in the unbound fraction. Thus, the fractionation process has essentially separated the 30–36-kDa protein class from the remainder of the proteins.

We note the presence of a 28-kDa protein in both the bound and unbound fractions. This species appears substantially more intense than the 30–36-kDa group in the unbound fraction. Ng et al. (1983) have observed similar patterns and attributed the presence of the 28-kDa protein in the unbound fraction to an unglycosylated species. In any case, the observation of a 30–36-kDa fraction, substantially glycosylated (see below), indicates that this class of protein corresponds to the predominant, SP-A, surfactant-specific class.

Endoglycosidase H Cleavage. Determination of total sugar from the orcinol/sulfuric acid assay following cleavage with endoglycosidase H showed that the bound fraction contained 0.25 mg/mg of protein, confirming its identifiation as a glycoprotein. These results support the observation (Benson et al., 1985; Ross et al., 1986) of variable N-linked glycosylation in the COOH-terminal region of the protein. Variation of molecular weight within the 30K–36K group then reflects differing degrees of glycosylation with a common amino acid primary sequence. It is noted that canine SP-A has been cloned and two possible glycosylation sites have been suggested (Benson et al., 1985).

Infrared Spectroscopy. (A) Intact Surfactant. Typical IR data for the C–H stretching region of surfactant in aqueous suspension are shown in Figure 2. In a previous study (Mautone et al., 1987), assignments have been given both for this spectral region and for the 1000–1200 cm^{-1} spectral region containing the phosphate stretching modes. The latter have been used (Mautone et al., 1987) for investigation of charged species such as Ca^{2+} ion with phospholipid polar regions. A detailed evaluation of the effect of surfactant protein on these modes is under way.

For the current study, the needed spectral features are the phospholipid acyl chain symmetric and antisymmetric CH_2 stretching modes near 2850 and 2920 cm^{-1} (Figure 2). Their band positions can be determined with a precision of better than 0.05 cm^{-1} (Cameron et al., 1982); thus, temperature-induced variation in frequency position provides a convenient method of monitoring lipid thermotropic behavior. Although frequency increases in these vibrational modes are small (1.5–4 cm^{-1}) during lipid melting events, the precision available allows a good representation of these processes to be obtained. The origin of the frequency increase upon melting has been traced by Snyder et al. (1982) to variations in interaction constants between C–H stretching coordinates on adjacent methylene groups when the lipid physical state is altered by the formation of gauche rotamers, for example. The spectral feature near 2850 cm^{-1} is better suited for melting curve construction as it suffers minimal interference from overlapping protein vibrations (Mendelsohn & Mantsch, 1986).

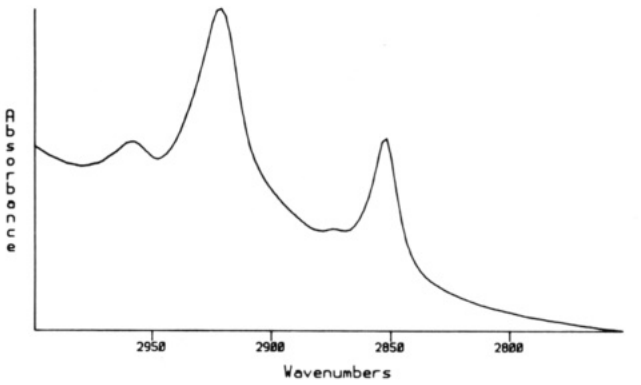

FIGURE 2: FT-IR spectra of the C–H stretching spectral region (3000–2700 cm^{-1}) in bovine surfactant. The plotted peaks arise mainly from the phospholipid components.

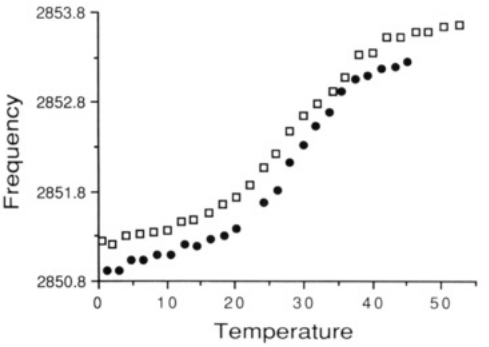

FIGURE 3: Effect of the surfactant proteins on the phase transition of bovine surfactant in buffer I (0.2 M Tris-HCl/0.5 M NaCl, pH 7.5). Curves of total surfactant (□) and its hydrophobic extract (●) are constructed from the temperature dependence of the CH_2 symmetric stretching frequencies of the lipid acyl chains near 2850 cm^{-1}.

The temperature dependence of the 2850 cm^{-1} frequency from intact bovine surfactant, and its hydrophobic extract (probably containing lipids as well as residual hydrophobic 3.7-kDa protein at levels of 1% or less by weight), is shown in Figure 3. A broad melting event with an onset temperature of about 19–20 °C and a completion temperature of about 37 °C is clearly discernible in the hydrophobic extract. Thus, at physiological temperatures, the lipids are mostly in the liquid-crystalline state. The melting curve for intact surfactant has the same general characteristics but differs in detail. At all temperatures, the CH_2 frequency is about 0.3 cm^{-1} higher than that for the extract, suggestive of a protein-induced disordering of the lipid acyl chains. In addition, the transition width appears to have been increased slightly by protein, although determination of a particular onset temperature for

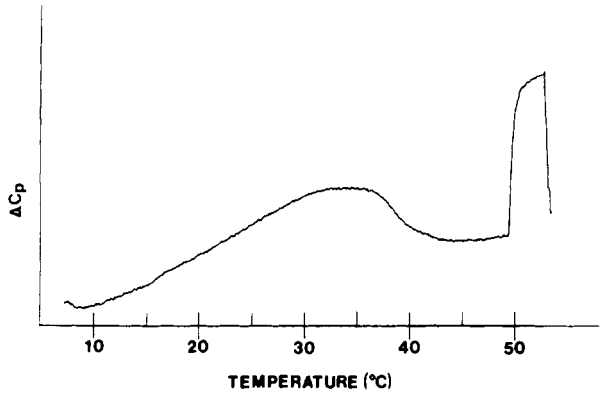

FIGURE 4: Differential scanning calorimetry (DSC) endotherm of bovine surfactant in buffer I. The feature beginning at 50 °C is a power pulse used to calibrate enthalpies.

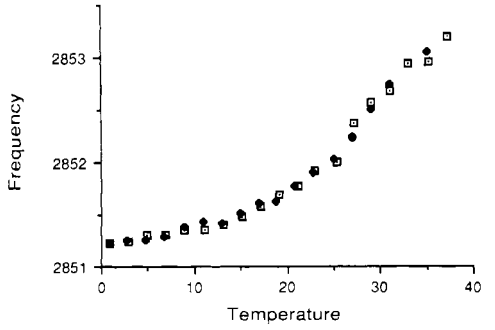

FIGURE 5: Thermotropic behavior of native bovine surfactant from 1 to 37 °C. Heating (⊡) and cooling (♦) curves are superimposable, showing the reversibility of the melting event as well as the reproducibility of data.

lipid melting in the presence of protein is difficult to pinpoint due to the continuously changing curvature of the melting profile in the onset region. The occurrence of a thermotropic event was confirmed by differential scanning calorimetry (Figure 4).

The reversibility of the broad melting event in native surfactant is demonstrated in Figure 5. The FT-IR melting profiles taken during heating and cooling cycles (1–37 °C) are coincident within the estimated precision of the experiment (0.05 cm^{-1}). The results of Figure 5 lend further credence to the observation in Figure 3 of protein-induced disordering of the lipids (as monitored through the 0.3 cm^{-1} increase in frequency), by showing that highly precise and reproducible sets of frequencies may be collected.

(B) Reconstituted Systems. The reconstitution protocols described above were initiated in an attempt to see how the various protein fractions, particularly SP-A, affect the lipid melting events. A simple lipid system, shown by King (1982) to be efficient in reconstitution experiments, was chosen to clarify interpretations of melting curves and to reduce ambiguities in establishing accurate onset and completion temperatures.

The bound (SP-A) and unbound fractions were reconstituted into binary lipid mixtures consisting of 85% acyl chain perdeuterated DPPC (DPPC-d_{62}) and 15% DPPG. The use of DPPC-d_{62} as one component of a binary mixture serves two purposes. First, it shifts the acyl chain frequencies into a spectral region (2000–2250 cm^{-1}) which is free from interference from other membrane components. Second, it permits (in experiments of good signal-to-noise ratio) the separate determination of the melting of each lipid component in binary lipid mixtures. The approach has been used by this laboratory (Jaworsky & Mendelsohn, 1985) and the Pezolet group

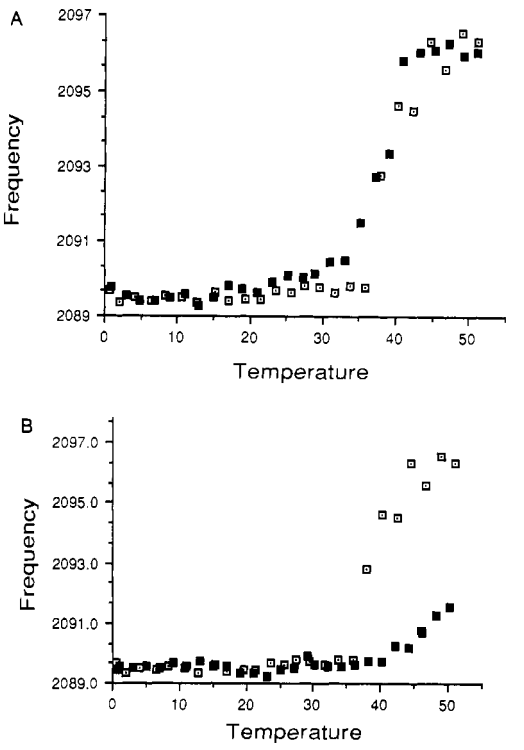

FIGURE 6: Temperature dependence of the CD_2 stretching vibration of the phosphatidylcholine (PC) component of reconstituted samples. Lipid model system (DPPC-d_{62}/DPPG, 85:15 w/w) (⊡) reconstituted with SP-A (■) at lipid:protein weight ratios of (A) 1:1 and (B) 1:2.

(Laroche et al., 1988) to study lipid/lipid and lipid/protein interactions.

Melting curves constructed from the perdeuterated acyl chain CD_2 symmetric stretching vibrations near 2090 cm^{-1} of the DPPC-d_{62} component in an 85:15 mole ratio binary lipid mixture with no added SP-A, and with SP-A added in weight ratios (lipid/protein) of 1:1 and 1:2, respectively, are shown in Figure 6A,B. The SP-A-free lipid component shows a melting event from about 36 to 39 °C. The effect of SPA at a 1:1 weight ratio on the melting of the DPPC-d_{62} component is small (Figure 6A). The onset temperature may be shifted down by about 1–2 °C (which is close to the precision of the temperature measurement), and the completion temperature is unaltered. In addition, the frequency parameter is at most only slightly affected by the presence of protein (over the temperature range 0–30 °C). Larger proportions of SP-A produced a more substantial perturbation of the lipid acyl chain melting (Figure 6B). At a 1:2 lipid:protein weight ratio, a substantial ordering of the lipid component is noted. The melting process begins near 42–43 °C (a 5–6 °C increase from the protein-free systems) and is not yet completed by 50 °C, the highest temperature used in these studies. Thus, at least a 10 °C increase in the completion temperature is noted.

In contrast, the unbound fraction containing a mixture of surfactant proteins, as previously described, produced very different perturbations of the acyl chain melting profiles, as shown in Figure 7A,B. The melting curve for a 1:1 lipid:protein weight ratio sample shows a clear onset temperature of 27–28 °C, shifted down markedly from the SP-A-free lipid which begins to melt at 36 °C. The completion temperature is unaltered from the control systems. The midpoint of the melting process occurs at 33 °C, shifted down by 4–5 °C from the control lipids. Increasing the protein level to a 1:2 lipid:protein ratio by weight (Figure 7B) causes a large lowering in transition cooperativity to the point where the increasing disorder in the acyl chains occurs continuously from 15 to 42

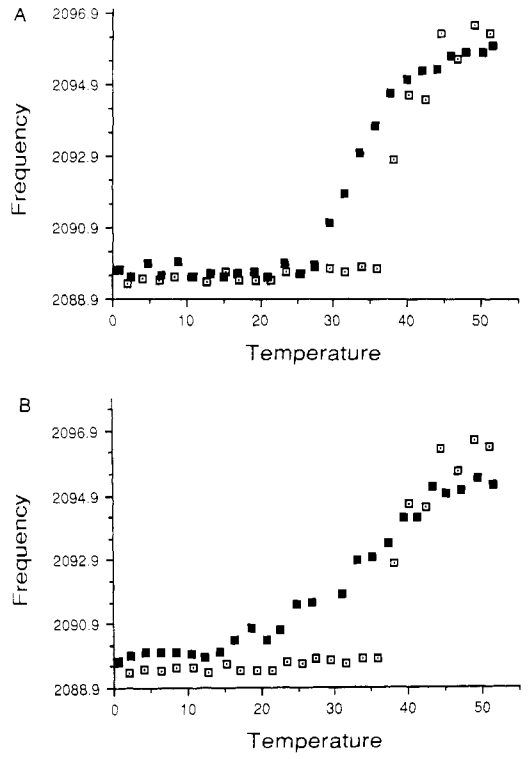

FIGURE 7: Temperature dependence of the CD_2 stretching vibration of the PC component of reconstituted samples. Lipid model system (DPPC-d_{62}/DPPG, 85:15 w/w) (⊡) with unbound protein fractions (■) at lipid:protein weight ratios of (A) 1:1 and (B) 1:2.

°C (midpoint, 29 °C). In addition, the CD_2 stretching frequencies are higher than those of the lipid control throughout the lipid gel phase, suggesting substantial protein-induced disordering. Similar progressive effects as protein levels are increased were observed for the CH_2 stretching modes of the DPPG component, albeit with increased scatter in the plots, due to the small fraction of proteated component (data not shown).

Discussion

Little is known about the molecular nature of the interaction of any of the surfactant proteins with phospholipids although the studies quoted in the introduction suggest possible physiological functions for this interaction. Binding studies (King, 1982) of SP-A with phospholipids suggested that the protein binds PC's that are in the gel state with greater affinity than it binds liquid-crystalline PC's. In addition, maximum binding was found to occur with 85:15 PC/PG mixtures. King suggested that apolar interactions might be important in the lipid/protein association, and further speculated that the PC's may be partially immobilized about the protein during the formation of the complex. The current results provide evidence in partial support of King's views. The FT-IR melting curves for phospholipid/SP-A complexes (Figure 6) are not consistent with a hydrophobic lipid/protein interaction, which would be expected (Mendelsohn et al., 1984) to progressively reduce the lipid melting temperature and cooperativity as the level of protein is increased. The current experiments indicate that the phospholipids become more ordered in the presence of high levels of SP-A. Whether our observed ordering of lipid is the result of a boundary layer with immobilized acyl chains, as suggested by King, or due to an interaction between SP-A and the phospholipid head groups is unclear and must await biophysical experiments in which rates of lipid motions can be examined in detail. We note that electrostatic interactions between cations or positively charged protein side chains and acidic phospholipids tend to increase the lipid transition temperatures (Kouaouci et al., 1985).

The lipid/protein interaction in native surfactant is characterized (Figure 3) by a slight disordering of the lipid in both the gel and liquid-crystalline lipid phases. As SP-A is the main surfactant protein and it tends to order the lipid molecules, the effect of the remainder of the protein fraction must be stronger, on a molar basis, in order to produce the observed overall disordering. The effect of the unbound fraction (Figure 7) on the melting of DPPC-d_{62}, which results in a disordering of the lipid, resembles a known hydrophobic interaction, namely, that of erythrocyte glycophorin on dimyristoyl-PC (Mendelsohn et al., 1981). Progressive incorporation of protein lowers the transition temperature and reduces the cooperativity of the melting event, as noted here.

There are at least two events which have been shown in vitro to be enhanced by SP-A. The first is the rate of Ca^{2+}-induced surfactant lipid aggregation and adsorption to air/water interfaces (Hawgood et al., 1985). The observed lipid ordering at high SP-A levels is not easily reconciled with an enhanced protein-induced spreading rate, which would probably require a disordering of the lipid by the protein, though we have not investigated the role of Ca^{2+} as to its ability to modulate the lipid/protein interaction. Introduction of disorder by one or more of the other surfactant proteins (the "unbound" fraction in the current work) may provide a mechanism for modulation of spreading rates. A possible mitigating factor arises in that the current experiments are carried out in the bulk phase. It is conceivable that the mechanism of lipid/protein interaction may be altered at air/water interfaces. The second in vitro event affected by protein is the reuptake of phospholipids into type II pneumocytes (Wright et al., 1987). Similar modulations of lipid order may also play a role in the reuptake pathway, which occurs in bulk phases.

Finally, the utility of molecular spectroscopy in general and FT-IR in particular for elucidating the moledular nature of interactions between surfactant components is demonstrated here. SP-A and the unbound fraction behave in opposite fashion as regards their ability to effect phospholipid order. Any model for their function must take these differences into account.

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Differential Sensitivity of Chicken Progesterone Receptor Forms to Sulfhydryl Reactive Reagents†

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ABSTRACT: DNA binding of chick progesterone receptor B form (PRB) has been examined and compared to that of the A form (PRA). We found that the elution profiles of the two receptors overlap on DNA–cellulose columns. Both PRA or PRB could bind to plasmid DNA equivalently as assayed by sedimentation velocity studies. However, DNA-binding activity of the two receptor forms showed differential sensitivity to reducing agents and to sulfhydryl (SH) reactive reagents. Reducing agents stabilized DNA-binding activity of PRA more efficiently than they stabilized PRB. Moreover, removal of reducing agents from receptor preparations caused preferential loss of DNA binding by PRB compared to the PRA. DNA-binding activity of PRA was readily destroyed by sulfhydryl modifying reagents such as *N*-ethylmaleimide and iodoacetamide while PRB was 3–4 times less sensitive to these reagents. We conclude the DNA-binding activity of PRB is less stable due to altered accessibility of SH groups despite the amino acid sequence identity of the DNA-binding domains of PRA and PRB.

The chicken progesterone receptor (PR)[1] exists in two forms: receptor A (PRA) of 78 kDa and receptor B (PRB) of 108 kDa (Birnbaumer et al., 1983). These receptor forms are products of two translation initiation sites of a single mRNA (Conneely et al., 1987a). In earlier studies, we found that PRA could bind tightly to DNA–cellulose (Schrader et al., 1972) and to soluble DNA (Compton et al., 1984). By contrast, PRB showed only weak binding to DNA–cellulose (Vedeckis et al.,

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[1] Abbreviations: PRB, progesterone receptor B form (108 kDa); PRA, progesterone receptor A form (78 kDa); SH, sulfhydryl; NEM, *N*-ethylmaleimide; DES, diethylstilbestrol; TAA, triamcinolone acetonide; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; βME, β-mercaptoethanol; DTT, dithiothreitol.

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