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Non-cooperative effects of lung surfactant proteins on early adsorption to an air/water interface

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Abstract

Two small hydrophobic proteins, SP-B and SP-C, are responsible for rapid adsorption of pulmonary surfactant to the air/water interface. Despite their physiological importance, the number of protein molecules required to trigger an absorption event remains unknown. To investigate this issue, we varied the protein content of calf lung surfactant extract (CLSE) by dilution with protein-depleted surfactant lipids (neutral and phospholipids, N&PL). Vesicles of a constant size and of composition ranging between 100% N&PL and 100% CLSE were generated by probe sonication. Their adsorption kinetics to an air/water interface were monitored at different temperatures using a Wilhelmy plate to measure surface tension. When plotted versus protein concentration, the adsorption rates during the initial change in surface tension exhibit a diphasic behavior, first increasing rapidly and linearly between 0% and 25% CLSE, and then more slowly at higher concentrations. Direct linearity at low protein content (0–5% CLSE ratio) was confirmed at 37 °C. These observations argue against cooperative behavior, for which the adsorption rate would first rise slowly with the protein content, and then increase suddenly once the critical number of proteins on each vesicle is reached. The apparent activation energy E_a and the free energy of activation ΔG_0^*, calculated from the temperature dependence of adsorption, further support the view that at least the early stages of protein-induced surfactant adsorption proceeds through a sequence of events involving not several, but a single surfactant protein.

Keywords: SP-B; SP-C; Vesicle; Fusion; Cooperativity; Monolayer

1. Introduction

Lung surfactant contains low concentrations of two small, very hydrophobic proteins, SP-B and SP-C. Their best defined activity is to promote rapid adsorption of surfactant to the air/water interface. By lowering surface tension, the surfactant interfacial film prevents alveolar collapse at the end of exhalation. In spite of their physiological importance, the mechanism by which SP-B and SP-C achieve their effect remains uncertain.

Interfacial adsorption shares a number of characteristics with bilayer/bilayer fusion. Both processes require major reorganization of a bilayer to fuse with another bilayer (fusion) or a monolayer (adsorption), and both depend on specialized proteins to proceed with any appreciable speed. Fusion and adsorption require intimate contact of the merging membranes, which is opposed by electrostatic and repulsive hydration forces [1–3]. The general similarities suggest that the molecular mechanisms in the two processes may also be similar.

One of the best-known fusion proteins is the Influenza hemagglutinin fusion protein (HA). HA trimers initiate fusion by binding opposing membranes and forcing them into close contact, overriding the repulsive hydration and electrostatic forces between bilayers [4]. Once in close proximity, a highly curved hemifusion intermediate joining the two outer leaflets presumably forms and induces fusion by a process controlled mostly by the membrane lipids [5]. In this proposed mechanism, several protein molecules acting cooperatively at the same locus should be more efficient than a single protein. Although the topic is still debated (see for example Ref. [6]), experimental evidence suggests that HA-induced fusion is indeed a cooperative process in which two to eight HA trimers are required to initiate one fusion pore [7,8].

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The surfactant proteins may also function cooperatively. In addition to promoting adsorption, SP-B induces fusion of lipid vesicles. The kinetics of fusion do not increase linearly with protein concentration, suggesting some degree of cooperativity [9]. The protein dependence of the more physiologically relevant process, interfacial adsorption, is unknown.

In this report, we determined whether the adsorption of pulmonary surfactant proceeds through a mechanism involving a single or multiple proteins by measuring adsorption kinetics at various protein-to-lipid ratios. The protein content of calf lung surfactant extract (CLSE) was altered by dilution with protein-depleted surfactant lipids (neutral and phospholipids, N&PL). Vesicles of a constant size and composed of increasing dilutions of CLSE in N&PL were generated by probe sonication. The kinetics of adsorption to an air/water interface were monitored at temperatures ranging from 10 to 50 °C during the initial change in surface tension. The activation energy E_a and activation free energy ΔG_0^* were calculated from the temperature dependence of k, the adsorption rate constant. In the absence of interactions between proteins, the adsorption rate and activation energy should vary linearly as a function of protein content, while ΔG_0^* should remain constant. Deviation from this behavior indicates cooperative behavior, and this approach can establish the minimum number of proteins required to promote interfacial adsorption.

2. Material

2.1. Chemicals

All chemicals used were of analytical grade. Solvents were of spectroscopic grade. Water with a resistivity greater than 17.3 MΩ/cm was obtained from a Firstream II water system (Barnstead, Dubuque, IA). CLSE extracted from material lavaged from calf lungs [10], was provided by Dr. Edmund Egan (ONY Inc., Amherst, NY). CLSE had a ratio of 6.67 μg protein/μmol phospholipid. Experiments compared material obtained from individual lots of CLSE. All lipid concentrations and CLSE/N&PL ratios were expressed in moles of phosphate assayed. We used as a standard buffer 150 mM NaCl, 10 mM Hepes, 1.5 mM $CaCl_2$, pH = 7.0 (HSC).

2.2. Biochemical assays

Phospholipid concentrations were determined by phosphate assay [11]. Protein content was assayed with amido black on material precipitated with trichloroacetic acid [12] using bovine serum albumin as the standard. Total cholesterol was assayed by reduction with ferrous sulfate [13].

2.3. CLSE fractionation

Fractionation of the hydrophobic components of CLSE used gel permeation chromatography [14]. The peaks containing the phospholipids and the neutral lipids (mostly cholesterol) were pooled to obtain the N&PL. Protein levels in N&PL were below the sensitivity of the assay (0.5 μg).

3. Methods

3.1. Small unilamellar vesicles

Lipids dried from chloroform solutions were resuspended in 1 ml of HSC buffer by extensive vortexing and then sonicated 10 min with a Branson tip sonicator (The Virtis Company, Gardiner, NY) at 10 W output. Sonication was carried out on ice to minimize lipid and protein degradation. Previously reported experimental data suggest that co-sonication with phospholipids has little influence on the secondary structure of SP-B [15]. Vesicle size, measured by dynamic light scattering (DynaPro LSR, Protein Solutions Incorporated, Charlottesville, VA), was 65 ± 10 nm radius regardless of the composition. Vesicles were kept at room temperature and used over a period of 8–10 h without significant change in measured size or adsorption kinetics.

3.2. Surface tension measurement

Adsorption was monitored by measuring surface tension at the surface of a thermostated 5 ml Teflon cup using a 1-cm wide Wilhelmy paper plate attached to a KSV 3000 force transducer (KSV Instruments Ltd., Helsinki, Finland). Signal was recorded versus time on a computer using KSV-supplied acquisition software. Kinetics of adsorption to an air/water interface were measured during the initial change in surface tension at temperatures between 10 and 50 °C and using a subphase concentration of 100 μM phospholipid. The initial rate of adsorption was measured as the slope of the initial linear segment of surface tension versus time [16].

3.3. Surface concentration calibration

The relationship between surface tension and surface concentration was established by incremental spreading of a known quantity of material, as described previously [16]. Isotherms of surface tension versus surface concentration were fitted between 68 and 32 mN/m surface tension by a straight line (data not shown). No significant differences were found between CLSE and N&PL. The surface concentration when surface tension first began to change at 70 mN/m was 1.5 μmol/m^2, approximately 50% of the maximum equilibrium concentration of 2.8 μmol/m^2.

3.4. Kinetic parameters

The adsorption rate constants k were calculated from the slope of the initial decrease in surface tension versus time. The rate constants were calculated assuming: (1) a unique relationship between surface tension and surface concentra-

tion; (2) interfacial adsorption that is irreversible; and (3) a reaction order of 1.6 [16],

$$V = kC^{1.6} \quad (1)$$

where V is the initial adsorption speed and C the surfactant concentration in the subphase. Previous studies found reaction orders of 1.41 ± 0.18 and 1.73 ± 0.18 for N&PL and CLSE adsorption, respectively [16]. An average value of 1.6 was chosen here to analyze adsorption of CLSE-N&PL mixtures. Because a constant concentration of 100 μM phospholipid was used, variations in the reaction order result only in modest changes of the reaction rates and have no influence on our conclusions.

The activation energies E_a were calculated for each composition from the slope of $\ln(k)$ versus $1/T$ (where T is temperature) on Arrhenius graphs (Fig. 5). The activation free energies ΔG_0^* of the transition state were calculated for each temperature from the rate constant using the expression:

$$\Delta G_0^* = -RT\ln[kh/(k_B T)] \quad (2)$$

where R, h and k_B are the universal gas constant, Planck's constant and Boltzmann's constant, respectively [17].

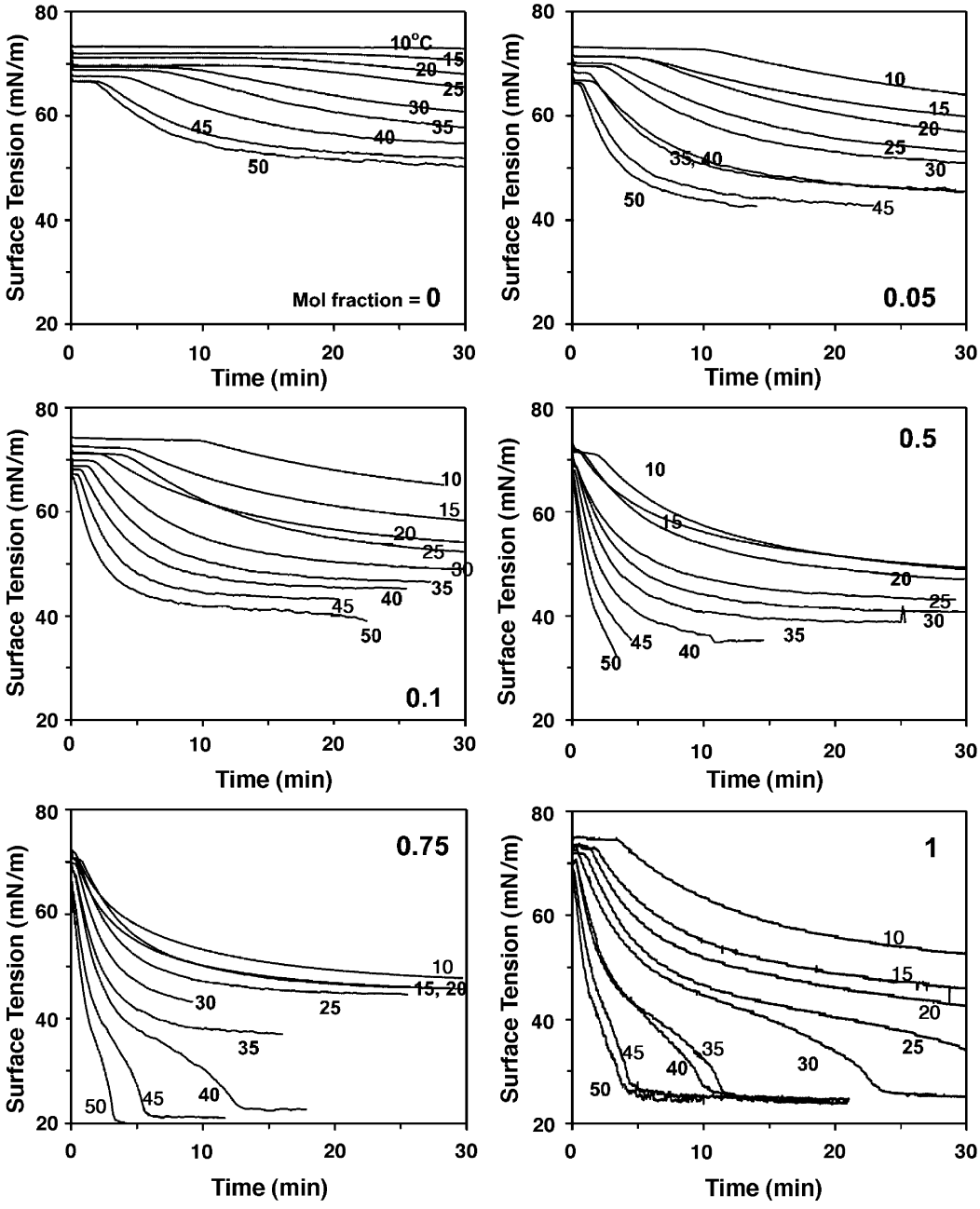

Fig. 1. Adsorption isotherms for mixed CLSE/N&PL vesicles. The mole fraction of CLSE is indicated on each panel. Data for CLSE mole fractions of 0.25 and 0.9 are omitted for clarity. Individual curves in each panel represent data for different temperatures (°C), specified on each curve. All experiments were performed with a subphase phospholipid concentration of 100 μM.

4. Results

To investigate the minimum number of proteins required to accelerate adsorption of the surfactant lipids, we measured the adsorption kinetics of probe-sonicated vesicles containing CLSE diluted with N&PL. Composition was expressed as the mole fraction of CLSE, or CLSE/(CLSE + N&PL), expressed in terms of phospholipid.

Adsorption isotherms obtained for CLSE mole fractions of 0 (pure N&PL), 0.05, 0.1, 0.25, 0.5, 0.75, 0.9 and 1 (pure CLSE) at different temperatures generally showed the expected variation with composition and temperature (Fig. 1). CLSE adsorbed more rapidly than N&PL at any given temperature, and the rate of adsorption increased at higher temperatures. Mixed CLSE/N&PL vesicles containing modest amounts of CLSE adsorbed significantly faster than N&PL, in particular at higher temperatures (Fig. 1). Only vesicles with a high mole fraction of CLSE, however, were able to reach equilibrium surface tension (around 25 mN/m) within the experimental duration of 30 min, and only at temperatures above 25 °C. The previously reported inflection point [16], at which adsorption ceases its expected progressive slowing and instead accelerates, again occurred for all samples that reached surface tensions below approximately 40 mN/m.

We used the initial fall in surface tension as the most readily available index of adsorption. The initial adsorption speeds, plotted in Fig. 2 versus temperature and CLSE content, reflected the trends observed from the adsorption isotherms. At temperatures above 30 °C, as little as 10% CLSE in N&PL provided speeds equivalent to 30–50% of those observed with pure CLSE. Furthermore, at physiological temperature, CLSE diluted by as much as 50% still maintained over 75% of the rate achieved by the pure sample (Fig. 2).

To calculate the kinetic rate constants, surface tensions were converted to surface concentrations. For CLSE ratios from 0 to 0.25, the initial adsorption rates increased almost linearly with protein concentrations (Fig. 3). Above 25% CLSE, adsorption rates also increased almost linearly, but with a lower slope. This difference in behavior above and below 25% CLSE was more evident at high temperature (35 °C and above), but was also apparent from 10 to 30 °C (Fig. 3).

The linear behavior observed over a broad range of protein concentrations left open the possibility that the critical number of proteins required for adsorption was below the lowest value investigated. Further experiments were conducted at lower protein content, from 0 to 0.05 CLSE fraction, at a temperature of 37 °C. Adsorption rates again showed no evidence of sigmoidal behavior, but instead reasonably fit a linear relationship with protein concentration (Fig. 4). The behavior established with higher protein content extended to these lower levels.

From the temperature dependence of k, we further calculated the apparent activation energy E_a from the slope of Arrhenius plots (Fig. 5). The activation free energy ΔG_0^* was calculated from the rate constants. Since ΔG_0^* varied much more with composition than with temperature, values at any composition were averaged over the full range of temperatures investigated. Within experimental

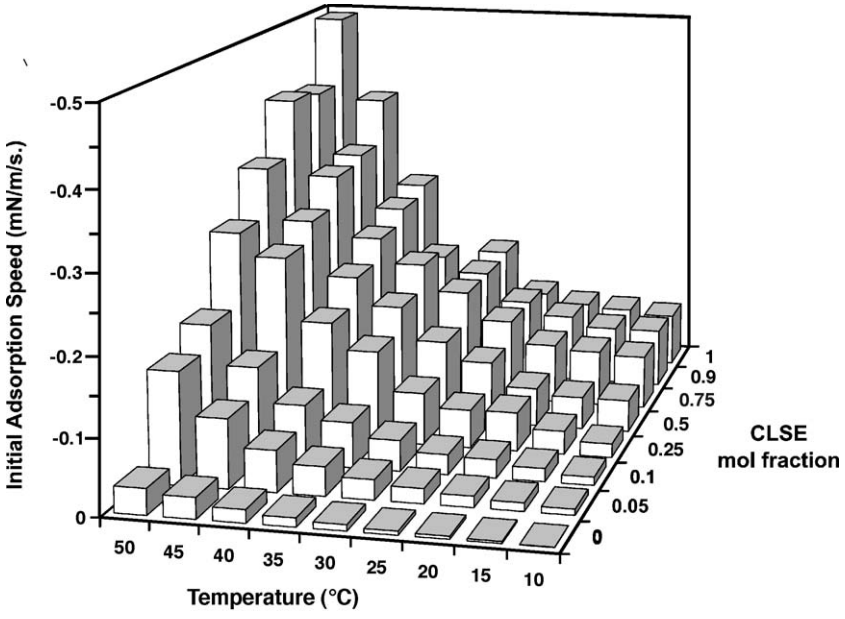

Fig. 2. Initial adsorption speed of CLSE/N&PL mixed vesicles (in mN/m/s) versus temperature and CLSE mole fraction.

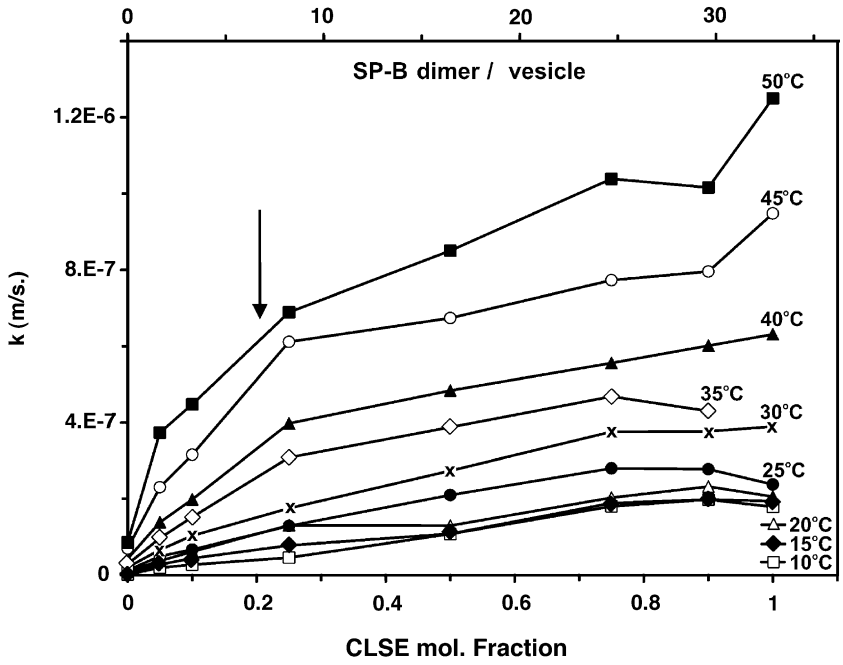

Fig. 3. Initial adsorption rates of CLSE/N&PL mixed vesicles at different CLSE mole fractions. Rates were calculated using surface calibrations and assuming an irreversible reaction with an order of 1.6 (see text). The upper abscissa indicates the estimated SP-B/phospholipid molar ratio. The number of SP-B dimers per vesicle was calculated assuming an equal amount of SP-B and SP-C present by weight in CLSE, molecular weights of 17,400 and 4200 Da for SP-B and SP-C, respectively, with an average vesicle radius of 65 nm and a phospholipid molecular area of 63 $Å^2$/molecule. Arrow indicates 4×10^{-5} SP-B/phospholipid molar ratio above which SP-B undergoes in-plane aggregation [31]. Temperature is indicated on each curve.

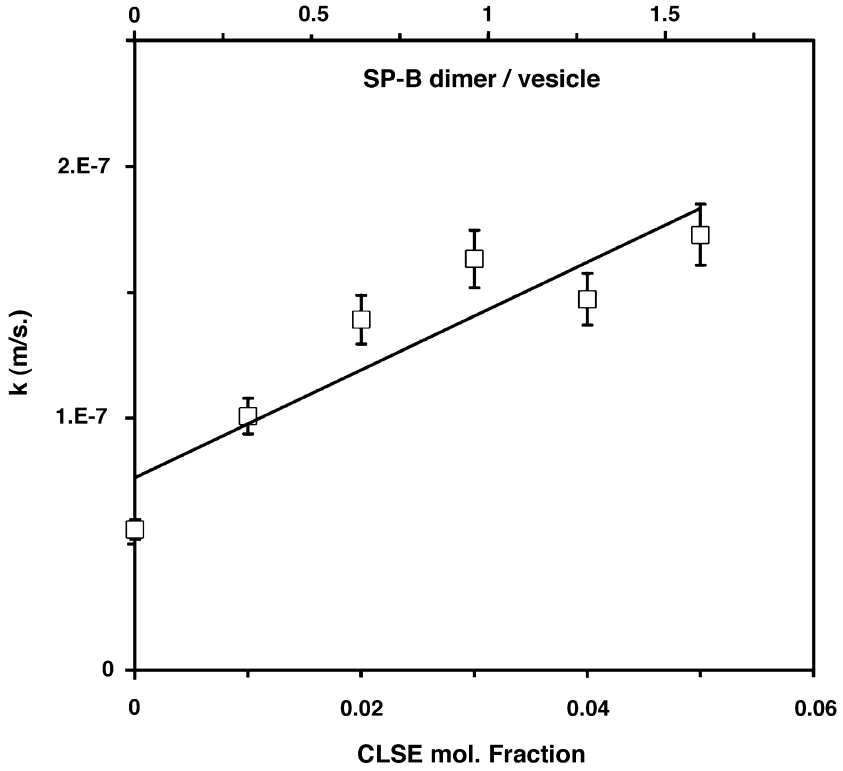

Fig. 4. Effect of protein content on initial adsorption rates for CLSE/N&PL mixed vesicles at low levels of protein. The upper abscissa indicates the number of SP-B dimer per vesicle. Data are mean ± standard deviations with best linear fit for six experiments performed on two different samples averaged for temperature = 37 °C.

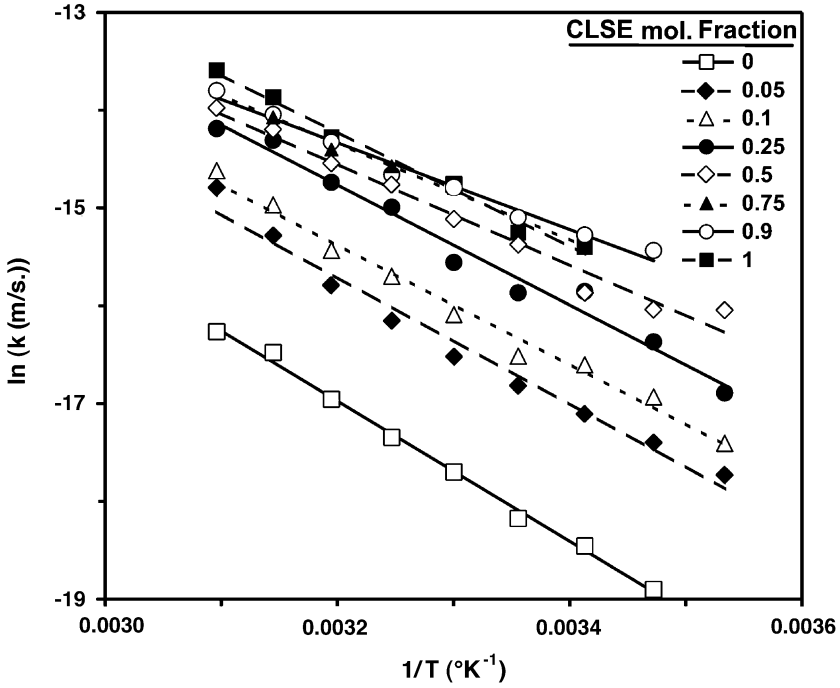

Fig. 5. Arrhenius graph of the initial adsorption rates at different CLSE mole fractions. Straight lines are linear regressions for each composition. Legend indicates CLSE mole fractions.

error, E_a varied linearly with the protein content, decreasing from 60 kJ/°K/mol for pure N&PL to 33 kJ/°K/mol for pure CLSE (Fig. 6). ΔG_0^*, which was greatest in the absence of protein, decreased sharply between 0% and 15% CLSE, and then more slowly with higher protein concentrations (Fig. 6).

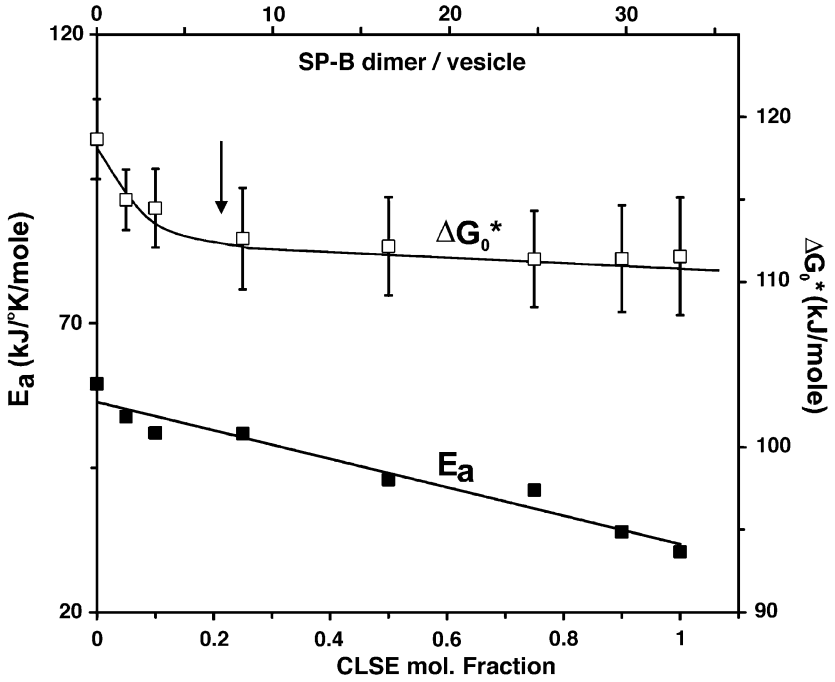

Fig. 6. Variation of the apparent activation energy (E_a) and free energy of activation (ΔG_0^*) for initial adsorption at different CLSE mole fractions. The upper abscissa indicates the estimated number of SP-B dimers per vesicle. Error bars on ΔG_0^* reflect changes with temperature. The curved line on ΔG_0^* is a guideline; the straight line on E_a is a linear regression on the activation energies. Arrow indicates 4×10^{-5} SP-B/phospholipid molar ratio above which SP-B undergoes in-plane aggregation [31].

5. Discussion

5.1. Vesicle composition

The extent to which the proteins act cooperatively could be studied with a variety of methods. In the experiments reported here, we vary the amount of protein by diluting CLSE with N&PL and compare the adsorption kinetics of probe-sonicated vesicles. This approach has the distinct advantage over reconstituting the purified proteins into surfactant lipids that the measured kinetics are unaffected by the rapid loss of activity of SP-B and SP-C once delipidated [18,19] or by the solvent system used for separation [20].

Vesicle composition, determined by the fraction of CLSE present in the mixture, may also be expressed as the molar ratios of SP-B to phospholipid or SP-C to phospholipid, or as the number of SP-B dimers and SP-C monomers per surfactant vesicle. The molar protein/lipid ratios are calculated from the known protein/phospholipid ratio present in CLSE, from the dilution factor for the different CLSE/N&PL mixtures, and from the presence of equivalent weights of SP-B and SP-C in extracted surfactants [20–22]. Using molecular weights of 17,400 and 4200 for SP-B and SP-C, respectively [23], the molar ratio of the two proteins in the native mixture is SP-B/SP-C 1:4. The number of protein molecules per vesicle may be computed from the measured vesicular radius (65 ± 10 nm) by assuming a molecular area of 63 $Å^2$ for each phospholipid in the surfactant bilayer [24]. The calculated values of CLSE mole fraction, protein-to-phospholipid molar ratio and number of proteins per vesicle are tabulated in Table 1 for the various mixtures. The calculated number of SP-B dimers per vesicle are also reported on the upper abscissa in (Figs. 3, 4 and 6).

While both proteins can accelerate adsorption, prior studies suggest that most of the effect of the combined proteins is actually contributed by SP-B alone. SP-B and SP-C promote adsorption independently [25], and SP-B is more active than SP-C on a weight basis [20,25–28]. Quantitative results diverge, probably because of different experimental protocols, but reasonable estimates indicate that per milligram, SP-B is three to five times more active than SP-C [20,26,27]. On a molar basis, the ratios of activities would then be 12–21. These prior results suggest that for any given sample in the present study, at least 75–83% of the initial adsorption is due to SP-B, while SP-C accounts for no more than 17–25% of the activity.

Table 1
Estimated ratios of individual proteins for different CLSE/N&PL mixtures

CLSE mole fraction	SP-B dimer/ phospholipid (μmol/mol)	SP-C monomer/ phospholipid (μmol/mol)	SP-B dimer/ vesicle	SP-C monomer/ vesicle
0.00	0.0	0.0	0.0	0.0
0.05	9.8	39	1.6	6.6
0.10	20	78	3.3	13
0.25	49	190	8.2	33
0.50	98	390	17	66
0.75	150	580	25	99
0.90	180	700	30	120
1.00	200	780	33	130

5.2. Cooperativity

If no interaction between proteins is required to trigger an adsorption event, then each additional protein in a vesicle increases the odds of adsorption by a specific amount. The macroscopic rate should therefore rise linearly with protein concentration. If a critical number of proteins is required, however, the increase in measured rate will be small until the cooperative size unit is reached. Addition of proteins well beyond the minimum cooperative unit size also would have little influence on the activity. Therefore, on a plot of activity versus protein concentration, cooperativity results in a sigmoidal shape, with an inflection point and a maximum slope indicating the size of the cooperative unit [29].

The adsorption kinetics of lung surfactant are neither linear nor sigmoidal in shape, but exhibit a diphasic dependence on protein concentration. Adsorption rates increase almost linearly up to 25% CLSE mole fraction (8 SP-B dimer per vesicle), and then more slowly at higher protein concentrations (Fig. 3). Even at very low protein concentrations from 0% to 5% CLSE (1.6 SP-B dimer per vesicle), our results show a strict linearity between adsorption rates and protein concentration (Fig. 4), suggesting a simple, non-cooperative behavior.

The thermodynamic variables of early adsorption provide further evidence against cooperativity (Fig. 6). The apparent activation energies, E_a, are obtained from the Arrhenius plots, all of which show the expected linear variation. For a non-cooperative process, each protein acts independently, and so E_a should fall linearly with increased protein. In a cooperative process, E_a should instead begin at one level for low amounts of protein, but then drop abruptly to a lower level when the critical number of proteins is reached. For adsorption, E_a shows the linear variation with protein content (Fig. 6) expected for a non-cooperative process, with no evidence for the discontinuous decrease that should occur with cooperativity. Furthermore, the range of protein contents over which E_a varies linearly, where the proteins should contribute independently, extends to vesicles without protein. Because this observation requires no assumptions concerning the relative activity of SP-B and SP-C, it represents perhaps our strongest evidence that the proteins act individually rather than cooperatively.

The Gibbs free energy of activation, ΔG_0^*, also indicates non-cooperative behavior. ΔG_0^* derives from transition state theory that considers the effect of temperature in terms of an equilibrium between reactant and an activated complex [17]. A smaller ΔG_0^* indicates a more stable intermediate or less stable reactants. This parameter is especially important since the surfactant proteins achieve

their acceleration of adsorption by reducing ΔG_0^* [16]. If more than one protein is required for adsorption, ΔG_0^* should vary little at low protein concentrations, but then drop significantly once the minimum size of the cooperative unit is reached. Instead of a moderate reduction preceding a marked decrease, however, ΔG_0^* exhibits a fast initial drop before decreasing more slowly at protein concentrations above 8 SP-B dimer per vesicle (Fig. 6). This behavior therefore differs from that expected for a cooperative process. Furthermore, since previous studies suggested that SP-B contributes most of the measured effect on adsorption (75–83%), our results suggest that as little as 2 SP-B dimer per vesicle reduce ΔG_0^* by 3.6 kJ/mol, increasing the adsorption rate 4-fold relative to N&PL [16,17]. SP-B is believed to adopt a peripheral membrane configuration [30]. For the sonically dispersed vesicles studied here in which SP-B presumably inserts equally into the inner and outer leaflets, two dimers per vesicle correspond statistically to a single dimer available for promoting interactions between the bilayer outer leaflet and the interface. These results again suggest that no more than a single SP-B dimer is involved in the induction of surfactant adsorption.

5.3. High protein concentration range

Above a CLSE mole fraction of 25%, the adsorption rates increase much more slowly with protein concentration (Fig. 3). The rate at which ΔG_0^* decreases is also less than at lower protein content (Fig. 6), suggesting that above this particular protein concentration, factors other than protein ratios are limiting the adsorption kinetics. If most of the measured adsorption is contributed by SP-B, one possible explanation involves the surface distribution of SP-B molecules in lipid bilayers. Fluorescence quenching experiments suggest that in fluid-phase bilayers, SP-B undergoes in-plane aggregation when the protein-to-lipid molar ratio exceeds 4×10^{-5} [31]. This value is in close agreement with a SP-B/phospholipid molar ratio of 5×10^{-5}, which corresponds to the 25% CLSE mole fraction (Table 1). Because deviations from the behavior at lower protein content begin at approximately this ratio, in-plane aggregation could explain the different behavior at high and low protein contents.

5.4. Qualifications

Our findings are subject to specific restrictions and uncertainties. Our studies focus on an early stage of adsorption when surface tension first begins to change. The unexpected acceleration of adsorption below 40 mN/m argues that the mechanism of adsorption must become more complex during that later stage. The simple insertion of vesicles into the interface should slow progressively as the density of the film rises, and so additional processes seem likely late in adsorption. Our results deal strictly with the fundamental mechanism by which individual vesicles fuse with the interface.

Our conclusions also depend on two assumptions concerning the amount of protein that can participate in the adsorption of our mixed vesicles. First, we assume that only protein in the vesicles can facilitate adsorption. Prior studies have shown that factors in preexisting films, including phospholipids as well as proteins, could accelerate adsorption, but only in films that significantly alter surface tension [26,32,33]. Our analysis uses rates measured at 65–70 mN/m where the effects of interfacial components should be minimal. The second assumption is that the amido black assay detects levels of hydrophobic protein that are generally accurate. Although the assay responds differently to different proteins, the protein-to-lipid ratio obtained for extracted surfactant ($\sim$ 1.5% w/w) is similar to values measured with methods such as amino acid analysis that determine protein more directly. Both assumptions have no effect on our finding of non-cooperativity over the range of proteins considered. The only question is the extent to which that range extends to circumstances in which only a single protein can participate.

6. Conclusions

Our findings indicate that the hydrophobic surfactant proteins promote the early adsorption of pulmonary surfactant to an air/water interface through a non-cooperative mechanism for which the initial number of proteins is low. Previous reports suggest that these proteins act by stabilizing a high-energy intermediate [16], and indicate that SP-C is inactive relative to SP-B [20,25–28,33]. Combined with our present results, these findings suggest that a single SP-B may promote adsorption by reducing the bending energy of a tightly curved intermediate structure.

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References

[1] R.P. Rand, V.A. Parsegian, Annu. Rev. Physiol. 48 (1986) 201–212.
[2] D.E. Leckband, C.A. Helm, J. Israelachvili, Biochemistry 32 (1993) 1127–1140.
[3] C.A. Helm, J.N. Israelachvili, Methods Enzymol. 220 (1993) 130–143.
[4] L.D. Hernandez, L.R. Hoffman, T.G. Wolfsberg, J.M. White, Annu. Rev. Cell Dev. Biol. 12 (1996) 627–661.
[5] L. Chernomordik, A. Chanturiya, J. Green, J. Zimmerberg, Biophys. J. 69 (1995) 922–929.

[6] S. Gunther-Ausborn, P. Schoen, I. Bartoldus, J. Wilschut, T. Stegmann, J. Virol. 74 (2000) 2714–2720.
[7] T. Danieli, S.L. Pelletier, Y.I. Henis, J.M. White, J. Cell Biol. 133 (1996) 559–569.
[8] J. Bentz, Biophys. J. 78 (2000) 227–245.
[9] F.R. Poulain, S. Nir, S. Hawgood, Biochim. Biophys. Acta 1278 (1996) 169–175.
[10] R.H. Notter, J.N. Finkelstein, R.D. Taubold, Chem. Phys. Lipids 33 (1983) 67–80.
[11] B.N. Ames, Methods Enzymol. VIII (1966) 115–118.
[12] R.S. Kaplan, P.L. Pedersen, Methods Enzymol. 172 (1989) 393–399.
[13] R.L. Searcy, L.M. Bergquist, Clin. Chim. Acta 5 (1960) 192–199.
[14] S.B. Hall, Z. Wang, R.H. Notter, J. Lipid Res. 35 (1994) 1386–1394.
[15] J. Pérez-Gil, A. Cruz, C. Casals, Biochim. Biophys. Acta 1168 (1993) 261–270.
[16] V. Schram, S.B. Hall, Biophys. J. 81 (2001) 1536–1546.
[17] H. Eyring, Chem. Rev. 17 (1935) 65–77.
[18] A. Cruz, C. Casals, K.M.W. Keough, J. Perez-Gil, Biochem. J. 327 (1997) 133–138.
[19] A. Cruz, C. Casals, I. Plasencia, D. Marsh, J. Perez-Gil, Biochemistry 37 (1998) 9488–9496.
[20] J.E. Baatz, Y. Zou, J.T. Cox, Z.D. Wang, R.H. Notter, Protein Expr. Purif. 23 (2001) 180–190.
[21] J. Perez-Gil, A. Cruz, C. Casals, Biochim. Biophys. Acta 1168 (1993) 261–270.
[22] M. van Eijk, C.G. De Haas, H.P. Haagsman, Anal. Biochem. 232 (1995) 231–237.
[23] J. Pérez-Gil, K.M. Keough, Biochim. Biophys. Acta 1408 (1998) 203–217.
[24] P.F. Almeida, W.L. Vaz, T.E. Thompson, Biochemistry 31 (1992) 7198–7210.
[25] Z. Wang, O. Gurel, J.E. Baatz, R.H. Notter, J. Lipid Res. 37 (1996) 1749–1760.
[26] M.A. Oosterlaken-Dijksterhuis, H.P. Haagsman, L.M.G. van Golde, R.A. Demel, Biochemistry 30 (1991) 8276–8281.
[27] M.A. Oosterlaken-Dijksterhuis, H.P. Haagsman, L.M.G. van Golde, R.A. Demel, Biochemistry 30 (1991) 10965–10971.
[28] K. Shiffer, S. Hawgood, H.P. Haagsman, B. Benson, J.A. Clements, J. Goerke, Biochemistry 32 (1993) 590–597.
[29] R. Garrett, C.M. Grisham, Saunders College Publ., Fort Worth, 1995, pp. 387–423.
[30] J. Pérez-Gil, Pediatr. Pathol. Mol. Med. 20 (2001) 445–469.
[31] R. Chang, S. Nir, F.R. Poulain, Biochim. Biophys. Acta 1371 (1998) 254–264.
[32] R.W. Walters, R.R. Jenq, S.B. Hall, Biophys. J. 78 (2000) 257–266.
[33] M. Ross, S. Krol, A. Janshoff, H.J. Galla, Eur. Biophys. J. Biophys. Lett. 31 (2002) 52–61.