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SHORT COMMUNICATION

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Direct measurement of molecular interaction forces in foam films from lung surfactant fraction

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Abstract Molecular interaction forces, operative in microscopic foam films obtained from the isolated hydrophobic fractions of porcine lung surfactant (AS-B) are investigated by monitoring film thickness h as a function of electrolyte concentration (C_{el}) and direct measurements of disjoining pressure/thickness ($\Pi(h)$) isotherms. The steep decrease of the common film thickness with the increase of C_{el} evidences the action of long-range electrostatic surface forces. The experimental $h(C_{el})$ curve indicates that non-Derjaguin-Landau-Verwey-Overbeek (DLVO) repulsive forces are operative at C_{el} where common black (CBF) and Newton black films (NBF) are obtained including the physiologically relevant $C_{el} = 0.14$ mol dm^{-3} NaCl. The action of additional non-DLVO forces is corroborated by the comparison of the experimentally measured $\Pi(h)$ isotherm with the DLVO theory. Considering the presence of proteins in AS-B and the formation of lipid-protein complexes it is inferred that steric type forces are operative in CBF and NBF.

Keywords Lung surfactant · Foam films · Surface forces · Molecular interactions

Introduction

The free thin liquid films (foam films) are a long known and useful model for investigation of long and short-range molecular interactions (surface forces) and the transition between them [1–3]. According to the classical theory of Derjaguin-Landau-Verwey-Overbeek (DLVO) for the stability of lyophobic colloids, the long range forces of electrostatic repulsion and molecular (van der Waals) attraction play a major role for the stability of common (CF) and common black (CBF) foam films. Non-DLVO type surface forces have also been found in some cases to play an important role in the stabilization of the foam films. Such are for instance the forces of steric origin found in foam films formed from aqueous solutions of block co-polymers [4] and some polyoxyethyleneglycol ethers of higher molecular weight [5]. The stability of the thinnest bilayer Newton black films (NBF) can be described by another approach based on a nucleation theory of fluctuation hole formation [6]. The good fit between theory and experiment corroborates this approach and demonstrates the importance of short-range molecular interactions for the stability of NBF similar to crystals [1, 7].

The advantages of the foam film as a model of two interacting air/water interfaces through a thin liquid core were successfully employed in studies of foam films formed from natural surfactants e.g. individual phospholipids [1, 8–10] and lung surfactant fractions [11–14]. The lung surfactant (LS) is a complex mixture of lipids and proteins lining out the hypophase (the thin water layer covering the epithelian cells) of the alveoli. LS plays a major role in maintaining the alveolar and lung integrity during the breathing process. Since the 1950s, the traditional model of the alveolar surface layer involves a surfactant monolayer adsorbed at the air/water interface, e.g. Ref. [15]. Recent ultrastructural [16, 17] and theoretical work [18] has challenged this view in favor of a functional unit formed of bilayers in continuum with a monolayer at the hypophase/air interface. The hypothesis for the in vivo formation of bilayer and multilayer films at the alveolar surface was advanced by Exerowa et al. [11] who proposed the foam film as a model for the study of the alveolar surface and stability. These authors were the first to obtain foam films formed from alveolar surfactant (AS) at the physiological conditions (pH, temperature, capillary pressure, electrolyte concentration in the aqueous solution). The application of the theory [6] for the stability and rupture of amphiphilic bilayers to the experimentally obtained parameters characterizing formation and stability of the bilayer NBF from LS components substantiated a new clinical diagnostic method for assessment of fetal lung maturity [12]. The effectiveness of this method has also been demonstrated in studies of black foam films stabilized with therapeutical preparations used for exogenous surfactant therapy of respiratory disorders of the lung [19].

So, the objective of this study is to get an insight of molecular interaction forces, operative in foam films obtained from isolated hydrophobic fraction of porcine lung surfactant and to find the ranges of action of long and short range molecular interaction forces and the transitions between them.

For this purpose we experimentally investigated equilibrium film thickness (h) as a function of electrolyte concentration (C_{el}) and directly measured the disjoining pressure/thickness isotherms ($\Pi(h)$) of different types of foam films—CF, CBF and NBF. Special attention is paid to the C_{el} and the capillary pressures corresponding to the physiological conditions in the lung.

Experimental

Materials

Porcine lungs from recently slaughtered animals were lavaged three times with 0.14 mol dm^{-3} NaCl solution. The collected alveolar lavage fluid was centrifuged at 220×g for 20 min at 4 °C, in order to remove cell debris. The supernatant was centrifuged again at 16,000×g for 60 min. The resulted pellet was resuspended in 0.14 mol dm^{-3} NaCl. Water-soluble proteins, including hydrophilic surfactant proteins SP-A and D were removed by extraction according to the method of Bligh and Dyer [20]. The chloroform phase was evaporated under nitrogen and the dry residue was dissolved in distilled water and lyophilized. Alveolar surfactant fraction (AS-B) consists mainly of a mixture (or complex) of surfactant phospholipids (60–80% phosphatidylcholine and 8–10% phosphatidylglycerol), neutral lipids, and the hydrophobic proteins SP-B and SP-C (approximately 4%). More detailed chemical composition of AS-B is presented elsewhere [14, 21].

Triple distilled water of specific electrical conductivity $\kappa \sim 10^{-6}$ S cm^{-1} and pH~6 was used for the preparation of the AS-B stock solutions. To remove any surface-active contaminations, NaCl (Merck, suprapur) was added to the solutions after heating for several hours at a temperature of 700 °C.

All measurements were performed with solutions of constant AS-B concentration, 410 µg ml. Conductivity measurements showed that κ of such a solution was equivalent to κ of an aqueous NaCl solution of $C_{\mathrm{el}} = 5.4\times10^{-3}$ mol dm^{-3}. After dialysis C_{el} was decreased to the equivalent of $C_{\mathrm{el}} = 5\times10^{-4}$ mol dm^{-3} NaCl.

Methods

Foam film thickness was measured employing the contemporary version of the microinterferometric method of Scheludko and Exerowa [1]. When working at constant capillary pressure the foam film with a radius of 100 µm is formed by withdrawing the liquid from the biconcave drop hanging in the glass tube (radius 2.5 mm) of the Scheludko–Exerowa measuring cell [1, 2]. In order to maintain equilibrium conditions in the cell, concerning temperature ($T = 23$ °C) and vapor pressure, the measurements were performed at least 2 h after the tightly closed cell was mounted on the microscope. In the course of this work the experimental points were determined as the mean values of five registered film thicknesses.

The porous plate method [22] was used to monitor disjoining pressure isotherms. In this case the foam films is formed in a hole with a radius 500 µm drilled in a sintered glass plate. Gradual and reversible change of the pressure in the porous plate cell is applied using the thin liquid film—pressure balance technique [23]. The increase of the pressure in the measuring cell compared to the atmospheric pressure is measured with an accuracy of ± 5 Pa.

The interferometrically obtained photometric data are used to assess the so-called equivalent film thickness h, which is found considering the film as homogeneous with a refraction coefficient n equal to the refraction coefficient of the solution from which the film is obtained. In our case n was constant and equal to 1.333. The accuracy of the so determined microscopic thin liquid film thickness is ± 0.2 nm.

The surface tension σ of the solutions was measured by the Wilhelmi method with a Pt plate using a tensiometer "Krüss T10". The equilibrium value of $\sigma = 25.3$ mN m^{-1} was reached in about an hour and a half and did not depend on C_{el} of the solution.

Results and discussion

Figure 1 shows the effect of NaCl concentration (C_{el}) on equilibrium thickness h of AS-B foam films. As seen, at $C_{\mathrm{el}} = 5\times10^{-4}$ mol dm^{-3} 94 nm thick equilibrium common films (CF), that are films with a thick aqueous core surrounded by the AS-B adsorbed layers, are obtained. Increased C_{el} leads to a decrease of h to 60 nm at $C_{\mathrm{el}} = 1.7\times 10^{-3}$ mol dm^{-3} and 48 nm at $C_{\mathrm{el}} = 5.4\times10^{-3}$ mol dm^{-3}. Black spot formation leading to the formation of 19.5 nm thick black foam films is first observed at $C_{\mathrm{el}} = 0.06$ -mol dm^{-3} NaCl. The thickness of these films decreases with the increase of C_{el} showing that these films are CBF. The range of CBF formation comprises the physiological $C_{\mathrm{el}} = 0.14$ mol dm^{-3} NaCl, where $h = 14.7$ nm. The slight decrease of h above $C_{\mathrm{el}} = 0.35$ mol dm^{-3} NaCl is within the experimental error of the measurement. So, we can consider that these films of h approximately 10.5 nm are NBF.

The comparison of the obtained curve with previous studies of films formed from synthetic detergents [5] and individual phospholipids [8, 10] allows to infer that the decrease of h of the CF and CBF with the increase of C_{el} evidences the action of repulsive electrostatic forces (electrostatic disjoining pressure Π_{el}) in the film. The continuous thinning of the AS-B films with the increase of C_{el} shown in Fig. 1, however, distinguishes them from those stabilized with phospholipids [8, 10] where the jump-like barrier transition to NBF evidences its electrostatic origin. Here it is possible to infer that the continuous film thinning is caused by the action of an additional positive component of the disjoining pressure. Considering the complex composition of AS-B, comprising not only phospholipids but also specific proteins, it is possible that forces of steric origin can be operative leading to the observed slow and continuous decrease of h with the increase of C_{el}. Besides, the charged phosphatidylglycerol is an important phospholipid component of LS and it could affect the properties of the obtained films as well.

So, to distinguish between the different types of films and the molecular interaction forces determining their stability, we measured $\Pi(h)$ isotherms for films obtained from AS-B at different C_{el}.

Figure 2 shows the obtained $\Pi(h)$ isotherm for films formed from AS-B solutions of ionic strength 5×10^{-4} mol dm^{-3}. As seen, the increasing Π causes a decrease of the thickness of the obtained CF film until it reaches 30 nm at $\Pi = 10^4$ Pa. This is the lowest h reached as the film ruptures at further increase of Π.

This experimentally obtained $\Pi(h)$ isotherm was compared with the equations of the classical DLVO theory. According to the basic equation of the classical DLVO theory at equilibrium $\Pi = \Pi_{\mathrm{el}} + \Pi_{\mathrm{vw}} = P_{\sigma}$, Π_{el} and Π_{vw} being the electrostatic and van der Waals components of the disjoining pressure. Π_{el} was evaluated by applying an approach described in detail in previous papers [5, 23] involving the numerical solution of the complete Poisson-Boltzmann equation for 1–1 valent electrolyte. The film was considered as homogeneous with a distance h between the planes of origin of the diffuse electric layers with potential ϕ_0. Π_{vw} was assessed using the complete Lifshitz equations for a homogeneous film as proposed in Ref. [24]. The theoretical calculations were performed at the limiting conditions of a constant potential $\phi_0 = 50$ mV and the respective constant charge density $\sigma_0 = 3$ mC m^{-2} (Fig. 2). This value of the ϕ_0-potential was determined from the experimental result for $h = 94$ nm obtained at the same C_{el} in the Scheludko-Exerowa measuring cell (Fig. 1).

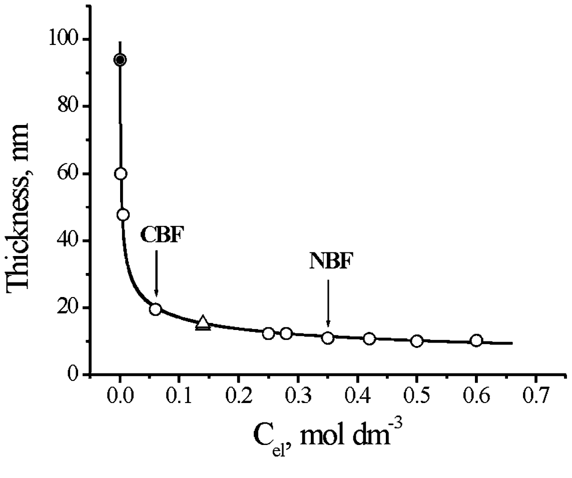

Fig. 1 Equilibrium thickness of AS-B microscopic foam film as a function of NaCl concentration (C_{el}) at 23 °C

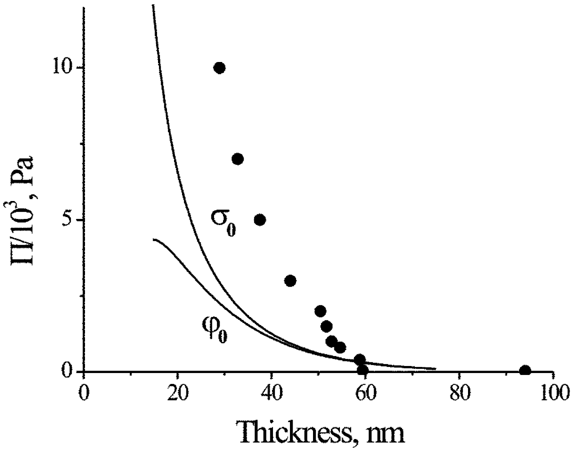

Fig. 2 ($\Pi(h)$ isotherm of films formed from AS-B solutions of ionic strength 5×10^{-4} mol dm^{-3} (black dot in Fig. 1). Dots experimental data; lines theoretical calculations at the limiting conditions of a constant potential $\phi_0 = 50$ mV and respective constant charge density $\sigma_0 = 3$ mC m^{-2}

As seen, a good fit is found between the theoretical curves and the experimental points only at $h > 50$–60 nm. The deviation of the experimentally obtained points from the theoretical calculations evidences the action of additional non-DLVO repulsive forces in the AS-B films formed at this C_{el}. It shows that the classical DLVO surface forces determine the film stability only at low Π where comparatively thick CFs are obtained.

The steep curves in Fig. 3 show the $\Pi(h)$ isotherms for CBF and NBF. The thickness of the CBF obtained at $C_{\mathrm{el}} = 0.06$ mol dm^{-3} NaCl decreases with 9 nm—from 19.5 (see Fig. 1) to 10.9 nm—with the increase of Π from 25 to 2.5×10^4 Pa. $\Pi = 25$ Pa (not shown in Fig. 3) equals P_σ of the film formed in the Scheludko-Exerowa's cell used for measurements at constant capillary pressure. It is noteworthy to mention that similar to CF the studied CBF film does not exhibit jump-like transition to NBF, though it does not rupture till the highest applied pressure. Besides, the lowest h of the films measured at the highest applied pressure does not reach that of the NBF, suggesting the action of additional repulsive forces in the film, presumably of steric origin.

In the case of the $\Pi(h)$ isotherm obtained for films formed from AS-B solutions with 0.6 mol dm^{-3} NaCl added, h remains practically constant with the increase of Π. This course of the isotherm demonstrates the lack of a free water core in the film and corroborates the assumption of NBF formation at high C_{el}.

The study of alveolar stability during breathing necessitates an investigation of the change of h of the film formed at the physiological $C_{\mathrm{el}} = 0.14$ mol dm^{-3} NaCl during a reversible variation of the applied pressure between 3×10^2 and 3×10^3 Pa, corresponding to the change of the pressure in the alveoli. The experimentally obtained $\Pi(h)$ isotherm (Fig. 4) shows no hysteresis of the foam film thickness during pressure increase and decrease. The horizontal arrows in the figure indicate the limiting values of the capillary pressure during inhaling and exhaling. We should note that h decreases with only 1 nm with increase of pressure to 10^4 Pa. Additional experiments at higher pressure corroborate the presumption of additional repulsive forces in the AS-B films as the lowest thickness measured at a pressure of 4×10^4 Pa was of 12 nm, i.e. higher than the NBF thickness.

Considering the physiological relevance of the experiments at $C_{\mathrm{el}} = 0.14$ mol dm^{-3} we measured the $\Pi(h)$ isotherm for the AS-B films at a temperature of 37 °C (data not shown). The obtained curve was practically the same as that measured at 23 °C. In this case again the same thickness values were obtained during increase and decrease of the applied pressure.

Concluding remarks

The present study allows getting more of an insight of the molecular interaction forces operative in foam films stabilized with hydrophobic fraction of lung surfactant. The steep decrease of h of the CF with the increase of C_{el} evidences the action of long-range electrostatic surface forces. The experimental results evidence also the action

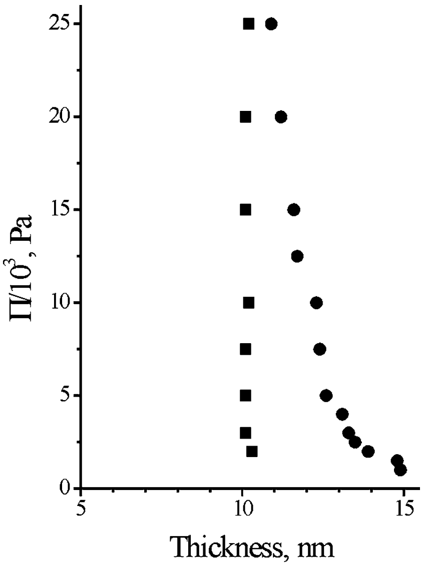

Fig. 3 $\Pi(h)$ isotherms of common and Newton black films: *dots* common black films, $C_{\mathrm{el}} = 0.06$ mol dm^{-3} NaCl; *squares* Newton black films, $C_{\mathrm{el}} = 0.6$ mol dm^{-3} NaCl

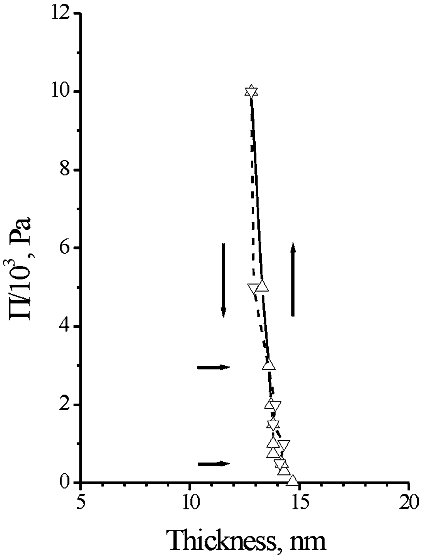

Fig. 4 $\Pi(h)$ isotherm of foam films formed at the physiological $C_{\mathrm{el}} = 0.14$ mol dm^{-3} NaCl during a reversible variation of the applied pressure. The horizontal arrows indicate the minimum (3×102 Pa) and maximum (3×103 Pa) pressure in the alveoli during respiration

of additional repulsive forces operative in the AS-B films and particularly in the obtained CBF and NBF including the physiologically relevant $C_{\mathrm{el}} = 0.14$ M NaCl. Considering the presence of proteins in AS-B and the formation of lipid–protein complexes we can assume that these forces are of steric origin. So, additional studies of films stabilized with isolated individual components of LS can further contribute to the elucidation of the origin, role and range of action of the DLVO and non-DLVO surface forces in the LS films.

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