Surface Pressure Hysteresis of Mixed Lipid/Protein Monolayers: Applications to the Alveolar Dynamics

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Mixed Lipid/Protein Monolayers, Alveolar Surfactant, Monolayer Models

Experimental data on surface pressure-surface area hysteresis of mixed serum albumin/dipalmitoyl lecithin/sphingomyelin monolayers in the Langmuir trough are presented. Several possible physicochemical mechanisms of the hysteresis are discussed: Marangoni effect, surface pressure relaxations, bulk-to-surface diffusion interchange, and collapse. Depending on the concrete conditions each of these mechanisms can be important. Possible applications of these results to the alveolar dynamics are presented and discussed on the basis of the balloon model of the alveolus. The main conclusions of biological importance are that 1) the alveolar stability depends on the DPL/SM ratio as well as on the protein content. Under normal breathing conditions the surface pressure hysteresis is small and does not play a decisive role in the alveolar dynamics. 2) At large extent of compression the collapse predominates in determining the hysteretic behavior of the alveolar surface.

Introduction

When the lung is inflated its membranes are stretched and stresses are induced in the alveolar lining layer (ALL) and in the underlying tissue. Because of the viscous energy dissipation in them and in the bronchial tree, the stresses at deflation differ from those at inflation at the same deformations. The dissipative processes in these three component parts lead to the double valued loop, observed in the pressure (P)-volume (V) relationship at breathing [1] as well as for lungs cyclically inflated and deflated with gas [2, 3]. The significance of the energy dissipation in the ALL in relation to that in the lung tissue and in the bronchial tree depends on the physicochemical and physiological conditions (frequency of the cyclic deformation, composition, pH, humidity, temperature, etc.). The objective of the present paper is the analysis of the physicochemical mechanisms underlying the surface pressure hysteresis in a lipid-protein monolayer on a saline subphase which simulates the ALL and its contribution to the P–V hysteresis.

A general theoretical analysis is applied, by way of illustration, to the mixed monolayers of one protein (serum albumin – SA) and two lipids (dipalmitoyl lecithin – DPL and sphingomyelin – SM) – a system which relates to the alveolar mechanics. Indeed, a number of investigators have stated the important role of DPL in reduction of the alveolar surface tension at deflation [e.g. refs. 4–7]. Since Gluck et al. [8] and Nelson [9] have established that the lecithin/sphingomyelin ratio shows a contrabalance behavior and provides an index of fetal lung maturity, the L/S ratio test has proved to have the best diagnostic valences. The possible presence and role of proteins at the surface of the ALL is still a controversial matter. In order to examine the influence of a protein to the properties of the alveolar surface we chose the serum albumin, which is the most abundant protein in lung washings [10] and whose surface properties are relatively well studied [11–18].

The role of the ALL for the stability and the dynamic behavior of the alveoli can be investigated by means of different model systems [19–27].

Models

The plane surface model

The equilibrium and dynamic behavior of monomolecular layers formed on a liquid subphase in a
Langmuir trough — the so-called “plane surface model” [2] has been intensively studied with regard to the properties of the alveolar surface. The surface equilibrium tension $\gamma$ can be measured for instance with the Wilhelmy plate. The static surface elasticity $E_s$ can be calculated from the surface pressure ($\pi$)-area $(A)$ isotherms as

$$E_s = \frac{d\pi}{d(ln A)} = -\frac{d\pi}{d(ln A)}.$$ 

The dynamic surface pressure hysteresis during cyclic deformation is defined as the difference between the values of the surface pressure at compression and at expansion at one and the same surface area. It can be represented as a sum of the following effects [23, 28]:

i) The Marangoni effect arising from the viscous friction with the liquid substrate. Its contribution is [28]

$$\Delta\pi_{\text{sys}} = -(4 \mu \frac{u_b}{h}) \left[ x - x^2/2L - L/3 \right]. \quad (1)$$

$\mu$ is the liquid viscosity, $x$ is the distance to the moving barrier, $L$ is the monolayer length, $h$ is the depth of the subphase, and $u_b$ is the velocity of the barrier.

ii) Surface pressure relaxations which result from the reorganization of the molecules in the monolayer (typical for lipid molecules) and conformational changes (typical for protein molecules) with the respective characteristic times. It was shown [24] that the relaxation of DPL/SA monolayers can be described by a bidimensional Maxwell body with two relaxation times. The second one can be neglected being too large ($\sim 10^5$ sec) to affect the dynamics of the ALL during the period of normal breathing. The surface pressure hysteresis due to the viscoelastic relaxation with one relaxation time $\theta$ is

$$\Delta\pi_{\text{sys}} = (E_{ds} \theta \frac{u_b}{L}) \left[ 1 - [2 - \exp(-T/2\theta)] \exp(-T/2\theta) \right], \quad (2a)$$

where $T$ is the periodic of the deformation cycle and $\theta$ can be estimated as described elsewhere [29] from the curves for the relaxation following dynamic compression. The dynamic elasticity $E_{ds}$ is given by

$$E_{ds} = \left[ \pi(T/2) - \pi(0) - E_s \frac{L}{L_0} \right] \left[ 1 - \exp(-T/2\theta) \right] \frac{L_0}{u_b \theta}. \quad (2b)$$

iii) The bulk-to-surface diffusion interchange of the soluble components of the alveolar surfactant yields a surface pressure hysteresis [30]:

$$\Delta\pi_{\text{sys}} = u_b E_s (T/2)^{1/2} \left( \frac{d\Gamma}{dc} \right)/(L \sqrt{D}), \quad (3)$$

where $D$ is the diffusion coefficient, and $\Gamma$ and $c$ are the surface and the bulk surfactant concentrations.

iv) The formation of three dimensional aggregates and/or alteration of the conformation of the surfactant molecules at the interface — the collapse, occurring at relatively high surface pressures.

The balloon model

The model of the alveolus as a bubble at the end of a capillary was introduced by von Neergard [19]. Clements et al. [21] used an empirical formula for the tissue elasticity forces, based on the saline diagram of the whole lung in order to determine the conditions for mechanical stability of the balloon model of the alveolus. Mead et al. have shown [31] that when a totally collapsed lung is inflated, the parallel balloon model is completely appropriate for description of the mechanics of the lung. Horn and Davies [32] calculated the surface tension change of a liquid shell at a given variation of its radius, but the alveolar volume was not determined as a function of the applied pressure. The equation of equilibrium and the condition for stability of the balloon model were derived by Dimitrov et al. [33, 34] as a solution of the rigorous equations of the elasticity theory. For the case of small deformations and thin membranes the condition of stability does not depend on the radius of the alveolus and the pressure difference:

$$E h + 2E_s \gamma > 0, \quad (4)$$

where $E$ is the Young modulus of the lung tissue and $h$ is the thickness of the alveolocapillary membrane. It is supposed that the pressure acting on the outer membrane surface $P_2$ is a periodical function of the time $t$:

$$P_2 = P_{20} - \Delta P(t) = P_{20} - \Delta P_m \sin \omega t. \quad (5)$$

$P_{20}$ is the initial static pressure, $2\Delta P_m$ is the maximum pressure change, and $\omega$ is the frequency. The inner pressure $P_1$ is represented as a linear function of the rate of the lung volume change (valid at small rates):

$$P_1 = P_{10} - k (dV/dT), \quad (6)$$
where $P_{io}$ is the pressure at the beginning of the bronchial tree and $K$ is an experimentally determined constant [35]. The dependence of the balloon volume change $\Delta V$ on the time has the form

$$\Delta V = \Delta V_m \sin (\omega t + \alpha) + C \exp (-t/\tau),$$  

(7)

where the maximum volume change $2 \Delta V_m$, the phase constant $\alpha$, the constant $C$ and the relaxation time $\tau$ of the system have different values for every concrete limiting case. In the case of a viscoelastic surface film, elastic tissue and negligible resistance in the bronchial tree (which case is under consideration in the present study), the following expressions for the functions and constants in the above equation were obtained [36]:

$$V_m = (\Delta P_m/\alpha) (1 + \phi^2)^{1/2} [1 + (1 + c)^2]^{-1/2};$$  

(8a)

$$\alpha = \arctg \phi - \arctg (1 + c)/\phi;$$  

(8b)

$$C = c(1 + c)^2/\{1 + (1 + c)^2\};$$  

(8c)

$$\tau = \theta (1 + c),$$  

(8d)

where

$$c = E_{ds} (E h + 2 E_s + \gamma)^{-1};$$  

(9a)

$$\phi = \phi \theta.$$  

(9b)

Here $\gamma$ is the initial surface tension, $E_{ds}$ is the dynamic elasticity of the surface film, and $\theta$ is the relaxation time of the surface pressure.

The value of the hysteresis loop area $\Delta S$ gives the energy dissipated for one cycle. A very characteristic value for the hysteretic behavior is the ratio $\Delta S/S$ of the hysteresis loop area $\Delta S$ to the half of its maximum value $S = 2 \Delta P_m \Delta V_m$ (at fixed $\Delta P_m$ and $\Delta V_m$) [36]:

$$\Delta S/S = - (\pi/2) \sin \alpha.$$  

(10)

The combined utilization of the plane surface and the balloon models enables us to evaluate the contribution of the properties of the alveolar surface to the $P-V$ hysteresis of the alveoli.

**Materials and Methods**

The surface pressure was measured by the method of Wilhelmy with a roughened glass plate suspended to an electronic balance “Beckman”. The depth of the subphase $h$ (0.7 cm) was much smaller than the length of the monolayer $L$ (70 cm), i.e. $h^2/L^2 = 10^{-4} \ll 1$ in conformity with their proportion in vivo ($h = 0.1 \pm 1 \mu m$, $L = 100 \pm 300 \mu m$ and $h^2/L^2 = 10^{-4}$). The Teflon barrier moved with constant velocity $u_b = 0.23 \, cm \cdot sec^{-1}$ at $2 \, dyn \cdot cm^{-1}$ steps and the equilibrium values of $\pi$ were waited for in order to obtain the $\pi(A)$ isotherms. Precautions were taken against leakage of the monolayer. In a groove running in the middle of the barrier was placed a roughened glass rod in contact with the subphase. The water barrier created that way prevents from leakage of lipid molecules. The water was twice destilled in an all glass apparatus and contained 0.01 M NaCl Merck purest quality (additionally purified by heating up to 600 °C). The water surface was swept 6 times with two barriers prior to spreading of the monolayer. The monolayer was obtained by successive spreading of the protein and the lipid mixture (after 15 minutes delay). The SA was spread from its aqueous solution with a concentration of $0.5 \times 10^{-3} \, g \cdot cm^{-1}$ in the presence of 1%o amyl alcohol [37]. The mixture of DPL and SM in chloroform (spectroscopically and chromatographically pure) was formed prior to spreading. Under these conditions the average reproducibility of the surface pressure was $0.5 \, dyn \cdot cm^{-1}$. The surface pressure was measured with an accuracy of $\pm 0.02 \, dyn \cdot cm^{-1}$ and the area with $\pm 0.01 \, m^2 \cdot mg^{-1}$. The SA crystallized and lyophilized was purified in the Department of Colloid Chemistry of the Moscow State University and was essentially globulin free. DPL was supplied by “Fluka” and SM (from bovine brain) – by “Koch Light”. Surface concentrations were determined as following fractions:

$$x_{DPL} = n_{DPL}/(n_{DPL} + n_{SM});$$

$$x_{SA} = n_{SA} z/(n_{SA} z + n_{DPL} + n_{SM}),$$

where $n$ stands for the number of molecules and $z$ is the number of monomeric units per SA molecule.

**Results and Discussion**

**Stability of the balloon model of the alveolus**

In order to determine the contribution of the surface forces we shall use equation (4) in the form

$$2E_s > \gamma.$$  

(11)

The surface elasticities $E_s$ needed to determine the interval of validity of (11) are calculated from the $\pi(A)$ isotherms at 20 °C; some of them are shown in
Table I. Upper values of the surface tension (in dyn/cm) for which the stability condition [15] is satisfied. The values in parenthesis indicate the respective surface pressures ($\pi = 72.8 - \gamma$).

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<th>0.00</th>
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<th>0.25</th>
<th>0.34</th>
<th>0.43</th>
<th>0.50</th>
<th>0.60</th>
<th>0.68</th>
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Fig. 1. Equilibrium isotherms of DPL/SM/SA monolayers for $x_{\text{DPL}} = 0.75$ and different $x_{\text{SA}}$ (indicated above the curves). The molecular areas are calculated for one amino acid unit in SA.

Fig. 1. Table I summarizes the upper limit values of the surface tension $\gamma$ for which the last equation holds (and the lower limit values of the surface pressure $\pi$, respectively). From the data in Table I it is evident that for all components tested Eqn. (11) is satisfied at relatively (sufficiently) low surface pressures. The slight but reliable increase of the surface pressure values for the mixed lipid monolayers (at $x_{\text{SA}} = 0$), compared with the case of pure lipids, is in accordance with the reported poor miscibility of the two lipids [38]. For the SM/SA monolayers an increase of the lower $\pi$-limit is observed for $x_{\text{SA}} > 0.75$, which corresponds to the transition from elastic to viscoelastic behavior during deformation [39]. A balloon with liquid surfaces covered with a monolayer from a DPL/SM mixture with $x_{\text{DPL}} < 0.75$ interacting with the bidimensional SA network (regardless of the $x_{\text{SA}}$ value) would be more stable than in other cases in the region of large dilution of the monolayer (of low $\pi$, respectively). For $x_{\text{DPL}} = 0.75$ a situation in the mixed lipid protein monolayer opposite to that one for the SM/SA monolayer is observed: the lower $\pi$-limit of stability is larger for $x_{\text{SA}} < 0.68$ than for $x_{\text{SA}} > 0.68$. It is worth noticing also that at $x_{\text{DPL}} = 0.75$ (characteristic for the fetal lung maturity according to the amniotic fluid test) and at $x_{\text{SA}} = 0.68$ (a value within the range of SA concentrations in the ALL if the lipid fraction is represented by DPL + SM) the stability is assured at very low surface pressure value, i.e. low surfactant density. A rough estimate of the tissue elasticity term $Eh$ gives a value of $10 \text{dyn} \cdot \text{cm}^{-1}$ (if $E = 10^4 \text{dyn} \cdot \text{cm}^{-2}$ [40] and $h = 10^{-3}\text{cm}$). Consequently, the results in Table I indicate that the balloon remains stable in the whole range of surface tensions.

Surface pressure – area hysteresis of the plane surface model

The breathing conditions can be modelled having in mind that according to physiological data $\Delta V = 500 \text{cm}^3$, $V = 3000 \text{cm}^3$ ($V$ – volume of the lung of an adult man) and 16 breathings are performed per minute under normal conditions. The relationship between the relative changes of the area and the volume of an alveolus can be written as $\Delta A/A = 2 \Delta V/3V$, assuming a spherical shape. This expression indicates that a relative change of the surface area $\Delta A/A$ of about 10% would account for the normal breathing. Consequently, at normal conditions the assumption for small deformations
(under which are derived most of the above presented equations) can be applied.

Figure 2 shows the hysteresis loop observed for the model system DPL/SM/SA with $x_{\text{DPL}} = 0.75$ and $x_{\text{SA}} = 0.68$ subjected to a cyclic deformation with a period $T = 12$ sec. The analysis of the effects contributing to the hysteresis shows that

1) At deformation rate $u_b = 0.23 \text{ cm} \cdot \text{sec}^{-1}$ (the liquid viscosity $\mu$ and the trough depth $h$ equal to $10^{-2} \text{ p}$ and 0.7 cm, respectively) the hysteresis value, due to the Marangoni effect, is $-0.04 \text{ dyn} \cdot \text{cm}^{-1}$, according to (1). It appears that under conditions such as in our experiment the hysteresis due to the Marangoni effect is small, but from (1) follows that when other parameters of the system are used (e.g. greater bulk viscosity or smaller substrate depth) this effect should be also accounted for.

2) The viscoelastic relaxation shift is determined by Eqn. (2a). The relaxation times calculated from the relaxation curve shown in Fig. 3 are equal to 13 and 262 sec. We describe the viscoelastic behavior of the monolayer taking into account only the $\theta = 13$ sec because the second relaxation time is too large compared to the first one and to the cycle period. The surface elasticity determined from the respective $\pi(A)$ isotherm in Fig. 1 is equal to 50.9 dyn $\cdot$ cm$^{-1}$. The dynamic elasticity is evaluated from Eqn. (2b): $E_{\text{ds}} = 46.0 \text{ dyn} \cdot \text{cm}^{-1}$. A hysteresis value of $-0.81 \text{ dyn} \cdot \text{cm}^{-1}$ is calculated for the contribution of the viscoelastic relaxation.

3) It has been shown that the used compounds behave as insoluble ones [30].

4) The loss of molecules from the interface does not influence the hysteresis because the cycle is performed rapidly in the region just below the collapse surface pressure of SA (20 dyn $\cdot$ cm$^{-1}$, [15]) and considerably below the collapse surface pressures of SM and DPL (56 dyn $\cdot$ cm$^{-1}$ and 68 dyn $\cdot$ cm$^{-1}$, respectively).

Thus, our estimation provides a value of $-0.85 \text{ dyn} \cdot \text{cm}^{-1}$ for the hysteresis shift compared to $-0.92 \text{ dyn} \cdot \text{cm}^{-1}$, which gives our experiment.

The role of the collapse is evident at longer cycling periods, particularly when they include...
values of the surface pressure, exceeding that of the collapse. Figure 4 shows the hysteresis for the same π range as in Fig. 2 but for a much longer period, long enough to enable some SA molecules to leave the surface or to undergo conformational changes. The collapse of SA is of limited extent below 20 dyn · cm⁻¹ but its contribution has a plus sign and reduces to a considerable extent the resultant hysteresis. The effect of the collapse is much more pronounced above π = 20 dyn · cm⁻¹ (Fig. 5) at one and the same surface area the concentration of molecules (and consequently the surface pressure) is larger in compression than in expansion. The hysteresis due to the collapse is prevailing upon that one due to the viscoelastic relaxation and the Marangoni effect at surface pressures above 40 dyn · cm⁻¹ (Fig. 6), which correlates to the data for lung surfactant [41] and DPL monolayers [4, 42].

The large hysteresis loop in Fig. 7 is obtained after deformation to 20% of the initial area and is obviously due mainly to molecules ejected from the monolayer. The kinetics of the collapse may be accounted for in computations of the hysteresis shift using for instance the approach of Mehta and Nagarajan [43].

**Pressure – volume hysteresis of the balloon model of the alveolus**

The contribution of the viscoelastic properties of the surface film to the $P - V$ hysteresis can be...
Fig. 8. Hysteresis loops calculated from (7)-(9) with $a = -0.05$ and $\Delta_m = -0.29$, corresponding to the physicochemical properties of the monolayer of Fig. 2.

Fig. 9. Dependence of the relative hysteresis loop area on the dimensionless frequency $\omega = \omega \theta$.

evaluated using the data presented above. For a cycle with a half period of 6 sec, involving a relative area change of about 6% and accomplished in the presence of a monolayer with the $\pi(A)$ hysteresis shown in Fig. 2, the phase constant $x$ is equal to $-0.05$, calculated by means of Eqn. (8) and (9). The dimensionless pressure – volume hysteresis shown in Fig. 8 is due to the viscoelastic behavior of the monolayer and is rather small. Consequently our model confirms that at conditions of normal breathing the main dissipation is in the bronchial tree and not in the ALL.

The maximum value of the hysteresis loop area occurs at $T_m = 2 \pi / \Delta m = 108$ sec ($\Delta m = 0.75$, Fig. 9). The corresponding value of the phase constant $x$ is $x_m = -0.29$. The extremum of the calculated hysteresis loop area is at a cycle period much longer than the period of normal breathing. $T_m$ can diminish and approach the period of breathing if the surface pressure relaxation time is much shorter. In general, proteins may lower the relaxation times, but similarly a plausible reason for a low $\theta$ may be the stress relaxations due to rearrangement in the bidimensional network of surface micelles of different lipids.

The considered effects illustrate the suggested approach. The matter would be settled in a more conclusive way if in a future work the model system approximates the physiological conditions in respect to composition, temperature, humidity, etc. Furthermore, it should be pointed out that in the real biological systems effects due to formation of surfactant micellar aggregates, slow surfactant adsorption, etc. can play a role, by analogy with similar phenomena in colloid systems (see e.g. refs. [12, 45]).


