

A Surface Chemical and Molecular Interpretation of Lung Surfactant Hysteresis

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Lung surfactant monolayers when compressed and expanded cyclically exhibit a reproducible hysteresis in the surface pressure–area behavior. A quantitative description of this hysteresis in terms of plausible molecular events occurring at the interface is proposed.

INTRODUCTION

The critical role of lung surfactants to pulmonary function is now well recognized, following the pioneering studies of Neergaard (1) and Pattle (2), and contributions from Clements (3), Avery (4), and others (5). The properties of these surfactants at the air–water interface are studied *in vitro* on monolayers in Langmuir troughs. When compressed and expanded cyclically, these monolayers exhibit (i) high surface pressures and (ii) a reproducible hysteresis in the surface pressure (π)–trough area (A) curve. In the qualitative understanding of the above behavior, only recently some progress has been made with important contributions from Bangham (6). Here we present a quantitative description of the π – A hysteresis in terms of plausible molecular events occurring at the air–water interface, each of which is amenable to independent experimentation. On the basis of our results, we conclude that such direct experiments would permit establishing a correlation between the various molecular events, the molecular structures of lung surfactant, and their role in pulmonary function.

MODEL

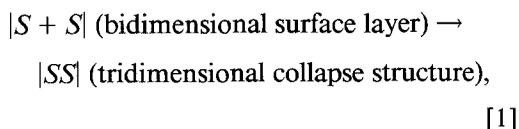
We depict the monolayer compression–expansion cycle in terms of the following

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events: (i) compression of the surfactant monolayer, (ii) monolayer collapse occurring near a critical surface concentration, leading to the expulsion of surfactant molecules from the interface to the aqueous subphase in the form of collapse structures, (iii) expansion of the monolayer, (iv) reentry of surfactant molecules into the monolayer from the aqueous subphase, (v) rearrangement of surfactant molecules at the interface, and, (vi) metabolic depletion and replenishment of surfactant molecules. Very few independent studies of these events have been reported in literature. All the kinetic data and the equilibrium π – A data on the same surfactant molecule needed to completely describe its dynamic compression–expansion behavior are not available for any molecule. Nevertheless, on the basis of scattered information reported in literature on one or more of the molecular events outlined here, for phospholipids or other surfactants, we now formulate a quantitative description of the events listed above.

Film collapse in the proximity of critical surface concentration has been studied for arachidic acid monolayer (7) on a Langmuir trough by measuring surface pressure as a function of time. The collapse is supposed to involve a nucleation and growth mechanism, leading to various types of collapse structures such as liposomes, lamellar bodies,

crystallites, etc. By treating the collapse process as analogous to a chemical reaction, viz.,



the rate of collapse has been formally described to be second order in surface pressure. The surface pressure is determined by surface concentration and their interrelation is described by a surface equation of state. Hence, we postulate the rate of monolayer collapse as second order in surface concentration with a rate constant k_1 .

The reentry of the phospholipid molecules into the monolayer from the aqueous subphase has been studied (8) using a Langmuir trough. The aqueous subphase consists of liposomes and singly dispersed surfactant molecules. It is found that the concentration of liposomes in the sublayer adjacent to the surface film is much larger than that in any other plane of the aqueous subphase. This sublayer of liposomes acts as a lipid reservoir for the interface via a lipid exchange process between the outer layer of liposomes and the monolayer. In turn, the sublayer of liposomes is rapidly replenished with lipid structures from the solution phase via a liposome diffusion exchange process. Because of the ready availability of surfactant molecules in the liposome sublayer, the lipid reentry into the monolayer would be unaffected by the diffusion exchange process between liposome sublayer and the aqueous subphase. Therefore, surfactant reentry occurs by interlayer exchange as long as the surface concentration is lower than its value at equilibrium. On the basis of this picture, we represent reentry as a first-order exchange process in surface concentration's approach to its equilibrium value, with an exchange coefficient k_2 .

The rearrangement of surfactant molecules in the monolayer due to compression, expansion or reentry, is assumed to occur quite rapidly compared to the time scales of the other events. This assumption is sup-

ported by the experimental observations of the absence of relaxation effects for phospholipids in liquid expanded monolayers (6). The metabolic depletion and replenishment of lipids are expected to occur over a time scale vastly greater than that of the other processes. Therefore, they do not contribute to π - A hysteresis. The rates of compression and expansion of the monolayer are constants determined by the conditions of the *in vitro* Langmuir trough experiments, or by *in vivo* lung expansion-contraction behavior.

We now incorporate the rate expressions discussed above in an overall description of the surfactant concentration at the interface. The time rate of change of surface concentration (C) is given by

$$\frac{dC}{dt} = (\pm) \frac{1}{A} \left[C \frac{dA}{dt} \right] - \alpha_1 k_1 C^2 + \alpha_2 k_2 (C_e - C). \quad [2]$$

The first term on the right-hand side represents the change in surface concentration due to rate of expansion (-) or rate of compression (+) of the monolayer; dA/dt is the constant expansion/compression rate and is given by

$$\frac{dA}{dt} = \frac{(A_{\max} - A_{\min})}{\tau/2}, \quad [3]$$

where τ is the period of the compression-expansion cycle and A_{\min} and A_{\max} are the minimum and maximum trough areas during the cycle. The second term represents the rate of change of surface concentration due to monolayer collapse. Since monolayer collapse occurs only near a critical surface concentration (C_{cr}), the factor α_1 is introduced to trigger the collapse process when the surface concentration C approaches a value close to the critical surface concentration, C_{cr} . Accordingly,

$$\begin{aligned} \alpha_1 &= 1 \quad \text{when } (1 - C/C_{cr}) < \epsilon, \\ \epsilon &\approx (0.05) \\ &= 0 \quad \text{otherwise.} \end{aligned} \quad [4]$$

The last term represents the rate of change

of surface concentration due to reentry of the surfactant molecule from the aqueous sub-phase to the monolayer. Here C_e is the surface concentration that would exist at equilibrium corresponding to the equilibrium spreading pressure π_e . The factor α_2 accounts for the fact that the lipid reentry into the monolayer from an infinite lipid reservoir occurs only as long as the surface concentration C is less than the equilibrium concentration C_e . Therefore,

$$\begin{aligned} \alpha_2 &= 1 & \text{when } C < C_e \\ &= 0 & \text{otherwise.} \end{aligned} \quad [5]$$

RESULTS AND DISCUSSION

Equation [2] is numerically solved to obtain the time periodic behavior of the surface concentration. For the various model parameters physically plausible values are assigned. The critical surface concentration (C_{cr}) refers to the surface concentration of a solid condensed state of the monolayer. For most components of the lung surfactant which contain two hydrocarbon chains per lipid molecule, the area per molecule corresponding to a solid condensed state is about $40 \text{ \AA}^2/\text{molecule}$. Here the model computations have been performed for a critical molecular area of $40 \text{ \AA}^2/\text{molecule}$ or equivalently for a critical surface concentration of $2.5 \times 10^{14} \text{ molecules/cm}^2$. The equilibrium surface pressure for dipalmitoyl lecithin has been determined to be about 48 dyn/cm (6). Therefore, the equilibrium surface concentration C_e is assumed to be $0.7C_{cr}$ corresponding to $\pi_e = 48 \text{ dyn/cm}$. The model parameter ϵ determines at what proximity of the surface concentration C to the critical surface concentration C_{cr} , the monolayer collapse would occur. It should have a value close to zero and the numerical simulations have been performed for a range of values up to $\epsilon < 0.10$.

The second-order rate constant k_1 for the monolayer collapse is assigned a value comparable to the experimental data obtained for

the monolayer of arachidic acid (7). The simulations have been carried out for values of k_1 spanning over one order of magnitude. The value of the first-order reentry rate constant k_2 is chosen on the basis of approximate experimental data available for phospholipids (8, 9). In future, for components of lung surfactant one should obtain the values of k_1 and k_2 directly from independent studies of the collapse and reentry processes.

For the compression–expansion cycle time τ , we have considered a range of values which includes both the *in vivo* cycle frequency of about 15 cycles/minute ($\tau = 4$ seconds) and a much lower frequency of 0.2 cycle/minute ($\tau = 300$ seconds) encountered in a typical *in vitro* Langmuir trough experiment. For the compression ratio A_{min}/A_{max} , the *in vivo* value of about 0.5 as well as a smaller value of 0.125 used in *in vitro* Langmuir trough experiments are considered. The initial trough area A ($t = 0$) and the initial surface concentration C ($t = 0$) can both be arbitrarily chosen. Of course, the choice of initial conditions would affect the transient surface concentration–area behavior but has no influence on its steady periodic behavior. The range of parameter values over which Eq. [2] is solved are summarized in Table I.

The numerical computations show that for a number of combinations of the model parameter values, Eq. [2] generates a reproducible surface concentration (C)–trough area (A) hysteresis. Illustrative hysteresis curves corresponding to *in vivo* and *in vitro* conditions are shown in Fig. 1, for the same values of model parameters k_1 , k_2 , C_{cr} , C_e , and ϵ , all of which depend on the nature of

TABLE I

Range of Parameter Values for Numerical Simulations

$C_{cr} = 2.5 \times 10^{14} \text{ (molecules/cm}^2\text{)}$
$0.125 \leq A_{min}/A_{max} \leq 0.5$
$0.02 \leq \epsilon \leq 0.1$
$4 \leq \tau \leq 300 \text{ (seconds)}$
$2 \times 10^{-15} \leq k_1 \leq 2 \times 10^{-14} \text{ (cm}^2\text{/molecules/second)}$
$0.01 \leq k_2 \leq 1.0 \text{ (second}^{-1}\text{)}$

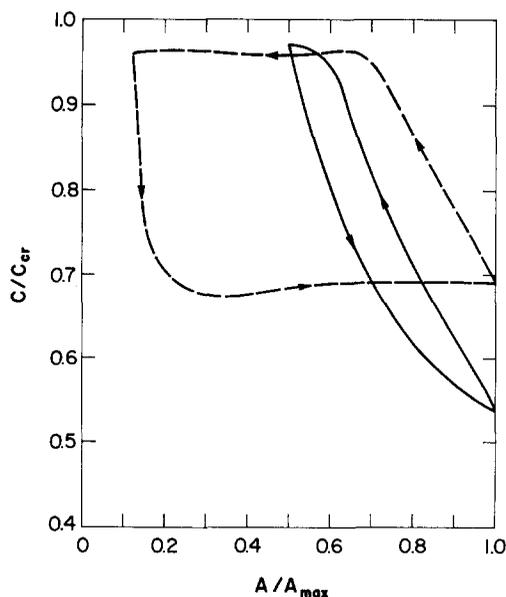


FIG. 1. Simulated C - A hysteresis curves for $\tau = 300$ sec, $A_{\min}/A_{\max} = 0.125$, corresponding to typical *in vitro* experiments (---) and $\tau = 4$ sec, $A_{\min}/A_{\max} = 0.5$, corresponding to *in vivo* conditions (—). Other parameter values are $k_1 = 2 \times 10^{-15}$, $k_2 = 0.5$, $C_{cr} = 2.5 \times 10^{14}$, $\epsilon = 0.03$, $C_e = 0.7C_{cr}$.

the lipid molecule, pH, temperature, etc. The two curves differ from one another only in the frequency of the compression-expansion cycle and the compression ratio.

The C - A hysteresis curves of Fig. 1 can be translated into surface pressure (π)-trough area (A) curves since the surface concentration C is related to the surface pressure π . As mentioned earlier, well-characterized and commonly accepted dynamic and equilibrium data for single-component systems are not readily available. Therefore, in order to show that our model generates hysteresis curves comparable to the *in vitro* experimental data, we have chosen the π - A data (10) for dipalmitoyl lecithin (DPL) at 23°C, although in this case the experimental hysteresis loop changes from cycle to cycle. Figure 2 shows the observed hysteresis curves for the first and the second compression-expansion cycles in a Langmuir trough experiment. This experimental data can be compared against π - A data simulated by the model

(also shown in the figure). The simulated π - A data is obtained by translating the simulated C - A data of Fig. 1 (corresponding to *in vitro* conditions) using experimentally obtained (11) equilibrium π vs $1/C$ curve. Both the calculated and experimentally observed hysteresis curves are qualitatively similar and straddle the same π - A region.

The model simulations also show that the area of the hysteresis loop depends upon the frequency ($1/\tau$) of the compression-expansion cycle. This is in qualitative accord with the observations made in Langmuir trough experiments (12). Further, the model displays the occurrence of a few transient hysteresis loops before a reproducible hysteresis

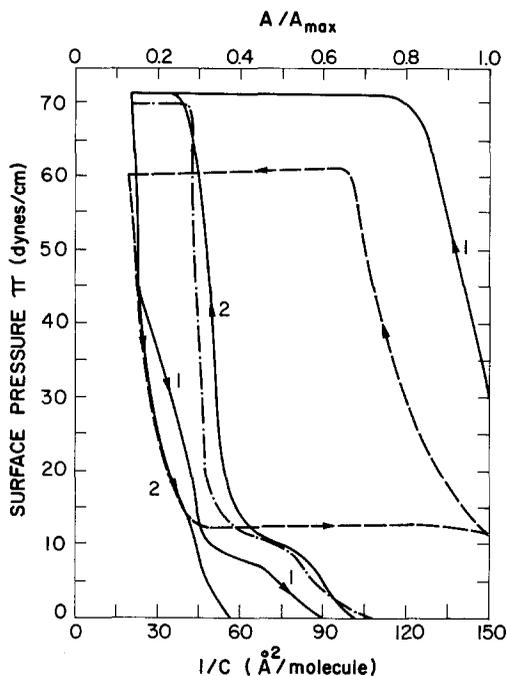


FIG. 2. π - A hysteresis curves generated from C - A hysteresis curves of Fig. 1 for the *in vitro* conditions $\tau = 300$ sec and $A_{\min}/A_{\max} = 0.125$. For comparison the hysteresis loops for the first and the second cycle of an *in vitro* experiment (10) with $\tau = 300$ sec and $A_{\min}/A_{\max} = 0.125$ are shown (—). ($\pi = \sigma_0 - \sigma$, where σ_0 and σ are the air-water interfacial tensions in the absence and presence, respectively, of surfactant molecules.) Also shown is the equilibrium π vs $1/C$ data (11) (-·-·-) used to translate C - A curves into π - A curves. The experimental data are for dipalmitoyl lecithin (DPL) at 23°C.

loop is established. This model behavior is also consistent with the results of *in vitro* monolayer experiments (13).

We anticipate that components of lung extracts may exhibit differing rates of monolayer collapse and of reentry into the monolayer. Also the highest surface pressures generated at maximum compression may differ from one another. However, it is the combined role of the different components which leads to the observed high surface pressures and the reproducible π - A hysteresis in the case of lung surfactant. Therefore, in light of our model we conclude that independent kinetic studies of the collapse and reentry processes for components of lung extracts hold promise for an improved understanding of lung surfactant behavior and in the rational design of synthetic lung surfactant analogs.

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