A Conformation Transition of Lung Surfactant Lipids Probably Involved in Respiration

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ABSTRACT X-ray scattering and freeze-fracture electron microscopy of a lung surfactant extract show the existence of a complex lamellar phase, L$_\gamma$, over a wide range of concentrations and temperatures. This lamellar phase, which consists of two bilayer motifs comprised of monolayers with stiff chains alternating with monolayers with disordered chains, allows us to propose a structural model of a collapse phase at the alveolar pulmonary interface. This model accounts for the increase in surface pressure during the compression as well as the easy respreading upon expansion of the interface during the respiratory cycle.

INTRODUCTION

The alveolar surface of the mammalian lung is lined with a film of a highly surface-active material. It plays a crucial role in the respiratory cycle (Von Neergard, 1929). A deficiency in this surfactant is responsible for the respiratory distress that occurs frequently in premature babies (Avery and Mead, 1959). The pulmonary surfactant is synthesized in pneumocyte II cells, processed, and packaged into lamellar bodies that are secreted, probably via the tubular myelin (Williams, 1977), into the hypophase of the alveolar air space and finally conveyed toward the alveolar air-interface. This tensioactive material lines the alveoli and ensures the variation in surface tension along with the variation in the interfacial area during the respiratory cycle (Pattle, 1955; Clements, 1956, 1962; Clements et al., 1958). Many physicochemical studies (Keough, 1992, and references therein) and in situ determinations have led to the conclusion that a few main properties are essential for the interfacial function: the ability to adsorb to the interface, the ability to sustain high surface pressure (or to decrease the surface tension to low value upon kinetic effects (Bangham, 1987)), and the ability to respread at the interface. These properties rely at least partly upon the chemical composition of the surfactant (King, 1984; Hawgood and Clements, 1990; Possmayer, 1988). It is a complex mixture comprised of a few percent of specific apoproteins (mainly SP-A, SP-B, SP-C, and SP-D; SP-B and SP-C are hydrophobic) and 90% lipids, essentially phospholipids (phosphatidylcholines, phosphatidylglycerol, phosphatidylethanolamine), among which phosphatidylcholine is largely predominant (70%) with 50% of them being fully saturated. DPPC represents the main component. The unusually high amount of DPPC, whose transition temperature is higher than physiological, is responsible for the considerable lowering of the interfacial tension during expiration. Schematically, one may consider the respiratory cycle as being composed of successive compression-decompression cycles of a tensioactive material at an air liquid interface in dynamic conditions. This material is able to withstand high surface pressure on compression and, following the collapse, to respread easily on decompression. It has been thought for some time that the lipid in the surfactant films is refined during compression by selective exclusion of non-DPPC materials to leave a film enriched in DPPC.

Extensive studies have shown that the ability of phosphatidylcholine films to sustain the high surface pressures requires a temperature below that of the gel to liquid crystalline phase transition of the corresponding bilayer. At physiological temperatures (below the transition temperature of DPPC) a DPPC monolayer can be compressed to a high surface pressure. This corresponds to a liquid-to-solid phase transition (Albrecht et al., 1978). Such a compressed solid film can form, at the collapse, a solid three-dimensional structure that is not able to respread easily upon decompression below the transition temperature. Conversely, above their transition temperature, lipids cannot be compressed to a high surface pressure and do not form a bidimensional solid, but they can respread easily (Tchoreloff et al., 1991; Philips and Hauser, 1974). Therefore, the combination of DPPC with other lipids and also the apoproteins is very likely an essential feature that contributes to the appropriate interfacial properties of the lung surfactant function. Despite numerous physicochemical approaches, the questions still remain concerning the mechanisms involved in the tensioactive function. It has been suggested that some selective exclusion or extrusion occurs during the compression and also that some nonbilayer arrays of lipids in the subphase might also play a role for the penetration of the lipids into the surface film (Keough, 1992). Respreading from the collapse phase could also be an important process in the lung. It has also been suggested that the alveoli show a critical

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Abbreviations used: DPPC, dipalmitoyl phosphatidylcholine; DOPC, dioleolyl phosphatidylcholine; LSE, lung surfactant extract.

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behavior at physiological temperature resulting from a phase transition occurring during the compression-decompression cycle (Teubner et al., 1983; Traüble et al., 1974).

Taking into account the variation of the surface tension during the respiratory cycle, it seems quite reasonable to look for an order-disorder lipid transition that might be associated with the structural properties of the lung surfactant. These properties should confer two apparently opposite properties: on the one hand, the ability to withstand high surface pressures resulting from a solid condensed layer with ordered paraffinic chains; on the other hand, the ability to adsorb and respread from a liquid state with disordered chains.

Compression of a monolayer of a lung surfactant extract (LSE) may result in the formation of 3D interfacial aggregates (Tchoreloff et al., 1991). This characteristic prompted us to investigate the structures formed by mixing a surfactant extract and water in various conditions, when the paraffin chains are in ordered and in disordered states.

It should be recalled that in lipid-water systems, lipid molecules may aggregate in a variety of structures exhibiting long-range 1D, 2D, or 3D organizations with disordered short-range organization usually referred to as the α conformation of hydrocarbon chains (as for example in lamellar, hexagonal, and cubic phases) (Luzzati, 1968; Luzzati and Tardieu, 1974). The ordered short-range organization is restricted (except for 3D crystals with very limited or no water) to a few lamellar-type phases showing 1D or 2D long-range organization with all the chains, or some of them, in an ordered conformation, referred to as β or β' when parallel or tilted with respect to the direction normal to the lamellar plane. These types of organization have been identified in 1D ($L\beta$, $L\beta'$, $L\alpha\beta$, $L\gamma$, $L\delta$) and 2D ($P\beta'$, $P\alpha\beta$, $P\gamma$, $P\delta$) phases (Tardieu et al., 1973; Gulik-Krzywicki, 1975).

In the present work we performed an x-ray diffraction and a freeze-fracture electron microscopy study on mixtures of a bovine LSE and water as a function of water content and temperature; we also studied a DPPC-DOPC mixture for the sake of comparison.

MATERIALS AND METHODS

LSE was an organic extract (obtained after a preliminary elimination of the cellular material) from bovine lung composed of lipids and a few percent of hydrophobic proteins (Jobe and Igekami, 1987; Bonanno et al., 1992). Mixtures of LSE and water in various proportions were prepared. The concentration c was defined by the weight ratio of lipid material to lipid plus water.

Usually in lipid systems the transition temperature is well defined, and the order-disorder transition occurs in a rather narrow range of temperatures. In the present work, the transition may extend over a broad range of temperatures; Tc denotes the temperature above which all the paraffin chains are disordered.

The x-ray scattering experiments were performed on a temperature-controlled Guinier camera using Kα1 radiation (λ = 1.54 Å) and a linear collimated beam. Positions in reciprocal space are specified by the parameter $s = 2\sin\theta/\lambda$, where 2θ is the scattering angle, and h or hk are the reflection indices of a 1D or 2D lattice. We refer to two distinct regions of the x-ray scattering spectra, one at low ($s < 0.15$ Å^{-1}), the other at high ($s > 0.15$ Å^{-1}) angles. Both small- and wide-angle scattering were recorded during the same experiment on the same photographic film.

For freeze-fracture electron microscopy small drops of the sample, containing 30% gycerol as a cryoprotectant, were placed on copper planchets and frozen in liquid propane. Freeze-fracture and replication were performed using a Balzers BAF 301 apparatus (Liechtenstein) equipped with an electron gun for platinum shadowing. Replicas were examined in a Philips 301 electron microscope (Eindhoven, The Netherlands).

RESULTS

X-ray diffraction

The LSE behavior was examined as a function of temperature at different water contents up to 40%. The results are summarized in Fig. 1. When all the paraffin chains were disordered (α conformation) above a temperature that depends upon the amount of water, there was only one phase, hexagonal for $c \approx 1$ or lamellar for $c < 0.9$. At lower temperature, when some of the chains were in an ordered conformation, two phases coexisted, a lamellar $L\beta$ or $L\beta'$ with a hexagonal for $c \approx 1$ and a lamellar $L\gamma$ with a lamellar $L\alpha$ for $c < 0.9$.

In the absence of added water or in the presence of small amounts of water, and below 60°C, two phases were observed, one hexagonal and the other lamellar. At 20°C the hexagonal phase was characterized by two sharp small-angle reflections having a spacing ratio of $1/\sqrt{3}$ corresponding to hk = 10 and 11 of a 2D hexagonal cell dimension of 47 Å. The high-angle region contains a wide-angle diffusion band around 4.5 Å. The lamellar phase was characterized by a set of sharp reflections of spacings h = 1, 2, 3, 4, 5, 6, 8, 9 corresponding to a dimension of 57.4 Å. The high-angle part was characterized by two reflections at 4.13 and 4.07 Å. This diffraction pattern corresponds to a $L\beta'$ lamellar phase with tilted stiff chains. Upon increasing the temperature, the amount of the hexagonal phase increased, whereas the $L\beta'$ was replaced by an $L\beta$ (nontilted stiff chains) phase (a = 56.6 Å at 45°C) as indicated by only one sharp reflection at 4.15 Å. Above 60°C, there was only a hexagonal phase characterized by a set of reflections with spacings $1/\sqrt{3}/\sqrt{4}/\sqrt{7}/\sqrt{9}$ (hk = 10, 11, 20, 31, 30) and a cell dimension of 45.6 Å.

Upon the addition of water and above a temperature ranging from 43 to 38°C according to the amount of water, one lamellar phase, $L\alpha$, was observed (h = 1, 2, 3, 4) and a

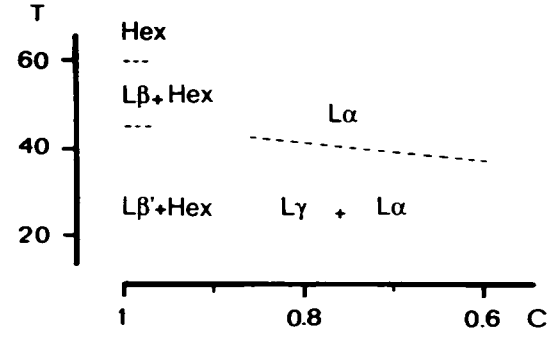

FIGURE 1 LSE phase domains as a function of temperature and water content.

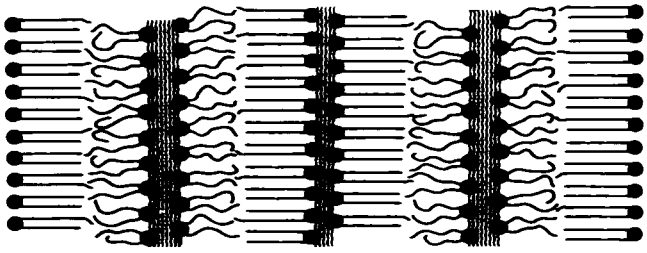

FIGURE 2 Structure of the Lγ phase. Note that the water is unevenly distributed between two fluid and two rigid layers.

diffuse band in the wide-angle region. Below these temperatures, two sets of sharp small-angle reflections were observed, corresponding to two lamellar phases of different types whose proportions varied with the water content and the temperature: one was a lamellar Lα identified by four reflections ($h = 1, 2, 3, 4$) that persisted at high temperature; the other corresponded to a lamellar repeating distance, which is about twice the Lα dimension having numerous small-angle reflections, $h = 2, 3, 4, 5, 6, 7, 8, 9, 10$ ($h = 1$ is absent or undetectable) and one wide-angle reflection at 4.11 Å typical of stiff β chains and a diffusion band. This unambiguously indicates the existence of an Lγ lamellar phase, which consists of a two-bilayer motif composed of monolayers with stiff chains alternating with monolayers with disordered chains (Ranck et al., 1980) (Fig. 2). The distribution of the intensities of the reflections is similar to that of the Lγ phase already described (Ranck et al., 1974). The distributions of the intensities of the reflections is analogous to that of the Lγ phase already described (Ranck et al., 1974). The data are shown in Table 1. An x-ray scattering profile is shown in Fig. 3 (photographic film and densitometry of the film).

The quantity of the Lγ phase decreased with increasing temperature and water content, whereas the quantity of the Lα phase increased. The dimensions of the Lγ phase increased with the water content and tended to be exactly twice that of the Lα at maximum hydration. This makes proper analysis of the diffraction patterns difficult, requiring a great deal of analysis of a lot of experimental data. As is usual for lipid systems, *Tc* decreases with increasing amounts of water. In the present case, *Tc* was about 38°C at maximum hydration.

To compare the behavior of the LSE with other lipids, we studied a mixture of DPPC-DOPC (1:1) with various amounts of water. At temperatures above *Tc*, one lamellar phase was present. Below *Tc* the behavior of the sample was more complex and depended upon the history (cycle of heating and cooling) of the sample and the water content. Generally, the ordered paraffin chains segregated into a separate phase with β or β′ conformation. Whatever the amount of water or the temperature, no Lγ phase was observed.

Freeze-fracture electron microscopy

We checked, by x-ray diffraction, the existence of the Lγ phase when using a mixture of 30% glycerol-70% water instead of 100% water. For $c = 0.6$ and at 20°C, two lamellar phases were observed; their dimensions are $d = 159.4$ Å for Lγ and $d = 78$ Å for Lα.

The samples of LSE were frozen beginning at room temperature. Freeze-fracture images show smooth surfaces typical of a lamellar structure. Often the fracture planes were associated in pairs (Fig. 4). This indicates the presence of two planes displaying different resistances to the fracture. This is typical of the Lγ phase (Gulik-Krzywicki, 1975).

DISCUSSION AND CONCLUSIONS

Lipids are known to exhibit a very rich structural polymorphism even below their transition temperature. Below that temperature, most often all the paraffin chains are ordered, and the lipids can be organized in various ways, either in 1D lamellar phases such as Lβ, Lβ′, and Lδ; or in 2D phases such as Pβ′ and Pδ (Luzzati and Tardieu, 1974). In other cases, the ordered and disordered chains can be associated within the same paraffin layer as in Lαβ and Pαβ. However, there is another possibility: the ordered and the disordered chains may belong to distinct layers such as in the Lγ phase, which contains four monolayers (Fig. 2). This phase is quite unusual; it has been found by x-ray scattering in the mitochondrial lipid-water system in a narrow range of temperature and at low water content (Gulik-Krzywicki et al., 1967). It has also been studied by neutron scattering (Ranck et al., 1980). The structure consists of two bilayers, each one formed by an α and a β monolayer (Fig. 2).

TABLE 1 Dimensions d (in Å) of the lamellar (Lγ and Lα) phases obtained from mixtures of LSE and water

	Concentration							
	0.86		0.75		0.66		0.60	
T(°C)	*d* (Lγ)	*d* (Lα)	*d* (Lγ)	*d* (Lα)	*d* (Lγ)	*d* (Lα)	*d* (Lγ)	*d* (Lα)
20	118.0	55.0	128.6	59.4	149.1	72.8	162.0	81.0
28	115.6	53.6	127.9	58.6	145.5	71.5	157.0	78.5
35	115.4	53.6	126.0	58.2	144.5	68.6	153.3	76.6
37–39	113.2	53.2	124.1	58.2	140.8	67.7	150.7	75.3
41	112.1	53.0		57.0		66.9		74.0
45		52.9						

Note that, as the water content increases, the difference between 1/2 *d* (Lγ) and *d* (Lα) tends toward zero.

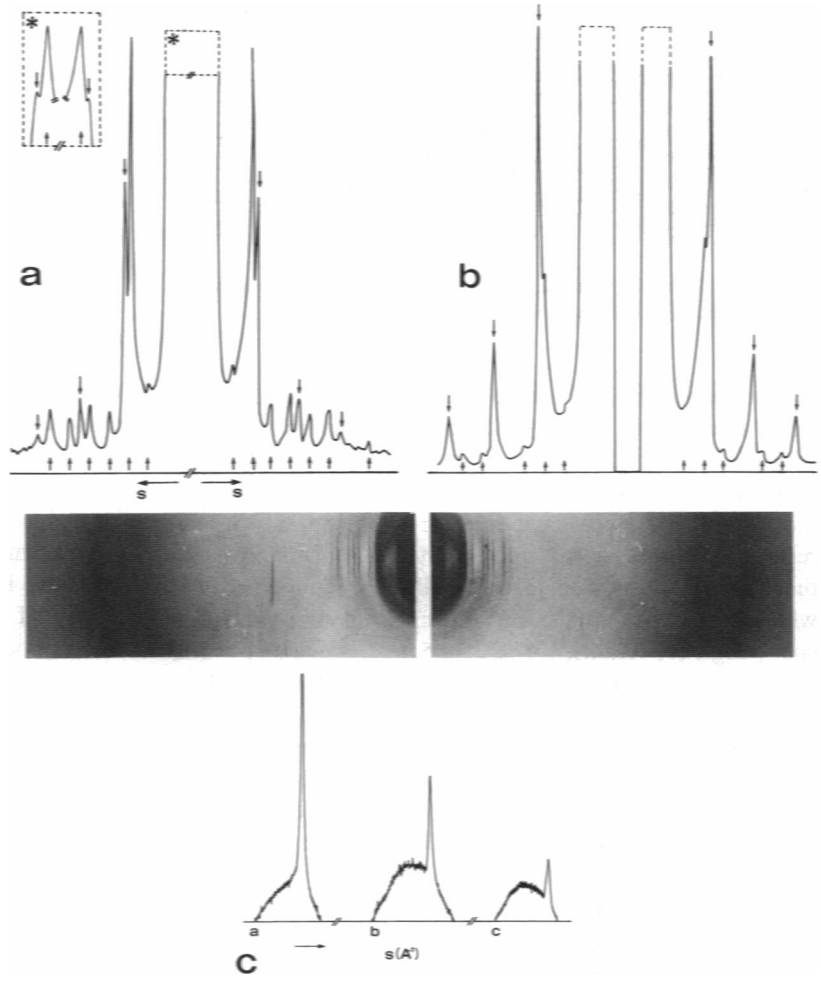

FIGURE 3 X-ray scattering of LSE. (*a*)Densitometry of the small-angle part of the film shown below (c = 0.75 at 25°C). Two series of reflections are clearly seen: at bottom, arrows for Lγ reflections; at top, arrows for Lα reflections. (*Insert*) The first reflections. (*b*) Densitometry of the small-angle diffraction pattern at 35°C. (*c*) Densitometry of the wide-angle scattering region as a function of temperature (left to right: 20°C, 35°C, 38°C). One can see the decrease of the sharp reflection corresponding to the stiff chains, whereas the intensity of the broad band corresponding to the disordered chains increases. The intensities of the corresponding small-angle reflections of the Lγ phase decrease concurrently, as can be seen by comparing (a) and (b).

Remarkably, the Lγ phase exists over a large range of concentrations and temperatures in the LSE system. This suggests the possible biological significance of this phase. A monolayer of DPPC, whose chains are stiff at a temperature higher than physiological, can thus be associated with a fluid layer. Near the physiological temperature in LSE-water mix-

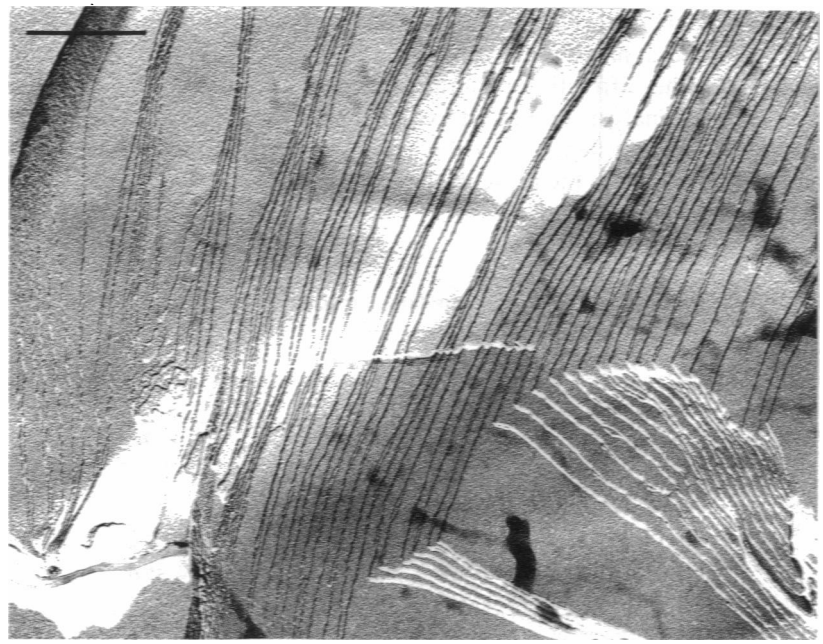

FIGURE 4 Freeze-fracture electron microscopy of the Lγ phase (20°C). Note the presence of smooth fracture planes associated in pairs. Bar = 400 nm.

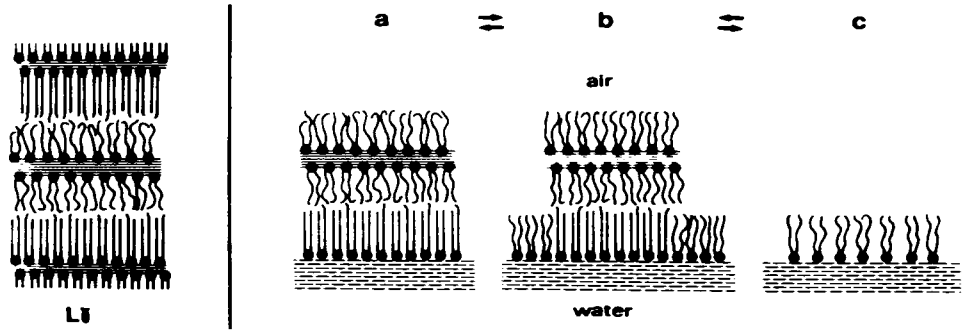

FIGURE 5 Structural interfacial transitions of lipids able to associate into an Lγ phase. (*Left*) Structure of Lγ in a bulk phase. (*Right*) At the air-water interface the number of lipid layers becomes odd, and the lipids, able to form an Lγ bulk phase, may be associated as indicated in (*a*). Upon expansion of the interfacial area, this type of association may easily and gradually transform into a fluid monolayer (*c*) through a series of steps as indicated in (*b*). It may also be as well compressed.

tures, most of the lipids are associated in the Lα form, and a few remain in the Lγ. In other words, some part of the DPPC molecules is still able to form a rigid monolayer associated with a fluid one. The temperature of about 38°C and the maximum uptake of water into the lamellar Lγ phase represent the conditions defining a kind of a critical point limiting the existence of Lγ. The extent of the phase may therefore enlarge or disappear with minor physicochemical changes in the vicinity of this point.

If one considers the phenomena that can occur at an air-water interface, the conditions of existence of a solid film, i.e., ordered paraffin domains, depend upon the lipid molecules, the temperature, and the rate of compression. In mixtures of DPPC and water the melting transition is limited to 41°C at maximum hydration; however, this temperature can be increased at an air-water interface. For example, at a rate of compression of 90 cm^2/min in a Langmuir trough, DPPC is still capable of sustaining a high surface pressure even at 47°C (Tchoreloff et al., 1991). This change of transition temperature is very likely due to a variation in the hydration of the polar head group (Tchoreloff et al., 1991; Goerke and Gonzales, 1981; Helm et al., 1991). It is well known that this temperature is strongly dependent, in the bulk phase, upon the water content. Therefore, compressing an interfacial film may be equivalent to decreasing its water content in bulk, which results in an increase in the transition temperature. During the respiratory cycle, the interface of lung sustains large variations in the surface area, which might be equivalent to a large variation in water content or transition temperature in a bulk phase. Thus, the quantity of lipid able to form an ordered layer can increase noticeably during the respiratory cycle. Given that such an ordered lipid layer is always associated with a disordered one in an Lγ phase, it is very likely that, near the collapse of the interfacial pulmonary surfactant, a solid monolayer is strongly associated with other lipids, which are in a fluid form. One could imagine a transient state at the interface between the two extreme configurations as shown in Fig. 5.

Such an organization could explain the ability of a collapse phase of the Lγ type to respread easily. When a monolayer of DPPC is compressed to the collapse in the solid form, it can form a 3D structure at the interface, which is unable to respread upon decompression. Conversely, above the melting transition the lipid cannot be compressed to a high surface pressure and can respread easily; at the collapse there is only one layer of lipids, and some of them may be irreversibly lost into the bulk phase. An association of a solid layer and a fluid layer, however, combine both properties required for the lung surfactant: the ability to sustain high surface pressure, and the ability to respread easily, depending upon the conditions of compression that vary during the respiratory cycle.

Although we have not considered all the factors that may take part in the functioning of the surfactant such as contractile forces, the nature of the alveolar hypophase, and the turnover of the lipids, the present results provide some clues to the mechanisms involved in the interfacial phenomena. They may help to understand the "easy breathing and the difficult surface chemistry" (Keough, 1992; Goerke and Clements, 1986). A γ-like interfacial film can form a solid layer that is bound to a liquid one and is able to respread upon relaxation into a complete fluid monolayer without irreversible loss into the subphase. The role of such a structure in the physiology of the lung may depend upon the particular lipid and apoprotein composition, and it might be of interest to look for exogenous artificial lung surfactants able to form Lγ structures.

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