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Biophysical Chemistry 89 (2001) 243–251

Biophysical Chemistry

www.elsevier.nl/locate/bpc

The role of the gel ⇔ liquid-crystalline phase transition in the lung surfactant cycle

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Received 3 August 2000; received in revised form 29 November 2000; accepted 29 November 2000

Abstract

Lipid polymorphism plays an important role in the lung surfactant cycle. A better understanding of the influence of phase transitions on the formation of a lipid film from dispersions of vesicles will help to describe the mechanism of action of lung surfactant. The surface pressure (or tension) of dispersions of DPPC, DMPC, and DPPE unilamellar vesicles was studied as a function of temperature. These aggregates rapidly fuse with a clean air–water interface when the system is at their phase transition temperature (T_m), showing a direct correlation between phase transition and film formation. Based on these results, an explanation on how fluid aggregates in the alveolar subphase can form a rigid monolayer at the alveolar interface is proposed.

Keywords: Lung surfactant; Vesicles fusion; Gel/liquid-crystalline phase transition; Monolayer formation; Surface tension; Surface pressure

1. Introduction

Lung surfactant [1,2] is a complex mixture of proteins (~10%) and phospholipids (~90%) whose main function is to reduce the surface tension at the alveolar air–liquid interface to low values (< 10 mN/m). This facilitates the absorption of oxygen and prevents alveolar collapse during the respiratory cycle, thus ensuring its physiological dynamics. Lack of a proper lung surfactant supply can lead to respiratory distress syndrome (RDS), which affects prematurely born infants [3] and even adults. A widely used methodology to study lung surfactant is the formation of a monolayer at the surface of a vesicle dispersion [4–7]. This spontaneous phenomenon has been observed in dispersions of both synthetic and natural phospholipids, and such experiments are

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0301-4622/01/$ - see front matter
PII: S0301-4622(01)00240-4

usually monitored by surface tension (γ) or surface pressure (π) measurements [8], using a tensiometer or a Langmuir balance, respectively. Notwithstanding the efforts of several groups [9] in elucidating the mechanism of action of lung surfactant, a number of important details are still to be described. Most works have focused on the function of the two major phospholipid components, dipalmitoyl phosphatidylcholine (DPPC) and phosphatidylglycerol (PG), which correspond to ~ 50 and $\sim 10\%$ of the total amount of lipids in the human lung surfactant, respectively [2,10]. It has been suggested that the function of PG, and other minor components, is to increase the fluidity of the membranes [11]. The influence of the surfactant proteins (SP) on the rate of vesicle disruption at the air/water interface (a/w) is also a widely studied subject. It was demonstrated that SP-A, -B, and -C accelerate the process of vesicle disruption and consequent monolayer formation at the a/w interface [12–18]. It was also shown that these proteins can shift the gel $\Leftrightarrow$ liquid-crystalline phase transition temperature (T_m) [19] of some synthetic membranes to a higher value [20–22].

At the T_m, vesicles undergo a structural modification in which the bilayer turns from a rigid state (gel) into a fluid one (liquid-crystalline). It was demonstrated that as the temperature of a vesicle dispersion approaches the T_m, an increase in the rate of vesicle fusion[1] ($\Delta\gamma/\Delta t$ or $\Delta\pi/\Delta t$, where t is time) with the a/w interface is detected [23–26]. Surprisingly, only a few works deal with a possible influence of the T_m on the fusion of vesicles with the a/w interface [27–30]. A better understanding of this process can throw light upon the correlation between membrane fluidity and spreading, which in its turn will help to clarify the functions of the individual components of the lung surfactant. Hitherto, some authors have studied the thermotropic phase transitions in lung surfactant dispersions and films [20–22,31–33], but there is not a direct correlation between lipid transitions and the mechanism of film formation inside the alveoli. The present work shows the influence of the T_m on the rate of fusion of pure synthetic vesicles with the a/w interface, in the absence of proteins, and points out the implications for lung surfactant dynamics.

2. Experimental procedures

2.1. Materials

Dipalmitoyl phosphatidylcholine/ethanolamine (DPPC/DPPE) and dimyristoil phosphatidylcholine (DMPC) were obtained from Sigma. NaCl and the buffer components (NaOH, Na_2HPO_4) were of analytical grade, purchased from Merck. The water used in all experiments was doubly distilled (glass) and passed through a MilliQ (Millipore) apparatus.

2.2. Vesicles preparation and characterization

Vesicle dispersions (5–10 ml) were sonicated for 15 min using a titanium tip sonicator (Braunsonic, 1510, B.Braun), keeping the samples 5–10°C above their respective T_m values. The concentrations of all vesicle dispersions are expressed in terms of the lipid monomer concentration, and are indicated in each case (figure legends). The subphases consisted of NaCl solutions with the same concentration of the surface tension (pressure) assays (see next topic) in order to ensure an isosmotic medium. The samples were centrifuged ($10\,000 \times g$ for 20 min) in order to separate the titanium particles. Vesicle size was determined by dynamic light scattering (He–Ne laser, 60 mW, EMI photomultiplier, Brookhaven BI90 autocorrelator). Vesicles hydrodynamic diameters were 440 ± 50, 540 ± 50 and 1700 ± 50 Å for DMPC, DPPC and DPPE, respectively.

[1] The process of fusion between membranes is catalyzed by fluctuations and defects in the bilayer structure, both which are enhanced at the T_m. From the results present here, it can be inferred that T_m plays a similar role in the disruption process of vesicles at the a/w interface, and such a process may involve the same mechanism of fusion between membranes. Although the disruption process has not been proved to be a real fusion yet, this name has been used before, and is the authors' visualization of the phenomenon.

2.3. Surface pressure and surface tension assays

π was measured based on the Wilhelmy plate method on a Langmuir Balance (KSV Instruments, model KSV 5000), placed in a cleanroom, without compression of the monolayer. $\Delta\pi/\Delta t$ was calculated taking into account the first minute. Measurements of γ were carried out on a tensiometer (Fisher Scientific Tensiomat, model 21) using the Ring method. In both cases, a hollow Teflon trough (diameter 6 cm, 2-cm deep) was connected to a water bath circulator system. The temperature was measured directly inside the trough using a Teflon-coated thermometer (0.5°C accuracy).

3. Results

After the injection of an aliquot of a vesicle dispersion into a subphase, a layer of vesicles will be formed right beneath the a/w interface [5,6]. Through an unknown mechanism, vesicles from the upper layer fuse with the interface, releasing monomers and forming a monolayer (Fig. 1). An equilibrium state is reached between vesicles close to the interface and those in the bulk, and a process of monomers exchange between vesicles and monolayers takes place [6]. Thus, a vesicle dispersion is characterized by a monolayer, a layer of vesicles right beneath it, and the remaining population of vesicles in the bulk. It was shown that the rate of vesicles fusion depends on the concentration of vesicles, but not above a threshold value [34,35]. Therefore, in order to study only the influence of the phase transition on the fusion rate, concentrated vesicle dispersions were used, yielding final concentrations of circa 10^{-4} M after injection into the subphase.

In a first approach, γ was measured consecutively as a function of temperature. Fig. 2 shows the results for a dispersion of DPPC, where a faster decay is found at 41.5°C, which corresponds to the T_m of the vesicles [19]. The same assay was carried out for dioctadecyldimethylammonium chloride (DODAC) vesicles (not shown), and a greater reduction in γ is found at the T_m region of these vesicles [29]. The same methodology was

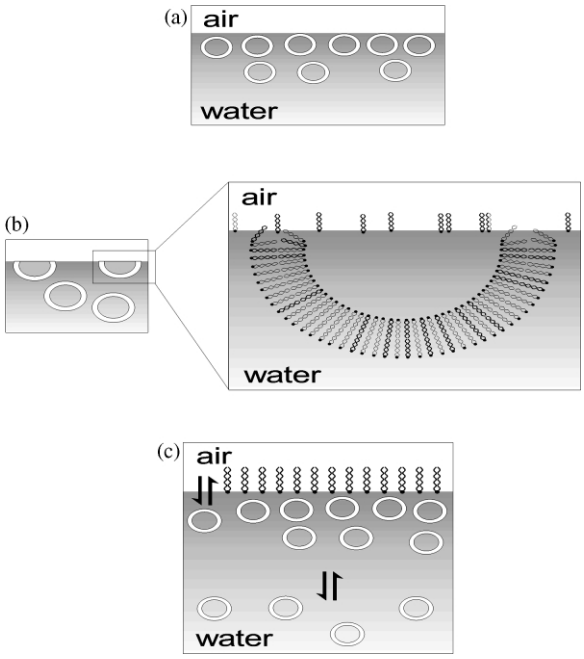

Fig. 1. Schematic representation of the distribution of vesicles in a dispersion. (a) Formation of a layer of vesicles beneath the a/w interface; (b) fusion of vesicles with the a/w interface and consequent monolayer formation; (c) vesicle diffusion and vesicle-monolayer lipid exchange after reaching the equilibrium state.

applied for a dispersion of DMPC vesicles, now using a Langmuir Balance. In this case, π was measured continuously as a function of temperature, and an abrupt change is detected at approximately 23°C (Fig. 3). The first derivative of this curve (inset) shows that π rises abruptly at 23.2°C, which corresponds to the T_m of DMPC vesicles [36].

In order to verify the rate of fusion with the a/w interface at selected temperatures, another approach was used, whose detailed description can be found elsewhere [30]. In short, γ (or π) was measured as a function of time at several temperatures, and $\Delta\gamma/\Delta t$ ($\Delta\pi/\Delta t$) was plotted against temperature. Fig. 4 shows the results for DPPC and DMPC vesicles, and it is clearly seen that the highest values of $\Delta\gamma/\Delta t$ correspond to the T_m of the vesicles.

The same technique was applied for studying the influence of salt on the rate of fusion of

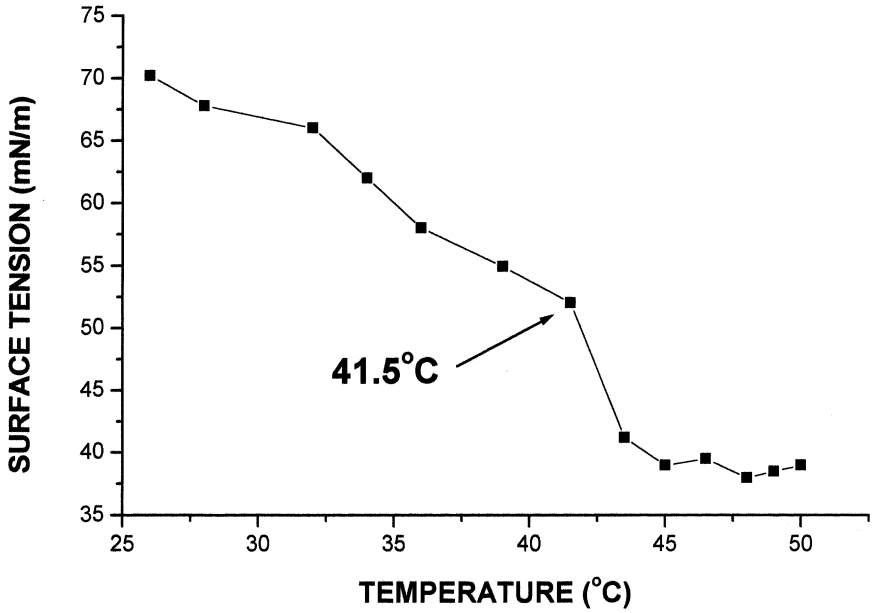

Fig. 2. Surface tension of a dispersion of DPPC vesicles as a function of temperature. Five milliliters of 5 mM of DPPC vesicles were injected into 40 ml of an aqueous subphase of 1 mM NaCl.

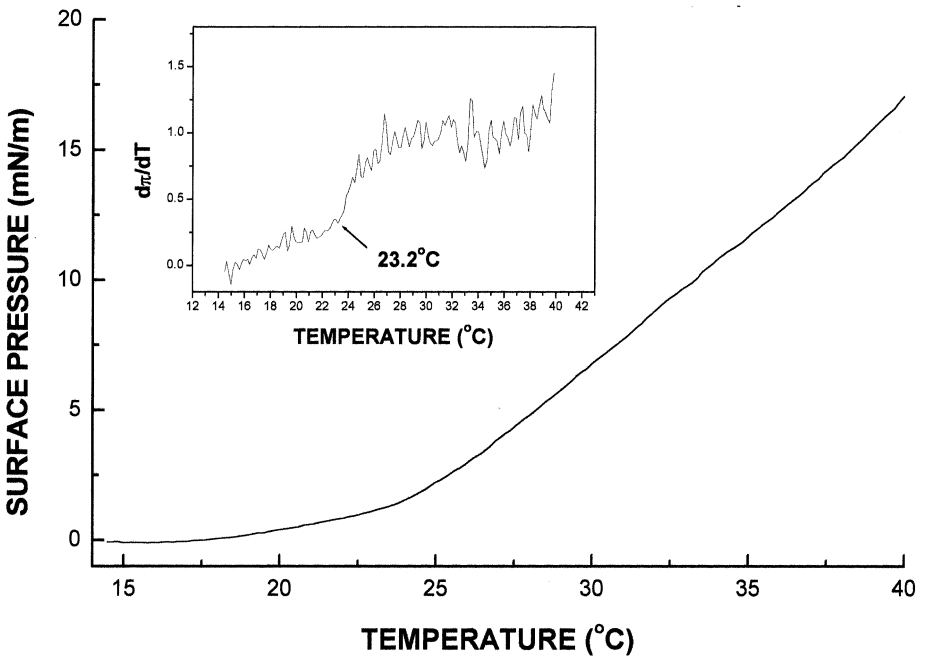

Fig. 3. Surface pressure of a dispersion of DMPC vesicles as a function of temperature. An aliquot of 2 ml of 2 mM of DMPC vesicles was injected into 40 ml of a subphase containing 1 mM NaCl. The inset shows the variation of the surface entropy as a function of temperature. A detailed description of this experiment is given in Gugliotti et al. [29].

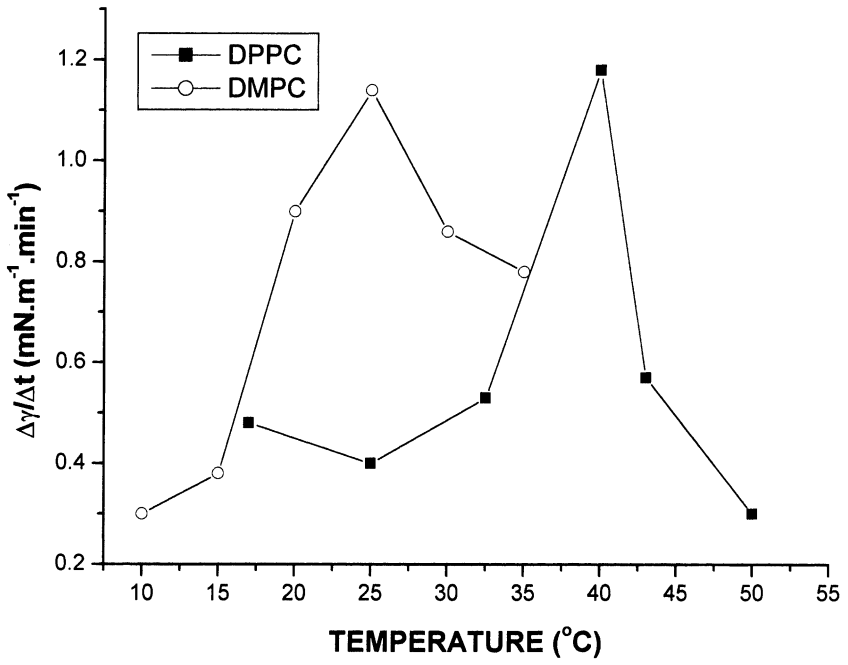

Fig. 4. Rates of vesicle fusion with the a/w interface for DMPC and DPPC vesicles within the first minute. Aliquots of 5 ml of 5 mM of DMPC and 5 ml of 2 mM of DPPC vesicles were injected into 40 ml of a subphase of 1 mM NaCl. The scales were adjusted in order to plot the results together (see Gugliotti et al. [30]).

charged DPPE vesicles at pH 12 (PE corresponds to ~12% of the total amount of lipids in the human lung surfactant [10]). The assays were carried out on a Langmuir Balance, and the results for $\Delta\pi/\Delta t$ as a function of temperature are shown in Fig. 5. For a subphase containing 20 mM of NaCl, $\Delta\pi/\Delta t$ increases continuously after reaching the point of 41°C, which is in good agreement with the value for the T_m of DPPE vesicles at high pH (42°C) [37]. In the case of 50 mM of NaCl, the pattern of the curve changed, not allowing an accurate correlation between $\Delta\pi/\Delta t$ and the T_m.

4. Discussion

The increase in the rate of vesicles fusion with the a/w interface at the T_m seems to be a general phenomenon. The results in Figs. 2 and 3 show clearly that vesicles can disrupt easily at the temperature corresponding to their T_m, making these assays a simple method for T_m determination. The inset in Fig. 3 gives additional information. A simple analysis shows that surface pressure (or tension), given, i.e. in N/m, is also a measurement of the surface energy G, for:

$$\pi = \frac{\mathrm{N}}{\mathrm{m}}\left(\frac{\mathrm{m}}{\mathrm{m}}\right) = \frac{\mathrm{Nm}}{\mathrm{m}^2} = \frac{\mathrm{J}}{\mathrm{m}^2} = G,$$

where m is meter, J is Joule, and G is the surface energy per unit of area [8]. It is known from the thermodynamics that the first derivative of the surface energy G as a function of temperature is the surface entropy S [8]. The inset in Fig. 3 shows an abrupt change in $\delta\pi/\delta T$ as a function of temperature at the transition, which corresponds to an abrupt change in S. According to the Ehrenfest classification of phase transitions [38], it can be concluded that this is a first-order phenomenon.

The methodology applied for obtaining the results in Fig. 4 shows that the rate of vesicles

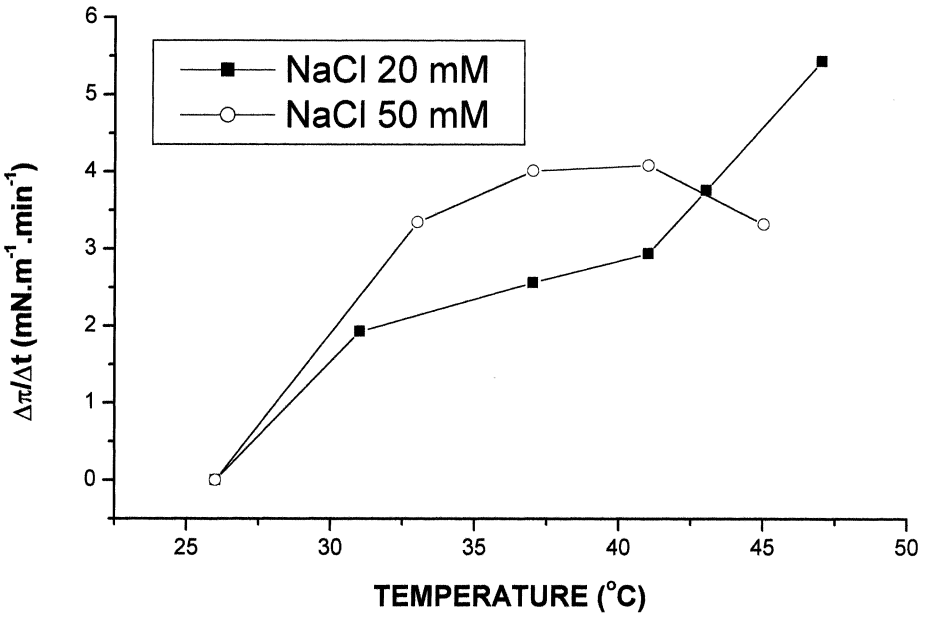

Fig. 5. Five milliliters of a 5-mM aliquot of DPPE vesicles at pH 12 were injected into 40 ml of NaCl 20 mM (squares) and 50 mM (circles) at the same pH. $\Delta\pi/\Delta t$ was calculated taking into account the first minute. At 20 mM of NaCl, an increase in the rate of vesicles fusion is detected at the T_m region of the vesicles. At 50 mM of NaCl the pattern of the curve changes dramatically (Gugliotti et al. [30] gives a detailed description of the methodology used).

fusion with the a/w interface is faster at 25 and 40°C for DMPC and DPPC vesicles, respectively, which are in good agreement with the values of 23.2 and 41.5°C obtained from Figs. 2 and 3. These temperatures correspond very well to the T_m of the vesicles, and the shape of the curves in Fig. 4 resemble those from permeability studies [39,40]. This suggests that the effect which leads to a faster fusion with the a/w interface at the T_m is the same that makes the vesicles more permeable at this temperature, that is, the coexistence of the both gel and liquid-crystalline domains in the membrane.

The influence of salt concentration on the rate of fusion of DPPE vesicles is presented in Fig. 5. The pattern of the curve changes dramatically when the concentration of NaCl is increased from 20 to 50 mM, which indicates the interaction of NaCl with the charged head groups [30].

It is known that the composition of lung surfactant plays an important role regarding the fluidity of the membranes. The changes in lung surfactant composition as a function of body temperature are understood as a need to keep the homeoviscosity [10,41,42]. The thermotropic behavior of fully hydrated lung surfactant lipids and hydrophobic extracts shows a transition below the physiological temperatures, which is changed by interaction with proteins [21,31]. Taking into account the above conclusions and the results presented here, it seems that these changes in composition would represent the need to equal the T_m of the lamellar aggregates to the body temperature, promoting a better condition for rapid monolayer formation at the alveolar interface. This leads to an interesting picture of the phenomenon inside the alveoli. The thermotropic state of vesicle structures seems to be regulated in order to make the T_m of these aggregates equal to the body temperature. This eases the deposition of monomers at the alveolar interface. Once the film is formed, compression during the respiratory cycle will decrease the alveolar interfacial tension to a minimum value, but only if the lipids

are in the gel state. DPPC lipids are in the gel state at physiological temperatures, only melting at 41°C. Thus, according to the suggested 'squeeze-out' mechanism, supported by several works ([43], and references therein), lipids in the fluid state are excluded from the monolayer, which would be enriched by DPPC ones, forming a monolayer in the gel state. A lipid film in the gel state can be highly packed at the a/w interface forming a 'solid state' film, which can resist to high surface pressures, thus decreasing the surface tension to very low values. Lipid monomers in the fluid state have a larger area per molecule than those in the gel state. This makes it difficult to pack the film at the a/w interface in the solid state, and during compression the lipids are easily ejected from the monolayer, which is known since the early works of Langmuir [44]. It is interesting to note that some synthetic lipids in the fluid state form vesicles easily [45] (for example, the process of sonication is generally carried out at temperatures above the T_m of the vesicles). This suggests that the fluid lipids that are excluded from the monolayer could return to the alveolar subphase in the form of three-dimensional structures in the liquid-crystalline state. In fact, this has been noticed for monolayers made of the major components of the lung surfactant after collapse [46]. It has also been suggested that phosphatidylcholines can inhibit the secretion of surfactant from the type II cells when they are in the gel state [47]. It appears that these cells can recognize the physical state of lipids. These features show the importance of the lipid phase transitions in the alveoli.

It is remarkable how the layers of vesicles represented in Fig. 1 correlate with the phenomenon inside the alveoli. It was shown that after spreading liposomes at the surface of water, approximately 75% of the phospholipid material remained close to the interface in the form of layers of vesicles [4–6]. An attempt to calculate the thickness of the vesicle layers yielded the value of 0.54×10^{-4} cm [48], which corresponds well to the value of 10^{-4} cm found by King and Clements in 1972 for 'the equivalent thickness of the stagnant layer' right beneath the surface film [49]. This indicates that a vesicle dispersion mimics well the phenomenon inside the alveoli, and that a dynamic equilibrium including monomers exchange may also occur (see Fig. 1).

The results presented in Figs. 2 and 3 show that the final values of γ are not so low (or high, in the case of π), since there is no compression of the monolayers formed. Also, the decrease in γ (or increase in π) is not so fast, but it should be taken into account that the experiments were carried out in the absence of proteins, which increase significantly the rate of vesicle fusion with the a/w interface. It is reasonable to think that, since some surfactant-proteins have positive charges and hydrophobic parts in their structure, they could act as 'fusion machines' [50,51], improving the rate of vesicle breakdown at the a/w interface. The effect of proteins might be detected by applying the methodology described here for studying natural samples of lung surfactant, which will be certainly a matter for further discussions.

5. Conclusions

The influence of the T_m on the rate of vesicle fusion with the a/w interface was demonstrated. At this temperature, DPPC and DMPC vesicles fuse easily with the a/w interface following a first order process. Charged DPPE vesicles showed a change in the rate of fusion as function of salt addition, indicating a large influence of the polar head group.[2] The simple methodology used makes these assays a good tool for studying membrane properties [52].[3]

The data presented here suggest a possible role

[2] Since DPPE lipids make pH sensitive vesicles largely used as drug delivery systems, it is important to ensure that the T_m of these aggregates will be higher than the physiological temperatures to avoid disruption before they reach the target cell. The results presented here may also be useful for the field of fusogenic liposomes, where a local heating would promote their faster disruption.

[3] There is an error in the abstract of Gugliotti et al. [52]. It should read '...vesicles fusion...', and not '...vesicles formation...'. Also, the definition of surface tension in the text is not correct. Surface tension is tangential to the plane of the surface, and not perpendicular.

for the main lipid phase transition with respect to the formation of a monolayer at the alveolar interface. Taking into account the influence of the T_m on the rate of vesicle fusion and the squeeze-out mechanism, it is possible to think of a lamellar system that is fluid in the subphase, and after fusion with the interface forms a rigid monolayer. These are important features for the lung surfactant cycle, and materials with such properties may become an alternative for RDS replacement therapy.

Acknowledgements

The authors wish to thank Prof. Dr José Atílio Vanin, Dept. of Química Fundamental, for helpful discussions, and Guilherme A. Marson, Dept. de Bioquímica, for the help with Fig. 1, both at the University of São Paulo.

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