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The main transition of dipalmitoylphosphatidylcholine monolayers: A liquid expanded to solid condensed high order transformation

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Abstract

We have investigated the orientation processes of a fluorescent probe, 12-(9-anthroyloxy)stearic acid (12-9-AS), embedded in a dipalmytoylphosphatidylcholine (DPPC) monolayer spread at the air/water interface. These orientation processes are represented by an orientation parameter defined as the ratio of two dimerization constants obtained from fluorescence experiments using two perpendicular linearly polarized incident light beams. The orientation parameter shows three particular domains of orientation during the compression of the DPPC film, these domains being separated by two drastic changes in the orientation parameter, one located at the LC/SC transition point (0.48 $nm^2 \cdot molecule^{-1}$) and the other at the end of the LE/LC transition region (0.58 $nm^2 \cdot molecule^{-1}$). Attribution of the phases implicated in the bidimensional liquid/solid transformation was thus revisited. Therefore, from the present results together with results gathered in the literature, we show that the main monolayer phase transition of DPPC implies a transformation from a molecular state having a characteristic area A_{LE} (phase LE) to a molecular state of characteristic area A_{SC} (phase SC) with an intermediate molecular state of characteristic area A_{LC} (phase LC). During the main monolayer transformation (the LE/SC phase transition) the highly condensed domain with a molecular area A_{SC} (0.48 $nm^2 \cdot molecule^{-1}$) are thus surrounded by a region of interfacial lipid of molecular area A_{LC} (0.58 $nm^2 \cdot molecule^{-1}$) distributed in a fluid phase of characteristic area A_{LE} (0.74 $nm^2 \cdot molecule^{-1}$).

Keywords: Dipalmitoylphosphatidylcholine monolayers; Phase transition

1. Introduction

It has been clearly shown, mainly by fluorescence microscopy, that the main phase transition (the so-called liquid expanded (LE) to liquid condensed (LC) phase transition) of a DPPC monolayer is heterogeneous in nature [1–3]. This observation—albeit the fact that the surface pressure isotherm has been reported by most authors, including those who performed the experiments in fluorescence microscopy, to be non-horizontal in the transition region—has become the major element to get to the conclusion that this transi-

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Abbreviations: DPPC – Dipalmitoylphosphatidylcholine; LE – liquid expanded; SC – solid condensed; 12-9-AS – 12-(9-anthroyloxy)stearic acid.

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tion is a first-order thermodynamic transition. Therefore, according to this model, the starting point of the main monolayer transition (π_t) in the π vs. A isotherm represents the initial point from which the transformation from a low density state (i.e. a pure LE phase) to a high density state is taking place. This high density state has been assumed, without much critical analysis, to be the pure LC state. The results presented below and discussed in the present work will lead us to reassess the nature of the condensed state really implicated in the main transition of DPPC monolayers.

In fact, the classical description of the main phase transition referred to above has been challenged and the debate on the true nature of this transition is still open. The reader is referred to the review of Cadenhead [4] for a thorough discussion on this matter. Since then, however, Pallas and Pethica [5] have shown that by successive additions of the DPPC molecules at the air–water interface, the LE/LC region seems to be horizontal. More recently, this result has been reproduced by Hifeda and Rayfield [6] using a continuous compression of the monolayer. However, even in these cases, one realizes upon careful observation, that in the zone of the lower area per molecule of the main transition region (i.e. around 0.52–0.54 nm^2 molecule^{-1}), there is no net break in the isotherm as required for a true first order thermodynamic transition. On the other hand, Baret et al. [7], from theoretical considerations, have proposed that the the main monolayer phase transition is rather second order in nature. These authors have based their study on the work of Lifschitz, Landau and Joly (see Joly [8]), who have first shown that the phase transformation in a monolayer film can result either from a change in the degree of symmetry of the film network (positional order) or from some changes in the degree of symmetry of the surfactant molecules themselves (e.g. conformational order). Thus, Baret et al. [7] have proposed that a second order phase transformation can result from a break of the rotational symmetry of the film, which gives rise to a preferred direction of the molecules within the plane of the interface. This phase transition has also been identified as an isotropic-nematic (anisotropic) second order phase transition by Dervichian [9,10].

More recent investigations by synchrotron X-ray diffraction and reflectivity studies have provided some insights into the nature of the ordered state of lipid films at the air/water interface and into the problem of phase transformation in phospholipid monolayers. In particular, the group of Möhwald has shown that the gel phase (or SC phase) is characterized by long range bond orientational order but short positional correlations [11]. This has led Flörsheimer and Möhwald [12] to propose that the main monolayer transformation of phospholipids results from superimposed orders of transition, i.e. a first order transition to which higher order transitions are superimposed.

In this context, we have investigated in the past few years the phase transition and molecular dynamics of DPPC monolayers at the air/water interface using the fluorescence recovery after photobleching technique. It has been shown that the lateral diffusion coefficient of the probe decreases dramatically in the liquid-condensed state region of the isotherm [13] and that the diffusion measurements can be used to determine more precisely the frontier of the LC state [14]. We have indeed demonstrated that the liquid-condensed state (LC) appears as a transition region between the LE/LC region and the solid (SC) state. In this liquid condensed state, the lipid molecules present a significant decrease in their lateral diffusion coefficient related to a structural reorganization of both their polar heads and their aliphatic chains. The characteristic molecular area of the LC state was found to be 0.58 nm^2 molecule^{-1}. At this area, we showed that the lipid molecules have about 10 water molecules tightly bound to their polar heads. Upon further compression, the DPPC molecules continuously lose water molecules until the SC state is reached at 0.48 nm^2 molecule^{-1}. We also showed that associated with this dehydration phenomenon in the LC region, there is significant steric hindrance arising between the adjacent hydrated polar heads wich gives rise to a decrease in the diffusion coefficient. Also, during this progressive loss of water molecules, fluorescence studies with polarized incident light [15] indicated that although the mean orientation of the polar heads at the glycerol level did not seem to change, the standard deviation for the distribution function of the orientations was larger in the liquid-expanded state and the LE/LC region relative to the SC state. This suggests that indeed a structural change is occurring in the region of the polar heads (glycerol region) of the phospholipid array during compression within the LC state.

Further informations on the orientational changes of the aliphatic chains of the DPPC molecules under compression are presented here. A fluorescent covalently labelled fatty acid (12-(9-anthroyloxy)stearic acid, 12-9-AS) is used with linearly polarized incident excitation light and we follow the behaviour of the dimerization constant of the probe photoreaction. The results obtained, together with observations gathered from the literature, yield us to reassess the nature of the condensed phase implied in the main monolayer transition, which is presented here as a global liquid (LE) to solid (SC) monolayer state transformation involving the LC phase as an interfacial region around the SC phase in the fluid LE phase.

2. Materials and methods

2.1. Chemicals

DPPC and 12-9-AS were purchased from Sigma (St. Louis, MO). Their purity was checked by thin layer chromatography and found to be satisfactory. They were thus used without further purification. The sodium chloride used was analytical grade. Ultrapure water (specific resistivity greater than 17×10^6 Ω cm) for the semiconductor industry was obtained from Motorola (Toulouse), France and was used throughout the experiments.

2.2. Monolayer and polarized fluorescence measurements

The interface fluorometer used has been described elsewhere [16,17]. In brief, the illumination is provided by a 100-W mercury HBO arc lamp (Orsam, Germany) and the illuminated area is 2 mm in radius. The fluorescence from the illuminated area was monitored at 45° with respect to the excitation for different compression states of the monolayer. The observation direction was perpenticular to the film surface. The through was milled in Plexiglas in order to minimize light-scattering problems. The dimensions of the trough (20 mm wide, 250 mm long, and 5 mm deep) were chosen such that a large range of compression states could be easily obtained by compressing the monolayer with a movable Teflon barrier. The trough was equipped with a platinum plate to record the surface pressure as the monolayer was compressed.

Wavelengths for illumination were selected by means of optical filters. For excitation, we used a Schott UG1 filter which selected the mercury line at 360 nm; for observation a MTO DH 525 c′ plus Kodak 2E broad band filter, transmitting light above 420 nm, was used. A prism was inserted on the excitation light beam.

Fluorescence was detected with a high-sensitivity photomultiplier tube, connected to a data acquisition unit. The fluorescence intensity was measured by means of a high sensitivity photomultiplier tube (EMI 9558, England) connected to a data acquisition unit. The results were displayed on a chart recorder or transferred to a microcomputer (Ohio Challenger, USA) via an A/D converter. The deconvolution of the photobleaching experiments was operated on-line with an acquisition speed of more than two data per second.

A typical bleaching experiment is carried out as follows. The background signal, due to the light scattered by the subphase (0.1 *M* NaCl), was set electronically as zero fluorescence. A mixture of the probe and lipid molecules (2% mol/mol) in chloroform/methanol (5/1, v/v) is then spread at the air/water interface. This is done in darkness to avoid photodimerization of the probe molecules. The solvent is then allowed to evaporate (10 min.) and the film is compressed at a rate of 3×10^{-4} nm^2 molecule^{-1} s^{-1} to a given surface pressure. It is kept at this surface pressure for 15 min. During this time period, absolutely no change of the surface pressure was recorded. The optical shutter is then opened for 15 s and the dimerization reaction is followed as a decrease of the fluorescence intensity that is produced in the presence of the linearly polarized light beam. Then a second 15 s illumination period follows with a light beam perpenticularly polarized compared to the one used during the first illumination period. This operation is repeated five times at fixed time intervals. From the first illumination experiment, K_{dx} is computed and K_{dyz} is computed from the results of the second illumination period. The orientation parameter (K_{dx}/K_{dyz}) is then calculated. The film is afterwards compressed to a new state and a new bleaching experiment is run.

3. Results and discussion

The surface pressure isotherm of a DPPC monolayer incorporating 12-9-AS as a fluorescent probe is presented in Fig. 1. The isotherm shown is obtained by a continuous compression of the surface film. The isotherm is identical to an isotherm recorded for the pure DPPC and shows a phase behaviour similar to that reported by various authors [18–21]. The various two-dimensional states in Fig. 1 have been assigned following the terminology of Adam [22] and based on an ideal surface behaviour initially presented by Joly [8]. In such an assignment, the limits between the various states are more or less established arbitrarily. For the moment, it is enough to note that the surface pressure isotherm of Fig. 1 shows the well-defined kink point at the end of the LE state (A_{LE}, π_t), a non-horizontal linear portion for the LE/LC transition region [23], a point at the end of the linear region that is not generally well defined and a strong curvature for the LC state (A_{LC}, π_{LC}) [14], and another kink (A_{SC}, π_{SC}) that characterizes the LC/SC transition (for details see Albrecht et al. [19]).

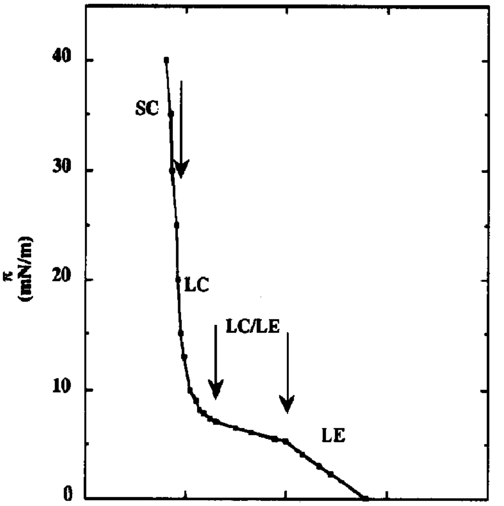

Fig. 1. The surface pressure isotherm of DPPC + 2% (mol/mol) 12-9-AS at the air/NaCl 0.1 M solution interface. $T = 21 \pm 1$°C. LE: liquid expanded state; LC: liquid condensed state; and SC: solid condensed state.

In fact, Dervichian [9] and Joly [8] were the first to show that the thermodynamic classification of Ehrenfest on phase transformations could be extended to a monolayer. They showed that the successive derivations of the surface chemical potential (μ^s) with respect to the surface pressure determined the thermodynamic order of the phase transformation. Inasmuch as the gas to liquid expanded (G/LE) or gas to liquid condensed (G/LC) transitions are concerned, the situation is quite clear since these transitions are characterized by a well defined kink and a horizontal portion in the surface pressure isotherm during the transition [9,24–26]. This behaviour during the transition arises from the fact that two different immiscible phases, corresponding to two different molecular states, coexist at equilibrium within the film.

However, inasmuch as DPPC is concerned, the situation is not that clear-cut. As we have shown in Fig. 1, the so-called LE/LC transition region does not seem to follow the ideal behavior observed for the G/LE or G/LC transition. This implies that the LE/LC transition does not seem to reflect a simple equilibrium between two immiscible phases, one corresponding to the LE state and the other to the LC state. On the other hand, Pallas and Pethica [5] and, more recently, Hifeda and Rayfield [6] have recorded a horizontal portion of the isotherm in the LE/LC region.

But these authors have used "highly purified" samples of DPPC that seem in fact to be dehydrated lipid samples. Thus, one may make the hypothesis that the horizontality of their isotherms in this region reflects this characteristic of their samples, as it is well known that rehydration of DPPC is a rather slow phenomenon [27]. It has also been shown from fluorescent micrography that the LE/LC region of the isotherm is biphasic [1–3], which thus gives support to the interpretation that a first order transition is indeed taking place in this region. However, when one carefully looks at the fluorescent micrography results published, one observes that the LC region itself seems to be biphasic, a situation that is rather surprising according to the classical phase behaviour described above.

The ratio of the two dimerization constants of the probe (12-9-AS) embedded at 2% (mol/mol) in the DPPC monolayer, as obtained from fluorescence experiments using crossed polarized excitation light beams, are shown in Fig. 2 as a function of the molecular area of the film. The orientation parameter ($K_{d_x}/K_{d_{yz}}$) varies in a complex manner as a function of the compression state of the monolayer. Indeed, $K_{d_x}/K_{d_{yz}}$ decreases during the LE/LC transition and reaches a minimum at the beginning of the pure LC phase (LC state), i.e. at 0.58 nm^2 molecule^{-1}. With further compression of the film, the value of the orientation parameter suddenly changes and increases to reach the high values previously observed in the LE state. Within the LC state, $K_{d_x}/K_{d_{yz}}$ decreases again and reaches a second local minimum at the end of the LC state, i.e. at 0.48 nm^2 molecule^{-1}. Finally, the value of the orientation parameter increases suddenly as soon as the monolayer enters the SC state. Figure 2 therefore shows that the orientation of the probe progressively changes inside the LC state and along the LE/LC region while the most drastic orientational transitions are observed at molecular areas characteristic of a phase transformation within the lipid film, i.e. at 0.48 nm^2 molecule^{-1} for the LC/SC transition, and at 0.58 nm^2 molecule^{-1} at the end of the LE/LC region [14]. These complex changes of the orientation parameter have been systematically reproduced during the various experiments. Thus, one therefore finds three orientational regions upon compression of the monolayer, corresponding to various orientational configurations of the probe microdomains within the monomolecular film [13].

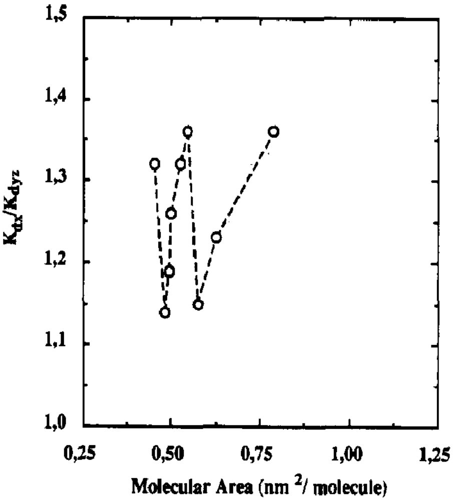

Fig. 2. Ratio of the dimerization constant as a function of the molecular area of DPPC+2% (mol/mol) 12-9-AS. $T = 21 \pm 1$°C. Subphase: NaCl 0.1 *M*.

A sudden change in the orientation parameter thus takes place at the LC/SC transition (i.e. at 0.48 nm^2 molecule^{-1}). Since the probe used is sensitive to the orientation of the aliphatic chains, it follows that the transition observed at 0.48 nm^2 molecule^{-1} is associated to an orientational transition and thus, is of second order in nature or higher [8]. This is indeed in agreement with Albrecht et al. [19] who have proposed this transition to be second order. Therefore at 0.48 nm^2 molecule^{-1}, a second order phase transition is ending, as it is well known that second order phase transformations end at the point where they are recorded [8]. But the important question that remains is the following: where does the molecular species characterized by an area of 0.48 nm^2 molecule^{-1} appear? At 0.58 nm^2 molecule^{-1} where a transition is clearly observed or even at higher molecular areas, i.e. in the region from 0.74 to 0.58 nm^2 molecule^{-1}?

The other sudden change of the orientation parameter, observed at 0.58 nm^2 molecule^{-1} takes place at the beginning of the LC state (i.e.

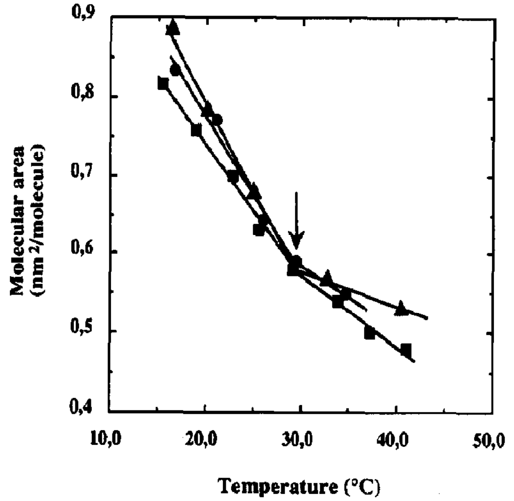

Fig. 3. The molecular area at the onset of the LE/LC transition as a function of temperature: (■) ref. [21], (●) ref. [18], and (▲) ref. [20].

at the end of the LC/LE region). At this point, for the reasons presented in the preceding paragraph, it is likely that one is also dealing with a second, or higher order phase transition. In order to validate the existence of the LC state initiated at 0.58 nm^2 molecule^{-1}, we have explored, from the data available in the literature, the variation of the molecular area with temperature. This is indeed a well-known technique to identify phase transition phenomena in monolayers [28]. Thus, the molecular area at the beginning of the main monolayer transition, A_t, has been plotted as a function of temperature. The results, taken from refs. [18] and [20,21] are presented in Fig. 3. They show indeed a clear discontinuity at 29°C, corresponding to a phase change occurring at about 0.58 nm^2 molecule^{-1}, i.e. at the entry of the lipid in the LC state. On the other hand, Fig. 2 showed that there was no change of the orientation parameter at the beginning of the LE/LC region, around 0.74 nm^2 molecule^{-1}, and that the parameter showed a continuous decrease until the area was 0.58 nm^2 molecule^{-1}. This raises the question on the phases that are in presence between 0.74 and 0.58 nm^2 molecule^{-1}.

In order to get informations on this matter as well as on the organization of the lipid molecules within these various regions, we turned to a study done by Heckl et al. [29]. These authors have determined, from an analysis of their fluorescence microscopy measurements, the crystallization degree (ϕ) of a DPPC film as a function of the molecular area. The crystallization degree (ϕ) is determined as the ratio of the dark surface of the micrography (i.e. the phase without probe molecules, corresponding to condensed phases of the film) over the total surface of the micrography, S, i.e. $\phi = 1 - (1 - S)\, A/A_{fl}$, where A is the mean molecular area and A_{fl} is the molecular area of the fluid state (liquid) at pressure π_t at the beginning of the so-called LE/LC transition. Figure 6a of ref. [29] shows that ϕ varies linearly as a function of the molecular area, A, for surface pressures larger than π_t. This linear region can be interpreted as a consequence of the coexistence of two phases, one with a characteristic molecular area of 0.74 nm^2 molecule^{-1}, corresponding to the molecular area at the beginning of the phase transition (A_{fl}), the other one, 0.48 nm^2 molecule^{-1} (A_{SC}), corresponding to the extrapolation of the first linear part shown in Fig. 6a of ref. [29] to 100% crystallization of the film. Thus, the fluid phase LE present in the non-horizontal transition in the surface pressure isotherm is characterized by a molecular area of 0.74 nm^2 molecule^{-1}, corresponding to a single molecular state, while the condensed phase with which it coexists thermodynamically is characterized by a molecular area of 0.48 nm^2 molecule^{-1}, characteristic of a molecule in the solid-condensed phase, SC. During a real first order transition, some of the molecules that occupy an area A_{LE} pass directly, upon compression, to an area A_{SC}.

However, when the film has reached a crystallization degree of about 75%, the slope of the linear relationship, ϕ vs. A, changes (Fig. 6a of ref. [29]). This occurs at a molecular area of about 0.58 nm^2 molecule^{-1}, i.e. at the beginning of the kink in the ϕ vs. A curve. In this case, the molecular area at which the crystallization is complete (100%) corresponds to a molecular area of about 0.42 nm^2 molecule^{-1}. Thus the newly formed condensed phase in this region of the surface pressure isotherm is characterized by an area of 0.42 nm^2 molecule^{-1}, corresponding to the molecular area of the collapse state of the film [28] while the area at the beginning of this new transition, 0.58 nm^2 $molecule^{-1}$, corresponds to the area at the beginning of the LC state. The molecular area A_{SC} (0.48 nm^2 $molecule^{-1}$) referred to here is actually higher than the area found at the collapse pressure as obtained on the surface pressure isotherm ($A_{collapse} = 0.42$ nm^2 $molecule^{-1}$, ref. [28]). This indicates a difference in the density of the condensed phase for the pressure range where it is found to exist [11]. Therefore, the change of slope in the ϕ vs. A curve observed at 35 mN m^{-1} (corresponding to an area of 0.58 nm^2 $molecule^{-1}$) supports the idea of a structural change of the LC state, as already discussed in our previous paper [14].

It has been observed above from the data of ref. 29 that the crystallization degree as a function of the molecular area suggested that a jump of the molecules occurred from a molecular state of area A_{LE} (A_{fl}) characteristic of the LE phase, found at 0.74 nm^2 $molecule^{-1}$, to a molecular state of area A_{SC} characteristic of the SC phase, found at 0.48 nm^2 $molecule^{-1}$, together with the formation of an intermediate molecular state of area A_{LC} (LC phase), as revealed by the kink in the ϕ vs. A curve at 0.58 nm^2 $molecule^{-1}$. This arrangement of the phases is illustrated in Fig. 4. Thus, we propose that the region of the phase transition observed on the surface pressure isotherm of DPPC between 0.75 and 0.48 nm^2 $molecule^{-1}$ is a consequence of an overall transformation from the LE state to the SC state. In this model, three types of lipid domains or phases coexist, i.e the solid condensed phase (SC), the liquid expanded phase (LE) and the LC phase, this latter phase being thus considered here as a domain of interfacial lipid molecules which form a transition region between the solid and the fluid phases.

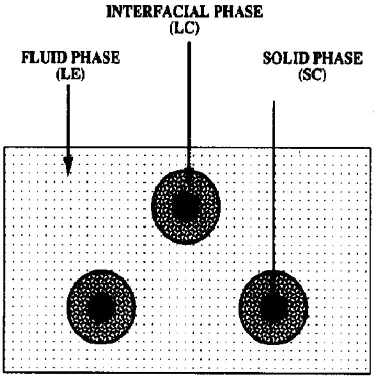

Fig. 4. Schematic view of the lateral organization of the interfacial lipids (LC) that form the intermediate phase between the fluid phase (LE) and the solid phase (SC).

Admittedly, additional informations should be given to bring support to this schematic representation of the liquid to solid phase transformation. For instance, the model proposed is compatible with the microscopic solid domains (of the order of about 10 μm in slow compression) observed in fluorescence microscopy in the course of the main monolayer transformation [30]. These solid domains can be attributed to the exclusion of the probe from both the highly condensed domains of area A_{SC} and the region of interfacial lipid of area A_{LC}. Thus, small domains of the solid condensed phase of the lipid could be present, and as a consequence, could contribute to some extent to the non-horizontality of the main phase transition [4,19].

The existence of an intermediate zone between the solid and the fluid phases in the monomolecular film along the liquid-to-solid transformation is also supported by the laws of kinetics. In fact, Cohen and Turnbull [31] have shown that a high fraction of the molecules (5–10%) implied in the three-dimensional liquid-to-solid transformation are always in an "activated state" (i.e. an intermediate state of high activation energy). By comparison, thus, the LC phase, considered as an interfacial phase, would possess a higher free energy than the LE or the SC phases as a result of its position at the interface between the ordered domains of the SC phase and the fluid regions of the LE phase. In addition, the laws of kinetics also predict that the transition from the liquid to the solid state is accompanied by an increase of the potential energy of the system arising from the use of the kinetic energy of its constitutive molecules in such a way that the transition state (i.e. the activated state) is obtained [32]. It is likely that the LC phase may represent such a high potential energy state. We have indeed shown elsewhere

[14] that the lateral diffusion coefficient of the probe decreases drastically upon entry in the LC state. This decrease may then reflect the fact that the system has used the kinetic energy of the molecules to reach the high potential energy LC phase and thus to accomplish the transition to the solid state.

Inasmuch as bilipidic organizations are concerned, it has also been proposed that interfacial lipids are involved. Structural models of the phase transition that take them into account have been presented in the literature [33,34]. Thus, if one agrees that the phase transition in monolayers and in bilipidic structures bears some similarities, the LE/SC phase transformation would first be characterized by the formation, within the fluid liquid-expanded phase, LE, of small islands of solid lipids (SC phase) of molecular area $A = 0.48$ nm^2 $molecule^{-1}$. While the transition is in progress, the growth of the lipid domains and their aggregation take the form of a cooperative change from a fluid lipid state to a solid one. However, one has to bear in mind that as soon as the SC phase is formed, it becomes surrounded by a peripheral region of lipids characterized by a molecular area of 0.58 nm^2 $molecule^{-1}$. We have discussed in a previous work [14] that at this molecular area, the polar heads of the lipids bear about 10 tightly bound water molecules and that the rehydration of the polar heads being particularly slow, these lipids are involved in the hysteresis phenomenon observed upon recompression of the DPPC monolayer [35]. Thus, in the present interpretation, the monolayer film at 0.58 nm^2 $molecule^{-1}$ does not present the character of a unique phase but rather represents an average area resulting from the coexistence of the pure SC phase, together with some residual LE phase (fluorescence microscopy does indeed reveal the presence of a liquid expanded phase at 0.58 nm^2 $molecule^{-1}$) and the boundary LC phase.

The situation of the monolayer film at 0.58 nm^2 $molecule^{-1}$, as just described, may appear complex, but a number of phenomena have been shown to occur in the literature at this molecular area that imply that things are indeed not simple there. For example, Villalonga [36] has shown that the lateral compressibility between 0.55 and 0.60 nm^2 $molecule^{-1}$ is abnormally high. Also, the incorporation in the monolayer film of various components dissolved in the subphase, is maximal at this molecular area. For instance, Thuren et al. [37] have shown that the activity of phospholipase A_2 is maximal at this area while Hendrickson et al., [38] have observed that the highest penetration of glucagon and melittin in the monolayer is found at this area, a phenomenon that is also observed with the incorporation of chlorpromazine [39] and β-naphtol [40]. Thus, similarly to what is found with multibilipidic systems [27], it seems that the simultaneous presence of the three phases at 0.58 nm^2 $molecule^{-1}$ results in some kind of defects in the molecular packing which would be responsible for the enhanced incorporation of components dissolved in the subphase. These defects may arise, as suggested elsewhere [41], from critical fluctuations of the surface density in the interfacial region between the various phases, thus enabling the components from the subphase to penetrate more favorably the monolayer.

Upon further compression of the monolayer, the surface pressure isotherm presents a roundish transition until the pure SC state, at 0.48 nm^2 $molecule^{-1}$, is reached (Fig. 1). Thus, from 0.58 to 0.48 nm^2 $molecule^{-1}$, the fluid phase completely disappears. The fact that this portion of the isotherm is curved implies that there is a progressive change from the LC state to the SC state. This progressive change from one state to the other has been put in evidence by various experimental observations. For example, it has been shown [36,42] that the surface viscosity of the DPPC film increases linearly throughout the main transition region (i.e. from 0.75 to 0.58 nm^2 $molecule^{-1}$) as well as in the SC region (from 0.48 to 0.42 nm^2 $molecule^{-1}$) but increases in a non-linear fashion in the LC region. Furthermore, FTIR spectroscopic experiments on DPPC monolayers indicated a progressive variation of the frequency of the antisymmetric CH_2 stretching band of the hydrocarbon chains of the lipid molecules within the LC region (i.e., between 0.48 and 0.58 nm^2 $molecule^{-1}$). This was interpreted as a change in conformation of the aliphatic chains from a more disordered state in the LE phase, to a more ordered state in the SC phase [43], i.e. a transition from chains with mainly gauche conformers in the LC state to chains mainly having an all-*trans* conformation in the SC state. This is also supported by ellipsometric observations that show a progressive increase of the thickness of the film in the LE–LC region and in the LC region as compared to a constant thickness in the SC state [44]. Finally, as shown in our previous work [14], the lipid molecules within the LC state present a significant structural reorganization of both their polar heads and aliphatic chains upon compression. More specifically, it was discussed that the polar head group undergoes progressive dehydration, from 20 to 5% as the monolayer is compressed from 0.58 to 0.48 nm^2 $molecule^{-1}$. These various changes thus drive continuously a DPPC molecule from the LC state to the SC state, such that the transition involved is more likely second order.

In the light of what has just been discussed, further insights into what actually happens at 0.58 nm^2 $molecule^{-1}$ may now be given. The actual dehydration of the polar head groups of the DPPC molecules in the LC state is responsible for the reduction of the lateral proton conduction, as we have already shown to be produced in this state [45]. There is therefore a disruption of the H-bond array in the polar head group organization and, as we have also shown, a high steric repulsion then prevails between the polar head groups at $A < A_{LC}$. On the other hand, at $A < A_{LC}$, the van der Waals attractive interactions of the all-trans organization of the chains contribute favorably to the organization of the film. In contrast, at $A > A_{LC}$, the polar head group are favorably hydrated and less repulsions prevail among them, but the aliphatic chains are in a conformation such that the attractive force is reduced. The polar head groups and the aliphatic chains are then in a mismatched organization with the consequence that an important fluctuation of the surface density of the film around A_{LC} occurs. Therefore, an enhanced incorporation of sub-phase material into the monolayers will be observed at this area, as it is the case experimentally.

The penultimate state of the monomolecular film is reached when the molecular area reaches 0.48 nm^2 $molecule^{-1}$ (A_{SC}). This area is characterized by a single state, the solid condensed state, and thus all the DPPC molecules occupy the same area. Finally, upon further compression, the collapse state is reached at $A_C = 0.42$ nm^2 $molecule^{-1}$ (not shown in Fig. 1). The transition to the collapse pressure is discontinuous in the sense that a molecule jumps directly from an area A_{SC} to an area A_C. The fact that this transition is not horizontal suggests a complete miscibility of the two molecular states.

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