Eur. Phys. J. B **6**, 1–4 (1998)

THE EUROPEAN PHYSICAL JOURNAL B

Rapid Note

Translational order in liquid-expanded lipid monolayers functionalized with nucleosides

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Received: 3 August 1998 / Revised: 16 September 1998 / Accepted: 21 September 1998

Abstract. The monolayer behavior of a lipid containing two unsaturated alkyl chains and a nucleoside derivative as polar headgroup has been investigated by the Langmuir technique. From the surface pressure *vs.* molecular area isotherm, the monolayer appears as a pure liquid-expanded phase and should be then considered as a two-dimensional liquid. However, grazing incidence X-ray diffraction experiments evidence a translational order that does not exist when the lipid headgroup is a choline moiety. Since unsaturated chains are expected to induce a fluid state of the monolayer at the temperature considered, this order is likely to originate from the natural tendency nucleosides have to establish among themselves π-stacking interactions between the bases. The collected X-ray data are consistent with the geometrical requirements for bases stacking.

PACS. 87.15.Kg Molecular interactions – 87.15.By Structure, bonding, conformation, configuration, and isomerism of biomolecules

The phase properties of lipid monolayers have been extensively investigated and many of their features are reasonably well understood [1–3]. Fluid monolayers, identified when their compression isotherm shows the liquid-expanded phase characteristics, are generally considered as two-dimensional liquids [1,4]. In this phase, the pressure *vs.* molecular density curve shows a small slope indicative of a high monolayer compressibility, and the molecular area is 1.5 to 3 times the cross sectional area of the compound when in the crystalline phase [1]. The structure of this phase has not received as much attention as the one of the liquid-condensed phase, mostly for the obvious reason that it presumably does not have any translational order. However, given the practical importance of the fluid character of monolayers in such processes as protein two-dimensional crystallization [5] or biological events involving cell membranes [6], the structural properties of liquid-expanded monolayers ought to be better understood. In protein two-dimensional crystallization on ligand-functionalized lipids for example, a lipid monolayer is spread at the air-water interface in the liquid-expanded state to give enough mobility to the lipids for allowing subsequent organization of the protein. In a second step, the protein is introduced into the aqueous phase and gets adsorbed onto the lipid film through ligand complexation. Finally, the protein located at the air-water interface laterally diffuses with the lipid attached to it and eventually organizes into two-dimensional crystals. It is clear in this case that the disordered and poorly cohesive lipid film confines the protein in a plane without restricting its lateral motion, while the final two-dimensional order is a consequence of the macromolecules interaction. We show in this letter, that although the lipid monolayer phases are generally imposed by the organization of the aliphatic chains, the headgroup of the lipids can truly play some role in particular cases.

This study on the structure of a liquid-expanded lipid monolayer was further motivated by surface forces measurements performed between monolayers of lipids with nucleoside headgroups known to associate in stacks or in pairs [7,8]. Such measurements of molecular recognition forces require monolayer fluidity to ensure the full accessibility of the molecules of one surface to those of the other one. This has been provided through the design of nucleoside functionalized lipids with unsaturated alkyl chains, which usually do not crystallize in monolayers, and by incorporating a flexible spacer between the lipid chains

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and the nucleoside headgroup [9]. The interactions between monolayers of these lipids were measured with a surface force apparatus (SFA) and an attraction of a much longer range than that of the van der Waals forces was observed. Forces between surfaces have received a great attention in the past decades, especially since the surface force apparatus was developed. No long-range attraction has ever been observed between surfaces that are electrostatically neutral except for hydrophobic surfaces made of crystalline lipid layers [10]. Tsao *et al.* have proposed an explanation for these forces that involves the presence of domains of ordered dipolar molecules which may correlate their orientation from one surface to another and generate attractions with a distance range of the same order as the domains size. However, this explanation does not exclusively require the surfaces to be hydrophobic, and the nucleoside hydrophilic surfaces might as well be relevant to the same scheme of interaction. That is supported for example by the fact that DNA bases have a tendency to set their planes parallel to each other in an arrangement called "π-stacking" which takes a substantial part in the structure stabilization of the DNA molecule [7]. In water, the interplane distance is 0.34 nm but may slightly vary in other environments [11], and organic solvents generally prevent stacking. This phenomenon has been extensively studied [7,11] and it has been observed that stacked bases often show little overlap of the aromatic rings in crystals as well as in DNA. Stacking has also been shown to occur in layers of free nucleotides [12] adsorbed at the mica-water interface [13]. The possibility of stacking of nucleoside lipids within monolayers however remains open since it might be prevented by the disorder introduced by the two unsaturated oleoyl chains of the lipid. In order to investigate that possibility, we have conducted some experiments with a lipid incorporating two unsaturated chains (oleyl) and a thymidine polar headgroup (DOT, for structure, see Fig. 1) [14]. The compression isotherm of DOT is very close to the one of DOPC and shows pure liquid-expanded phase characteristics with a fairly high collapse pressure around 39 mN/m (Fig. 1). No first order transition plateau or kink that might indicate some transition to an ordered state has been observed. Brewster angle microscopy (lateral resolution 1 μm) [15] did not show any evidence of two-dimensional domains at any pressure, indicating therefore that the fluctuations of the chains prevent the stacking of the hydrophilic head groups. Nevertheless, numerical simulations [16] of monolayers of these particular lipids gave some indications that stacking might occur in the monolayers and additional experimental data was definitely required. X-Ray diffraction experiments have been previously described to detect and analyze ordered phases in lipid monolayers. These experiments have so far been carried out in the grazing incidence geometry to reveal translational ordering between the hydrophobic chains of lipids [2]. Some difficulties arise from the observation of the headgroups in a lipid monolayer because the integrated electron density of the headgroup is smaller than that of the chains, and therefore the expected signal is weaker. Absence of the diffraction signal from

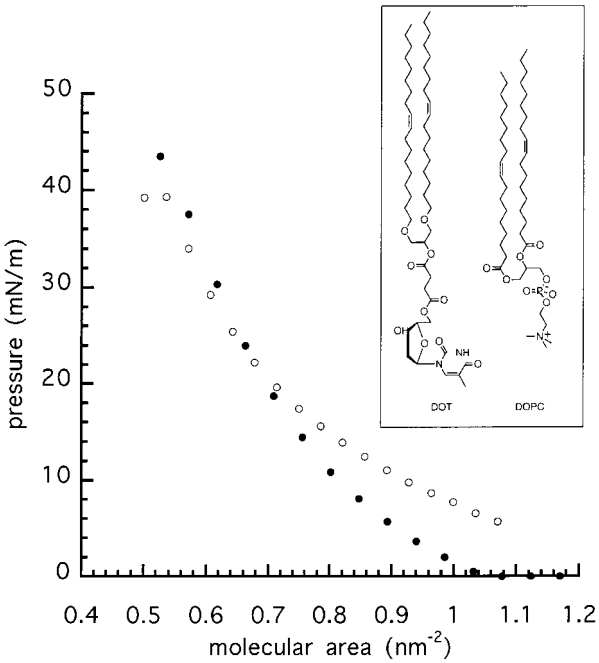
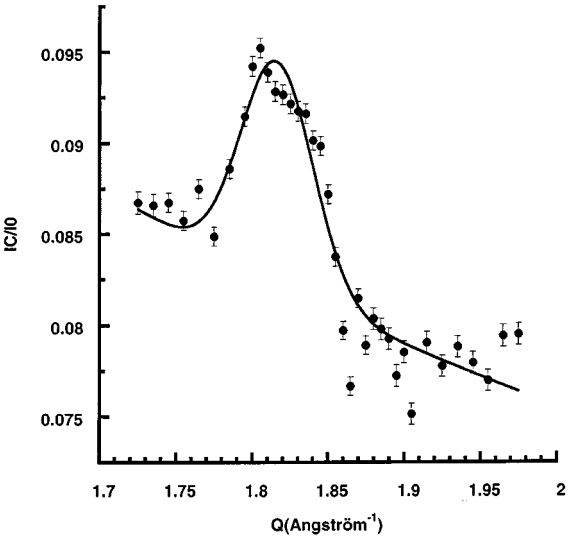


Fig. 1. Isothermal surface pressure *vs.* area curves for DOPC (closed circles) and DOT (open circles) monolayer at the air-water interface; insert: DOPC and DOT molecular structures.

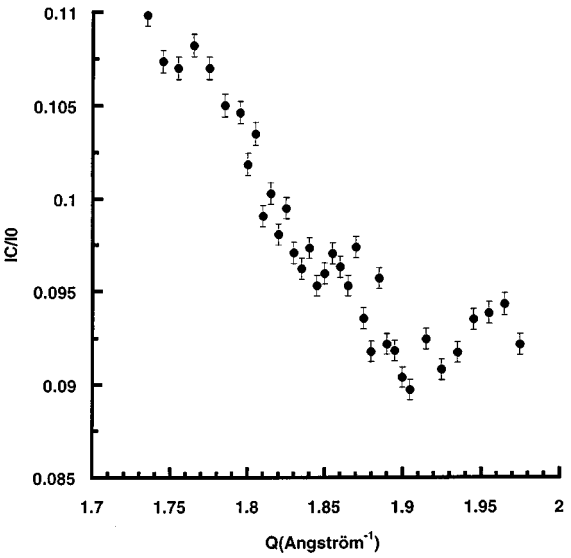
headgroups is a common experimental fact. These unfavorable conditions imposed to use the technique near its limits by minimizing the noise and maximizing the counting time while preserving the integrity of the monolayer with respect to a possible degradation induced by X-rays. Control experiments were carried out on DOPC monolayers.

The experiments were performed on the D41B beam line at the LURE synchrotron source (Orsay, France). The experimental setup has been described elsewhere [17]. We used a 1.488 Å wavelength with a 5" Sollers collimator and a xenon filled PSD detector. The lipid monolayers were deposited in a home made Langmuir trough (area: 700 cm^2, compression rate: 3.8 Å^2/molecule/minute). The surface pressure was measured by the Wilhelmy method with an "R&K" surface tensiometer. After deposition, 15 minutes were allowed for the solvent to evaporate. Then the layer was compressed to the desired pressure (36.5 mN/m). The surface pressure and water level in the trough were maintained constant during the diffraction measurements. In order to reduce the noise signal, experiments were conducted in a sealed box and helium was circulated for 3 hours to replace air before the monolayer was irradiated by X-rays and the diffraction measurements started. The counting time was 10 minutes per point and the layer was changed after 6 hours of measurements. As the signal was collected after a single scan, we added several diffraction spectra to improve the signal to noise ratio.

Diffraction peaks were looked for at many wave vector regions. A peak was found between 1.7 and 2 Å^{-1} and the measurements of the diffraction spectrum of DOT were consequently focused in this q region. This spectrum is



(a)



(b)

Fig. 2. X-ray diffraction spectra for (a) DOT and (b) DOPC monolayers. The line is a fit of the peak (Gaussian fit) and of the baseline (Lorentzian fit). IC/I0 is the relative diffraction intensity. Q is the wave vector in $\mathrm{\AA}^{-1}$.

presented in Figure 2. It shows a peak at the wave vector $q = 1.83 \pm 0.03$ $\mathrm{\AA}^{-1}$ with a full width $\Delta q = 0.05$ $\mathrm{\AA}^{-1}$. The weakness of the signal did not allow to make a reliable q_z analysis. As usual, the peak is superimposed on a sloping baseline attributed to the diffusion by the air-water interface. We did not observe any other diffraction peak on such monolayers. In contrast to these results, the DOPC scan does not present any diffraction peak in this q region. Some evidence for DOPC degradation could be observed on spectra after extended X-ray irradiation time (8 hours) while DOT did not seem to be affected, indicating a higher stability of DOT relative to DOPC. That is consistent with the high chemical stability exhibited by DOT and molecules of related structure [18]. When the DOT monolayer was compressed to the collapse pressure and then set back to 36.5 mN/m, the diffraction peak was much weaker but still observable, as if some possible multilayer formation were disrupting the film structure. The peak characteristics obtained for DOT correspond to a repeat distance d_0 : $d_0 = 2\pi/q = 0.34$ nm and to a correlation length ξ : $\xi = 2\pi/\Delta q = 12$ nm which corresponds to about 35 molecules. This value of the correlation length explains why no domain was observed by Brewster angle microscopy whose resolution is in the micrometer range. The repeat distance measured is indeed equal to the 0.34 nm internucleoside stacking distance in DNA. Since the headgroups are made up of such DNA bases [12], this confirms that the measured order in the fluid monolayer should be induced by the stacking interaction between bases which tends to set the bases planes parallel. Though our results do not constitute a direct proof that the bases are stacked in the monolayer, they provide anything but a very strong evidence for it.

A crude unidimensional Boltzmann model of the base stacking in long chains may relate the correlation length to the stacking energy. Starting from a chain end, and assuming a probability p that the next molecule is stacked, the probability for a chain to comprise n molecules is: $P(n) = (1-p)p^{n-1}$. The average chain length $\langle n \rangle$ is $(1-p)^{-1}$. The probability p is related to the stacking energy e (in k_BT units) by Boltzmann law $p = \exp(e)/(1+\exp(e))$ and therefore $e = \ln(\langle n \rangle - 1)$. Here, with $\langle n \rangle = 35$, we deduce $e = 3.5$ k_BT which is close to the values found in the literature and related to the enthalpy of stacking for two thymidine molecules (4.3 k_BT, [7]). It now seems understandable that the fluctuations of the chains cannot prevent thymidine stacking.

This diffraction experiment does not give any detail on the way thymidine groups are arranged in the plane of the monolayer. It has been observed [8] that stacked bases often show little overlap of their aromatic rings in crystals and in DNA, but Boland *et al.* have observed a high overlap of the aromatic rings in a two-dimensional arrangement at the mica-water interface [13]. The d_0 value depends on the self-assembly properties of the nucleosides. Therefore, while ξ should depend on the surface pressure, d_0 should not. To ascertain that point, some investigation of the evolution of the repeat distance d_0 and its correlation length with respect to the surface pressure would be of great interest.

To our knowledge, no observation of some translational ordering within a liquid-expanded phase has ever been reported in the literature. Monolayers of lipids bearing large anisotropic headgroups may display the coexistence of ordered headgroups with disordered chains. However this was so far always evidenced in the compression isotherm by a plateau transition. For instance, Flament *et al.* [19] used lipid monolayers in which large headgroups occupied an area of 0.58 nm^2 at 10 mN/m, in the liquid condensed phase, area at which the two chains of the lipid are likely to be disordered.

This report of a translational ordering within liquid expanded lipid monolayers changes the vision of the two-dimensional liquid character generally attributed to these monomolecular films. This organization results from some lipid headgroup cohesion (nucleoside stacking interaction) which gets over the chains fluctuations, although they limit the value of the correlation length and prevent the isotherm from giving indications of this order. The long distance attraction observed between such layers [8,16] may be a consequence of this hidden structure. We conlude that monolayers may not be as liquid like as one might deduce from compression isotherms, and that any attraction between headgroups may significantly affect their mobility even in liquid expanded monolayers.

The authors wish to thank S. Henon and J. Meunier for useful discussions and for the observations with Brewster angle microscope.

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