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Interdigitation in spin-coated lipid layers in air

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ABSTRACT

In this study, we show that dry saturated phospholipid layers prepared by the spin-coating technique could present thinner regions associated to interdigitated phases under some conditions. The morphological characteristics of lipid layers of saturated phosphocholines, such as dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC), have been measured by Atomic Force Microscopy and revealed that the presence of interdigitated regions is not induced by the same parameters that induce them in hydrated samples. To achieve these results the effect of the lipid hidrocabonated chain length, the presence of alcohol in the coating solution, the spinning velocity and the presence of cholesterol were tested. We showed that DPPC and DSPC bilayers, on the one side, can show structures with similar height than interdigitated regions observed in hydrated samples, while, on the other side, DLPC and DMPC tend to show no evidence of interdigitation. Results indicate that the presence of interdigitated areas is due to the presence of lateral tensions and, hence, that they can be eliminated by releasing these tensions by, for instance, the addition of cholesterol. These results demonstrate that interdigitation in lipid layers is a rather general phenomena and can be observed in lipid bilayers in dry conditions.

1. Introduction

Model lipid membranes have been widely used to facilitate the comprehension of natural membranes properties by reducing complexity and simplifying their preparation [1–7]. They allow, under experimentally well controlled conditions, the reconstruction of membrane structures, the study of the interactions between specific membrane components and also the effect of other elements (e.g. ions, proteins, drugs, etc.).

Apart from these fundamentals studies, model lipid membranes have also been used as platforms for other applications, such as lipid-assisted assays and biosensors [8–11]. In these applications, the resistance and morphological stability in dried or low humidity environments is an important feature. But, at present, relatively little information is available on the nanostructure of dry lipid bilayers, as most of the studies performed so far have focused on the production and analysis of hydrated lipid bilayers.

In recent years, the use of the spin-coating technique has allowed obtaining high-quality and morphologically stable dry lipid layers in air [12–17] on different supports [18–20]. This advancement opened the possibility to address its nanoscale and nanomechanical properties in a reliable and relatively simple way. Dry and stable single component lipid layers of DOPC and SM have been produced as well as binary and ternary mixtures containing cholesterol, including lipid raft models such as DOPC/ SM/ Chol [21,22]. The study of these systems showed the presence of liquid disordered, liquid ordered and gel phases in dry lipid samples, with morphological properties (e.g. layer thicknesses) in similarity to those observed on the same systems under hydrated conditions and prepared by different techniques such as, the vesicle fusion [23], Langmuir-Blodgett/Schäfer [24], microcontact printing [25] or lipid dip-pen nanolithography [26].

In the present study we investigate a subtler phenomena, i.e. the presence/absence of thinner regions associated to interdigitated phase ($L_{\beta}I$) in saturated phosphocholines in dry conditions. Interdigitated phases have been widely observed on hydrated lipid bilayers. This phase consists of a thinning of the bilayer height, which could be partial or of the total area [27,28], due to the interpenetration and disorder or tilting of the acyl chains and tilt of their angle [27,29–33]. Derived from that there is also an expansion of the bilayer area laterally [29,34,35]. This phase is produced in phosphatidylcholines in solution due the presence of alcohols [27,28,32,34,35], volatile anesthetics [36,37] or by the presence of an abnormal pressure, such as, the

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interaction with the substrate or the presence of lateral tensions [28,38,39]. Moreover, it is known that Cholesterol and other sterols play a role in the interdigitating process enhancing or reducing the effect of ethanol depending on the sterol content [38,40–42].

The existence of an interdigitated phase in dry lipid layers has not been reported to date. In this study, we precisely show that dry saturated phospholipid layers prepared by the spin-coating technique can present thinner regions associated to interdigitated phase in some conditions. Atomic Force Microscopy (AFM) offers the appropriate lateral and vertical resolution to characterize lipid bilayers in different media, air or liquid, and to distinguish heights corresponding to different lipid phases (gel, L_β; liquid disordered, L_α; liquid ordered, L_o; interdigitated phase, $L_\beta I$ or interdigitated phase due to lateral tension $L_\beta'I_T$) [38,43,44]. In fact the value of these characteristic heights, has been used extensively in literature to estimate the phase present in a sample and also the presence of interdigitated regions [28,35]. In this study, we investigated by AFM the morphology of lipid layers prepared by the spin-coating technique in dry air and the influence of different parameters in the presence of these thinner regions, such as, the phospholipid chain length, and consequently of different transition temperatures T_m, the presence of alcohols in the coating solution, the spinning velocity and the cholesterol concentration.

2. Experimental section

2.1. Materials

Lipid layers were prepared with 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) purchased from Sigma-Aldrich and used as received without further purification. Hexane, LC–MS grade (Sigma-Aldrich), Isopropanol (Sigma-Aldrich) and Methanol, HPLC grade (Sigma-Aldrich), were used as solvent in the experiments. Hi-grade freshly cleaved mica substrates (Ted Pella,Inc) were used as support.

2.2. Sample preparation and AFM imaging

Air-stable lipid layers have been obtained by the spin-coating technique following the methodology developed by Simonsen and Bagatolli [12] further optimized to produce ultrathin (single) bilayer samples [21]. In here, the concentration of lipid used in the coating solution is 1 mM. Briefly, a small volume of lipid stock solution was deposited on the high-grade freshly cleaved mica and spun with a spinner (WS-650MZ-23NPP/LITE, Laurell Technologies Corp.) for 1 min immediately after the deposition of the solution. The speed used was 3000 rpm unless is directly specified in the text. Next, the samples were placed under vacuum in a desiccator during 15–20 h to fully evaporate the solvents. Fresh lipid solutions were prepared on the day of each experiment to avoid solvent evaporation and change in the lipid concentration.

AFM imaging was performed at 25 °C in dry environment in a closed chamber with N_2 stream and relative humidity RH ~ 0%. Calibrated AFM probes (PPP-CONTR, Nanosensors, nominal spring constant 0.2 N/m, tip radius < 7 nm) and a commercial AFM (Nanotec Electronica S.L) were used. Images were processed by using the WSxM software [45]. Height analysis was performed using histogram analysis of the pixels over image areas of 1000 nm × 1000 nm (N = 3–7). The small size of the areas is selected to avoid systematic errors in height determination associated with image flattening. The obtained value is the mean of the values extracted at different areas and the error corresponds to the standard deviation (SD) of the means. By using the spin-coating technique it is possible to form incomplete layers that allow measuring directly the bilayer thickness. Bare mica was demonstrated to be not observable in samples prepared by this method at these lipid concentrations, since the bottom layer consists of a uniform lipid monolayer, as we showed earlier [21]. This fact does not affect the determination of the bilayer heights.

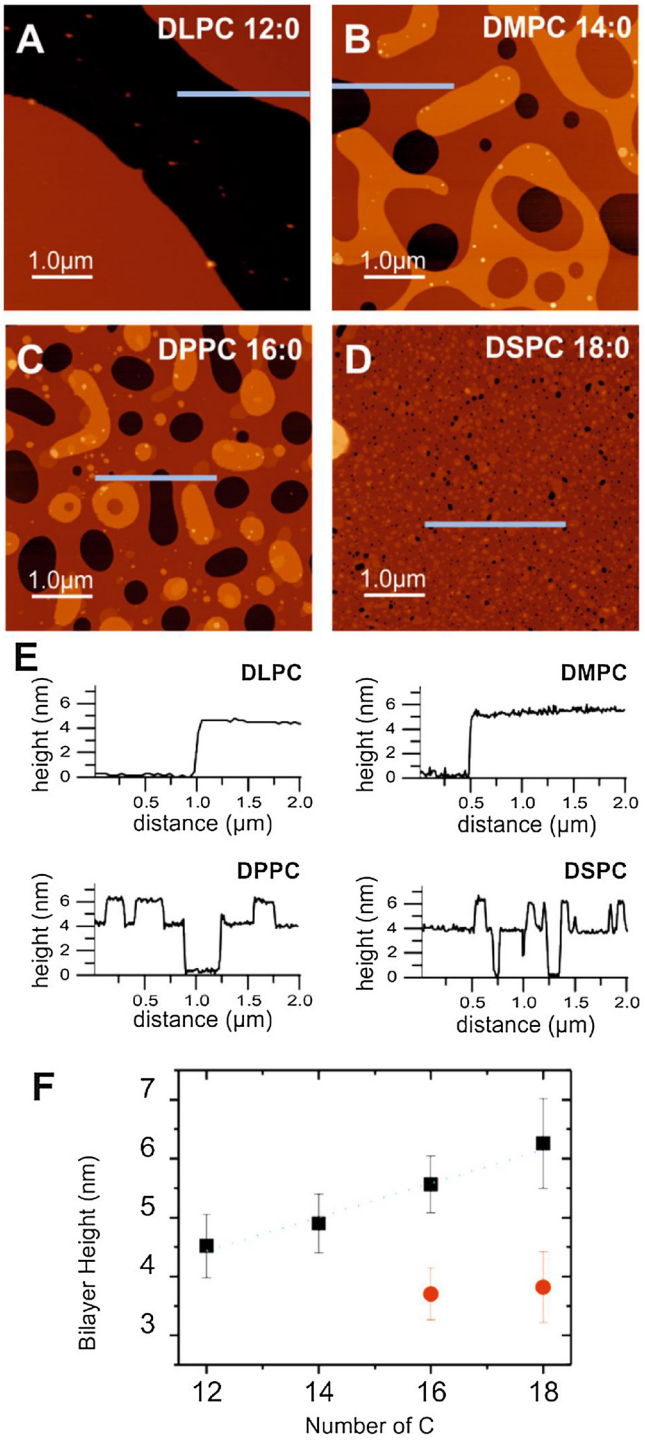

Fig. 1. A–D) AFM topography image of spin-coated samples of DLPC (A), DMPC (B), DPPC (C) and DSPC (D). Z-scale = 30 nm. Figure E) Height profile of images A–D. Figure F) Plot representation of the bilayer height extracted from the topography images vs number of C of the acyl chain of the lipids used: DLPC (12C), DMPC (14), DPPC (16) and DSPC (18). Black squares = higher regions, red dots = lower regions, dashed line = bilayer height tendency as a function of the number of C.

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