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Structural and nanomechanical effects of cholesterol in binary and ternary spin-coated single lipid bilayers in dry conditions

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ABSTRACT

We investigate the effects of Cholesterol (Chol) in the structural and nanomechanical properties of binary and ternary spin-coated single lipid bilayers made of Dioleoylphosphatidylcholine (DOPC) and Sphingomyelin (SM) in dry conditions. We show that for the DOPC/Chol bilayers, Chol induces an initial increase of the bilayer thickness, followed by decrease for concentrations above 30% Chol. The mechanical properties, instead, appear practically insensitive to the Chol content. For the SM/Chol bilayers we have observed both the thinning of the bilayer and the decrease of the force necessary to break it for Chol content above 40 mol%. In both binary mixtures phase separation is not observed. For ternary single bilayers of DOPC/SM/Chol, Chol induces phase segregation and the formation of domains resembling lipid rafts. The domains show a thickness and mechanical response clearly distinct from the surrounding phase and dependent on the relative Chol content. Based on the results obtained for the binary mixtures, DOPC- and SM-enriched domains can be identified. We highlight that many of the effects of Chol reported here for the dry multicomponent single lipid bilayers resemble closely those observed in hydrated bilayers, thus offering an additional insight into their properties.

1. Introduction

Model lipid membranes have been extensively used to mimic natural membranes minimizing simultaneously their complexity [1–3]. They allow for instance the reconstruction of structures such as domains, as well as, the study of the interactions between specific membrane components under conditions which facilitates their experimental access. Different model membranes developed so far include, among others, free-standing membranes [3] as well as, supported planar lipid bilayers (SLBs) [4–7].

In spite of the extensive literature on SLBs, still at present relatively little is known about their physico-chemical properties under dry conditions. The study of dry SLB has been hampered by the difficulty of preparing air stable SLBs. It is well known that simply drying hydrated SLB produces and irreversible damage in their structure and morphology. To overcome this limitation, some techniques have been explored to avoid the effect caused by the dehydration. Adding molecules such as lyoprotectants or proteins has been proposed to preserve the bilayer during the dehydration [8–10] but they show a tendency to precipitate during dehydration. So, the protectant layer could mask the membrane and difficult its characterization under certain techniques. Freeze-drying of lipid layers has shown to give good-quality layers that preserve phase-separated lipid domains [11–13], but this approach is not easily reproducible without expertise.

Only recently, air stable lipid bilayers have been obtained by using the spin-coating technique, a widely applied technique that is used to produce hydrated single lipid bilayers of high quality [14–19], but in which intermediate phase consists of dry stable lipid layers [16,20–22].

Knowing the physic-chemical properties of spin-coated dry SLBs in the limit of ultrathin layers corresponding to a single lipid bilayer can be of interest for several reasons. On one hand, the understanding and control of properties of this intermediate state prior to the formation of the fully hydrated bilayer can provide a route to control and understand the properties of the latter. On the other hand, since this intermediate state is stable under dry conditions, it is directly accessible to a number of techniques that cannot access the final hydrated state. Among other techniques, we cite nanoscale techniques such as Secondary Ion Mass Spectrometry (Nano-SIMS), which probe material composition at the nanoscale, and nanoscale electrical characterization techniques such as conductive Atomic Force Microscopy (C-AFM) and Scanning Tunneling Microscopy (STM), which measure electrical conductivity or Nanoscale Impedance Microscopy (NIM) and Electrostatic Force Microscopy (EFM), which probe dielectric properties at the

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nanoscale. All these techniques cannot be applied in a liquid environment and hence have not been applied to nanoscale investigations on hydrated supported lipid bilayers. The information they could provide on the dry state could be of interest to understand some of the properties of the hydrated state, but which are not directly accessible. This latter fact can be of interest in the discussion of the raft problem since the characteristics dimensions of rafts in natural systems are in the sub-100 nm range, and hence require the use of nanoscale characterization techniques. Only very recently, we have investigated the structural and mechanical properties of monocomponent spin-coated DOPC single bilayers in dry conditions [23], but no investigation has been reported so far for the case of multicomponent spin-coated single bilayers.

In this study, we precisely fill this gap and investigate by Atomic Force Microscopy and Force Spectroscopy the effects of Cholesterol in the nanoscale structural and mechanical properties of binary and ternary spin-coated single bilayers of Dioleoylphosphatidylcholine (DOPC) and Sphingomyelin (SM) in dry conditions. We will show that air stable spin-coated multicomponent single bilayers containing Chol can be formed and that Cholesterol has a remarkable effect in both the structural properties of the bilayers (e.g. thickness and phase separation) as well as in their mechanical response. Importantly, we show that many of the effects reported here resemble those reported on hydrated single lipid bilayers, thus shedding further insight on the phenomena that drive the lateral organization and mechanical properties of lipid bilayers.

2. Materials and methods

2.1. Materials

The lipid layers have been prepared with 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), Egg Sphingomyelin (SM) and Cholesterol (Chol) purchased from Sigma-Aldrich and used as received without further purification. A 98:2 (v/v) solution of Hexane, LC-MS grade (Sigma-Aldrich), and Methanol, HPLC grade (Sigma-Aldrich), was used as solvent in all the experiments. Lipid layers were formed on hi-grade freshly cleaved mica substrates (Ted Pella, Inc).

2.2. Sample preparation, AFM imaging and force spectroscopy measurements

Air-stable lipid layers have been obtained by the spin-coating technique following the methodology developed by Simonsen et al. [16] further adapted to produce dry ultrathin (single) bilayer samples as described in reference [35]. In here, the concentration of lipid used in the coating solution is 1 mM. Using this concentration the spin-coated sample of lipids is composed by a homogeneous monolayer background with a single inverted bilayer on top forming patches or rims, and occasionally some multilayer patch [23]. At higher concentrations the number of layers increases [14,16,23]. Both binary mixtures of Chol and DOPC or SM, as well as ternary mixtures of DOPC/SM/Chol, have been prepared in order to elucidate the effect of Chol. We have obtained binary mixtures combining DOPC or SM with Cholesterol from 10 mol% to 50 mol%. Ternary mixtures (DOPC/SM/Chol) were prepared using the ratios 1:1:1, 2:1:1 and 2:2:1. These samples were prepared using the same stock solutions to avoid discrepancies in their characteristics. Sample preparation by spin-coating were performed following the methods that we previously detailed [23].

AFM imaging was performed in dry environment maintained by a N_2(g) flow, relative humidity RH ~ 0%, with calibrated AFM probes (PPP-CONTR, Nanosensors, spring constant 0.2 N/m, tip radius < 7 nm) using a commercial AFM (Nanotec Electronica S.L).

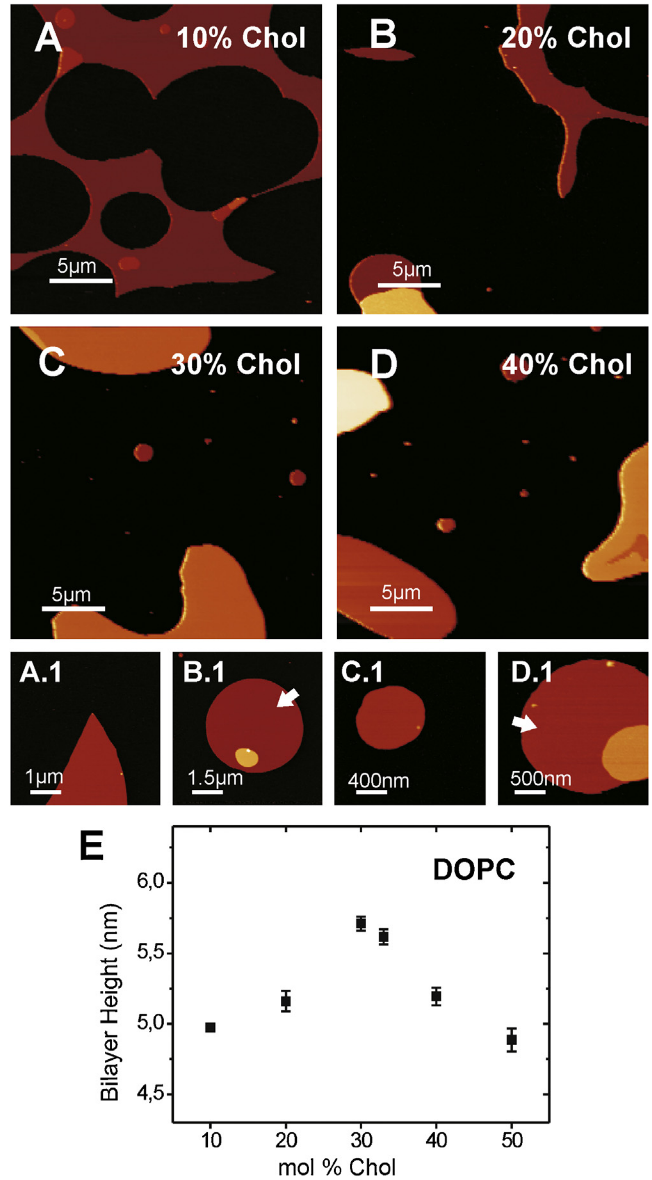

Fig. 1. Representative AFM topography images of spin-coated samples of DOPC/Chol in air with different concentrations of Chol. (A) 10 mol% of Chol, (B) 20 mol% of Chol, (C) 30 mol% of Chol and (D) 40 mol% of Chol (25 μm × 25 μm, *Z*-scale: 40 nm). (A.1–D.1) Zoomed topography images on specific regions of DOPC/Chol samples for different Chol concentrations showing the presence of single bilayers (marked with an arrow) (*Z*-scale: 20 nm). (E) Bilayer height as function of the Cholesterol concentration in the coating solution.

Height analysis was performed using histogram analysis of the pixels over image areas of 500 nm × 500 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. Additionally, force curves (Fz) were taken individually on selected locations of single bilayers of the sample to obtain the local mechanical properties (approach velocity 1.6 μm/s). They were systematically taken at different locations to avoid the effects of any plastic deformation of the sample. Fz curves were measured with two different probes to check the reproducibility of the data. Breakthrough forces, defined as the maximum force the layer is able to withstand before its rupture, have been determined from the force curves. This parameter is a direct measure of the mechanical stability of the