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Effect of externally applied electrostatic fields on the surface topography of ceramide-enriched domains in mixed monolayers with sphingomyelin

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Abstract

Lipid and protein molecules anisotropically oriented at a hydrocarbon–aqueous interface configure a dynamic array of self-organized molecular dipoles. Electrostatic fields applied to lipid monolayers have been shown to induce in-plane migration of domains or phase separation in a homogeneous system. In this work, we have investigated the effect of externally applied electrostatic fields on the distribution of the condensed ceramide-enriched domains in mixed monolayers with sphingomyelin. In these monolayers, the lipids segregate in different phases at all pressures. This allows analyzing by epifluorescence microscopy the effect of the electrostatic field at all lateral pressure because coexistence of lipid domains in condensed state are always present. Our observations indicate that a positive potential applied to an electrode placed over the monolayer promotes a repulsion of the ceramide-enriched domains which is rather insensitive to the film composition, depends inversely on the lateral pressure and exhibits threshold dependence on the in-plane elasticity.

Keywords: Electric field; Lipid monolayer; Sphingomyelin; Ceramide

1. Introduction

Lipid and protein molecules anisotropically oriented at a hydrocarbon–aqueous interface such as that existing in biomembranes configure a dynamic array of self-organized molecular dipoles. These can act as sensitive local and long-range sensors of the electrical properties along and across the membrane interface [1]. Intrinsic electrostatic features, intermolecular packing and interactions determine a resultant dipole moment density that in conjunction with line tension forces are major factors responsible for the individual morphology of coexisting phase domains as well as their lattice organization along the surface [2].

Constant or alternating electromagnetic fields of different intensities can induce dramatic effects in biosystems [3]. This is not surprising in view of the marked molecular and supramolecular anisotropy that is inherent to polarized molecules in biomembranes. Varied effects include dynamic modifications of membrane topology [4–6], cellular function [7,8], protein phosphorylation [9] as well as activation of membrane-associated enzymes [10–15]. The formation and response of selective domain morphology with boundary defects and lattice super-structuring through the control of dipole-generated electrostatic fields along the lateral and across the transverse planes of the membrane surface appear as important regulatory mechanisms for lipase catalysis [11,12,16–18], phospholipase interfacial location [19], phase transitions and lateral domain migration [20–23], and channel conductance [24–26]. Regarding shingolipids, changes of hydration, charge and/or molecular tilting in galactocerebroside- and sulfatide-containing films can be amplified to alterations of the phase state and domain topography [27–29]. We have previously shown that reversible surface reorganization of galactocerebroside occurred with marked hysteresis depending on the sign and magnitude of the electrostatic potential applied [30]. In a recent work, it was also demonstrated the importance of the dipole moment density difference among the immiscible sphingomyelin and ceramide monolayer, coupled to the domain boundary line tension, to establish both the characteristic domain morphology and their

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lattice organization in premixed binary films and in those generated by the action of sphingomyelinase [2]. In the present work, we have investigated the effect of externally applied electrostatic fields on the distribution of the condensed ceramide-enriched domains in mixed monolayers with different sphingomyelin proportions.

2. Experimental section

2.1. Materials

Bovine brain sphingomyelin (Sm), ceramide (Cer) and the lipophilic fluorescent probe L-α-phosphatidylethanolamine-*N*-(lissamine rhodamine B sulfonyl)–Ammonium Salt (RhoPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Solvents and chemicals were of the highest commercial purity available. The water used for the subphase was double distilled in an all-glass apparatus. Lipid monolayers were prepared and characterized in an automated equipment as described elsewhere [31]. Several measurements were performed for the compression isotherms; the results shown represent average values and reproducibility was within 5%.

2.2. Methods

2.2.1. Brewster angle measurements

Brewster Angle Microscopy (BAM) observations and image analysis were carried out as described before [32]. At the Brewster angle, the relative thickness at a defined surface pressure can be derived from relative reflectance measurements:

$$\frac{d}{d_{\pi=0}} = \sqrt{\frac{I}{I_{\pi=0}}} \quad (1)$$

where I and d are the reflectance and the film thickness at a given lateral pressure, respectively. $I_{\pi=0}$ and $d_{\pi=0}$ are the intensity and the thickness at 0 mN m^{-1}.

2.2.2. Epifluorescence microscopy of monolayers

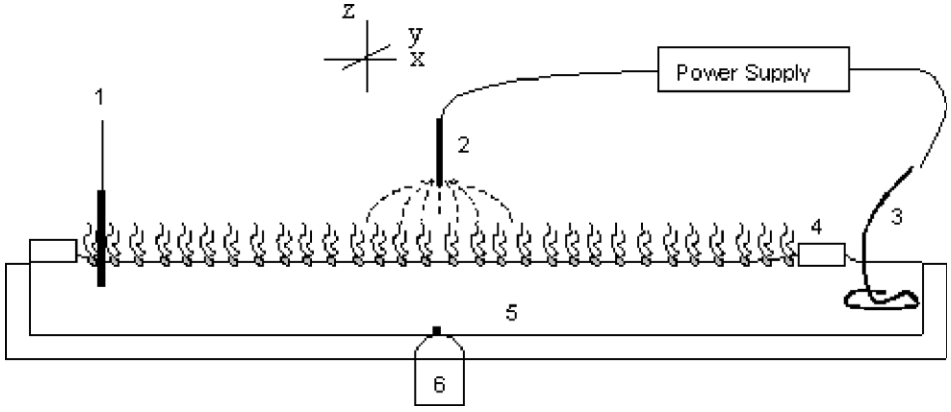
RhoPE was incorporated into the lipid solution before spreading (0.5 mol%). The monolayer was compressed up to 35 mN m^{-1} (a high pressure at which the layer is in an all-condensed state, with high cohesion, but still well before collapse), decompressed to 0 mN m^{-1} and then taken to the desired lateral pressure. A micrograph was taken at the chosen pressure before applying the electrostatic field. The observations were carried out at room temperature (24 ± 1 °C), using a glass through (microthrough, Kibron, Helsinki, Finland). An open-end Teflon mask with lateral slits covering the objective and extending through the film into the subphase was used to restrict lateral monolayer flow under the field being observed. A Zeiss Axiovert-200 (Carl Zeiss, Oberkochen, Germany) epifluorescence microscope with a source of radiation provided by a mercury lamp HBO 50 and a rhodamine filter set were used. Images were registered by a CCD video camera AxioCam HRc (Zeiss) commanded through the Axiovision 3.1 software of the Zeiss microscope. Objectives of 20×, 5.6× and 3.2× were used.

2.2.3. Electrostatic field setup

The experimental setup for applying the electrostatic field was similar to that used in Heckl et al. [22]. It consists in a Pt wire inserted in the subphase and a metal wire of 30 μm in diameter held at 150–200 μm of the subphase (see Scheme 1); while the monolayer topography is continuously observed from above, the upper electrode can be manipulated over the monolayer by moving it into three orthogonal directions with a micromanipulator (Carl Zeiss, Oberkochen, Germany) to an accuracy of 10 μm. The electrode can be charged to apply potentials of up to ±300 V with respect to the subphase electrode. This was performed with a BioRad pac 300 constant power supply.

2.2.4. Computational analysis of surface topography

The lipophilic fluorescent probe RhoPE shows preferential partition in the Sm-enriched zones of the lipid monolayer [33]. In Sm : Cer mixed monolayers, the Cer-enriched domains are in a more condensed and ordered state than the Sm-enriched zones, thus excluding the fluorescent probe. In the images recorded before applying the electrostatic field, segmentation of RhoPE-depleted areas was achieved by interactive image



Scheme 1. Experimental set up (not in scale). A Langmuir monolayer is spread on an aqueous surface in a glass trough that is mounted on an inverted microscopy. 1 – Wilhelmy plate, 2 – upper electrode and qualitative electric field lines. The electrode can be displaced in the three orthogonal directions with a micromanipulator: 3 – Pt electrode, 4 – PTFE barriers, 5 – subphase : electrolytic solution, 6 – microscope objective.