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Miscibility and phase separation in mixed erucylphosphocholine–DPPC monolayers

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ABSTRACT

Binary Langmuir monolayers composed of 1,2-dipalmitoyl-sn-phosphatidylcholine (DPPC) and erucylphosphocholine (ErPC) – a new generation anti-cancer drug of phospholipids-like structure, have been studied with classical Langmuir technique complemented with Brewster angle microscopy (BAM) and Grazing Incidence X-ray Diffraction (GIXD). In the course of surface pressure (π)–area (A) isotherms for film containing mole fraction of ErPC (X_{ErPC}) equal to 0.1, 0.2 and 0.3, two collapses appear – the value of the first one is close to the collapse pressure observed for pure ErPC, while the second one occurs at the pressure similar to that for pure DPPC. Such a behavior could imply that the investigated system is immiscible – the expulsion of a component collapsing at a lower pressure (ErPC) from mixed monolayer occurs at the first transition while DPPC (collapsing at a higher pressure value) is expelled at the final collapse. To confirm this hypothesis further analysis has been performed. Namely, thermodynamic analysis based on the calculation of the excess free enthalpy of mixing (ΔG_M^{exc}) evidenced for mutual miscibility of DPPC and ErPC in the region of surface pressures below the first collapse, which has been additionally confirmed with BAM images. On the other hand, at high surface pressures, above the first collapse (50 mN/m) GIXD experiments complemented with BAM images proved phase separation of the investigated lipids and 2D crystalline domains formation. The interpretation of the X-ray scattering results enabled us to propose a possible model of molecular packing, according to which only condensed domains of the 1:1 lipids ratio are periodically ordered; whereas at the other proportions the lipids are located randomly within the domains.

1. Introduction

Miscibility or phase separation occurring in membranes are of utmost importance in many physiological processes, including transport and fusion, and affect a number of membrane properties such as water permeability, bilayer potential, and reactivity of membrane proteins (for details see [1] and references therein). Therefore, it is fundamental to study the miscibility both between membrane constituents themselves and between biomolecules and membrane components. The latter are of particular importance in understanding the mode of action of drugs acting at membrane level [2]. Using one of biomembrane models (among which Langmuir monolayers are most popular in addition to liposomes/vesicles [3]), the information on the miscibility and interactions were of much help in elucidating the importance of membrane sterols and phospholipids in the mechanism of action of polyene antibiotics [4,5] and their toxicity [6], chemotherapeutics [7] and other drugs [8–12] as well as enabled to study the influence of various biomolecules on membrane organization [13–16].

In 2D systems (Langmuir monolayers) the miscibility between components can be interpreted in terms of both the additivity rule of mean molecular areas and the 2D phase rule, applied to the collapse pressures (π_{coll}) [17]. The latter approach leads to phase diagrams for total miscibility, partial miscibility and immiscibility both in the monolayer phase as well as in the collapsed phase (very well developed and compiled in [18]). The experimental results of the collapse pressure (π_{coll}) vs composition plots enable us so to get insight into the miscibility between film components [17,19,20] although in some cases other methods (optical and/or diffraction) in addition to collapse pressure determination with classical surface manometry are necessary to be performed in order to have a clear view on the phase situation in the investigated system [21].

This paper is aimed at analyzing the behavior of erucylphosphocholine, ErPC (Scheme 1) – a synthetic alkylphosphocholine, being a new generation antitumor drug [22] – in model membrane environment. Our investigations were inspired by the fact that although it is clear that ErPC acts on membrane level, still little is known how

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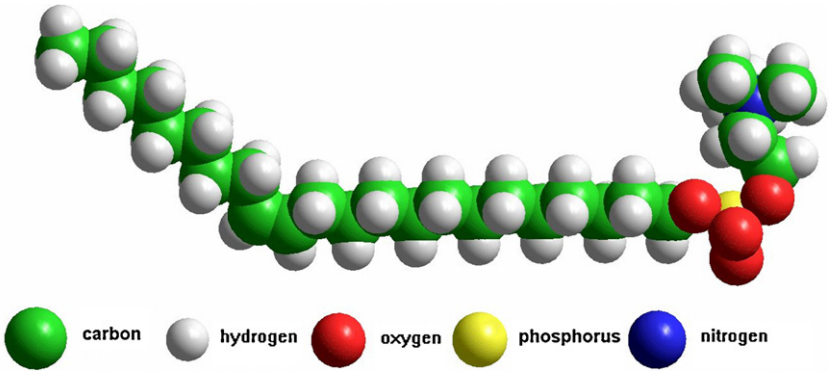

Scheme 1. Chemical structure of ErPC.

this molecule penetrate through the membrane and which membrane component is important in this process. For this purpose we have performed miscibility studies of ErPC with a representative lipid that is highly populated in the outer membrane of different cell lines. 1,2-Dipalmitoyl-sn-3-phosphatidylcholine (DPPC) perfectly fulfills this requirement.

In this contribution the results obtained for binary Langmuir monolayers formed by ErPC and DPPC, covering the whole range of molar fractions, are presented. We have employed the classical Langmuir technique, i.e. surface pressure–mean molecular area (π–A) isotherm registration, complemented with visualization of the monolayers with Brewster angle microscopy (BAM). Based on the π–A isotherms, the thermodynamic analysis of the 2D-mixtures was also performed. π–A isotherms and BAM images provide the monolayer characterization in the micrometer scale. To gain information about the molecular packing with Angström resolution, the Grazing Incidence X-ray Diffraction (GIXD) method was used. The application of this technique was found to be especially useful for understanding the behavior of the investigated binary systems at high surface pressure region.

2. Experimental

2.1. Materials

DPPC (1,2-sn-dipalmitoylphosphatidylcholine) of the >99% purity was purchased from Avanti Polar Lipids; whereas the sample of 1-erucylphosphocholine (ErPC) was a generous gift of Aeterna Zentaris. The lipids were applied for investigation without any further purification. Chloroform of spectroscopic purity stabilized by ethanol and the p.a. methanol were provided by Sigma–Aldrich. The ultrapure water of the resistivity $\geq$18.2 MΩ cm was produced by the Millipore system. The lipids were dissolved in a chloroform/methanol 9/1 mixture and the concentrations of the solutions were ca. 0.2 mg/ml. Mixed solutions of various composition (ErPC ratio ranged from 0 to 1 with the increment of 0.1) were prepared from the stock solutions. Spreading solutions were deposited onto the water subphase with a Hamilton microsyringe, precise to 2.0 μl.

3. Methods

3.1. Brewster angle microscopy and Langmuir monolayer experiments

BAM experiments were performed with ultraBAM instrument (Accurion GmbH, Goettingen, Germany) equipped with a 50 mW laser emitting p-polarized light at a wavelength of 658 nm, a 10$\times$ magnitude objective, polarizer, analyzer and CCD camera. The spatial resolution of the BAM image was 2 μm.

The BAM instrument was installed over a KSV 2000 700 cm^2 double-barrier Langmuir trough (KSV, Helsinki, Finland). The temperature of the trough was controlled to be 20.0 $\pm$ 0.1 °C by a circulating water bath. The number of the spread lipid molecules was kept constant on the level of 7×10^{16} molecules per monolayer. The monolayers were compressed with the compression rate of 20 cm^2/min. Surface pressure was measured to within 0.1 mN/m using a Wilhelmy plate made from ashless chromatography paper (Whatman Ch1). Each isotherm was repeated at least three times to ensure reproducibility of the curves to $\pm$2 Å^2.

3.2. Grazing Incidence X-ray Diffraction

GIXD experiments were performed at the BW1 beamline at the HASYLAB DESY synchrotron source (Hamburg, Germany) using a dedicated liquid surface diffractometer [17–19] with an incident X-ray wavelength λ = 1.303 Å. A thermostated Langmuir trough made of one block of Teflon, equipped with a movable barrier for monolayer compression (Riegler&Kirstein, Potsdam, Germany), was placed in a gastight container and mounted on the diffractometer. After spreading the lipid solution onto the subphase, the cover of the container was screwed tightly and at least 30 min were allowed for the trough container to be flushed with helium to reduce the scattering background and to minimize beam damage of the monolayer during X-ray scans. Afterward, the films were compressed to the surface pressure of 30 mN/m (or to 50 mN/m in one experiment discussed in the text) and after stabilization of the monolayer the GIXD experiments were performed.

The detailed construction of the BW1 liquid surface diffractometer, the applied scintillation detectors, and the data reduction are described with details in a multitude of experimental and review papers can be found elsewhere [23–27]. Here, we would like only to remind the most important definitions enabling the calculation of the parameters listed in Tables 1 and 2.

The scattering vector Q is a sum of two components: the vertical Q_{xy} and the horizontal Q_z. A monolayer can be treated as a large conglomerate of differently organized 2D domains, so it is analogous to a powder sample. Therefore, it is impossible to separate the Q_x and Q_y components and they are referred together as Q_{xy} – the scattering vector component in monolayer plane [28]. The Q_{xy} component is defined by the formula:

$$Q_{xy} = \frac{4\pi}{\lambda} \sin\left(\frac{2\theta_{xy}}{2}\right) \quad (1)$$

where $2\theta_{xy}$ is the angle between the incident and the diffracted beam projected on the liquid surface. The Q_z component is defined by the formula:

$$Q_z = \frac{2\pi}{\lambda} \sin \alpha_f \quad (2)$$