Applied Surface Science 328 (2015) 596–605

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Contact angle hysteresis and phase separation in dry phospholipid films with cholesterol deposited on mica surface

Małgorzata Jurak*

Department of Physical Chemistry – Interfacial Phenomena, Faculty of Chemistry, Maria Curie-Skłodowska University, Maria Curie-Skłodowska Sq. 3, 20031 Lublin, Poland

ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form
12 December 2014
Accepted 15 December 2014
Available online 23 December 2014

Keywords:
Contact angle hysteresis
Phase separation
Model membranes

ABSTRACT

A series of apparent advancing and receding contact angles of water and diiodomethane was measured on the phospholipid/cholesterol monolayers physisorbed on the mica surfaces. It was found that the contact angles and their hystereses vary significantly depending on the lipid film composition and mutual miscibility of both components. These changes were much greater for water than diiodomethane. When the phase separation occurred, the hysteresis of water contact angle significantly decreased whereas the diiodomethane contact angle hysteresis increased considerably. Different behavior of both liquids may result from different mechanisms of the liquid droplets penetration/retention and points to structural changes that occur within the monolayers, including molecules rearrangement when exposed to water. The structure of the studied monolayer surfaces was confirmed by means of the microscopic techniques. The images are a visual evidence of cholesterol precipitation out the binary films at their specific stoichiometry. The results provide a new insight into the advancing/receding contact angles origin (and contact angle hysteresis) of polar and apolar liquids depending on the phospholipid/cholesterol monolayer composition, as well explanation of the origin of the contact angle hysteresis on the model biological surfaces, which are molecularly smooth.

1. Introduction

The Young's equation assumes only one thermodynamically stable contact angle for a liquid drop on a solid surface. However, in real systems, the contact angle of a drop observed at its advancing edge, i.e. when the contact line starts to expand, is usually different from that appearing at its receding edge, i.e. when the contact line starts to shrink/recede [1,2]. The difference between the advancing (θ_A) and receding (θ_R) contact angles is known as the contact angle hysteresis (CAH) [3]. Although this phenomenon has been studied extensively, its origin is still not fully understood. Initially, contact angle hysteresis was attributed to surface roughness [4–8] and surface heterogeneity [9–15]. Moreover, it results from the line tension considerations [16,17] that the contact angle hysteresis should vanish if the lateral dimension of patches on a heterogeneous and rough surface is of the order of 1 μm. Progress in the preparation and investigation techniques of solid surfaces has facilitated the production of extremely smooth and homogeneous surfaces on which, however, the contact angle hysteresis also appears. Therefore, such surfaces have become helpful in identifying several other reasons for hysteresis: molecular level topography and rigidity of molecules [18], molecular organization of the solid surface [19], liquid penetration and surface swelling [20–22], liquid sorption and liquid retention [21,23], size and shape of liquid molecules [24], interactions at the solid/liquid interface [25], rate of motion of the three-phase contact line on the solid surface [26], and finally the observed hysteresis can be due to the liquid film present right behind the three-phase solid surface/liquid drop/gas (vapor) contact line [27–29], receding contact line pinning [30], and the presence of Derjaguin pressure [31].

As mentioned above several authors [18] have shown that the molecular film topography is responsible for the changes of contact angles which contributes to contact angle hysteresis. The others found that static advancing contact angle is not affected by the surface roughness if its height is less than 10–30 nm [31]. Therefore the regularly structured surfaces provide useful models for quantitative evaluation of the relationship between the contact angle , its hysteresis, and surface structure. The solid supported lipid films prepared by the Langmuir–Blodgett technique are highly organized nanostructures used as models of the biological membranes [32,33]. Cholesterol (Chol) which is an important component of most biological membranes exhibits the solubility limit in these lipid films. When its content is exceeding its

* Tel.: +48 81 5375547; fax: +48 81 533 33 48.
E-mail address: malgorzata.jurak@poczta.umcs.lublin.pl

http://dx.doi.org/10.1016/j.apsusc.2014.12.104
0169-4332/

maximum solubility, the excess cholesterol molecules precipitate as monohydrate crystals [34,35].

There are several models of structural organization of phospholipid/cholesterol (PL/Chol) membrane and Chol precipitation. For example, in the superlattice model (or regular distribution model) [36–38] cholesterol molecules are regularly distributed in the phospholipid matrix tending to follow a regular pattern (superlattice). This model is consistent with the observation of non-monotonic variations of the physical properties of binary mixtures and assumes that at critical concentrations the cholesterol molecules exhibit a specific long-ranged ordering. Both attractive van der Waals interactions and repulsive interactions between similarly charged lipids are a driving force for the superlattice formation. On the other hand, in the umbrella model [34,35,39] the nonpolar part of cholesterol molecule is preferentially screened by the polar headgroups of phospholipid. The umbrella model predicts the preferential association of cholesterol with some membrane molecules due to the mismatch between the small cholesterol polar headgroup with its large nonpolar body. Moreover, regular distribution of cholesterol in the phospholipid membrane, as well as the condensing and ordering effects of cholesterol on it, are also considered. When a defined amount of Chol is gathered, the shielding breaks down, and the excess Chol precipitates out. Next, the condensed-complexes model [40–43] assumes that the mixtures of cholesterol and saturated phospholipids form stoichiometric ordered condensed complexes having fixed stoichiometry. Their formation is independent of the overall cholesterol content. At a defined cholesterol concentration, the equilibrium exists between these condensed phospholipid–cholesterol complexes and the ordinary lipid molecules. Only at a characteristic composition, at which the complex is formed, neither excess of cholesterol nor excess of phospholipid exists, and the two components mix leaving no individual phases. Recently, a mesoscopic water–lipid–cholesterol model has been developed in which the condensation effect is a direct consequence of particular changes in the phase behavior induced by cholesterol [44]. Another model claims formation of alloy like mixed domains of short-range order where vertical orientation of cholesterol molecules in the phospholipid membrane depends on its content [45]. Thus following these models one may expect formation of strictly organized and regularly packed PL/Chol monolayers, and/or, the films of separated domain structure, depending on the cholesterol content. Anyway, the lipid stoichiometry has an essential significance for the characteristic structure of the model membranes.

Thus, the aim of this present work was to study how the composition of well-defined phospholipid/cholesterol monolayers and possible phase separation affect the measured contact angles and their hystereses of different liquids. The surface topography was characterized using a number of complementary techniques, including optical microscopy, profilometry and atomic force microscopy (AFM). These methods provide insight into the structural characterization and organization of the molecules in monolayers. Because biological membranes are not isolated systems but are in contact with substances of different character, it is believed that these results can be helpful for better understanding functionality of biological membranes in processes occurring at the membrane surface or those associated with cell membranes, such as transport of water molecules, some ions, and substances across the membrane.

2. Experimental

2.1. Materials

Phospholipids: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DPPG) and cholesterol (Chol) were supplied by Sigma with purity 99%. Solvents: chloroform (p.a.) and ethanol (96%, p.a.) were purchased from POCH (Poland) and used without further purification. The phospholipids (excluding DPPG) and cholesterol solutions were prepared in chloroform while DPPG was dissolved in chloroform/ethanol 4:1 (v/v) mixture. From the respective stock solutions (concentration 1 mg/ml), their mixtures of desirable compositions were prepared. Ultrapure water with resistivity of 18.2 MΩ cm was obtained from the Milli-Q Plus system and used as the subphase for the lipid monolayer formation and as the probe liquid, together with diiodomethane (99%, Aldrich), for the contact angle measurements. Freshly cleaved mica plates (Continental Trade, Poland) of 38 mm × 26 mm × 1 mm size were used as a support for the lipid monolayers.

2.2. Solid supported film preparation

Surface pressure versus mean molecular area (π-A) isotherms were obtained in a Langmuir–Blodgett KSV 2000 trough (KSV Instruments, Ltd., Finland) placed on an anti-vibration table in a plexi-glass box. Surface pressure was measured with the accuracy 0.1 mN/m by using a platinum Wilhelmy plate. In order to prepare a stable Langmuir monolayer, spreading solutions were dropped onto the water subphase with the Hamilton microsyringe at 20.0 ± 0.1 °C. Once spread, the monolayer was left for 10 min to ensure complete evaporation of the solvent and then the symmetric compression was started with the barrier speed of 10 mm/min to the desired surface pressure of 35 mN/m which corresponds to the biological membrane pressure regime [46]. Then the spread monolayer was transferred vertically by pulling a mica plate through the compressed film out the subphase at a speed of 5 mm/min, keeping the film pressure and temperature constant. In this way "physisorbed" lipid surfaces called Langmuir–Blodgett (LB) monolayers were obtained. The samples obtained in this way were dried in a vacuum apparatus, under pressure of 117 mbar, for about 20 h at room temperature.

2.3. Contact angle measurements

The advancing and receding contact angles of probe liquids were measured on the prepared films with Contact Angle Meter Digidrop GBX (France) by means of the sessile drop method which yields "apparent contact angle". All measurements were performed in a closed chamber at 20 ± 0.1 °C under constant flow of nitrogen where the samples were placed horizontally on the stage. A 1.5 ml syringe was located in an automatic (motor-driven) manipulator which was used to adjust carefully the position of the needle tip of the syringe above the sample. The motorized syringe was then operated at a constant speed which was controlled. The droplet volume was 3 μl. For the receding contact angle measurement, the drop volume was decreased by sucking 1 μl of liquid back into the syringe.

An image of the drop settled on the sample surface was created using a digital camera, and it was magnified by an optical lens. To prevent the changes in the monolayers due to their long exposure to bulk water, the advancing angles were measured within the first few seconds after the droplet deposition. The contact angles were calculated by averaging from at least three, and up to eight, repeated measurements, which were conducted on three samples prepared independently. Relatively reproducible advancing and receding macroscopic contact angles were measured.