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# AFM surface morphology and friction force studies of microscale domain structures of binary phospholipids

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ABSTRACT

We have studied friction forces on binary mixtures of phospholipid monolayer films. The phospholipid monolayer films have been prepared via the Langmuir–Blodgett (LB) technique on mica. The two-component phospholipids are distearoylphosphatidylcholine (DSPC) and dilauroylphosphatidylcholine (DLPC). At 25 °C the LB monolayer films give the gel-state DSPC domains surrounded by the liquid crystalline DLPC matrix. The friction forces measured on the DSPC domain region are significantly greater than those on the DLPC matrix region at this temperature. An increased temperature results in a decreased friction of the DSPC domain region, and above the gel-to-liquid crystalline phase transition temperature of DSPC, the difference in the friction forces measured on the two phospholipids becomes negligible. This means that the phase state is a key factor in determining friction forces on the phospholipid monolayer films.



## 1. Introduction

Understanding the factors that influence the formation of phospholipids domains within the lipid bilayer is of significant interest due to their potential participation in transport and signalling in cellular membranes. The domains are expected to be in a liquid-ordered phase that is characterized by closely packed chains and a high degree of lateral mobility [1,2]. Lipid model membranes have been typically investigated by a wide range of surface analytical techniques such as scanning probe microscopy, ellipsometry, neutron reflectivity, time-of-flight secondary ion mass spectrometry and electrochemical impedance spectroscopy. Solid-supported phospholipid films prepared using the Langmuir–Blodgett or Langmuir–Schaefer technique have been used to investigate the formation and structure of lipid microdomains or rafts [3–6], to probe peptide/lipid interactions [7–9], to generate biologically addressable surface patterns [10,11], and to identify the forces which govern cell adhesion processes [12,13].

The formation of condensed phase structures in the two-phase co-existence region of lipid monolayers at the air/water interface has provided a wealth of information on the 2D growth of micrometer domains [14,15]. This is because the molecular density and phase state of these Langmuir monolayers can be determined via surface pressure–area ($\pi$–$A$) isotherms, and readily controlled by varying the area per molecule, subphase temperature and ionic conditions on a Langmuir film balance. Such precise control on the lipid molecular area and phase is not possible when using bilayer vesicles or solid-supported bilayers formed by lipid vesicle fusion. For this reason, much has been learned from previous investigations with use of Langmuir monolayer [4,16,17].

In our current work, we have studied surface morphology and friction forces of binary mixtures of phospholipids as a function of temperature, in order to understand effects of sample temperature on friction forces in the system where lateral phase separation arises from a difference in the hydrocarbon chain length of the phospholipids. Friction force microscopy (FFM) measures simultaneously normal and lateral forces on the scanning tip with the laser beam deflection [18–20]. As far as we are aware, this is the first report focusing on friction forces on such phase-separated phospholipid monolayers from the viewpoint of their phase states. The phospholipid mixtures employed in this study consist of L-α-distearoylphosphatidylcholine (DSPC) and L-α-dilauroylphosphatidylcholine (DLPC), which exhibit low miscibility with each other and hence form a phase-separated monolayer film at the air/water interface [21]. Such low miscibility results from the difference in the phase states between the two phospholipids at room temperature: the main gel-to-liquid crystalline phase transition temperatures of DSPC and DLPC are reported to be 80 °C and 0 °C, respectively [22]. The binary phospholipid monolayers with various composition ratios are prepared at the air/water interface under controlled surface pressure (by using a Langmuir trough) and transferred on mica.

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## 2. Experimental

### 2.1. Materials

The phospholipids used in this study (DSPC and DLPC) were kindly supplied from the NOF Corporation and their purities are reported to be >99.6% by the supplier. Chloroform (Spectrochemical Anal., Wako) was used without further purification. Mica plates (Nilaco) were used as an LB film substrate after cleaving. The water used in this work was filtered with a reverse osmosis membrane after deionization and disinfectant with a Barnstead NANO pure Diamond UV System.

### 2.2. Preparation of DSPC/DLPC mixed films

The phospholipids were first dissolved in chloroform, and then the DSPC/DLPC chloroform solution (approximately 100 μL) was dropped on water. At this stage the surface pressure was adjusted at 32 mN m$^{-1}$, using an HBM700LB trough with a glass Wilhelmy plate (Kyowa Interface Science). The prepared monolayer film was transferred onto a cleaved mica surface according to the horizontal-lifting method, and hence the hydrophobic chains of the lipids face the outer surface of the substrate. $\pi$–$A$ isotherm data were measured using the same apparatus with a computer controller.

### 2.3. Measurements

DSC measurements were carried out using a Rigaku DSC8230 calorimeter with a stainless vessel. The measurement conditions were set at 1 °C min$^{-1}$ for the scanning rate, 20–80 °C for the scanning range and 0.42 mJ s$^{-1}$ for the sensitivity, respectively.

An SPI4000 AFM system with an E-Sweep (SII NanoTechnology Inc.) was used for measuring surface morphologies and friction forces on the DSPC/DLPC lipid films. Cantilevers with a silicon tip (SII NanoTechnology Inc. SI–AF01, nominal spring constant = 0.2 N m$^{-1}$, nominal torsional spring constant = 81.3 N m$^{-1}$) were used for the contact mode AFM in air at room temperature (approximately 25 °C). Based on this nominal torsion spring constant value, friction forces are evaluated as 1 mV (torsion count) = ca. 0.022 nN.

Friction forces on the LB Film were measured by the use of torsion of the cantilever. The sample surface was moved back and forth in a direction perpendicular to the cantilever, and the torsion count against position coordinate was measured. In the current work, we set the scan range and scan speed as 4 μm and 4 μm s$^{-1}$, respectively, and the load of the cantilever was fixed at 0 nN.

## 3. Results and discussion

### 3.1. Surface morphology and $\pi$–$A$ isotherm data

A typical topographic AFM image of a mixed DSPC/DLPC monolayer film is presented in Fig. 1a. The DSPC/DLPC mixed molar ratio is set at 0.5/0.5 for this particular case. One can clearly see ellipsoidal phase separation in this image. The diameter of the domain region is measured to be ca. 3–7 μm and the step height between the two phases is 0.8 ± 0.1 nm (Fig. 1b). We have confirmed that the area fraction of the domain region increases linearly with increasing DSPC mol fraction (data not shown), and hence, we assume that DSPC forms the domain region whereas DLPC exists in the matrix. Taking the difference in the hydrocarbon chain length into consideration, the step height measured here is deemed to be within an acceptable range [23]. The spherical DSPC domain may result from its greater intermolecular van der Waals interaction as well as the gel structure at this temperature. Sanchez and Badia have studied the surface morphology of binary mixtures of DPPC and DLPC, transferred onto a mica surface, and demonstrated a very similar phase-separated domain structure of DPPC surrounded by DLPC matrix [24].

The lipid monolayer films, prepared by the LB technique, were transferred onto cleaved mica, and friction forces were measured. Before presenting the resultant friction force data, the $\pi$–$A$ isotherm data are shown in Fig. 2. In the case of the DSPC single system, gas-to-solid film transition occurs at the occupied area of 0.55 nm$^2$. The limit area per molecule and collapsed pressure of the film was 0.38 nm$^2$ and 52 mN m$^{-1}$, respectively. On the other hand, DLPC forms a liquid-expanded film, which is distinguishable from the DSPC monolayer film. The shapes of the isotherms indicate that the DSPC monolayer is characterized as a two-dimensional (2D) solid-like organization, whereas the DLPC monolayer shows a 2D liquid-like behavior.

In our current study, the LB monolayer films were transferred on the cleaved mica at 32 mN m$^{-1}$, which is close to the biomembrane pressure. The values of the averaged occupied area per molecule, estimated at 32 mN m$^{-1}$, are summarized in Fig. 3 as a function of DSPC composition. The occupied area is decreased linearly with increasing DSPC mol fraction, which is predicted by the additive rule [24] as follows. The area of a two-component monolayer at a given surface pressure is comparable to that of the pure components:

$$\bar{A} = x_1 A_1 + x_2 A_2 \quad (1)$$

where $\bar{A}$ is the mean molecular area of the two-component film, $x_1$ and $x_2$ are the mol fraction of components 1 and 2 in the mixed film, and $A_1$ and $A_2$ are the molecular areas of 1 and 2 in pure monolayers.

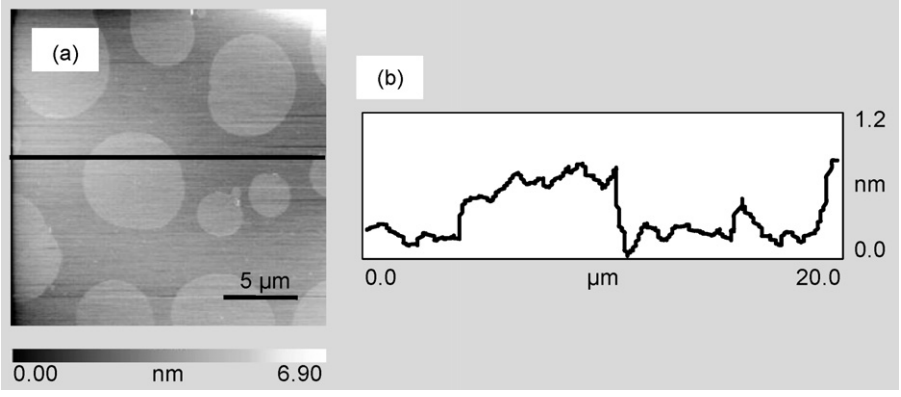


**Fig. 1.** (a) An AFM topographic image (20 μm × 20 μm) of the mixed DSPC/DLPC (molar ratio = 0.5/0.5) monolayer transferred on mica. (b) The section analysis along the solid line in (a).