Phase Transitions in Phospholipid Monolayers Studied by Atomic Force Microscopy and Langmuir-Blodgett Technique

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The phase behaviour and surface structure of dipalmitoyl phosphatidyl choline (DPPC) monolayers at the air/water interface, in the absence and the presence of procaine, have been investigated by Langmuir-Blodgett (LB) technique and atomic force microscopy. The LB films were transferred on mica, at a controlled surface pressure, characteristic for the expanded liquid to condensed liquid phase transition of pure DPPC monolayers. The results indicate that procaine penetrates into and specifically interacts with phospholipid monolayers stabilizing the lipid membrane interface.

Keywords: DPPC, procaine, LB monolayers, AFM

In 1985 Professor Petre T. Frangopol started long term collaboration with Professor Emil Chifu and Chifu’s research group at Babes-Bolyai University of Cluj-Napoca, on the investigation of physical and chemical properties of anesthetics and their effects on lipid membrane models [1-12], such as monolayers or multilayers of fatty acids, cholesterol or phospholipids. Later, he was nominated visiting professor at the Babes-Bolyai University of Cluj-Napoca, where he carried out research on numerical analysis of compression isotherms of diverse lipid monolayers [13] and on the interfacial phenomena involved in the molecular mechanism of anesthesia, which is still ongoing [14].

From a perspective point of view, Dr. Frangopol’s scientific interests cover a broad set of subjects, from model membranes, such as monolayers, micelles and liposomes to the cell membranes and drug delivery systems. A comprehensive account on his scientific achievements in the field of interfacial phenomena and anesthetics can be found in a series of publications [1-16]. Recently, Dr. Frangopol has developed novel approaches to investigate the interaction between drugs and lipid membrane models by atomic force microscopy in combination with Langmuir-Blodgett (LB) self assembled films [14]. He also helped his research group at Babes-Bolyai University of Cluj-Napoca to purchase a state of the art scanning probe microscope (AFM, JEOL, Japan) and the Langmuir-Blodgett film equipment (KSV, Langmuir System, Finland).

More specific, we have reported that the local anesthetics (e.g. procaine) expand the lipid monolayers, spread at the air/water interface, depending on the pH's and ionic strengths [1-12]. Also, we have done thermodynamic studies to determine the binding curves of procaine on the lipid monolayers [10-12] at fluid interfaces. The binding curves have shown that procaine is preferentially bound to the expanded liquid state of the lipid monolayers. The molecular mechanism results from the adsorption on and the penetration of procaine into lipid membrane models in substantial agreement with other related published data [17-24].

As a continuation of our efforts to elucidate the molecular mechanism for the local anesthesia, the effects of procaine on the expansion and on the phase transition of lipid monolayers are investigated in the present study. We have chosen a synthetic phospholipid, namely L-α dipalmitoyl phosphatidyl choline (DPPC), which forms stable monolayers at fluid interfaces. Here, we have focused on the change in the phase behaviour and in the surface structure of DPPC monolayers spread on aqueous solution of 10⁻³ M procaine using LB equipment combined with atomic force microscopy (AFM).

Experimental Part

Materials

Synthetic L-α-dipalmitoyl phosphatidyl choline, i.e., 1,2-dipalmitoyl-sn-glycero-3-phosphocholine: DPPC, and procaine, 2-(diethylamine)ethyl-para-(amine) benzoate, hydrochloride (P- HCl), were purchased from Sigma Chemical Co., and used without further purification. For the study of the procaine effect, P- HCl was first dissolved in two-distilled water, giving a 10⁻³ M procaine in aqueous solution.

Preparation of Langmuir-Blodgett (LB) film

DPPC was dissolved in a mixture of chloroform: ethanol (9:1), giving a 1 mM phospholipid in organic solution. The lipid solution was spread at the air/water interface with a microsyringe both in the absence and the presence of procaine in aqueous solution. After spreading, the system was allowed to stand for 10 min, without causing the surface disturbance. Then, the compression isotherms in terms of surface pressure versus mean molecular area of DPPC were recorded. All measurements were performed with KSV equipment. The speed of the compression was 10 mm min⁻¹. For AFM observations, single layer LB films were transferred on the freshly cleaved mica, using a vertical dipping method at a surface pressure of 8 mN/m. The LB film transfer took place at about 5 mm min⁻¹.

AFM observations

Atomic force microscopy (AFM) is a surface imaging technique with a nanometer-scale resolution [25-27]. AFM studies were performed using the AFM JEOL. The cantilevers were made of Si₃N₄, with a resonance frequency of 250 - 350 kHz. Triplicate samples were prepared for each monolayer composition and at least four separate areas were imaged for each sample. Through this study, AFM

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images were obtained at a surface pressure of 8 mN/m in order to examine the effect of procaine on the phase transition from expanded liquid to condensed liquid in pure DPPC monolayers.

**Results and discussion**

The surface pressure versus mean molecular area isotherms of the DPPC monolayer in the absence and the presence of procaine, at the air/water (pH 5.6) interface, for 20°C, were reported in our previous paper [10]. For pure DPPC monolayer, the compression isotherms showed a phase transition at a surface pressure (8 mN/m) from expanded liquid to condensed liquid state. The compression isotherms showed a collapse phenomenon at very high surface pressures, characterized by collapse area ($A_c$) and collapse pressure ($\pi_c$). The collapse characteristics and the limiting molecular areas ($A_0$), characterizing the condensed liquid state of DPPC monolayers [10], are given in table 1.

From table 1, it is observed the expanded effect of procaine, namely the limiting molecular area ($A_0$) for liquid condensed state and the collapse pressure ($\pi_c$) are much higher in the presence of procaine than in pure DPPC monolayers. These data indicate that procaine has a remarkable effect both on the DPPC phase transition and on the collapse of DPPC monolayers, at the air/water interface. Due to the same collapse areas, ($A_c$), it is found that the procaine is excluded from DPPC monolayers at collapse, but still remains adsorbed on DPPC monolayer surface thus increasing the monolayer collapse pressure and stability. This effect might correspond to pressure-driven exclusion of procaine from zwitterionic DPPC monolayers. The topography of pure DPPC monolayers, transferred at 8 mN/m on mica, is shown in the AFM images in figures 1.1 and 1.2 for two different scanned areas. The 2D topographic images show a heterogeneous DPPC monolayer with characteristic features, irregular in shape and of different size, for expanded liquid and for condensed liquid phase. The maximum height of the pure DPPC monolayer is 4.3 nm, which was measured through spontaneously arising holes. Also, domain boundaries are commonly observed and are thought to be the boundaries between DPPC areas with different tilt directions.

Brighter areas (figs. 1.1 and 1.2) are observed for high domains of the DPPC in condensed liquid state, but darker areas correspond to lower domains characteristic for expanded liquid DPPC layers. Thus, AFM images reveal a phase separation, namely the DPPC condensed liquid phase is practically immiscible with the DPPC expanded liquid phase. The phase contrast image, given in figure 1.3, shows the morphological character more clearly than the corresponding topography (fig. 1.2) due to the difference in the surface physical and chemical properties between the condensed and expanded DPPC monolayers. We suggest that the lighter domains correspond to well organized probably vertically oriented DPPC molecules, while the darker areas correspond to less orderly DPPC molecules.

Further, the AFM images for DPPC monolayers in the presence of procaine transferred on mica at the same lateral surface pressure (8 mN/m) were examined (figs. 2 and 3) and compared with the AFM images of pure DPPC monolayers (fig. 1). Although the phase transition was not detected on compression isotherms [10], a phase separation was still found in the AFM images for DPPC monolayers in the presence of procaine (figs. 2 and 3) for different scanned areas.

![Fig. 1. AFM images of the pure DPPC monolayer, transferred onto mica at 8 mN/m, by Langmuir-Blodgett technique, from the air/water interface; (1) and (2) are 2D-topographies at the scanned area of 3 x 3 $\mu$m$^2$ and 1 x 1$\mu$m$^2$, respectively; marked area in figure 1.1 is rescanned in figure 1.2.; (3) the phase image corresponds to the topography given in figure 1.2.](image1)

![Fig. 2. AFM images of mixed DPPC and procaine monolayer at a lateral surface pressure of 8 mN/m, transferred by Langmuir-Blodgett technique from the air/water interface on mica; (1), (2), (3) represent 2D-topography images at the scanned area of 5 x 5 $\mu$m$^2$, 3 x 3 $\mu$m$^2$ and 1 x 1 $\mu$m$^2$, respectively; marked area in figure 2.1 is rescanned in figure 2.2.; marked area in figure 2.2 is rescanned in figure 2.3.](image2)
In mixed DPPC: procaine monolayers, the condensed brighter domains became smaller and rounded, or slightly elongated, or as dots (fig. 2 and figs. 3.1 and 3.3) when compared to those found in the pure DPPC monolayers (fig. 1). The change in shape and size of DPPC domains is related to the interaction of procaine and the DPPC phases. We suggest that procaine coexists with less organized DPPC molecules and is preferentially located at the domain boundaries. This would indicate that the lighter domains correspond to almost highly oriented DPPC molecules. The dark domains probably correspond to less organized DPPC molecules mixed with procaine. Thus, AFM images reveal a phase separation between DPPC condensed phase (high areas) and DPPC expanded phase enriched in procaine (low areas).

This situation might be related to the fact that the procaine adsorbs and penetrates preferably at the boundaries between DPPC domains, with a different tilt direction, and in the expanded DPPC phase, as also found by epifluorescent microscopy [18]. However, the binding of procaine molecules to the surface of the DPPC condensed domains could also perturb the lipid chains organization. In this regard, the maximum height in mixed monolayers is about 3.8 nm as determined in figure 3.3. This height corresponds to the mixed DPPC: procaine condensed domains, and is decreased as compared to the height of the condensed domains existing in pure DPPC layers (fig., 1, where maximum height of 4.3 nm was found). The phase contrast image, given in figure 3.2, shows also clearly the morphological character corresponding to the topography (fig. 3.1).

The tertiary amine procaine is an amphiphilic molecule which exists in the charged form [8] at the working pH of about 5.6. The hydrophilic amine moiety is responsible for water solubility and DPPC membrane surface binding and the hydrophobic moiety appears to control the organization within the DPPC membrane model. The interaction of procaine molecules with zwitterionic DPPC molecules may lead to an ordering effect on the DPPC monolayer interface and to a disordering effect on the hydrocarbon interior of DPPC monolayers, in substantial agreement with AFM observations.

In order to explain the mode of action of procaine, positively charged under the working conditions, on the zwitterionic phospholipids monolayers, we assume that positively charged procaine molecules adsorb onto the lipid membrane surface. This will allow the hydrophobic portion of procaine molecule to be embedded into the hydrocarbon part of the expanded liquid phase and at the domain boundaries of lipid monolayers. The positively charged amine group of procaine molecules can interact electrostatically, at the monolayer/water interface, with the negatively charged group of the zwitterionic DPPC molecules. The electrostatic interaction will appear in both the condensed and the expanded liquid state of DPPC monolayers increasing the stability of the lipid membrane.

The findings in this study suggest that procaine accumulates in the lipid phase of cell membranes, and thus might change the physical properties of the membrane lipid and consequently influence the protein conformation. The effect of procaine and other local anesthetics on DPPC membrane organization at various lateral surface pressures is under further investigation in our laboratories.

Conclusions

We have used Langmuir-Blodgett (LB) self-assembly technique and atomic force microscope (AFM) as tools to investigate the effect of procaine, at a drug concentration of 10^{-4} mol dm^{-3} in the aqueous phase, on DPPC monolayers spread as Langmuir film at the air/water interface.

The LB self-assembled monolayers were transferred from Langmuir films onto mica, at controlled surface pressure characteristic for the phase transition in pure DPPC monolayers (i.e. 8 mN/m), by using vertical transfer method. In the presence of procaine, the stability of DPPC films is highly increased as it is reflected by the increased collapse pressures of DPPC monolayers at the air/water interface (table 1).

At the phase transition highly ordered structures and less organized features have been directly observed in the pure DPPC monolayers (fig. 1). In the presence of procaine the condensed liquid domains (figs. 2 and 3) are smaller than in the pure DPPC monolayers. The results reveal some specific molecular interactions between these biologically relevant biocompounds in agreement with our previous thermodynamic studies [10, 11]. The different micro- and nano-structures visualized by AFM measurements on LB mixed films of DPPC and procaine are in substantial agreement with molecular interactions and with molecular structure of these biocompounds.

References


Fig. 3. AFM images of mixed DPPC and procaine monolayer transferred at the same lateral surface pressure of 8 mN/m on mica: (1): 2D-topography at the scanned area of 3 x 3 mm²; (2): Phase image; (3): 3D-topography corresponds to figure 3.1
15. FRANGOPOL, P. T., MIHAILESCU, D., Colloids and Surfaces B: Biointerfaces, 22, 2001, p. 3
26. HODA, K., IKEDA, Y., KAWASAKI, H., YAMADA, K., HIGUCHI, R., SHIBATA, O., Colloids and Surfaces B: Biointerfaces, 52, 2006, p. 57

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