THE LANGMUIR MONOLAYER: AN EFFICIENT MODEL FOR STUDYING INTERFACIAL PROPERTIES OF BIOMEMBRANES

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Received 09May 2012; accepted 11 May 2012.

1. Introduction

The Langmuir monolayer is a very suitable model for the study of self-assembly processes in two dimensions. The water phase provides ideally planar and smooth surface as a substrate. A pair of thermodynamic quantities – temperature and surface pressure – can be easily controlled. Surface pressure can be changed by moving barrier over the surface. Such mechanical compression, which is analogous to hydrostatic pressure in 3D systems, is not available in other 2D molecular systems and arrangements. In addition, the interaction between molecules in the monolayer can be systematically affected by chemical modification of their polar and nonpolar counterparts (e.g. the chain length can be varied by sub-nanometer steps) or by changes of pH or ionic strength and composition of the water subphase. This way of molecular nanotechnology evokes many interesting aspects of physics in two dimensions as well as some technological relevance. The Langmuir monolayers are also convenient model of biomembranes, because they can be considered as two superimposed lipid monolayers. The monolayer, being half a membrane, is a very wel-defined planar system to study intermolecular interactions between the membrane constituting molecules: between lipids and proteins or other compounds adsorbed on the membrane surface.

In this communication, we describe aspects of monolayer technology by focusing on effects of calcium ions on physical properties of phospholipid monolayers using results of measurements of surface pressure, x-ray reflectivity and AFM. These experiments are motivated by the search for lipid−DNA complexes with high transfection efficiency but without toxicity which might be a promising tool in gene therapy. In each part methodological importance is stressed and its specificity for studying molecular interactions at a lipid monolayer.

2. Materials and Methods

2.1 Chemicals

The phospholipid used was 1,2-dipalmitoyl-sn-glycero-3-phosphocholine mono-hydrate (DPPC) purchased from Sigma-Aldrich. The lipid was dissolved in chloroform at the stock concentration 0.5 mg/ml and spread on the subphase using a microsyringe (Hamilton, USA). As subphases, pure water (bidistilled deionized water, 18 MΩ·cm) and solutions of $CaCl_2$ (Sigma-Aldrich) were used; with pH ~ 7 and NaCl at a concentration of 5 mM. The subphase was thermostated at the temperature of 17 °C. The monolayer was allowed to

equilibrate and solvent to evaporate for 15 minutes. This time was sufficient for chloroform to evaporate and monolayer to stabilize.

Highly polymerized calf thymus DNA (Sigma Chemicals Co., USA) was dissolved in 0.5 mM NaCl, pH ~ 7, at concentration ~ 2 mg/ml. The precise value of the concentration was determined by measuring the absorbance A_λ at $\lambda = 260$ nm. The purity of DNA was checked by measuring the absorbance A_λ at λ = 260 nm and 280 nm, the ratio A_{260}/A_{280} = 1.8 was obtained [1].

2.2 Experimental methods

The methods of surface pressure measurements versus molecular area were chosen for investigations. The isotherms were measured during the continuous compression of the monolayer, using a computer–controlled Langmuir trough (model 611, Nima Technology, UK). The total working area of the trough was 600 cm^2 and the compression rate was 50 cm^2/min., which corresponds to 0.68 $Å^2$/s per one lipid molecule. The surface pressure-area isotherms were measured by the Willhelmy plate method, using Surface pressure sensor PS4 (Nima Technology, UK), with accuracy of 0.05 mN/m.

For further determination of structure, Langmuir-Blodgett (LB) films of DPPC bilayers were prepared by a deposition on silicon substrate. During the deposition, a surface pressure of 30 mN/m was maintained, *i.e.* the monolayer was in the LC state. Two different calcium ions concentrations in the subphase were used: 0 mM and 15 mM.

The LB films were analyzed by x-ray reflectivity. The data were collected on a Bruker D8 X-ray diffractometer by specular scans (□/2□) using Cu K□ radiation (λ=1.54 Å).

3. Results and discussion

Surface pressure – area isotherms recorded for various calcium ions concentrations in the subphase are shown in Fig. 1. The π - *A* isotherms are of a typical shape for DPPC on the

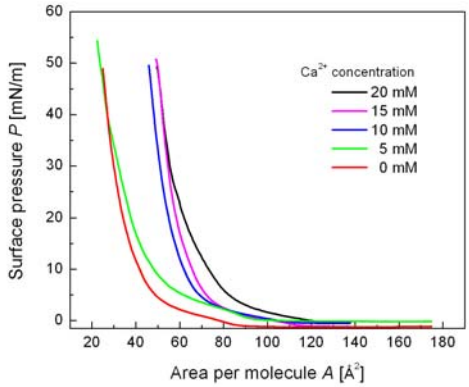

Fig. 1: *Surface pressure – area isotherms of DPPC monolayer. The individual curves correspond to various Ca^{2+} ion concentrations in the subphase (see the inset).*

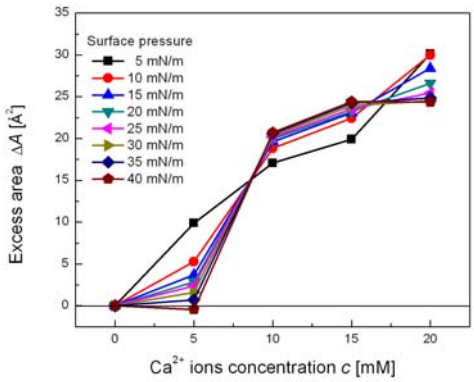

Fig. 2: *Excess area of DPPC monolayer as a function of Ca^{2+} concentration in the subphase.*

subphase at pH 7 [2]. It can be seen that monolayers of DPPC are in the LE phase even at a high area per molecule (>120$Å^2$) and at a surface pressure of $\pi \approx 10$ mN/m they turn into the solid phase.

The information about the structural and/or conformational changes, which are represented by the occupied area per molecule, may be obtained from the excess in surface area as a function of the molar ratio of calcium ions to DPPC. The excess area of monolayer ΔA is defined as

$$\Delta A = A_{Ca^{2+}} - A_{water} \quad (1)$$

where A_{water} and $A_{Ca^{2+}}$ represent the area per molecule at the surface pressure π of the monolayer situated on the pure water surface and on the calcium solution surface, respectively. Excess areas of the DPPC monolayer as dependences on calcium ion concentration are shown in Fig. 2. If no interaction of the ions with the monolayer occurred the excess area ΔA would be equal to zero. The weak condensing effect of the ions situated in the subphase on the molecules in the monolayer is observed at low concentrations, below 5 mM. By increasing the concentration the excess area passes into positive values, the area per a molecule in the monolayer in the presence of the ions in the subphase becomes larger due to the electrostatic repulsion of ions bound to the head groups of phospholipids.

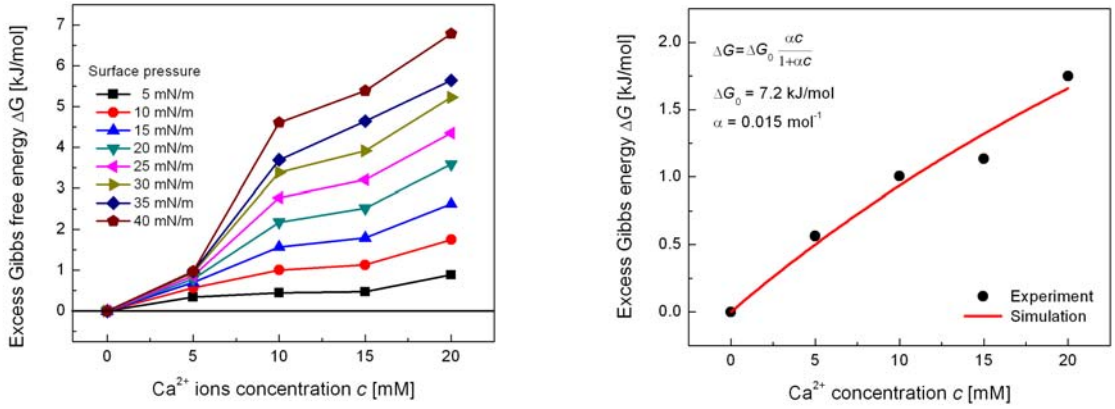

Fig. 3: *Excess Gibbs free energy vs. function of Ca^{2+} ions concentration (left panel) for various surface pressures. Fit of the Langmuir adsorption isotherm (right panel) for a surface pressure of 10mN/m.*

The stability of the monolayer can be also determined by evaluating excess Gibbs free energy [3] by integrating of the surface pressure – area isotherm up with respect to the π variable up to the selected surface pressure π of the monolayer situated on the pure water surface and the subphase containing calcium ions:

$$\Delta G = \int_0^{\pi} A_{Ca^{2+}} \mathrm{d}\pi - \int_0^{\pi} A_{water} \mathrm{d}\pi \quad (2)$$

The value of ΔG provides information whether the particular interaction is energetically favorable ($\Delta G < 0$) or not ($\Delta G > 0$), while $\Delta G = 0$ holds for non-interacting species. The value of ΔG as a function of calcium ions concentration for various values of surface pressure is shown in Fig. 3 (left panel).

For the evaluation of adsorption the Langmuir equation can be used. The coverage ratio Θ depends on the concentration of calcium ions c obeying the relation

$$\Theta = \frac{\alpha c}{1 + \alpha c} \quad (3)$$

α is the Langmuir constant. Then the excess of Gibbs energy represents a superposition of two energy states and can be expressed as

$$\Delta G = \Theta \cdot \Delta G_0 \quad (4)$$

where $\Delta G_0 = G_{Ca^{2+}} - G_{water}$. The agreement of model fit (ΔG_0 = 7.20 kJ/mol, α = 0.015 mM) with experiment is shown in Fig. 3 (right panel) for a surface pressure of 10 mN/m.

For X-ray reflectivity (/2 scan), a DPPC bilayer was deposited on the silicon substrate at the surface pressure of 30 mN/m. The ion concentration in the subphase was 15 mM. For determination of bilayer parameters, the Parratt32 program was used. A summary of parameters obtained for a DPPC bilayer is in Tab.1.

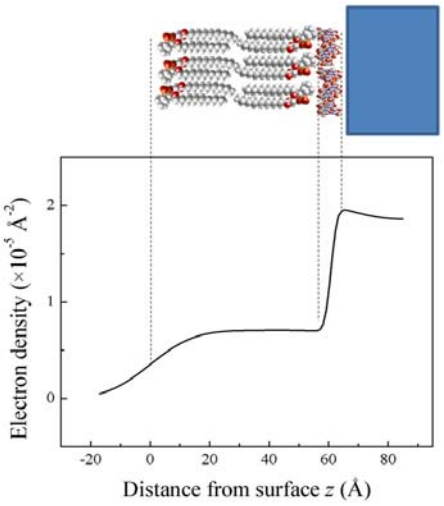

Fig. 4 *Electron density profile calculated on the basis of fitting the x-ray reflectivity pattern of DPPC bilayer formed on a subphase with Ca^{2+} ions concentration of 15 mM. Sketch of assumed structure is shown above.*

Tab. 1 *Parameters of layers as obtained from x-ray reflectivity*

	layer thickness (**Å**)	roughness (**Å**)
DPPC	60,78	11,613
DNA	8,09	1,667
SiO_2	-	7,702

We have shown the possibility of formation of the cationic lipid monolayer-DNA complex on a water surface. The increase in the area per a lipid molecule was documented in case if DNA molecules were added into the subphase. After the subsequent deposition of the monolayer onto a solid substrate (silicon wafer), x-ray reflectivity revealed an interlayer corresponding to a compact layer of DNA between the lipid layer and the substrate surface (Fig. 4). Its thickness was approx. 0.81 nm. Interestingly, the thickness of the DNA layer was markedly thinner than the geometry of the cylindrical DNA molecule. Thus, the anomalously thin thickness was due to some experimental treatments, such as deposition on a solid substrate and/or drying. The structure of the monolayer and monolayer-polymer complex on the solid substrates in a dried state is not the same as that on a water surface [4]. The possibility of some dynamic fluctuation in its structure may occur.

Acknowledgements
This publication originates from the project "National Center for Research and Applications of Renewable Energy Sources" (IMTS 26240120028) of Operational program Research and Development financed by European fund of Regional Development and the work was supported by the Scientific Slovak Republic grant agency VEGA, project No. 1/0879/11.

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