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Modification of structure and pattern of lipid monolayer on water and solid surfaces in presence of globular protein

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Abstract. Langmuir monolayers of phospholipids at the air-water interface are well-established model systems for mimicking biological membranes and hence are useful for studying lipid-protein interactions. In the present work, phases and phase transformations occurring in the lipid (DMPA) monolayer in the presence of globular protein (BSA) at neutral subphase pH (≈7.0) are highlighted and the corresponding in-plane pattern and morphology are explored from the surface pressure (π) – specific molecular area (A) isotherm, Brewster angle microscopy (BAM) and atomic force microscopy (AFM) both at air-water and air-solid interfaces. Films of pure lipid and lipid-protein complexes are deposited on solid surfaces by Langmuir-Blodgett method. Due to the presence of BSA molecules, phases and domain pattern changes in comparison with that of the pure DMPA. Moreover, accumulations of globular proteins in between lipid domains are also visible through BAM. AFM shows that the mixed film has relatively bigger globular-like morphology in comparison with that of pure DMPA domains. Combination of electrostatic and hydrophobic interactions between protein and lipid are responsible for such modifications.

Keywords: Lipid monolayer; Lipid-Protein complex; Langmuir-Blodgett films; phase transitions; BAM; AFM.
PACS: 68.47.Pe, 68.37.Ps, 68.18.Jk, 68.55.J-

INTRODUCTION

Cell membrane can be considered as two-dimensional sheet made up of lipid bilayer and proteins. Supported lipid bilayers and Langmuir monolayers are useful model systems to mimic such biological membranes [1]. Studies on lipid monolayer and its interaction with proteins are useful from both biological as well as physical point of view as physical phenomena differ from 2D to 3D. Proteins having heterogeneous surface charges can interact differently with membranes. They can get adsorb to the lipid headgroup region or partially penetrate to the hydrophobic core, however, in most of the cases it is observed that the protein interacts with both the hydrophilic headgroup and hydrophobic interior of the membrane. A suitable matching between the hydrophobic parts of membrane proteins and the surrounding lipid molecules is believed to play a significant role in forming the complex structures and controlling the biological activity of the proteins and lipid-protein complexes [2-8]. The presence of hydrophobic protein in a lipid layer can induce local segregation of proteins which is enveloped by appropriate lipids [4]. All these phenomena shows structural and morphological modifications in the lipid environment around the protein, which are reflected through various structural, conformational, mechanical and thermodynamic properties of the lipid membrane [3,4,7-9]. Phases and phase transitions of such 2D lipid layer on water in presence of proteins can be identified from surface pressure (π) – specific molecular area (A) isotherms. Variation of different physicochemical properties, such as surface pressure, temperature, pH, etc. explore different phases, phase transitions and domain patterns of lipid and lipid-protein complexes at the air-water interface which can be identified by Brewster angle microscopy (BAM), whereas, different morphologies formed on the deposited surfaces are obtained by atomic force microscopy (AFM).

In this article, phospholipid DMPA (1, 2-dimyristoyl-*sn*-glycero-3-phosphate) is used for preparing model membrane at air-water interface and lipid-protein complex was formed using globular protein BSA (bovine serum albumin). We have explored the phases and corresponding domain patterns in absence and presence of BSA at air-water interface and the corresponding morphology at air-solid interfaces. The reason of such domain pattern and morphology variations has been proposed.

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EXPERIMENTAL DETAILS

Bovine Serum Albumin (BSA) (catalog No. A2153) was purchased from Sigma-Aldrich. The isoelectric point of the BSA protein is ≈ 4.8. Phospholipid DMPA (catalog No. 830845P) was purchased from Avanti polar lipids. It has a net charge of -1.3 at pH ≈ 7.4. BSA solution of concentration 1 mg/ml was prepared in solution of Milli-Q water (resistivity 18.2 MΩ.cm) and DMPA solution of concentration 0.5 mg/ml was prepared in chloroform. Pure DMPA monolayer was formed after spreading desired amount of DMPA solution on water surface in a Langmuir trough (Apex Instruments). To form a complex monolayer desired amount of BSA solution was carefully spread with the help of a syringe on the water surface and after that equal volume of DMPA was spread on that. Any changes in the surface tension were well recorded through a Wilhelmy plate connected to an electro balance. Monolayers were compressed at a constant speed of 5mm/min for isotherm as well as for film deposition. Depositions were carried out at a speed of 2 mm/min. Prior to the deposition, Si (001) substrates were made hydrophilic after placing it in a mixed solution of ammonium hydroxide (NH_4OH, Merck, 30%), hydrogen peroxide (H_2O_2, Merck, 30%), and Milli-Q water (H_2O: NH_4OH: H_2O_2:: 2:1:1, by volume) for 5–10 minutes at 100˚C.

Domain patterns were collected by means of BAM (nanofilm_EP4). The instrument consists of a standard 50 mW solid state laser, emitting *p*-polarized light at a wavelength of λ ≈ 658 nm by use of a high-quality polarizer. The reflected light is imaged to a computer controlled high quality, monochrome GigE CCD camera through a 10x magnification objective, yielding a spatial resolution of ≈ 2 µm. Surface topography of all the deposited films were studied by AFM (NTEGRA Prima, NT-MDT Technology) in semi-contact mode using silicon cantilever having spring constant ≈ 11.8 N/m.

RESULTS AND DISCUSSION

Fig. 1 shows the π – *A* isotherms of pure DMPA and DMPA-BSA complex monolayers. DMPA on pure water shows a major transition at ≈ 5.0mN/m and the monolayer collapses at ≈ 51mN/m. Here it is seen that the gaseous (G) phase directly condenses to liquid condensed (LC) phase on compression without passing through the intermediate liquid expanded (LE) phase and finally through better ordered solid (S) phase monolayer collapse, i.e., two- to three-dimensional phase transition takes place. In case of DMPA-BSA complex monolayer, gaseous phase compressed into the LE phase with barrier compression and with further compression through the first order phase transition goes to the liquid condensed (LC) phase. The plateau in the isotherm corresponds to the LE and LC phase coexistence region. Actually, it is a pseudoplateau region formed in between 18 and 25mN/m. This pseudoplateau can be understood as a conformational change of the BSA molecules from an unfolded configuration with all amino acid segments located at the air/water interface, to a coiled configuration due to the folding of the amino acid chains with the polar groups of BSA immersed in the subphase and the hydrophobic regions oriented toward the air.

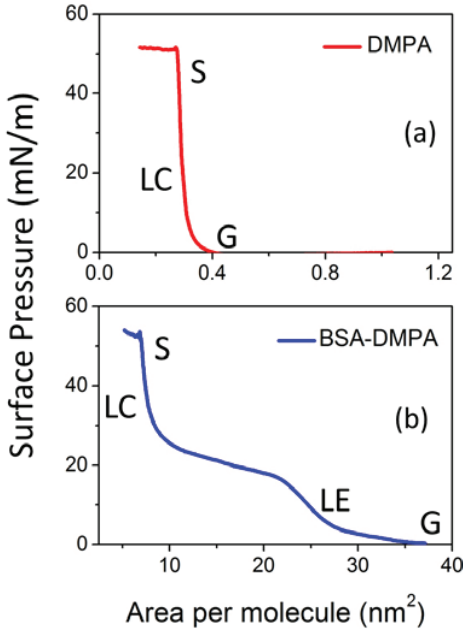

FIGURE 1. Surface pressure-film area (π-*A*) isotherms of (a) pure DMPA and (b) mixture of BSA-DMPA at the air - water surface.

BAM images of DMPA and DMPA-BSA complex monolayer on water surface at surface pressure of 20mN/m are shown in Figure 2 to show the variation of domain patters. The images obtained show flower-like small LC domains in case of the pure DMPA monolayer, but for DMPA-BSA complex monolayer it appears that all the domain gets surrounded by the proteins. Thus it has been observed that there is a structural as well as pattern modifications in the lipid environment in the presence of protein around their vicinity.

One-layer LB films of DMPA and DMPA-BSA were deposited on Si substrates, at a surface pressure of 20mN/m, and characterized with AFM. AFM

images depicting the surface topography of the DMPA and DMPA-BSA films are shown in Figure 3a and 3b respectively.

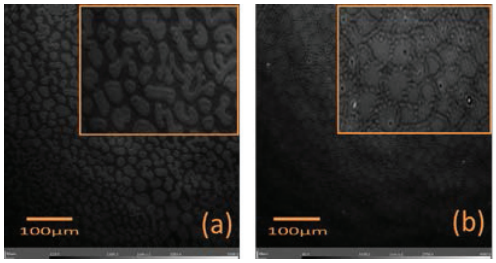

FIGURE 2. BAM images of (a) pure DMPA and (b) of DMPA-BSA complex monolayer at the air -water interface.

Line profiles are also shown in the insets of the corresponding figures to reveal the height information of the deposited films, which are nearly of 2 to 3 nm. Although, domain patter exists in optical length scale, but in micrometer length scale both pure DMPA and DMPA- BSA films show nearly smooth morphology, except some globular like features and the size of the globules for DMPA-BSA film is relatively bigger than that of pure DMPA. The average roughness of DMPA and DMPA-BSA complex film are nearly same (≈ 1Å).

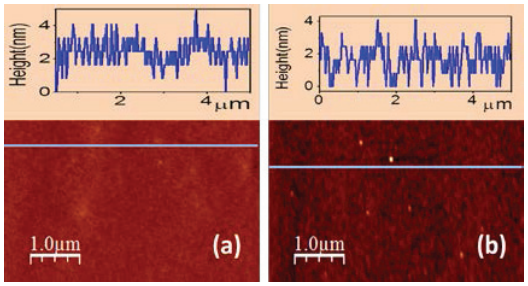

FIGURE 3. AFM images of (a) DMPA and (b) DMPA-BSA complex monolayer deposited at pH ≈ 7.0 on Si. Scan size: 5μm×5μm. Insets are for the corresponding line profiles.

CONCLUSIONS

To conclude, we have examined the lipid behavior with the introduction of the globular protein at neutral pH in a 2D system. We have shown from the isotherm and BAM images that DMPA structure and pattern gets altered from its usual in the presence of BSA on water surfaces. As of the case on solid surfaces AFM shows relatively larger globule structure for hybrid DMPA-BSA film compared to smooth DMPA film, hereby suggesting the modifications on solid surfaces too. The reason for such modifications is the combined effect of electrostatic and hydrophobic interactions between protein and lipid.

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