Surface pH induced hysteresis behavior of lipid (DMPA)-protein (BSA) complex monolayer

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Abstract. Compression-decompression cycles of the surface pressure (π) - specific molecular area (*A*) isotherms of lipid (DMPA)-protein (BSA) monolayers show pH dependent reversible/irreversible hysteresis. Below the isoelectric point of BSA (pH ≈ 4.0), i.e., when the protein molecules have effective positive charge and is weakly hydrophobic show reversible hysteresis whereas for pH above the isoelectric point of BSA (pH ≈ 7.0) when molecules have effective negative charge and relatively higher hydrophobicity show irreversible hysteresis indicating a permanent transformation in its morphology which was also evident from the Brewster angle microscopy (BAM). From FTIR spectra it is clear that the BSA protein in protein-lipid complex did not undergo any major conformational changes during the compression-decompression cycle for both the pH conditions. Competitive electrostatic and hydrophobic-hydrophobic interactions are probably responsible for such hysteresis behaviors.

INTRODUCTION

Proteins and lipids are the major constituents of biological membrane and the interfacial behaviors of protein depend on the presence of lipid at solution or at the interface [1]. It is well known that membrane constituents like lipid and protein can be organized in lateral micro-domain, reflecting region based functional specialization of different parts of the membrane [2]. Protein-lipid interaction forms the basis of numerous biological processes including intercellular communication, cell fusion, molecular recognition and cell adhesion. Protein-lipid interaction also influences taste, texture of food and stabilize colloidal structures [3]. Studies on protein-lipid interaction at air-water interface require apparatus with high resolution and sensitivity. Langmuir-Blodgett technique is one of the versatile tools to study model membranes in two-dimension. In Langmuir monolayer physiochemical properties such as temperature, pH, ionic strength, etc. can be tuned easily. Additionally, control over the variation of molecular area and surface pressure helps to have a specific molecular orientation, arrangement and structure of the films. Langmuir monolayers of phospholipids are well established model systems to study the biological membrane at air-water interface.

Proteins and lipid can interact in different ways. Protein can interact with hydrophobic tail or hydrophilic head of lipid. Proteins biological activity depends on suitable matching between hydrophobic cores of protein and surrounding lipids [4]. Physiochemical properties like pH also play a significant role in determining the hydrophobicity of the protein and for example bovine serum albumin (BSA) tends to be less hydrophobic in a strong acidic environment (pH < 4.0) compared to neutral (pH ≈ 7.0) or alkaline environment (pH ≈ 9.0) [5]. All these phenomenon leads to the structural modification by the lipid environment around the protein.

In this article we have used phospholipid DMPA (1, 2-dimyristoyl-sn-glycero-3-phosphate) and BSA protein to form the lipid-protein complex at air-water interface. The compression-decompression surface pressure (π) - specific molecular area (*A*) isotherms for lipid (DMPA)-protein (BSA) complex are obtained at two different subphase pH≈ 4.0 and 7.0 (i.e. below and above the isoelectric point of BSA). Comparative hysteresis behavior was obtained from the compression-decompression isotherm cycle. Brewster angle microscope (BAM) was used to visualize the

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monolayer at the air-water interface for different surface pressure conditions during the compression and expansion cycle. The attenuated total reflection–Fourier transform infrared (ATR-FTIR) peaks obtained in the amide-I band (1700–1600 cm^{-1}) were used to study the conformational modifications of protein in lipid-protein complex film at different subphase pH conditions.

EXPERIMENTAL DETAILS

Bovine Serum Albumin (BSA) (catalog No. A2153) was purchased from Sigma-Aldrich. Phospholipid DMPA (catalog No. 830845P) was purchased from Avanti polar lipids. The BSA and DMPA solutions were spread on the water surface contained in a double-barrier Langmuir trough made up of Teflon (Apex Instruments). Depositions were carried out at a constant speed of 2 mm/min. Before deposition, Si (001) substrates were properly cleaned and made hydrophilic. The pH of the water subphase was maintained at ≈ 4.0 and 7.0 for different experimental conditions.

Visualizations of BSA thin films at different subphase pH were performed by means of Brewster angle microscopy (BAM) using a nanofilm_EP4 BAM. The instrument consists of a standard 50 mW solid state laser, emitting *p*-polarized light at a wavelength of 658 nm using a polarizer. The reflected light coming from the water or BSA film surfaces are imaged to a computer controlled high-quality CCD camera which is attached to a real time frame grabber with 1392 × 1040 pixels through a 10x magnification objective, yielding a spatial resolution of ~2 μm. The attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy was also performed over the mixed films. Data were taken using spectrophotometer NICOLET 6700 (Thermo- Fisher) within the wavelength range of 380- 4000 cm^{-1} at 4cm^{-1} resolution.

RESULTS AND DISCUSSION

Compression-decompression cycles of DMPA-BSA complex at air-water interface are shown in Fig. 1(a) and (b) for pH ≈ 4.0 and 7.0 respectively, i.e., below and above the isoelectric point of BSA (*p*I ≈ 4.8). Here 'area per molecule' refers to the average area occupied by all the lipid and protein molecules. On water surface equal amount, i.e., 75 μl of DMPA and BSA molecules were spread having a concentration of 0.5 mg/ml and 1 mg/ml, one after another in a time lapse of about 10 minutes. At pH ≈ 4.0, surface pressure increases from $A \approx 65$ nm^2 and at pH ≈ 7.0, pressure starts to rise from $A \approx 53$ nm^2. Compression-decompression cycle shows that for pH ≈ 4.0 the surface pressure obtained during decompression nearly follows the compression path finally reaching the starting point introducing a small amount of hysteresis. However, in case of pH ≈ 7.0, compression-decompression cycle shows huge hysteresis and as a result of large gap exists between two *A* values at zero surface pressure, i.e., $A \approx 53$ nm^2 and ≈ 36 nm^2 are obtained at zero surface pressure. Hysteresis values are obtained from the compression-decompression isotherm cycles of the complex films, which are ≈ 17.9 × 10^{-21} N•m and 87.4 × 10^{-21} N•m for pH ≈ 4.0 and 7.0 respectively. Thus, below the isoelectric point of BSA (pH ≈ 4.0), when the net charge on BSA is positive and is weakly hydrophobic, nearly reversible hysteresis is observed but irreversible hysteresis is observed above the isoelectric point of BSA (pH ≈ 7.0), when BSA is negatively charged on average and relatively more hydrophobic.

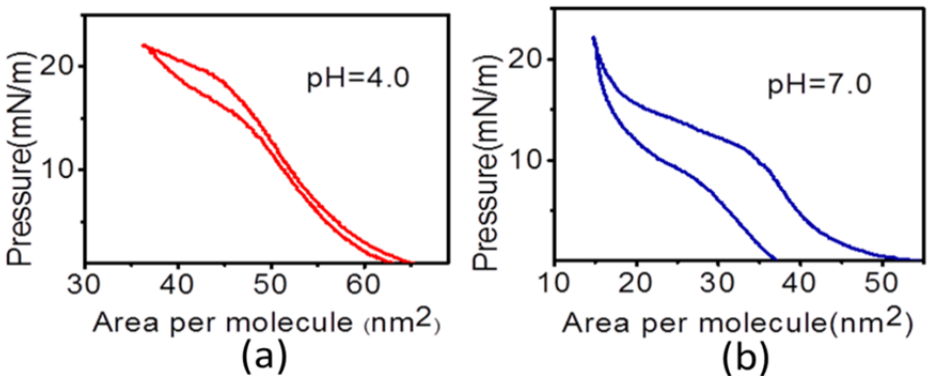

FIGURE 1. Compression-decompression isotherm cycle of DMPA-BSA complex at (a) pH ≈ 4.0 and (b) pH ≈ 7.0.

Figure 2 shows the BAM images at two different pH conditions for the DMPA-BSA complex., i.e., at pH ≈ 4.0 and ≈ 7.0 during the first and second compression at a surface pressure of 10 mN/m. Image obtained for pH ≈ 4.0 during the first compression, i.e., Fig. 2(a) shows domains those are linked together and within the domains the presence of globule-like dots probably suggest the aggregation of proteins. While during the second compression at the same surface pressure (10 mN/m) the domains seem to retain the confined protein within themselves, however the compactness of the domains is enhanced as shown in Fig. 2(b).

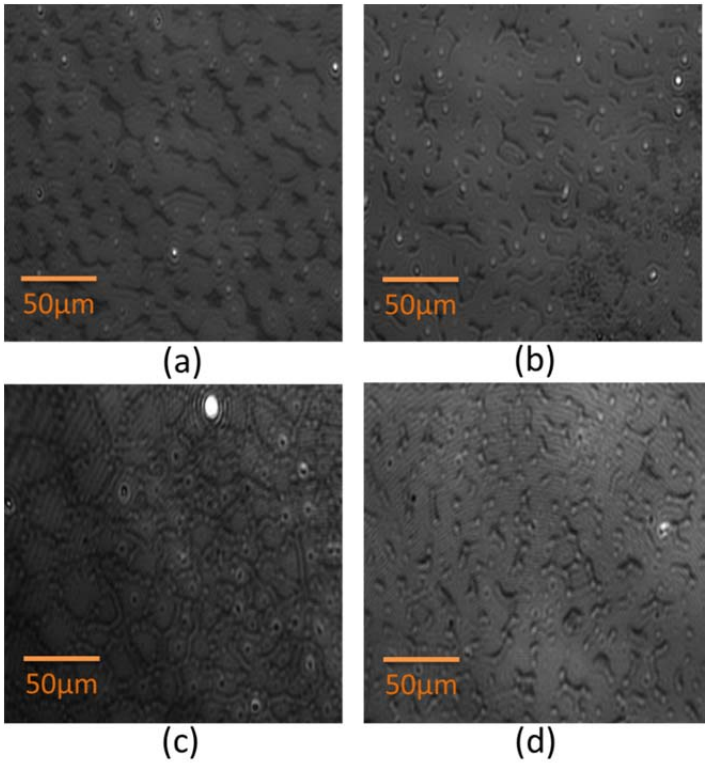

FIGURE 2. BAM images of DMPA-BSA complex monolayers during first compression and second compression at lower surface pressure ($\pi \approx 10$ mN/m) at two different pH values, i.e., at pH ≈ 4.0 (a and b) and 7.0 (c and d).

On the other hand, at pH ≈ 7.0, during the first compression, domains are surrounded by the protein aggregates forming a continuous network at a surface pressure of 10 mN/m as shown in Fig. 2(c), whereas Fig. 2(d) shows that during the second compression at the same surface pressure hardly any domains are visible and the film appeared to be compact with the embedded proteins along with the irregular cleavages.

Conformations of proteins inside lipid-protein mixed film are identified from the ATR-FTIR peaks occurring in the amide-I band (1700–1600 cm^{-1}) as it is considered as the most sensitive spectral region for obtaining secondary structure information of proteins. Within amide-I band 14 peaks are observed as shown in Fig. 3. Peaks found at 1623, 1627, 1636 and 1697 cm^{-1} are related with beta sheet and the peaks found at 1669, 1674, 1684 and 1687 cm^{-1} are related to beta turn. Other peaks observed at1603, 1607, 1615, 1646, 1654 and 1662 cm^{-1} correspond to side chain vibration, intermolecular beta strand, intra molecular beta strand, random coil, alpha helix and 310 helix respectively. For both the first and second compression at 10 mN/m surface pressure, the peak positions remain unchanged indicating that the same secondary structures are present during first and second compressions, however, their relative amount varies.

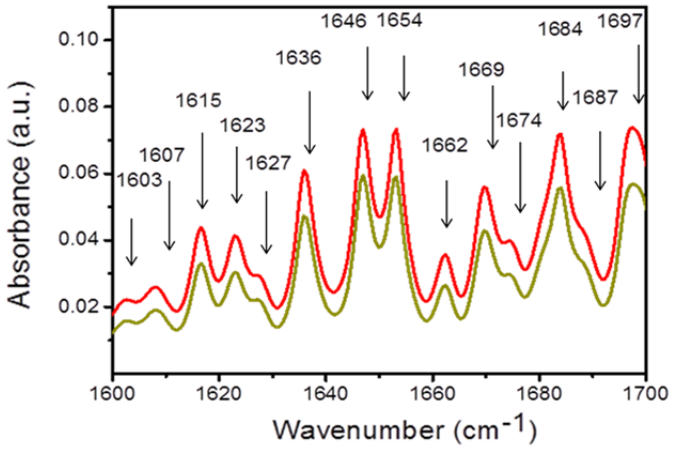

FIGURE 3. ATR-FTIR spectra of amide-I region obtained from BSA and DMPA mixed films during first (red) and second (dark yellow) compression at 10 mN/m surface pressure at pH ≈ 7.0. Arrows indicate the corresponding peak positions.

Nearly the similar information is obtained about protein secondary structures from the lipid-protein mixed film at pH ≈ 4.0. This work thus shows that in addition with the surface pressure, subphase pH also plays a significant role in determining the mutual lipid-protein interactions which modifies the physiochemical properties of the lipid-protein mixed film.

CONCLUSION

Hysteresis behaviors of lipid-protein (DMPA-BSA) thin films are obtained for two different subphase pH conditions (pH ≈ 4.0 and 7.0) at air-water interface. DMPA-BSA complex monolayer shows reversible hysteresis at pH ≈ 4.0, i.e., below the isoelectric point of BSA, when the net charge on BSA is positive and is weakly hydrophobic, but it demonstrate irreversibility in the hysteresis at pH ≈ 7.0, i.e., above the isoelectric point of BSA, when BSA is negatively charged on average and relatively more hydrophobic. Thus, the pH dependent hydrophobic and electrostatic nature of BSA is crucial for showing the reversible/irreversible hysteresis behaviors of lipid-protein complex thin films.

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