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Monolayer Behavior of Human Serum Albumin (HSA) at Air-Water Interface

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Abstract. Langmuir monolayers of human serum albumin (HSA) are formed on water surface at neutral pH (≈ 7.0) in absence and presence of Ca^{2+} ions in aqueous subphase. Compression-decompression surface pressure (π) - specific molecular area (A) isotherm cycle for both the conditions are recorded to compare the corresponding hysteresis behaviours. It is seen from the isotherms that in pure water compression curve of HSA monolayer tries to follow the decompression curve, whereas it is almost similar in presence of Ca^{2+} ions. Topographical features of the HSA monolayer at the air-water interface are obtained from Brewster angle microscopy (BAM). Protein films are also deposited in Si (001) substrates at lower and higher surface pressures, i.e., at 5 and 18 mN/m and are investigated by Atomic Force Microscopy (AFM) to explore out-of-plane structure and surface morphology. Although nearly homogeneous layer is formed by the HSA protein at the air-water interface before and after interaction with calcium ions, but slight conformation variation of protein takes place in presence of ions and accordingly the elastic behavior of monolayer changes under mechanical compression and expansion.

INTRODUCTION

Proteins are biomolecules which play an important role in living organisms. Behaviour of proteins and their responses can be controlled by varying the pH, temperature, strength of ions, addition of various additives, etc. as their functions are structure dependent. In addition with different biological functions proteins can also be used as an emulsifier in food industry with many other practical applications. Adsorption of proteins on various interfaces have found its applications in the manufacturing of medical devices, food processing, drug delivery, biosensors, chromatographic purifications of various drugs, peptides and antibodies, etc [1-3]. Globular proteins, which are soluble in water, can form thick monolayer by adsorption at the air-water interface. Spreading of protein solution on water surface [4] or injection of protein molecules into the aqueous subphase [5] results in the formation of protein films at the air-water interface. Protein films can be deposited on solid substrates by using various techniques like self-assembly, sol-gel process, drop casting, Langmuir-Blodgett (LB) technique, etc. [6, 7]. Among all these techniques, LB method is widely used as this technique enables precise control over the monolayer structure, homogenous deposition over substrates, multilayer structures with varying thickness, etc.

Among all the proteins, globular protein human serum albumin (HSA) is found abundantly in blood plasma (typical concentration of 42g/L). HSA structure consist of nine loops having 17 disulphide bridges which forms a repeated pattern of 3 alpha-helical homologous domains and these are numbered as I-II-III from the amino terminus. Various physiological and pharmacological functions like pH and osmatic blood pressure are maintained by HSA [8, 9]. On the other hand, it has a capacity to bind different substances, metabolism, distribution and excretion of drugs. Interaction of protein-lipid complex with ions such as Ca^{2+} is well-known. Presence of calcium in the lung surfactant may affect the function and structure of the surfactant. The lipid-protein interaction in the surfactant lipoprotein complexes may also be affected by the presence of Ca^{2+} ions through changes in its conformation, charge and hydration state of the polar headgroups of the phospholipids [10]. Neutralization of

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surface charges of lipoprotein vesicles and hence modification of electrostatic forces between vesicles in the subphase and surface film is also an act of Ca^{2+} ions.

In the present article, by compression and decompression cycle of the surface pressure (π)–specific molecular area (A) isotherms, the reversible/irreversible behaviour of HSA monolayer are shown both in pure water and in presence of Ca^{2+} ions at neutral pH ≈7.0. The film patterns on water surface are investigated through Brewster Angle Microscopy (BAM) at different surface pressures. Atomic Force Microscopy (AFM) is used to study the surface morphologies of the protein films deposited on Si (001) substrates. Isotherm nature, hysteresis behaviours of protein layer, and its structure and pattern in presence of Ca^{2+} ions are also explored.

EXPERIMENTAL DETAILS

Human Serum Albumin (HSA) (catalog No. A3782) was purchased from Sigma-Aldrich. The average molecular weight is about ~66.5 kDa and it was used as received without any purification. The isoelectric point of the protein is approximately 4.7. HSA solution of concentration 1 mg/ml was prepared in Milli-Q water (resistivity 18.2 MΩ.cm). A volume of HSA solution was carefully spread with the help of a syringe onto the subphase of Milli-Q water in a properly cleaned double-barrier Langmuir trough made of Teflon (Apex Instruments). Any changes in the surface tension were well recorded through a Wilhelmy plate connected to an electro balance. Before compression, the system was allowed to attain some equilibrium. All through the experiment monolayers were compressed at a constant speed of 5mm/min for isotherm measurements. Depositions were carried out at a speed of 2 mm/min. Prior to the deposition, the nature of Si (001) substrates were made hydrophilic after placing 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.

Monolayer/multilayer visualization at air-water interface was done 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 λ =658 nm by using of a high-quality polarizer. The reflected light is imaged to a computer controlled high quality, monochrome GigE CCD camera with 1392 × 1040 pixels through a 10x magnification objective, yielding a spatial resolution of ~2 μm. A black wedge-shaped glass plate is placed at the bottom of the trough to reflect any light transmitted through the subphase out of the optical axis and to minimize the convection on the trough. In-plane surface morphology and film thickness of the deposited films were obtained through atomic force microscope (AFM) (NTEGRA Prima, NT-MDT technology) in semi-contact mode using silicon cantilever having spring constant of ≈ 11.2 N/m.

RESULTS AND DISCUSSION

The successive compression and decompression surface pressure-mean molecular area (π-A) isotherms obtained from the HSA monolayer formed at the air-water interface in pure water and in the presence of Ca^{2+} ions (1.4 mM/L concentration) at neutral pH ≈ 7 are shown in the Fig. 1. From the isotherms it is seen that the surface pressure starts to increase nearly at A ≈ 50 nm^2 for pure water as well as for the subphase containing Ca^{2+} ions. A plateau-like feature is observed for each isotherm nearly at 13 mM/m in both the cases. Such plateau-like feature is a confirmation of the fact that a structural rearrangement process is taking place around that point.

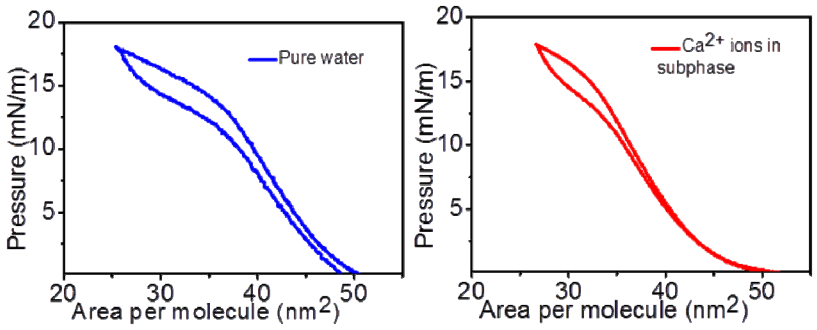

FIGURE 1.Compression-decompression isotherm cycle of HSA monolayer at pH ≈ 7.0 (a) in pure water and (b) in presence of Ca2+ ions in water subphase.

With increase in surface pressure or barrier compression the HSA molecules come closer to each other and hence molecular packing starts to form on the air-water interface. On the other hand the surface pressure starts to decrease with decompression and try to follow the compression path by introducing a small amount of hysteresis. A close look into the isotherm suggests that in case of pure water subphase the compression and decompression curve is separated by a small distance indicating small structural modification. But in presence of calcium ions the decompression curve merges over the compression curve indicating the occurrence of negligible changes in the monolayer.

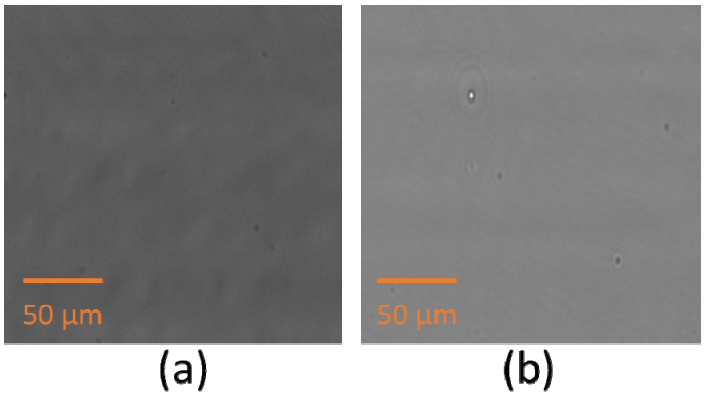

FIGURE 2. BAM images of HSA monolayer at (a) lower pressure ($\pi \approx 5$ mN/m) and (b) higher pressure ($\pi \approx 18$ mN/m) at pH ≈ 7.0.

Hysteresis obtained from the compression-decompression cycle for pure water subphase is nearly 33.37×10^{-21}N•m and in the presence of Ca^{2+} ions it was found to be 17.0×10^{-21}N•m.Figure 2 shows the BAM images of the HSA monolayer at the air–water interface. Large numbers of images are collected but only two selected images are shown as most of them are closely similar. HSA monolayer did not show any variation in presence of calcium ions. In both the cases (pure water and in presence of Ca^{2+} ions) homogeneous adsorbed films are observed at the air-water interface. However with surface pressure variation the intensity of the reflected laser light changes, which implies the thickness variation of the monolayer. Fig. 2(a) and 2(b) shows the HSA monolayer at lower ($\pi \approx 5$ mN/m) and higher ($\pi \approx 18$ mN/m) pressures respectively.For high pressure the observed intensity is higher in comparison to its lower pressure counterpart, which indicates that the film thickness is increased with increasing surface pressure. Probably such thickness increment is related with the molecular tilting.

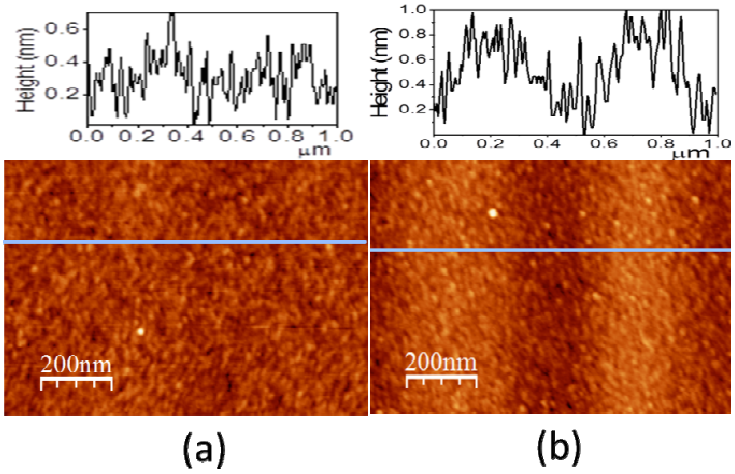

FIGURE 3. AFM images of HSA films deposited on Si surfaces at two different surface pressure values, i.e., at (a) $\pi \approx 5$ mN/m (lower pressure) (b) $\pi \approx 18$ mN/m (higher pressure). Scan size: 1µm x 1µm. Insets are for the corresponding height profiles.

Protein films are also deposited from air-water interface on hydrophilic Si (001) substrates in single upstroke to study the out-of-plane structures. The transfer ratios of all the deposited films are within the range of 0.75-1.10 which implies the satisfactory transfer of HSA molecules from the subphase surface to the solid substrates. Fig. 3 shows the AFM images depicting the topography of the HSA film at lower ($\pi \approx 5$ mN/m) and higher ($\pi \approx 18$ mN/m) pressures at pH ≈ 7.0. AFM images show homogeneously distributed protein films which is well in agreement with the BAM images. For lower pressure the average height and roughness are nearly about 0.52 nm and 0.12 nm respectively, however, for higher pressure the average height and roughness becomes 0.83 nm and 0.21 nm respectively. Similar images are obtained from the HSA films in presence of Ca^{2+} ions in the water subphase. Our study thus shows that although nearly homogeneous layer is formed by the HSA protein at the air-water interface both in absence and presence of calcium ions, but slight conformation variation of protein takes place in presence of ions and accordingly the monolayer behaviours changes under mechanical compression and expansion.

CONCLUSIONS

Hysteresis behaviours of HSA Langmuir monolayers at neutral pH (≈ 7.0) both in absence and presence of Ca^{2+} ions are studied. HSA molecules shows complete reversible hysteresis in presence of Ca^{2+} ions, whereas in pure water it shows less hysteresis as the compression and decompression path is slightly separated from each other indicating a small modification in its conformation. BAM and AFM images show the formation of homogeneous thin films at the air-water interface both in presence and absence of calcium ions. Thus, the minute variations in HSA protein layer after interaction with Ca^{2+} ions are visible only by surface pressure measurement through mechanical compression and decompression.

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