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Cite as: AIP Conference Proceedings **2265**, 030266 (2020); https://doi.org/10.1063/5.0017722
Published Online: 05 November 2020

Bijay Kumar Sah, and Sarathi Kundu

Surface Pressure and pH Dependent Structure and Optical Emission Behaviors of Protein (BSA) Thin Films

Bijay Kumar Sah[a)] and Sarathi Kundu

Soft Nano Laboratory, Physical Sciences Division, Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragoan, Garchuk, Guwahati, Assam 7810035, India.

[a)]Corresponding author: way2bksah@gmail.com

Abstract. Langmuir monolayers of Bovine serum albumin (BSA) are formed at the air-water interface nearly at (pH ≈ 5.0) and below (pH ≈ 4.0) the isoelectric point (pI ≈ 4.8) of BSA. The thin films are deposited on Si (001) substrates for different physiochemical conditions using Langmuir–Blodgett method. Photoluminescence spectroscopy is used to study the optical emission behavior of deposited BSA thin films. In-plane morphology and out-of-plane structure are obtained from the atomic force microscopy and X-ray reflectivity respectively from the deposited protein thin films. Below the isoelectric point of BSA, i.e., when BSA possesses some net surface charge, it exhibits reversible structural and optical emission behaviors. But at isoelectric point, i.e., when net surface charge on BSA is nearly zero the reversibility behaviour ceases.

INTRODUCTION

Proteins have gained lots of attention in the field of material science as its properties can be altered or tuned by varying the external physiochemical conditions [1]. Proteins are considered as unique biopolymers as they display intrinsic fluorescence [2]. The amino acids responsible for the proteins fluorescence nature are tryptophan, tyrosine and phenylalanine as they are capable of absorbing and emitting light [3]. Among the three amino acid residues, tryptophan emission shows most intense fluorescence and also it is highly correlated with its local environment. Physiochemical parameters such as pH condition, substrate binding, ionic environment, solvent polarity, etc. interfere with the microenvironment surrounding the tryptophan residues and hence it shows change in the protein emission [3]. Studies have shown that fluorescence emission from the tryptophan modifies due to the interaction of the protein molecules with different types of compounds [4-6]. And any conformational change in protein structure can be indirectly monitored through the fluorescence emission spectra [7].

Protein adsorption on solid surfaces has wide range of biomedical and industrial applications such as food processing, drug delivery and bio molecular devices [8]. Protein molecules in thin film configuration are arranged in a two-dimensional (2D) sheet of interacting particles with properties such as enhanced thermal and chemical stabilities [9]. Although there are numerous approaches to prepare protein thin films, but the Langmuir-Blodgett (LB) technique is one of the promising tools for building protein thin film as it can precisely control many parameters having high degree of order on desired substrates. Bovine serum albumin (BSA) is a globular protein, has two tryptophan residues and it has an oblate ellipsoidal shape of dimensions 39Å × 39Å × 9Å [10]. Isoelectric point of BSA is ≈ 4.8 [9], therefore, above and below the isoelectric point it possesses a net negative and positive surface charge respectively. It is well known that BSA forms monolayer at air-water interface but the surface pressure dependent structures and corresponding optical behavior is yet to be explored.

In this article we have examined the structural and optical behaviour of BSA thin film at and below the isoelectric point of BSA under different surface pressure and monolayer compression conditions. Photoluminescence spectroscopy is used to study the optical properties of BSA thin films. In-plane morphology and

DAE Solid State Physics Symposium 2019
AIP Conf. Proc. 2265, 030266-1–030266-4; https://doi.org/10.1063/5.0017722
Published by AIP Publishing. 978-0-7354-2025-0/$30.00

out-of-plane structure are obtained from the atomic force microscopy (AFM) and X-ray reflectivity (XRR) respectively.

EXPERIMENTAL DETAILS

BSA (catalog No. A2153) is purchased from Sigma Aldrich and used without any further purification. BSA solution of concentration 1 mg/ml is prepared. The BSA solution is spread on the water surface containing double-barrier Langmuir trough (Apex Instruments). Depositions were carried out at a constant speed of 2 mm/min using Langmuir – Blodgett (LB) method. Before deposition, Si (001) substrates were properly cleaned and made hydrophilic. The pH of the water subphase was maintained at ≈ 4.0 and 5.0 for different experimental conditions respectively. The fluorescence emission spectra is taken by JASCO FP-8500 fluorescence spectrofluorometer recorded in the range 300 – 600 nm with the excitation wavelength of 273 nm for BSA. The slit width used for both excitation and emission monochromator is 5 nm. Surface topography was studied through an atomic force microscope (NTEGRA Prima, NT-MDT Technology). All the scans were taken with scan area of 5 μm × 5 μm in semi-contact mode using silicon cantilever having spring constant of ≈ 11.8 N/m. X-ray reflectivity (XRR) of BSA thin film was taken using an X-ray diffractometer (XRD) setup. Diffractometer (D8 Advanced, Bruker AXS) has a copper (Cu) source sealed in a tube followed by a Göbel mirror for the selection and enhancement of the Cu K_α radiation (= 1.54 Å). NaI scintillation detector was used for detecting the scattered beam. Data were taken in specular condition. And as result a non-vanishing wave-vector component (q_z) exists which is given by ($4\pi/\lambda$) sinθ. Analysis of XRR data was pursued using Parratt's formalism. However, to analyze the XRR data, surface and interfacial roughness's have been included. XRR data provides the electron-density profile (EDP) which is in-plane (x–y) average electron density (ρ) as a function of depth (z) with high resolution.

RESULTS AND DISCUSSION

BSA thin films at air-water interface are prepared for two different pH conditions, i.e., at pH 4.0 and 5.0 respectively. The thin films were deposited on the Si (001) substrate using the LB technique. During the first compression of the BSA monolayer, the films were deposited at and 19 mN/m surface pressures. After the decompression process the BSA film was again compressed up to the surface pressure of 5 mN/m and the deposition was carried out at this condition.

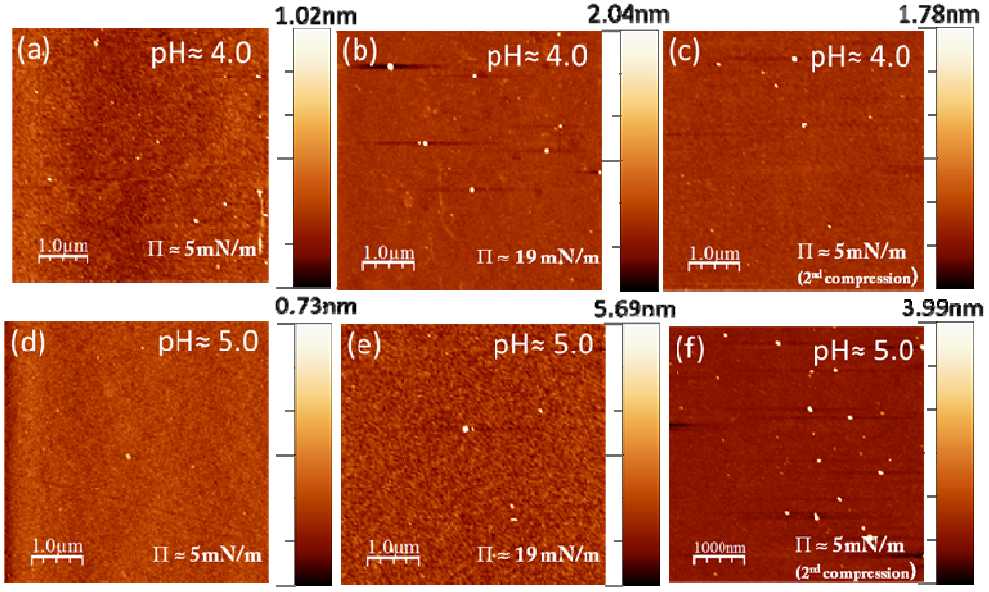

FIGURE 1.AFM images of BSA films deposited on Si surfaces at two different pH values, i.e., at pH ≈ 4.0 (a-c) and 5.0 (d-f). Scan size: 5μm x 5μm. Corresponding z- scale is also shown along with the images.

Fig. 1 shows the surface morphologies of the BSA thin films deposited at pH ≈ 4.0 (a-c) and 5.0 (d-f) respectively. Fig. 1(a, d) and (b, e) shows the film morphology for the lower (5 mN/m) and higher (19 mN/m) pressures during the firstcompression, while Fig. 1(c, f) shows for the lower pressure (5 mN/m) during the second compression.Irrespective of the pH conditions, the surface shows smooth morphology for the pH, surface pressure and compression cycle conditions. From the z scale profile it is evident that for higher surface pressure the thickness of the film is relatively higher compared to the lower pressure conditions. The average surface roughness of the film varies from 1.5 to 10 Å.

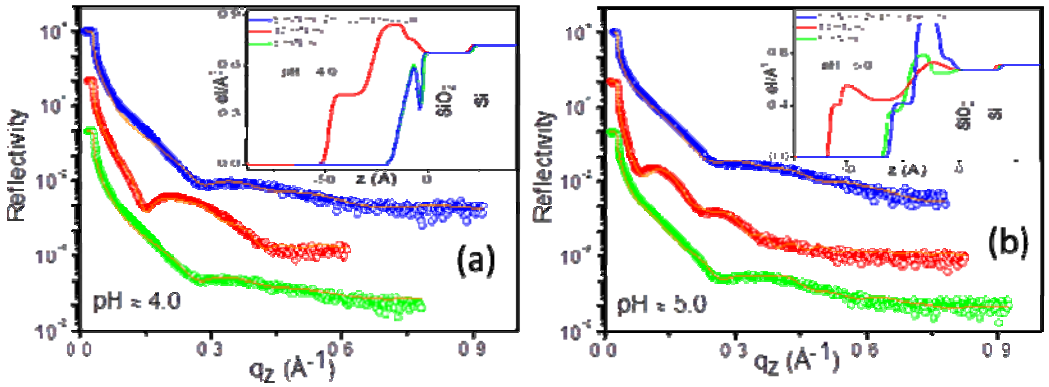

FIGURE 2.X-ray reflectivity data (circle) and the corresponding fitted curves (solid line) obtained from BSA films deposited at pH ≈ 4.0 (a) and 5.0 (b) for three different surface pressure condition 5 mN/m (green), 19 mN/m (red) during first compression and 5 mN/m during second compression (blue). Inset: corresponding electron density profiles extracted from the fitting of the reflectivity data.

To obtain the out of plane structures of the BSA films deposited on the Si (001) substrates, XRR is performed over the films. Fig. 2 shows the XRR data (open circles) and the corresponding fitted curves (solid lines) for pH ≈ 4.0 (a) and 5.0 (b) respectively. The insets show the corresponding electron density profiles (EDPs), which is obtained by fitting the XRR data. EDP at lower surface pressure shows that BSA molecule forms monolayer such that at pH ≈ 4.0 the semi major axis of the BSA molecules arrange themselves parallel to the Si (001) substrate. But for pH ≈ 5.0, the molecules form monolayer in a tilted configuration. At higher surface pressure, EDPs show that for pH ≈ 4.0 a dense monolayer is observed such that the molecules are tilted to the substrate surface but in case of pH ≈ 5.0 a bimolecular layer is observed, in relatively more tilted state. After full decompression to a 0 mN/m, again when the BSA film is compressed to a surface pressure of 5 mN/m then BSA achieves a parallel configuration as before for pH ≈ 4.0. However, for pH ≈ 5.0, the bilayer does get transformed to monolayer but it could not completely get back to its initial tilting state. Thus, a permanent structural transformation is seen depending on the surface pressure and subphase pH conditions.

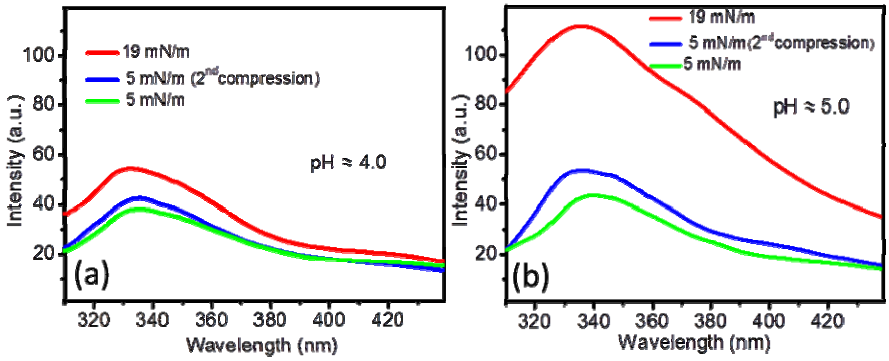

FIGURE 3. Emission spectra of BSA thin film at two different pH values, i.e., at pH ≈ 4.0 (a) and 5.0 (b) for three different surface pressure conditions: 5 mN/m (green), 19 mN/m (red) during first compression and 5 mN/m during second compression (blue).

Fluorescence emission spectra of BSA thin film at pH ≈ 4.0 and 5.0 are shown in Fig. 3(a) and (b) respectively. The emission peak is observed in the range between 335 to 340 nm for all the BSA thin films. Fig. 3(a) shows that the emission spectra for lower surface pressure (5 mN/m) in nearly same for first and second compression, however there is a slight increase in the intensity of the emission spectra for higher surface pressure. On the other hand, for pH ≈ 5.0, an increase in the peak intensity is observed at lower surface pressure during the first and second compression of the film. Moreover, at higher surface pressure the peak intensity significantly rises obtaining roughly twice the value to that of the lower pressure counterparts. These results too come in agreement with the XRR and AFM results. Hence at pH ≈ 4.0 when BSA acquires positive surface charge, under sufficient compression, it undergoes some change in its structure, however, due to the electrostatic-electrostatic interaction BSA restores to its initial state. But as the surface charge is neutral in case of pH ≈ 5.0, the molecules could not attain its initial organization, once the molecule gets structurally transformed under external surface pressure. Thus, BSA structural and optical behaviors in thin film configuration are dependent on the subphase pH as well as on the surface pressure conditions.

CONCLUSIONS

Langmuir thin films of BSA are studied at two different subphase pH conditions, at and below the isoelectric point of BSA, i.e., at pH ≈ 5.0 and 4.0 respectively. Protein molecules in thin film configuration show reversible structural behaviour in presence of net surface charge on the molecule due to the electrostatic interaction. The optical emission behaviour also dictates the similar behaviour. However, this behavior vanishes for the neutral surface charge condition of the BSA molecules. Thus, BSA thin film displays structural and optical emission behaviours depending on the surface pressure and subphase pH conditions.

ACKNOWLEDGMENT

The work is supported by Department of Science and Technology (DST), Nano Mission, India (Grant No.SR/NM/NS-1035/2013(G)).B.K.S. acknowledges Council for Scientific and Industrial Research (CSIR), Govt. of India for CSIR-SRF fellowship (Grant No: 09/ 835(0027)/2019-EMR-I).

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