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AFM investigation of the adsorption process of bovine serum albumin on mica

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Abstract

Bovine serum albumin molecules have been adsorbed onto mica surfaces from aqueous solutions at pH 3 and 6. The adsorbed surfaces prepared at different adsorption times have been observed by atomic force microscopy. At pH 3, monolayer adsorption proceeded with time, and almost all of surface was covered after 20 min. Although the adsorption at pH 6 was monolayer at first, multilayer adsorption proceeded after 1 min. The adsorbed molecules arranged "side-on" at both pH values. © 1997 Elsevier Science B.V.

Keywords: Atomic force microscopy; Bovine serum albumin; Mica; Protein adsorption

1. Introduction

The investigation of protein adsorption and interactions at interfaces is important medically, biotechnologically, and industrially. In particular, blood protein adsorption is focussed on in relation to blood clotting. The adsorption on solid surfaces from dilute aqueous solutions of serum albumin, which is one of the main proteins in blood, has been investigated using scanning tunneling microscopy (STM) [1], surface force measurement [2–4], energy-resolved X-ray photoelectron spectroscopy (XPS) [3], adsorption kinetics [5,6], and time-resolved evanescent wave-induced fluorescence spectroscopy [7].

The bovine serum albumin (BSA) molecule has ~66 000 molecular weight. The BSA molecule in water takes on a prolate ellipsoidal shape with a 116 Å long axis and a 27 Å short axis [3]. The molecular chain is held by intramolecular bridges of disulfide linkages, forming three domains [8]. Human serum albumin, which has a similar molecular weight and structure to BSA, adsorbed on solid surfaces. Adsorbed proteins were observed by STM [1]. The size of the adsorbed molecule was consistent with the theoretical one in a direction horizontal to the solid surface, although the height from the surface was not deduced. The adsorption kinetic data of protein molecules on the surface were analyzed by a two-step mechanism [6]. BSA adsorption was also discussed in relation to the molecular orientation [2–4]. BSA molecules adsorb on the solid surface by two kinds of orientation, so-called "end-on" and "side-on" with major and minor axes, respectively, perpendicular to the surface. However, which is the more likely is not yet elucidated, although both orientations have been suggested by different groups [2–4].

In this work, BSA molecules are adsorbed on a mica surface, and the adsorption process and the

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surface profile are examined by atomic force microscopy (AFM). The BSA orientation and its adsorption coverage are discussed in relation to adsorption time and solution pH.

2. Experimental section

BSA was purchased from Sigma Chemical Co. Mica was a product of RADD Research Industry Co. Ltd. Water was redistilled and regassed as usual. Aqueous BSA solutions of 0.002 mg cm^{-3} were prepared at pH 3 and 6. The solution pH was adjusted by using a standardized 0.1 N HCl solution.

Freshly cleaved mica was dipped into an aqueous BSA solution. The mica was picked out after constant adsorption times of 0.5, 1, 5, and 20 min. After residual water was drained from the mica surface, the mica was dried for more than 3 h in vacuo.

AFM observation was carried out in the tapping mode on a Digital Instruments Nanoscope III at room temperature (~25°C). Vertical distances and surface roughness were measured by section analysis.

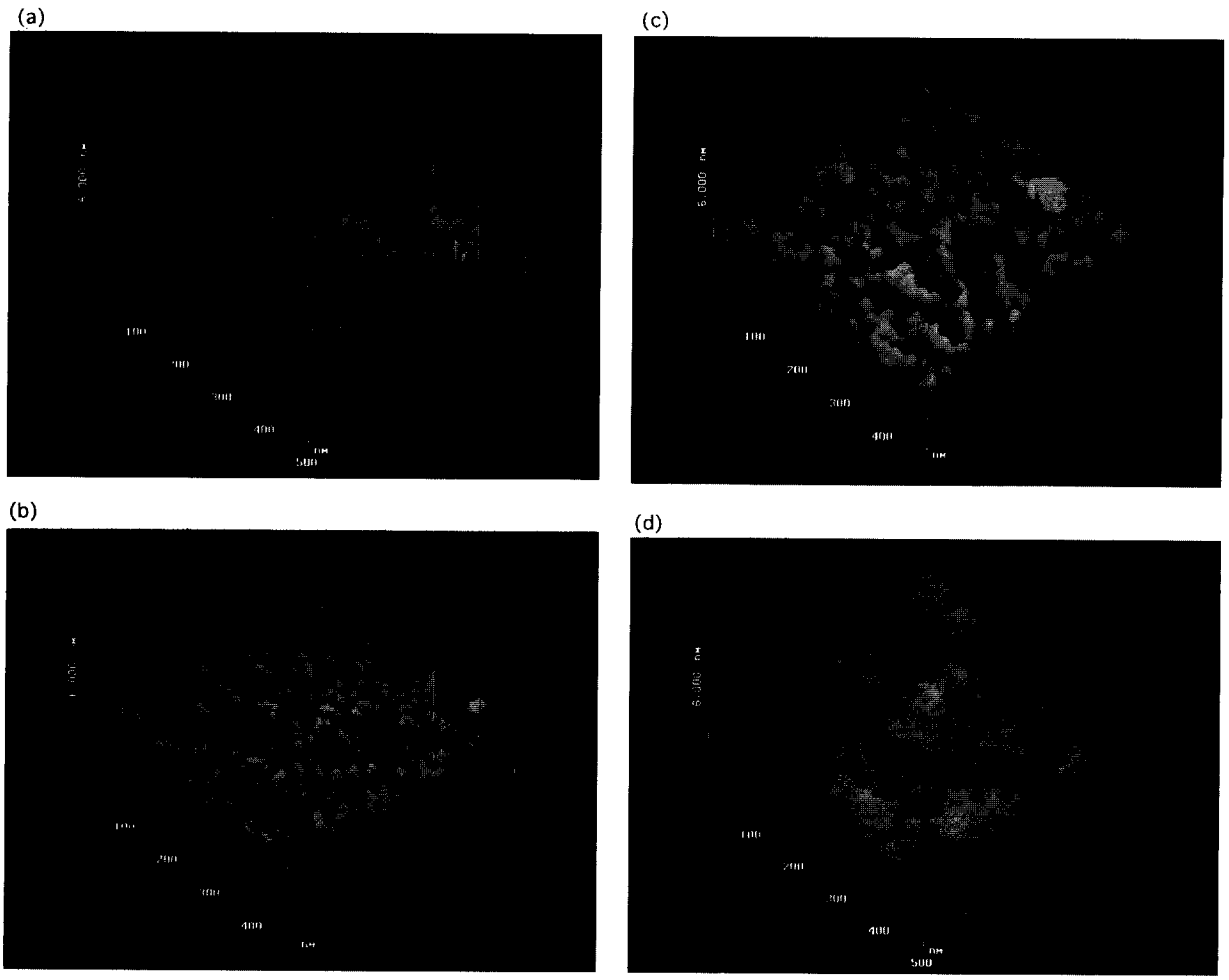

Fig. 1. AFM photographs of BSA molecules adsorbed on mica surfaces from an aqueous solution of pH 3. Adsorption time: (a) 0.5 min; (b) 1 min; (c) 5 min; (d) 20 min.

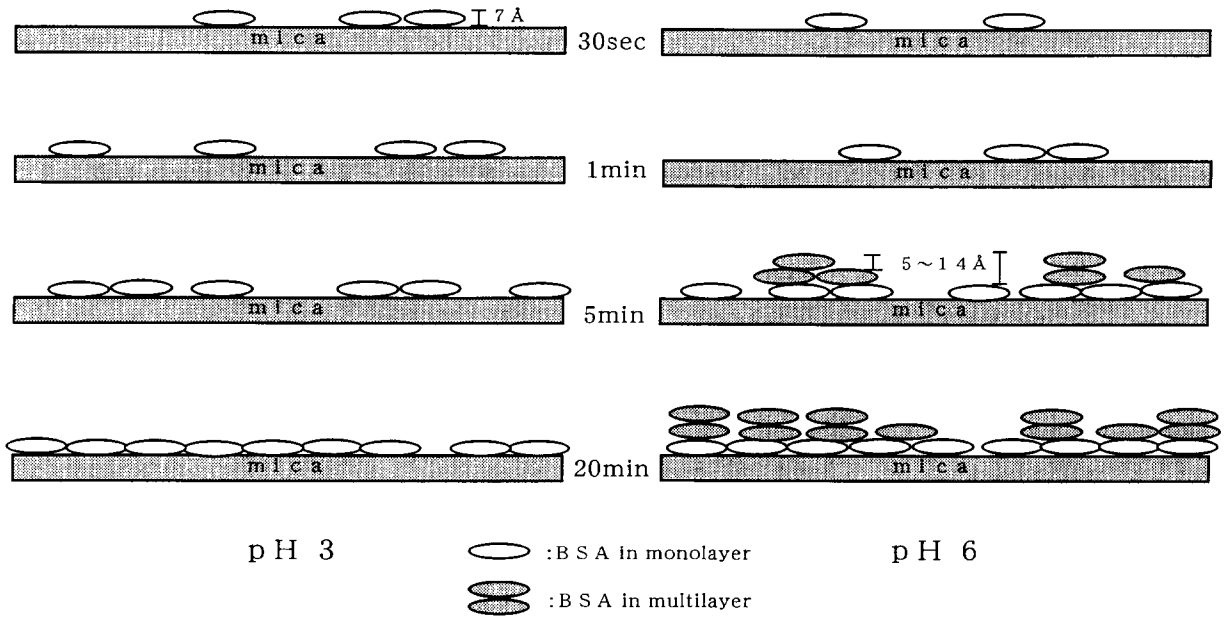

Fig. 2. Schematic representation of the adsorption process of BSA molecules on mica surfaces as a function of adsorption time.

3. Results and discussion

Fig. 1 shows AFM photographs of BSA adsorption on mica from an aqueous solution of pH 3 at different adsorption times. Figures are shown at 500 × 500 nm scanning size and 6 nm height. The adsorption process of BSA molecules on the mica surface, as a function of time, was estimated on the basis of AFM observation, and a schematic representation is given in Fig. 2. Although the adsorption of BSA was less after 0.5 min, adsorption coverage increased with increasing time. The flatter surface image after 20 min implies a high fractional coverage. The height sectional analysis displayed a one-step height difference, indicating that monolayer adsorption of BSA is maintained even after 20 min adsorption. It was concluded that the monolayer adsorption occurred at pH 3, and the fractional coverage increased with adsorption time. After 20 min, most of the mica surface was covered.

Fig. 3 represents an AFM image of a BSA molecule adsorbed on mica for 0.5 min from a solution of pH 3. Scanning was carried out in the 40 × 40 nm region and for 2 nm height. Three domain regions were observed, consistent with the three-dimensional model previously estimated [8]. Similar submolecular domains as well as individual molecules were reported by Feng et al. [1]. As seen in Fig. 3, the horizontal texture of adsorbed BSA with major and minor axes was similar to those of the estimated prolate ellipsoidal model of BSA. However, the molecular height of BSA, calculated from the sectional analysis on mica was apparently far shorter than the value estimated from the model. Native BSA may shrink during specimen preparation by drying in vacuo. The other possibility is protein denaturation during adsorption. Krisdhasima et al. [6] demonstrated a kinetic model for protein adsorption. The model included an initial, reversible adsorption step, followed by an irreversible adsorption step. For BSA, the initial step adsorption was overcome by the second-step adsorption, which yielded a surface-induced conformational change; that is, denaturation.

The AFM photographs of mica surfaces adsorbed from a solution of pH 6 are shown in Fig. 4. The number of BSA molecules adsorbed on a mica surface increased with adsorption time.

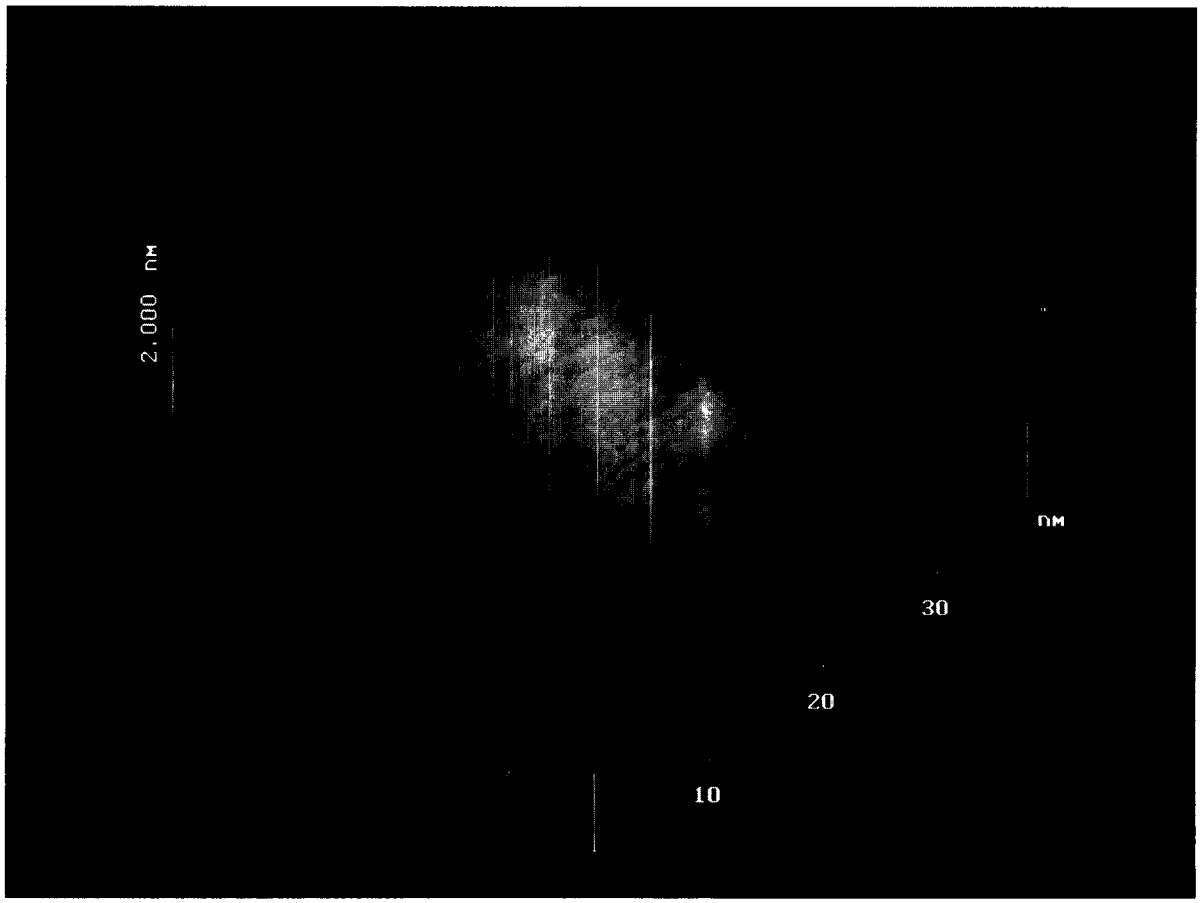

Fig. 3. An AFM image of BSA molecules adsorbed on mica surfaces for 0.5 min from an aqueous solution of pH 3.

On adsorption for 1 min, BSA molecules formed a monolayer, as in the case of pH 3. However, sectional analysis of the AFM photograph for 5 min adsorption displayed three kinds of height difference. Each height difference was the same as the monolayer height at the initial adsorption. This suggests that, after BSA molecules adsorbed as a monolayer, the second and third adsorption layers are formed on the monolayer. The uncoveraged adsorption sites in the monolayer, which was found at 5 min adsorption, was occupied at 20 min adsorption, and adsorption at the second and third layers increased simultaneously. The adsorption process at pH 6 is represented in Fig. 2. Krisdhasima et al. [6] reported that the adsorbed mass of BSA on silanized silica surfaces reached 80% within 5 min. Similarly, it was indicated above that most of the coverage by BSA on a mica surface was accomplished within 5 min. The BSA adsorption was carried out on other solid surfaces besides mica. Evidence for multilayer adsorption of protein on polystyrene latex surfaces was not established at higher coverage [5].

Each multilayer at pH 6 had the same height as a monolayer at pH 3, indicating the same orientation of BSA at pH 3 and 6. As shown in Fig. 2, since BSA molecules adsorb with major axes horizontal to the mica surface, molecules take "side-on" orientation but not "end-on". The thickness of adsorbed BSA layers between two solid mica plates was measured by Gallinet and Ganthier-Manuel [4] using a surface force apparatus,

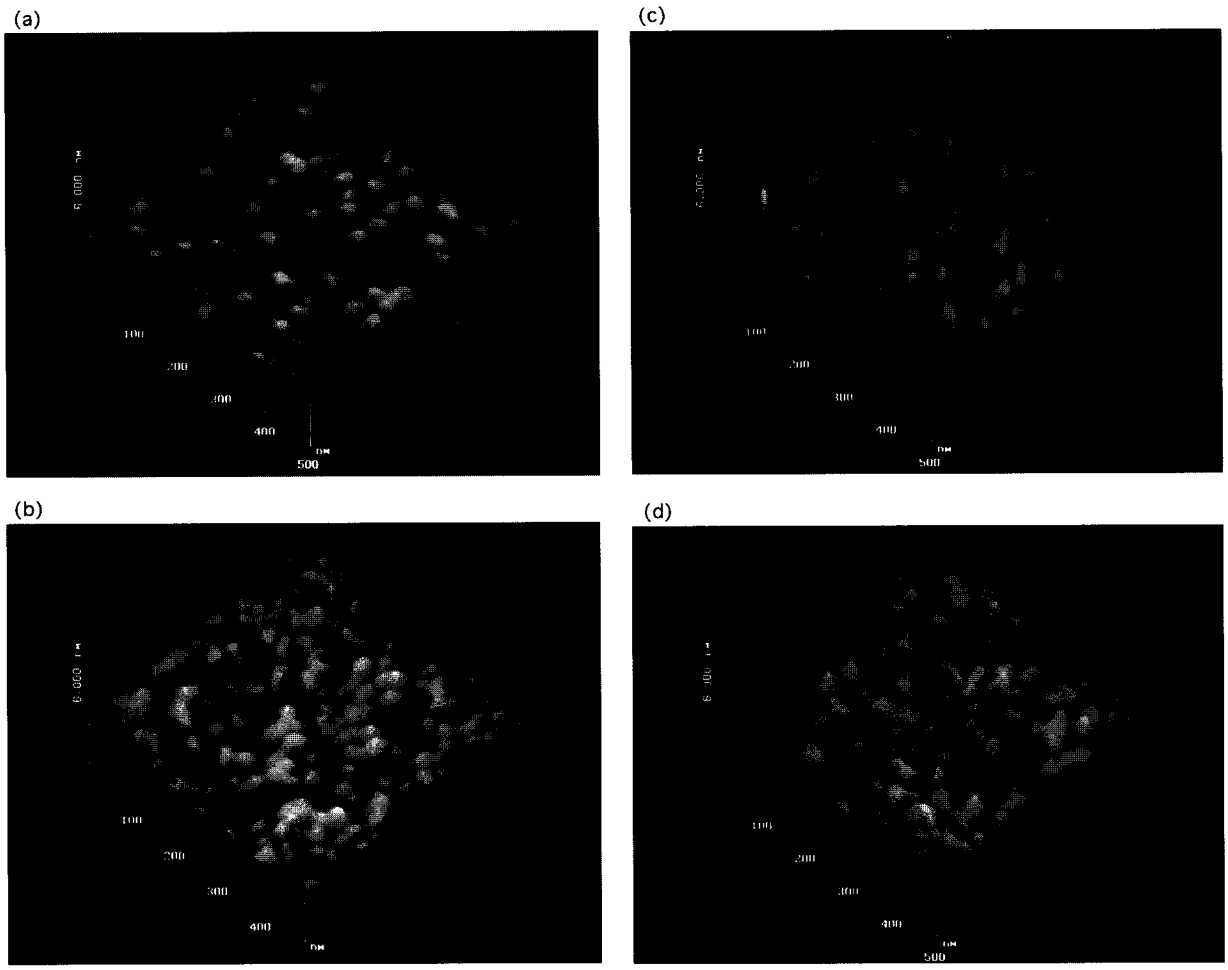

Fig. 4. AFM photographs of BSA molecules adsorbed on mica surfaces from an aqueous solution of pH 6. Adsorption time: (a) 0.5 min; (b) 1 min; (c) 5 min; (d) 20 min.

although the pH of the solution was not clarified. The molecular layer thickness was in excellent agreement with the minor diameter of the BSA molecule. This indicates "side-on" adsorption in the monolayer. From the experiment by Blomberg et al. [2], the BSA adsorption on mica was "side-on" at low concentrations and "end-on" at high concentrations. Fitzpatrick et al. [3] reported that coverage by adsorption of BSA molecules on a mica surface, resulting in "end-on" orientation at pH 5.5, decreased with decreasing pH, although they explained this result in terms of the conformational change of BSA at the lower pH. These results are consistent with that discussed in the present paper, if the "end-on" is redescribed as the "triple-layered side-on" which has a similar height to the "end-on".

The mica surface has a slightly negative charge in water owing to the dissociation of potassium ions. Thus, BSA-mica interactions can affect BSA coverage and orientation in the first adsorption layer. Since the isoelectric point of BSA is pH 4.7 [4], BSA has a positive effective charge at pH 3 and a negative one at pH 6. Therefore, BSA molecules at pH 3 interact electrostatically with mica more than BSAs at pH 6 do. In fact, it is apparent from the comparison of AFM images in Fig. 1 and Fig. 4 that the coverages at 0.5 and 1 min

adsorption are larger at pH 3 than at pH 6. Further multilayer adsorption does not occur at pH 3, and the monolayer adsorption coverage is accomplished, because the intermolecular electrostatic repulsion overcomes other affinities. In contrast to the case of pH 3, BSA molecules at pH 6 interact with mica by an adsorption affinity superior to the intermolecular electrostatic repulsion. Fitzpatrick et al. [3] compared the interaction forces between mica surfaces bearing an adsorbed layer of BSA at pH 3.5 and 5.5. They indicated the presence of a longer-range steric interaction at pH 5.5. Such an interaction can induce the multilayer adsorption, as seen in the case of pH 6 in the present work.

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