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Local Modification of the Silicon Surface with Protein Molecules

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Abstract — Adsorption of bovine serum albumin, horse radish peroxidase, and green fluorescent protein on the hydrophilic silicon surface was studied. The possibility of preparing microstructured one-, two-, and three-component films on the solid surface by combining the methods of microcontact printing and self-arrangement of proteins from solution was demonstrated.
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The development of new methods for local modification of the solid surface is topical for microelectronics, biochemistry, and medicine, allowing solution of problems associated with miniaturization of electronic devices, development of multifunctional biosensors, etc. Much promise is shown by the method of microcontact printing suggested for the first time by Whitesides [1] in 1993 as an alternative to photolithographic methods for preparing structured monolayers of alkanethiolates on gold as resists in the subsequent chemical etching of the surface [2]. Today the applications of microcontact printing are considerably wider, e.g., in microelectronics [3–5], production of sensors [6, 7], heterogeneous catalysis [8–10], microseparation [11], formation of functional structures for microliquid chips [12, 13], and also for local immobilization of proteins [14], DNA [15, 16], cells [17], and bacteria [18, 19]. By now, local immobilization of protein molecules on such substrates as gold, modified borosilicate glass, and silicon, and also on various polymeric materials (polystyrene, polyethylene, polymethyl methacrylate) has been fairly well studied. The goal of this study is to demonstrate the possibility of preparing multicomponent films of various proteins by microcontact printing followed by self-arangement of the substance on local areas of the surface, in particular, of the silicon surface. We examined the possibility of preparing one-component and multicomponent microstructured protein films on the surface of standard silicon wafers without using any auxiliary sublayers.

We examined by atomic-force microscopy (AFM) the surface morphology of continuous films of a green fluorescent protein (GFP), horse radish peroxidase (HRP), and bovine serum albumin (BSA), deposited on the surface of hydrophilic silicon by adsorption of the proteins from the corresponding solutions. The monolayer thickness was estimated by analyzing the profile of the cross section through an artificial defect formed in the structure of a protein film by repeated scanning (Fig. 1). For example, for a monomolecular BSA film prepared by adsorption of the protein on the hydrophilic silicon surface from solution, the depth of the artificial defect was ~6.0 nm. The result obtained correlates with published data [20] according to which the mean thickness of the BSA monolayer is 6.0 ± 2.0 nm.

By a similar procedure we characterized the GFP and HRP films, for which the results of AFM analysis of the monolayer thickness are also consistent with the molecular size of these proteins (layer thickness ~5.0 nm) evaluated by scanning tunneling microscopy and X-ray diffraction analysis [21]. Indeed, the GFP molecule has a structure of a hollow cylinder with a diameter of approximately 2.4 nm and a height of 4.2 nm. According to in situ scanning tunneling microscopy, the area occupied by one peroxidase molecule at monolayer adsorption is no less than 25 nm^2 [22]. On the whole, the results of examining the continuous films show that the monolayer thickness for all the proteins under consideration is $\sim 5.0 \pm 1.0$ nm.

Figure 2a shows the AFM pattern of a microstructured HRP film formed on the surface of a polydimethylsiloxane die and transferred onto hydrophilic silicon by microcontact printing. Analysis of the cross section

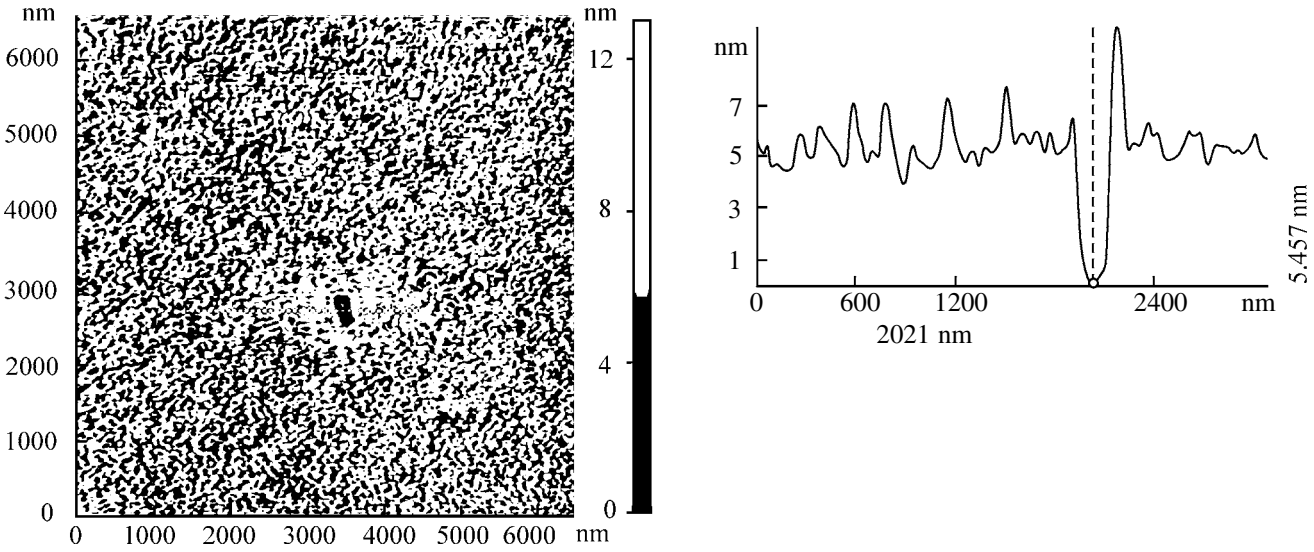

Fig. 1. AFM pattern of the surface and results of analysis of the cross section profile for a BSA monolayer.

profile shows that the thickness of the microstructured HRP film on silicon is also about 5.0 nm. Our results indicate that films deposited from solutions are mainly formed by monomolecular adsorption of horse radish peroxidase on the surface of polydimethylsiloxane die, with the subsequent quantitative transfer of the protein monolayer onto the silicon surface. Indeed, in the course of microcontact printing, the protein ("ink" from the die) is transferred onto the substrate surface completely, because repeated printing on a clean silicon support leaves no traces of the protein film.

Thus, both deposition of BSA from solution in the form of a continuous film and transfer of a microstructured HRP film by microcontact printing onto the surface of hydrophilic support result in adsorption of the protein in the form of a 5.0 ± 1.0-nm-thick monomolecular film.

Figure 2b shows an AFM pattern of a microstructured HRP film on hydrophilic silicon after treatment of the surface with a BSA solution. It is seen that the protein molecules are deposited only on free areas of

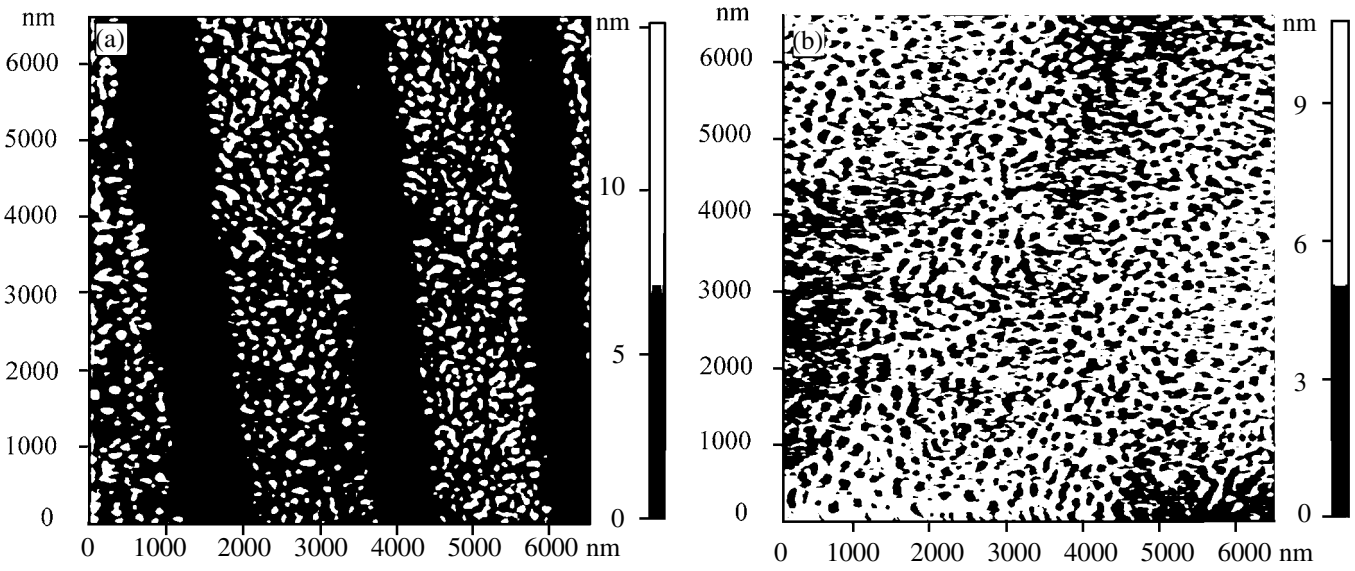

Fig. 2. AFM pattern of a microstructured HRP film on the hydrophilic silicon support (a) before and (b) after treatment with a BSA solution.

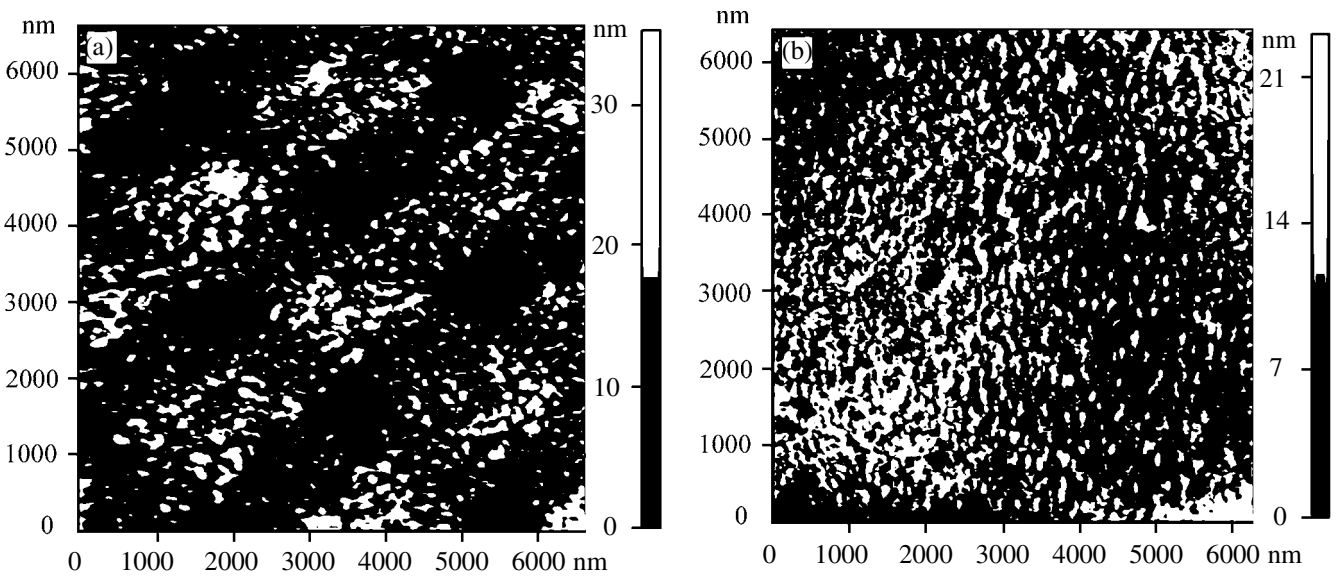

Fig. 3. AFM pattern of a two-component microstructured film of HRP and BSA, formed on hydrophilic silicon by cross microcontact printing (a) before and (b) after treatment with a GFP solution.

the surface, and no adsorption of BSA from solution occurred on areas already occupied by an HRP monolayer. Apparently, in this case adsorption of the second protein (BSA) from solution results in "healing" of microrelief voids between the strips of HRP transferred onto the silicon surface by microcontact printing. This conclusion is also supported by the fact that the resulting protein film consists of regions of equal height but different morphology. These differences may be due to the fact that with BSA there was spontaneous adsorption from solution, and with HRP, forced transfer of the protein monolayer from the hydrophobic surface of polydimethylsiloxane onto the hydrophilic surface of silicon.

An alternative to the above-described method for the formation of bicomponent protein films is cross microcontact printing. In this case, the areas of the resulting film that correspond to different proteins do not differ essentially in the height and morphology (Fig. 3a). Furthermore, free areas of hydrophilic silicon, suitable for deposition of a third protein from solution, remain on the surface. As seen from an AFM pattern of a bicomponent microstructured HRP–BSA film, treatment of the surface with a GFP solution (Fig. 3b) results in GFP arrangement only on free areas of the silicon surface.

Thus, we have demonstrated the possibility of formation of microstructured one-, two-, and three-component films based on bovine serum albumin, horse radish peroxidase, and green fluorescent protein on the surface of hydrophilic silicon by combining microcontact printing with self-arrangement of proteins from solution.

EXPERIMENTAL

Horse radish peroxidase (Sigma, RZ ~2.7) and bovine serum albumin (Sigma, ≥99%) were used without additional purification. Green fluorescent protein was prepared by heterological expression in E. coli cells, followed by isolation and purification to the homogeneous state by metal affine chromatography. The protein purity was estimated by SDS electrophoresis and spectrophotometrically.

As a template for preparing a die we used a TGZ3 calibration grating for an atomic-force microscope (relief height 540±2 nm, pitch 3 μm). To prepare the die, the commercial prepolymer and catalyst (Syglard 184, Dow Corning, Midland) were mixed in 10 : 1 weight ratio, the mixture was deaerated with a water-jet pump for 15–20 min, after which it was applied as a ~1-mm-thick layer onto the template surface, with the subsequent thermal polymerization at 100±5°C for 60 min. After cooling to room temperature, the die was separated from the template and either used within a day or stored in ethanol to prevent polymer sweating on the die surface.

To modify the die and silicon supports, we used solutions of bovine serum albumin, horse radish peroxidase, and green fluorescent protein (1 mg ml^{-1}) in potassium–sodium phosphate buffer (pH ~7.4). A drop of the solution was applied onto the surface

of the die placed in a clean Petri dish, the dish was closed with a lid and allowed to stand for 20–40 min, and the die was washed with double-distilled water and dried in a nitrogen flow. The modified microdie was contacted with the substrate surface (hydrophilic silicon support) to transfer the protein film onto the support in sites of direct contact of the die and sample.

Prior to experiments, silicon supports were cleaned by hydrophilization in a mixture of concentrated sulfuric acid and 30% hydrogen peroxide (volume ratio 1 : 1) at ~70°C for 10–15 min, followed by fivefold washing with distilled water.

The specific features of the relief and morphology of the coatings were studied with a FemtoScan 001 atomic-force microscope (Center for Advanced Technologies, Moscow State University, Moscow, Russia). The surface images were obtained in the contact mode using Si_3N_4 needles with a rigidity constant of 0.32 $N\,m^{-1}$. The scanning rate was varied within 1–3 Hz, and the force applied during scanning, from 1 to 10 nN. The information density was 512×512 dots.

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