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PHYSICAL CHEMISTRY OF SURFACE PHENOMENA

Patterns of the Adsorption of Bovine Serum Albumin on Carboxymethyl Dextran and Carboxymethyl Cellulose Films

I. V. Paribok[a], A. E. Solomyanskii[a], and G. K. Zhavnerko[b]

[a] *Institute of the Chemistry of New Materials, National Academy of Sciences of Belarus, Minsk, 220072 Belarus*
[b] *UP IZOVAC Technologies, Minsk, 220040 Belarus*
e-mail: i_paribok@yahoo.com

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Abstract—Patterns of the adsorption of bovine serum albumin on carboxymethyl dextran and carboxymethyl cellulose films are studied by means of microcontact printing, atomic force microscopy, and quartz crystal microbalance. It is shown that both the charge of polysaccharide macromolecules and the technique for deposition of their films onto the surface (via adsorption from a solution or covalent cross-linking) are factors that determine the degree of nonspecific adsorption of the protein on such films.

Keywords: thin films, polyelectrolytes, nonspecific adsorption of proteins.
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INTRODUCTION

Preventing the nonspecific adsorption of proteins is an important task in designing immunochips, biosensors, medical devices, targeted drug delivery systems, and so on [1]. The physical adsorption of proteins on solid surfaces is known to occur spontaneously and is influenced by entropic effects, Van der Waals interactions, electrostatic forces, and the formation of hydrogen bonds [2]. The contributions from each of these components depend on the given protein-surface system. Self-assembled monolayers based on oligo(ethylene glycols) containing terminal SH-group are most commonly used to prevent the nonspecific adsorption of protein on gold [3]. Recent studies have proven the effectiveness of multilayer polyelectrolyte films for this purpose [4, 5]. The aim of this work was to determine the possibility of using films based on carboxylated polysaccharides to control the nonspecific adsorption of bovine serum albumin onto such solid surfaces as silicon and gold.

EXPERIMENTAL

Bovine serum albumin (BSA, Sigma), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, Sigma), dextran (M_w of approximately 60000, Pharmacosmos), carboxymethyl cellulose sodium salt (CMC, M_w of approximately 250000, Aldrich), polyethyleneimine (PEI, M_w = 60000, Aldrich) and cystamine hydrochloride (CAH, Sigma) were used without additional purification. Carboxymethyl dextran (CMD) was synthesized via the reaction between dextran and bromoacetic acid according to [6]. Dextran (10.0 g) was dissolved in 50 mL of solution containing 4.0 g of NaOH and 0.87 g of bromoacetic acid, kept for 24 h. The resulting solution was purified via successive dialysis against a 0.1 M HCl solution and distilled water with subsequent lyophilization.

The ζ-potentials of macromolecules of polyelectrolytes (PEs) were calculated using the Smoluchowski equation and the data obtained from measuring their electrophoretic mobility in aqueous solutions with a Zetasizer Nano ZS analyzer (Malvern, UK).

PE films were deposited onto silicon substrates preliminarily purified in a mixture of concentrated H_2SO_4 and 30% H_2O_2 solution (ratio, 7 : 3 by volume) at a temperature of approximately 70°C for 10–15 min followed by washing for five times with distilled water. Microstructured PEI films were formed via microcontact printing (mCP) according to [7, 8]. CMC and CMD were subsequently deposited on PEI bands via their spontaneous adsorption from the solution. The polysaccharides were not adsorbed on the negatively charged surface of the silicon substrate.

Features of microstructure and morphology of the resulting coatings were investigated by means of atomic force microscopy (AFM) with on a Multi-Mode Nanoscope IIID instrument (Veeco, United States). AFM images were obtained in contact mode with Nanoprobe cantilevers made of Si_3N_4 with a spring constant of 0.12 N m^{-1}, and processed and analyzed using the Nanoscope 5.31r1 software. The surface roughness was estimated from the root mean

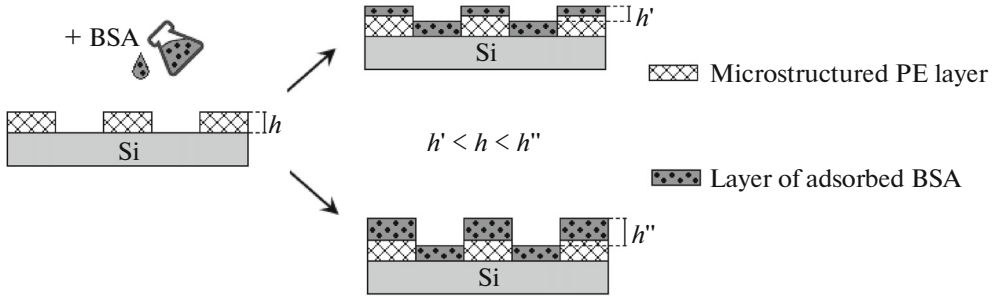

Fig. 1. Scheme for determining the height of PE bands before and after BSA adsorption.

square value (R_{ms}) with a scanning window size of 5 × 5 μm.

Microgravimetric analysis was performed with a QCM200 quartz crystal microbalance (Stanford Research Systems, United States).

The mass of the films deposited on the surface of our resonator was calculated using the Sauerbrey equation,

$$m = -\frac{\Delta F}{C_f},$$

where m, μg cm^{-2}, is the mass of the film; ΔF, Hz, is the change in the oscillation frequency of piezoelectric resonator; C_f is the sensitivity factor of piezoelectric resonator. The use of the Sauerbrey equation for calculations in the studied systems is acceptable, as the equation correctly describes the dependence of the resonator frequency change on its mass if the polymer coating is dense; i.e., any change in the resonance resistance tends to zero, and the thickness of the coating does not exceed 40 nm [9].

Measurements were performed in a liquid flow cell. Polished quartz resonators with gold electrodes characterized by a self-resonant frequency of 5 MHz were used. The resonators were purified before use by heating in $H_2O : H_2O_2 : NH_4OH$ (ratio, 5 : 1 : 1 by volume) mixture for 10–15 min at a temperature of approximately 70°C, followed by thorough washing with distilled water and drying under a stream of nitrogen. In order to functionalize the gold surface with cystamine, the resonators were kept in a 10 mg mL^{-1} aqueous solution of CAH for 10–12 h, and layer deposition was used to form the positive PE sublayer of PEI.

The films of polysaccharides (CMC and CMD) were deposited onto the surfaces of resonators using two techniques:

(1) adsorption from the solution on the PEI sublayer;

(2) the covalent binding of polysaccharide carboxyl groups activated with EDC to free amino groups of CAH grafted on the gold surface.

The carboxyl groups of polysaccharides were activated with EDC following the familiar procedure in [10, 11].

RESULTS AND DISCUSSION

The AFM method for measuring the thickness of a film deposited on a solid substrate generally consists of determining the depth of an artificial defect by scanning a small area of a sample's surface (approximately 400 × 400 nm) with a tip at a force no less than 10 nN. The formation of defects shows that the coating is completely (down to the substrate) removed. The mCP technique allows us to form bands of PE that alternate with areas of unmodified silicon on the surface, thereby ensuring correct determination of the thickness of PE layer. In this work, the approach was used for a comparative assessment of the efficiency of BSA adsorption on microstructured PE films of varying compositions. It was assumed that the thickness of the protein layer between the PE bands (in the areas of hydrophilic silicon) was always the same. The difference between changes in the height of the PE bands caused by BSA adsorption thus depended only on the nature of the PE that was used (Fig. 1).

The AFM data shown in Fig. 2 indicate an increase in the height of PEI bands by approximately 10 nm after treatment with the BSA solution. The isoelectric point of BSA is known to be at a pH ranging from ~4.7 to 4.9 [12], thus leading to the overall negative charge of the protein macromolecules in an aqueous salt-free solution at a pH of approximately 6.0 (ζ-potential, -22 ± 2 mV). The adsorption of BSA on PEI (ζ-potential, 11 ± 2 mV) is therefore mainly due to the electrostatic interactions between the protein molecules and the positively charged PE molecules.

Similar measurements were performed for microstructured PEI/CMD and PEI/CMC films. The height of PEI/CMD bands grew by approximately 7 nm after treatment with the BSA solution and remained virtually the same for PEI/CMC (Table 1). The blocking of the adsorption of BSA on PEI/CMC bands would seem to be due to the electrostatic repulsion of macromolecules of protein and polysaccharide

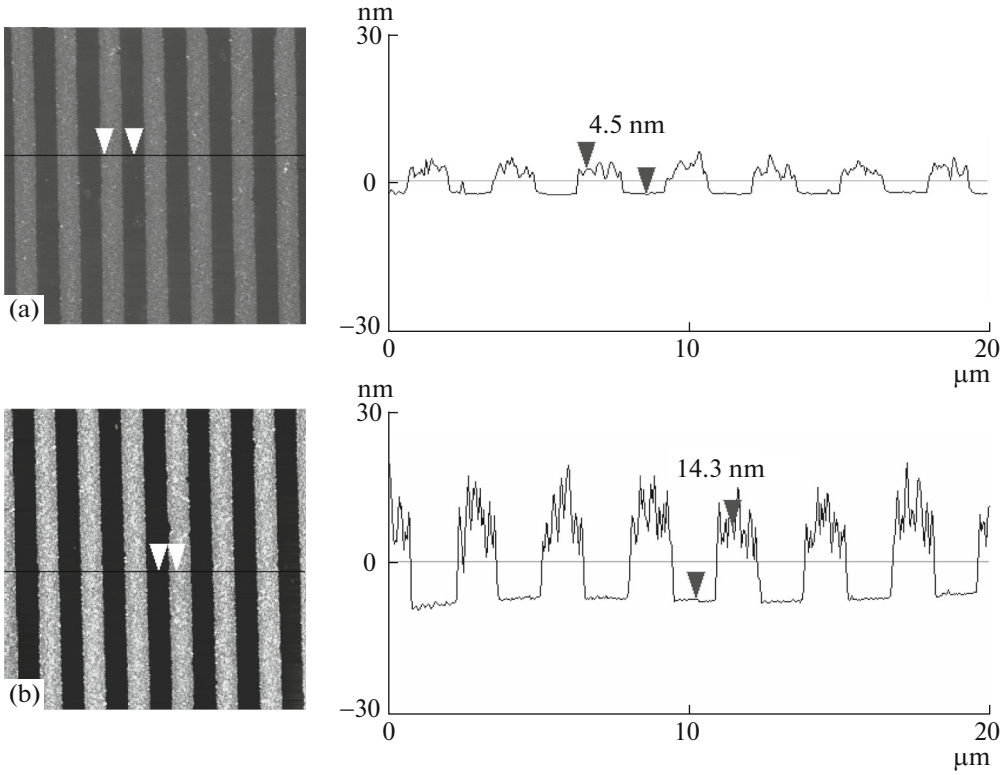

Fig. 2. AFM images of microstructured PEI film on silicon (a) before and (b) after BSA adsorption.

charged with the same sign (the ζ-potential of CMC is −47 ± 2 mV). With CMD (ζ-potential, approximately zero) conformational changes in polysaccharide chains that ensure the penetration of BSA molecules between them and interaction between the molecules and the PEI sublayer could be a factor that limits adsorption [13].

Analysis of the surface roughness of continuous (unstructured) PEI/CMD and PEI/CMC films before and after treatment with BSA solution confirms the data obtained for microstructured films. The R_{ms} value of 0.7 ± 0.1 nm thus does not change for PEI/CMC after protein adsorption, while it grows from 0.4 ± 0.1 to 1.7 ± 0.1 nm for PEI/CMD films.

The quantitative dependences of the adsorption of polysaccharides and BSA on gold-plated quartz resonators were determined via microgravimetric analysis. CMC and CMD were deposited onto the surface in two ways: due to adsorption from the solution (onto the PEI sublayer) or to covalent binding (onto the CAH sublayer). The dependence of the change in the oscillation frequency of the resonator during the formation of PEI/CMD/BSA film on the time is shown in Fig. 3. The mass of films of polysaccharide and protein were calculated based on the $\Delta F'$ and $\Delta F''$ values, respectively (Fig. 3).

Similar measurements were performed for PEI/CMC/BSA, CAH/CMD/BSA, and CAH/CMC/BSA films (Table 2). For the sake of comparison, the mass of BSA film deposited onto an unmodified resonator (i.e., onto the gold surface) was 0.60 ± 0.05 μg cm^{-2}. The results from our microgravi-

Table 1. Height (h, nm) of PE bands before and after BSA adsorption

PE layer	Initial	After adsorption
PEI	4.5 ± 0.5	14.3 ± 1.5
PEI/CMD	4.8 ± 0.5	11.6 ± 1.0
PEI/CMC	6.7 ± 0.5	6.5 ± 0.5

Table 2. Results from microgravimetric analysis

Polysaccharide	Sublayer	m, μg cm^{-2}	m_{BSA}, μg cm^{-2}
CMD	PEI	0.25 ± 0.03	1.94 ± 0.15
	CAH	0.19 ± 0.02	0.21 ± 0.02
CMC	PEI	0.14 ± 0.02	0.39 ± 0.04
	CAH	0.16 ± 0.02	0.19 ± 0.02

The polysaccharide films were deposited via adsorption from solution (with PEI) or by covalent cross-linking (with CAH); m is the mass of polysaccharide, and m_{BSA} is the mass of BSA.

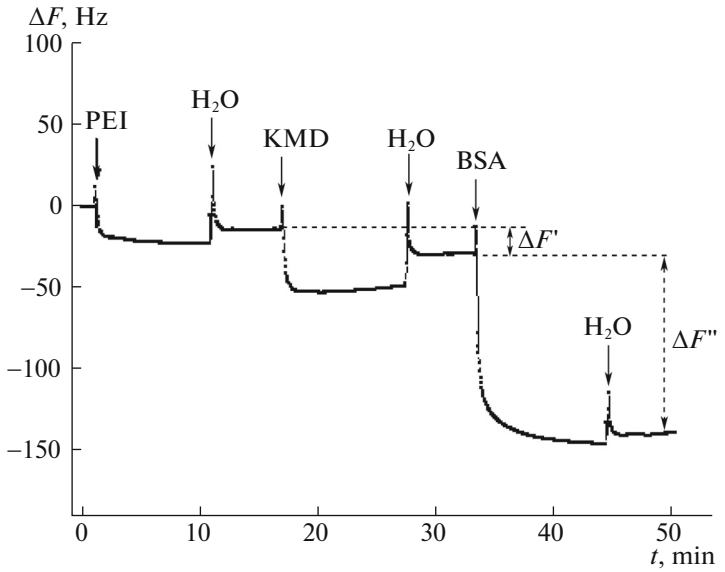

Fig. 3. Changes in the resonant frequency of oscillations during the formation of the PEI/CMD/BSA film.

metric analysis of PEI/CMD/BSA and PEI/CMC/BSA films agree with those obtained via mCP and AFM. Thus, CMD promotes protein adsorption, while CMC slightly blocks adsorption. Meanwhile, the covalent binding of carboxylated polysaccharides to the gold surface (CAH/CMD and CAH/CMC films) leads to a significant (approximately threefold) reduction in the mass of deposited BSA.

CONCLUSIONS

CMC and CMD films can be used to control the nonspecific adsorption of BSA on silicon and gold. The amount of protein deposited on CMD and CMC films depends on both the charge of macromolecules of polysaccharides and the method for immobilizing them on their surfaces. CMC and CMD films prepared by chemical sorption reduce nonspecific BSA adsorption most effectively (by a factor of approximately three).

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