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Formation and characterization of protein patterns on the surfaces with different properties

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Abstract

The different protein patterns with varied surface coverage are formed by the adsorption of a protein, bovine serum albumin (BSA), on six types of surfaces and characterized with atomic force microscope (AFM). The surfaces include a gold film and five self-assembled monolayers (SAMs) on gold films. Five SAMs are prepared by the immersion of gold plates in solutions: 16-mercaptohexadecanoic acid (MHA), 1-octadecanethiol (ODT), and the mixtures of MHA and ODT at mole ratios of 1:1, 1:10 and 10:1, respectively. The mechanism of protein adsorption on the six surfaces is discussed.

Keywords: Self-assembled monolayers; Atomic force microscope; Bovine serum albumin

1. Introduction

Protein adsorption on solid surface is a very important and active area of research due to its potential applications from fundamental studies in cell biology to the development of various "biochip" platforms [1,2]. There are mainly two types of methods to study protein adsorptions. First type is to fabricate selective templates for protein adsorption to form micro- to nanoscale protein patterns using techniques such as photolithography [3], micromaching [4], microfluidic channel networks [5,6], microcontact printing [7,8] and AFM-based nanotechnology, nanografting [9,10] and dip-pen nanolithography [11–16]. The second type for immobilizing protein in micro- or nanoscales is through self-assembled monolayers (SAMs) [17,18]. Both types of methods are based on the modification of substrate surface property to realize selective adsorption.

Gold, mica, glass and SiO_2 are most often used as substrate materials for protein adsorption because their surface properties are easily modified by forming SAMs with high degree organization and physical robustness. Materials used to prepare SAMs on gold contain two terminated groups. One is –SH, which forms Au–S covalent bond on gold [19]. The other terminated group with special property is exposed to the surface for protein adsorption due to the electrostatic interaction, hydrophobic interaction or formation of chemical bond between protein and the other terminated group.

George et al. firstly reported the preparation of mixed SAMs by immersing gold in the solution containing two types of thiols [20–24]. They further studied the protein adsorption on the mixed SAMs and found the SAMs of thiol containing oligo(ethylene glycol) have high resist to protein adsorption. They prepared mixed SAMs containing oligo(ethylene glycol) moiety to selectively immobilize protein [25–27]. Chemically immobilizing protein on mixed carboxylate-terminated (–COOH) SAMs through chemical reactors to form S–RCO–NH-protein covalent bond was also reported [28,29].

Serum albumin is one of the most abundant proteins in blood, and it is capable of affecting blood coagulation [30]. Therefore, serum albumin adsorption on self-assembled monolayers [31,32], mica [33], chemically modified silicon

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[34], titanium oxide film [35] and polymer surfaces [36] was extensively studied.

In this work, we prepared SAMs and mixed SAMs with two long chain thiols, CH_3-terminated 1-octadecanethiol (ODT) and carboxylate-terminated 16-mercaptohexadecanoic acid (MHA) at various molar ratios. We studied the adsorption of a protein, bovine serum albumin, on gold film and the prepared SAMs, compared the different protein patterns on surfaces with different chemical properties, and studied the mechanism for the protein adsorption on these surfaces.

2. Experimental

2.1. Materials

1-Octadecanethiol (Aldrich), 16-mercaptohexadecanoic acid (Aldrich), 2-butanol (International Laboratory), and bovine serum albumin (Sigma) were used as received.

2.2. Preparation of the polycrystalline gold film

A 10 nm Ti and then 30 nm gold are coated on *n*-Si(1 0 0) wafer by sputtering with Explorer^{TM} 14 Denton Vacuum.

2.3. Preparation of SAMs

Fresh gold surfaces were immersed in one of the five solutions: 1 mM ODT in 2-butanol, 1 mM MHA in 2-butanol, the mixture of the above solutions with the mole ratios of MHA to ODT as 1:10, 1:1, 10:1) for 48 h. Then the samples were taken out and copiously rinsed with 2-butanol and ethanol followed by distilled deionized water and blown dry with pure nitrogen to form different SAMs on gold surfaces. We name the three mixtures of SAMs as the mixture 1:10 SAMs, 1:1 SAMs, and 10:1 SAMs, respectively.

2.4. Protein adsorption

SAMs on gold were immersed into aqueous BSA solution (2.5 μg/μl) for 1 h, copiously rinsed with distilled deionized water to remove weakly adsorbed BSA and blown dry with pure argon.

2.5. Characterization of protein patterns

The adsorbed protein was characterized with a commercial AFM (Solver P47H, NT-MDT, Russia) in tapping mode. The typical force constant, tip curvature radius and resonance frequency of the silicon cantilever are about 0.30 N/m, 10 nm, 21 kHz (MicroMasch), respectively. AFM images were obtained in ambient condition (about 20 °C and at relative humidity of 50%) and at a scan rate of 2 Hz.

3. Results and discussions

3.1. Gold film and SAMs

The surfaces of gold film and SAMs are shown in Fig. 1. All surfaces are very flat and the roughness of all surfaces is less than 0.5 nm. Fig. 1a–c are the typical topography of gold surface, ODT SAMs and the mixture 1:1 SAMs, respectively. The MHA and ODT form uniform SAMs on gold through S–Au covalent bond [19], exposing hydrophilic –COOH group and hydrophobic $-CH_3$ group on the surface, respectively. Nearly the same chain length, 3 nm of MHA and ODT [37] results in flat surface of mixture SAMs.

3.2. Proteins patterns and the mechanism for protein adsorption

3.2.1. On gold film

Fig. 2 shows the protein pattern immobilized on gold film. Fig. 2a is the topography of the absorbed protein. The protein densely absorbed on gold has the shape of belts, lines and points. The width of the belts and lines is in the range of 80–700 nm and the diameter of the points is in the range of 30–250 nm. Densely coalesced streptavidin pattern on gold was also obtained by Kim et al. [38]. The protein pattern is probably caused by the solution concentration, adsorption time, the interaction between protein molecules and between protein molecules and gold substrate [32,36]. Each

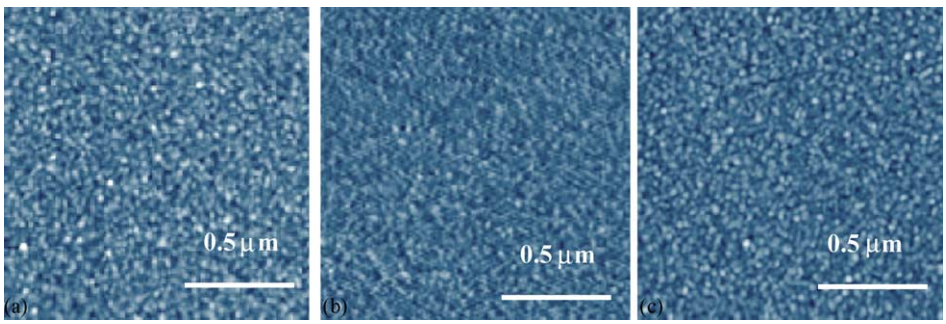

Fig. 1. Topography of gold film and SAMs: (a) topography of gold film, (b) topography of ODT SAMs, (c) topography of the mixture 1:1 SAMs.

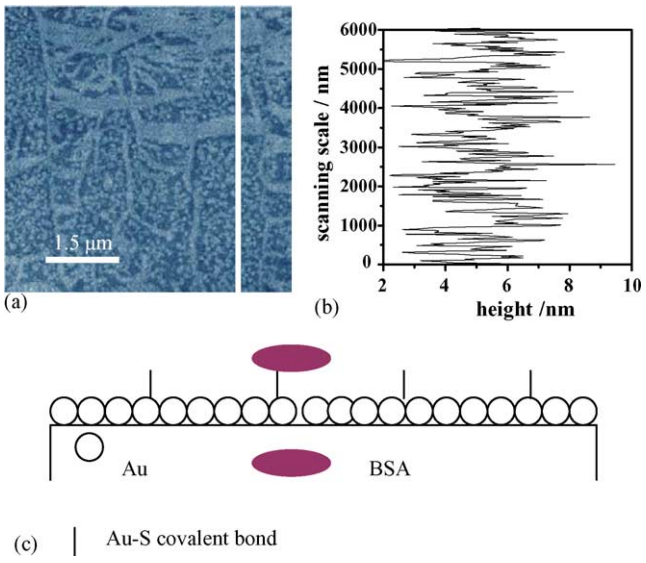

Fig. 2. Protein immobilization on gold film: (a) topography of gold after absorb BSA, (b) cross section of the line in (a), (c) schematic illustration for the protein immobilization on gold film.

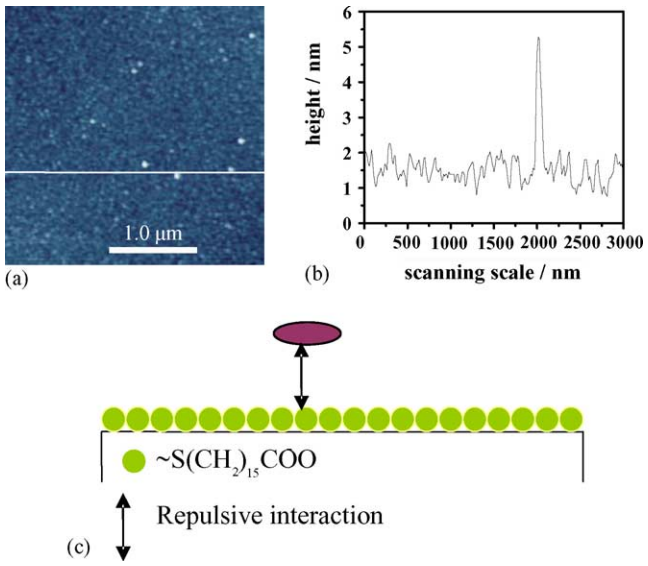

Fig. 3. Protein adsorption on MHA SAMs: (a) topography of protein pattern on MHA SAMs, (b) cross section of the line in (a), (c) schematic illustration for the adsorption MHA SAMs.

BSA molecule contains 582 amino acids. It is a prolate ellipsoid. The major and minor axes of the ellipsoid are 14 and 4 nm, respectively [39]. BSA molecules adsorb on solid surface by "side-on" or "end-on" orientation with minor or major axes perpendicular to the surface [33]. The surface coverage of the protein is about $81 \pm 2\%$, estimated by an image analyzer (Clemex Technologies Inc.). Fig. 2b is the line cross section in Fig. 2a. The height of the patterns in Fig. 2a is about 4 ± 0.5 nm, which is close to the minor axes of BSA molecule. The result suggests that BSA monolayer is formed on gold surface by side-on orientation. BSA molecule contains 17 disulfide valence (R–S–S–R′) and one free –SH [39]. Both disulfide and free –SH have strong affinity with gold through formation of Au–S covalent bond [19]. The Au–S bond is illustrated in Fig. 2c. The covalent Au–S bond is much more stable than intermolecular interaction, resulting in protein immobilization on gold surface with single layer.

3.2.2. On MHA SAMs

Fig. 3 shows the protein pattern absorbed on MHA SAMs. Fig. 3a is the topography of the protein absorbed on MHA SAMs. As shown in Fig. 3b, some spherical patterns with the height of about 4 ± 0.5 nm and lateral dimension of about 18 ± 2 nm sparsely distribute on the surface, demonstrating individual BSA molecules on the MHA SAMs. The spherical pattern other than ellipsoid [39] is caused by the convolution effect of the tip. The typical radius of the tip used in the present study is about 10 nm, greater than the BSA molecular scale. Therefore, the BSA molecule is extended [40]. Globular structures of albumin were also obtained with AFM by other research groups [36,41]. The surface coverage of the protein on MHA SAMs is very low, about $2 \pm 0.4\%$. The absorption of protein on MHA SAMs is probably due to repulsive electrostatic interaction between COO^- and protein molecules [4]. The pH of the used aqueous BSA solution in the present experiment is about 6, higher than the reported isoelectric point (p*I*) of BSA (4.6–4.7) [42], therefore, the protein molecules have net negative charge. MHA SAMs surface is also negatively charged due to ionization [43], thus produce repulsive electrostatic interaction between BSA molecules and MHA SAMs. Protein adsorption on MHA SAMs is illustrated in Fig. 3c.

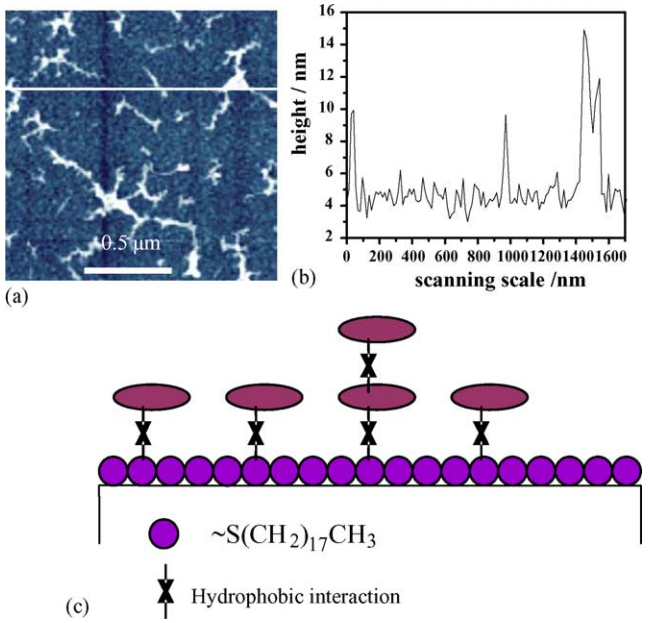

Fig. 4. Protein adsorption on ODT SAMs: (a) topography of protein pattern on ODT SAMs, (b) cross section of the line in (a), (c) schematic illustration for the protein adsorption on ODT SAMs.

3.2.3. On ODT SAMs

Fig. 4 shows the protein pattern absorbed on ODT SAMs. Fig. 4a is the topography of protein adsorbed on ODT SAMs. Protein molecules form non-uniform and branched patterns. The heights are nearly 8 ± 1.0 nm at the knot of the branches and about 4 ± 0.5 nm at the branches, shown in Fig. 4b, indicating that two protein molecules aggregate at the knots. The surface coverage of protein on ODT SAMs is about $9 \pm 1\%$, which is greater than that on MHA SAMs. This result confirms the observations from previous reports [34,36,44], i.e., the amount of BSA adsorbed on CH_3-terminated surface is greater than that on COOH-terminated surface. Protein adsorption on ODT SAMs is mainly due to hydrophobic interaction between hydrophobic moiety of protein molecule and the hydrophobic surface through reverse phase chemistry [25,45], as illustrated in Fig. 4c. Protein aggregation formed at the knot is probably caused by the hydrophobic interaction, which further increases the hydrophobic interaction between protein molecules.

3.2.4. On the mixture 1:1, 1:10 and 10:1 SAMs

Fig. 5 shows the protein pattern absorbed on the SAMs with the mixture 1:1 SAMs. Fig. 5a is the topography of the SAMs after the adsorption of protein. Protein on the SAMs with the mixture also forms branched but much dense patterns. The surface coverage of protein is about $28 \pm 1.5\%$. As shown in Fig. 5b, the pattern height is about 4 ± 0.5 nm, indicating a monolayer of protein molecules on the mixture 1:1 SAMs.

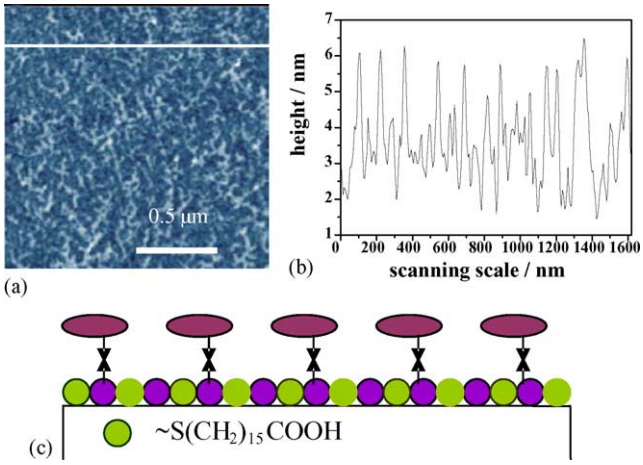

Fig. 5. Protein adsorption on the mixture 1:1 SAMs: (a) topography of protein pattern on the mixture 1:1 SAMs, (b) cross section of the line in (a), (c) schematic illustration for the protein adsorption on the mixture 1:1 SAMs.

The sum of the coverage of proteins on ODT SAMs and MHA SAMs is about 11%, which is less than that on the mixture 1:1 SAMs (28%). This result indicates that the protein adsorption on mixed SAMs is not simply mathematical sum of MHA SAMs and ODT SAMs. The increased adsorption of protein on mixed SAMs is probably due to coordination of hydrophobic group ($-CH_3$) and hydrophilic group (COOH). The pK_a of carboxylic acids in mixed SAMs where the other component is hydrophobic can be shifted to much higher values [46]. At pH 6, the carboxylic acid groups in the mixed SAM are likely to be predominantly protonated and

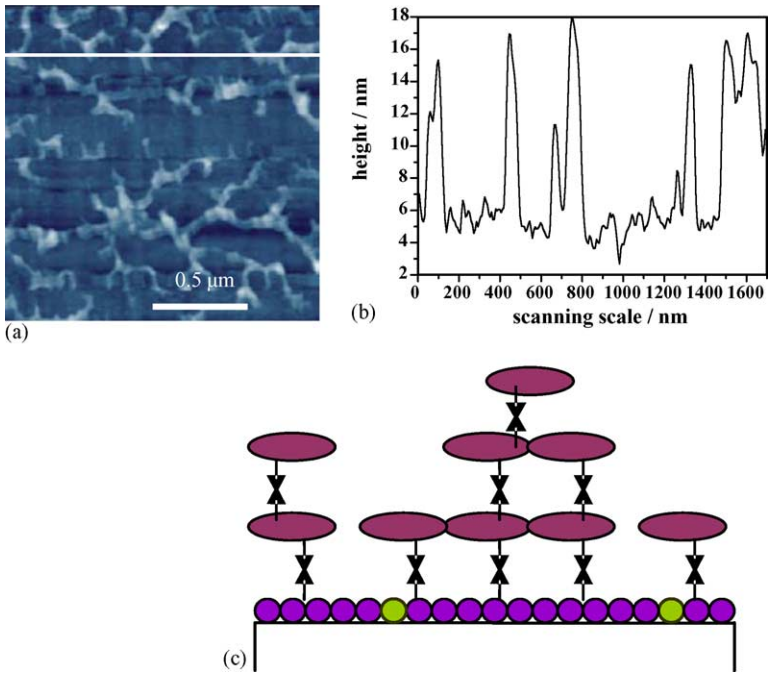

Fig. 6. Protein adsorption on the mixture 1:10: (a) topography of protein pattern on the mixture 1:10, (b) cross section of the line in (a), (c) schematic illustration for the protein adsorption on the mixture 1:10 SAMs.

repulsive interactions between BSA and the SAM should decrease, which leads to the increased protein adsorption. The adsorption of proteins on the mixture 1:1 SAMs is illustrated in Fig. 5c.

Fig. 6 shows the protein pattern absorbed on the mixture 1:10 SAMs. Fig. 6a is the topography of the protein pattern on the mixture 1:10 SAMs. Network pattern of protein is obtained. Dupont-Gillain et al. obtained net-like structure of collagen adsorbed on poly(methyl methacrylate) with slow drying procedure, they thought a chemically heterogeneous surfaces are produced at slow drying rates [47]. In present study, the mixed SAMs surface was blown dry with argon, which has a faster drying rate. The net-like protein pattern is mainly caused by the mixed SAMs surface properties and the interplay between protein–SAMs interaction and protein–protein interaction. Surface coverage of protein on the mixture 1:10 SAMs is about $19 \pm 1.5\%$. As shown in Fig. 6b, the heights of network are about, 4, 8 and 12 nm, integer increments of the protein molecule size. The protein pattern on the mixture 1:10 SAMs in Fig. 6a is similar to that on ODT SAMs in Fig. 4a. Nevertheless, the amount of proteins absorbed on the surface of the mixture 1:10 SAMs is increased, and proteins coalesce to form networks. The line width and height of the network on the mixture 1:10 SAMs (Fig. 6b) are greater than those on ODT SAMs (Fig. 4b). The amount of protein molecules on the mixture 1:10 SAMs is greater than that on the mixture 1:1 SAMs. Adsorption of protein on the mixture 1:10 SAMs is illustrated in Fig. 6c. The protein pattern on the mixture 10:1 is similar to that on MHA SAMs in Fig. 3a, indicating that the high concentration of MHA has the dominant effect on adsorption patterns of protein.

4. Conclusions

The various protein patterns with different coverage on six different surfaces are formed. Dense but non-uniform protein pattern with single layer is immobilized on gold film through formation of Au–S covalent bond. Sparsely distributed, individual protein molecules are adsorbed on MHA SAMs surface due to repulsive electrostatic interaction. Branched protein pattern is formed on ODT SAMs with aggregates at knots via hydrophobic interaction. In the mixed SAMs of MHA and ODT with large concentration differences, the adsorption properties of the mixture SAMs are close to that of the dominant component. In the mixed SAMs of MHA and ODT with little concentration difference, the combination properties of the mixture SAMs are significantly changed. The amount of protein adsorbed on mixed SAMs is greater than that on the SAMs with single component due to coordination. The amount of protein adsorbed on six different surfaces decrease according to the following order: gold film > the mixture 1:10 SAMs > the mixture 1:1 SAMS > ODT SAMs > MHA SAMs (the mixture 10:1 SAMs).

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