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Competitive Adsorption between Bovine Serum Albumin and Collagen Observed by Atomic Force Microscope

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Abstract: Atomic force microscopy (AFM) was used to study the competitive adsorption between bovine serum albumin (BSA) and type Ⅰ collagen on hydrophilic and hydrophobic silicon wafers. BSA showed a grain shape and the type Ⅰ collagen displayed fibril-like molecules with relatively homogeneous height and width, characterized with clear twisting (helical formation). These AFM images illustrated that quite a lot of type Ⅰ collagen appeared in the adsorption layer on hydrophilic surface in a competitive adsorption state, but the adsorption of BSA was more preponderant than that of type Ⅰ collagen on hydrophobic silicon wafer surface. The experiments showed that the influence of BSA on type Ⅰ collagen adsorption on hydrophilic surface was less than that on hydrophobic surface.

Keywords: Atomic force microscope, protein competitive adsorption, collagen, bovine serum albumin.

Many studies of competitive adsorption of proteins on different substrates have been carried out[1, 2]. Bovine serum albumin (BSA) is often used as passivating agents to prevent the adhesion of cells. When cancer cell transport and invade, it must first adhere to the extracellular matrix (ECM) proteins (collagen, laminin, fibronectin). The cell adhesion can be better understood by studying the competitive adsorption between collagen and BSA. Here we present AFM images of type Ⅰ collagen, BSA, and the mixture of type Ⅰ collagen and BSA adsorbed on silicon wafer to study the competitive adsorption between type Ⅰ collagen and BSA.

Experimental

The silicon wafers were made hydrophilic and hydrophobic[3]. Proteins were dissolved in PBS buffer (0.1 mol/L, pH=7.40) to the concentration of 1 mg/mL (BSA) or 0.5 mg/mL (collagen). The incubation time in all adsorption was 30 min. An AutoProbe CP Research Scanning Probe Microscope (Park Scientific Instrument, CA) was utilized. The images were made in IC-AFM mode (Park Scientific Instrument, CA). All the measurement were performed at 25℃, 30-40% relative humidity in air.

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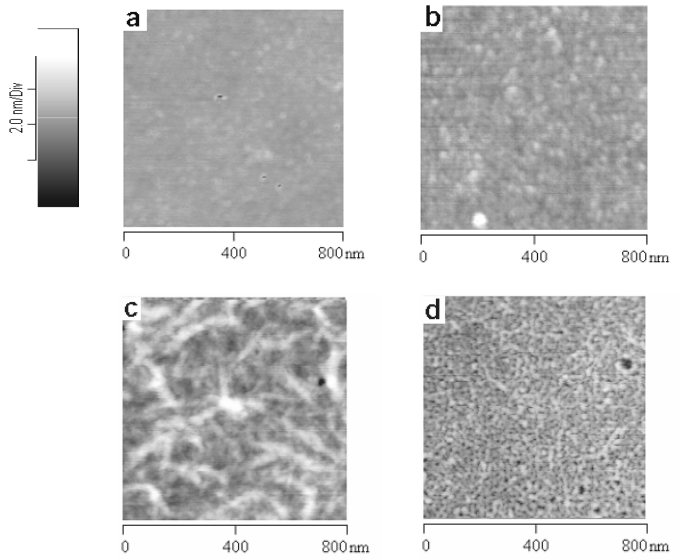
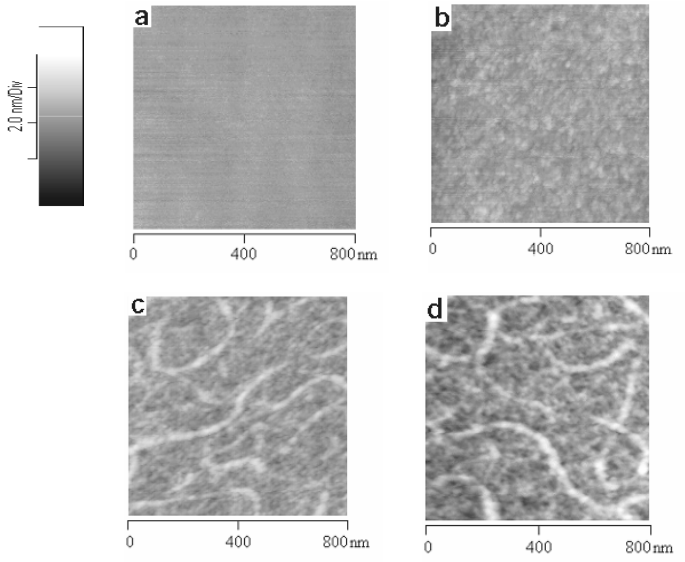
Figure 1 AFM images of hydrophobic silicon surface with adsorbed proteins



a) substrate; b) BSA; c) type Ⅰ collagen; d) mixture of type Ⅰ collagen and BSA. Scale bar = 800 nm. 10 nm black to white.

Figure 2 AFM images of hydrophilic silicon surface adsorbed with proteins



a) substrate; b) BSA; c) type Ⅰ collagen; d) mixture of type Ⅰ collagen and BSA. Scale bar = 800 nm, 10 nm black to white.

Results and Discussion

The contact angles for hydrophilic and hydrophobic surface were about 5±1° and 80±1°, respectively. The AFM images of hydrophobic silicon surface adsorbed BSA, type Ⅰ collagen, and mixture of type Ⅰ collagen with BSA were showed in **Figure 1**. The BSA adsorbed on hydrophobic silicon surface showed a grain shape. Type Ⅰ collagen displays fibril-like molecules with relatively homogeneous heights and widths, characterized by clear twisting (helical formation) in **Figure 1** (c). The coverage of type Ⅰ collagen adsorbed on hydrophobic surface is more than that on hydrophilic surface. The AFM image of competitive adsorption was displayed in **Figure 1** (d). **Figure 1** (d) showed that few type Ⅰ collagen was adsorbed on hydrophobic surface and most part of the hydrophobic surface was occupied by BSA. It meant that the adsorption of BSA was more preponderant than that of type Ⅰ collagen on hydrophobic silicon surface. **Figure 2** (a) illustrated the image of hydrophilic silicon surface. The hydrophilic silicon surface is a glaze with about 0.5 nm undulations, so the proteins can be shown on it very well. **Figure 2** (b), (c) showed the images of BSA and type Ⅰ collagen adsorbed on hydrophilic surface. BSA was similar to **Figure 1** (b). The AFM image of competitive adsorption was displayed in **Figure 2** (d). The coverage of type Ⅰ collagen in **Figure 2** (d) is similar with **Figure 2** (c), which shows that quite a lot of type Ⅰ collagen appeared in the adsorption layer.

Competitive adsorption on hydrophobic surface

The hydrophobic interaction was thought to be the major interaction on hydrophobic surface. When proteins arrived at the hydrophobic surface, structure rearrangement might occur in which the inner hydrophobic groups of proteins are exposed to interact with surface. In general, less stable molecules are more surfaces active[2]. BSA is a flexible protein that easily denatured after adsorption[4,5]. Collagen molecule is a helical coil of three polypeptides, which is non-flexible and rather rigid[6]. Globular BSA should be easier to make a conformation rearrangement to exposure its inner hydrophobic groups of proteins. Those inner hydrophobic groups of proteins have a strong hydrophobic force with hydrophobic surface. The hydrophobic interaction of BSA with hydrophobic surface was stronger than that of type Ⅰ collagen. Adsorption of BSA was more preponderant than that of type Ⅰ collagen on hydrophobic surface.

Competitive adsorption on hydrophilic surface

The experiments showed that the influence of BSA on type Ⅰ collagen adsorption on hydrophilic surface was less than that on hydrophobic surface. Binding affinity is the major factor influencing the proteins competitive adsorption. The low affinity proteins adsorbed initially are easier to be replaced by scarcer proteins of high affinity[1]. The normal binding affinity in protein adsorption is recognized includeing H-bonding,

electrostatic, and hydrophobic interaction. For the hydrophilic surface of silicon with Si-OH, the hydrophobic interaction should be inexistent. The interactions between proteins and hydrophilic surface mainly include H-bonding and electrostatic interactions. The charge of the surface is believed to be an important factor[7]. On hydrophilic surface, the rearrangement or orientation of adsorbed molecules might take place whereby the mobile regions of positive charge are brought near the hydrophilic silica to enable the molecule to bind relatively tightly to the surface[2]. If the electrostatic interaction is the major interaction, the electrostatic interaction between type Ⅰ collagen and hydrophilic surface should be stronger than that between BSA and hydrophilic surface. The difference of different proteins binding affinity on different surfaces made the competitive adsorption of BSA on hydrophobic was stronger than on hydrophilic surface.

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