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Immobilization of Protein Molecules on Step-Controlled Sapphire Surfaces and Characterization of the Adhesion Forces

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1. Introduction

In the recent biotechnology, sensing techniques using solid surfaces attract much attention because electronic functions based on semiconductor device technology can be integrated with bio-sensing units. In those devices, immobilization of bio-materials on solid surfaces is one of the most critical issues because the biological activities are often inhibited through the immobilization process. In bio-sensors, on the other hand, non-specific adsorption of bio-molecules are often serious problems. In both cases, properties of the interface between bio-molecules and the solid surfaces play crucial roles towards high-performance bio-sensing devices on solid surfaces.

In this paper, we describe avidin molecule immobilization on well-defined sapphire surfaces. Avidin is a protein derived from egg-white and interacts specifically with biotin, a kind of vitamins. Sapphire is a chemically stable material as a substrate for bio-sensing devices. Moreover, the step arrangement can be controlled. We show that a sapphire substrate is suitable for arrangement control of the bio-molecules by using well-controlled steps. A well-defined surface is also suitable for exact measurement of the adhesion force between an bio-molecule and a solid surface. In this paper, we show adhesion forces of avidin molecules under various conditions.

2. Experiments

We used three kinds of sapphire C surfaces: a surface with randomly-distributed atomic steps (randomly-stepped surface), a surface with regularly-ordered single steps whose height is about 0.2 nm (single-stepped surface), and a surface with bunched steps (multi-stepped surface). A sapphire surface was chemically cleaned using a H_2SO_4 and H_2O_2 mixture. After this treatment, the surface is terminated with -OH groups.

In order to immobilize the avidin molecules by chemical bonds, we used 2-(carbomethoxy)ethyltrichlorosilane (CMETS) as a coupling molecule between the sapphire surface and the avidin molecules. After deposition of CMETS in the dipping process, the sample was immersed in the concentrated HCl for 24 hours to carboxylate the surface. Then, N-hydroxysuccinimide (NHS) was reacted with the COOH groups in a buffer solution of PH 7.4 to form an NHS-terminated surface. In this process, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was added to promote the dehydration reaction. Finally the sample was immersed in the avidin solution for 1 hour. In this step, the density of avidin molecules on the surfaces was controlled by changing the avidin mole fraction from 20 nM ([M] = [mol/l]) to 160 nM.

For the adhesion force measurements, both hydrophilic and hydrophobic surfaces were prepared. A hydrophilic surface is obtained by the chemical treatment using a H_2SO_4/H_2O_2 mixture. To form a hydrophobic surface, we used self-assembled monolayers of octadecyltrichlorosilane (OTS). Avidin molecules were physically adsorbed on these surfaces by immersing the substrates in the avidin-containing water.

The avidin-immobilized substrates were observed in air or in liquid by the dynamic force mode (DFM) of an AFM. The adhesion force was qualitatively estimated from whether any movement of the molecules is observed or not during the frictional mode scanning by regulating the set force, that is the vertical force of the tip with respect to the surface.

3. Results and Discussion

Figure 1 shows DFM images of the sapphire surfaces taken in air before and after avidin-immobilization. Figure 1(a) shows a typical morphology of a single-stepped surface indicating that the surface is atomically flat. Figures 1(b) and 1(c) show the avidin-immobilized surfaces when the avidin densities of the solution used for the deposition were 20 nM and 160 nM, respectively. Preferential adsorption of avidin molecules on the step edges is observed in Fig. 1(b). This suggests the possibility of arrangement control of bio-molecules by surface structure of the substrate. When the avidin density of the solution was 160 nM, avidin molecules were immobilized also on the terrace as well as the step edges, as shown in Fig. 1(c). Figure 1(d) is a DFM image of the avidin-immobilized randomly-stepped surface for the 20 nM solution where the molecule density is much higher. This result indicates that randomly-stepped surfaces are suitable for high-density immobilization compared with the single-stepped surfaces. The bio-activity of the immobilized avidin molecules was found to be retained from phase-shift images of avidin-immobilized surfaces taken using a biotinylated cantilever. Because a biotinylated cantilever interacts with the avidin molecules due to the avidin-biotin reaction, a phase-shift takes place only on the avidin molecule positions. Since no phase shift is observed when a normal cantilever is used, we can examine the bio-activity of the immobilized avidin molecules.

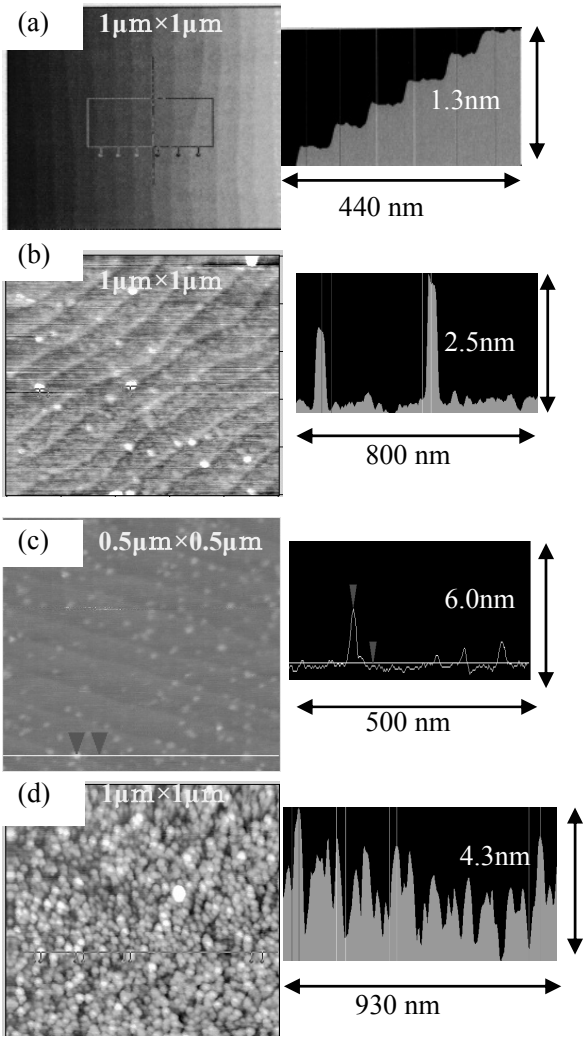

Fig. 1 DFM images and height profiles of the sapphire surfaces. (a) Single-stepped sapphire surface. (b) and (c) Avidin-terminated single-stepped surfaces where avidin densities in the solutions were 20 nM and 160 nM, respectively. (d) Avidin-terminated randomly-stepped surfaces where avidin density in the solution was 20 nM.

Figure 2 shows an example of the avidin molecule movement in aqueous environment when the surface was hydrophobic. Figure 2(a) is the original surface and Figs. 2(b) and 2(c) are the surfaces after a scan in the frictional mode with the set force (pressure from the tip) of 0.044 nN and 0.45 nN, respectively. The molecule in the black circle did not move for the set force of 0.044 nN, but moved for 0.45 nN. Table I shows the summary of the set forces that the molecule movement was first observed when the set force was increased. The avidin molecules are tightly bound on the surface in air, but the adhesion force on the hydrophilic surface is smaller than the hydrophobic surfaces. Avidin molecule movement in water was much different. On the hydrophilic surfaces, the whole avidin molecules were removed from the scanning area after the contact-mode scan even when the tip force was 0.044 nN. This is because a mobile water layer exists between the

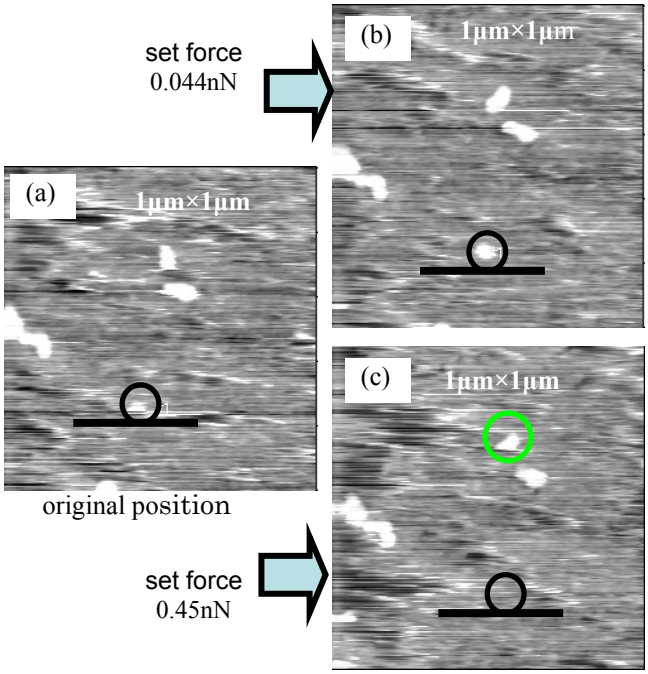

Fig. 2. DFM images of the sapphire surfaces (a) before the scan of the frictional force mode, (b) after the scan with the set force of 0.044 nN, and (c) after the scan with the set force of 0.044 nN.

avidin molecule and the hydrophilic surface. On the hydrophobic surfaces, on the other hand, the avidin molecules were weakly bound. After the scan, it was often observed that some avidin clusters became larger. This is probably due to the detachment of the molecule from the tip and the attachment to the cluster. Although the physical adhesion forces are generally small in liquid, the avidin molecules immobilized by chemical bonds are tightly fixed to the surface as shown in Table I.

Table I Set forces when movement of the avidin molecules were observed by a scan in the frictional force mode.

environment	hydrophilic/hydrophobic	set force
air	hydrophilic	18 nN
air	hydrophobic	>22 nN
liquid	hydrophilic	0 nN
liquid	hydrophobic	0.45 nN
liquid	immobilized by chemical bond	>22 nN

4. Conclusions

Avidin molecules were immobilized on well-defined sapphire surfaces and the adhesion forces were characterized. We have found that the sapphire surfaced is suitable to control the arrangement of protein molecules and the adhesion forces.

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