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Selective Adsorption of Protein Molecules on Atomic-Structure-Controlled Sapphire Surfaces

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1. Introduction

Non-specific adsorption of protein molecules on solid surfaces is one of the serious issues in bio-sensors and bio-chips [1] because it hides the target specific reaction behind the other materials. Therefore, the studies on protein adsorption have been focused on development of inert materials for biological molecules and a passivation technique of the device surfaces using those "bio-compatible materials". In these studies, attentions were not paid to a difference in adsorption properties depending on structures and properties of the proteins.

In this paper, we report on the selective adsorption of protein molecules on sapphire surfaces.

2. Experimental Procedures

Substrates

Sapphire substrates [2] were used for the selective adsorption of protein molecules. The sapphire (0001) surface was miscut about 0.15 degree and the misorientation direction was tilted slightly from the direction of a stable atomic step on this surface. We refer to "tilting angle" for this angle. The wafers were annealed at 1400 °C. Under these conditions, the surface is covered with bunched steps of about 1.0 nm height and the crossing steps generated by the tilting from the stable step direction. Hundreds-nanometer scaled terraces are grown on the areas surrounded by the bunched steps and the crossing steps.

Protein adsorption

The sapphire substrates were treated with an H_2SO_4 - H_2O_2 solution. To examine the effect of the acid treatment, we also prepared thermally treated substrates which were annealed in air at 700 °C. On these surfaces, protein molecules, such as bovine serum albumin (BSA), ferritin, fibrinogen, and avidin, were adsorbed in a 100 mM (M=mol/l) N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/NaOH (pH7.0) buffer solution. After the adsorption process, the extra protein molecules were removed from the buffer solution.

Characterization

The sapphire surfaces after the acid treatment or the thermal annealing were characterized by the topographical mode and the frictional force mode in atomic force microscopy (AFM) in air. A frictional force is measured from the twist of the cantilever whereas the topograohy from the bending. Therefore, both images can be taken simultaneously. Protein adsorption patterns were observed by the tapping mode in AFM in buffer solutions to retain the biological activity of the proteins.

To investigate the surface chemical states after the acid treatment, OTS (octadecytrichlorosilane, $C_{18}H_{37}SiCl_3$) self-assembled monolayers were deposited by the dipping method.

3. Results and Discussion

Figure 1 shows topographical and frictional images on sapphire surfaces. On this surface, the tilting angle was small and the density of the crossing steps was small, as shown in Fig. 1(a). The topographical image after the thermal treatment was same as that after the acid treatment. As shown in Figs. 1(b) and 1(c), however, the frictional images were different between the treatment methods. In Fig. 1(b), elliptic domains with less frictional force was clearly observed, whereas there was no specific pattern after the thermal treatment, as shown in Fig. 1(c).

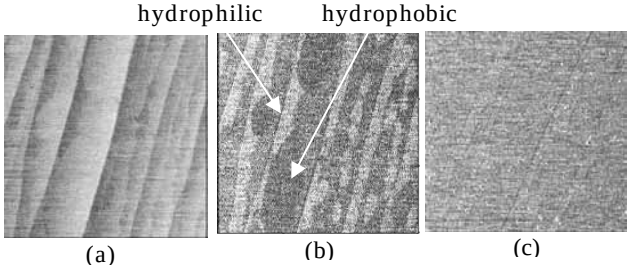

Fig. 1 AFM images on the sapphire surface. (a) Topographical and (b) frictional images of the acid-treated surface, and (c) frictional image of the thermally treated surface. Images are 15 x 15 μm^2.

Generally, the difference in the frictional force is generated by the amount of adsorbed water. The sapphire surface becomes hydrophilic after the acid treatment because the surface is terminated with OH-groups. To investigate the spatial distribution of the OH group, an OTS SAM was deposited. Figure 2 shows an OTS pattern deposited on a sapphire surface observed by AFM. Since OTS molecules are chemically fixed only on the OH terminated areas, the image shown in Fig. 2 clearly demonstrates that the OH-group density on the elliptic areas is smaller than the outside areas. In other words, a phase separation into hydrophilic and hydrophobic domains takes place on the acid-treated surface.

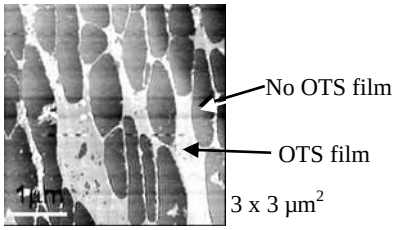

Fig. 2 OTS deposition pattern on an acid-treated surface.

Figure 3 shows AFM images of the sapphire surfaces after ferritin molecule adsorption (a) on the acid-treated surface and (b) on the thermally treated one. On the acid-treated surface, the ferritin molecules are preferentially adsorbed on the smaller frictional force domains whereas no specific pattern is observed on the thermally treated surface. This pattern formation shows that the acid-treated surface consists of two domains and that the ferritin molecules are preferentially adsorbed on the more hydrophobic domains. Generally, protein molecules tend to be preferentially adsorbed on a hydrophobic surface rather than a hydrophilic one. The result shown in Fig. 3(a) coincides with such general phenomenon. On the thermally treated surface shown in Fig. 3(b), on the other hand, the hydrophilicity is uniform over the surface. Therefore, a uniform adsorption of ferritin molecules takes place.

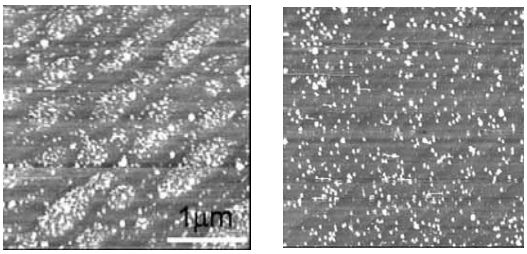

Fig. 3 Adsorption patterns of ferritin molecules on (a) the acid-treated (3 x 3 μm^2) and (b) the thermally treated sapphire surfaces (5 x 5 μm^2).

Figure 4 shows adsorption patterns for various proteins. In this series of experiments, sapphire surfaces with a larger tilting angle were used. This means that the density of crossing steps are higher. The hydrophobic (smaller density of OH group) domains are rather circular and isolated from each others. Therefore, the adsorption patterns can be more clearly demonstrated. In Fig. 4(a) and 4(b), albumin and ferritin exhibits similar patterns where the hydrophobic domains are the preferential adsorption sites. Avidin molecules, however, are preferentially adsorbed on the hydrophilic domains, as shown in Fig. 4(d), which is opposite to albumin and ferritin cases. In the case of fibrinogen, no clear adsorption pattern appeared.

We describe the correlation of the adsorption patterns and the molecular structures. Albumin and ferritin molecules are mainly composed of α-helixes whereas avidin is of β-sheets. Generally, an α-helix prefers hydrophobicity to hydrophilicity if compared with β-sheets, as demonstrated in the configuration of membrane proteins buried in the cell membrane. Although the origin of the selectivity should be quantitatively described by considering hydrophobic attractive force, hydrophilic repulsive force, van der Waals force, and the electrostatic force generated by the charges on the substrate surface and the protein molecules, the observed selectivity shows a possibility of the protein selection by a simple method.

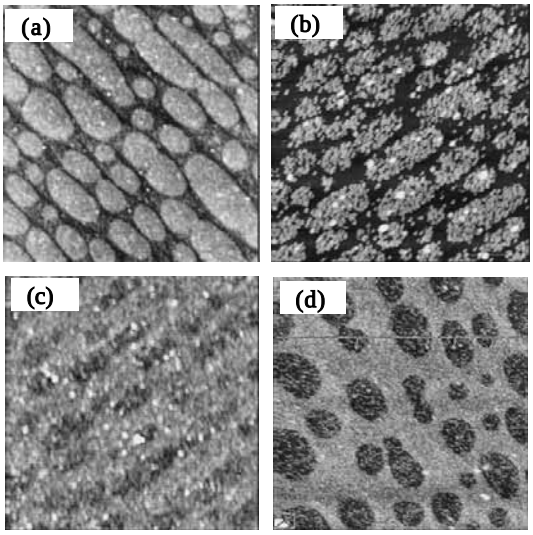

Fig. 4 Adsorption patterns of various protein molecules on the acid-treated sapphire surfaces. (a) Albumin, (b) ferritin, (c) fibrinogen, and (d) avidin. Protein molecules are preferentially adsorbed on the bright domains.

4. Conclusions

We have found that a domain structure is self-organized on step-controlled sapphire surfaces where hydrophilic and hydrophobic areas appear after the acid treatment. Protein molecules exhibit specific adsorption patterns on this surface. The observed adsorption patterns seem to be related to the molecular structures. The present surface can be obtained simply by acid treatment if the step arrangement is carefully designed. Therefore, this surface is promising for protein selection chips. The present finding also suggests that molecular recognition by a solid surface is also possible.

Acknowledgements

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References

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[2] Y. Shiratsuchi, et al., Jpn. J. Appl. Phys., 41 (2002) 5719.