Fabrication of a New Substrate for Atomic Force Microscopic Observation of DNA Molecules from an Ultrasmooth Sapphire Plate

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ABSTRACT   A new stable substrate applicable to the observation of DNA molecules by atomic force microscopy (AFM) was fabricated from a ultrasmooth sapphire (α-Al_2O_3 single crystal) plate. The atomically ultrasmooth sapphire as obtained by high-temperature annealing has hydrophobic surfaces and could not be used for the AFM observation of DNA. However, sapphire treated with Na_3PO_4 aqueous solution exhibited a hydrophilic character while maintaining a smooth surface structure. The surface of the wet-treated sapphire was found by x-ray photoelectron spectroscopy and AFM to be ~0.3 nm. The hydrophilic surface character of the ultrasmooth sapphire plate made it easy for DNA molecules to adhere to the plate. Circular molecules of the plasmid DNA could be imaged by AFM on the hydrophilic ultrasmooth sapphire plate.

INTRODUCTION

Atomic force microscopy (AFM) has been extensively applied to the atomic-scale observation of biological molecules (Ratneshwar and Scott, 1994: Ushiki et al., 1996: Yang and Shao, 1993).

In particular, many groups have investigated the structure of deoxyribonucleic acid (DNA) molecules in air and/or water by AFM (Hansma et al., 1995: Vesenka et al., 1992: Yang and Shao, 1993). So far, cleaved mica has mainly been used as the substrate for the AFM observation of these molecules because it has atomically smooth surfaces. Because mica contains potassium as a component, the surface structure of a mica plate is considered to be unstable when exposed to air or water. It is important for the precise atomic-scale analyses of biomolecules to develop a new substrate that is stable in air and water for a long period.

Recently we succeeded in obtaining an atomically flat sapphire (α-Al_2O_3) with atomic steps on its surface by high-temperature annealing in air (Yoshimoto et al., 1995). The ultrasmooth sapphire was found to be useful for the perfect epitaxial growth of oxide thin films (Maeda et al., 1997). The ultrasmooth surface structure is strikingly stable in water as well as in air for some months. However, the hydrophobic character of ultrasmooth sapphire makes it difficult to use this material as a substrate for adhering hydrophilic organic molecules such as DNA. For this reason it is essential to develop a hydrophilic surface for sapphire.

In this study we investigated the effect of chemical treatments by using several solutions on the hydrophilic surface property of the sapphire. We also investigated the difference in the stabilities of the surface structures of a mica plate and a hydrophilic ultrasmooth sapphire plate. We will show the capabilities of a hydrophilic ultrasmooth sapphire plate as a new substrate for AFM observation of DNA.

EXPERIMENTAL

The sapphire (0001) plate (Shinkosha Co. Ltd., Yokohama, Japan) were subjected to thermal annealing at 1000°C for 1 h in air to create an ultrasmooth surface with atomic steps. The ultrasmooth sapphire obtained was wet-treated with various kinds of aqueous solutions of NaCl (2 mol/liter), Na_2CO_3 (2 mol/liter), $MgCl_2$ (1 mol/liter), or Na_3PO_4 (0.5 mol/liter), and then rinsed with distilled water and dried in air. The contact angle of water was measured on the wet-treated samples. The surface morphology of the wet-treated sapphire was also examined by AFM. The AFM observation was carried out in air with an SPI 3700 probe station (Seiko Instruments, Chiba, Japan). All images were obtained in noncontact mode, using cantilevers with a silicon tip (Martin et al., 1987).

The chemical composition of the sapphire surface was characterized by x-ray photoelectron spectroscopy (XPS) (JEOL model JPS-80). XPS spectra were recorded by using Mg K_α radiation (1253.6 eV) under a pressure of 4×10^{-7} Pa.

To compare the stability of the surface structure of a mica plate with that of a wet-treated sapphire plate against water exposure, the surface roughnesses of mica and sapphire were measured by AFM as a function of period after dipping into water followed by drying in air. Surface roughnesses were measured for samples of mica and wet-treated sapphire plate that were exposed to air after the deposition of distilled water and drying with compressed air, and samples that were exposed to water.

Double-stranded plasmid DNA with a ring structure (pUC18, 2868 bp) was diluted with distilled water to a final concentration of 50 ng/μl. The DNA was deposited on the center of a wet-treated sapphire plate. Then the wet-treated sapphire plate was blown dry with compressed air. AFM imaging of DNA was carried out in air in noncontact mode.

RESULTS AND DISCUSSION

Fig. 1 shows the AFM image on the surface of the annealed sapphire (0001) plate and its line profile. The annealed sapphire plate had ultrasmooth terraces with atomic steps 0.2 nm in height. The surface morphologies of the plates
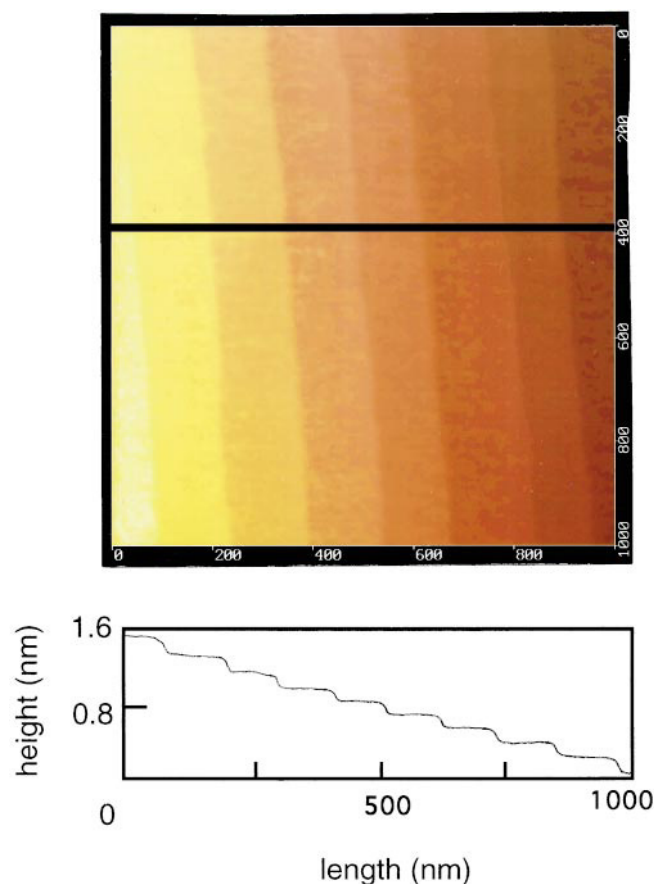
FIGURE 1 AFM image and a line profile on the sapphire annealed at 1000°C in air.

FIGURE 2 AFM image and a line profile on the surface of the sapphire annealed and then wet-treated with Na_3PO_4 aqueous solution.

wet-treated with several solutions were also examined by AFM. The surface of the sapphire treated with NaCl or $MgCl_2$ solution had many particles a few nanometers in height on the terraces. However, the sapphire treated with Na_3PO_4 or Na_2CO_3 solutions maintained atomic steps and ultrasmooth terrace structure. Fig. 2 shows the AFM image of the surface of a Na_3PO_4-treated sapphire plate and its line profile. It indicates that the height of the individual steps is 0.2 nm, even after Na_3PO_4 treatment. The surface smoothness of each terrace was found to be better after the Na_3PO_4 treatment than after the Na_2CO_3 treatment.

The contact angles of dropped water on the surfaces of the annealed sapphire plates treated with NaCl solution and $MgCl_2$ solution were all $\sim 80°$ (Fig. 3 a), indicating their hydrophobic character. On the other hand, the contact angle for the sapphire plate treated with Na_2CO_3 or Na_3PO_4 solutions was changed to $\sim 20°$ (Fig. 3 b), which indicates the hydrophilic character of the plate surface.

To examine the surface changes after wet treatment, the sapphire plate treated with Na_3PO_4 solution and the annealed sapphire were subjected to XPS, focusing on the signals of Al 2s, Na 1s, and P 2p. The photoelectron intensities were evaluated as the integrated areas of the XPS peaks. Although the peaks of Na and P were not detected in the case of the annealed sapphire, the peaks of Na and P
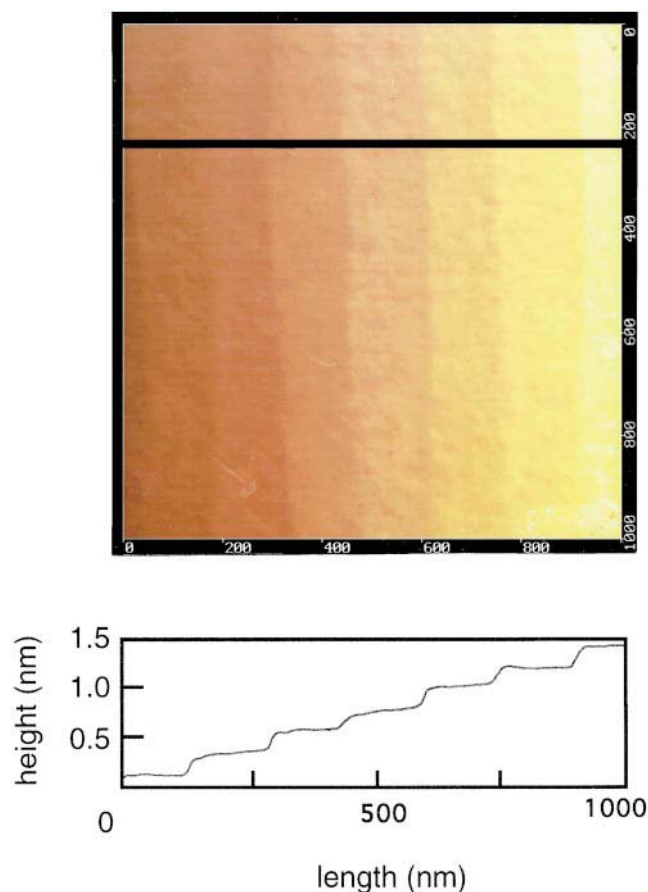
were clearly observed for the wet-treated sapphire. The peak intensity of Al from the wet-treated sapphire is smaller than that from the nontreated sapphire. It means that the surface of the sapphire plate treated with Na_3PO_4 solution was covered with the compound including Na and P elements.

The thickness of the compound layer on the sapphire plate treated with Na_3PO_4 solution was estimated to be ~ 0.3 nm from the difference in the peak intensity of Al 2p between the nontreated and wet-treated sapphires. The composition ratio of Na to P was determined from the XPS intensities of Na 1s and P 2s to be 2:1.

Table 1 shows a comparison of binding energies of P 2p and Na 1s peaks for the sample wet treated with Na_3PO_4 solution with values reported for some oxides including Na and P (Ikeo et al., 1991). From the results on the composition ratio of Na to P and binding energies for the wet-treated sample, it is concluded that the surface of the sapphire plate treated with Na_3PO_4 aqueous solution was uniformly covered with a 0.3-nm-thick Na_2HPO_4 compound layer.

Fig. 4 shows the time dependence of surface roughness of a mica plate and that of a wet-treated sapphire plate. The surface of mica that was exposed to air and water became rough in a few hours. On the other hand, the surface roughness of a wet-treated sapphire plate that was exposed to air
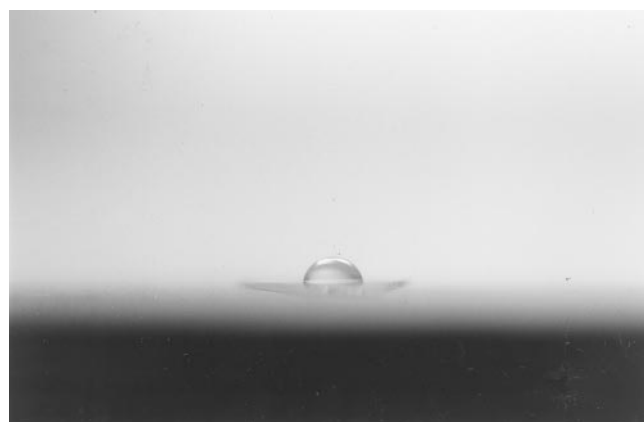
(a)
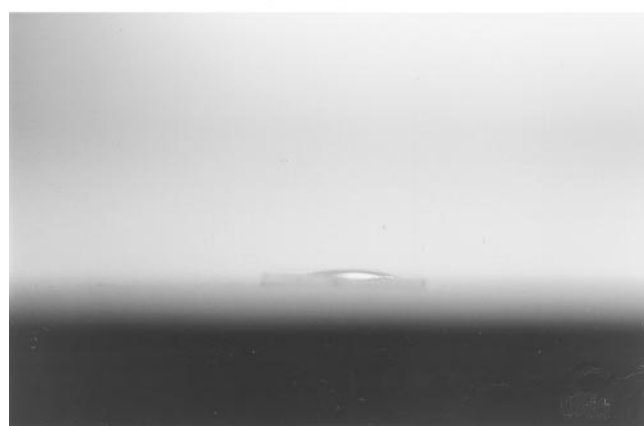
(b)

FIGURE 3 Photographs showing a contact angle of a drop of water on the as-annealed sapphire substrate (*a*) and on the substrate annealed and then wet-treated with Na_3PO_4 Aqueous solution (*b*).
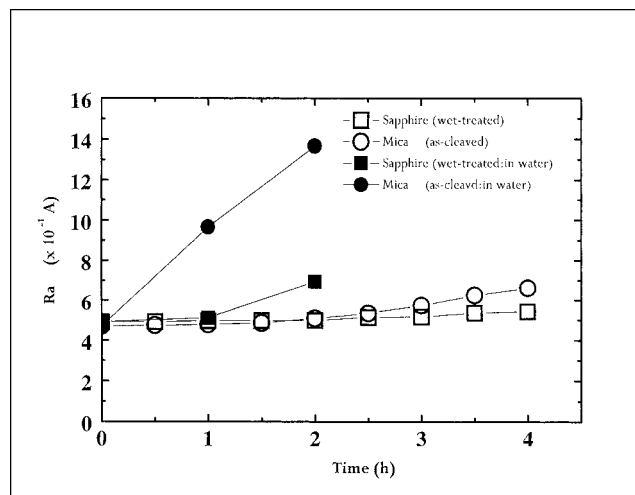

FIGURE 4 Time dependence of surface roughness (Ra) of mica plate and wet-treated sapphire plate.

Fig. 5 shows the AFM image of plasmid DNA on an ultrasmooth sapphire plate treated with Na_3PO_4 aqueous solution. The ring structure of the plasmid DNA molecule could be clearly observed, although the double-helix structure was not. The observed height and width of the DNA molecules were 1–1.5 nm and 7–15 nm, respectively.

SUMMARY

A new substrate for the observation of DNA molecules by AFM was fabricated by using a ultrasmooth sapphire plate treated with Na_3PO_4 aqueous solution. Wet treatment of an ultrasmooth sapphire obtained by high-temperature annealing changed its surface character from hydrophobic to hydrophilic while maintaining its smooth surface structure. The surface of the wet-treated substrate was found from XPS analyses to be covered with Na_2HPO_4 layers with a thickness of ~0.3 nm.

The surface structure of wet-treated sapphire plates is also found to be stabler than that of mica. The hydrophilic surface character of ultrasmooth sapphire plates made it easy for DNA molecules to adhere to the substrate. With a hydrophilic ultrasmooth sapphire plate as an AFM substrate, plasmid DNA molecules were clearly and stably observed by AFM in air.

and water changed less than the mica plate. This indicates that the surface structure of a wet-treated sapphire plate is stabler than that of cleaved mica in air and water. Furthermore, Fig. 4 suggests that a wet-treated sapphire plate would be very useful for AFM observation of DNA molecules in water.

Using the hydrophilic ultrasmooth sapphire plate obtained in this work, we tried to observe plasmid DNA by AFM.

TABLE 1 XPS binding energies of P 2p and Na 1s peaks from the sapphire substrate wet-treated with Na_3PO_4 aqueous solution and the values for an oxide

XPS peak	Binding energy (this work)	Binding energy (compound) (reported values)
P 2p	132.5 eV	132.2 eV (Na_3PO_4)
		133.1 eV (Na_2HPO_4)
Na 1s	1071.5 eV	1071.1 eV (Na_3PO_4)
		1071.9 eV (Na_2HPO_4)

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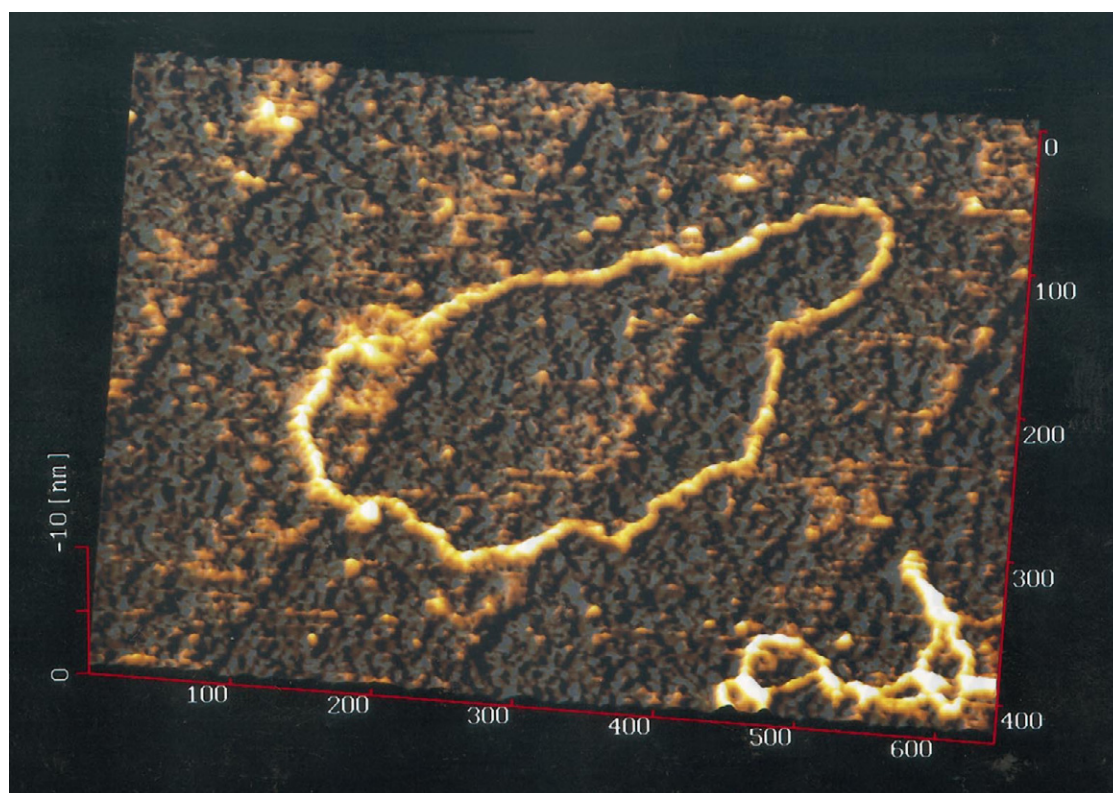
FIGURE 5   AFM image of plasmid DNA with a ring structure on the ultrasmooth sapphire substrate wet-treated with Na_3PO_4 aqueous solution (image size: 670 nm $\times$ 450 nm).

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