Scanning tunneling microscopy imaging and manipulation of DNA oligomer adsorbed on Cu(111) surfaces by a pulse injection method

Hiroyuki Tanaka and Tomoji Kawai a)

The Institute of Scientific and Industrial Research, Osaka University, Mihogaoka, Ibaraki, Osaka 567, Japan

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We have developed a pulse injection method of DNA oligomer aqueous solution onto Cu(111) surfaces as a new sample-preparation technique for scanning tunneling microscopy (STM) studies of biomolecules. The STM images revealed that the surface of Cu(111) retains its atomic flatness even after the injection of DNA-containing solution and that the deposition of intact DNA oligomers without aggregation is possible. The observed internal structure of deposited DNA suggests the promising possibility of sequencing DNA by means of STM. In order to examine the possibility of manipulation of DNA, translational positioning of DNA oligomer has been performed by a lateral pushing of the DNA with scanning tip. © 1997 American Vacuum Society.

I. INTRODUCTION

The scanning tunnel microscopy (STM) imaging of DNA is the first step toward the sequencing and manipulation of DNA by means of STM. This goal, however, has not been achieved up to now. This is partly due to the difficulty to obtain reliable images at high spatial resolution. One of the major difficulties is the development of a reliable sample preparation method that can provide a deposition of DNA, the biological molecule, onto a surface without aggregation. It is well known that conventional methods such as air evaporation, electrochemical deposition, and dropping of DNA-containing solution directly onto a substrate, result in the deposition of aggregated DNA. Moreover, it is clear that molecular manipulation by STM cannot be achieved unless a reliable sample preparation method is obtained. In order to achieve the reliable sample preparation method, many new techniques such as pulse deposition, electrostatic spray, adsorption to chemically modified surfaces, and electrochemical deposition have been explored.

In this article, we describe a pulse injection technique for the adsorption of DNA oligomers onto a Cu(111) substrate in vacuum system. We have performed STM experiments in ultrahigh vacuum (UHV), because imaging in vacuum tends to afford the highest resolution for DNA. We have chosen the Cu(111) surface because it has low corrugation of the potential for the surface diffusion on atomically flat terraces, as one of the close-packed face-centered cubic (111) noble metal surfaces. This offers us an appropriate adsorption condition to perform translational manipulation of deposited DNA oligomers by lateral pushing by the scanning tip. Under UHV conditions, the common method of deposition of solid organic molecules such as nucleic acid bases is performed by thermal evaporation. This method, however, cannot be applied to large biomolecules such as DNA oligomers due to pyrolytic decomposition. From this reason, the common droplets method has been applied to the deposition of aggregated DNA under (at ambient pressure) conditions.

By pulsed injection of DNA containing solution onto the Cu(111) surfaces in UHV, using a high-speed solenoid pulse valve, a codeposited solvent (H2O) will volatilize leaving DNA as the species onto the surface before aggregation of DNA occurs. The word “aggregation” is defined in this article as “to form large particles.” In this deposition process, we found that a Cu(111) substrate retains its atomic flatness at the STM level even after the exposure of the injection of DNA-containing aqueous solution and that aggregation of DNA is prevented. We show results of STM imaging of deposited DNA oligomer on Cu(111) by the pulse injection method and also positioning of deposited DNA oligomer by lateral pushing by the scanning tip.

II. PROCEDURE

A schematic of the apparatus is shown in Fig. 1. The UHV apparatus basically consists of a STM chamber and a preparation chamber. After repeated sputtering and annealing cycles in the preparation chamber, we obtained a clean flat Cu(111) surface. The single-stranded DNA oligomer purified by HPLC, pAAAAA, containing five adenines was purchased from Vector Research Co. (Japan). The distilled water, purified by passage through a Barnstead II purifier, was used in this study. The concentration of the sample was 0.1 mM/l. In order to make aggregated DNA dissociate, the pulse valve (General Valve Co., Series 9) was warmed by a hand-made electric heater at ~90 °C for a few minutes before the injection. The conditions of the pulse injection were the following. The temperature of the Cu(111) substrate was room temperature. The height of the pulse valve above the substrate surface was 130 nm. The temperature of the sample (pAAAAA, 0.1 mM/l) was ~90 °C. The amount of injected sample was ~0.1 ml for 10 shots of the pulse with each pulse duration of 1.5 ms. Under these conditions, we estimate an approximate coverage of pAAAAA at around 100 of the molecules on the area of 100×100 nm². After the deposition by the injection, the Cu(111) substrate was transferred to the STM chamber to perform STM observation.

a)Author to whom correspondence should be addressed; Electronic mail: kawai@sanken.osaka-u.ac.jp
The STM measurements were performed using USM-401U (Unisoku, Japan), using electrochemically etched W tips. The images were typically taken with a sample bias $V_s$ of $-0.5$ to $-3$ V and a tunnel current of 10 pA. The positioning of the deposited DNA oligomer was performed by lowering the tunnel voltage: $V_t$ to $-1$ mV, similar to that reported in the literature.  

III. RESULTS AND DISCUSSION

Figure 2 shows a typical STM image ($V_s = 2$ V, $I_t = 10$ pA, $100 \times 100 \times 0.4$ nm$^3$), obtained after the injection of the aqueous sample solution. One can see the distribution of bright objects on an atomically flat terrace in this image. Some of them are preferably located at the step edges (not shown). These objects did not appear when control experiments (injection of H$_2$O without DNA) were performed. This result indicates that the surface of Cu(111) retains its atomic flatness even after the exposure to the injection of DNA-containing aqueous solution. These bright objects have topographical heights of 0.2–0.3 nm. The heights of 0.2–0.3 nm is relatively larger than those usually observed for the height of nucleic acid bases adsorbed on Cu(111) substrate, typically 0.1–0.2 nm. Assuming that deposited single-stranded pAAAAA ($\sim 3$ nm long, when straight) takes flat (rather than steric) conformation to the substrate plane, the height of 0.2–0.3 nm may be explained. Although there is a distribution of apparent shapes and widths of these objects, smaller objects tend to have round shape with $\sim 3$ nm width. On closer inspection, they appear to have an internal structure of several bright maxima. However, the resolution of the images is not high enough to determine a definite structure model. We have observed by STM self-assembly of DNA bases adsorbed on Cu(111) and found that individual molecules are difficult to be resolved at room temperature but can be resolved at liquid nitrogen temperature. Since STM imaging was performed at room temperature in this experiment, we consider that limited resolution is due to the thermal perturbation at room temperature. We presume that imaging at low temperature enable us to obtain higher resolution to sequence DNA. Thus we think that round bright objects with $\sim 3$ nm are isolated pAAAAA and larger objects are clusters of the pAAAAA, which may be formed during surface diffusion before losing translational energy. We tentatively propose a model for the observed isolated pAAAAA, as shown in Fig. 3. In this model, the hydrophilic chain of the each sugar and phosphate is located to form a circle.
inside, while each hydrophobic adenine is located towards outside the circle. The diameter of this model is \( \sim 3 \) nm, in good agreement with the observed value of \( \sim 3 \) nm. It should be emphasized that the deposition of intact DNA oligomer without aggregation is possible using pulse injection of DNA-containing solution.

Gimzewski et al. have positioned C\(_{60}\) adsorbed on Au(110) by a lateral pushing action of the tip of STM at lowered sample voltage: \( V_s = -4 \) mV.\(^{11-13}\) In order to examine a possibility of manipulation of DNA, positioning of the pAAAAA DNA oligomer has been performed by a lateral pushing of the scanning tip. Figure 4(a) is an image of a region \( (40 \times 28 \times 0.4 \text{ nm}^3) \) taken at \( V_s = -2 \) V and \( I_t = 10 \) pA prior to the manipulation. Then, manipulation was performed with the STM tip coming approximately 0.8 nm closer to the surface by scanning at \( V_s = -1 \) mV and \( I_t = 10 \) pA on a region \( (10 \times 9 \text{ nm}^2) \), as indicated with a white open square in Fig. 4(a). An image simultaneously recorded during this manipulation is also shown in the inset of Fig. 4(a), indicating a trajectory (negative contrast) of the molecule in manipulation. After this operation, an image of the same region was obtained, as shown in Fig. 4(b). This image reveals a lateral displacement of the molecule, as indicated with a white arrow. This attempt indicates that the positioning of the DNA oligomer has been achieved by means of STM as a step towards further manipulation.

IV. CONCLUSIONS

We have investigated pulse injection of aqueous solution containing DNA oligomer onto Cu(111) surfaces as a novel sample-preparation technique for STM studies of biomolecules. We found that the surface of Cu(111) retains its atomic flatness even after the exposure to the injection of aqueous solution and that the deposition of intact DNA oligomer without aggregation is possible. We have proposed a model for the observed isolated pAAAAA in which hydrophilic chain of each sugar and phosphate is located to form a circle inside, while each hydrophobic adenine is located towards outside the circle. In order to examine the possibility of manipulation of DNA, translational positioning of DNA oligomer is achieved by a lateral pushing with the scanning tip. A rather simple sample-preparation method may contribute to the studies on molecules which cannot be evaporated or deposited without degradation or aggregation.

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