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High-resolution scanning tunneling microscopy imaging of DNA molecules on Cu(111) surfaces

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Abstract

Using the pulse injection method, single-stranded DNA and double-stranded plasmid DNA have been deposited on well-defined Cu(111) surfaces under ultrahigh vacuum (UHV) conditions to obtain high-resolution scanning tunneling microscope (STM) images. These particular UHV-STM images have revealed that DNA molecules are adsorbed directly onto a clean Cu(111) surface and exhibited the detailed structures of DNA, which has not been resolved previously. The single-stranded DNA oligomers have exhibited the images of individual internal base molecules and the helix structures made of complementary base sequences. For the double-stranded plasmid DNA, the images have shown the Watson–Crick double-helix structure.

Keywords: Biological molecules; DNA; Copper; Single crystal surfaces; Low index single crystal surfaces; Scanning tunneling microscope (STM)

1. Introduction

Since the discovery of the double helix structure of DNA by Watson and Crick in 1953 [1], direct observation of DNA with real-space atomic/molecular resolution using microscopy has been extremely intriguing. After the advent of the scanning tunneling microscope (STM), many reports aiming at the observation of DNA appeared [2,3]. Among them, the highest resolution STM images were reported in 1989–1990 on DNA adsorbed on highly oriented pyrolytic graphite substrates prepared by dropping DNA solution in air [4–7]. However, later reports have identified these 'DNA images' as grain boundaries of graphite [8,9]. After these reports, almost no high-resolution STM images have appeared in scientific journals. The problem is considered to lie in the lack of a sophisticated sample preparation technique by which DNA molecules can be deposited on an atomically flat well-defined substrate surface without contamination. Since an ultrahigh vacuum (UHV) condition is one of the most well-defined conditions, we have performed observation of DNA by UHV-STM using a clean single crystal Cu(111) surface as an artifact-free well-defined substrate. We have developed a pulse injection method as a new sample preparation technique

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applicable to bio-molecules such as DNA to avoid decomposition. Here we report reproducible and reliable high-resolution STM images of DNA deposited on a well-defined Cu(111) substrate under UHV conditions using the pulse injection method. The STM images clearly exhibit the sub-molecular structures, i.e. double helix and individual bases, for the first time.

2. Experimental

Three kinds of DNA molecule have been observed: single-stranded DNA oligomers purified by HPLC, (i) pAAAAA (containing five adenines), (ii) pAAAAAAATTTTTTT (containing seven adenines and seven thymines), and double-stranded plasmid DNA of 3k base-pairs. The concentrations of the DNA samples were 0.1 mM, 0.03 mM and $1.2\,\mu g\,ml^{-1}$ for pAAAAA, pAAAAAAAATTTTTTT and 3k-base-pair plasmid DNA respectively. The plasmid DNA solution contains $\sim$0.05 mM of Tris and $\sim$0.005 mM of EDTA as buffer species. The UHV apparatus consists of an STM chamber and a preparation chamber as shown in Fig. 1a [10]. After repeated sputtering and annealing cycles in UHV in the preparation chamber, we obtained a clean flat Cu(111) surface. The DNA molecules were deposited using the pulse injection method as follows (see Fig. 1a). The temperature of the Cu(111) substrate was room temperature. The orifice of the pulse valve (General Valve Co., Series 9) is 0.8 mm, and the distance between the orifice and the Cu(111) substrate surface is 130 mm. The total amount of injected sample solution was $\sim$0.1 ml for five shots of the pulse with a pulse duration of 1.5 ms. Upon the injection of the DNA solution, the base pressure of the preparation chamber changes from $\sim 1\times10^{-8}$ Torr to $\geq 1\times10^{-3}$ Torr. After the base pressure of the preparation chamber becomes better than 1×10^{-6} Torr, the Cu(111) sample is transferred to the STM chamber whose base pressure is $\leq 1\times10^{-10}$ Torr. The STM measurements were performed using models USM-401U and USM-602S2 (Unisoku, Japan), at room temperature for the pAAAAA and at liquid nitrogen temperature($\sim$80 K) for the pAAAAAAATTTTTTT and the plasmid DNA in order to suppress thermal disturbance for the improvement of the resolution of STM images.

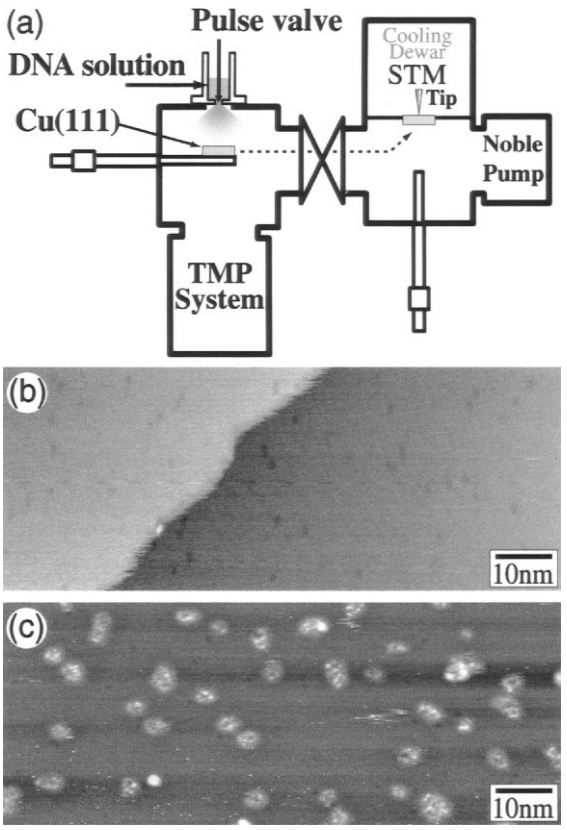

Fig. 1. Schematic diagram of the pulse injection and STM apparatus used for this study (a), comparison of STM images of Cu(111) substrate surfaces obtained before (b) and after (c) the injection of the DNA, pAAAAA. Imaging parameters: (a) $V_s=-2$ V, $I_t=10$ pA, (b) $V_s=-3$ V, $I_t=10$ pA. The x, y, z scales of both images are $100\times40\times0.4$ nm^3. Both images were observed at room temperature. Bright objects that have not been observed before the injection are seen on an atomically flat terrace (b). Dark features observed in (b) are possibly copper defects or carbon [11].

3. Results and discussion

A typical STM image of a clean Cu(111) substrate surface showing two terraces separated by a single atomic step is shown in Fig. 1b. Upon the injection of single-stranded pAAAAA aqueous solution on to the Cu(111) surface, we observed

circle/arc-shaped adsorbates, as shown in Fig. 1c; these are not observed after the injection of pure water in a control experiment. These bright protrusions have a topographic height of $\sim$0.2 nm and an apparent diameter of $\sim$2 nm, and a series of high magnification three-dimensional images are exhibited in Fig. 2a–c. In these images, there appear to be five bright maxima corresponding to internal molecular structures. The observed height of $\sim$0.2 nm is close to the typical values usually observed for the height of base molecules adsorbed on Cu(111) substrate [12,13]. The contrast of the STM images for adsorbed molecules corresponds to the density of states near the Fermi level arising from hybridization of the metallic states with states of adsorbed molecules [14,15]. Among the three molecular components in DNA, base, sugar and phosphate, the bases that have π electrons mainly contribute to the density of states near the Fermi level. Therefore, the observed images of DNA molecules (Fig. 2) may contain information on the molecular orbitals of base molecules; the deposited single-stranded DNA oligomers take up a flat conformation with their bases lying flat rather than in steric conformation on the Cu(111) surface. In order to examine the validity of the above assumption, we have constructed a structural model which reproduces the observed shape and dimensions of the STM images. A possible model is shown in Fig. 2d. In this model, the hydrophilic sugar–phosphate chain is located inside to form a circle/arcs, whereas the hydrophobic bases are located outside of the circle/arcs. It should be noted that when the hydrophilic sugar–phosphate chain is located outside and the hydrophobic bases are inside, the bases must be upright from the substrate surface due to the steric repulsion between neighboring bases. This would make the diameters of the circle/arcs much smaller and the topographic height a much larger value than the 0.2 nm, which is close to the typical values observed for the height of base molecules lying flat on a Cu(111) substrate [12,13]. Therefore, the proposed structural model reproduces the dimensions of the observed single-stranded DNA molecules. The circular structure of the single-stranded DNA is not common in an aqueous environment where water molecules are involved. However, in our UHV deposition system, where water molecules present around adsorbed DNA molecules are pumped out, a single-stranded DNA molecule may be adsorbed on the substrate surface with as many parts of its constituent elements (sugar–phosphate chain and lying-flat bases) as possible being adsorbed in order to reduce the total energy. As a result, the circular structure of single-stranded DNA is formed.

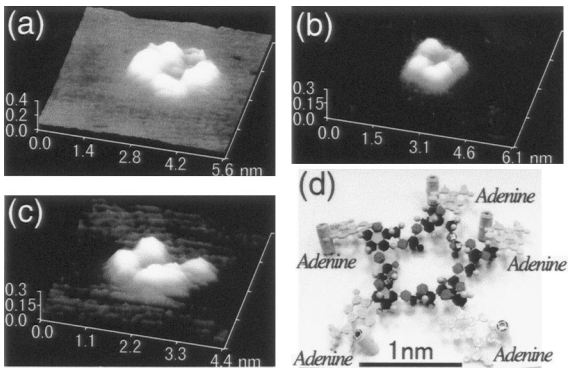

Fig. 2. A series of high magnification STM images (a)–(c) and a proposed model (d) of pAAAAA adsorbed on Cu(111) surfaces. (a)–(c) The molecules appear to have five maxima corresponding to internal molecular structures. (d) The hydrophilic sugar–phosphate chain is located inside to form a circle, whereas hydrophobic bases are located lying flat outside the circle.

A longer single-stranded DNA containing seven adenine and seven thymine bases is of interest, because this DNA has a longer and a complementary base-sequence, and it may have an adsorbed structure different from that of pAAAAA. Fig. 3 shows a typical STM image of a pAAAAAAATTTTTTT deposited on a Cu(111) surface by the pulse injection method. We found a Z-shaped adsorbed structure (Fig. 3a), as well several other types of adsorbed structure, such as circled and clustered shapes. As can be seen in the STM image (Fig. 3a) and corresponding structural model (Fig. 3b), the single-stranded DNA, pAAAAAAATTTTTTT molecule, does not form a simple circled structure as the pAAAAA does. This result itself may not be surprising, because a DNA molecule with a longer sequence should possess a larger number of conformations or adsorbed structures. A common result is that the

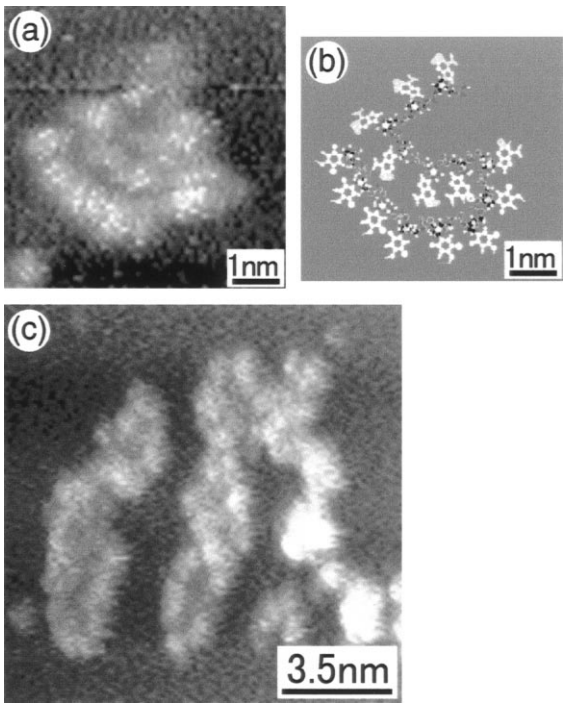

Fig. 3. High magnification STM images, (a) and (c), and (b) a proposed model of single-stranded DNA oligomer, pAAAAAAATTTTTTT molecule, obtained after the injection of the sample on a clean Cu(111) substrate and observed at liquid nitrogen temperature (~80 K). (a) The single pAAAAAAATTTTTTT appears to take a Z-shaped adsorbed structure. (b) A proposed structural model for the observed image in (a). (c) A paired pAAAAAAATTTTTTT, showing double-helix structures. Among paired or clustered DNA molecules, the presence of a paired DNA double-helix has been found. Imaging parameters: (a) $V_s = -3$ V, $I_t = 10$ pA, $6 \times 6 \times 0.2$ nm^3; (c) $V_s = -2$ V, $I_t = 10$ pA, $13 \times 13 \times 0.2$ nm^3.

both pAAAAA and pAAAAAAATTTTTTT oligomers take a flat conformation as the adsorbed structures.

Besides the single oligomer adsorbates, we have observed characteristic helix structures of pAAAAAAATTTTTTT, as shown in Fig. 3c. Apparently, a pair of mono-oligomers forms a double helix with one and a half turns. This structure has a pitch of helical periodicity ~3.5 nm and height of 0.16–0.36 nm. The corresponding values of Watson–Crick DNA in the biological condition are ~3.4 nm and ~2 nm respectively [1,16]. It is known that the single turn of the Watson–Crick DNA consists of approximately ten base-pairs. Since the single-stranded DNA used here is a 14mer, this DNA should have nearly one and a half double-helix turns. This relationship agrees well with the observed double-helix structure (Fig. 3c) having one and a half turns. We should emphasize the fact that the single-stranded DNA, 14mer, can form a double-helix structure and that structure is clearly visualized using the STM.

For the direct observation of a double-helix structure, double-stranded plasmid DNA was deposited on a clean Cu(111) by the pulse injection method. Fig. 4a–d shows a series of STM images of 2739 base-paired double-stranded plasmid DNA deposited on Cu(111) surfaces. The steps and dislocations native to the Cu(111) substrate surface can be seen as background, which adds validity to the images. White dots dispersed on the surfaces could come from buffer species, such as EDTA. Although the plasmid DNAs in the images are partly entangled, they appear as circled whole

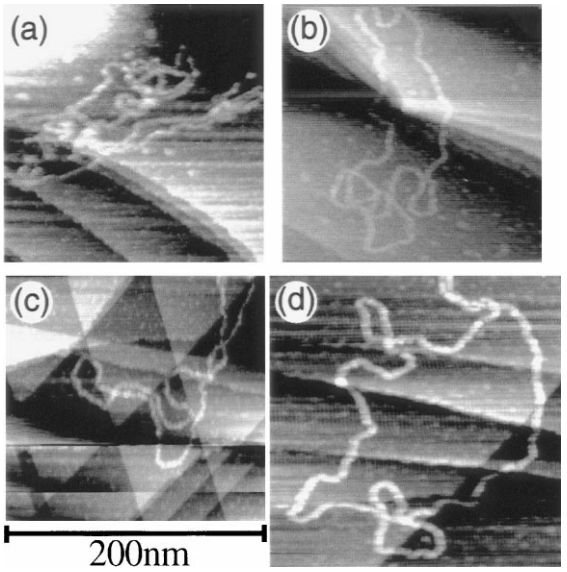

Fig. 4. A series of STM images of 2739 base-paired double-stranded plasmid DNA, obtained after the injection of the sample on clean Cu(111) substrates observed at ~95 K. Imaging parameters: $V_s = -0.75$ V, $I_t = 10$ pA for all. (a) and (c) $\sim 200 \times 200 \times 0.8$ nm^3. (b) $\sim 200 \times 200 \times 1.0$ nm^3. (d) $\sim 225 \times 225 \times 0.6$ nm^3. In addition to the DNA, a background of steps and dislocations of the Cu(111) substrate surface can also be seen.

plasmid DNA molecules. Moreover, no open-circled or fragmented plasmid DNAs were found, even after searching hundreds of sites on the sample surface. Thus, by using this particular pulse injection method, we have successfully deposited 2739 base-paired plasmid DNA on clean well-defined Cu(111) surfaces under UHV without breaking the DNA strand. The observed plasmid DNA molecules have a topographic height of 0.2–0.5 nm and a strand diameter of about 2–4 nm, depending strongly on the STM tip conditions. In high-resolution images, internal structures with a periodicity of 2.6–3.7 nm along the strand are resolved, as shown in Fig. 5a and its magnified image in Fig. 5b. A histogram for the periodicities along the chain is also indicated in Fig. 5a. The histogram has a broad peak in the range 2.6–3.7 nm centered at 3.2 nm. The observed periodicity of 2.6–3.7 nm covers that of Watson–Crick DNA, ~3.4 nm. This rather wide-ranging periodicity may be due to the interaction between the DNA and the substrate and the flexibility of the DNA structure. From Fig. 5 it is obvious that each pitch of the double-helix structure of DNA is resolved by the STM. The height of 0.2–0.5 nm is slightly higher than that observed for the single-stranded DNA oligomers, and is still much lower than that expected for Watson–Crick DNA, ~2 nm. A possible reason for the low apparent height can be explained as follows. As discussed above for the pAAAAA system, for the observed molecules the contrast of the STM images corresponds to the density of states near the Fermi level arising from hybridization of the metallic states with the states in the molecules [14,15]. Assuming the STM visualizes such electronic states, the height of the molecule in the image does not necessarily correspond to the actual height of the atomic skeleton.

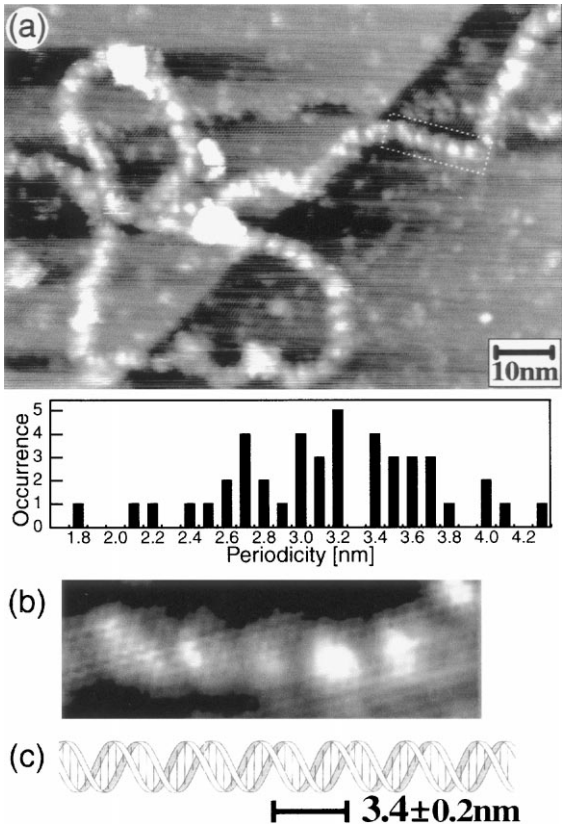

Fig. 5. High-resolution STM images, (a) and (b), of 2739 base-paired double-stranded plasmid DNA observed at ~95 K, and a schematic illustration of the Watson–Crick DNA (c). For (a) the imaging parameters are $V_s = -0.75$ V, $I_t = 10$ pA, $100 \times 70 \times 0.5$ nm^3. A histogram for the periodicities along the chain is shown below the image (a) and shows a broad peak in the range of 2.6–3.7 nm. (b) A magnified image (~$19.3 \times 6.4 \times 0.4$ nm^3) of the region indicated by a dotted white rectangular frame in (a), showing an internal structure with periodicity of 2.6–3.6 nm along the strand. (c) For direct comparison between the magnified image (b) and the illustration (c), they are fitted to the same scale.

4. Conclusions

We have successfully deposited single-stranded DNA and double-stranded plasmid DNA on well-defined Cu(111) surfaces under UHV condition using the pulse injection method. The high-resolution STM images are observed at room temperature and at liquid N_2 temperature. These particular UHV-STM images have revealed that DNA molecules are adsorbed directly onto a clean Cu(111) surface and exhibited the detailed structure of DNA, which has not been resolved previously. The single-stranded DNA oligomers have exhibited the images of individual internal base molecules and the helix structures made of complementary base sequences. For the double-stranded

plasmid DNA the STM images have shown the Watson–Crick double-helix structure.

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