Extended Structure of DNA Oligomer and Nucleotide Imaging Studied by Scanning Tunneling Microscopy

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DNA oligomers deposited on Cu(111) surfaces were observed at liquid nitrogen temperature using a scanning tunneling microscope. The observed oligomers were *pAAAAAAATTTTTTT* (14mer), *pTTTGGTTAACCAAA* (14mer), *pGGGGGTTTTTTTTT* (15mer), and *pAAAAAAAAAATTTTTTTTTT* (20mer). The structure of the isolated oligomers adsorbed on the Cu surface varies with the length of the molecular chain. The isolated 20mer is adsorbed to aggregate three-dimensionally. The isolated 14mer and 15mer are extended on the surface, and the almost entire molecular chain touches the surface. A highly resolved image of the 20mer shows bright spots aligned in a row along a single-stranded DNA with the same periodicity as that of the nucleotide units, demonstrating that each bright spot is a nucleotide.

Introduction

A number of studies on DNA observations in a solution or under an atmospheric condition using a scanning tunneling microscope (STM) have been published.[1−10] The imaging in a solution is highly significant, since it allows us to investigate the structures of DNA existing in a environment similar to that within an organism. Conversely, observations made in an atmosphere are useful because of the simplicity of the STM operation. Under these conditions, however, the high resolution required to image the fine structures of DNA has not been achieved to date. We observed DNA oligomers adsorbed on Cu(111) surfaces at a temperature of approximately 80 K using an ultrahigh vacuum STM (UHV-STM). We expected the imaging conditions to enhance the resolution due to the reduced thermal motion of DNA and the clean, well-defined substrate surface. The observed DNA oligomers were *pAAAAAAATTTTTTT* (14mer),[11,12] *pTTTGGTTAACCAAA* (14mer), *pGGGGGTTTTT-TTTTTT* (15mer), and *pAAAAAAAAAATTTTTTTTTT* (20mer), abbreviated to *A7T7*, *T3G2T2A2C2A3*, *G5T10*, and *A10T10*, respectively.

In this article, we present the structural difference of the isolated oligomer depending on the chain length, the conformation of the isolated 14mer and 15mer, and the proof that the bright spots seen in the images of DNA oligomers are the individual nucleotides.

Experimentation

A clean Cu(111) surface was prepared by several cycles of annealing at 550 °C and sputtering with Ar^+.[13,14] We used the pulse injection technique[12,15] in order to deposit the DNA oligomers on the Cu(111) surface. In this technique, the Cu-(111) surface was placed in a preparation chamber (at 10^{-7} Torr) and sprayed with a DNA aqueous solution using a pulse valve. In each observation, the concentration of the DNA aqueous solution was approximately 10^{-5} mol/L. After the DNA oligomers were deposited on the Cu surface, the sample was transferred to an STM chamber (10^{-10} Torr) and cooled to around 80 K for STM measurements. All images were obtained in a constant current mode. The images shown here were obtained at a sample bias voltage between −2 and −3 V with a tunneling current of 4−10 pA. These various tunneling conditions resulted in no differences in the images.

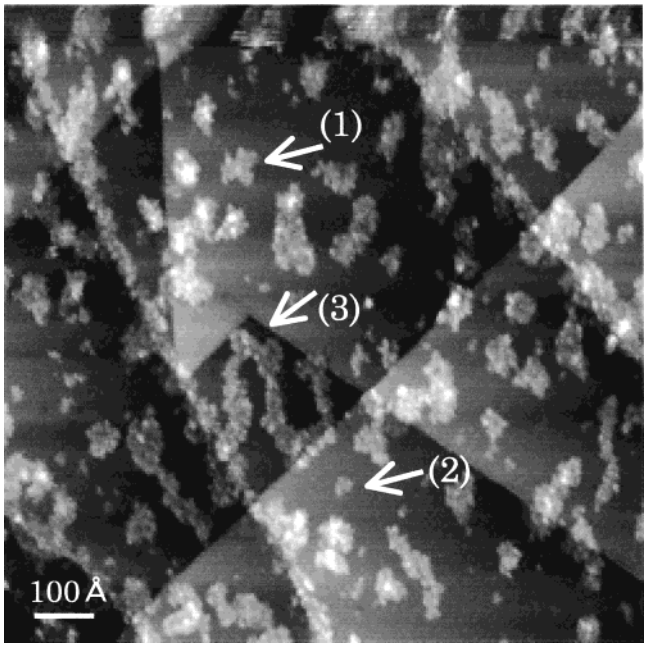

Figure 1. STM image of *A7T7* taken at a sample bias voltage of −3 V and a tunneling current of 5 pA. Arrows (1), (2), and (3) indicate a single oligomer, a shortened molecule, and a cluster, respectively.

A typical image of the DNA oligomers is shown in Figure 1, specifically an image of A7T7. The transverse lines across the surface are Cu steps. In the prior studies, we showed that the structures of the oligomers on the Cu surface are categorized into the three types, i.e., the isolated oligomer, the shortened molecule, and the cluster.[11] In Figure 1, the bright images marked with arrows (1), (2), and (3) are identified as an isolated

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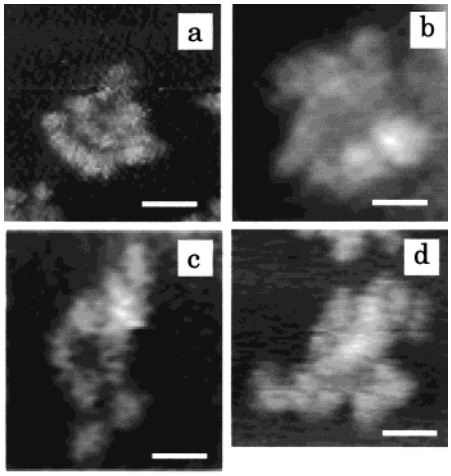

(e)

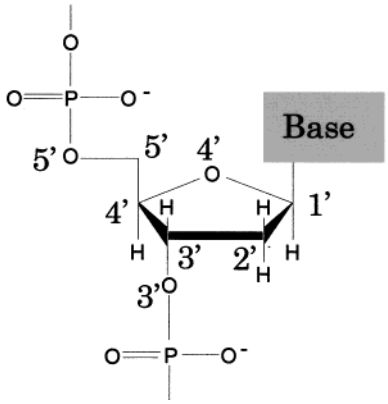

Figure 2. (a)−(d) STM images of isolated molecules taken with tunneling currents of 5−10 pA and a sample bias voltage of −3 V. All scale bars are 20 Å, and all images have the same magnification. (a) Image of *A7T7* (14 mer). (b) Image of *T3G2T2A2C2A3* (14 mer). (c) Image of *G5T10* (15 mer). (e) Image of *A10T10* (20 mer).

oligomer, a shortened molecule, and a cluster, respectively. The apparent heights of the DNA images are approximately 2 Å.

Results and Discussion

The images of the isolated *A7T7* (14mer), *T3G2T2A2C2A3* (14mer), *G5T10* (15mer), and *A10T10* (20mer) are shown in Figure 2a−d, respectively.

The isolated oligomers of 14mer and 15mer were identified according to the following criterion. The molecular images are categorized into the large, the medium, and the small sizes. The large molecular images show the complicated structures such as parallel two chains or twisted chains as described elsewhere.[11] And we have found that the ends of the molecular chains in the large structure cannot be found owing to the complexity of the structure. We call them the cluster. While, the medium and the small structures have the two ends of the chain which are easily found, and they can be traced from end to end along the chain. Every medium structure in a given image shows the uniform length; on the other hand, the small structure shows the diverse length which is smaller than that of the medium structure. We identify the medium and the small structures as the isolated oligomer and the shortened molecule, respectively. We note that the medium structures (i.e., the isolated oligomer) can show the different area and chain length when they are extracted from the different images, because the size of the molecular image strongly depends on the resolution of the image. Because of this, the sizes of the oligomer images shown in Figure 2 are not equal to one another; the chain length of the oligomers shown in Figure 2a (14mer), 2b (14mer), and 2c (15mer) are 116 Å, 141 Å, and 137 Å, respectively.

Though we could use the size and the chain length to identify the isolated 14mer and 15mer, we used only the size of the molecular image to identify the isolated 20mer, because the 20mer is adsorbed so that the molecular chain has a large number of overlapping parts and cannot be traced from end to end as discussed below.

Figure 2 reveals that the adsorbed structure of the 20mer is different from those of the 14mer and 15mer. The molecular chains in the 14mer and 15mer images can be traced from end to end, indicating that 14 mer and 15mer are adsorbed so that their almost entire chains touch the surface. Conversely, the 20mer chain images cannot be traced from end to end: it has a large number of three-dimensionally overlapping parts. This indicates that the stable adsorbed structure varies with the chain length. It is expected that the adsorbed structures depend on the adsorption process. The 14mer and 15mer entangled in solution would be flattened on the surface by the forces given to the oligomer during adsorption. The forces at the adsorption, however, are too weak to completely unfasten and flatten the entangled 20mer.

To better understand the conformation of the isolated 14mer and 15mer, we examined the apparent height and the chain length. The isolated 14mer and 15mer show the apparent height of ca. 2 Å which is relatively consistent with that of the DNA base molecule lying flat on the Cu(111) surface (1−2 Å),[16] suggesting that the base planes contained in the oligomers directly touch the surface and lie flat. To further interpret the conformation, we compared the chain length of the observed oligomers with that of the extended oligomer. We considered an extended structure of a nucleotide shown in Figure 3b, in which the conformations originating from the rotation around the single bonds constituting the P−$O_{5'}$−$C_{5'}$−$C_{4'}$−$C_{3'}$−$O_{3'}$−P main chain (Figure 3a) are all anti-conformations. In such a structure all atoms in the main chain are put on the identical plane. The values of the bond angle and bond length obtained by analyzing a crystal structure[17] were used for the calculation, and the phosphorus−phosphorus distance of the nucleotide unit was found to be 7.8 Å. The chain length of the extended 14mer was therefore calculated to be 109 Å (i.e., 7.8 Å × 14). The chain length of 14mer (*A7T7* and *T3G2T2A2C2A3*) obtained from the STM images was 116−141 Å, which agrees with the length estimated for the extended oligomer (109 Å). This agreement suggests that the isolated molecule is adsorbed so that the chain structure is extended.

A length greater than 109 Å would be caused by the variable position of the base and the structure of the tip apex. The π orbital located on the base probably produces the brightest image of all the orbitals surrounding the base, the sugar, and the phosphoric acid, as the local state density brought by the base π orbital possesses the highest energy level. This is verified by studies confirming that aromatic rings are imaged even higher than alkyl chains in images of liquid crystal molecules.[18,19] If the base contributes most significantly to the imaging, the chain length will depend on the direction in which the base is pointing toward the P−$O_{5'}$−$C_{5'}$−$C_{4'}$−$C_{3'}$−$O_{3'}$−P main chain. When the oligomer is adsorbed as the P−$O_{5'}$−$C_{5'}$−$C_{4'}$−$C_{3'}$−$O_{3'}$−P main chain draws an arc, the curved line linking the bases together becomes longer than the main chain when the bases lie outside the arc of the main chain. Moreover, when the tip apex is large,

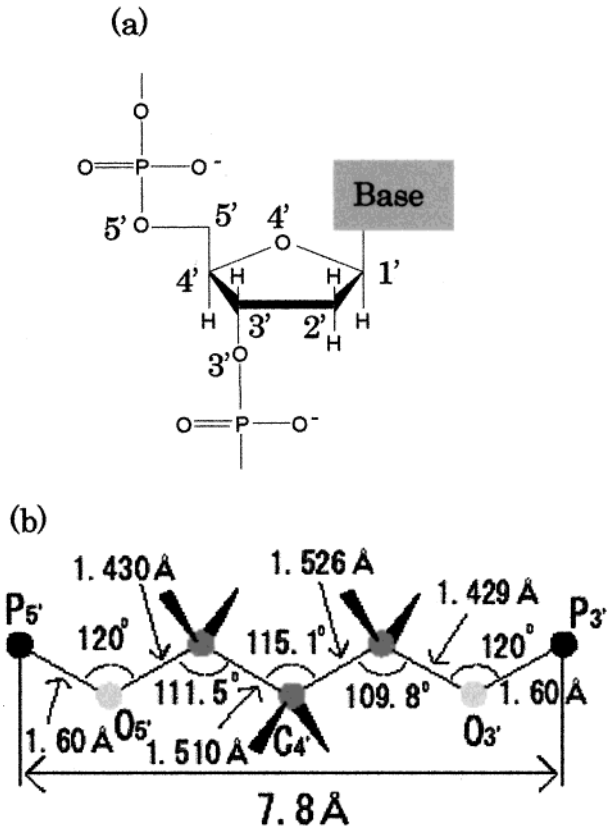

Figure 3. (a) Chemical structure of a nucleotide. (b) The extended nucleotide. The conformations originating from the rotation around the single bonds constituting the $P-O_{5'}-C_{5'}-C_{4'}-_{3'}-O_{3'}-P$ main chain are all anti-conformations.

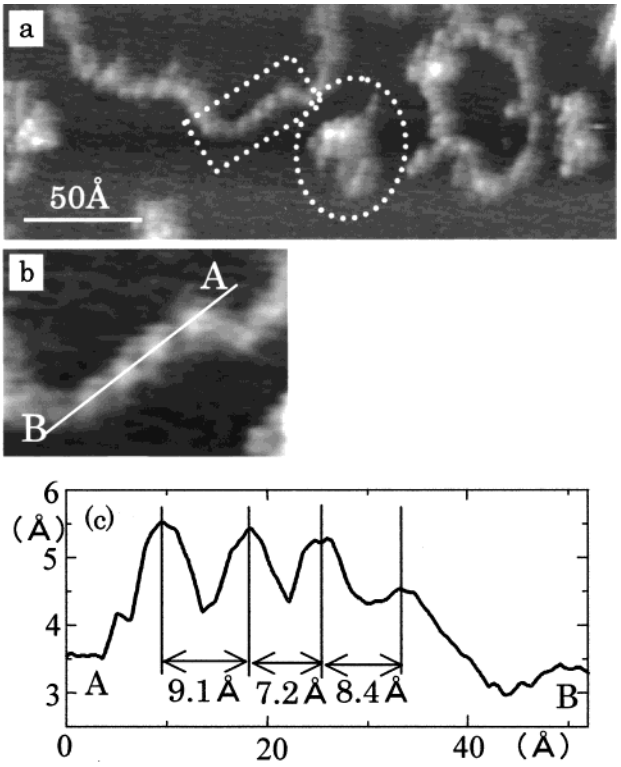

Figure 4. (a) STM image of a chain structure of *A10T10* taken at a tunneling current of 5 pA and a sample bias voltage of −3 V. An isolated oligomer is enclosed by an oval. The chain structure enclosed by a rectangle has been identified as a single strand. (b) Expanded image of the area enclosed with a rectangle in Figure 4a. (c) Profile along the line AB shown in Figure 4b.

the molecular image becomes blurred, resulting in a longer chain length in the STM image.

The maximum value of the measured chain length of the 14mer (141 Å) is greater by 25 Å than the minimum value (116 Å). The distribution within 25 Å is a result probably from the different resolution and the different orientation of the bases. There is also a possibility that the molecule identified as the isolated molecule has lost a few nucleotides in truth.

The image shown in Figure 4 was taken in the observation of *A10T10*. We find the chain-shaped structure and the isolated molecule which is enclosed by an oval. The chain-shaped structure has been determined to be a single strand, because the width of the chain image is the same as that of the isolated oligomer. When the resolution is high, all kinds of observed oligomers are imaged as gatherings of small bright spots as shown in Figure 4b, which is the expanded image of the area enclosed by the rectangle in Figure 4a. In Figure 4b, the four protrusions are aligned in a row with a separation of 9.1, 7.2, and 8.4 Å along the cross-section AB as shown in Figure 4c. These separations agree closely with the assessed phosphorus−phosphorus distance of the extended nucleotide unit (7.8 Å), demonstrating that the single strand shown in Figure 4b has been extended, and each protrusion corresponds to a nucleotide unit aligned in the extended single strand.

Conclusions

The DNA oligomers adsorbed on the Cu(111) surfaces were observed with high resolution at a temperature of approximately 80 K using UHV-STM. The observed oligomers were *A7T7* (14mer), *T3G2T2A2C2A3* (14mer), *G5T10* (15mer), and *A10T10* (20mer).

We found that the structure of the isolated oligomer varies with the length of the molecular chain. The isolated 20mer is adsorbed so that the molecular chain has a large number of overlapping parts. On the other hand, the isolated 14mer and 15mer are hardly overlapped, and almost the entire chain touches the surface. The apparent height and the chain length have shown that the isolated 14mer and 15mer are extended and their bases lie flat on the surface.

A highly resolved image of the 20mer shows bright spots aligned in a row along the single molecular chain with the same periodicity as the length of the extended nucleotide, demonstrating that each bright spot is a nucleotide.

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