SCANNING TUNNELING MICROSCOPY OF DNA

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ABSTRACT

Uncoated DNA deposited on graphite surface was imaged in air with the scanning tunneling microscope (STM). The images revealed a helical pitch of 3.6 nm whereas the average width was 2.3nm. Periodic alternation of major and minor grooves were resolved with high resolution. Our STM images were in reasonable agreement with the B- form DNA structural model derived from X- ray diffraction studies. It was concluded that the STM could be useful for local structural studies of biomolecules.

Keywords: STM B- DNA

Ⅰ. INTRODUCTION

Since G.Binnig and H.Rohrer published their first STM image of DNA in 1984[1], extensive efforts were concentrated on DNA due to its characteristic feature of periodic right- handed double helix as well as its biological significance. STM images were visualized in vacuum, water, air and with metal- shadowing[2-9]. Despite the promise of high resolution and the capability of operating in close to native conditions, researchers are still exploring conditions in which STM could be used to resolve fine structures of DNA. In this report, we present the high resolution STM image revealing the major and minor grooves of DNA.

Ⅱ. MATERIAL AND METHODS

Calf thymus DNA (USB, 99.9%) were purified and dissolved in a buffer of 10 m · mol/l Tris- HCl (pH7.8), with 0.5 m · mol/l EDTA, to a concentration of 20 μ g/ml. The concentration of DNA solution was carefully controlled in order to avoid sample aggregation, which would elude stable STM imaging due to its lower conductivity. The solution was mixed with 0.8% glycerin in order to preserve the hydration of DNA molecules under air- drying conditions. 2μ l of the solution was mounted onto a freshly cleaved highly oriented pyrolytic graphite (HOPG) surface and was air- dried at 0°C for 15 minutes, then it was used directly for microscopy.

We constructed an STM with a tubulor piezo rather than a tripod to scan the tip[3,5,9].The tip was mechanically cut from a 0.25mm Pt(0.8)/Ir(0.2) wire. The sample bias was 100mV and a constant feed- back current was kept at 200pA. The

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atomic topograph of the HOPG graphite surface was measured in advance to check a lateral resolution of 0.2– 0.3nm and a vertical resolution of 0.01 nm.

Ⅲ.RESULTS AND DISCUSSION

Fig.1*a* showed a typical STM image of DNA. From the picture, three DNA

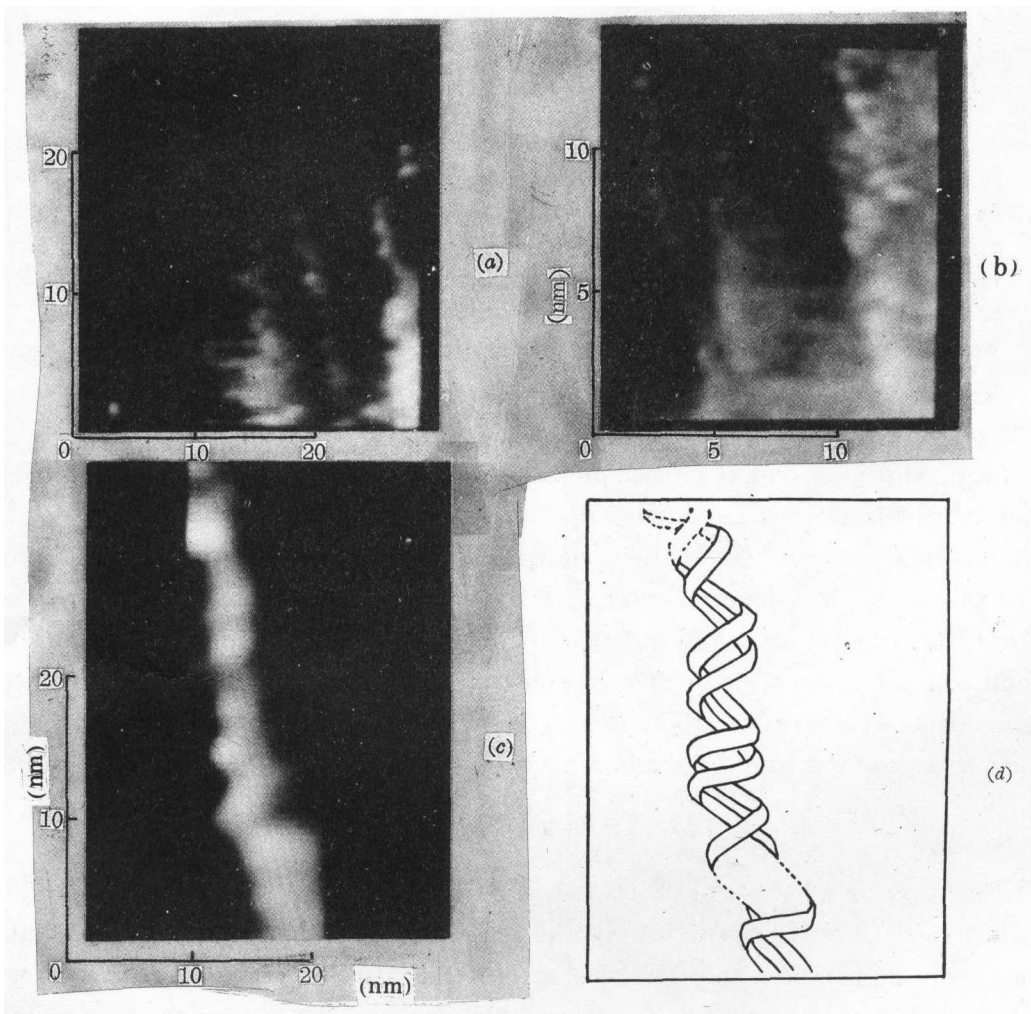

Fig.1 STM images of uncoated calf thymus DNA

Height information was encoded in the gray scale, where the brightest point represented the highest point in *Z* direction. (*a*) Image shows three molecules. (*b*) Image obtained from different region showing aggregation of DNA, two isolated fragments could be seen in the upper– left corner. (*c*) Magnified image showing fine structures of one DNA duplex. (*d*) The schematic drawing of DNA according to the magnified image.

molecules was seen making some convolutions on the graphite surface. The DNA molecule in the left side of the image was obviously deformed by tip– sample interaction. In the middle of Fig.1*a*, the DNA duplex was less distorted and its

periodic bumps along the duplex were resolved, but the lower portion of the molecule was widened along the duplex. This indicated that the molecular conformation could be deformed or modified by the strong tip- sample interactions in some cases.

Image from different regions of the sample was shown in Fig.1*b*. Overlapping and aggregating DNA molecules covered most parts of the region. In the upper- left corner of the image, two isolated DNA duplexes were seen convoluting from the aggregation. The image was stable from scan to scan. Since there were no reactive binding sites like defects and step edges in the region, Fig.1*b* demonstrated that DNA aggregation might be difficult to be displaced by the tip and could be constantly imaged. The magnified view of the upper- left region was shown in Fig. 1*c*, which revealed one isolated DNA fragment. The periodic structure of the major- minor groove alternation of double helix was seen in the image. A schmatic drawing of Fig.1*c* was shown in Fig.1*d*. The average space of the major- minor pair, which was measured from Fig.1*c*, was 3.6 nm across the duplex, and its average width was 2.3 nm. The height of structures was about 1.8 nm. According to the measurement of X- ray crystallography, the B- form DNA in crystalline state with a relative humidity of 95%, was characterized by wide major grooves and narrow minor grooves with a helical pitch of 3.4 nm and a width of 2.0 nm. Therefore, the observed conformation in our STM images was in reasonable agreement with the B- DNA structural model.

IV. CONCLUSIONS

In this study, we showed that STM allowed reliable high- resolution imaging of DNA with following advantages: simplicity of operation, small amount of sample, a direct visualization and a preservation of high resolution even in air or liquid. The sample deformation and modification of the molecules could be found in some cases, and were attributed to the considerable local tip- sample interaction. This indicated that the interpretation of STM images should be carefull to avoid ambiguous analysis.

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