

## VARIANT STRUCTURES OF DNA OBSERVED BY SCANNING TUNNELING MICROSCOPE\*

BAI CHUN-LI (白春礼), YIE JIAN (叶坚), GONG LI-SAN (龚立三),  
DAI CHANG-CHUN (戴长春), WANG DA-WEN (王大文),  
GAO FAN (高凡) AND GU JIAN-GUO (顾建国)  
(*Institute of Chemistry, Academia Sinica, Beijing 100080, PRC*)

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Deoxyribonucleic acid (DNA), as the essential genetic substance in life process, may undergo structural changes in various environments. For instance, the unwinding of the original double helix of DNA could occur after being heated.

Recent reports on the direct structural observation of single-stranded DNA and double helices of naked B, A and Z-DNA in air or in ultra-high vacuum by scanning tunneling microscope (STM)<sup>[1-4]</sup> prove that STM is a powerful technique for the structural research on nucleic acids. To our knowledge, however, no direct observation on the changed structures of native DNA has been reported until now. Here we report some experimental results of variant structures of DNA, which undergoes denaturational treatment, obtained by the STM developed in our laboratory<sup>[5-7]</sup>.

The sample used was lambda phage DNA-Hind III (fragment sizes: 23130, 9416, 6557, 4361, 2322, 2027, 564, 125) produced by Sino-American Biotechnology Company. Aqueous solution of the sample was heated up to 100°C over its  $T_m$  and kept for about 15 min, then cooled sharply down to 0°C. A droplet of this dilute solution was deposited on freshly cleaved pyrolytic graphite surface, then observed directly with STM in air at room temperature after being dried.

Fig. 1a is the STM topographic image of DNA triple-stranded braid-like structure with 30 Å in width, which is in striking contrast to the right-handed double helical structure of native DNA without denaturational treatment. The joining structures between a short right-handed double helical section and a triple-stranded braid-like section (Fig. 1b) and between the right-handed and left-handed double helical sections (Fig. 1c), and the spring-like tertiary structure with 27 Å in pitch and 59 Å in width (Fig. 1d) were also

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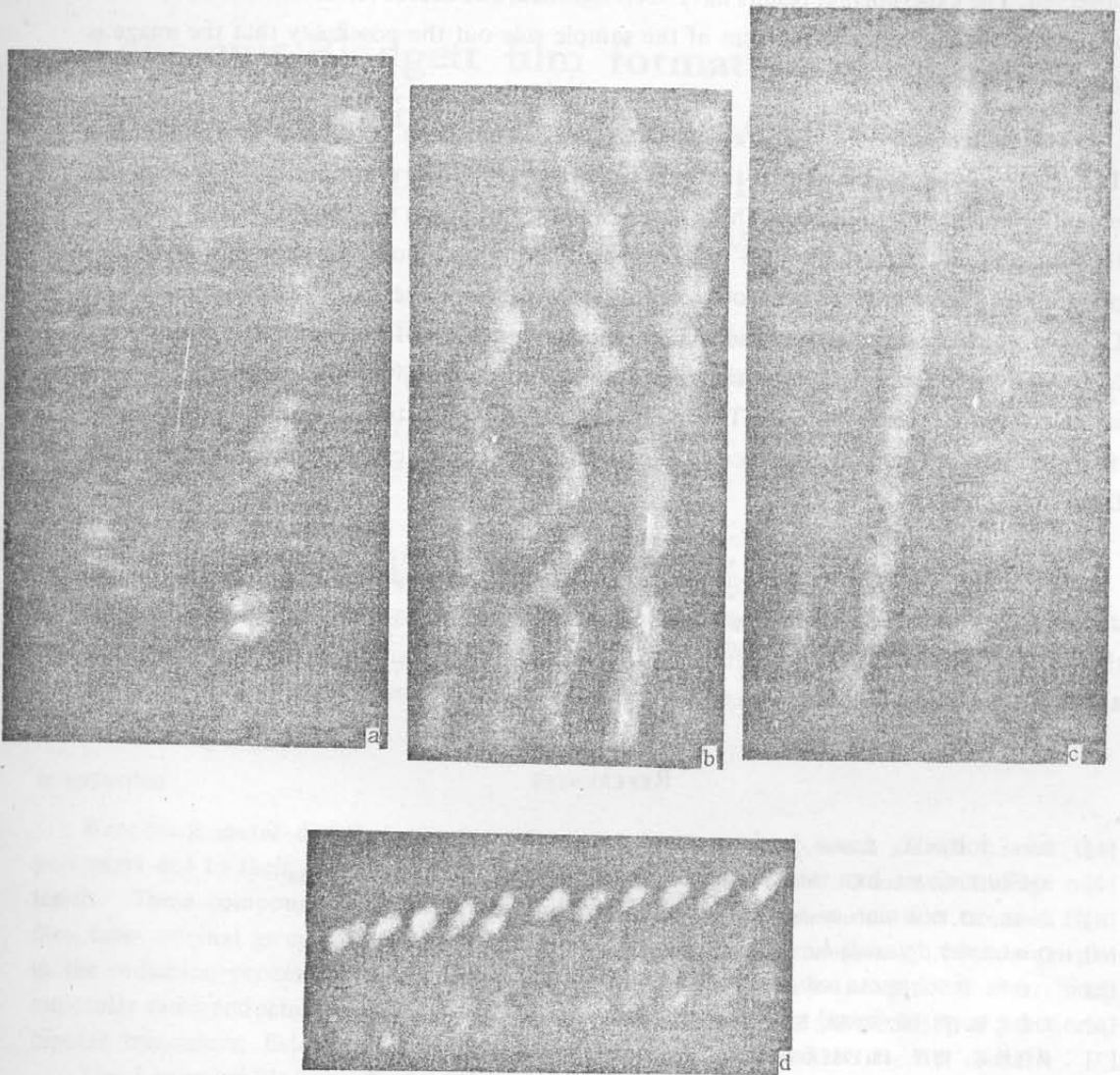


Fig. 1. Variant structures of denatured DNA.

a, Braid-like triple-stranded structure, scanning area:  $47 \times 96 \text{ \AA}^2$ ; b, the joining structure between a right-handed double helical section and a triple-stranded braid-like section, scanning area:  $61 \times 147 \text{ \AA}^2$ ; c, the joining structure between the left-handed and right-handed double helical sections, scanning area:  $93 \times 177 \text{ \AA}^2$ ; d, the tertiary structure with helical characteristics, scanning area:  $319 \times 98 \text{ \AA}^2$ .

observed. The experimental results have been regained, and moreover the measurements and analysis of the barrier-height images of the sample rule out the possibility that the image is graphite artifacts.

The existence of the triple-stranded DNA *in vitro* has been known for several decades. The recent studies, which suggest that the triple-stranded DNA exists *in vivo*, have shown its potential biological significance. This has renewed the interest in its structure<sup>[8]</sup>. However, little structural information about it has been obtained. Up to now, the structure of the triple-stranded DNA has not been observed directly although the model of the triple helical DNA in which the third strand winds along the major groove of the underlying double helix has been derived from polynucleotide fiber diffraction<sup>[9-11]</sup>, NMR<sup>[8]</sup> and molecular mechanics calculations. Moreover our STM image of DNA triple-stranded braid-like structure does not support the model mentioned above. Our results may cause a new insight into the DNA structures.

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