Molecular Structure of DNA by Scanning Tunneling Microscopy

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Uncoated DNA molecules marked with an activated tris(1-aziridinyl) phosphine oxide (TAPO) solution were deposited on gold substrates and imaged in air with the use of a high-resolution scanning tunneling microscope (STM). Constant-current and gap-modulated STM images show clear evidence of the helicity of the DNA structure: pitch periodicity ranges from 25 and 35 angstroms, whereas the average diameter is 20 angstroms. Molecular structure within a single helix turn was also observed.

THE STM HAS PROVED USEFUL NOT only for imaging surfaces of metals and semiconductors (*1, 2*) but also for important biological systems such as biomembranes (*3*), bacteriophage particles (*4*), and DNA molecules (*5*). Recent STM observations of non–metal-shadowed DNA that clearly show the helicity of the molecule (*6*) have greatly increased the interest of STM applications in biology. We report STM observations of unshadowed DNA that show features of the molecular structure within single helix turns.

The STM images were taken in air simultaneously in the constant-current and gap-modulated modes. In the latter mode the sample-tip distance is sinusoidally modulated by biasing the *z*-piezo high-voltage controller with a suitable ac voltage. The frequency is set at a value higher than the feedback cutoff in order to keep the tip-sample mean distance constant. Since the tunneling current can be expressed as $I \sim \exp(-\phi^{1/2}s)$, the modulated current divided by the current is approximately [neglecting the term $d\phi/ds$ (*1, 7*)] $d(\ln I)/ds \sim \phi^{1/2}$; ϕ is the local effective barrier height and s the sample-tip gap. Thus the amplitude of the ac part of the tunneling current, as measured by a lock-in amplifier, is roughly proportional to the square root of the tunnel barrier height (*1*). The barrier height depends on the local value of the work function so that the method is sensitive to the chemical structure of the sample.

The specially designed STM used is described elsewere (*8–10*), as is the technique for the shaping of the tungsten tip (*11*). The reliability and stability of this instrument have been tested by imaging graphite C(0001) surfaces with a lateral resolution better than 0.5 Å. The images shown were obtained from raw data without any post-electronic image treatment.

Plasmid circular DNA in aqueous solution (concentration 1 mg/ml) was used as a standard solution. The DNA was marked with activated tris (1-aziridinyl) phosphine oxide (TAPO) by mixing 20 μl each of DNA and activated TAPO solutions for 1 hour at room temperature (*12*). The solution was then deposited on a gold-plated aluminum stub and dried at room temperature for several hours. The possibility of preserving naked untreated DNA suitable for STM observation is still an open question. DNA molecules deposited on conducting substrates frequently collapse and a stable tunneling condition is not achieved (*13*). The success of the present experiment seems to suggest that naked DNA molecules are

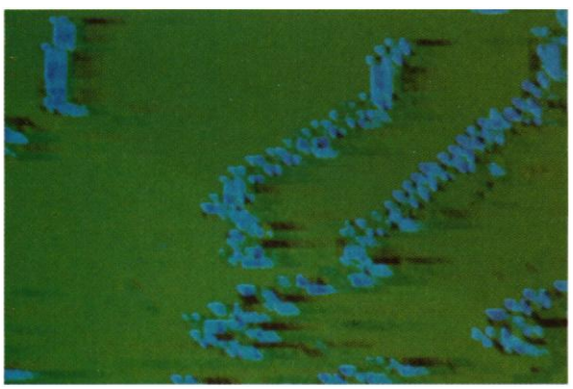

Fig. 1. A $d(\ln I)/ds$ image (480 Å by 320 Å) of DNA molecule deposited on gold. A three-dimensional top-view image was obtained with simulated illumination; gap voltage, 20 mV (sample positive); tunneling current, 1.0 nA; modulation frequency, 11 kHz; and modulation amplitude, 0.5 Å.

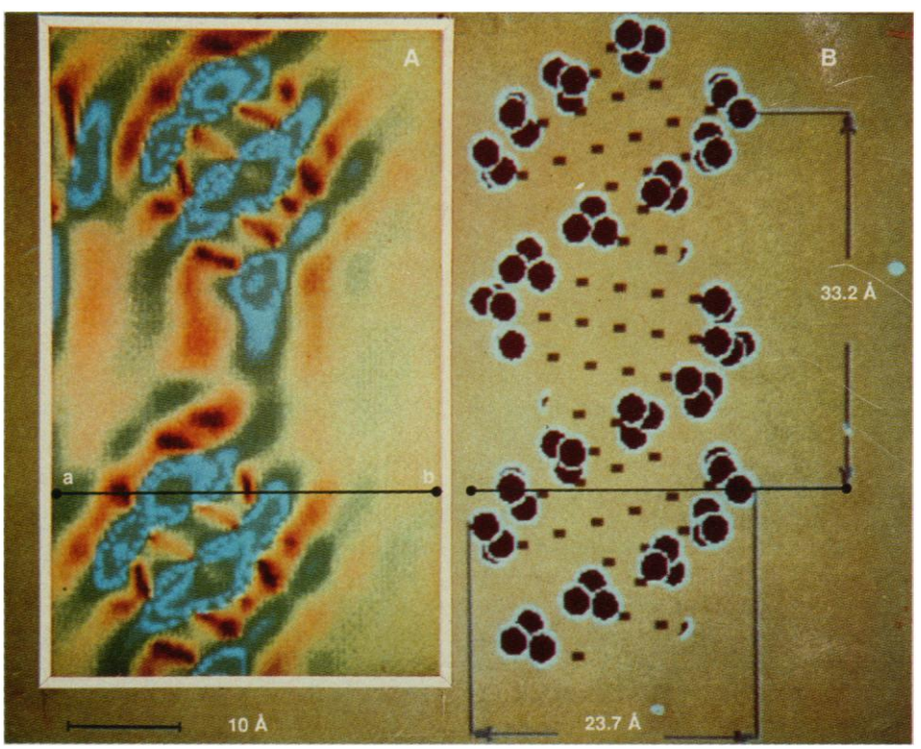

Fig. 2. (**A**) $d(\ln I)/ds$ high-magnification image (60 Å by 36 Å) of DNA molecule. Three-dimensional top view of shading obtained by simulated illumination at 65° from the surface. Colors represent the various values of the cosine of the angle between the direction of light and the local normal to the surface ordered in the following sequence: purple, red, yellow, green, and cyan. (**B**) Standard model of B-DNA with phosphate molecules aligned along the DNA backbones and inner bases (dashed lines).

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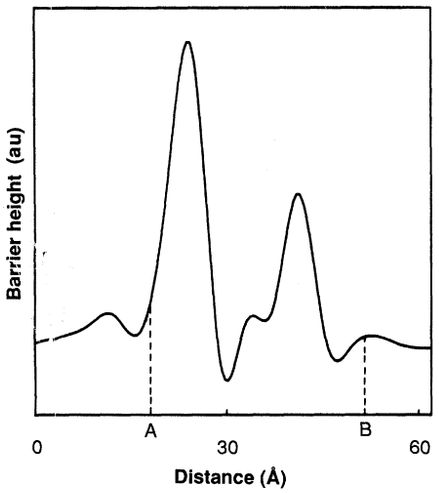

Fig. 3. Plot of $d(\ln I)/ds$ along the line A–B of Fig. 2A. The two highest peaks should correspond to phosphate molecules of DNA backbones, whereas the small peak in the center may be due to the shallow bases inside the minor groove.

well preserved, presumably since the ends of the DNA segments are fixed by TAPO. Pure TAPO solution on a similar gold substrate can be imaged only in the constant-current mode and appears as a crystalline structure of squares 3 Å on a side. The absence of imaging in the gap-modulated mode is probably due to a negligible modification of the work function of gold by TAPO.

In Fig. 1, which is a $d(\ln I)/ds$ map, two long, partially overlapping segments of double-stranded DNA molecules are shown. The two segments of DNA molecules show a typical helical conformation. The periodicity of the helix ranges from 25 Å for the lower segment to 35 Å for the upper one. Reproducible results have been obtained in several samples. At low magnification (as in Fig. 1), images in the constant-current mode are essentially the same, except for minor details in those segments where the molecule is marked by TAPO.

In Fig. 2A, which is a $d(\ln I)/ds$ map, a section of the DNA molecule is shown at a much greater magnification (60 Å by 36 Å) and is a three-dimensional top-view representation with simulated illumination at an angle of 65° from the plane. The various colors represent the shading and their sequence has been chosen to increase the contrast. The molelcular structure of the DNA shows up dramatically. The two sections of approximately rectangular shape (20 Å by 15 Å) in Fig. 2A form an angle of 40° with the molecular axes and clearly represent the shallow minor grooves of the helix separated by an imperfectly imaged region that corresponds to the deep major groove. The poor imaging of the major groove is probably due to the tip trying to enter the groove and drawing current from its sides, thus blurring the image. Due to the illumination technique used, the purple-red color spots correspond to local maxima. This kind of image representation enhances the small peak clearly visible in the middle of Fig. 3, which corresponds to the purple-red spots at the center of the rectangles, whereas in a relative height map representation it would not be resolved. A standard model of the B-DNA double helix is shown in Fig. 2B for comparison. The model is composed of phosphate molecules aligned along the DNA backbones and inner bases (dashed lines). In order to make a direct connection with the experimental result, the line A–B in Fig. 2A has been redrawn in the approximate location on the model. The agreement is remarkable: the periodicity of the helix is approximately 35 Å; the width of the minor groove is 12 to 15 Å. The slight difference in the helix angle with respect to the model is due to local tilting that is probably caused by interaction with the substrate. A plot of $d(\ln I)/ds$ is shown in Fig. 3 along the line A–B of Fig. 2A.

The association of the structure of $d(\ln I)/ds$ to the different molecules or molecular groups is a much harder task. A possible interpretation is that phosphate molecules of the backbone, which are negatively charged and thus increase the local work function, are associated with the purple-red spots on the long side of the rectangles corresponding to the two highest peaks of Fig. 3. The larger blue-green colored structures would then be associated with sugar molecules and bases. Five purple-red bars corresponding to the intermediate peak in Fig. 3 should also be noticed on the long axes of the rectangles: they might be due to the shallow bases inside the minor groove.

The image of Fig. 2A presumably shows naked DNA, although the possibility of the presence of water molecules and TAPO bound to the DNA cannot be ruled out. Further experimental work is needed for a complete understanding of such a problem. However, the results reported here already show the great potential of the method for the characterization and possible sequentiation of the DNA.

REFERENCES AND NOTES

1. G. Binnig and H. Röhrer, *IBM J. Res. Dev.* **30**, 355 (1986).
2. ——— Ch. Gerber, H. Weibel, *Phys. Rev. Lett.* **50**, 120 (1983).
3. J. A. N. Zasadzinski, J. Schneir, J. Gurley, V. Elings, P. K. Hansma, *Science* **239**, 1013 (1988).
4. A. M. Baró *et al.*, *Nature* **315**, 253 (1985).
5. G. Travaglini, H. Röhrer, M. Amrein, H. Gross, *Surf. Sci.* **181**, 380 (1987).
6. T. P. Beebe *et al.*, *Science* **243**, 370 (1989).
7. B. Marchon *et al.*, *Phys. Rev. Lett.* **60**, 1166 (1988).
8. S. Selci, A. Cricenti, R. Generosi, E. Gori, G. Chiarotti, *Inst. Phys. Conf. Ser.* **93** (vol. 1), 281 (1988).
9. A. Cricenti, S. Selci, R. Generosi, E. Gori, G. Chiarotti, *J. Microsc.* **152**, 789 (1988).
10. S. Selci, A. Cricenti, R. Generosi, E. Gori, G. Chiarotti, *Surf. Sci.* **211**, 143 (1989).
11. A. Cricenti, S. Selci, R. Generosi, E. Gori, G. Chiarotti, *Solid State Commun.* **70**, 897 (1989).
12. W. Djaczenko and C. C. Cimmino, *J. Cell Biol.* **57**, 859 (1973).
13. M. Amrein, A. Stasiak, H. Gross, E. Stoll, G. Travaglini, *Science* **240**, 514 (1988).
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Aerosols, Cloud Microphysics, and Fractional Cloudiness

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Increases in aerosol concentrations over the oceans may increase the amount of low-level cloudiness through a reduction in drizzle—a process that regulates the liquid-water content and the energetics of shallow marine clouds. The resulting increase in the global albedo would be in addition to the increase due to enhancement in reflectivity associated with a decrease in droplet size and would contribute to a cooling of the earth's surface.

TWOMEY *et al.* (*1*) ARGUED THAT INcreases in aerosols due to either natural or man-made causes can increase cloud reflectivity by increasing the number of cloud condensation nuclei (CCN). Because there may be few CCN over the oceans away from continental influence, any increase in the number of CCN may have a significant impact on the microphysics of clouds and thus climate. Ship trails (*2, 3*) provide evidence that under proper conditions increases in aerosol concentrations can locally increase the reflectivity of shallow marine stratocumulus clouds. Charlson *et al.* (*4*) discussed the possible interaction between cloud reflectivity and the production of dimethylsulfide (DMS) by phytoplank-

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