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APPLICATION OF SCANNING TUNNELING MICROSCOPY TO STRUCTURAL STUDIES OF DNA

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Abstract: Uncoated DNA molecules marked with an activated tris 1-aziridinyl phosphine oxide (TAPO) solution were deposited on gold substrates and imaged in air with high resolution Scanning Tunneling Microscope (STM). Constant-current and barrier-height STM images show a clear evidence of the helicity of the DNA structure: pitch periodicity ranges between 25 and 35 Å while the average diameter is 20 Å. Pure TAPO solution on a similar gold substrate can be imaged only in the constant-current mode and appears as a crystalline structure of centered hexagons of 3.1 Å side.

1. Introduction

Organic molecules or macromolecules have always been a great deal for scientists who have tried to understand their molecular structure using several techniques (X-ray diffraction, Transmission Electron Microscopy, Scanning Electron Microscopy etc.). The success of these techniques relies upon removal of the water component of biological samples. Since water is the natural environment of molecules that participate in biological processes, the observation in the subnanometer range of biological samples in a close to native conformation or even the

observation of their functional activity, requires a technique that operates successfully also in the presence of water.

In this frame the recently developed STM technique [1] is ideally suitable for biologically imaging applications provided that the problems associated with the poor conductivity and the physical mobility of biological samples can be solved.

STM has been applied to several biological important systems [2-4]. A special effort has been dedicated to the study of DNA: DNA has been imaged by STM in vacuum [5], in water [6], in air [7] and with metal-shadowing [8]. However, all these STM experiments were not able to resolve structural details of DNA but only rough contours of the molecule. This limited resolution can be attributed either to an insufficient conductance or to a high mobility of DNA molecules, for naked material, and to the grain size of the related coated film, for metal-coated DNA.

In this paper we report on STM observations of native non-metal-shadowed, TAPO marked DNA deposited on a gold substrate. The idea is to attach one end of a conductive organic molecule to the DNA molecule and to chemically anchor the other end of the molecule to the gold substrate. This approach provides a mechanical tie as well as a conducting path. The TAPO molecule [9] is well suited to this task due to 3 ethylenimine groups that react strongly with sugar groups of the DNA [9] and a phosphorus oxide group which can provide a link to the gold substrate. Constant-current and barrier-height STM images show a clear evidence of the helicity of the DNA structure: the pitch periodicity ranges between 25 Å and 35 Å while the average diameter is 20 Å. Pure TAPO solution on a similar gold substrate can be imaged only in the constant-current mode and appears as a crystalline structure of centered hexagons of 3.1 Å side.

2. Experimental apparatus

The STM microscope used in the present experiment is described elsewhere [10-12] as well as the technique for shaping of the Tungsten tip [13]. The figures shown in this paper are obtained from raw data without any post-electronic image treatment. STM images have been taken in air simultaneously in

the constant-current, which reflects the true topography only when the surface is electronically homogeneous, and barrier-height modes. The latter consists in modulating the sample-tip distance (ds) and measuring the current variation (dI/ds) which is proportional to the square root of the local 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. Tip-sample voltage and tunneling current were set at 20 mV (sample positive) and 1.0 nA, respectively. The Z-piezo modulating frequency was set at 11 kHz with an amplitude (ds) of 0.5 Å.

Plasmid circular DNA in aqueous solution, concentration 1 mg/ml, was used as base solution. DNA was marked with activated Tris 1-Aziridinyl Phosphine Oxide (TAPO) by mixing 20 μ l each of DNA and activated TAPO solutions for one hour at room temperature [14]. The solution was then deposited on a gold plated Aluminum stub and dried at room temperature for several hours .

3. Results

Fig.1 is a dI/ds image (500 Å by 500 Å) in three-dimensional representation with simulated illumination. Two long segments of double-stranded DNA molecules may be clearly seen.

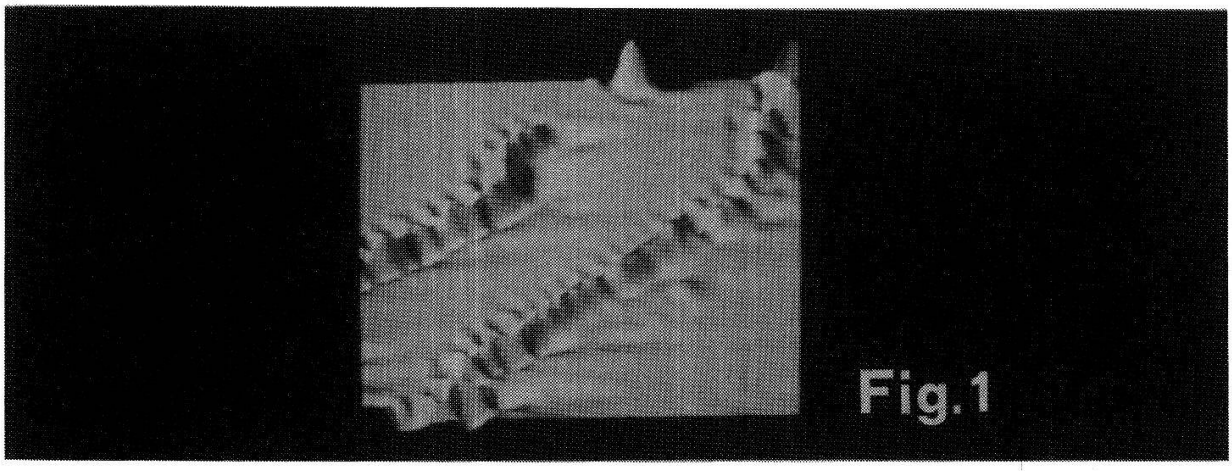
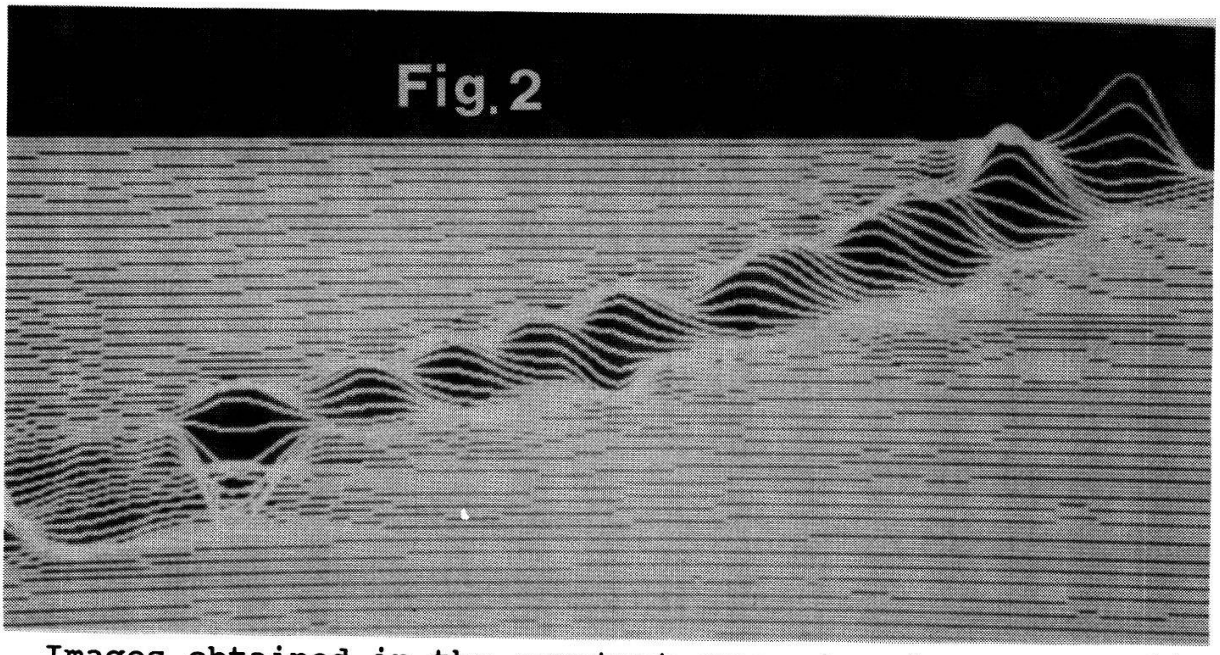
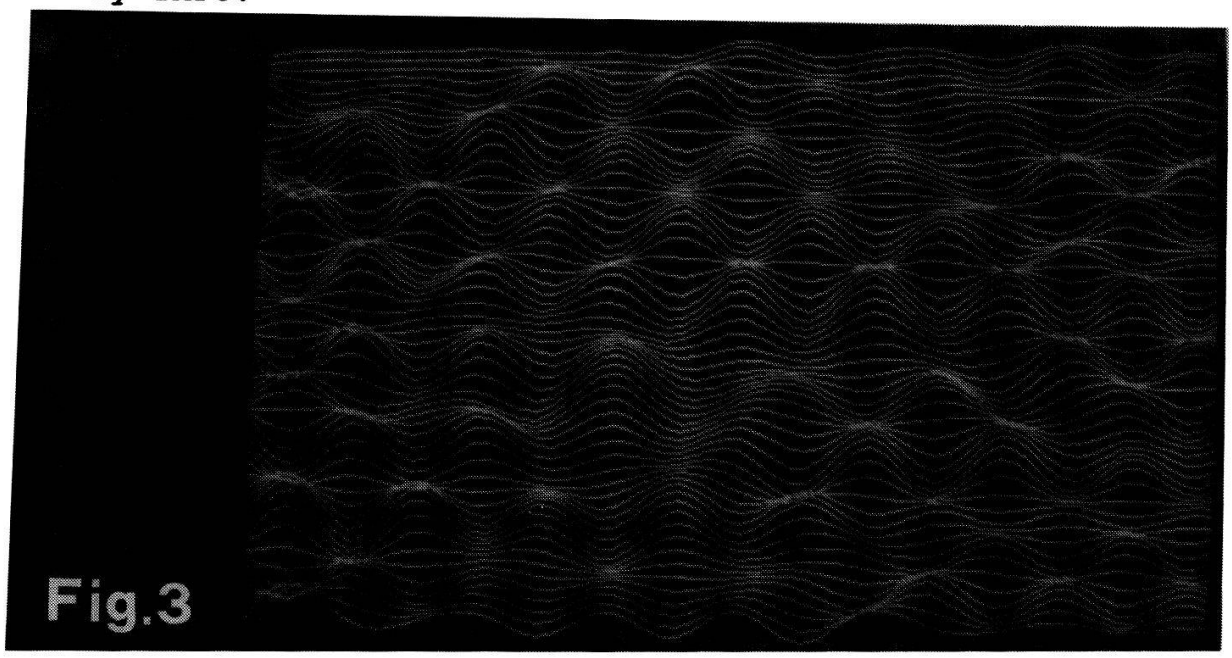


Fig.2 is a dI/ds image (300 Å by 300 Å) in amplitude traces representation, as it appears on the computer display during data acquisition. The image is formed of regularly occurred bumps along the DNA molecule with a mean spatial periodicity of 30 Å and full width half maximum of 20 Å. Bumps correspond to ten turns of the DNA helix.



Images obtained in the constant-current mode are essentially the same as in barrier-height mode, except for minor details in segments where the molecule is presumably marked by TAPO.

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 /8/. The success of the present experiment seems to suggest that naked DNA molecules are well preserved, presumably since ends of 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 centered hexagons of 3.1 Å side. Fig.3 is a constant-current image (25 Å by 25 Å) of TAPO molecules in amplitude-trace representation as it appears on the computer during data acquisition. The absence of imaging in the barrier-height mode is probably due to a negligible modification of the work function of gold by TAPO.

4. References

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