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INVESTIGATION OF DNA STRUCTURES WITH SCANNING TUNNELING MICROSCOPE**

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Scanning tunneling microscopy (s.t.m.) has attracted special attention of biologists because it can resolve surface details down to the atomic level with non-distractive probes. There are serious difficulties in imaging biological samples such as low conductivity of electrons and also technical difficulty in deposition of the sample on conducting surface.

Many research groups have attempted to visualize biological samples directly without making a replica, however only a limited number of biological macromolecules, including DNA, could be visualized with the s.t.m. system [1 - 6].

In this paper we present s.t.m. observations of the native and thermally denatured DNA molecules.

The fully computerized scanning tunnelling microscope was used. The microscope was constructed in the Department of Solid State Physics, Institute of Physics University of Łódź and was supplemented with the CAMAC controlling unit. For the imaging head, the bimorph type coarse approach system was used. The scanning system was "Tripol" and the

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computer was connected to the microscope in the IBM PC/AT clone equipment with an SVGA (800 × 600) graphics card and Multisymec color monitor (Mitsubishi).

S.t.m. system was operated in air, in the constant height mode. A voltage of 1.2 - 1.5 V was used and a tunneling current of the medium value of 1.5 nA. Samples (put on a vacuum sublimated gold film) were negative with respect to the tip. All images were obtained using electrochemically etched tungsten tips.

Highly polymerized sodium salt of calf thymus DNA was dissolved in 10 mM NaCl at a concentration of 1 mg/ml. The DNA sample was not sonicated. Denaturation was achieved by boiling samples in a water bath at 100°C for 10 min and rapidly cooling in an ice bath. For the s.t.m. observation a droplet of the solution was allowed to dry in air for 2 h on a gold-plated glass.

DNA could exist in at least two forms: B and A, wherein the base pairs have a different tilt and are displaced outward with respect to the axis of the helix [8 - 10].

Figure 1 shows a fragment visualized of the DNA molecule with a high resolution scanning tunneling microscope.

The right handedness of the molecules is clearly visible. The periodicity of the helix ranged from 30 to 40 Å. The width of the DNA was approximately 20 - 25 Å. Therefore the image shown in Fig. 1 could fit the A or B models of DNA. Under dehydratation, DNA is known to adopt the A conformation [8 - 10]. Since the DNA molecules were dried in air and one would anticipate that it is in the A form, characterized by a pitch of 28.2 Å and a width of 23 Å. However, we cannot exclude local variations in hydration of the environment and the presence of a DNA fragment in B conformation. The groove which can be seen may represent either the major groove characteristic for the B form or the wide minor groove of A conformation. S.t.m. images of DNA molecules prepared in a similar way were interpreted by others either as conformation B or A [2, 5].

The observed deviation of DNA parameters may be a result of a finite size of the tip (the tips used were not atomically sharp) and/or inperfect processing of the images.

Gaps observed at the bottom and the upper part of Fig. 2 are possibly due to DNA molecule tilt by the device tip.

Control sample (10 mM NaCl without DNA) subjected to identical treatments showed no topographic structures. When DNA was dissolved in

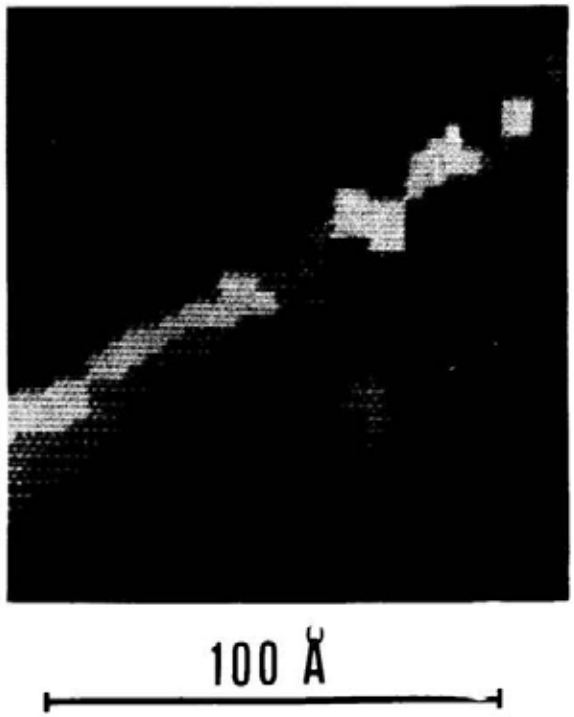

Fig. 1. Calf thymus DNA imaged by s.t.m. at a higner magnification

Fig. 3. Fragments of ssDNA

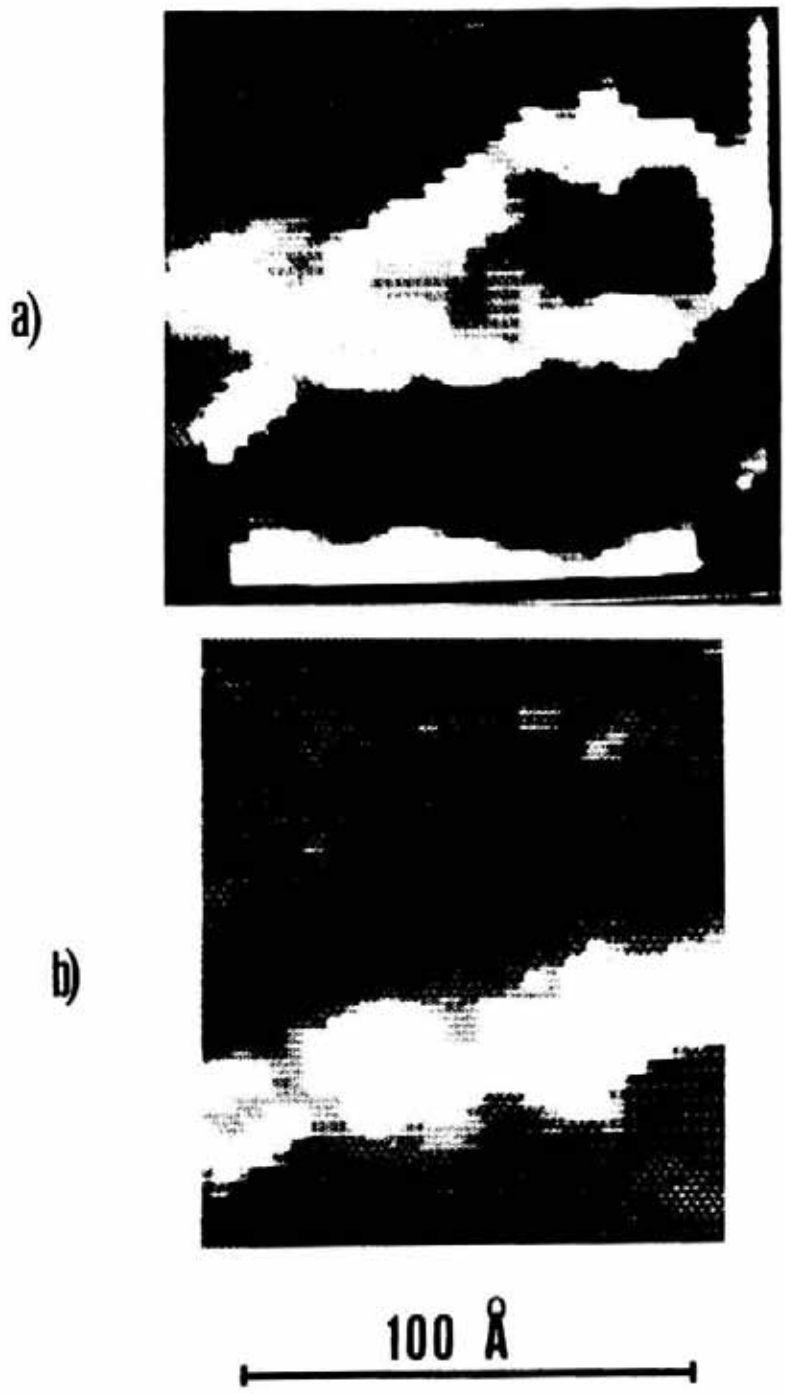

Fig. 2. S.t.m. image of thermally denatured DNA. The magnification was the same as in Fig. 1

solutions of low salt concentration (up to 50 mM NaCl) the observed images revealed the same structural parameters but the background was considerably greater. Higher salt concentration (above 50 mM) made impossible any observation due to a strong background. When DNA was thermally denatured it was impossible to find any of the features characteristic for the native molecule (Fig. 2). The observed structures may represent either aggregated fragments of ssDNA or inaccurately renatured DNA molecules. However, some characteristic features of denatured, single stranded DNA are also visible in Figs. 2 and 3 marked with arrows, where short strands can be seen of about 10 Å wide, with the steps which may represent nucleotide bases, spaced approximately 5 Å apart. These parameters are in good agreement with the values presented for poly(dA) and single stranded 25 mer DNA images obtained by applying s.t.m. and atomic force microscopy [7].

The experiments reported here demonstrate the great potential of the s.t.m. technique for precise characterization of DNA and possible investigation of the alteration in DNA conformation induced by different physico -chemical agents.

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