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A close encounter with DNA

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Abstract Atomic force microscopy, associated with surface science, has the potential to resolve the secondary structure of DNA in liquid form with unusually high resolution and with unprecedented accuracy.

Keywords Atomic force microscopy · DNA

Introduction

Although the "current tunnel" phenomenon was theoretically demonstrated in 1928, it was not until 1982 that this phenomenon gave rise to the first laboratory microscope: the scanning tunnelling microscopy (STM), which allowed direct imaging of atoms (Binning et al. 1982). The exceptional resolution of the STM lead to the development of a set of techniques based on an analogue principal and grouped together under the name of scanning probe microscopes. Of these techniques derived from the STM, the atomic force microscopy (AFM) is the most developed. Recent technological progress in several domains has been essential to the development of the AFM, whose performance in terms of the spatial resolution is four or five times greater than that of the classic mechanical sensor, although it has been conceivable since Newton's time.

Since the beginning of the 1980s, DNA has been a subject of interest to physicists and has caused a great deal of excitement, especially in the field of scanning probe microscopy, not only for its biological importance, but also because of its role as an experimental model for the behaviour of ideal polymer chains. At the time of the famous discovery by Watson and Crick (1953) of the structure of DNA, the most efficient tool to investigate the detail of molecular structures was X-ray diffraction. More recently, many people have tried to obtain images of DNA using scanning probe microscopy (about 200 papers). Only 10–15% of published articles investigate DNA in aqueous solution, which seems to be a fair representation of the relative effort. However, since the results published by Hansma et al. (1992; Hansma 2001) and Mou et al. (1995), no significant improvement, in term of resolution, has been made regarding the structure of DNA in solution by AFM. In general, the resolution achieved is not sufficient to visualize the helical structure directly, although, quite often, good images show features compatible with the turns of the helical structures. We report here reproducible, high-resolution observations of DNA in aqueous solution using the AFM (Nanoscope).

The technical method used to prepare the oriented DNA on a silicon wafer surface was inspired by the results obtained by Safinya's group (Rädler et al. 1997) on the mixtures of DNA with binary mixtures of lipids that contained DOPC (dioleoylphosphatidycholine) as the neutral co-lipid and DOTAP (dioleoyltrimethylammonium propane) as the cationic liposome.

Materials and methods

Materials

The plate was first washed with 1:1 chromosulfuric acid/water for at least 30 min. The Teflon trough was then washed with pure water and knocked until it was dry. The Teflon trough had a diameter of 1.5 cm a depth of 1 cm.

Methods

DOPC and DOTAP were prepared at a concentration of 0.1 mg/mL in a 2:1 mixture of chloroform/methanol. In step 1 (Fig. 1), the Teflon trough was filled with water and 0.1 μL of the mixture was placed on top. The solvent evaporated after 10–15 min, leaving a lipid monolayer. The hydrophobic carbon side chains are oriented to the outside and the hydrophilic head groups are oriented to the

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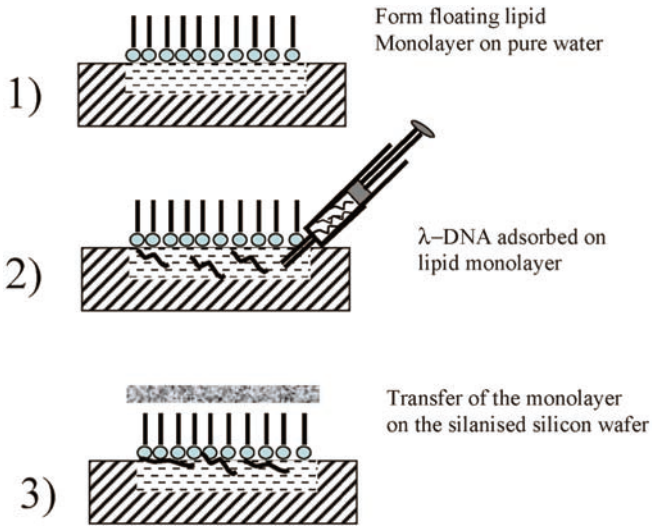

Fig. 1 Scheme for preparation of DNA on a solid surface

aqueous solution. In step 2, 12 μL of a λ-DNA (48,502 bp, contour length 16.5 μm) solution in water at a concentration of 0.005 mg/mL was injected carefully under the liquid monolayer with a Hamilton syringe. Then, the Teflon plate was left for 8 h in a saturated atmosphere to avoid evaporation of the water. In step 3, the hydrophobic silicon wafer was placed carefully on the monolayer with the adsorbed λ-DNA (Schaefer method). This is an important step, because the quality of the DNA layer is strongly dependent on the manner in which it is transferred onto the surface. The silicon wafer was silanized with dimethyldichlorosilane.

The sample was examined in a liquid cell in pure water using an AFM in tapping mode (Nanoscope III). The cantilever used here was from Olympus, with a very sharp tip (5 nm), a resonance frequency of 300 kHz, and a spring constant of 42 mN/m.

Results and discussion

Figure 2 shows AFM images of long DNA oriented on the surface. This orientation may be due to the expected local demixing of the cationic and neutral lipids and the shearing of the water after transfer. The length of the λ-DNA is 16 μm. The diameter and the height of some of the molecules are larger than the diameter of single DNA molecules. This is due to the aggregation of several molecules, which leads to an increase in the persistent length. Note that the persistent length of a DNA molecule is about 500 Å.

The high magnification images of a single DNA molecule (Figs. 3 and 4) show a typical helical conformation of DNA with a diameter of 30 Å. Since 1992, a series of DNA images using AFM have been obtained. However, this is the first time that such a resolution has been obtained in water. It is now possible to accurately measure the periodicity of the helix, as revealed by analysis of the section along the DNA axis (Fig. 3B). The value of the helix pitch is equal to 33 ± 2 Å. The spacing of the helix turns is exactly the spacing of the major groove in B-DNA. The size of the minor groove is 8 Å. For the moment it is not possible to resolve the structure of the minor groove. The flatten filter has been used for

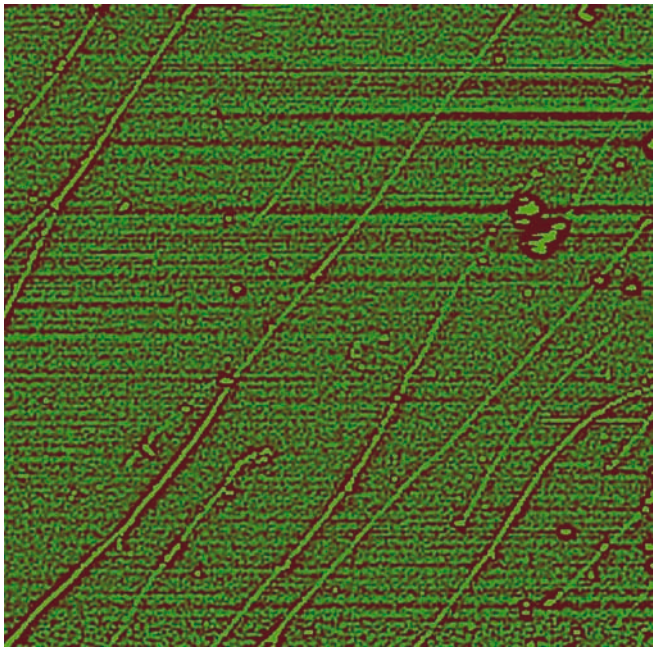

Fig. 2 AFM images of λ-DNA in water at a large scale. DNA molecules are oriented on the surface. Some molecules are larger than the others, showing the aggregation of several molecules after the transfer (size 600×600 nm)

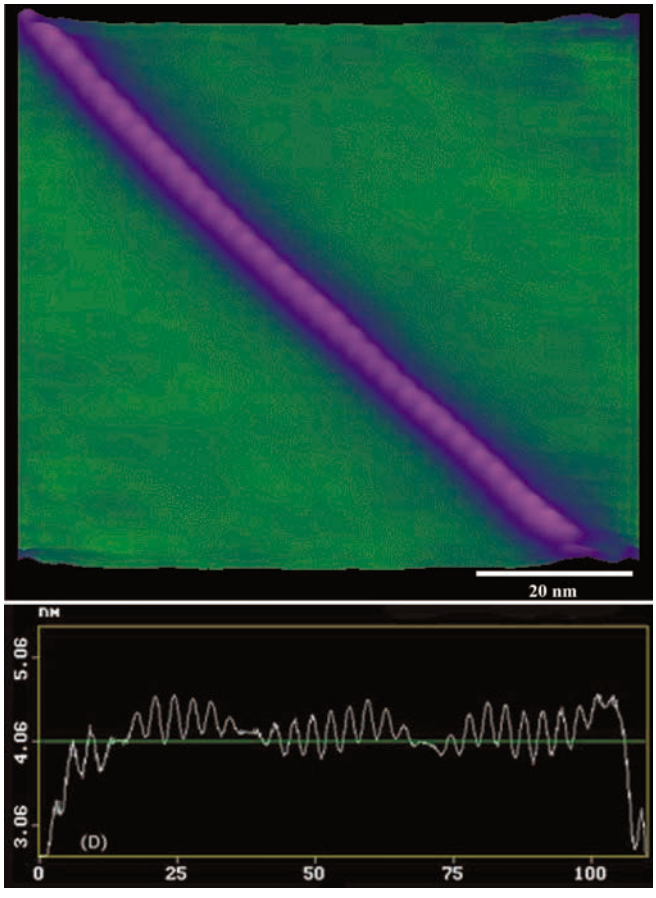

Fig. 3 **A** Nanometer resolution of DNA showing the major grooves. **B** Section of the image along the DNA axis. The periodicity of the helix is 33 Å

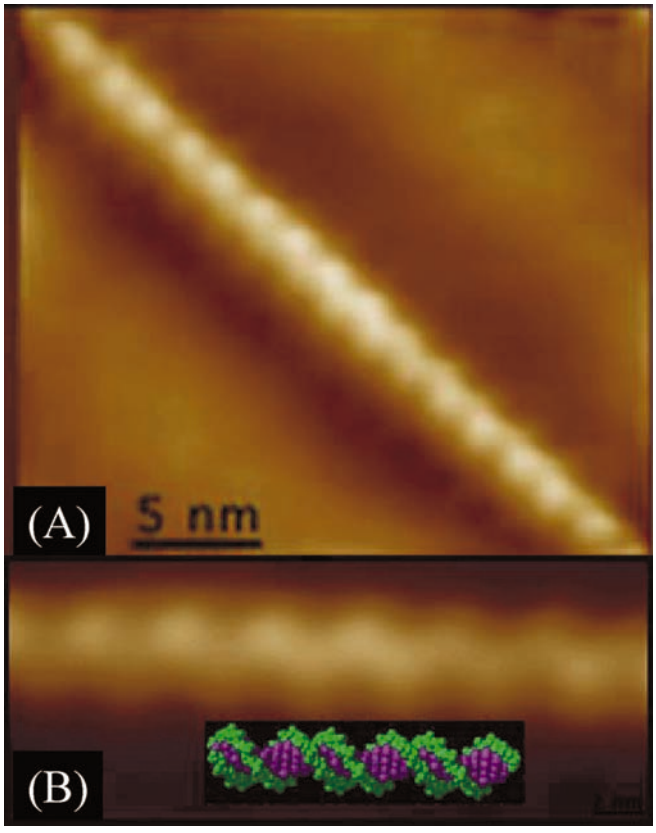

Fig. 4 **A** High-resolution image of DNA showing the right-handed double helix. **B** Standard model of the B-DNA double helix is shown for comparison purposes with the experimental result

all images. The AFM resolution of DNA has actually been good enough to resolve the major groove of DNA. There are, however, a few examples of unusually good resolution for DNA. Matsumoto et al. (1999) using ultra-high vacuum (UHV) non-contact AFM obtained a single image of DNA with approximately two turns of the double helix. However, the spacing seems to be 3.3 nm for only one-half of a helix turn instead of a full helix turn. AFM of packed DNA molecules on a bilayer of positively charged lipids has resolved features with the spacing and right-handedness of the major groove of the DNA double helix (Yang et al. 1996; Fang and Yang 1997a, 1997b).

Compared to previous studies, the chosen experimental conditions lead to a better resolution due to (1) the lower mobility of the lipids in a monolayer than in a bilayer and (2) the favorable orientation of the DNA with respect to the scanning direction. Figure 4 demonstrates the exceptional resemblance between the AFM images and the standard model of B-DNA in terms of the periodicity and the helix angle. These results show that the DNA molecules have been well preserved. The unique quality of this set of images broadens the potential of AFM imaging in solution. It points the way to a possible means of investigating structural defects and molecular recognition along DNA and brings us one step nearer to the goal that excited experts in the field of microscopy at the beginning of the 1990s: possible sequencing of DNA using AFM.

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