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A Study of the Probe Effect on the Apparent Image of Biological Atomic Force Microscopy

Xue Mei WANG[1,2]*, Lei QIN[2], Long BA[2], Zu Hong LU[2]

[1]The State Key Lab of Coordination Chemistry, Nanjing University, Nanjing 210093
[2]National Lab of Molecular and Bio molecular Electronics, Southeast University, Nanjing 210096

Abstract: The probe effect on the apparent image of biological atomic force microscopy was explored in this study, and the potential of AFM in conformational study of gene related biological processes was illustrated by the specific nanostructural information of a new antitumor drug binding to DNA.

Keywords: Atomic force microscopy (AFM), absorption, DNA recognition.

The atomic force microscopy (AFM) possesses high spatial resolution and it is compatible with liquid environments. AFM can provide possibility to study a wide range of biological problems at the molecular level and acquire topological information at nanometre resolution under physiological conditions[1,2]. However, a major problem for image reconstruction of biological specimens is that structures of most biological molecules are very soft and delicate, which could be easily deformed and damaged under the probe force. Besides, many biological macromolecules could only be weakly adsorbed to the substrate surface so that they might be readily displaced by a scanning tip. In view of this, some factors concerning with the application of AFM technique in biological study have been explored in this study, and the potential of AFM in structural research of gene related biological processes has been illustrated by DNA conformational study, including study of interaction of the antitumor drug binding to DNA.

In the experiments, the specimen was prepared in tris buffer (pH 7.8) with double-distilled water. A Nanoscope III (Digital Instruments) was utilized for AFM measurements. The experiments were performed in air at 20°C in the tapping mode with a conventional silicon tip and/or an ultrasharp Si_3N_4 tip (radius of curvature less than 30 nm) (Silicon MDT).

To make sure that DNA adhibits to the supported solid substrate (like mica) strongly enough, the specimen was deposited onto a Mg^{2+} pre-soaked mica. Before observation, the prepared specimen was rinsed several times and dried by evaporation in ambient air. Each sample was imaged many times in different places in order to obtain reliable measurements.

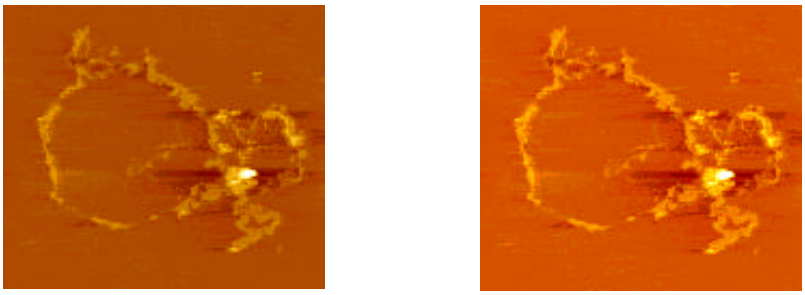
The results of our AFM study indicate that the relative humidity and the geometry

* E-mail: xuewang@seu.edu.cn

of the tip used in the DNA conformational study play an important role to achieve topological information with high spatial resolution. As reported in the literature that a water layer may weaken the adsorption of the specimen, we also observed that low humidity is beneficial for the AFM of DNA specimen. Besides, the choice of suitable cantilevers is also vitally critical to acquire DNA topological information at nanometre resolution. **Figure 1b** is a representative AFM image of CT-DNA obtained with an ultrasharp Si_3N_4 tip, while **Figure 1a** is that of the same sample observed with conventional Si tip. The other experimental conditions are identical. It is clear that nanometre resolution structures of the DNA could be observed much more clearly if using the ultrasharp Si_3N_4 tip in AFM study.

Figure1 Top view of AFM image of CT-DNA (scan size 2500 nm×2500 nm) (10 mmol/L) with (a) conventional Si tip and (b) ultrasharp Si_3N_4 tip

(a) (b)



In view of the above study, we have further utilized AFM to acquire nanostructural information of DNA-drug interactions under physiological conditions. The DNA binding properties of a new and potentially efficient antitumor drug dacarbazine (DTIC) have been explored in this work. Initial evidence for the interaction of DTIC with DNA comes from electronic absorption and NMR study, which indicate that non-intercalation is a typical mode of the binding of DTIC to DNA. Especially, the AFM nanostructural information at the molecular level by using the ultrasharp cantilevers further illustrates the recognition specificity and binding affinity of DTIC to DNA.

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