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MAC Mode AFM Studies of Zinc-Induced DNA Kinking

Application Note

Y. Judy Zhu, Wenhai Han[1,2], Mensur Dlakic, S.M. Lindsay[1,2], and Rodney E. Harrington
[1]Agilent Technologies
[2]Arizona State University

Introduction

The bending of DNA strands is a common phenomenon either intrinsic or induced by protein binding and/or other changes in the physiological environment (such as local ion concentrations). It is believed to play an important role in the control of gene expression, replication, recombination, and packaging in the nucleus. Kinking, roughly defined as abrupt bending, is a distinct category of DNA bending and is often observed in protein-DNA complexes. Numerous methods are available to detect structural fluctuations in DNA. To clearly distinguish between smoothly distributed bending and highly localized kinking, however, a spatial resolution on the order of at least 1 nm is required.

Among many high-resolution imaging tools, the atomic force microscope (AFM), with easy sample preparation and close to native imaging conditions, stands out as one of the best options for biologists. Its near-atomic resolution makes it useful for in situ imaging of conformational changes in DNA molecules. Due to tip-broadening effects, however, the width of DNA molecules obtained with atomic force microscopy has been on the order of 5 nm.

In this study, we employed Agilent's patented MAC Mode (Figure 1) atomic force microscopy, which uses a magnetically-driven oscillating probe with an oscillation amplitude significantly smaller than that of fluid tapping mode, or Tapping Mode. MAC Mode (Figures 2 and 3) was shown to image DNA molecules at a much higher resolution (about 1 nm) than other AFM techniques. We utilized MAC Mode to investigate DNA minicircles constructed from phased sequence motifs known to bend DNA.

The following 42-bp sequence was used for T4 ligase-mediated ligation:

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CCCAAAAAGGGCCAAAAAGGGCCCAAAAAGGGCCAAAAAGGG
    TTTTTCCCGGTTTTTCCCGGGTTTTTCCCGGTTTTTCCCGGG
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Linear and circular molecules were separated with two-dimensional gels. A 168-bp DNA circle containing four of the above fragments was used for AFM study. DNA samples were diluted to a final concentration of 0.5 µg/ml using deionized water. Before imaging, $MgCl_2$ and/or $ZnBr_2$ were added to the DNA solution and the mixture was deposited on freshly cleaved mica surface in the liquid cell of an Agilent AFM.

In a previous study using the same sample and technique, the 168-bp circles were shown to obtain dramatically different conformations in different cations. When imaged in 1 mM $MgCl_2$, the DNA circles were smoothly bent, all nearly circular. In 1 mM $ZnBr_2$, about 4 kinks on the average were observed in each molecule. In this work, we were mainly interested in the cation centration-dependence of the DNA kinking phenomenon. In particular, what is the Zn^{2+} concentration threshold for its induced kinking?

To answer this question, $ZnBr_2$ concentration was gradually decreased from 1 to 0.05 mM in a series of experiments.

High $ZnBr_2$ (1 mM)

DNA was imaged in either 1 mM $ZnBr_2$ alone or in both 1 mM $ZnBr_2$ and various concentrations of $MgCl_2$. In all cases, regardless of the $MgCl_2$ concentration (which was as high as 10 mM, as shown in Figure 1), the DNA molecules were clearly kinked. The fact that zinc-induced kinking remained even when Mg^{2+} was tenfold more concentrated suggests that the binding of these two ions to DNA molecules is independent of one another. This result agrees with a well-accepted mechanism in which Zn^{2+} interacts predominantly with bases, whereas Mg^{2+} associates primarily with phosphates.

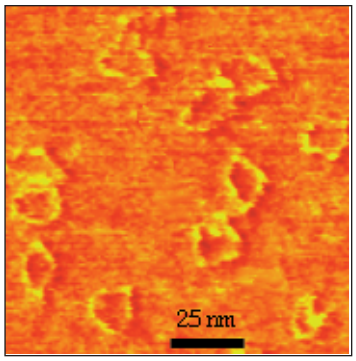

Figure 1. MAC Mode AFM image of the 168-bp circles in 1 mM ZnBr2 / 10 mM MgCl2.

Medium $ZnBr_2$ (100 µM)

Figure 2 shows an AFM image of the 168-bp circles still kinked in 100 µM Zn^{2+} and 1 mM $MgCl_2$, although both kinked and circular molecules have been observed under these conditions. The number of kinks was estimated to be 4. An interesting feature is the presence of "islands" around each molecule. They are likely salt clusters on which DNA molecules often deposit.

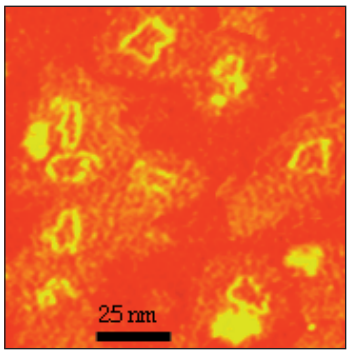

Figure 2. MAC Mode AFM image of the 168-bp circles in 100 µM ZnBr2 / 1 mM MgCl2.

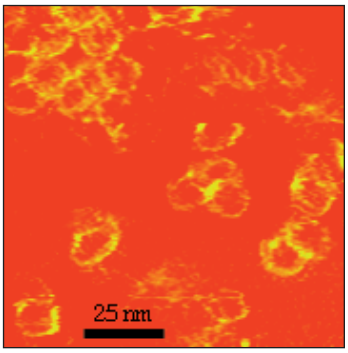

Figure 3. MAC Mode AFM image of the 168-bp circles in 50 µM ZnBr2 / 1 mM MgCl2.

Low ZnBr2 (50 µM)

When Zn^{2+} concentration was further reduced to 50 µM in 1 mM $MgCl_2$, very few kinked molecules remained and almost all were circular (Figure 3). This result is similar to that of DNA in Mg^{2+} alone.

Based on the above experiments, we conclude the following.

The threshold

As there was a clear conversion from kinked to circular DNA when the Zn^{2+} concentration was reduced from 100 to 50 µM, the threshold for zinc-induced kinking evidently lies between these two values. Previous solution studies suggested that the effects of Zn^{2+} persist even to lower concentrations (10 µM) than observed here. Since Zn^{2+} must interact somewhat with the mica surface as well as DNA; however, it is possible that the activity of the Zn^{2+} is reduced substantially near the surface. It is safe to say that our threshold estimate defines an upper limit value.

Kinking mechanism

The fact that images from the same original preparation of circular DNA samples show both kinked and non-kinked structures suggests that the observed kinks cannot be attributed to nicks arising from incomplete ligation. Furthermore, when Zn^{2+} concentration varied, we did not observe any intermediate state in which the number of kinks per molecule lies between 1 and 4. Therefore, the zinc-induced kinking on a DNA molecule appears to be a cooperative process. The 4-kinks-per-molecule state is probably much more energetically favored than that of 2 or 3 kinks in this particular molecule.

Image contrast

We found an interesting correlation between the amount of salt and the apparent contrast of the DNA. As ion concentration varied from Figure 1 to Figure 3, the image contrast improved from only 0.2 nm in Figure 1 to about 1.5 nm in Figure 3. These results suggest a significant role of salt in AFM image contrast of DNA samples.

Summary

Using a unique AFM technology, MAC Mode, we characterized zinc-induced DNA kinking under different physiological conditions. For the first time, we were able to explore the ionic strength threshold of the kinking in a fairly direct fashion. We also showed that MAC Mode combines a major improvement in image resolution with an ability to image DNA that is relatively gently bound to the substrate. Future experiments in which a fluid-flow system is added to the MAC Mode AFM in order to obtain "online" changes of ionic environment could allow real-time kinetic studies of the kinking process.

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Revised: 11/08/06

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Printed in USA, February 14, 2007
5989-6275EN

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