Damage of DNA ends induced by mechanical force during AFM nano-manipulation

DUAN Na[1,2] WANG Xinyan[1] ZHANG Chen[1,2] ZHANG Yi[1] HU Jun[1,*]

[1]*Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China*

[2]*University of Chinese Academy of Sciences, Beijing 100049, China*

Abstract An experimental and statistical study was carried out to explore the effects of mechanical forces on the ends of linear double-stranded DNA (dsDNA) fragments. Mechanical force was applied onto individual DNA molecules during atomic force microscope (AFM)-based picking-up manipulation. By comparing the PCR efficiency of two DNA fragments with primers either at ends or at the inner regions, it was found that the ends of DNA fragments were damaged during picking-up process.

Key words Nanomanipulation, DNA damage, Mechanical force, Atomic force microscope (AFM)

1 Introduction

Since its invention in the 1986 by Binnig *et al*.[1], atomic force microscope (AFM), being able to measure surface topography with nanometer-resolution under different environmental conditions, has made great contributions to fundamental studies. And this is especially true in nano-biology, such as high-resolution imaging of protein[2-6] and nucleic acid[7,8], high sensitivity protein force spectroscopy[9,10], and elastic measurement-combined fluorescence imaging on cells[11]. In addition, AFM can apply a tiny mechanical force to a sample through its tip, hence the possibility of using AFM as a micromanipulation tool to dissect biological samples, in microscopic levels from cells down to individual molecules[12-16]. Efforts were made to isolate biological objects[17-20] with AFM. Several AFM operation modes are employed to isolate DNA fragments. For instance, dissection and subsequent extraction of genetic materials from chromosomes were demonstrated by driving an AFM tip to contact with the target[14,21]. Occasionally, DNA fragments could be picked up from the surface with the AFM tip engaged in the force mapping mode[18,22]. By using the negative lift mode AFM, our group developed a serial of techniques to construct artificial DNA patterns[23], and to isolate individual DNA molecules from a solid surface[24], which may be applied in ordered DNA sequencing[25] and direct molecular haplotyping of genomic DNA[24,26].

However, an AFM tip applies a mechanical force of tens of nano-newton on DNA molecules during the AFM nanomanipulation[23]. And whether the isolated DNA fragment can keep its biological activity after such a process is a natural question. Especially, the ends of a DNA fragment, which are less bonded, are less stable than its inner regions, and DNA ends play an important role in DNA sequencing, ligation and other biological process. Therefore, the possible damage to DNA ends during AFM manipulation should be investigated.

The polymerase chain reaction (PCR)[27] conducted after the isolation of DNA fragments provides a suitable means for exploring mechanical force effects on the DNA ends. Normally, the isolated DNA fragments attached on the AFM tip should be amplified to provide sufficient copies for subsequent biochemical analysis. The PCR amplification on a single-molecule level is delicate, and any damage on the bases stops replication in the first run. So, a

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* Corresponding author. *E-mail address:* hujun@sinap.ac.cn

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successful amplification of the isolated DNA fragment rules out measurable damages to the DNA molecules in the AFM manipulation process.

In this paper, PCR efficiency of two DNA fragments picked up by AFM nanomanipulation is compared: One is amplified from its ends, and another from its inner part. The first DNA fragment is of a significantly lower PCR efficiency than that of the second, indicating that DNA ends were damaged during AFM nanomanipulation.

2 Materials and methods

Template DNA was prepared from PBR322 (TaKaRa Bio. Inc., Dalian, China) by amplifying with the Ex Taq DNA polymerase Hotstart (TaKaRa Bio. Inc.), followed by purification by Takara DNA fragment Purification Kit Ver2.0. Two sets of primers in Fig.1 (Sangon Co., China) were used: p1: 5'-CTCTTACTGTCATGCCATCCG-3', p2: 5'-CCGTGTCGCCCTTATTCC-3'; Ex-p1: 5'-GTCGTTTGGTATGGCTTCA-3', Ex-p2: 5'-GACAATAACCCTGATAAATGCT-3'. The PCR procedure was carried out using a Mastercycler gradient (Eppendorf). After denaturing at 95°C for 5 min, 35 cycles of 95°C/30 s − 63°C/30 s− 72°C/30 s, with an additional extension at 72°C for 10 min, were performed. Sterilized Milli-Q H_2O was used as a blank control. Owing to the low copy number of DNA templates, the product of PCR was used as template and a second round PCR was conducted. For each sample, 5 μL PCR products were separated by 1.5% agarose gel electrophoresis and stained by ethidium bromide.

Fig.1 Schematics of the DNA templates. The fragments of pBR322 from the 3799th site to the 4137th site, and from the 3632th site to the 4200th site, were picked up for PCR amplification.

Mica substrate was treated with 0.5% aqueous solution of 3-aminopropyl-triethoxysilane (APTES, Sigma Co.) for 4 min under humidity of 40%−60% at 20°C−25°C, washed with double-distilled water, and dried with clean air. Soon afterwards, the mica was baked at 120°C. A drop of 1 μL DNA solution was placed onto the mica substrate and stretched with a modified molecular combing technique[28,29]. The samples were dried with clean air.

Imaging and nanomanipulation of DNA were performed with a multimode AFM (Nanoscope V, Veeco/Digital Instruments, Santa Barbara, CA) equipped with a J scanner. Silicon cantilevers (NSC-11, MikroMasch) with a spring constant of 48 $N \cdot m^{-1}$ were used. All operations were carried out under the conditions of 22°C and 40% relative humidity. Images were collected with tapping mode AFM. Details of manipulating and picking up individual DNA fragments can be found elsewhere[24,26]. Finally, the AFM tip with DNA fragments was put into a PCR tube for PCR.

3 Results and discussion

3.1 Substrate preparation

Preparing a proper mica substrate is a key to the success of picking up DNA fragments from the substrate. In this work, the mica substrate was modified by APTES and its capability of adsorbing DNA molecules was modulated by varying baking time of the mica substrate at 120°C. It was found that, a modified mica baked for just 2 h adsorbed a DNA molecule so firmly that it could neither be rolled up into a round feature, nor to be picked up from the substrate, and the substrate would be damaged in the nanomanipulation process, too; while a DNA molecule adsorbed on an APTES-modified mica baked for 4 h could be pushed around on the substrate by the AFM tip (Figs.2A−2C), in most cases. And baking the APTES-mica substrates for 3 h at 120°C was of suitable absorbability to DNA molecules, which could be rolled up and picked from the substrate (Figs.2D−2F).

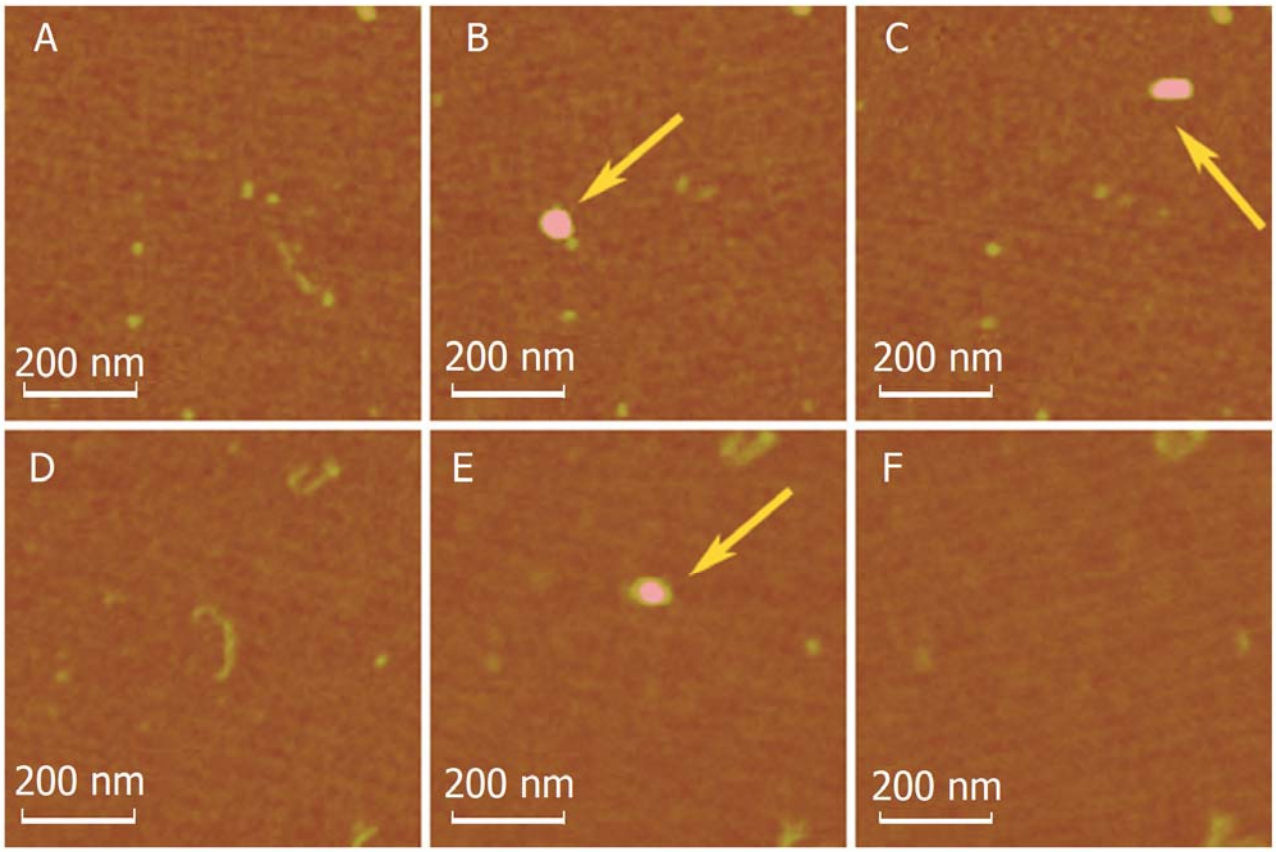

Fig.2 Tapping mode AFM images in manipulating a target DNA molecule on APTES-modified mica baked for 4h (A, before manipulation; B, the target DNA indicated with an arrow was rolled up to a round feature; C, the round-shaped target DNA was moved to another location, indicated with an arrow) and baked for 3 h (D, before manipulation; E, the target DNA was rolled up to a round feature; F, after DNA pickup, the target DNA disappeared from the surface). Height scale: 3 nm.

3.2 DNA template amplification

As shown in Fig.1, the 338 bp DNA fragments (pBR322 original sites of 3799th–4137th), and the 569 bp DNA fragments (pBR322 original sites of 3632th–4200th), were amplified from plasmid pBR322 by using the primers p1/p2 and Ex-p1/Ex-p2, respectively. The PCR products were purified and analyzed by electrpheresis (Fig.3). Sequences of the DNA products are as expected (data not shown), indicating that the DNA products are pure and were well prepared.

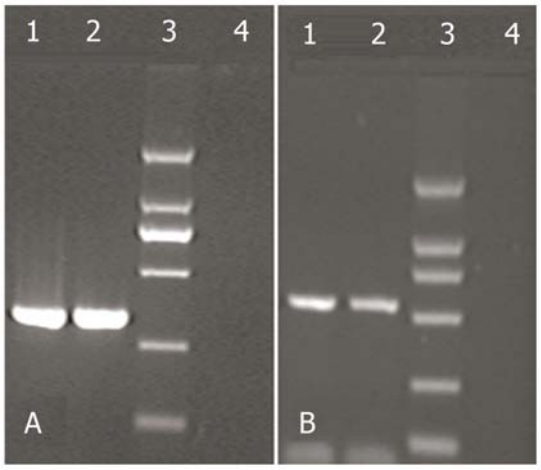

Fig.3 Electrophoresis results of the PCR products. Lanes 1 and 2 for DNA fragments of the 338bp (A) and 569 bp (B); Lane 3, DL2000 DNA marker (from top to bottom 2000, 1000, 750, 500, 250, 100 bp); Lane 4, blank controls.

3.3 Pick-up of DNA fragments and PCR

The DNA samples shown in Fig.3 were deposited on APTES-mica (baked at 120°C for 3 h) and were used for AFM nanomanipulation. The DNA molecules picked up by the AFM tips were amplified using the primers p1/p2. The PCR efficiencies for the two sets of DNA templates were compared.

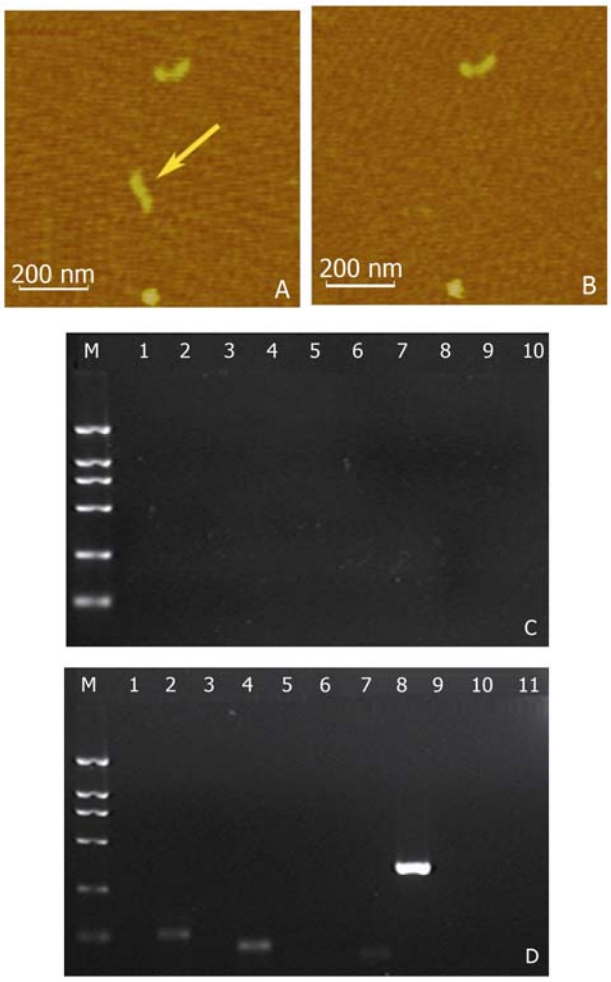

Fig.4 Pick-up and amplification of 338 bp DNA fragments. (A) AFM image of 338 bp DNA fragments deposited on APTES-mica. The target DNA fragment was indicated with an arrow. (B) AFM image showing that the target DNA fragment was picked up. Height scale: 3 nm. (C) Electrophoresis result of the products of the first-round PCR. Lanes 1 and 2, blank controls; Lanes 3−9, DNA fragments picked up by AFM tips and amplified by PCR; Lane 10, negative control by the AFM tip for imaging and manipulating the DNA fragment before its pick up; Lane M, DL2000 DNA marker (from top to bottom 2000, 1000, 750, 500, 250, 100 bp). (D) Electrophoresis results of the products of the second-round PCR. Lane 1, blank control; Lanes 2 and 3, negative controls of the first PCR; Lanes 4−10, DNA fragments picked up by AFM tips; Lane 11, negative control by the AFM tip for imaging and manipulating the DNA before its pick up; Lane M, DL2000 DNA marker (from top to bottom 2000, 1000, 750, 500, 250, 100 bp).

AFM images of the 338 bp DNA fragments on the APTES modified mica were shown in Figs.4A and 4B. The length of the 338 bp DNA fragments was about 115 nm in the AFM images. After AFM manipulation, the target DNA fragment disappeared, as shown in Fig.4B, indicating the target DNA was picked up by the AFM tip.

The AFM tip with 1 to 3 isolated DNA fragments was transferred into a sterile tube, and PCR experiment was carried out. Some typical results of the PCR products detected by 1.5% agarose gel electrophoresis are given in Figs.4C and 4D, for the first- and second-round PCR, respectively. Fig.4C does not show any product band, while there is a PCR product in Fig.4D. In our primary demonstration, 1 among 13 tips with expected DNA fragments were detected positive after amplification, while no false positive was found in the negative controls.

Fig.5 revealed the pick-up and amplification of 569 bp DNA fragments. The target DNA fragment on APTES modified mica was shown in Fig.5A, which disappeared after AFM manipulation (Fig. 5B). The length of 569bp DNA fragments (~200 nm) was longer than the 338 bp DNA fragments.

Some typical results of the PCR products analyzed by 1.5% agarose gel electrophoresis are given in Fig.5C and Fig.5D, for the first- and second-round PCR, respectively. In total, 6 among 16 tips with expected DNA fragments were detected positive after amplification, while no false positive was found in the negative controls.

3.4 Statistical analysis

A statistical analysis was done on the PCR efficiencies of the two different DNA fragments. For comparison of the two samples, the equilibrium is:

$$z = \frac{p_1 - p_2}{\sqrt{pq(1/n_1 + 1/n_2)}}$$

where, n_1 and n_2 are the total numbers of the two compared samples; p_1 and p_2 are the successive proportion of the two compared samples, $p=(x_1+x_2)/(n_1+n_2)$, with x_1 and x_2 being the successive times, and $q = 1-p$. For PCR amplification of the 338 bp DNA fragments, 1 of 13 samples could be amplified. For the PCR amplification of 569 bp DNA 6 of 16 samples were detected positive after

amplification. With these data we get z=−1.867 at the P value of 0.05, indicating that the PCR efficiencies are significantly different between the two samples.

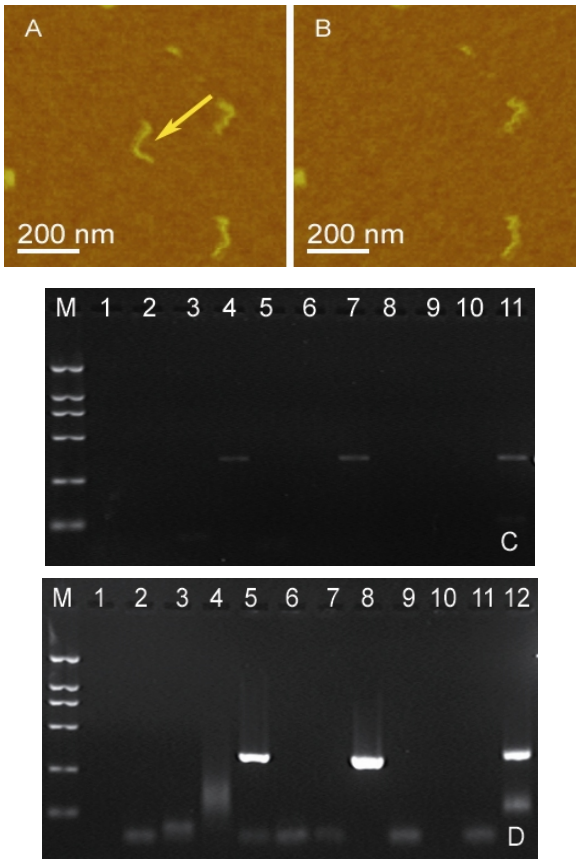

Fig.5 Pick-up and amplification of 569 bp DNA fragments. (A) AFM image of 569 bp DNA fragments deposited on APTES-mica. The target DNA fragment was indicated with an arrow. (B) AFM image showing that the target DNA fragment was picked up. Height scale: 3 nm. (C) Electrophoresis results of DNA products after the first-round PCR. Lanes 1−3, blank controls; Lanes 4−8 and 10−12, DNA fragments picked up by AFM tips; Lane 9, negative control by the AFM tip for imaging and manipulating the DNA fragment before its pick up; Lane M, DL2000 DNA marker (from top to bottom 2000, 1000, 750, 500, 250, 100 bp). (D) Electrophoresis results of the products of the second-round PCR. Lanes 1−3, blank controls; Lanes 4−8 and 10−12, DNA fragments picked up by AFM tips; Lane 9, negative control by an AFM tip for imaging and manipulating the DNA fragment before its pick up; Lane M, DL2000 DNA marker (from top to bottom 2000, 1000, 750, 500, 250, 100 bp).

3.5 Mechanism discussion

The low PCR efficiency of the 338 bp DNA fragment indicates that the DNA ends were damaged during the AFM nanomanipulation, which in turn reduced the rate of base pairing between the DNA ends and primers. Base pairs located at the ends of dsDNA molecules fluctuate into open states much more frequently than those in the dsDNA interior[30], even at temperatures well below temperature of melting T_m. The dsDNA breathing enhances its flexibility and instability on short length scales[31], which may be the cause that the ends of dsDNA molecules are easy to be damaged by the force exerted by the tip during AFM nanomanipulation.

4 Conclusion

By comparing the PCR efficiency of two DNA fragments with primers either at the ends or at the inner regions, we deducted that the AFM manipulation process would induce DNA damage at its ends. Our results suggest that, to avoid mechanical force-induced damage during AFM nanomanipulation, it is necessary to subtle design the DNA template and the PCR primers.

References

1 Binnig G, Quate C F, Gerber C. Phys Rev Lett, 1986, **56:** 930−933.

2 Shekhawat G S, Lambert M P, Sharma S, *et al*. Appl Phys Lett, 2009, **95:** 183701.

3 Bippes C A, Muller D J. Rep Prog Phys, 2011, **74:** 086601.

4 Niedzwiecka A, Lekka M, Nilsson P, *et al*. Biophys Chem, 2011, **158:** 141−149.

5 Schabert F A, Henn C, Engel A. Science, 1995, **268:** 92−94.

6 Karrasch S, Hegerl R, Hoh J H, *et al*. P Natl Acad Sci USA, 1994, **91:** 836−838.

7 Klinov D, Dwir B, Kapon E, *et al*. Nanotechnology, 2007, **18:** 225102.

8 Klinov D V, Neretina T V, Prokhorov V V, *et al*. Biochemistry−Moscow, 2009, **74:** 1150−1154.

9 Moy V T, Florin E L, Gaub H E. Science, 1994, **266:** 257−259.

10 Zhang X, Halvorsen K, Zhang C−Z, *et al*. Science, 2009, **324:** 1330−1334.

11 Rhode S, Breuer A, Hesse J, *et al*. Cell Biochem Biophys, 2004, **41:** 343−356.

12 Falvo M R, Washburn S, Superfine R, *et al*. Biophys J, 1997, **72:** 1396−1403.

13 Schoenenberger C A, Hoh J H. Biophys J, 1994, **67:** 929−936.

14 Thalhammer S, Stark R W, Müler S, *et al*. J Struct Biol,

1997, **119:** 232−237.

15 Hansma H G, Vesenka J, Siegerist C, *et al*. Science, 1992, **256:** 1180−1184.

16 Henderson E. Nucleic Acids Res, 1992, **20:** 445−447.

17 Dupres V, Alsteens D, Andre G, *et al*. Nano Today, 2009, **4:** 262−268.

18 Kufer S K, Puchner E M, Gumpp H, *et al*. Science, 2008, **319:** 594−596.

19 Kufer S K, Strackharn M, Stahl S W, *et al*. Nat Nano, 2009, **4:** 45−49.

20 Puchner E M, Kufer S K, Strackharn M, *et al*. Nano Letters, 2008, **8:** 3692−3695.

21 Stark R W, Thalhammer S, Wienberg J, *et al*. Appl Phys A, 1998, **66:** S579−S584.

22 Xu X−M, Ikai A. Anal Chim Acta, 1998, **361:** 1−7.

23 Hu J, Zhang Y, Gao H B, *et al*. Nano Letters, 2002, **2:** 55−57.

24 Lu J H, Li H K, An H J, *et al*. J Am Chem Soc, 2004, **126:** 11136−11137.

25 Lu M, Shi B C, Li X L, *et al*. Prog Biochem Biophys, 2006, **33:** 660−664.

26 An H J, Huang J H, Lu M, *et al*. Nanotechnology, 2007, **18:** 225101.

27 Saiki R K, Gelfand D H, Stoffel S, *et al*. Science, 1988, **239:** 487−491.

28 Bensimon A, Simon A, Chiffaudel A, *et al*. Science, 1994, **265:** 2096−2098.

29 Hu J, Wang M, Weier H U G, *et al*. Langmuir, 1996, **12:** 1697−1700.

30 Jose D, Datta K, Johnson N P, *et al*. P Natl Acad Sci USA, 2009, **106:** 4231−4236.

31 Lee O C, Jeon J−H, Sung W. Phys Rev E, 2010, **81:** 021906.