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Positioning Isolation and Biochemical Analysis of Single DNA Molecules Based on Nanomanipulation and Single-Molecule PCR

Jun-hong Lü,[†] Hai-kuo Li,[‡] Hong-jie An,[‡] Guo-hua Wang,[†] Ying Wang,[†] Min-qian Li,[†] Yi Zhang,[†] and Jun Hu*[,†,‡]

Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China, and Bio-X Life Science Research Center, Shanghai JiaoTong University, Shanghai 200030, China

Received May 17, 2004; E-mail: jhu@sjtu.edu.cn

Traditionally, isolation and chemical analysis of DNA are always performed on a large number of molecules. Recently, it has been recognized that the analysis of single DNA molecules is of critical importance in fundamental genetic research, clinical analysis, and industrial biotechnology development.[1−6] Currently, some methods attempting to isolate single molecules have been developed[7−9] which are usually based on the dilution of sample and limited to the molecules that are suspended in aqueous solutions. The fact that all solution-based methods have lost the "spatial resolution" in handling molecules makes it very difficult to isolate directly a particular molecule in a complex system,[10] and calls for a "positioning isolation" technique. Recently, scanning probe microscopy, including scanning tunneling microscopy (STM) and atomic force microscopy (AFM) that are usually used for mapping the surface topography, have been further developed into operative tools, with which manipulation of single atoms and molecules becomes possible and positioning chemistry has been realized.[11−19] But to pick up a particular biomolecule is still a big challenge. During recent years, several research groups have attempted to isolate biological objects with AFM tips.[20] For instance, Heckl et al. demonstrated dissection and subsequent extraction of genetic materials from chromosomes.[21,22] Xu and Ikai reported that AFM tips could occasionally pick up plasmid DNA from the mica surface.[23] Guthold et al. tried to use AFM to isolate single aptamer molecules.[24] Osada et al. presented a method to extract mRNAs from living cells using AFM tips.[25] All these efforts have shown that, in principle, it is possible to isolate biological materials with AFM. However, the above approaches have not been able to realize the positioning isolation which is requested for practical applications such as ordered DNA sequencing[1−3] and direct molecular haplotyping of genomic DNA.[4,5] Novel techniques have to be developed to integrate the whole process including positioning, dissecting, and picking up with sufficient spatial resolution together with the subsequent single-molecule PCR to indeed achieve the isolation of a particular fragment from a genome DNA molecule.

In our previous work, we have shown how to precisely cut, push, and fold single DNA molecules by using AFM.[26] Here we demonstrate that single DNA fragments dissected from long DNA molecules at the designated positions can be further isolated by a precise AFM nanomanipulation, and the isolated DNA fragments can be amplified by single-molecule PCR.

As proof of concept, pBR322 DNA molecules were first deposited and stretched on a substrate. The single DNA fragment was cut at the designated positions on one pBR322 DNA and then isolated (or picked up) by a special operation mode called "kneading". All the operations including imaging, dissecting, and picking up were done with one AFM tip.

A Nanoscope IIIa AFM system (Veeco/DI, Santa Barbara, CA) was used in the experiment. Commercial silicon tips (MikroMasch Co., Russia) with force constant of 23−91 N/m and resonance frequency of 260−420 kHz were used.[27] All the operations were performed in air under relative humidity of 20−30% at the room temperature.

In a typical experiment, *Pst*I-linearized pBR322 DNA (4362 bp, 1483 nm in theoretical length) molecules were first deposited and stretched by a modified "molecular combing" technique[28,29] onto a mica substrate, which was pretreated with (3-aminopropyl)triethoxysilane (APS, purchased from United Chemical Corp.) for several minutes [30] and further treated in a clean oven under a temperature of 120 °C for 4 h. After an image was taken of the aligned DNA molecules in tapping-mode AFM, a specific DNA molecule was selected as ready for dissection, as shown in Figure 1A. A DNA fragment was then cut off according to the methods reported previously,[26] as shown in Figure 1B. The following isolation process, which we named as "kneading", is similar to that of "pushing" in ref 26. The differences are that kneading manipulation needs less loading force, typically about 50% less, and slower scan rate, usually 1 Hz, and is limited in a small scan region (300 nm × 300 nm or less). After the tip scanned over the area with the target DNA fragment in the continuous kneading mode, the AFM was restored to the imaging mode (tapping mode) and the isolation result was examined. Clearly, the dissected DNA fragment in Figure 1B has been picked up, leaving a gap at the middle region of the DNA molecule (Figure 1C).

One may argue that the DNA fragment could be swept away by the AFM tip, since the AFM was working in contact mode during the kneading process. However, in most cases we could not find the DNA fragments or their residuals on the substrates by imaging in a bigger region, implying that the DNA fragments were stuck to the tip. This was further confirmed by single-molecule PCR amplification.

The isolated single DNA fragments have to be amplified to provide sufficient copies for subsequent biochemical analysis. Scientists have shown that single-molecule PCR, in which single DNA molecules were prepared by serial dilution, is possible.[4−5,23,31−39] However, it is not clear if the DNA molecules can keep their biochemical activities under those mechanical manipulation with a force around tens of nano-Newtons since the PCR process on a single-molecule level is quite delicate and any damage on the bases will stop the replication in the first run. In our experiments, the AFM tip containing an isolated DNA fragment was transferred into a sterile tube, and a single-molecule PCR experiment was carried out (Supporting Information). The PCR products were detected by 1.5% agarose gel electrophoresis. Some typical results are given in Figure 2. In our primary demonstration, 7 among 20 tips with expected DNA fragments were detected positive after amplification,

[†] Chinese Academy of Sciences.
[‡] Shanghai JiaoTong University.

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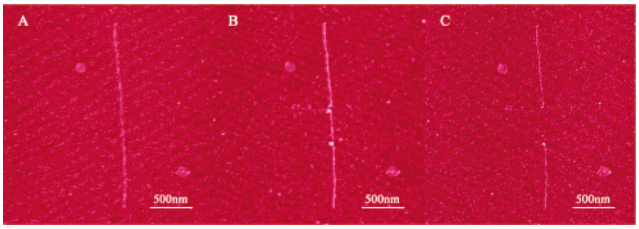

Figure 1. Positioning dissection and isolation of single DNA fragments. (A) AFM image of one pBR322 DNA molecule deposited and stretched on APS-mica. (B) The DNA molecule was cut at the selected positions. (C) AFM image showing that the small DNA fragment has been isolated.

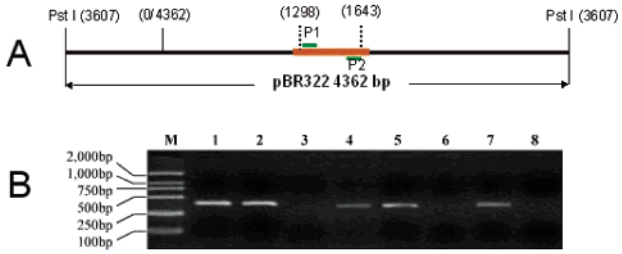

Figure 2. (A) Schematic drawing of the *Pst*I-linearized pBR322 DNA. The orange bar indicates the position of the DNA fragments to be isolated. The two green bars (P1 and P2) show the positions of up-river and down-river primers. In our experiments, the amplified DNA molecules is 346 bp in length, and is designed to locate at the center region of the linear pBR322 DNA molecule. The isolated DNA fragments were usually longer than that designed for PCR amplification to ensure that the target fragment was included. (B) Electrophoresis results of amplified products. Lane M, DL2000 DNA marker (TaKaRa Bio. Inc.); lanes 1 and 2, controls with 10 and 1 copies of pBR322 DNA prepared by serial dilution, respectively; lane 3−7, single DNA fragments isolated by the AFM tips; lane 8, negative control by an AFM tip that was used to image and cut (but not pick up) pBR322 DNA.

while no false positive was found in the negative controls. The fact that the isolated DNA fragment could be successfully amplified indicates that the kneading process causes no or very little damage to the DNA molecules, which will be vital for the subsequent biochemical analysis.

In summary, we have developed a unique and novel method for positioning, dissection, and isolation of individual DNA molecules. Single-molecule PCR amplification verifies that the DNA fragment is kept active during the isolation process. This method adds spatial information to the conventional biochemical analysis of DNA with nanometer resolution at the single-molecule level. Future work will be focused on improvement of the isolation efficiency to fit specific practical applications. Given the convenient nature of the present method, great applications will be expected in many fields of biology and medicine.

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Supporting Information Available: Detailed description of the single-molecule PCR. This material is available free of charge via the Internet at http://pubs.acs.org

References

(1) Namasivayam, V.; Larson, R. G.; Burke, D. T.; Burns, M. A. *Anal. Chem.* **2003**, *75*, 4188−4194.
(2) Lü, J. H.; Wu, S. Y.; Wang, G. H.; Lei, X. L.; Kong, X. Y.; Chen, R. S.; Zhu, L. H.; Li, M. Q.; Hu, J. *J. Chin. Electron Microsc. Soc.* **2003**, *22*, 185−188.
(3) Mizuno, A.; Katsura, S. *J. Biol. Phys.* **2002**, *28*, 587−603.
(4) Ruano, G.; Kidd, K. K.; Stephens, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6296−6300.
(5) Ding, C.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7449−7453.
(6) Eigen, M.; Rigler, R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5740−5747.
(7) Zhao, X.; Tapec-Dytioco, R.; Wang, K.; Tan, W. *Anal. Chem.* **2003**, *75*, 3476−3483.
(8) Hashiguchi, G.; Goda, T.; Hosogi, M.; Hirano, K.; Kaji, N.; Baba, Y.; Kakushima, K.; Fujita, H. *Anal. Chem.* **2003**, *75*, 4347−4350.
(9) Shortreed, M. R.; Li, H.; Huang, W.; Yeung, E. S. *Anal. Chem.* **2000**, *72*, 2879−2885.
(10) Yamamoto, T.; Kurosawa, O.; Kabata, H.; Shimamoto, N.; Washizu, M. *IEEE Trans. Ind. Appl.* **2000**, *36*, 1010−1016.
(11) Gimzewski, J. K.; Joachim, C. *Science* **1999**, *283*, 1683−1688.
(12) Oyabu, N.; Custance, O.; Yi, I.; Sugawara, Y.; Morital, S. *Phys. Rev. Lett.* **2003**, *90*, 176102.
(13) Dudko, O.; Filippov, A. E.; Klafter, J.; Urbakh, M. *Nano Lett.* **2003**, *3*, 795−798.
(14) Eigler, D. M.; Schweizer, E. K. *Nature* **1990**, *344*, 524−526.
(15) Jung, T. A.; Schlittler, R. R.; Gimzewski, J. K.; Tang, H.; Joachim, C. *Science* **1996**, *271*, 181−184.
(16) Piner, D.; Zhu, J.; Xu, F.; Hong, S.; Mirkin, C. A. *Science* **1999**, *283*, 661−663.
(17) Pascual, J. I.; Lorente, N.; Song, Z.; Conrad, H.; Rust, H. P. *Nature* **2003**, *423*, 525−528.
(18) Jacobs, D. C. *Nature* **2003** *423*, 488−489.
(19) Yamachika, R.; Grobis, M.; Wachowiak, A.; Crommie, M. F. *Science* **2004**, *304*, 281−284.
(20) Fotiadis, D.; Scheuring, S.; Müller, S. A.; Engel, A.; Müller, D. J. *Micron* **2002**, *33*, 385−397.
(21) Thalhammer, S.; Stark, R. W.; Müller, S.; Wienberg, J.; Heckl, W. M. *J. Struct. Biol.* **1997**, *119*, 232−237.
(22) Stark, R. W.; Thalhammer, S.; Wienberg, J.; Heckl, W. M. *Appl. Phys. A* **1998**, *66*, S579−S584.
(23) Xu, X. M.; Ikai, A. *Anal. Chim. Acta* **1998**, *361*, 1−7.
(24) Guthold, M.; Cubicciotti, R.; Superfine, R.; Taylor, R. *Biophys. J.* (Annual Meeting Abstracts) **2002**, *82*, 163.
(25) Osada, T.; Uehara, H.; Kim, H.; Ikai, A. *J. Nanobiotech.* **2003**, *1*, 2.
(26) Hu, J.; Zhang, Y.; Gao, H. B.; Li, M. Q.; Hartmann, U. *Nano Lett.* **2002**, *2*, 55−57.
(27) To reduce the risk of any contamination, the AFM tips were immersed in chloroform for 30 min and then H_2SO_4:H_2O_2 (7: 3) for 30 min, washed thoroughly with Milli-Q water, and dried in air. The tips were further sterilized using UV radiation and kept under sterile conditions before use.
(28) Bensimon, A.; Simon, A.; Chiffaudel, A.; Croquette, V.; Heslot, F.; Bensimon, D. *Science* **1994**, *265*, 2096−2098.
(29) Ouyang, Z.; Hu, J.; Chen, S. F.; Sun, J. L.; Li, M. Q. *J. Vac. Sci. Technol. B* **1997**, *15*, 1385−1387.
(30) Hu, J.; Wang, M. H.; Weier, U. G.; Kolbe, W.; Ogletree, D. F.; Salmeron, M. *Langmuir* **1996**, *12*, 1697−1700.
(31) Lagally, E. T.; Medintz, I.; Mathies, R. A. *Anal. Chem.* **2001**, *73*, 565−570.
(32) Li, H.; Xue, G.; Yeung, E. S. *Anal. Chem.* **2001**, *73*, 1537−1543.
(33) Nakano, M.; Komatsu, J.; Matsuura, S.; Takashima, K.; Katsura, S.; Mizuno, A. *J. Biotechnol.* **2003**, *102*, 117−124.
(34) Ohuchi, S.; Nakano, H.; Yamane, T. *Nucleic Acids Res.* **1998**, *26*, 4339−4346.
(35) Mitra, R. D.; Church, G. M. *Nucleic Acids Res.* **1999**, *27*, e34.
(36) Wabuyele, M. B.; Soper, S. A. *Single Mol.* **2001**, *1*, 13−21.
(37) Nakano, H.; Kobayashi, K.; Ohuchi, S.; Sekiguchi, S.; Yamane, T. *J. Biosci. Bioeng.* **2000**, *4*, 456−458.
(38) Rungpragayphan, S.; Kawarasaki, Y.; Imaeda, T.; Kohda, K.; Nakano, H.; Yamane, T. *J. Mol. Biol.* **2002**, *318*, 395−405.
(39) Grace, M.; Buzard, G. S.; Hughes, M. R.; Gore-Langton, R. E. *Anal. Biochem.* **1998**, *263*, 85−92.

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