Single DNA molecular manipulation with atomic force microscopy

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Abstract Nanomanipulation of DNA molecules or other biomolecules to form artificial patterns or structures at nanometer scale has potential applications in the construction of molecular devices in future industries. It may also lead to new insights into the interesting properties and behavior of this fantastic nature-selected molecule at the single-molecular level. Here we present a special method based on the combination of macroscopic "molecular combing" and microscopic "molecular cutting" to manipulate DNA molecules and form complex patterns at nanometer scale on solid surfaces. A possible strategy for ordered DNA sequencing based on this nanomanipulation technique has also been proposed.

Keywords Nanomanipulation, DNA, Atomic force microscopy (AFM), Ordered sequencing

CLC numbers Q523, Q336

1 Introduction

Nanotechnology, which aims at the ideal miniaturization of device and machines down to atomic and molecular sizes has been a recent hot topic as a promising high technology.[1] In order to approach this goal, many attempts have already been focused on constituting artificial features directly from atoms or molecules.[2-4] There are several strategies to make molecular devices and one of them is to utilize the products of life. Life is supported by highly sophisticated organisms composed of assemblies of proteins and nucleic acids, which work as molecular machines. Biomolecular machines show many interesting characteristics and they could be modified by modern biotechnology in varied functions. Manipulation of biomolecules provides a way to realize important breakthrough in making nanometer-size devices or functional structures based on principles completely different from what the present technologies are based on. Among the biomolecules, DNA seems particularly suitable because of its stability and many special properties. DNA is not only a unique carrier of information that occurs with a large structural variety, but also displays a comparably high mechanical rigidity and physico-chemical stability. Recent findings also inspire the imagination with respect to the employment of DNA to constitute optical, electrical, or other functional circuits at the nanometer scale, [5] e.g. the preparation of DNA strands according to a predesigned pattern in order to realize circuit construction and device design.[6]

The nanomanipulation of DNA molecules or other biomolecules to form artificial patterns or structures has potential applications in the construction of molecular devices in future industries. Besides, it may also lead to new insights into the interesting properties and behavior of this fantastic nature-selected molecule at the single-molecule level. On the biology side, many essential biological processes, such as protein folding, RNA folding, transcription and translation, exhibit complex kinetics and dynamics. They often

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involve a rich set of kinetic paths and transient states. A complete understanding of this process depends crucially on our ability to detect and characterize these dynamic features. Recent advances in the detection and manipulation of single molecules offer great promise for enhancing our understanding of behavior of individual biological macromolecules even in living cells. Scanning probe techniques allow the imaging of single molecules on surfaces, and special optical techniques enable their characterization in complex environments. Such measurements allow one to look beyond the ensemble average, measure time-trajectories of individual molecules and determine the exact distributions of molecule's properties.

Although scientists have made considerable progress in arranging atoms, small molecules, and even nanoscale liquid droplets,[7-14] manipulation of biomolecules has not been realized with sufficient precision because of many problems.[15] For example, in the case of DNA, it is difficult to manipulate its soft and elongated molecule to obtain a regular artificial pattern from its natural random-coil state by solely treating it with a scanned probe.

In this paper, a new approach of manipulating DNA molecules to form fairly artificial patterns at nanometer on solid surfaces was introduced. This method is based on the combination of macroscopic "molecular combing" and microscopic "molecular cutting".

2 Materials and methods

2.1 Sample preparation and deposition

Lambda DNA (Sigma, St. Louis, Missouri) was diluted with distilled-deionized (dd) water to a concentration of 1ng/μL. The samples were prepared by first depositing 5 μL of this DNA solution onto a clean coverslip. The coverslip was then carefully laid on the top of mica substrate, which had been coated with a self-assembled monolayer of 3-aminopropyl triethoxysilane (AP, United Chemical Technology Co., Bristol, PA).[16] Subsequently, the DNA molecules were extended on the AP-mica substrate with the modified molecular combing technique.[17,18] After waiting for a few minutes, the coverslip was removed and the AP-mica surface was rinsed with dd water and blown dried with clean compressed air or nitrogen.

2.2 AFM imaging and lambda DNA nanomanipulation

A nanoscope IIIa AFM with E and J scanners (Digital Instruments Inc., Santa Barbara, CA) was used in all experiments. Scanning tips used were 'force modulation probes' (Digital Instruments Inc.) made of silicon. Their force constants varied from 1 to 5 N/m and the resonant frequency from 60 to 100 kHz. The cantilevers were usually 225 μm in length.

All images were collected in tapping mode in air. The relative humidity during the experiments was controlled at 30%~40%. The scan speed was 2 Hz. Prior to the nanomanipulation the DNA target area was localized by scanning the AFM tip in the tapping mode. After scanning back to the desired position, the tip was lowered down onto the surface by a setting distance resulting from the preliminary line scan. The DNA strand would be manipulated after several such scans at a certain site when the setting distance, which corresponds to the load on the surface, was large enough. After manipulation, the setting distance was returned to the previous level for tapping mode AFM, and further images were taken to check whether the procedure was successful.

3 Results and discussion

The principle of this method is similar to that of digital painting in which a complex pattern could be obtained by the connections of small squaire pixels. At first, a matrix consisting of a 2D DNA network is generated by molecular combing (Fig.1). The advantage of this method is that DNA strands could be aligned straight in parallel in one direction in a very large scale over hundreds of micron. The orientation of the aligned DNA strands depends on the direction of fluid flowing during the process. The size of the unit cell of the network could be controlled at nanometer scale by adjusting the DNA concentration in the solution. We first align the DNA strands in one direction and then another with a selected angle to the first one. After this 2D molecular combing, DNA molecules were aligned on a 3-aminopropyl triethoxysilane -coated mica (AP-mica) surface with uniform features down to nanometer scale.[18]

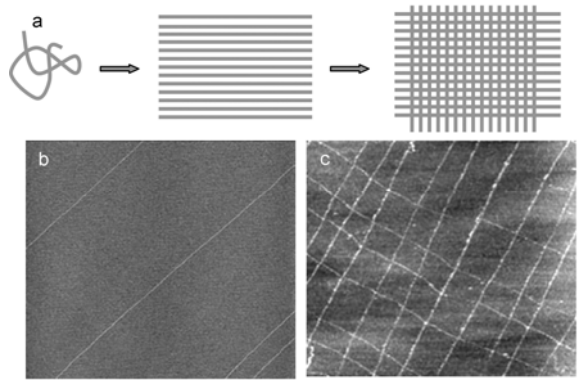

Fig.1 Stretching DNA molecules and constructing 2D network. (a) Schematic presentation of the whole process; (b) AFM image of one-dimensional DNA strands aligned on the AP-mica. DNA samples were linear lambda-DNA from Sigma with length of 48.5 kb in TE buffer (pH 8.0). The scanning size is 7μm×7μm; (c) AFM image of two-dimensional DNA network aligned on the AP-mica. The scanning size is 2.7μm× 2.7μm. AFM used in this experiment was from Digital Instrument (Santa Barbara, CA). The tips were normal tapping mode tips or force modulation tips. All the experiments were operated in air at room temperature.

After DNA molecules were arranged on the substrate in a fairly uniform way, single DNA molecules can be manipulated by AFM tip with nanometer precision, as shown in Fig.2. One of the nanomanipulation modes is cutting, that is, DNA strands can be cut by an AFM tip at a sufficient load. During each line scan, the tip first scanned across the surface in the standard 'tapping mode' and then after scanning back it was lowered down enough to execute cutting operation. If the tip scans at a load smaller than the cutting threshold, we can perform pulling and pushing modes. By a pulling operation, a straight DNA strand was manipulated by the AFM tip to form the wavy structure. Interestingly, the DNA strands do not shrink back but stay on the surface after the deformation process. This might have been caused by an overstretching of the strands leading to an irreversible change of the DNA helix structure. During performing pushing manipulation, DNA can be made into nano particles and nano threads. One interesting phenomenon we found in our experiment was that DNA could fold up by pushing it with AFM tip. Small DNA pieces could fold up into particles and long strands into thick threads. Threads were ordered since there were several peaks along the threads.

Based on the combination of macroscopic "molecular combing" and nanomanipulation, several artificial DNA patterns are locally constituted at nanometer scale. For example, three characters "D" "N" "A" can be formed with DNA strands themselves, as shown in Ref. [5].

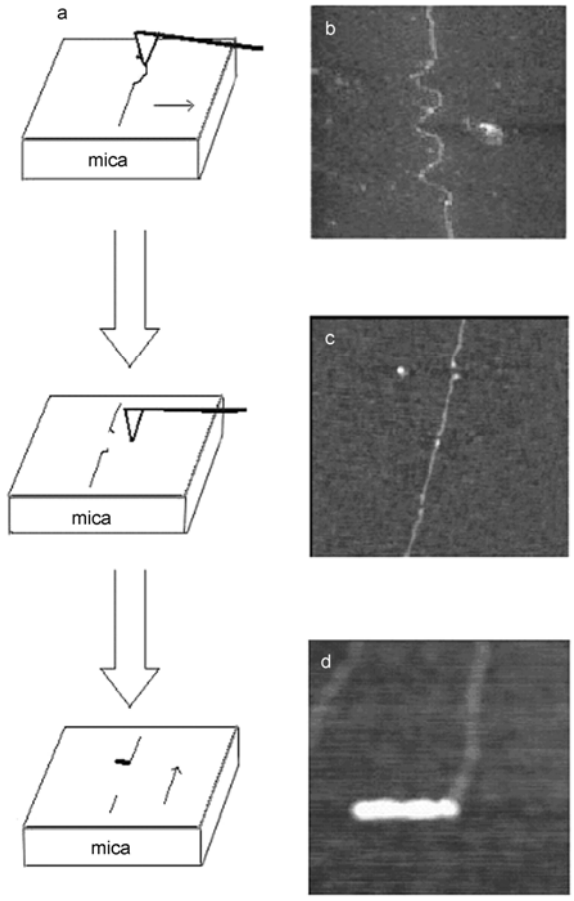

Fig.2 Some images of single DNA molecules manipulated by AFM tip. (a) Schematic presentation of the nanomanipulation modes; (b) AFM image of the wavy structure after performing pulling mode nanomanipulation. The image size is 500nm× 500nm; (c) AFM image of broken DNA strand after performing cutting mode nanomanipulation. The image size is 500nm× 500nm; (d) AFM image of DNA stimulated by pushing mode nanomanipulation. The image size is 300nm×300nm.

Nanomanipulation of single biomolecules has great potential application both in the constitution of molecular devices and the development of new strategy in genome research. As a demonstration of application in gene research, based on MutS protein recognition of heteroduplex DNA, an approach combining AFM imaging and DNA-stretching manipulation has been developed for directly detecting DNA mutation,[19,20] which can afford not only single mutation detection but also direct determination of mutation sites on long DNA strands.

Concerning the well-developed DNA manipulating technique, the method described in this paper might be used in genomic sequencing project. Fig.3 is

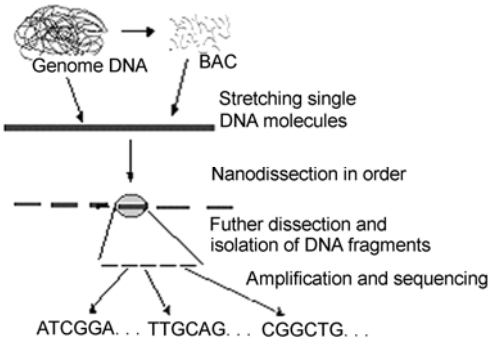

Fig.3 Flow chart of our elementary strategy for ordered sequencing based on nanomanipulation.

a flow chart of a new strategy proposed for ordered DNA sequencing based on nanomanipulation. There are some shortcomings in the current disordered sequencing technologies and methods. For example, the existence of gaps caused by non-cloned region and/or repeat sequences results in the incomplete sequencing. Lots of time and money are cost to solve these problems. The strategy we have proposed here, has many advantages: full coverage of the genome DNA, fast and cheap. More important, the method is based on nearly matured technology from different fields. The possible challenges lie in the efficiency of single-molecule PCR amplification and the accuracy and efficiency of nanomanipulation, but the problems could be solved by tip array and automatic computer controlled operation.

References

1 Reed M A. Proc IEEE, 1999, **87**: 652-658

2 William O G, Barbour L J, Atwood J L. Science, 1999, **285**: 1049-1052

3 Yurke B. Nature, 2000, **406**: 605-608

4 Quake S R, Scheer A. Science, 2000, **290**: 1536-1540

5 Hu J, Zhang Y, Gao H B *et al*. Nano Lett, 2002, **2**: 55-57

6 Di Mauro E, Hollenberg C P. Adv Mater, 1993, **5**: 384-386

7 Eigler D M, Schweizer E K. Nature, 1990, **344**: 524-526

8 Crommie M F, Lutz C P, Eigler D M. Science, 1993, **262**: 218-220

9 Boland J J. Science, 1993, **262**: 1703-1706

10 Mo Y W. Science, 1993, **261**: 886-888

11 Hu J, Xiao X D, Ogletree D F *et al*. Science, 1995, **268**: 267-269

12 Postma H W C, Sellmeijer A, Dekker C. Adv Mater, 2000, **12**: 1299-1302

13 Falvo M R, Taylor II R M, Helser A, *et al*. Nature, 1999, **397**: 236-238

14 Derycke V, Martel R, Appenzeller J *et al*. Nano Lett, 2001, **1**: 453-456

15 Bustamante C, Keller D. Phys Today, 1995, **48**: 32-38

16 Hu J, Wang M, Weier H U G *et al*. Langmuir, 1996, **12**(7): 1697-1700

17 Bensimon A, Simon A, Chiffaudel A *et al*. Science, 1994, **265**: 2096-2098

18 Ouyang Z, Hu J, Chen S F *et al*. J Vac Sci Technol B, 1997, **15**(4): 1385-1387

19 Tanigawa M, Gotoh M, Machida M *et al*. Nucleic Acids Res, 2000, **28**: e38

20 Zhang Y, Lu Y T, Hu J *et al*. Surf Interface Anal, 2002, **33**: 122-125