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Positioning Scission of Single DNA Molecules with Nonspecific Endonuclease Based on Nanomanipulation

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Controlling chemical and biochemical reactions on nanometer scale is one of the hotspots in the fields of chemistry and biology due to its possibility of exploring new phenomena and/or unraveling novel mechanisms that are inaccessible in traditional bulk systems.[1] For instance, positioning chemical reactions have been realized,[2] and reaction kinetics and pathways at the single-molecule level have been revealed.[3]

A variety of methods, such as nanopipette,[4] nanovesicle,[5] and nanomanipulation,[6] have been developed to perform chemical or biochemical reactions in limited volumes or areas. Among them, nanomanipulation based on scanning probe microscopy (SPM) has unique features that can image and manipulate materials on surfaces with high spatial resolution. Using SPM, spatially resolved chemical coupling reaction,[7] enzymatic nanolithography,[8] nanowelding,[9] controlled atomic doping of a single molecule,[10] and targeted delivery of single molecules[11] have been realized.

Herein, we report that a positioning scission reaction of a single DNA molecule can be realized using our recently developed technique termed combined-dynamic mode dip-pen nanolithography (CDDPN).[12] The key point that makes the CDDPN different from the conventional DPN[13] is that its whole nanolithographic procedures, including reading and writing, are performed through a direct exchange between AFM operation modes.[14] Therefore, soft materials with irregular shapes and even single biomolecules can be modified based on positioning with desired chemical reagents by CDDPN.

Traditionally, DNase I is known as a type of enzyme that digests DNA in a nonspecific manner when Ca^{2+} or Mn^{2+} are present.[15] In this paper, however, we demonstrate for the first time that a positioning reaction of digestion with DNase I can be carried out on a single genome DNA molecule using CDDPN.

AFM (Multi-mode Nanoscope IIIa, Vecco/Digital Instruments, Santa Barbara, CA) equipped with a J scanner was used in all CDDPN experiments. A commercially available silicon tip (Nanosensors Co., Germany) with a force constant of ~42 N/m and resonant frequency of ~330 kHz was used to both write and read the resulting structures. The AFM tip was coated by dipping it into a solution of 0.1−0.2 unit/μL DNase I (Sigma) in 5−10% glycerol, 20 mM Tris-HCI, pH 8.3, 2 mM $MgCl_2$, and 2 mM $CaCl_2$ for 1 min. Then the tip was air-dried. The detailed procedures of the CDDPN process are as follows. First, an image was obtained with the tapping mode, and an exact site in the image was selected. No protein would be deposited at this step. Second, lift mode was turned on. Instead of lifting up the AFM tip from the surface in normal electrical or magnetic measurements, we brought the tip close to the surface by setting a negative LIFT HEIGHT value. The tip stayed for a while once it touched the surface for ink deposition. Finally, the resulting feature was revealed by scanning the area again with tapping mode.

In a typical experiment (Figure 1), a freshly cleaved mica surface was treated with (3-aminopropyl)triethoxysilane (APTES, 0.2% (v/v) in water) for 5 min to attach positively charged amine groups for fixing DNA.[16] This was then heated in a clean oven at a temperature of 120 °C for 2 h. Using a modified "molecular combing" technique,[17] linear λ DNA (Sigma) was extended on the APTES−mica surface. After an individual DNA molecule was selected in the AFM image, DNase I ink was locally deposited at designated sites by the CDDPN process. Then, rather than being immersed in a buffer solution, the sample was put in a sealed box and incubated under a relative humidity of 80−90% at a temperature of ~37 °C for about 30 min. To remove the deposited ink, the sample surface was rinsed 10 times, each time with 5 μL of deionized water that was added on the surface and adsorbed away by filter papers. The sample was dried before AFM imaging.

Figure 2 shows a series of topographical images of the above process. Before CDDPN (Figure 2a), the width (full width at half-maximum) and height of DNA strands were 15.6 ± 2.0 and 0.45 ± 0.03 nm, respectively. After CDDPN (Figure 2b), there were nine spots of DNase I embellishing the single DNA molecule. The linear distance between the neighboring spots was controlled to be about 300 nm. The measured width of the spots was 40 ± 14 nm, and the height was 5.3 ± 2.2 nm on average. The sizes and locations of the spots could be changed according to experimental requirements. After digesting and rinsing (Figure 2c), no enzyme spots remained on the surface, and cleaved DNA fragments were present. A sketch indicating the spots' positions and gap sites is presented in Figure 2d. It clearly shows that most of the sites deposited with DNase I were digested, resulting in many DNA fragments. It is worth mentioning that depositing enzymes on DNA strands would not always result in observable gaps on the DNA strands. In a set of experiments, statistically ~50% of 48 enzyme spots on four single DNA molecules in three different samples generated detectable gaps.

To exclude any possibility that the observed gaps would be caused by the mechanical force applied by the AFM tip,[18] CDDPN was performed on the DNA molecules with DNase I ink without Mg^{2+}. In this case, no significant gaps in the DNA strands were observed, clearly indicating that the DNase I solution with Mg^{2+} ions was responsible for the digestion of the immobilized DNA molecule.

The process of the APTES modification of mica surface is very important. On the freshly cleaved mica, DNA molecules were hardly extended, and deposited enzymes liked to diffuse on the surface. In addition, to perform the reaction in air is critical to realize positioning digestion. If the CDDPN generated samples were directly immersed into the reaction buffer, we found that the

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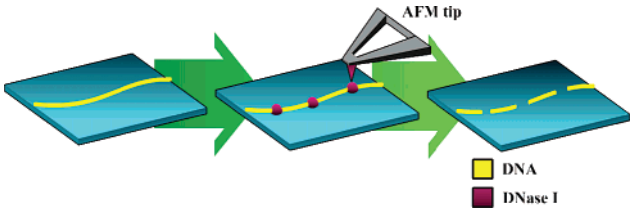

Figure 1. Schematic illustration of the positioning digestion with DNase I on a single DNA molecule by CDDPN. Three steps are involved, including choosing an individual molecule, depositing enzymes at the desired sites with the DNase I coated AFM tip, and finally, characterizing the results ex situ after digestive reaction.

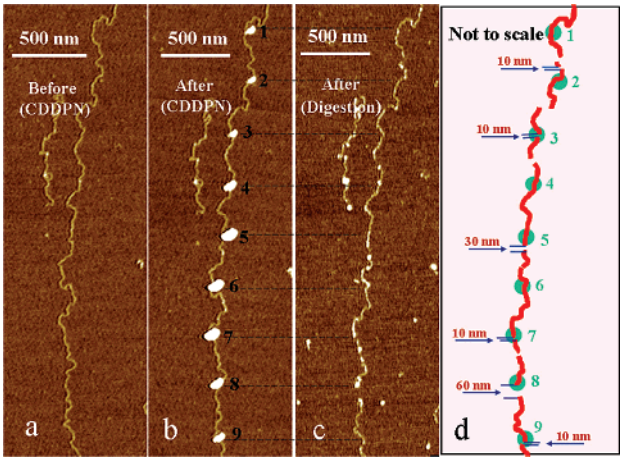

Figure 2. The AFM images of an individual λ DNA molecule at different stages during positioning digestion. The DNA molecule before (a) and after (b) DNase I deposition. DNA fragments after digestion at desired sites by DNase I (c). A sketch for demonstrating the controlled sites of the digestion (d). (The green circles represent the enzyme spots, and the red lines represent the resulting DNA fragments after digestion.)

digestion of DNA was hardly observed, or, if any occurred, it was not limited at the enzyme spots.

In fact, that the digestion of DNA by DNase I is effective under such a harsh condition on an air−liquid−solid interface is quite surprising and beyond our imagination. According to conventional knowledge, the activity of enzymes is very sensitive to incubation temperature, buffer ionic strength, pH, and Mg^{2+} and glycerin concentration. Thus, those factors have to be finely controlled to guarantee a reaction happened. However, in our experiments, the reaction was conducted in ambient air with a humidity of about 90% and a temperature of 37 °C, far from the optimal reaction conditions for DNase I digestion in bulk solutions. Under such conditions, the nanodrops deposited on the DNA samples might undergo a dehydration process, which would result in a variation of buffer ionic strength, pH, and Mg^{2+} and glycerin concentration to its standard ones. The observed digestion events may be attributed to a high effective concentration of DNase I when confined in the nanospots.

In conclusion, we conducted experiments resulting in a spatially controlled single biomolecule reaction. Precise positioning of DNase I and its digesting activity within controlled fragments, not limited to sequences, of a single DNA molecule on surface in air was demonstrated. This approach is important for single DNA molecular research and might be helpful for genetic applications. In addition, this method can also be extended to other biomolecular systems.

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