Stretching Short Single-stranded DNA Adsorbed on Gold Surface by Atomic Force Microscope*

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Abstract The manipulation and direct mechanical measurement of single DNA molecule could give much information about its elastic properties. Single stranded DNA (ssDNA) of 100 bases was adsorbed onto flat gold surface fabricated by depositing gold onto mica surface. Atomic force microscope (AFM) was used to observe the surface topography with different ssDNA concentration. Then the ssDNAs were stretched by AFM tip and in 50% cases ssDNAs could be stretched. Various kinds of force curves have been observed due to the different interaction between AFM tip and ssDNAs.

Key words gold surface, DNA, atomic force microscope, force spectrum

DNA molecules have unique mechanical properties due to its unique structure as one dimensional nanowire. Recent progresses of technology make it possible to study the mechanics of single DNA molecule using optical tweezers [1], micropipette [2], as well as force spectrum based on atomic force microscopy (AFM) [3~6]. The studies of single-molecule DNA mechanics not only help us to understand the nature of DNA more deeply, but also give more information on the mechanism involved in DNA copying, transcribing and packaging[7].

The studies of force spectrum of single DNA molecule fall in two categories. One was to study the elasticity of long DNA molecules (usually as long as 1 μm)[5]. The results suggested that the force spectra of double-stranded DNA (dsDNA) in physiological buffer could be described with worm-like Chain model with persistent length about 50 nm, while B-DNA transformed to S-DNA at forces above 65 pN[5,7,8]. For single-stranded DNA (ssDNA) or RNA, it is more flexible with persistent length about 18 nm [7]. Other studies focus on the unzipping and refolding of dsDNA (usually as short as 100 bp), in attempt to measure sequence-specific force variations [6,9]. An unbinding force of (20±3) pN for G-C base pairs and of (9±3) pN for A-T base pairs has been revealed[3,4,6].

However, the elasticity of short DNA (compared with its persistent length) is rarely reported, especially short ssDNA. For this kind of DNA, the worm-like model would not work and may give new information about DNA elasticity. In this paper, we tried to stretch short ssDNA of 100 bases, using force spectrum based on AFM.

1 Experiment methods

A scheme of experiment setup is shown in Figure 1. Short ssDNA oligomers were adsorbed onto gold surface by thiol-gold interaction. An individual DNA strand may be stick to the AFM tip by applying a contact force of several nano-newtons (nN) when tip extending to the surface. It is reported that using this attachment protocol the AFM tip could hold the DNA molecules under forces of up to 1 nN[5,10]. Then the tip

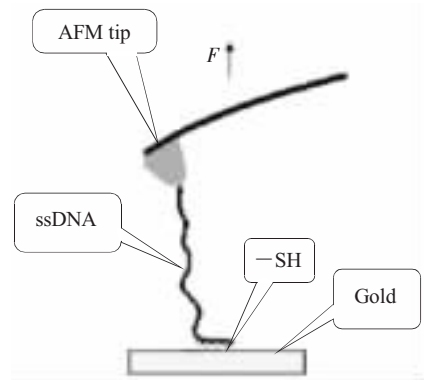

Fig. 1 Scheme of stretching ssDNA with force spectra technique based on atomic force microscope

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was retracted, the oligomer was stretched between gold surface and tip. Meanwhile, a force curve (cantilever deflection *vs*. piezo displacement) was recorded. The force curves were further transformed into force spectra (force *vs*. tip-surface separation) by multiplying the spring constant of the cantilever and subtracting cantilever deflection from piezo displacement. However, in this paper the force *vs*. piezo displacement curves were demonstrated for clarity.

Flat gold surface was prepared as described by Ling[9]. First 200 nm gold was deposited onto freshly cleaved mica by thermal evaporation. Then, the gold surface was adhered on a supporting steel disc by two-component epoxy glue. Finally, the mica was stripped to expose a flat gold surface. The exposed gold surface could be inspected with an ohmmeter to show whether the mica has been fully stripped.

The ssDNA oligomers of 100 bases with random sequence were purchased from BioAsia.Co., which contains 50% AMP and 50% TMP, with first five nucleotides were thiol-functioned. The oligomers was dissolved to different concentration with buffer solution (100 mmol/L KCl, 10 mmol/L Tris-HCl, pH 7.6). A 20 μl drop of the ssDNA sample was deposited on gold surface, allowed to stand for 30 min in air. Then the gold surface was washed with three 200 μl aliquots of double distilled water.

All force spectra experiments were performed in liquid with a pico-force MultiMode AFM (Veeco Instr., USA). A 10 μm scanner (type E) and a glass liquid cell without o-ring were used for the experiments. Untreated oxides sharpened silicon-nitrogen tips (NP-S) were used and its spring constant of each cantilevers were independently measured by a thermal tune method[11]. After mounting the sample, 30 μl buffer was injected. Then, cantilever deflection versus distance curves were recorded at tip velocities ranged from 80 nm/s to 8 μm/s. A relative trigger of 2 nN was used to control the maximal force of the tip against the surface. Force curves were recorded continuously at different spots, usually more than 100 force curves were captured for each samples. The data were processed using Nanoscope software V6.12.

2 Results and discussions

In order to stretch ssDNA by AFM tip, it is necessary that ssDNAs were adsorbed onto flat gold surface first. But the commonly thermal evaporated gold surface is relatively rough, as shown in Figure 2a. It composed of gold particles ranged from 10 to 50 nm. The measured roughness (root-mean-square value, RMS) was about 2.8 nm. However, a much flat gold surface could be obtained by first depositing gold onto mica surface and then stripping the mica off. Figure 2b shows the AFM topography of gold surface prepared using this method. The measured roughness (RMS) was 0.27 nm, ten times less than that of Figure 2a.

The ssDNA molecules were adsorbed onto gold surface through thiol-gold interaction. The samples were imaged with tapping-mode AFM in air to verify the adsorption of ssDNA. Figure 3 shows the surface topography when different concentration of ssDNA was used. The concentration of ssDNA for Figure 3a and 3b is 5 mg/L and 50 mg/L respectively. Although the surface roughness is almost the same, 0.29 nm for Figure 3a and 0.28nm for Figure 3b, the topographies are very different as compared with bare gold surface (Figure 2b). The results suggest that a much dense ssDNA layer formed with 50 mg/L ssDNA, while dispersed ssDNA adsorbed to gold surface with concentration of 5 mg/L. The density of adsorbed ssDNA is very important parameter for force spectrum experiment as demonstrated below.

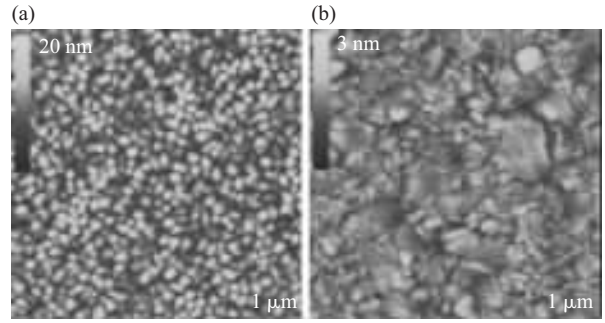

Fig. 2 AFM topography of gold surface

(a) Gold surface prepared by commonly thermal evaporation. (b) Gold surface prepared by first depositing gold on mica surface and then stripping the mica.

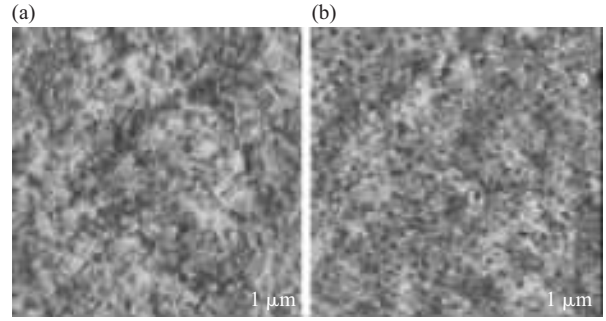

Fig. 3 AFM topography of gold surface with ssDNA adsorbed

(a) Monolayer adsorption of ssDNA with its concentration of 5 mg/L. (b) Multi-layer adsorption of ssDNA with its concentration of 50 mg/L. The *Z* range of the color scale is 3 nm.

The force spectra were firstly taken on freshly exposed gold surface for control. Figure 4a shows ten representative force curves. Each curve shows a single peak before the tip was fully disengaged from the surface. The peak corresponds to the pull-off process between the tip and the gold surface[12]. It is measured that the pull-off force ranged from several tens pN to more than 100 pN. Our experiments also suggest that the pull-off force is almost independent of retract velocity of the tip ranged from 80 nm/s to 8 μm/s.

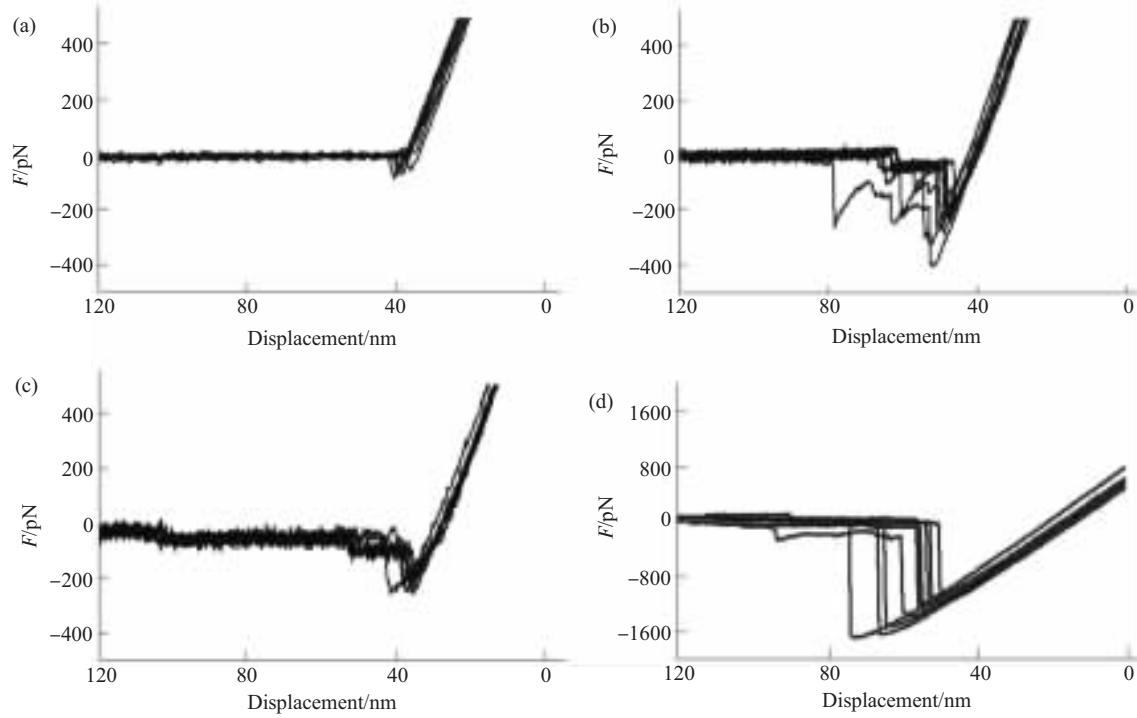

Fig. 4 Force spectra obtained on different surfaces

(a) Freshly exposed gold surface. (b,c) Surface with 50 mg/L ssDNA adsorbed. (d) Surface with 5 mg/L ssDNA adsorbed. The tip velocity is 800 nm/s for (a,b,d) but 8 μm/s for (c).

When the same tip was used to take force curve on ssDNA monolayer adsorbed on gold surface (the same sample of Figure 3a), much more complex force curves were obtained. Figure 4b shows ten representative force curves taken with tip velocity of 800 nm/s. Many different kinds of force curves were observed. We attribute the various of force curves to different interactions between AFM tip and ssDNA adsorbed on gold surface and some of them reflect the stretching of ssDNA by the tip. The curves were further analyzed in Figure 5.

However, when the tip velocity increased, the variety of force curves diminished. Figure 4c show ten representative force curves obtained with tip velocity about 8 μm/s. Most of the curves are similar to each other, with a pull-off force about 200~300 pN. It suggests that the ssDNA may also been stretched by the AFM tip, but the noise is relatively large which make the analysis the data much complex.

When force cures were taken on dense ssDNA layer on gold surface (the same sample of Figure 3b), a very large pull-off force usually existed, as shown in Figure 4d. That suggests a strong interaction between the AFM tip and the dense ssDNA layer, which results from the binding of many DNA molecules to AFM tip. The strong interaction prevents the observation of the stretching of single DNA molecules.

The results above indicated the AFM tip may stretch ssDNA adsorbed on gold surface as monolayer with tip velocity of about 800 nm/s. We further analyzed 216 force curves taken with the same parameter, and found that the curves can be divided into three categories. About 50% of the curves has only a pull-off peak, just like the curves in Figure 4a; 40% of the curves has a smooth step extended more than 20nm, as shown in Figure 5a; and there are 10% of curves have one or more peak in addition to the pull-off peak, as shown in Figure 5c.

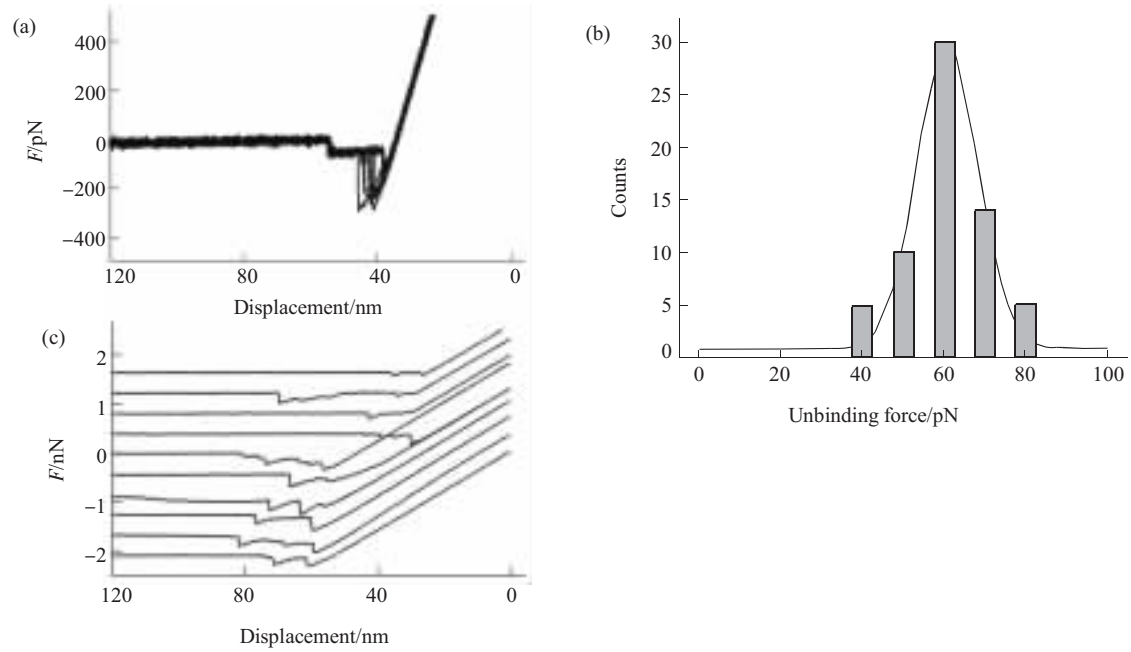

Fig. 5 Different force curves obtained on gold surface with 5 mg/L ssDNA adsorbed

(a) Ten representative force curves with a step about 70 pN. (b) The force of 64 step-break events and a histogram was drawn. (c) Ten representative force curves with Multi peaks.

Figure 5a shows ten representative force curves with a step. The ssDNA was stretched to about 24 nm (point A), and then break off from the AFM tip. We measured the force of 64 step-break events and a histogram was drawn in Figure 5b. Gauss distribution was used to fit the histogram and the acquired that the average force of step-break was 60 pN. It should be pointed out that the resolution of force measurement was no better than 10 pN due to the thermal noises of cantilever. We assume that the steps were results from a weak binding of DNA molecules to AFM tip.

Figure 5c shows ten typical force curves with one or more peak in addition to the pull-off force. The curves were shown in cascade manner for clarity. Each peak represents a process that ssDNA was stretching by the AFM tip beyond 25 nm and then break off from the tip with a force about 100~300 pN. In our results, the worm-like model do not give a good fit to each peak which indicate that the elasticity of short ssDNA was not obey the rule any longer.

The experiments demonstrated that AFM force spectrum techniques could be used to study the elasticity of short ssDNA. With same strategy, the method can also be used to study short dsDNA. However, compared with stretch of long DNA[5], the multiform force curves observed make them difficult to compare with each other. More studies are needed to improve the control of tip-sample interaction and to reduce noise.

3 Conclusion

Flat gold surface with roughness about 0.3 nm was prepared to allow the adsorption of short ssDNA of 100 bases. Then force spectrum technology was used to stretch the ssDNA. The ssDNA stretching by AFM tip were observed on ssDNA monolayer with tip velocity about 800 mm/L. Results suggested that the tip could hold ssDNA with force about 60 pN in 40% cases, and stretch it beyond 25 nm in 10% cases.

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原子力显微镜拉伸吸附在金膜表面的短单链 DNA 分子 *

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摘要 对单根 DNA 分子的操纵和拉伸可以直接研究 DNA 的弹性等力学性质. 首先通过将金沉积到云母表面制备了表面粗糙度小于 0.3 nm 的金膜，然后一段硫代的单链 DNA (100 bases) 吸附到金膜表面. 利用原子力显微镜观察不同浓度的 DNA 吸附在金膜上的表面形貌. 进一步用原子力显微镜的力曲线模式拉伸 DNA 分子，在 50%的情况下 DNA 可以被针尖拉伸，观察到了由于针尖和 DNA 分子间作用力的不同导致的多种不同力曲线.

关键词 金膜，DNA，原子力显微镜，单分子力谱

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