Radial compression elasticity of single DNA molecules studied by vibrating scanning polarization force microscopy

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The radial compression properties of single DNA molecules have been studied using vibrating scanning polarization force microscopy. By imaging DNA molecules at different vibration amplitude set-point values, we obtain the correlations between radially applied force and DNA compression, from which the radial compressive elasticity can be deduced. The estimated elastic modulus is ~20–70 MPa under small external forces (<0.4 nN) and increases to ~100–200 MPa for large loads.

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The elastic properties of single DNA molecules have attracted tremendous attention in the past 15 years because of their importance to numerous biological processes [1–4]. By overstretching and overtwisting B-DNA, structural transitions from B-DNA to S-DNA and to P-DNA have been observed experimentally [5–10]. Many theoretical models have been proposed to explain the mechanical responses of DNA under an external force [11–13]. It has been recently recognized that DNA responds to protein binding through a sequence-dependent kinking and intercalation [14,15]. While at this stage the stretching and twisting elasticity only provide base-pairs-averaged information on a single DNA strand, the knowledge of radial compression properties is necessary in order to provide local information along the DNA strands and to give new opportunities for understanding more deeply the biological functions such as gene regulation and DNA repair [16,17]. On the other hand, as a model molecule DNA's radial compression elasticity is of great interest and importance to polymer physics [18].

Radial compression is carried out by pressing on a DNA molecule lying on a mica surface where the compression is perpendicular to the DNA strand. The difficulty in exploring the radial compressive elasticity of DNA is partially due to the challenge in applying a small and controlled force (<0.5 nN with an accuracy of ~0.1 nN) on such a small object (~2.0 nm) while precisely measuring the resultant radial deformation (<1.0 nm with an accuracy of ~0.1 nm). Atomic force microscopy (AFM) is perhaps a unique technique that allows the measurement of both the force and resulting deformation at the nanometer scale [19]. The force-distance curve (including force volume) in contact mode AFM is widely used to reveal the local mechanical properties [20–23]. Due to its intrinsic limitations [23,24], however, the applied load and the resultant deformation of the sample cannot be extracted simultaneously with high accuracy. Using tapping mode AFM (TM-AFM), the radial deformability of some soft materials like carbon nanotubes has been successfully studied thanks to the reduction of lateral forces [25]. Unfortunately, the typical tip-sample force in a conventional tapping mode is still too large for a DNA molecule and results in a severe deformation. The measured height of a DNA molecule in tapping mode is ~0.7–0.9 nm, far smaller than the normal value (~2.0 nm) [26,27]. Recently, we have developed a method called vibrating scanning polarization force microscopy (VSPFM) [26,28]. The biggest advantage of VSPFM is its stable performance both in the noncontact and the tapping modes, which renders VSPFM potentially useful for imaging soft specimens with much smaller force. Using this method, we have reliably measured the heights of bio-macromolecules deposited on mica surfaces [26].

In this Brief Report we report our study on the radial compressive elasticity of single DNA molecules with VSPFM. Commercially available λDNA solution with an original concentration of 450 ng/μl was purchased from Sino-American Biotechnology Company (Shanghai, China). The solution was first diluted to 10 ng/μl with a TE buffer containing 100 m*M* NaCl, 10 m*M* Tris (*p*H 8), and 10 m*M* EDTA. A drop of this diluted solution was then deposited on a mica surface pretreated by Ni^{2+}. The sample was extensively washed with deionized water (Millipore water 18.2$M\Omega$ and dried in air [26].

We have modified a NanoScope IIIa SPM system (Veeco Instruments, Inc., Santa Barbara, CA) into VSPFM operation as described in Ref. [26]. A NSC12/Ti—Pt rectangular cantilever (MikroMasch, Russia) was used with a typical force constant of ~4.5 N/m and a typical resonant frequency of ~170 kHz. The images were taken at the temperature of 25–30 °C under the relative humidity of 20–30 %. Although the water layer may have a large effect on the AFM measurement, in many cases, particularly in contact mode [29], we could avoid the water layer influence by using the tapping mode with a fast vibrating AFM tip under the above humid-

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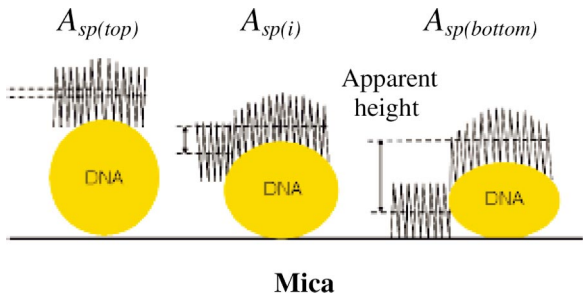

FIG. 1. (Color) A schematic description of the tip/DNA interaction at different A_{sp} values ($A_{sp(top)}$, $A_{sp(i)}$, and $A_{sp(bottom)}$).

ity environment [23,28,30]. The height of DNA was checked with no change within this humidity range as well.

The core concept in VSPFM is the utilization of polarization force during imaging in a dynamic vibrating mode [26]. Briefly, a conductive tip is biased to a certain ac voltage so that an electric polarization field can be induced in the sample. Since the range of electrostatic force is much longer than that of the van der Waals force, by carefully adjusting the tip vibration amplitude set point (A_{sp}), VSPFM can perform in the far noncontact, the near noncontact and the tapping regimes [26,28], achieving the control of tip-sample interaction in a wide range. That is very crucial for the measurement of compressive elasticity. By decreasing A_{sp} stepwise, the tip can be first adjusted to slightly touch the top of a DNA molecule (defined as the top image) and then gradually lowered until the tip touches the substrate surface (defined as the bottom image), as illustrated in Fig. 1. The corresponding A_{sp} were labeled as $A_{sp(top)}$ and $A_{sp(bottom)}$, respectively. In this fashion, a series of images corresponding to different loads can be taken at different A_{sp} values. The height D_{ij} of a given point (i) on the DNA molecules after compressing jth step could be calculated by

$$D_{ij} = D_{ij(app)} + \alpha(A_{sp(ij)} - A_{sp(bottom)}), \qquad (1)$$

where $D_{ij(app)}$ denotes the apparent height of a DNA molecule, $A_{sp(ij)}$ is the amplitude of tip vibration, and α is a conversion coefficient [31].

Figure 2 shows a series of images that illustrate the radial deformation of a single DNA molecule at different A_{sp} values. A1–A3 were obtained with the VSPFM operated at sequentially decreasing A_{sp} values (0.934 V, 0.922 V, 0.910 V). A4 was obtained by returning A_{sp} to 0.934 V at the end of the sequence. B1–B4 were the corresponding phase images, indicating the interaction is repulsive between tip and DNA molecules [32]. These images show clearly the large deformation of DNA molecules under small forces and the deformation is remarkably reversible. For comparison, we have also measured the deformability of a colloidal gold particle. Its height changes very little with the change of A_{sp} [Fig. 3(a)].

Unlike stretching or twisting, which measure the base-pairs-averaged properties of a single DNA strand [5–10,33], we found that the compression properties of single DNA molecules strongly depend on the locations along the strand, indicating that the radial elasticity entails much larger deviations of the local structure. Figure 3(a) shows three typical

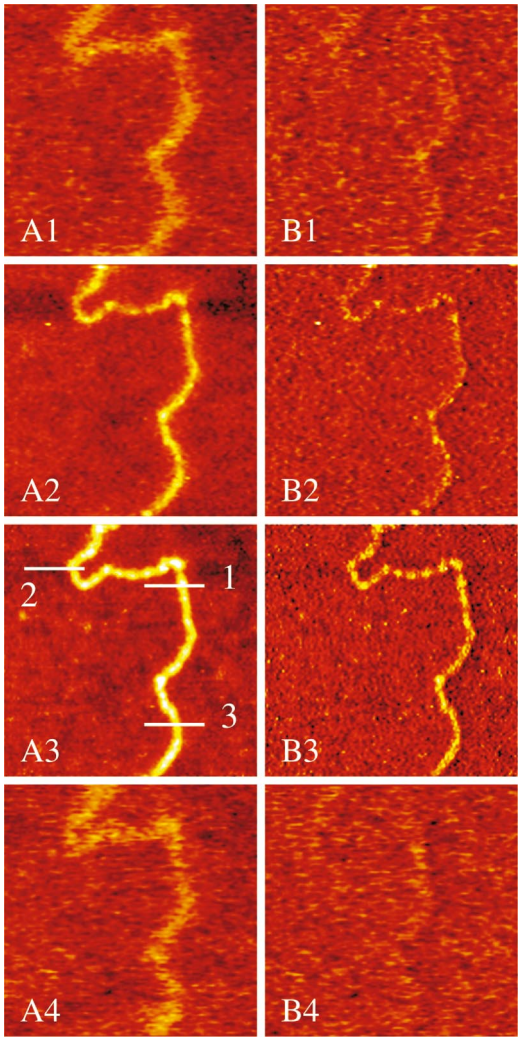

FIG. 2. (Color) Images showing the processes of compressing a single DNA molecule deposited on a mica surface at different A_{sp}. A1–A4 and B1–B4 are, respectively, topographic and phase images taken at A_{sp}=0.934, 0.922, 0.910, and 0.934 V. Image size: 200 nm×200 nm. The horizontal bars at points 1, 2, and 3 specify positions along the DNA molecule at which height-force curves discussed in later figures were taken.

height-force curves corresponding to three different sites on one DNA molecule as indicated in Fig. 2 (A3), where the force calculation is detailed in [34]. The measured large initial height (~1.6 nm) of DNA molecules demonstrates the advantage of VSPFM in contrast to TM-AFM and makes the small compressive deformation measurement possible. Each height-force curve for the DNA molecule in Fig. 3(a) comprises a process starting from the tip touching the top of the DNA molecule, then to compressing the DNA molecule step by step with decreasing A_{sp} and finally finishing with the tip touching the mica surface. The height-force curve for the reverse process is also included in Fig. 3(a). The height-force curve for location 1, frequently observed in our measurements, shows a typical radial deformation of a well-shaped DNA molecule. The remarkable reversibility over ~50% radial deformation in the height-force curves between the approaching and retracting is in strong contrast to most solid

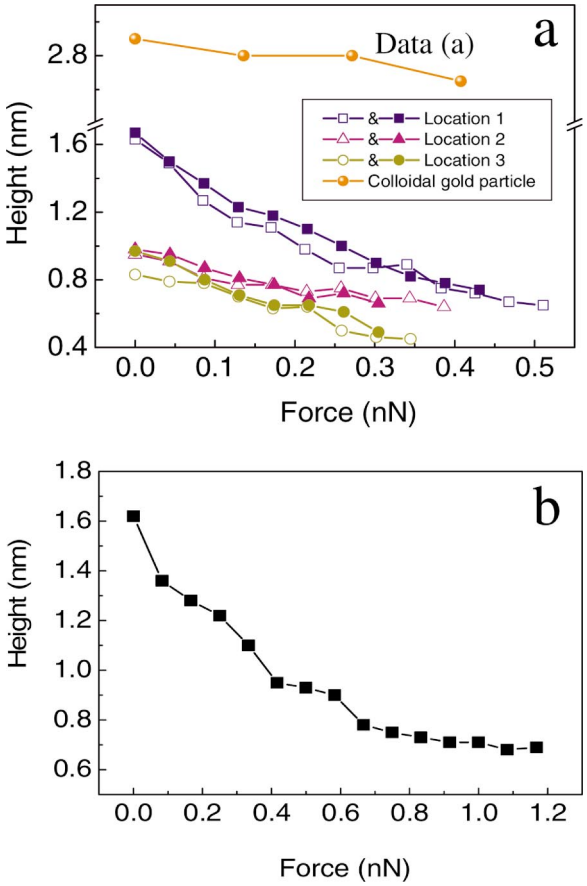

FIG. 3. (Color) (a) Height-force curves taken at three different locations along a DNA molecule as indicated in Fig. 2 (A3). The open and the corresponding solid square, triangle, and circle denote approaching and retracting processes, respectively. Data (a) on the top is for a colloidal gold particle. (b) The height-force curve usually reaches a relatively flat region under a larger load.

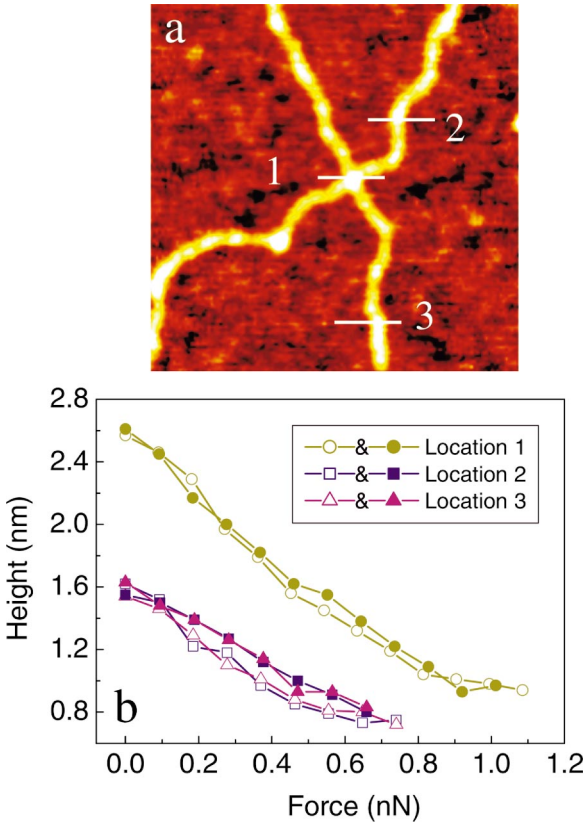

FIG. 4. (Color) (a) Topography of two crossed DNA molecules with $A_{sp}=0.810$ V. Image size: 200 nm$\times$200 nm. (b) The height-force curves taken at three different locations as indicated in (a). The open and the corresponding solid square, triangle, and circle denote approaching and retracting processes, respectively.

materials, for which the elastic compression regime only extends to a few percent of deformation. The relatively high stiffness (correctly speaking, we should compare the slope in force-strain curves [35]) for location 2 may have to do with the sharp bending nearby, similar to carbon nanotubes for which an abrupt bending would result in a much tighter structure and lead to a sudden change in its local stiffness [25]. Similar to other reports in the literature [36], the local morphology was found to vary greatly along a DNA strand in VSPFM images. For example, the topography in Fig. 2 (A3) shows that the DNA strand at section 3 is somewhat narrower in width and lower in height than that at section 1, suggesting that the molecule has a different local structure. Interestingly, this large morphological difference between sections 1 and 3 only results in a small variation in the slope of the height-force curves. From our above results, we find that the compressive elasticity of individual DNA molecules varies from site to site, consistent with the phenomenon reported in a recent paper by Keller *et al.* that single-molecule measurements often yield scattered values [33].

The height-force curve 1 in Fig. 3(a) shows an interesting nonlinearity of compression with applied loads. In the small force region ($F<0.4$ nN), the curve is approximately linear with a slope of $\sim$0.4 nN/nm, indicating the softness of DNA molecules under light compression in the initial deforming stage. Other biomolecules are also found "soft" at room temperature by neutron-scattering measurement in air, where a force constant $\sim$0.1–0.3 nN/nm was deduced [37,38], quite similar to our value. It is likely that the forces maintaining biological structures in this deformation regime are mainly through hydrogen bonding, electrostatic, and van der Waals interactions. The energies associated with these "weak interactions" are comparable to the thermal energy at room temperature and are expected to lead to a small force constant (DNA is soft) [39]. As the average force is increased to $F>0.4$ nN, the tip usually encounters a much stronger repulsive force and the height-force curve reaches a relatively flat region as more clearly showed in Fig. 3(b) in a large-force regime. We speculate that the space between backbones is now greatly reduced and the external force is mainly exerted on the much stiffer sugar-phosphate backbones.

To investigate the influence of the substrate, we have studied the compression properties of two crossed DNA molecules, and the topography is shown in Fig. 4(a). Figure 4(b) plots the height-force curves corresponding to three different locations as indicated in Fig. 4(a). The measured height of section 1 (crossing point) is $\sim$1.0 nm in TM-AFM, but reaches $\sim$2.6 nm in VSPFM. We have not found a pronounced difference in the slope of force-strain curves, espe-

cially in the small-force regime (<0.4 nN) between section 1 and sections 2 and 3 [35], suggesting that the radial compressive elasticity has not been significantly influenced by the substrate.

It is worthwhile estimating the range of the elastic modulus for the purpose of making a comparison with other materials and of providing a direct description on the softness of a DNA molecule. Taking the nonlinearity of the height-force curve into consideration, the effective radial elastic modulus is given by [40]

$$E = (dF/dh)(h/S) \tag{2}$$

where F is the normal force applied on DNA, S and h are, respectively, the contact area and the molecular height at the given load.

To calculate the contact area S between a spherical AFM tip and a DNA molecule, we follow the usual method when determining the contact area between an AFM tip and a cylindrical nanotube [40,41]. From curve 1 in Fig. 3(a) the effective elastic modulus of DNA is estimated to be $\sim 20-70$ MPa in the small-force region ($F<0.4$ nN), which is comparable to the Young's modulus of human cartilage (~ 24 MPa). In the large-force region the effective elastic modulus can increase to $\sim 100-200$ MPa, close to some polymers like leather ($\sim 120-400$ MPa). The calculation on 30 other DNA fragments agrees with these values within a factor of 2–3.

In summary, with VSPFM we have studied the local radial compression properties of single DNA molecules, particularly in the small-force regime. Besides biophysical importance, our data may also be interesting to DNA "molecular surgery" and DNA nanobiotechnology, where DNA is used as a building block [42,43]. In order to completely understand its deformation mechanism, a systematic experimental investigation in conjunction with theoretical simulation is necessary on how the compression properties of DNA are affected by humidity, temperature, and base-pair sequence. It is obvious that our method opens a door to characterize the local elastic properties of many other single biomolecules such as RNA, proteins, as well as soft polymers.

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