Visualization during Stick-Release and Plateau Responses of Collapsed DNA

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We have observed the elastic response of single DNA molecules and visualized it during the collapsing transition induced by trivalent cation, spermidine (SPD). A fluorescent dye, YOYO-1, was used for the visualization. While the contour length of DNA increases as the YOYO concentration increases, the contour length decreases as SPD concentration increases. This indicates that YOYO-SPD exchange occurs on the single DNA molecule. We have observed bright spots in the fluorescence intensity profile of collapsed DNA during stretching, which may correspond to the collapsed parts within the single DNA. The decrease of the intensity of the spots in stretching implies the mechanical unfolding of collapsed parts.

§1. Introduction

DNA molecules in aqueous solution containing mono-valent cations repel each other, however, multivalent cations induce the DNA molecules to collapse or condense. This phenomena is well known as DNA condensation and has been widely studied for over 25 years.[1] Recent single molecule techniques provide new interests in DNA condensation at single molecule level.[2)–4)] While an elastic response of a single DNA in an elongated coil state shows the entropic elasticity of an ideal worm-like chain (WLC),[5)] the response in a collapsed state is quite different from the WLC behavior. The elastic response depends on multivalent cation concentration.[4)] At low concentration of trivalent cation, spermidine (SPD), force-extension (f-x) curves show force plateau in a wide range of the extension. With increasing the SPD concentration, a sawtooth pattern called as a stick-release pattern appears; the force first gradually increases with increasing extension, then abruptly decreased periodically during stretching. The periodicity of stick-release patterns may reflect the unfolding of a toroidal condensate in well defined quanta,[4),6),7)] however, there is no direct evidence to explain so far. In the present study, we have visualized single DNA molecules using a fluorescent dye, YOYO, and measured elastic responses of single DNA molecules at various YOYO and SPD concentrations to elucidate the mechanism of collapsing and uncollapsing process at single molecule level.

§2. Materials and methods

Both ends of λ-Phage DNA of 48.5 kilobasepairs were attached to streptavidin-coated beads of 2.0 μm diameters, via biotinylated oligonucleotides that hybridized to the single-stranded ends of λDNA.[8)] The resulting DNA-beads complexes were diluted with buffer solution (10 mM Tris-HCl, 1 mM EDTA, 2 mM NaCl, pH 7.0), and the final nucleotide concentration of DNA was 10–100 nM. All solutions used

Millipore water (18.2 MΩcm). We introduced the DNA-beads solution into a flow chamber; a silicon rubber (thickness 0.5 mm) was sandwiched between two glass coverslips (thickness 0.12–0.17 mm). We used a dual-trap optical tweezers[4] to stretch individual DNA molecules tethered beads. First, we searched bead-DNA-bead complexes in the flow chamber and each bead was trapped at each focus and buffer solution was introduced to remove other beads and DNA molecules. Then a solution containing an appropriate amount of YOYO or SPD in buffer was introduced. We captured the two beads images by a CCD camera and analyzed on a computer. The distance between the two beads centers determines the extension of DNA and the displacement of one bead from the laser beam spot determines the force acting on DNA. When we visualized DNA molecules a fluorescent dye YOYO was used and 0.1 mg ml^{-1} glucose oxidase, 0.04 mg ml^{-1} catalase, 0.4% glucose and 1% β-mercaptoethanol were added to retard photo-bleaching. We observed fluorescence images of single DNA molecules using a cooled CCD camera (Hamamatsu, HiSCA).

§3. Results and discussion

First, f-x curves at various concentrations of YOYO were measured. The dye is often used as a fluorescent probe in DNA analysis because of its high affinity and low background. It is a bis-intercalator and has four positive charges in aqueous solution. The binding of YOYO to DNA influences not only elastic properties but also the collapsing transition by multivalent cations. The intercalation of YOYO increases the contour length and the persistence length of DNA.[8] Moreover, the high affinity of YOYO prevents DNA from the collapsing;[9] the critical concentration of multivalent cations in collapsing transition increases. Figure 1(I) shows that the contour length of DNA increases from 16.6 to 25.7 μm as the YOYO concentration increases from 0 to 0.5 μM. The contour length is obtained by fitting the measurements using WLC model.[5] Next, the concentration of YOYO and SPD was varied by exchanging solutions, and f-x curves for an identical DNA was measured at each concentration. As shown in Fig. 1(II), the contour length increases (a $\rightarrow$ b) when the solution is exchanged to 0.1 μM YOYO. However, the contour length decreases as the SPD concentration increases (b $\rightarrow$ c $\rightarrow$ d), though the same amount of YOYO is contained. In the absence of YOYO, the contour length does not change when the SPD concentration is varied (data not shown). The decrease of the contour length in Fig. 1(II) is caused by the detaching of YOYO from DNA, which indicates that exchanges of counterions on a single DNA molecule is occurred. Both YOYO and SPD are positively charged and behave as counterions for DNA's negative charges. YOYO molecules binding to DNA are detached and SPD molecules bind instead when the SPD concentration increases. Similar effects have been observed in bulk systems;[10),11] fluorescence intensities decrease due to detaching of dyes from DNA molecules as multivalent concentrations increase. At 10 mM SPD, the WLC behavior disappears and a typical stick-release pattern of a collapsed DNA appears. In the present conditions, the critical concentration of the collapsing transition is about 1–10 mM. This confirm that YOYO prevents DNA from the collapsing, because the disappearance of WLC behavior can be observed at 200–500 μM SPD without YOYO.

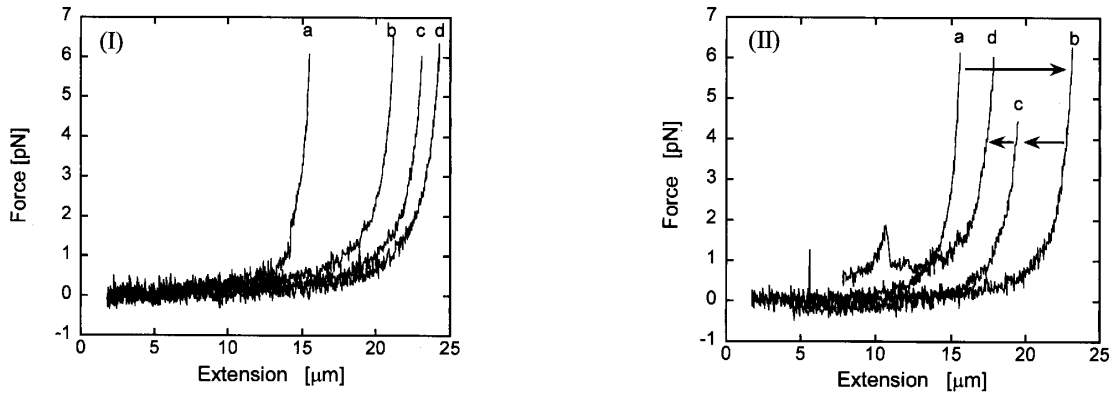

Fig. 1. (I) F-x curves at various YOYO concentrations. a: 0 μM, b: 0.01 μM, c: 0.1 μM, d: 0.5 μM YOYO. (II) F-x curves at various SPD concentrations. a: no SPD and no YOYO, b: 0 mM, c: 5 mM, d: 10 mM SPD. 0.1 μM YOYO are contained in b, c and d.

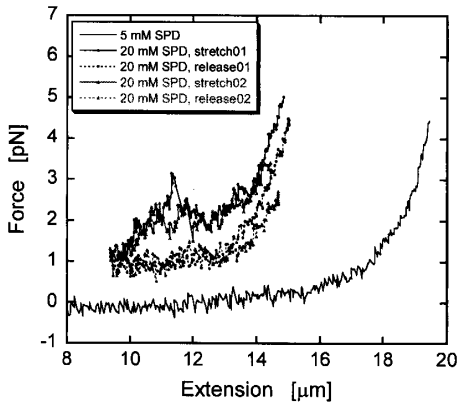

Fig. 2. F-x curves at 5 and 20 mM SPD. 0.1 μM YOYO are contained in both cases. Stretching and release are repeated for an identical DNA molecule.

Figure 2 shows f-x curves at 20 mM SPD and 0.1 μM YOYO. The stick-release response in stretching and force plateau of about 1 pN in release can be observed repeatedly. It clearly indicates that the DNA molecule is in collapsed state at this condition. The f-x curves show WLC behavior and there is no hysteresis in stretching or release when DNA is in an elongated coil state. In a collapsed state, however, hysteresis in stretching or release has been observed.[4] Such hysteresis in f-x curves has been observed also in numerical simulations.[7),12),13)]

Third, fluorescence images of single collapsed DNA molecules in stretching were obtained to verify the mechanical unfolding processes. Figures 3 and 4 show the fluorescence images and time series of the fluorescence intensity profiles at 0 and 20 mM SPD, respectively. The images were captured every 1 sec with 1 sec of exposure time and only six picked up images are shown. We can see the evidence of collapsing at 20 mM SPD from fluorescence images. The motions of DNA during stretching are clearly different between no SPD and 20 mM SPD cases, though it is difficult to distinguish the difference by static images. While DNA highly fluctuates in perpendicular direction to the stretching at no SPD, there are almost no fluctuations in that direction at 20 mM SPD. At no SPD, there are no bright spots at fixed positions in fluorescence intensity profile. At 20 mM SPD, however, we can see bright spots at around 14 and 18 μm in Fig. 4, which may correspond to the collapsing parts within single DNA molecule. The decrease of the fluorescence intensity of spots implies the mechanical unfolding of the collapsed parts, which are related to the stick-release response in f-x curves, though we need simultaneous measurements of fluorescence images and elastic responses to conclude.

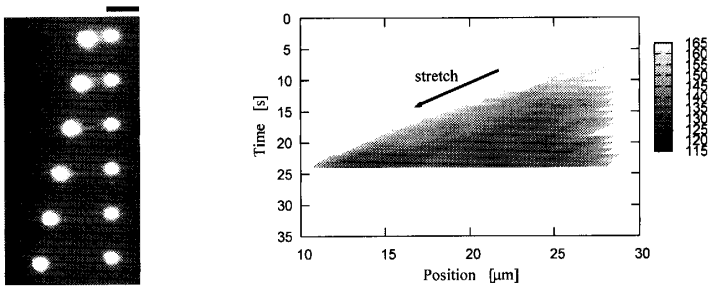

Fig. 3. No SPD and 0.1 μM YOYO. (Left): Fluorescence images of single DNA molecule in stretching. The scale bar is 10 μm. Large bright circles at both ends are beads. (Right): Time series of the fluorescence intensity profile along the DNA. Gray scale (arbitrary unit) indicates the fluorescence intensity.

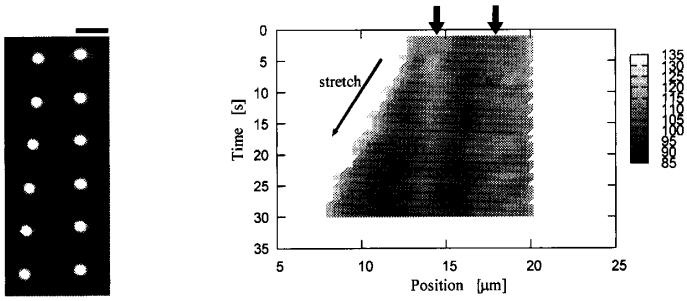

Fig. 4. 20 mM SPD and 0.1 μM YOYO. (Left): Fluorescence images. The scale bar is 10 μm. Large bright circles at both ends are beads. (Right): Time series of the fluorescence intensity profile. Thick arrows on the top indicate the positions of bright spots.

To summarize, we have observed the elastic responses of single DNA molecules at various concentration of YOYO and SPD. The counterion exchange on a single DNA molecule was observed as the change of DNA contour length. The decrease of the fluorescence intensity of the spots within a single DNA may reflect the mechanical unfolding of collapsed parts.

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References

1) V. A. Bloomfield, Biopolymers **44** (1997), 269.
2) K. Yoshikawa, M. Takahashi, V. V. Vasilevskaya and A. R. Khokhlov, Phys. Rev. Lett. **76** (1996), 3029.
3) C. G. Baumann, V. A. Bloomfield, S. B. Smith, C. Bustamante, M. D. Wang and S. M. Block, Biophys. J. **78** (2000), 1965.
4) Y. Murayama, Y. Sakamaki and M. Sano, Phys. Rev. Lett. **90** (2003), 018102.
5) J. F. Marko and E. D. Siggia, Macromolecules **28** (1995), 8759.
6) I. M. Kulic and H. Schiessel, Phys. Rev. Lett. **92** (2004), 228101.
7) H. Wada, Y. Murayama and M. Sano, Phys. Rev. E **72** (2005), 041803.
8) S. R. Quake, H. Babcock and S. Chu, Nature **388** (1997), 151.
9) N. Yoshinaga, T. Akitaya and K. Yoshikawa, Biochem. Biophys. Res. Comm. **286** (2001), 264.
10) G. Krishnamoorthy, G. Duportail and Y. Mély, Biochemistry **41** (2002), 15277.
11) Y. Murayama and M. Sano, Biopolymers **77** (2005), 354.
12) H. Wada, Y. Murayama and M. Sano, Phys. Rev. E **66** (2002), 061912.
13) N. Yoshinaga, K. Yoshikawa and T. Ohta, Eur. Phys. J. E **17** (2005), 485.