

Force Measurements of a Single DNA Molecule in the Collapsing Phase Transition

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The elastic force for a single DNA molecule (15.7 kilobasepairs) during a transition between an elongated coil and a collapsed globule state was measured by dual-trap optical tweezers. Under highly diluted conditions, the DNA concentration was 2 nM in nucleotides, the force change in a collapsing process and the elastic response in the collapsed state were observed at the single molecule level. To induce the transition, a trivalent cation, spermidine, was used. Two types of force changes in different timescales occurred in the intramolecular collapsing process; a fast force change from about 0.2 to 0.9 pN within 20 s, and a slow force change from 0.9 to 1.5 pN for 100 s. After the force change, a force plateau at about 1.8 pN was observed in a stretching process; however, after the process, the elastic response was the same as the one in a coil state. This hysteresis indicates the existence of a metastable state such as a supercooling liquid state.

KEYWORDS: DNA, force measurement, coil-globule, transition, intramolecular collapse, hysteresis, metastable state

It is known that multivalent cations induce the transition in the higher-order structure of a single DNA molecule between an elongated coil and a collapsed globule state.^{1,2)} Many experimental³⁻⁶⁾ and theoretical⁶⁻⁹⁾ studies have examined the interplay between multivalent cations and DNA. Although theory predicts that the transition can be discrete for stiff polymers,^{10,11)} including double-strand DNA molecules, experimental results have indicated that it becomes very steep but is still continuous with respect to the concentration of condensing agents.^{1,2)} Single molecule observations by fluorescence microscopy have shown that the transition is discontinuous, i.e., a first-order phase transition at the single molecule level, and it is continuous for the ensemble average.¹²⁻¹⁴⁾ These experiments verify the discrepancy between theory and experiment. Since the elastic force and the extension of a polymer chain are considered as the pressure and volume of gas/liquid, respectively, the coil-globule transition in a polymer chain resembles a gas-liquid phase transition. Although single molecule observation by fluorescence microscopy is a powerful method to investigate the coil-globule transition at the single molecule level, it is difficult to determine the practical force which the molecule is subjected to in the transition. Force measurements of the single DNA molecule enable the investigation of the elastic behavior during the transition and permit the calculation of the energy required for collapsing the molecule directly from the measurements.

In the present study, the elastic force for a single DNA molecule in the coil-globule transition induced by spermidine, a trivalent cation, was measured using dual-trap optical tweezers. Two types of force changes in different

timescales were observed during an intramolecular collapse. After the intramolecular collapse, a force plateau was observed in the molecule stretched process at first. The force plateau indicates the existence of a coil-globule bimodal state in a single DNA molecule. However, after the first stretching, the extension response to the force in the stretched and relaxed processes was the same as that in a coil state. It is expected that a metastable state such as the one of supercooling liquid was observed.

DNA molecules were prepared by PCR amplification and tethered between two kinds of protein-coated polystyrene beads to prevent both ends of a single DNA molecule from attaching to the same bead.¹⁵⁾ Both ends were labeled using 5' end-labeled primers; one was labeled with biotin (Takara, Japan) and the other was labeled with digoxigenin (Takara, Japan). The reaction was performed using DNA polymerase LA-Taq (Takara, Japan) and λ -phage DNA as a template. After agarose gel electrophoresis for amplified fragments, DNA molecules whose contour length $L = 5.3 \mu\text{m}$, 15.7 kilobasepairs (kbp), were purified. Streptavidin (Vector Laboratories, USA) was coupled to 2.0- μm -diameter carboxylated polystyrene beads (Polyscience, USA) according to the manufacturer's protocol and anti-digoxigenin (Boehringer Mannheim, Germany) was coupled to 3.0- μm -diameter beads by the same method. DNA fragments were mixed with two kinds of protein-coated beads in buffer solution (10 mM Tris-HCl, pH 7.0); the ratio of the number of beads to DNA molecules was 1:1~1:10. After 1 h incubation at room temperature, the mixed solution was highly diluted by the same buffer solution to prevent a multimolecular aggregation. The final concentration of DNA was 2 nM in nucleotides. During force measurements, DNA-beads solution was introduced into a sample cell made of two glass coverslips

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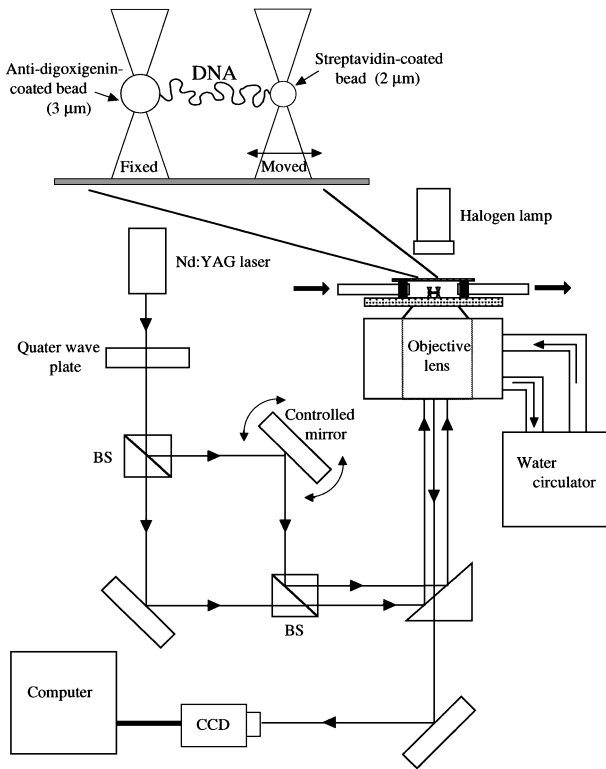


Fig. 1. Schematic diagram of experimental setup. To generate dual optical traps an infrared Nd:YAG laser (1064 nm, 450 mW in max., CrystaLaser, USA) was used. The laser beam was passed through a quarterwave plate and divided in two by polarizing beam splitters (BS). The position of one beam was fixed and the other one was moved by altering the angle of the mirror with a two-axis controller (Sigma Koki, Japan). A $100\times$ oil-immersion objective lens (NA 1.4, Plan Apochromat, Zeiss, Germany) was used. The temperature around the trapping spots was controlled using a water circulator through the objective lens and immersion oil. In the present experiments, the temperature was maintained at 23°C .

and a spacer. The spacer was a nitrile rubber *o*-ring (diameter 17.5 mm, thickness 1.5 mm) and two nylon tubes were connected to the spacer to exchange solution using a microsyringe. To minimize the adsorption of DNA into the glass surface, coverslips were washed in 0.1 M KOH, distilled water ($\rho \geq 18 \text{ M}\Omega\cdot\text{cm}$) and ethanol. Spermidine-3HCl (Sigma, USA) was prepared as 0.1 M stock in distilled water and the final concentration was prepared in buffer solution.

A single DNA molecule attached to beads was stretched using dual-trap optical tweezers.^{15,16} A schematic diagram of the experimental setup is shown in Fig. 1. A $3\text{-}\mu\text{m}$ -diameter bead was always trapped by a fixed beam, and DNA was stretched through a $2\text{-}\mu\text{m}$ -diameter bead which was trapped by a moving beam. Bead images were captured and analyzed on a computer using NIH image software (developed by the US National Institutes of Health). The extension of DNA was determined from the distance between the two bead centers by subtracting the bead's radius, and the force acting on the molecule was determined from the displacement of the $3\text{-}\mu\text{m}$ -diameter bead from the fixed beam spot. The force was calibrated against the viscous drag on a bead using Stokes law.

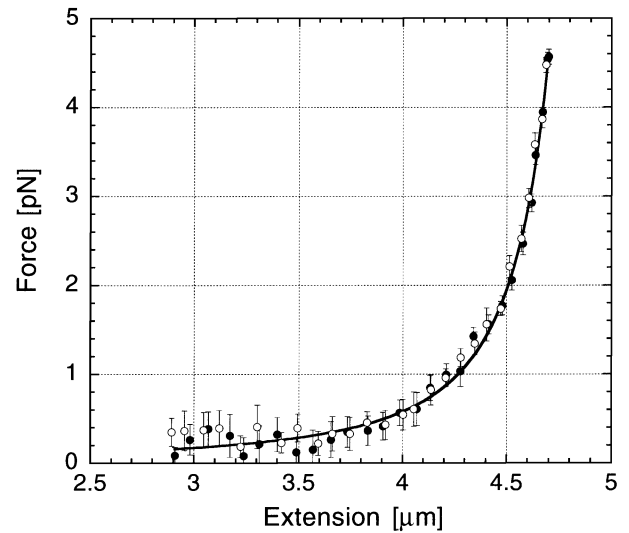


Fig. 2. The $f-x$ curve of a single DNA molecule (15.7 kbp) in buffer solution in stretched (solid circle) and relaxed (open circle) processes. The extension response to an applied force is well described as a WLC with $P = 44 \text{ nm}$ and $L = 5.1 \mu\text{m}$ (solid curve). Each data point is the average value for 10 measurements within 10 s and the error bar is its standard deviation.

It has been confirmed that the elastic behavior of a single DNA molecule is well described by the wormlike chain (WLC) model.^{17,18} An interpolation formula for the WLC force f versus extension x is

$$\frac{fP}{k_{\text{B}}T} = \frac{1}{4(1-x/L)^2} - \frac{1}{4} + \frac{x}{L}, \quad (1)$$

where k_{B} is the Boltzmann constant, P is the persistence length and T is the absolute temperature. Figure 2 shows the force-extension ($f-x$) curve for a single DNA molecule in the absence of spermidine. The data points are fit by eq. (1) with $P = 44 \text{ nm}$ and $L = 5.1 \mu\text{m}$. In this case, when the DNA molecule was stretched and relaxed there is no hysteresis on the $f-x$ curve.

Next, for the same DNA molecule, after stretching the molecule with the force $\sim 5 \text{ pN}$, the molecule was relaxed at $2.9 \mu\text{m}$. The elastic force in response to the extension at this position was less than 0.4 pN . Then, the solution in the sample cell was exchanged for 2 mM spermidine using a microsyringe. This exchange was introduced at $\sim 0.6 \mu\text{l/s}$. The force as a function of time during the exchange of solution is shown in Fig. 3. During the exchange, a fast change of the force from about 0.2 to 0.9 pN was observed within 20 s . After the fast change, the force slowly increased to about 1.5 pN during $\sim 100 \text{ s}$. The solution exchange was finished at 360 s . The amount of exchanged solution was less than the capacity of the sample cell. If the solution was homogenized in the sample cell, the concentration of spermidine was $700 \mu\text{M}$; the exact concentration around the measured molecule might be higher than this value. This concentration of spermidine is sufficient to collapse the DNA molecule; for T4DNA (166 kbp) it is about $200 \mu\text{M}$ in 10 mM Tris, 1 mM NaCl at pH 5.2¹²) and the value increases as the salt concentration increases.¹) Since the molecule was stretched at the extension of about 55% of

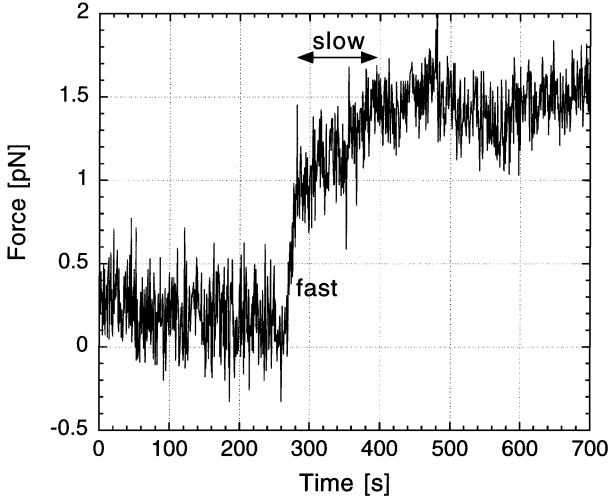


Fig. 3. The force as a function of time every 0.7 s during and after the exchange of the solution in the sample cell from only buffer solution to 2 mM spermidine. The force was almost constant for the first 260 s during the exchange. After about 270 s, two types of force changes in different timescales were observed: a fast change within 20 s and a slow one during ~ 100 s. The solution exchange was finished at 360 s.

the contour length and the interaction between molecules was negligible under the highly diluted condition, this change of the force is due to the effect of an intramolecular collapse.

Figure 4 shows the force-extension curve before and after the solution exchange. The above force change was observed at (A \rightarrow B). When the DNA molecule was stretched after the solution exchange a force plateau at 1.6 \sim 2 pN was observed at (B \rightarrow C). It is considered that the force plateau indicates a coil-globule bimodal state due to an intramolecular collapse. After the molecule was stretched at D, the molecule was relaxed. In this relaxed process, the force plateau could not be observed (D \rightarrow C \rightarrow A). Then, the stretched and relaxed processes were repeated; however, the force plateau could not be observed (A \rightleftharpoons C \rightleftharpoons D). In this case, the $f-x$ curve is well fit in eq. (1) with $P = 27$ nm and $L = 5.0$ μ m. After the intramolecular collapse, the hysteresis on the $f-x$ curve was observed in the stretched and relaxed processes.

On the basis of the above results, the work for extending the molecule against the tension was calculated both for the coil-globule bimodal state (W_b) and the coil state (W_c).

$$W_{b(c)} = \int_0^{x^*} f dx, \quad (2)$$

where x^* denotes the extension at which the force response coincides in both states ($x^* = 4.25$ μ m). Assuming that a 1.8 pN force plateau can be observed at $x < x^*$ for the coil-globule bimodal state and the $f-x$ response is described as WLC in eq. (1) with $P = 27$ nm and $L = 5.0$ μ m for the coil state, $W_b - W_c \approx 1.6 \times 10^3 k_B T$. This value corresponds to 0.10 $k_B T$ /bp. Since the force is only due to the elastic entropy for the coil state, the excess work for the coil-globule bimodal state is due to the attractive force between the molecule segments.

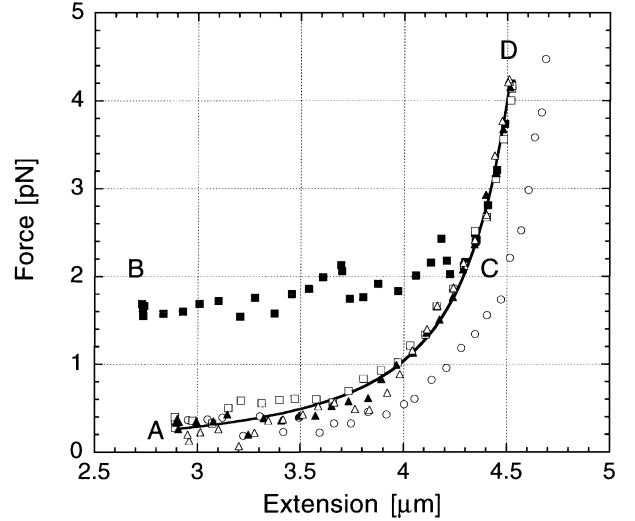


Fig. 4. The $f-x$ curve before and after the exchange of the solution in the sample cell from buffer solution to 2 mM spermidine. Before the exchange (open circle) the DNA molecule was stretched at A. After the exchange the force increased at B, and then the DNA molecule was stretched (solid square) to D following the force plateau (B \rightarrow C). When the molecule was relaxed (open square) the force plateau could not be observed (D \rightarrow C \rightarrow A). After reaching A, though the stretched (solid triangle) and relaxed (open triangle) processes were repeated, the force plateau could not be observed (A \rightleftharpoons C \rightleftharpoons D). The solid line describes the WLC in eq. (1) with $P = 27$ nm and $L = 5.0$ μ m.

The two types of force changes that occurred in different timescales are considered as follows. In the presence of applied force, several globule structures might be formed along a molecule, as seen in the presence of an external electric field.¹³ When the concentration of spermidine reaches the threshold value where a molecule is collapsed, the nucleation of the collapsed globule can be induced at some positions along the molecule. The intramolecular collapse proceeds at each position and small collapsed modules are formed along the molecule. Then, these modules interact with each other and further collapsed structures are formed at several positions. It is understood that the first process is faster than the last one as the effect of the molecule thermal fluctuation is taken into consideration. The process of DNA condensation with multivalent cations (i.e., spermine and spermidine) is observed by light scattering; there are two processes, a fast reaction in the millisecond time range with the intramolecular collapse and a slow reaction with the time constants of ~ 100 s which is assigned to an intermolecular condensation.¹⁹ In our experiments, since the concentration of DNA molecules was very low, the interaction between molecules are negligible. However, assuming that many small collapsed modules are formed initially, the interaction between these modules can be considered as multimolecular interactions which cause a slow condensation.

The force plateau which was observed in the first stretched process indicates a coil-globule bimodal state in single DNA, because the molecule was prevented from fully collapsing. This indicates that the coil-globule transition of a single DNA molecule induced by multivalent

cations can be described as the same as that of the gas-liquid phase transition. Recently, a force plateau was observed by Baumann *et al.* in the presence of spermidine and hexaammine cobalt (III).²⁰⁾ According to their report, the force plateau was observed in both stretched and relaxed processes for λ -DNA (48.5 kbp) and both plateau and stick-release pattern for plasmid DNA (3.8 kbp). In the present experiments, however, the hysteresis on the $f - x$ curve was observed in the stretched and relaxed processes although the salt concentration and buffer solution are different from Baumann *et al.*'s. The state between A and C in Fig. 4 represents a metastable state such as a supercooling state of liquid; in stretching and relaxing a polymer chain, it would be a superrelaxed state. If this state is metastable, however, once the intramolecular collapse is induced by some perturbation such as a small vibration or larger thermal fluctuation, the force plateau would be observed again. In fact, the reversible force plateau can be observed under the same solution condition at high temperature in our experiments.²¹⁾ Moreover, the stretched and relaxed processes were in a range much larger than the diameter of the fully collapsed state which is expected. If the molecule is more relaxed, since the thermal fluctuation of the polymer chain increases, the intramolecular collapse will reoccur.

To conclude, we measured the elastic force of a single DNA molecule in the coil-globule transition induced by a trivalent cation, spermidine. Two types of force changes in different timescales were observed with an intramolecular collapse. The hysteresis behavior in the elastic response in a coil-globule bimodal state indicates the existence of a metastable state.

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