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COMMUNICATIONS

Published on Web 11/04/2006

Compaction Dynamics of Single DNA Molecules under Tension

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The compaction of DNA by multivalent cations has been the subject of many investigations, not only because it is biologically important, but also because it poses a fascinating challenge to our understanding of semiflexible polymers.[1−5] Polyelectrolyte theory has figured out that the attractive potential that leads to DNA compaction originates from correlated fluctuation of counterions shared between DNA segments.[6] Experimental observations have revealed that DNA condensates have generally a toroidal geometry with a typical size of ∼100 nm in diameter. It is generally believed that DNA within a toroid is organized in a hexagonal close-packed lattice,[7] but how such a structure is formed is still not fully understood.

Single-molecule measurements have proven helpful to understanding the nucleation and growth of DNA condensates.[8−13] A few recent experiments concentrated on the kinetics of DNA compaction. They showed that DNA condenses continuously and linearly with time once the compaction process begins.[14−17] The results seemed to support the widely accepted opinion that the toroid is formed by continuously absorbing DNA to a primary loop randomly nucleated on DNA. The temporal resolution of the experiments was, however, relatively low. They might not be able to resolve the time trajectories of the DNA compaction.

Exerting forces on DNA is a useful way to study processes relevant to DNA.[18] The force may slow down the dynamical process so that details can be observed using an apparatus of finite temporal resolution. Here we report single-molecule studies on the dynamics of hexaammine cobalt chloride-induced DNA compaction under tension. It turns out that the compaction process is more sophisticated than the static structural model has suggested. DNA condenses into toroid in a quantized manner.

The measurements were performed using transverse magnetic tweezers similar to the one recently developed by Yan and Marko.[19] Two micrometer-sized beads are tethered to the two ends of a λ-phage DNA (16.4 μm). One bead is fixed in space by a micropipette, and the other is free in solution. A magnetic rod is inserted into the solution to generate a constant force to the free magnetic bead. The experiments were done in phosphate-buffered saline (10 mM PBS, 5 mM NaCl, pH = 7.4). After adjusting the force on DNA, 10 μL of 10 mM hexaammine cobalt chloride solution was added into the sample cell. The final concentration of the trivalent cations was 100 μM.

Figure 1a shows the time course of DNA compaction at a force $F = 0.5$ pN. Time courses measured at other concentrations of the trivalent cations (from 35 to 200 μM) are quite similar. After addition of the cations, a long induction period was observed before the compaction started. The induction time becomes longer when a less concentrated solution of trivalent cations is added. The compaction is discontinuous and stepwise. The two big steps in Figure 1a consist of multiple small steps. Such discontinuous compactions were also observed at other forces (Figure 1b,c). The

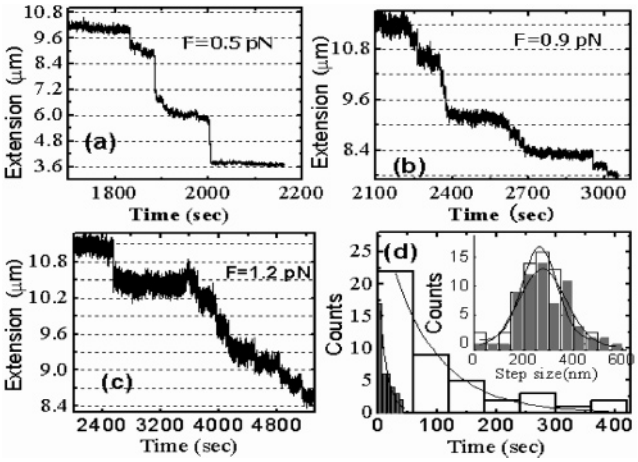

Figure 1. Compaction of DNA under different forces: (a) $F = 0.5$ pN, (b) $F = 0.9$ pN, and (c) $F = 1.2$ pN. (d) Un-normalized distributions of waiting time for $F = 1.2$ pN (white) and for $F = 0.9$ pN (gray). (Inset) Corresponding histograms of the step sizes. The distribution in (d) is each from more than 15 curves.

long time courses allowed us to study the statistics of the waiting time between two successive compaction events (Figure 1d), and the compaction step sizes (Inset, Figure 1d). The average waiting time is found to be 79 s at $F = 1.2$ pN and 13 s at $F = 0.9$ pN. The histograms of the compaction steps for both forces were found to peak at ∼270 nm. The maximum force that allowed us to see the discontinuous stepwise DNA compaction is 1.8 pN.

To study the mechanical properties of the above toroids, we performed constant force unraveling experiments on them. Parts a and b of Figures 2 are two unraveling time courses at 6 and 16 pN, respectively. The unraveling steps are almost of the same size as the compaction ones. At 16 pN, we observed very well-defined steps. Most of the step sizes are around 300 nm. At 6 pN, the steps are less well defined. However, we could still see distinct jumps: besides the 300-nm jumps, many jump sizes are less than 200 nm. These smaller jump sizes may represent the incomplete unwrapping of DNA from the toroid. The frequency to see these smaller jumps became less when the force was increased. At very high forces (>20 pN), >600-nm jumps could be seen (e.g., Figure 2c, 23 pN time course). These large jumps may represent that two or three turns of DNA were unwrapped from the toroid at the same time. The minimal force that allowed us to see unraveling steps is 6 pN. The critical force and the step size observed in our experiments agree with the earlier optical tweezers experiments in which both ∼100- and ∼300-nm steps were observed.[20,21]

We failed to obtain good statistics for the steps at a specific force as in Figure 1d because the unraveling process can readily get impeded. We never found that, in the time scale of 1 h, any force below 25 pN could completely unwrap DNA (i.e., to get the full DNA length back). A typical unraveling behavior is shown in Figure 2c; starting from a compact DNA of ∼9 μm long, a 12-pN

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10.1021/ja064305a CCC: $33.50

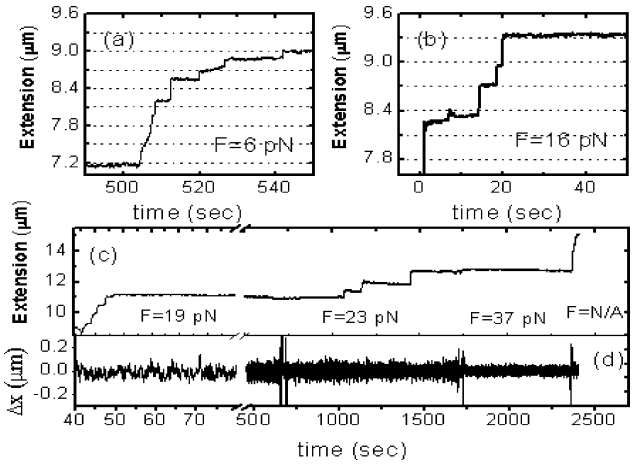

Figure 2. Unraveling of DNA toroids at different forces. (a) $F = 6$ pN. (b) $F = 16$ pN. (c) Typical full unwrapping course of single DNA. (d) The transverse fluctuation of the paramagnetic bead determines the forces in the unwrapping course in (c).

force may unwrap the DNA, with the step size similar to that observed in the 16-pN unraveling process in Figure 2b. The unraveling then stopped at the DNA extension ~11 μm. The extension remained unchanged for the subsequent 10 min. By increasing the force to 23 pN, we observed some further DNA unwrapping steps before it stopped again. Finally, the DNA was almost completely unwrapped at a force of 37 pN before it was broken.

Recently Kulić and Schiessel[22] developed a general theory describing the nonequilibrium behavior of unraveling DNA spools under tension. The total energy of the spool is contributed from the interaction between the DNA and the toroid surface, the deformation of the DNA backbone, and the work done by the tension. They found that the unraveling problem can be described by two angles: α, describing the adsorption of DNA on the spool surface, and β, describing the out-of-plane tilting of the spool. The total energy of the system was derived to be

$$E = 2R(\epsilon - F)\alpha + 2FR\left[\cos\beta \sin\alpha - \left(\frac{H}{2\pi R}\right)(\pi - \alpha)\sin\beta\right]$$

where ϵ is the effective adsorption energy density of DNA, R (~50 nm) the radius of the toroid, and H (~5 nm) the pitch height of the helical path of DNA. Note that we have ignored a term which is negligible for large thin spools discussed here.[22] The authors pointed out that one turn of DNA unwrapping from the spool is a force-induced transition from a metastable state M1 ($\alpha = -\arccos(1 - \epsilon/F)$, $\beta = 0$) to another metastable state M2 ($\alpha = \pi - \arccos(1 - \epsilon/F)$, $\beta = \pi$), via a saddle point S ($\alpha = 0$, $\beta = \arccos(1 - \epsilon/F)$). Assuming $\epsilon = 0.3-0.4\ k_BT$/nm, the authors predicted that the unfolding frequency is $0.3-50\ s^{-1}$ for $F = 8$ pN. The prediction agrees with our observations of the unraveling process in this force range.

If it is assumed that the unwrapping and wrapping of DNA follow the same but reversed pathway, the above theory may also be applied to the wrapping transition. One turn of DNA wrapping onto a toroid is then understood as a transition from state M2 to state M1 via the saddle point S. The energy barrier for the wrapping transition is $\Delta E = E(S) - E(M2)$.We found, however, that the model cannot explain the measured average waiting time in Figure 1d, unless we assume that ϵ depends on the applied force in the compaction process. We found that $\epsilon(1.2\text{ pN}) = 0.40k_BT$/nm and $\epsilon(0.9\text{ pN}) = 0.29\ k_BT$/nm may explain the result. Intuitively, this means that DNA in a toroid is more irregularly packaged if it condenses more rapidly, just like the observation that a crystal possesses more defects if it grows faster. Our experiments therefore suggest that the degree of regularity of DNA in a toroid depends on the compaction kinetics.

The discontinuous unwrapping behavior of the toroids (Figure 2) is due to the strong kinetic protection from mechanical disruption upon applied tension.[20−22] But the kinetic protection is not strong enough to explain why forces larger than 25 pN are needed to fully unravel the toroids in our force-clamp measurements. A plausible explanation, which is in accordance with the conclusion drawn from the waiting time measurements in Figure 1, is that the DNA segments in the toroid are not regularly arranged. As a consequence, a segment, which ought to be on the outmost surface of the toroid being unraveled, might be incorrectly overlain by another one. It is hard to strip off such a segment because its outgoing is impeded by the overlying segment.

In summary, we have studied the compaction dynamics of single DNA molecules invoked by hexaammine cobalt chloride. The observations suggest that the folding/unfolding events are transitions between two metastable structural states which are separated by a tension-dependent energy barrier. One turn of DNA is wrapped to (unwrapped from) the toroid in a folding (unfolding) event. Analysis of the waiting time revealed that the degree of the package ordering of DNA in a toroid depends on the compaction kinetics. The compaction of DNA is therefore more sophisticated than the static structure of toroid has suggested. Obviously, more accurate theoretical models and computer simulations are needed to explain the quantized compaction of DNA.

Acknowledgment. This work was supported by grants to M.L. from the National Natural Science Foundation of China (Grant Nos. 10334100 and 10325419), and by a grant to J.Y. from National University of Singapore (Grant No. R144000143112).

Supporting Information Available: Experimental setup and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA064305A