Role of Tension and Twist in Single-Molecule DNA Condensation

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Using magnetic tweezers, we study in real time the condensation of single DNA molecules under tension. We find that DNA condensation occurs via discrete nucleated events. By measuring the influence of an imposed twist, we show that condensation is initiated by the formation of a plectonemic supercoil. This demonstrates a strong interplay between the condensation transition and externally imposed mechanical constraints.

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Despite its stiffness and high charge density, double-stranded DNA (ds-DNA) is condensed *in vivo* into highly compact structures by positively charged proteins. Morphologies and packing densities similar to those observed in sperm nuclei and in certain viruses [1,2] can be reproduced *in vitro* using a broad array of simple tri- or quadrivalent cations [3]. The latter provide ideal experimental systems for testing theoretical ideas on microscopic mechanisms for like-charge attraction in electrolytes [4–9]. This marks an important step toward understanding more complex forms of DNA packaging since multivalent polyamines are associated with stages of the cell cycle during which chromatin is highly compact [10].

Most physical studies of DNA condensation to date have concentrated on free molecules in bulk solution. This neglects the biologically relevant influence of mechanical constraints such as DNA supercoiling and forces exerted by the cellular machinery [11,12]. For example, while the force exerted by a DNA molecule as a function of its end-to-end distance was recently measured under condensation conditions using optical tweezers [13–15], the consequences of the imposed tension on the condensation transition were not explored.

Here we investigate DNA condensation by multivalent ions at the single-molecule level using magnetic tweezers [16]. This technique allows applying a constant pulling force F on a DNA molecule while measuring its elongation in real time. Importantly, a controlled, sign-specific twist can be applied to the molecule to study the role of torsion in DNA condensation dynamics. We show that condensation occurs via a series of force-dependent nucleation events initiated by the formation of a plectonemic supercoil (loop) in the DNA.

Our experimental setup [16] consisted of an 8 kbp ds-DNA molecule tethered between a glass surface and a 2.8 μm paramagnetic bead. Translating and rotating nearby magnets allowed tuning the magnitude of the force F and rotating the bead in the plane parallel to the surface, respectively. The extension of the DNA was monitored by measuring the position z of the bead above the surface.

A typical experiment is shown in Fig. 1. Starting from a large applied force F, we measured the DNA extension z as a function of time t while gradually lowering F. The measurements were performed in TRIS buffer (10 mM Tris Hydroxymethylaminoethane) with pH 7.5 and a variable concentration c of multivalent salt. Figure 1(a) shows that for $c = 0$ (no multivalent ions), the DNA acted as an entropic spring. A fit to the worm-like-chain (WLC) model [17] [Fig. 1(a)] yields a persistence length $p = 52$ nm and a contour length $L = 2.85$ μm, as expected for 8 kbp nicked DNA in monovalent-salt buffer.

Figure 1(b) shows $z(F)$ for the same DNA molecule for $c = 1$ mM of the trivalent cation cobalt sepulchrate ($[CoC_{12}H_{30}N_8]^{3+}$, cosep). At high forces this curve was similar to the data without multivalent ions, but at $F =$ 3–4 pN a sudden, rapid drop in z occurred. No changes in the bead position in the horizontal plane (x, y) occurred

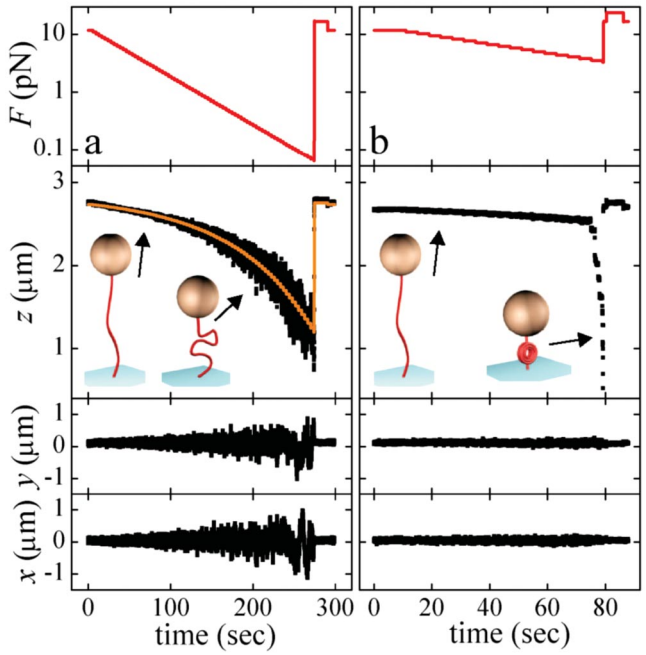

FIG. 1 (color). Measurement of DNA extension z and bead position in the horizontal plane (x, y) while gradually lowering the force F in (a) a monovalent buffer and (b) the same buffer with 1 mM cosep added, for the same 8 kbp nicked DNA molecule. The orange line is a fit to the WLC model.

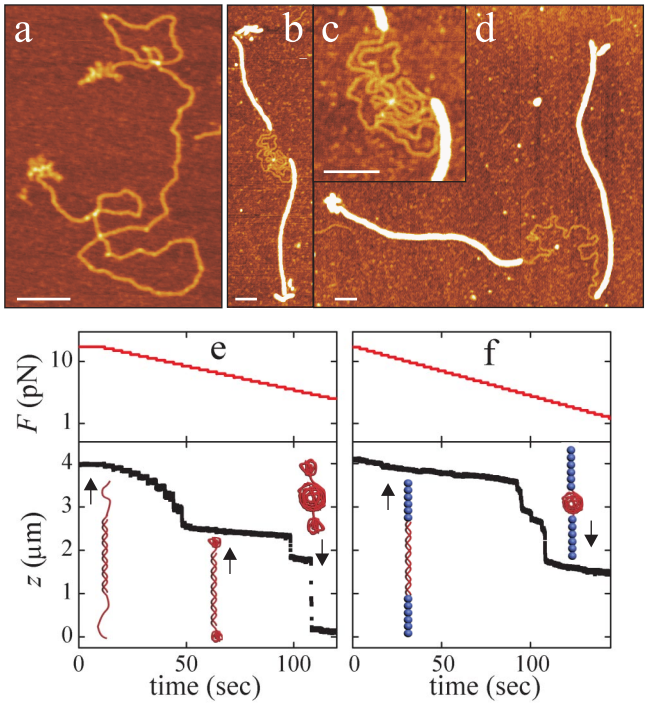

FIG. 2 (color). Tapping-mode AFM images of the ss-ds-ss-DNA (a) before and (b)–(d) after coating the single-stranded ends with RecA. (c) is a magnification of (b). Because of the large flexibility of ss-DNA, the ss ends in (a) appear as small globular structures. Scale bars represent 100 nm. (e),(f) Extension in 1 mM cosep of ss-ds-ss-DNA before (e) and after (f) RecA filament formation (different molecules).

during this drop. We tentatively interpret this behavior as condensation of the DNA molecule, consistent with the previous finding by imaging individual fluorescently-labeled DNA molecules that condensation is an abrupt transition between two stable states [18]. We define the condensation force F_c as the force at which the first sharp step was observed in $z(t)$, $F_c = 3.5$ pN in Fig. 1(b).

Another possible cause for the sudden collapse, however, is sticking of the DNA to the bead or glass surface. Multivalent ions mediate an attraction between like-charged DNA strands, and could similarly result in attraction between DNA and the nearby negative surfaces. This potential experimental artifact has not been thoroughly addressed in previous studies [13–15].

To test whether sticking plays a role, we performed measurements on ds-DNA kept away from the bead and surface by stiff, rodlike spacers that consist of RecA-coated single-stranded DNA (ss-DNA). RecA is a DNA-binding protein that promotes DNA strand exchange during homologous recombination in bacteria [19]. It can polymerize on ss-DNA and ds-DNA, but the rate of binding to ds-DNA is negligible compared to that to ss-DNA at *p*H 7.5 [19,20]. Figure 2(a) shows an atomic force microscope (AFM, Digital Instrument NanoScope IV) image of a DNA molecule consisting of ss ends of length 1.7 kb and 1.9 kb and a ds middle of length 7 kbp (ss-ds-ss-DNA) on

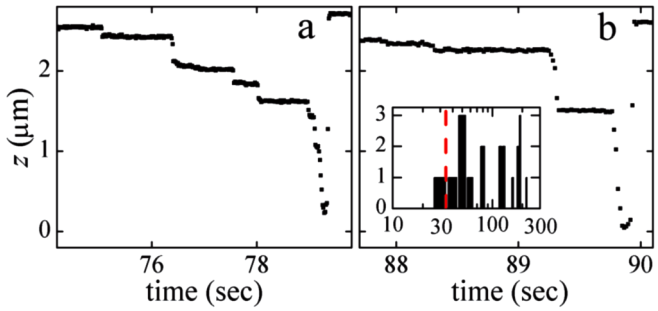

FIG. 3 (color online). (a) Magnification of Fig. 1(b). (b) Repeated measurement on the same molecule. The inset shows a histogram of the observed step sizes (in nm) for both measurements and a third consecutive measurement, where the dashed line indicates the size of a single loop at $F = 3.5$ pN.

mica [21]. Figures 2(b)–2(d) show ss-ds-ss-DNA on poly-L-lysine coated mica after filament formation on the ss ends, showing stiff filaments with RecA-free ds-DNA in between. The more compact appearance of the ds-DNA in Figs. 2(b)–2(d) is due to the different imaging surface.

Figure 2(e) shows the results of a condensation experiment in 1 mM cosep using ss-ds-ss-DNA without RecA. For $15 > F > 8$ pN, z decreased progressively with decreasing F. This decrease corresponds to that of a 3.6 kb (1.7 + 1.9 kb ends) ss-DNA molecule (data not shown), as expected since $z(F)$ for ds-DNA is almost constant over this range of forces. From $F = 8$ to 3.6 pN, z was ~ 2.4 μm, corresponding to the length of the 7 kbp ds-DNA. At 3.6 pN, z dropped suddenly as for ds-DNA condensation. The ss-ds-ss-DNA thus behaved as a 3.6 kb ss-DNA and a 7 kbp ds-DNA molecule attached end to end.

Figure 2(f) shows $z(F)$ for a RecA-modified ss-ds-ss-DNA with 1 mM cosep. The contraction of the ss-DNA from 15 to 8 pN was now absent. At $F = 3.1$ pN there was an abrupt reduction in z similar to that of a pure ds-DNA molecule. Experiments on four constructs gave an average F_c of 2.9 ± 0.4 pN, in good agreement with F_c of nicked ds-DNA molecules under the same conditions ($F_c = 3.2 \pm 0.4$ pN). Condensation stopped when the bead was about 1.8 μm from the surface, corresponding to the total length of the RecA filaments (0.85 and 0.95 μm for fully-coated 1.7 and 1.9 kb ss-DNA molecules, respectively [19,20]). We conclude that under the conditions investigated, the observed collapse of DNA was not due to multivalent-ion-induced DNA-surface interactions.

Having established that our experiment probes intrinsic DNA condensation, we further investigated the dynamics of this process. Figure 3(a) shows the rapid $z(t)$ transient of Fig. 1(b) in more detail. The decrease in z consisted of several sudden steps separated by ~ 1 sec plateaus. Condensation could be reversed so as to repeat the experiment with the same molecule. Decondensation was hysteretic, typically requiring a force ≥ 15 pN to return the molecule to its original state. No backward steps were observed unless such high forces were applied. For a given

molecule, the pattern of the steps was slightly different each time that it was measured [Fig. 3(b)], while F_c typically varied by no more than 20%. Similar results were obtained using condensing agents cobalt hexamine ($[Co(NH_3)_6]^{3+}$) and spermine ($[C_{10}N_4H_{30}]^{4+}$). These condensation dynamics and hysteresis indicate that DNA condensates are formed via a first-order process [18] in which activation through an energetically unfavorable transition state limits the rate of condensation.

Determining the nature of the transition state is a key step in understanding the condensation dynamics. Based on the size of toroid-shaped DNA condensates, it has been proposed that condensates nucleate as loops [22,23]. Although bending the DNA molecule into such conformations costs energy, it permits attractive short-range electrostatic interactions to come into play. The transition state has not been probed directly, however.

Assuming that condensation starts with loop formation, condensation is observed within our experimental time scale when the free energy barrier for condensation $G^{\ddagger} = U_{\mathrm{loop}} + U_{\mathrm{tw}} + G_{\mathrm{add}}$ is below a critical value G_{crit}. Here U_{loop} and U_{tw} are the energy for bending and twisting the DNA, as discussed below, while G_{add} represents any additional contribution to the energy barrier arising from, e.g., electrostatic repulsion between the two parts of the molecule being brought into contact.

The force-dependent energy for forming a plectonemic supercoil consisting of a circle of radius R is $U_{\mathrm{loop}} = \pi k_b T p/R + 2\pi RF$, where k_bT is the thermal energy. The first term represents bending energy and favors large loops while the second term represents work against the force F and favors small loops. This influence of the force is an important difference between our and bulk experiments. The energetically most favorable loop radius is $R_0 = \sqrt{k_bTp/2F}$ [16]. Note that R_0 only characterizes the transition state: it does not necessarily dictate the ultimate size of the condensate, nor does it imply that the condensate will be a toroid once fully formed. For the conditions in Fig. 3, the smallest expected steps are $2\pi R_0 = 34$ nm (using $p = 50$ nm and $F_c = 3.5$ pN), consistent with the observed step-size distribution [inset to Fig. 3(b)]. Because of the work against the external force, the nucleation loops that we observe are smaller than those deduced from toroid structures of free DNA [22,23].

The above discussion of loop formation holds for nicked DNA molecules (as used for Figs. 1–3), which are torsionally unconstrained. For unnicked molecules with fixed ends, forming a loop also implies twisting the molecule by an angle 2π. The free energy associated with introducing this twist is $U_{\mathrm{tw}} = -4\pi^2 C|n|/L$, where C is the DNA torsional modulus and $2\pi n$ is the twist angle already present in the molecule [16]. The negative sign corresponds to a loop that relieves existing twist; we neglect energetically unfavorable loops of the opposite sign.

The rotational capabilities of magnetic tweezers permit a stringent test of the loop hypothesis for a DNA molecule under tension. Twisting the DNA prior to condensation (increasing $|n|$) makes U_{tw} more negative and allows condensation at larger values of U_{loop} and F for a given value of $G^{\ddagger}$. Solving $U_{\mathrm{loop}}[F_c(n)] - U_{\mathrm{loop}}[F_c(n=0)] = |U_{\mathrm{tw}}|$ yields the expected dependence of F_c on n,

$$F_c(n) = F_c(n=0)\left(1 + \frac{2\pi C}{L\sqrt{2k_bTpF_c(n=0)}}|n|\right)^2. \quad (1)$$

The actual transition state is presumably more complex than an ideal circular plectonemic supercoil with a single contact point. In our simple description, these additional details and the values of G_{crit} and G_{add} enter into the experimentally determined parameter $F_c(n=0)$. This calculation assumes that G_{add} is independent of n and F. Insofar as this approximation is valid, Eq. (1) makes a nontrivial, experimentally testable prediction that must hold if the transition state contains a loop.

We performed an experimental test of Eq. (1) using 8 kbp unnicked DNA molecules. Before each condensation measurement the magnet was rotated n times at $F \geq 15$ pN. No plectonemic supercoils were introduced at such high forces. Figure 4 shows $F_c(n)$ for 1 mM cosep and spermine. At $n = 0$, both the value of F_c and the condensation dynamics were similar for torsionally constrained and unconstrained molecules, as expected. For $n \neq 0$, three different regimes were observed. (i) For negative n, F_c was practically independent of n except for a slight increase between $n = 0$ and $n = -20$. (ii) For small positive n, F_c increased monotonically with n. (iii) Around $n = 35$ for cosep and 45 for spermine, F_c reached a plateau and became independent of n.

Imposing a negative twist with $F > 0.3$ pN [regime (i)] does not elastically deform the DNA, but transforms regions of the molecule from B-form DNA into an alternate structure [24]. Similarly, regime (iii) can be explained by the transition of part of the DNA to P-DNA with 2.6 bases per turn. This has been reported to occur at $F \geq 3$ pN and a degree of supercoiling $+0.037$ [24], corresponding to $n = 30$ for 8 kbp DNA [25].

In regime (ii), B-DNA retains its structural integrity, the molecule can be described as an elastic rod, and Eq. (1) is expected to apply. Figure 4 shows fits to Eq. (1) using $T = 293$ K, $C = 86$ nm $\times k_bT$ [16], $L = 2.7$ μm, and $p = 50$ nm (values of p from 40 to 60 nm were consistent with the data). $F_c(n=0)$ was the sole fitting parameter. The good quantitative agreement between Eq. (1) and the data provides strong evidence that condensation is limited by the formation of plectonemic supercoils [26].

It has been proposed that the short-range attraction responsible for condensation is a consequence of the helical structure of ds-DNA [5]. We, however, observed that the condensation force is essentially independent of negative twist, even if sufficient twist is introduced as to completely transform the molecule from B-form DNA to an alternate structure ($n \approx -800$ for our 8 kbp DNA, see insets to Fig. 4). This suggests that the helicity of ds-DNA does

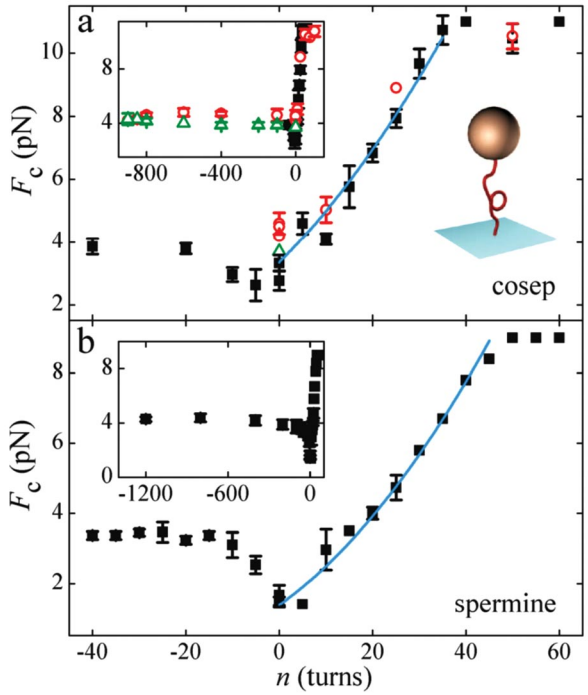

FIG. 4 (color). Condensation force F_c versus rotations n in 1 mM cosep (a) and spermine (b) solutions. The different colors in (a) correspond to different molecules. The blue lines are fits to Eq. (1). The insets show data at large negative n.

not play a fundamental role in its condensation, contrary to the assumption of this model.

Other microscopic mechanisms based on spatial correlations between multivalent ions [8,9] have mostly focused on DNA condensation as a transition between equilibrium phases. In particular, optical-tweezers experiments [15] were interpreted by assuming that condensates form in a continuous, reversible process and that the applied force exactly opposes a "condensation force" equal to the condensation free energy per unit length g_{cond} [8,15]. An analytical model for $F_c(c)$ based on the strongly correlated liquid (SCL) hypothesis [8] was found to be consistent with the optical-tweezers study of Ref. [15]. Our experiments, however, show that condensation of DNA under tension is an activated process that is irreversible on experimental time scales. The simple reversibility assumption $F_c = |g_{\mathrm{cond}}|$ thus does not hold under experimental conditions. Instead, decreasing F lowers the barrier for nucleation and the measured F_c represents the applied force at which nucleation becomes fast enough to be detected. The measured F_c thus cannot be directly compared to theoretical models for g_{cond} [8,9]. The condition $|g_{\mathrm{cond}}| > F$ remains a prerequisite for condensation to be energetically favorable, however, and the measured F_c thus represents a lower bound for $|g_{\mathrm{cond}}|$. This might partly explain why the simple equilibrium SCL model still provides an adequate qualitative description of the data of Ref. [15]. A more likely explanation, however, is that multivalent ions, by affecting the effective charge of the DNA, modify the barrier height for condensation through the term G_{add} as discussed above.

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[1] N. V. Hud *et al.*, Biochem. Biophys. Res. Commun. **193**, 1347 (1993).
[2] M. E. Cerritelli *et al.*, Cell **91**, 271 (1997).
[3] V. A. Bloomfield, Biopolymers **44**, 269 (1997).
[4] For a general review, see W. M. Gelbart *et al.*, Phys. Today **53**, No. 9, 38 (2000).
[5] A. A. Kornyshev and S. Leikin, Phys. Rev. Lett. **82**, 4138 (1999).
[6] I. Rouzina and V. A. Bloomfield, J. Phys. Chem. **100**, 9977 (1996).
[7] F. Oosawa, Biopolymers **6**, 1633 (1968).
[8] R. Zhang and B. I. Shklovskii, Physica (Amsterdam) **349A**, 563 (2005).
[9] R. Golestanian and T. B. Liverpool, Phys. Rev. E **66**, 051802 (2002).
[10] T. Thomas and T. J. Thomas, Cell Mole Life Sci **58**, 244 (2001).
[11] W. S. Ward and D. S. Coffey, Biol. Reprod. **44**, 569 (1991).
[12] M. G. Poirier and J. F. Marko, J. Muscle Res. Cell Motil. **23**, 409 (2002).
[13] C. G. Baumann *et al.*, Proc. Natl. Acad. Sci. U.S.A. **94**, 6185 (1997).
[14] C. G. Baumann *et al.*, Biophys. J. **78**, 1965 (2000).
[15] Y. Murayama, Y. Sakamaki, and M. Sano, Phys. Rev. Lett. **90**, 018102 (2003).
[16] T. R. Strick *et al.*, Prog. Biophys. Molec. Biol. **74**, 115 (2000).
[17] C. Bouchiat *et al.*, Biophys. J. **76**, 409 (1999).
[18] K. Yoshikawa *et al.*, Phys. Rev. Lett. **76**, 3029 (1996).
[19] A. I. Roca and M. M. Cox, Prog. Nucleic Acid Res. Mol. Biol. **56**, 129 (1997).
[20] M. Hegner, S. B. Smith, and C. Bustamante, Proc. Natl. Acad. Sci. U.S.A. **96**, 10 109 (1999).
[21] See EPAPS Document No. E-PRLTAO-98-018706 for supplementary information on preparation methods. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[22] C. C. Conwell, I. D. Vilfan, and N. V. Hud, Proc. Natl. Acad. Sci. U.S.A. **100**, 9296 (2003).
[23] N. V. Hud, K. H. Downing, and R. Balhorn, Proc. Natl. Acad. Sci. U.S.A. **92**, 3581 (1995).
[24] J.-F. Allemand *et al.*, Proc. Natl. Acad. Sci. U.S.A. **95**, 14 152 (1998).
[25] Regime (iii) could also occur if F exceeds the free energy of condensation per unit length g_{cond}. This would be consistent with F_c being close to the decondensation force and the occasional backward steps in this regime.
[26] The small increase in $F_c(n)$ for spermine for $n < 0$ can be fitted well with Eq. (1). $F_c(n)$ is symmetric around $n = 2$, indicating a small offset induced by spermine or by the flushing prior to the experiments. The same holds for cosep with a small negative offset; however, the change in $F_c(n)$ for $n < 0$ is too small to reliably fit to Eq. (1).