JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Overstretching DNA at 65 pN Does Not Require Peeling from Free Ends or Nicks

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Supporting Information

ABSTRACT: DNA exhibits a remarkable mechanical transition where its extension increases by 70% at 65 pN. Notwithstanding more than a decade of experimental and theoretical studies, there remains a significant debate on the nature of overstretched DNA. We developed a topologically closed but rotationally unconstrained DNA assay, which contains no nicks or free ends. DNA in this assay exhibited the canonical overstretching transition at 65 pN but without hysteresis upon retraction (v_{stage} = 5 μm/s). Introduction of a controlled nick led to hysteresis in the force-extension curve. Moreover, the degree of hysteresis increased with the number of nicks. Hence, the generation of single-stranded DNA from free ends or nicks is not an obligatory step in overstretching DNA, but rather a consequence.

The mechanical properties of single DNA molecules have been extensively studied.[1] At low-to-moderate force (<40 pN), measurements of DNA's elasticity[2] are quantitatively described by theory.[3] At slightly higher forces, DNA exhibits a mechanical transition where its extension increases by 70% at 65 pN.[4,5] While this basic experimental observation has been confirmed in many experiments across multiple platforms, including optical traps,[4,6] magnetic tweezers,[7,8] and atomic force microscopy,[9,10] a consensus description of overstretched DNA has yet to emerge. The two leading models are (*i*) a force-induced melting of the double-stranded (ds) DNA into single-stranded (ss) DNA[6,11] and (*ii*) a ladder-like dsDNA, called S-DNA, in which the base pairs remain intact but the rise per base pair is altered.[5,12,13]

Mounting evidence[6] supports the force-induced melting description, including studies of the overstretching force (F_o) as a function of pH,[14] salt,[15] and temperature.[16] The mechanism for melting could include peeling to generate ssDNA or internally melted states. In 2009, an elegant experiment directly visualized distinct ssDNA and dsDNA domains within the overstretch transition region.[17] Overall, this work strongly supports the force-induced melting hypothesis by detailing a mechanism whereby ssDNA is formed or "peeled" from unconstrained ends or nicks. This conclusion is based upon the assumption that the formation of such peeled ssDNA is an obligatory step in overstretching.

The debate on the nature of overstretched DNA continues. Recent simulations argue for S-DNA,[18] while recent experimental work puts forth a model where both S-DNA and ssDNA exist in a narrow force window at F_o.[19] The key experimental results were that changes in F across the overstretching transition lead to a concurrent change in DNA extension followed by a slower time-varying component as well as persistent hysteresis above a certain force. The authors conclude that the rapid change in extension corresponds to the formation of S-DNA, while the slower time component corresponds to the conversion of dsDNA into ssDNA based on its sensitivity to ionic conditions, temperature, force, and GC-content of the DNA.

Given the ongoing uncertainty, we sought to directly test if peeling from free ends or nicks is necessary for overstretching DNA and thereby test the leading mechanism for overstretching DNA.[17,20] However, to date, limitations in substrates have led to a discontinuous transition between stretching DNA that has no free ends (F_o = 110 pN, Figure 1a)[9,12] and DNA with nicks (F_o = 65 pN, Figure 1b,c) or free ends because the assay containing no free ends was both topologically closed and torsionally constrained. Torsional constraint was achieved by anchoring both ends of the DNA to mechanical supports by both strands.[9,12] This discontinuity has hampered the field because it precludes probing the overstretching process at 65 pN in a substrate without any free ends or nicks.

We developed a topologically closed but rotationally unconstrained assay by binding DNA at one end to a surface by both strands and the other end to a bead via an internal biotin embedded in a 5-nucleotide loop [Figure 1d (inset); see also Figure S1]. The resulting DNA was stretched with an optical trap by moving the stage (v = 5 μm/s) until $F \geq$ 80 pN. As shown in Figure 1d (*black*), overstretching of this DNA was indistinguishable from DNA with one nick, both showing F_o = 65 pN in 150 mM NaCl at room temperature.

The most visually distinct change between this new substrate and a typical DNA (*i.e.*, DNA with nicks or free ends) was observed during the relaxation after the overstretching (Figure 1; *blue*). Numerous studies of DNA containing nicks or free ends report that, after overstretching at 65 pN, the force-extension curves during retraction show significant hysteresis (*e.g.*, lower F at the same extension).[4,9,21] As shown in Figure 1b, DNA containing multiple nicks ($n \approx 2-3$) showed the expected hysteresis. Consistent with nicks playing a crucial role in hysteresis, we observed a significant decrease in hysteresis with a substrate containing only one nick (Figure 1c). Interestingly, the topologically closed but rotationally free assay exhibited no hysteresis at 65 pN (Figure 1d; see also Figure S2), similar to the known lack of

Received: October 4, 2010
Published: January 05, 2011

dx.doi.org/10.1021/ja108952v | *J. Am. Chem. Soc.* 2011, 133, 3219–3221

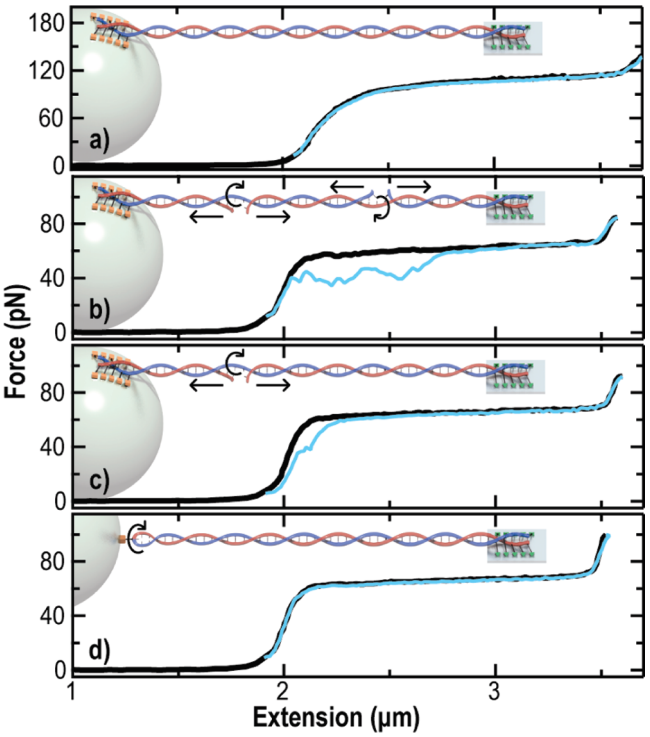

Figure 1. (a) Force-extension curve (FEC) of torsionally constrained DNA that was stretched (*black*) and then immediately relaxed (*blue*). (Inset) Cartoon of a taut DNA anchored to a surface via multiple digoxigenins (*green*) and to a bead via multiple biotins (*yellow*). (b) FEC of a similarly formed DNA but containing a few nicks ($n \approx 2-3$). The straight and curved arrows indicate peeling and twisting possibilities, respectively. (c) FEC for the same DNA but with a single nick. (d) FEC of DNA in a topologically closed but rotationally free assay using a biotin embedded in a 5-nucleotide ssDNA loop.

hysteresis at 110 pN in the torsionally constrained (and topologically closed) experiments (Figure 1a).[9]

To demonstrate that our assay was topologically closed, we provide three additional lines of evidence: (*i*) we measured different constructs produced with the same ligation protocol (see Supporting Information); (*ii*) we probed the effect of a single nick introduced into the torsionally constrained DNA to make it akin to DNA affixed to a surface by a single strand; and (*iii*) we verified the continuity of the DNA used in the topologically closed assay with a denaturing gel.

Given the same ligation protocol for making all of our substrates, we would expect a similar success rate in substrates sensitive to a single nick. Indeed, we observed that 84% of torsionally constrained DNA showed the expected elevated F_o ($N = 50$) and 83% of the records in the topologically closed assay showed no hysteresis ($N = 12$). Taken together, these results suggest that the vast majority of these substrates contained no nicks. Moreover, such correctly formed DNAs were easily identified by distinct features in the force-extension curve.

To make a DNA construct with a single nick, we started with the torsionally constrained DNA and used a sequence-dependent nuclease with a single binding site on the DNA. Such a protocol allowed for a quality control test on each DNA preparation by verifying F_o = 110 pN before nicking. After incubation with the sequence-dependent nuclease, overstretching occurred at $F_o = 65$ pN and retraction showed hysteresis similar to prior results.[4,9,21]

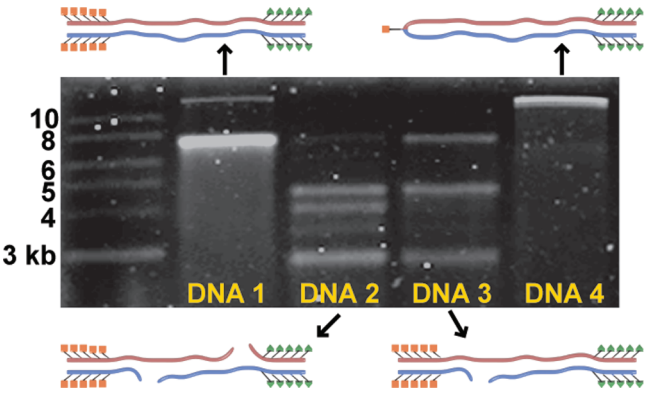

Figure 2. Denaturing alkaline agarose gel analysis of the DNA constructs shown in Figure 1a−d, where DNA 1 is for torsionally constrained DNA, DNA 2 is same as DNA 1 but with a few nicks ($n \approx 2-3$), DNA 3 has a single nick, and DNA 4 is for the topologically closed assay.

A definitive biochemical test to ensure the continuity of the DNA's phosphodiester backbone is to denature the base pairs and analyze the resulting ssDNA (Figure 2). Gel analysis of the DNA for the topologically closed assay showed a single band running at ~2× the molecular weight as the torsionally constrained DNA. Thus, both single-molecule and biochemical results demonstrate the continuity of our substrate for the topologically closed assay.

Having established confidence in the substrates, we show that the overstretching forces (and therefore the energies) of DNA 3 and 4 are statistically indistinguishable. Quantitatively, the topologically closed assay overstretched at 65.2 ± 0.5 pN (mean ± S.E; $N = 10$) while DNA with one nick overstretched at 64.8 ± 0.5 pN ($N = 12$). These forces agree with published values,[4,5] including prior work on the same instrument used here but stretching DNA with two free ends.[22] This quantitative agreement argues for the same mechanism when rapidly (5 μm/s) stretching DNA 3 and DNA 4; any proposed mechanistic differences are now bounded by this small observed difference (<1%).

In summary, DNA in the topologically closed assay overstretched at the canonical 65 pN but lacked hysteresis. Hence, ssDNA generation from nicks or free ends, the leading mechanism for overstretching DNA,[17,20] is not a compulsory step in overstretching. Rather, such peeling is the primary cause of hysteresis in the force-extension curve. Integrating this mechanistic insight with the prior work that visualized ssDNA and dsDNA domains seconds after overstretching suggests a model in which DNA is overstretched, followed by a conversion of overstretched DNA into distinct domains.

Several formal possibilities for this initial overstretched DNA include S-DNA and internally distributed ssDNA induced by force. We disfavor a model in which ssDNA is concurrently generated at a point of free rotation, since rapid stretching of DNA 3 and DNA 4 led to the same F_o, and this model requires tension distributed on one ssDNA strand for DNA 3 and two strands for DNA 4. Further insight can be provided by high-time resolution experiments with sub-pN force precision and stability.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, including DNA constructs, gel analysis, and optical-trapping assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

We thank Wayne Halsey for DNA preparation and Rebecca Montange for critical reading of the manuscript. This work was supported by NIST. T.T.P. is a staff member of NIST's Quantum Physics Division.

REFERENCES

(1) Bustamante, C.; Bryant, Z.; Smith, S. B. *Nature* **2003,** *421*, 423.
(2) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992,** *258*, 1122.
(3) Marko, J. F.; Siggia, E. D. *Macromolecules* **1995,** *28*, 8759.
(4) Smith, S. B.; Cui, Y.; Bustamante, C. *Science* **1996,** *271*, 795.
(5) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J. L.; Chatenay, D.; Caron, F. *Science* **1996,** *271*, 792.
(6) Williams, M. C.; Rouzina, I.; Bloomfield, V. A. *Acc. Chem. Res.* **2002,** *35*, 159.
(7) Strick, T. R.; Allemand, J. F.; Bensimon, D.; Croquette, V. *Biophys. J.* **1998,** *74*, 2016.
(8) Vlassakis, J.; Williams, J.; Hatch, K.; Danilowicz, C.; Coljee, V. W.; Prentiss, M. *J. Am. Chem. Soc.* **2008,** *130*, 5004.
(9) Clausen-Schaumann, H.; Rief, M.; Tolksdorf, C.; Gaub, H. E. *Biophys. J.* **2000,** *78*, 1997.
(10) Liu, N.; Bu, T.; Song, Y.; Zhang, W.; Li, J.; Shen, J.; Li, H. *Langmuir* **2010,** *26*, 9491.
(11) Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2001,** *80*, 882.
(12) Leger, J. F.; Romano, G.; Sarkar, A.; Robert, J.; Bourdieu, L.; Chatenay, D.; Marko, J. F. *Phys. Rev. Lett.* **1999,** *83*, 1066.
(13) Konrad, M. W.; Bolonick, J. I. *J. Am. Chem. Soc.* **1996,** *118*, 10989.
(14) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2001,** *80*, 874.
(15) Wenner, J. R.; Williams, M. C.; Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2002,** *82*, 3160.
(16) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **2001,** *80*, 1932.
(17) van Mameren, J.; Gross, P.; Farge, G.; Hooijman, P.; Modesti, M.; Falkenberg, M.; Wuite, G. J.; Peterman, E. J. *Proc. Natl. Acad. Sci. U.S. A.* **2009,** *106*, 18231.
(18) Whitelam, S.; Pronk, S.; Geissler, P. L. *Biophys. J.* **2008,** *94*, 2452.
(19) Fu, H.; Chen, H.; Marko, J. F.; Yan, J. *Nucleic Acids Res.* **2010,** *38*, 5594.
(20) Williams, M. C.; Rouzina, I.; McCauley, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2009,** *106*, 18047.
(21) Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Nat. Struct. Biol.* **1999,** *6*, 346.
(22) Paik, D. H.; Seol, Y.; Halsey, W. A.; Perkins, T. T. *Nano Lett.* **2009,** *9*, 2978.