Structural Transitions of a Twisted and Stretched DNA Molecule

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(Received 10 December 1998)

We report results of a micromanipulation study of single double-helical DNA molecules at forces up to 150 pN. Depending on whether the DNA winding is allowed to relax, or held fixed, qualitatively different structural transitions are observed. By studying the transitions as a function of winding the different DNA structures underlying them are characterized; this allows us to report the first estimate of *S*-DNA helicity. A model is introduced to describe these transitions; in addition to *B*-DNA, we find that four DNA states are needed to describe the experiments.

PACS numbers: 87.14.Gg, 36.20.Ey, 64.90.+b

Development of techniques to apply picoNewton forces to single biomolecules has led to study of the elasticity of single double-stranded DNAs (dsDNA). DNA is remarkably flexible: it can be stretched in excess of 1.7 times its (Watson-Crick) *B*-form length [1,2]. The twisting of a dsDNA may also be controlled, and it has been shown that DNA may be undertwisted or overtwisted by several times its natural helicity of one turn per 10.5 base pairs (bp) [3–5]. As DNA is stretched and/or twisted, it can transform to new states, some of which are relevant to specific DNA-protein interactions [6].

The first new state discovered by micromanipulation experiments, *S*-form, was obtained by stretching DNA to 1.7 times its *B*-form length [1,2]. Another new state of DNA (*P*-form) was later discovered in stretching-twisting experiments [5]; *P* is supposed to have exposed bases outside of a double phosphodiester backbone. Many questions concerning these transitions remain unanswered. Is *S*-DNA completely untwisted (like a ladder) or helical? What is the phase diagram of a single dsDNA subjected to both stretching and twisting? Our aim here is to address these questions with experimental results interpreted with the help of a simple statistical-mechanical model.

In our experimental setup, a λ-EMBL3 dsDNA (44 kb; *B*-form length $L_0 \approx 15.1\ \mu$m) is linked at one end to a small dsDNA fragment (700 bp), each strand of which has $\approx$25 biotin labeled bases. The other end is linked to a similar dsDNA fragment containing digoxygenin labeled bases (details of dsDNA preparation are published elsewhere [6]). With these functionalized ends, the molecule can be attached with its biotinilated end on a Streptavidin coated bead (3 μm diameter), and with its other end, on an antidigoxygenin-coated optical fiber acting as a force transducer. This is all immersed in 100 mM phosphate buffer at *p*H 7.4 with 500 mM of added NaCl. Force-extension measurements are done with a previously described setup [6]: a micropipette mounted on a piezoelectric translator holds the bead by suction and imposes fixed extension to the molecule; the stretched molecule exerts a force on the optical fiber and deflects it. A laser beam going through the fiber, focused on a two-quadrant detector with a 40× microscope objective, allows the deflection (proportional to the tension in the molecule) to be recorded. Rotating the micropipette around the micropipette-dsDNA molecule common axis changes DNA winding. Since both strands at each end of the molecule are attached to either optical fiber or bead, the DNA is torsionally constrained.

Torsional constraint of the molecule ends constrains the linking number Lk of the two strands of the DNA. Lk is the sum of the twist (Tw, the number of times one phosphodiester backbone wraps locally about the other) and the writhe (Wr, the average number of nonlocal self-crossings of the double helix) [7]:

$$\mathrm{Lk} = \mathrm{Tw} + \mathrm{Wr}\,.$$

An experiment begins with capture of a bead, on which a single dsDNA is grafted with Lk close to its natural number of helical turns, $\mathrm{Lk} = \mathrm{Lk}_0 = \mathrm{Tw}_0 \approx 4200$. Each micropipette turn changes Lk by ± 1. We may describe linkage in terms of fraction of turns added or removed:

$$\sigma = \frac{\mathrm{Lk} - \mathrm{Lk}_0}{\mathrm{Lk}_0}\,.$$

Rotation of the micropipette at zero extension selects σ; then a force-extension measurement is done. Many of the excess turns are stored as Wr when the molecule is unextended (e.g., as plectonemic supercoils); as the molecule is stretched, Wr is converted into Tw. For high extension, the Tw of an extended (and thus unwrithed dsDNA) is essentially under experimental control.

Some molecules had broken covalent bonds in their backbones ("nicks"); such molecules are not under torsional constraint. Nicked molecules behave qualitatively differently from unnicked molecules since they may torsionally relax as they are stretched. Figure 1 shows the force versus extension for a nicked DNA. As previously observed [1], there are four elastic regimes: first, up to extension $\approx$15 μm, only small forces ($<$10 pN) are necessary to remove thermal bending from the random coil. Once these bends are removed, the force rises linearly for

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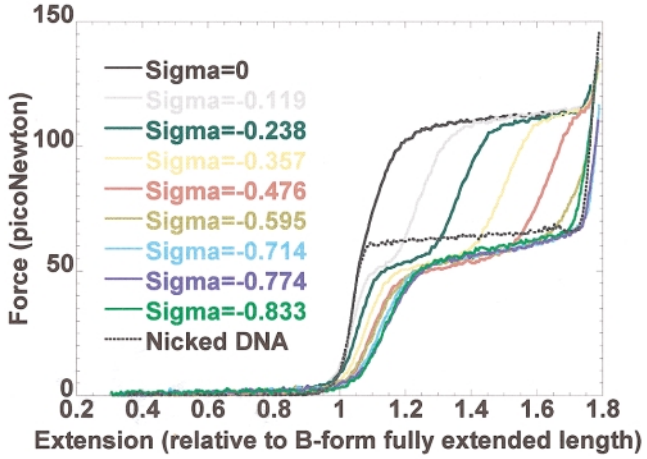

FIG. 1 (color). Force versus extension for underwound dsDNA (σ from 0 to -0.833 according to the color code given in inset), in comparison with force extension diagram of a nicked molecule (dotted black line).

extensions 15 to 16.5 μm, and a linear stretching elastic constant can be measured ($\gamma = 220$ kT/nm $= 900$ pN) [8]. Near 16.5 μm and 65 pN a force "plateau" occurs, as the dsDNA abruptly stretches to 1.7 times its *B*-form contour length. Beyond 26 μm, force increases again quickly with further extension. The 65 pN plateau has been interpreted as a cooperative transition from usual *B*-DNA to a new stretched *S*-form [1]. *S*-DNA is 1.7 times longer than *B*-DNA, but its precise structure is unknown; although a ladderlike form has been proposed [2,9], *S*-DNA is less than the 2 times *B*-form length expected for complete unwinding of the double helix. This suggests that *S*-DNA has nonzero helicity, and we will present in the following a rather direct way to determine this important parameter.

Figure 1 also shows force versus extension for an unnicked dsDNA with $\sigma = 0$; a plateau occurs at 110 pN, at nearly double the force as for nicked molecules. Both elastic responses are reversible on a one minute time scale. The next question is how dsDNA elasticity changes with σ (e.g., is there a continuous evolution from one type of curve to another as the dsDNA is progressively unwound?), and whether one can measure the unwinding needed for a fixed-twist molecule to exhibit an elastic behavior similar to the one of a free twist.

For $\sigma = 0$ to $\sigma = -0.8$, the force-extension curves of Fig. 1 are obtained. At $\sigma \approx -0.1$, a $\approx$50 pN plateau appears, increasing from zero width as more negative turns are made; a second plateau follows at 110 pN. For $\sigma = -0.72 \pm 0.05$, the 110 pN plateau disappears. Our interpretation is that when we underwind to, e.g., $\sigma = -0.24$, the turns are first stored in an untwisted double helix. Upon application of force, the molecule partially transforms to the *S*-form once a force of 50 pN is applied. When the underwinding is consumed by *S*-DNA, the remainder of the molecule will be *B*-DNA with $\sigma = 0$, and will display a transition at $\approx$110 pN. Further unwinding progressively narrows the 110 pN plateau while widening the one at 50 pN; a progressively larger fraction of the molecule can transform to *S*-form at 50 pN. The length increase of the first plateau is proportional to $|\sigma|$ until $\sigma = 0.72$. Combined with the observation that the elasticities of nicked DNA and unnicked DNA with $\sigma = -0.72$ are the same beyond the end of their plateaus, we conclude that the DNA in both situations is in the same state, i.e., entirely in *S*-form. Thus we are able to give the first estimate of *S*-DNA helicity: *S*-DNA has $\sigma = -0.72 \pm 0.05$, and is a helix with a pitch of 22 nm and 37.5 base pairs per turn.

Our $\sigma > 0$ data (Fig. 2) reproduces observations by Allemand *et al.* of a new plateau near 25 pN, proposed to be due to straightening of plectonemically supercoiled *P*-DNA [5]. Overwinding from $\sigma = 0$ to $\sigma = +1.07$, the molecule fraction which must transform to unwrithed *P*-DNA increases, widening the 25 pN plateau (from initially zero width) while narrowing the 110 pN plateau. Extrapolating our high-force results to $\sigma \approx +3$, the 110 pN plateau should disappear since the whole molecule would be *P*-DNA via overwinding, and there would be no need for creation of *P* by force. The end of the 25 pN plateau should likewise be located near extension 1.6 for $\sigma \approx +3$. We therefore predict *P*-DNA to have $\sim$2.62 bp/turn (close to an estimate of 2.4 bp/turn from Allemand *et al.*) and an extension 1.6 times that of *B*-DNA (slightly smaller than the factor 1.75 determined by Allemand *et al.*; these differences may be due to the differing buffers used).

We argue the 110 pN plateau of unnicked $\sigma = 0$ DNA to involve creation of coexisting *S*- and *P*-DNA, as follows. Beyond 50 pN, *B*-DNA cannot transform to

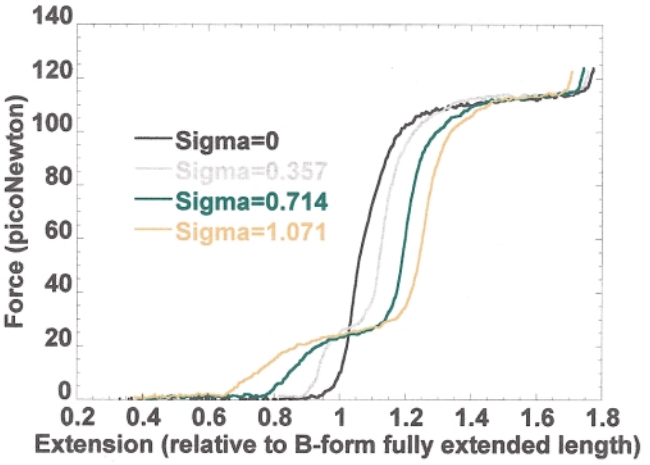

FIG. 2 (color). Force versus extension diagram for positively supercoiled dsDNA (σ from 0 to 1.071). Helicity of *P*-DNA of $\sigma \approx +3$ is determined from where extension at low force is extrapolated to vanish.

S-DNA without unwinding. However, if a compensating amount of P-DNA is created along with S, the molecule may lengthen while keeping its net linkage at zero. The proportion of each structure is fixed by this compensation ($\frac{1}{5}$ P-DNA to $\frac{4}{5}$ S-DNA at $\sigma = 0$). This is in accord with the observed overextension of 1.66 times the λ-EMBL3 B-length, the appropriately weighted average of the S- and P-form extensions.

To justify this interpretation we present a model of the transitions. Position along our molecule is described using B-form contour length s ($\Delta s = 0.34$ nm corresponds to 1 bp). Linkage density is described by $\Omega(s)$ (in excess of the B-form twist) in rads/nm, and stretching is described using the local strain $u(s)$ [8]. For relaxed B-DNA, $u = \Omega = 0$; if doubled in length, $u = 1$, and if entirely untwisted to form a ladder $\Omega = -1.85$ rad/nm ($-2\pi/3.4$ nm). Linkage is added up using $d\sigma = [\Omega(s)/2\pi]ds$.

As a function of contour length s we consider the coarse-grained energy

$$\frac{E}{k_BT} = \int_0^L ds\Bigg[\frac{w}{2}\left(\frac{du}{ds}\right)^2 + \frac{d}{2}\left(\frac{d\Omega}{ds}\right)^2 - \sum_{i=0}^{4} A_i \exp\left(-\frac{(u - \langle u_i\rangle)^2}{2\delta_i^2}\right) \times \exp\left(-\frac{(\Omega - \langle\Omega_i\rangle)^2}{2\varepsilon_i^2}\right)\Bigg],$$

which describes five distinct DNA states which can be slightly deformed (parameters for the five Gaussian wells are in Table I). Large deformations lead to a large free energy cost (the zero-energy level from which all the wells hang). Domain-wall stiffnesses ($w = 10$ nm as in Ref. [8]; also $d = 10$ nm^{-3}) define a minimum length to droplets of the various states. We consider equilibrium statistical mechanics of this model, but before we describe the results, we describe how the many parameters are constrained and fit.

The five states are B-DNA ($\Omega = 0$, $u = 0$), S-DNA ($\Omega = -1.33$, $u = 0.7$), P-DNA ($\Omega = 5.6$, $u = 0.6$), Z-like DNA ($\Omega = -3.9$, $u = 0.13$), and sc-P DNA ($\Omega = 6.0$, $u = -1.0$). The B, S, and P states have been previously observed; our Z state represents the known Z-DNA structure which is a left-handed double helix of helix repeat and length similar to B-form. Since the B, S, P, and Z states are considered to be fully extended and unwrithed, the (u, Ω) locations of these wells are fixed by known extensions and twists. sc-P DNA is a simple model of plectonemically supercoiled (therefore zero extension, or $u = -1$) P-DNA; sc-P DNA has already been proposed to explain the behavior of overtwisted DNA for <50 pN [5]. Z-like (undertwisted and near-B length) and sc-P-like (overtwisted and collapsed) states are essential to avoid creating S- and P-DNA at forces <20 pN by unwinding and overwinding, and thus are needed to allow double plateaus as in Figs. 1 and 2.

In each well, the extensional elastic constants are A_i/δ_i^2 and the torsional rigidities are A_i/ε_i^2; for the DNA case ($i = 0$) our choice of parameters for the $i = 0$ well gives the rigidities $\gamma = 320$ nm^{-1} and $C = 110$ nm^{-1} known for B-DNA [8]. The remaining states have extensional elasticities which we have fit using the high-force slopes of Figs. 1 and 2; the twisting elasticities were chosen to be comparable to B-DNA. Further constraint is put on the amplitudes and widths of the wells by the requirement that the transitions occur at the experimentally observed forces.

We treat the statistical mechanics of our model exactly with continuum transfer matrix techniques [8], with forces and torques added conjugate to u and Ω. Note that our model does not include the double helix entropic elasticity [8]; we describe only the DNA configurations expected for large forces (>10 pN), either straightened out, or tightly plectonemically supercoiled. Figure 3 shows the results. For nicked DNA (zero applied torque) a single plateau occurs near 60 pN, as B-DNA converts to S-DNA. For fixed

TABLE I. Parameters for the five-state DNA elasticity model of the text. Each state (B, S, P, Z, and sc-P) is described by a free energy amplitude A, an average stretching $\langle u\rangle$, an average twist $\langle\Omega\rangle$, and stretching and twisting fluctuation widths δ and ε.

	B-DNA	S-DNA	P-DNA	Z-DNA	sc-P DNA
A_i (nm^{-1})	50	44	9.0	41	45
$\langle u_I\rangle$	0	0.70	0.60	0.13	-1.0
δ_I	0.40	0.27	0.15	0.32	0.24
$\langle\Omega_i\rangle$ (nm^{-1})	0	-1.33	5.55	-3.85	6.0
ε_i (nm^{-2})	0.67	0.28	0.20	0.52	0.31

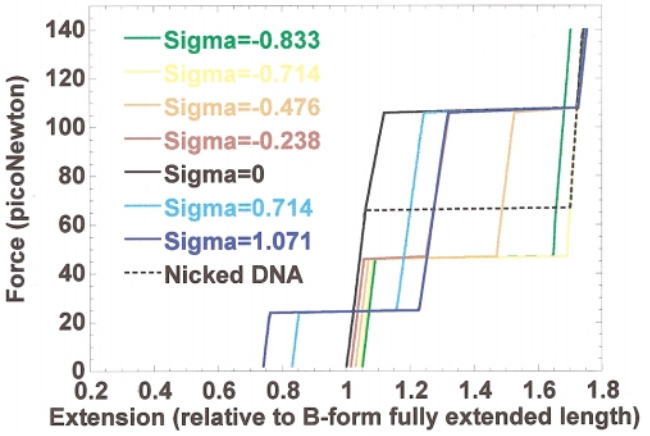

FIG. 3 (color). Theoretical force versus extension for overwound ($\sigma > 0$), underwound ($\sigma < 0$), and nicked (dashed line) DNA for the five-state model discussed in the text (parameters in Table I). The model shows four distinct transitions, and the double-step force curves, observed experimentally.

$\sigma = 0$, such a transition is not possible; instead a mixed $S + P$ state is created at 110 pN, with zero net linkage. For $\sigma < 0$, at low forces Z-DNA appears; then as force is increased, $Z + B$ transforms to $S + B$ near 50 pN, and finally $S + P$ appears at 110 pN. For $\sigma = -0.72$ (i.e., that of S) no P is needed, and a single transition to S occurs at 50 pN. For larger underwindings, the high-force state involves coexisting $Z + S$, and is slightly shorter than pure S-DNA.

Overwinding leads to a similar sequence of transitions: at low force, sc-$P + B$ appears, transforming first to $B + P$ at 25 pN and then to $S + P$ at 110 pN. For overwinding compatible with that of P-DNA ($\sigma = +3$), a single transition from B + sc-P to P occurs at 25 pN. The model is in accord with experimental data over a large extension and linkage range, justifying our experimental interpretation.

The model fails to reproduce some experimental results: there is no "softening" of B-DNA extensional modulus with underwinding as is apparent in Fig. 1; also, the broadening of the transitions from $Z + B$ to $S + B$ in the theory are slightly different from those seen experimentally. In addition, our model does not describe DNA elasticity for forces <10 pN. These shortcomings follow from the lack of any accounting for bending fluctuations of the extended states, and our use of simple symmetrical shapes for the wells defining the different states (note our model omits the microscopic twist-stretch coupling expected from the chirality of B-DNA, although there is an effective coupling of this type due to the asymmetry of where the different potential wells are placed). Finally, the present model ignores the sequence inhomogeneity of the molecules, which can be expected to broaden the transitions.

Our model shows that the rather abrupt and multiple-step force-distance curves can occur in a one-dimensional system, and also establishes that the 110 pN plateau seen for $\sigma = 0$ DNA can occur via simultaneous creation of S- and P-DNA. Although the many parameters cannot all be considered as fully determined by comparison with experiment, the basic "phase diagram" of DNA states in this model is robust to changes in the potential.

In conclusion, we have explored the mechanical response of twist-constrained DNA to large forces, which allows us to give an overall picture of dsDNA conformations. We find that B-DNA transforms to a mixture of S-DNA and P-DNA at a force near 110 pN if stretched with $\sigma = 0$. We find that two more states are required to explain the data at all σ, plausibly supercoiled P-DNA, and an unwound (Z-like) double helix. This overall picture of dsDNA conformations allows us to determine that S-DNA has $\approx$38 bases per right-handed turn and a helical pitch of 22 nm.

The work at LDFC was supported by the Fondation pour la Recherche Médicale, the DRET-Ministère des Armées, the CNRS-MPCV, the University Louis Pasteur in Strasbourg, and NATO; the work at UIC was supported by the Petroleum Research Foundation, the NSF through Grant No. DMR-9734178, by Research Corporation, and by The Whitaker Foundation.

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