Phase coexistence in a single DNA molecule

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Abstract

A DNA molecule subjected to stretching or torsional stress can undergo cooperative phase transitions to new structures. For a supercoiled molecule, these structures coexist with canonical Watson-Crick B-DNA over a large range of forces and degrees of coiling. We will describe these stress-induced transitions and discuss the new structures and their possible biological relevance.

1 Introduction

New micromanipulation techniques now enable physicists and biologists to study the behavior of single biomolecules such as DNA or proteins. The initial studies were carried out on torsionally unconstrained DNA at low forces, so that the work of stretching went mostly into a reduction of the configurational entropy of the DNA polymer. The resulting force vs. extension curves fit very nicely the predictions of the Worm Like Chain (WLC) model appropriate for semi-flexible polymers [1]. In fact, stretching experiments are the most accurate method for estimating the persistence length of DNA [2]. This excellent agreement now serves as a basis for the calibration of most single molecule force experiments, at least while an entropic elasticity regime persists. This is indeed the case of proteins in denaturating conditions [3,4] and of DNA. Further experiments have shown that overstretching DNA [5,6] or subjecting it to a torsional stress [7,8] might induce the molecule to undergo a transition from the classical Watson-Crick double-helical B form [9] to new structural forms. The purpose of this paper is to describe these new structures of stressed DNA and to discuss their possible biological relevance. In a previous paper [10] we have described the stretching behavior of coiled DNA at low forces, here we shall concentrate on what happens to the molecule at high forces and high torsional stresses.

To describe DNA under torsional stress it is first necessary to introduce some topological concepts. The number of times the two strands of the DNA double-helix are intertwined - the linking number of the molecule (Lk) - is a topological constant, the sum of two geometrical characteristics of the system, its writhe (Wr) and its twist (Tw): $Lk =$

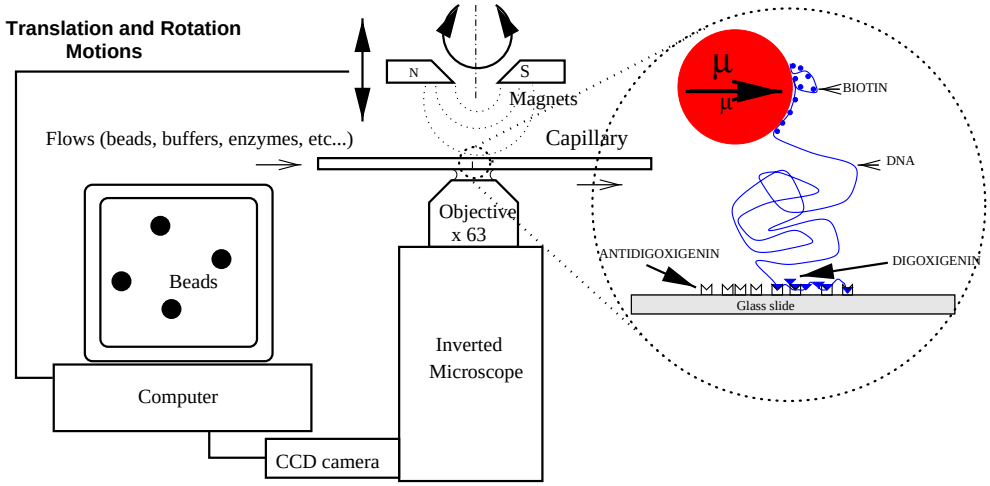

Fig. 1. Schematic view of the apparatus used to twist and stretch single DNA molecules. DNA molecules were first prepared with *biotin* attached to one end and *digoxigenin (dig)* bound to the other. These end-labeled DNA molecules are incubated with *streptavidin*-coated magnetic beads and then flowed into a square glass capillary coated with an antibody to *dig*, *antidig*. The DNA molecules bind specifically to the bead via *biotin/streptavidin* coupling and to the glass via *dig/antidig* coupling. The capillary is placed above an inverted microscope. Magnets are placed above the capillary. By approaching the magnets we increase the pulling force on the bead and thus on the molecule. By rotating the magnets the molecule is twisted at constant force. A frame grabber installed in a PC allows tracking of the Brownian fluctuations $\langle \delta x^2 \rangle$ of the bead. The determination of $\langle \delta x^2 \rangle$ and of the molecule's extension l leads to a measure of the stretching force $F = k_B T l / \langle \delta x^2 \rangle$.

$Wr + Tw$. Wr is a measure of the coiling of the DNA axis about itself, like a twisted cord forming interwound structures in order to relieve torque. Tw reflects the helical winding of the two strands around each other. For unconstrained linear DNA molecules, assuming the absence of any spontaneous local curvature, $Lk = Lk_0 = Tw_0$ (= the number of helical turns) [11]. One defines the relative change in linking number, or degree of supercoiling, $\sigma = (Lk - Lk_0)/Lk_0 = \Delta Lk/Lk_0$. The value of σ for most circular molecules isolated from cells or virions is roughly -0.06. At fixed Lk the ratio Tw/Wr depends on the force pulling on the molecule, the writhe being suppressed by high forces. As a consequence, pulling on a molecule increases its effective torque.

2 Single molecule micromanipulation

The typical forces at the molecular scale are of order $k_B T/\text{nm} \sim 4$ pN. This is typically the stall force of a single molecular motor such as myosin (4 pN [12]) or RNA-polymerase (15 pN [13]). It is also the typical force needed to unpair the DNA bases (about 15 pN [14]). At a force of 75 pN DNA overstretches [5,6]. To produce and measure such forces on a DNA molecule we use a single molecule manipulation technique. Briefly, it consists of stretching a single DNA molecule (λ-DNA $\sim$ 50000 base-pairs $\sim 16\mu$m is often used) bound at one end to a surface and at the other to a magnetic bead (see Fig.1). Small magnets, whose position and rotation can be controlled, are used to pull on and rotate

the bead and thus stretch and twist the molecule. As one turn of the magnets implies a change of one turn of the molecule, we have simply $\Delta Lk = \pm n$, where $\pm n$ is the number of turns the magnet rotates. The tethered bead ($1 - 4.5\mu m$ in diameter) exhibits Brownian motion whose amplitude gives access to the force applied to the molecule: the stronger the force, the smaller the fluctuations. This system allowed us to apply and measure forces ranging from a few femtoNewtons to nearly 100 picoNewtons (see [15]).

3 Stretched DNA

Fig. 2 shows the force versus extension curve of single DNA molecules in the range $0.06\text{pN} < F < 100\text{pN}$. As can be seen at low forces ($F < 6\text{pN}$) the WLC model (continuous curve) fits the data very well over three decades in force. This model describes a DNA molecule as a semi-flexible polymer chain of length l_0 and bending modulus B (or persistence length $\xi = B/k_BT$). The energy $\mathcal{E}$ of a given configuration $\vec{t}(s)$ of a molecule stretched by a force F along the $z-$axis is:

$$\mathcal{E} = \frac{B}{2}\int_0^{l_0}(\frac{\partial\vec{t}}{\partial s})^2 ds \; - \; F\int_0^{l_0}\vec{t}\cdot\hat{z}ds \tag{1}$$

where $\vec{t}(s)$ is the local tangential vector at curvilinear coordinate s. The first term is the bending energy, while the second sets the extension l of the molecule: $0 < l < l_0$. The free energy of the polymer $\mathcal{F} = -k_BTln\mathcal{Z}$ is obtained, as usual, by computing the partition function $\mathcal{Z}$, the Boltzman-weighted sum over all configurations of a molecule at extension l:

$$\mathcal{Z} = \sum_{\{\vec{t}(s)\}} e^{-\beta\mathcal{E}} \tag{2}$$

This sum is dominated by the lowest configurational energy contributions. To compute it, J. Marko and E. Siggia [1] have used the formal analogy between this problem and the quantum propagator of an electric dipole in an electric field. They were able to find the ground state of the associated Schrödinger equation. Its energy g_{WLC} is a function of a single dimensionless parameter: $u = F\xi/k_BT$. Hence the free energy of a stretched DNA chain is:

$$\frac{\mathcal{F}}{k_BT} = g_{WLC}(F\xi/k_BT) \tag{3}$$

The relative extension of the molecule at a given force is: $x = l/l_0 = -\partial g_{WLC}/\partial u$. Inverting this relation, the exact solution can easily be computed [16]. A convenient and very accurate approximation (within 0.1%) is:

$$u = \frac{F\xi}{k_BT} = x - \frac{1}{4} + \frac{1}{4(1-x)^2} + \sum_{i=2}^{7} a_i x^i \tag{4}$$

with $a_2 = -0.5164228$, $a_3 = -2.737418$, $a_4 = 16.07497$, $a_5 = -38.87607$, $a_6 = 39.49944$, $a_7 = -14.17718$ [16].

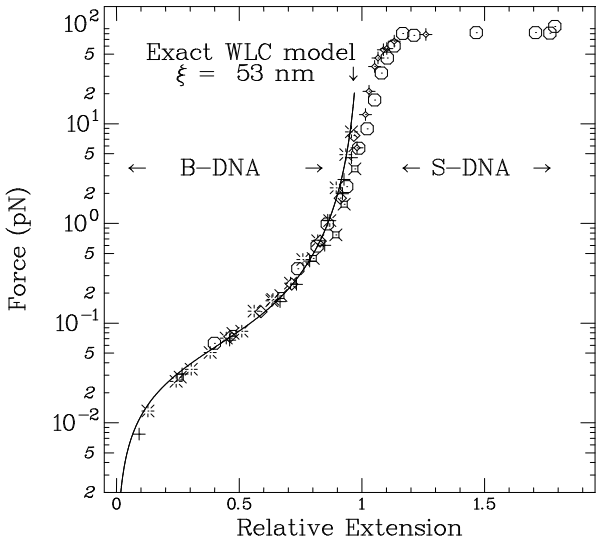

Fig. 2. Force versus relative extension curves of single DNA molecules. The dots correspond to several experiments made over a wide range of forces. The force was measured using the Brownian fluctuation technique [7]. The full line curve is a best fit to the WLC model for forces smaller than 5 picoNewtons. At high forces, the molecule first elongates slightly, as would any material in the elastic regime. Above 70 pN, the length abruptly increases, corresponding to the appearance of the new S-DNA phase.

Fitting the force vs. extension data to this theoretical prediction yields the most accurate estimate of the DNA's persistence length ξ and allows the study of its dependence on ionic conditions [17].

Beyond this entropic regime, i.e. from $\sim$ 6 pN to about 70 pN, DNA behaves like an elastic rod with stiffness $EA \sim 1000$ pN [6] (where E is the Young modulus of DNA and A its effective cross-sectional area [18]). Neglecting entropic contributions, the force vs. extension curve follows a simple Hookean law: $F = EA(x-1)$ (with $x > 1$). Notice that there exist some ad-hoc formula interpolating between the entropic and Hookean regimes, e.g. replacing the term $(1-x)^2$ in Eq.4 by $(1-x+F/EA)^2$.

Finally, at $\sim$ 70pN, a very interesting cooperative (i.e. quasi-first order) transition is observed from a slightly stretched ($\sim$ 10%) B-DNA phase to a highly stretched ($\sim$ 80%) phase, called S-DNA [5,6,19,20]. Although there are, as yet, no crystallographic data on the structure of S-DNA, very old experiments [21] (pre-dating Watson and Crick's discovery of the double helix structure [9]) have shown that stretched DNA fibers (made up of many side by side molecules) undergo a birefringent transition apparently due to a tilt of the DNA bases upon stretching. Recent numerical calculations indeed suggest that a tilted structure for S-DNA is one of two possibilities (the other being a straight ladder) depending on precisely how the strands are pulled [5,22].

A phenomenological Ising-like description of the B-DNA $\rightarrow$ S-DNA transition has been proposed [5,19], where the force plays the same role as the magnetic field in a ferromagnetic context. In this model, the observed sharpness of the transition (its high cooperativity) is associated with a large interfacial energy between the B and S phases, suggesting that the typical size of domains is about 100 bases long [19].

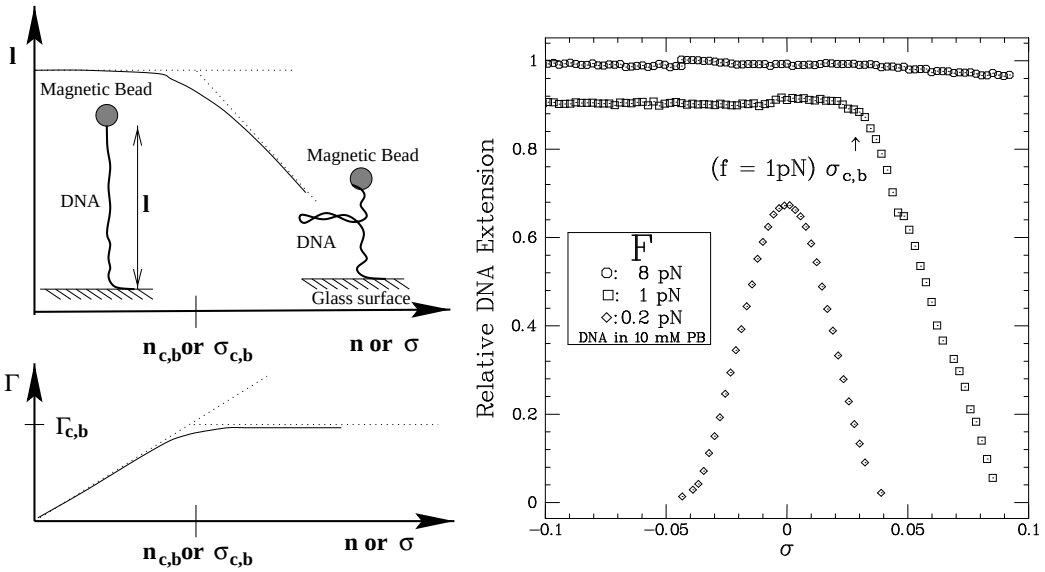

Fig. 3. LEFT: schematic view of the buckling transition of a twisted rubber tube. We display both the tube length and the applied torque at a constant pulling force, F. At first the tube length remains constant while the torque increases linearly with the number of turns applied. At a critical value $n_{c,b}$, which scales as $\sqrt{F}$, the length begins to decrease and the torque stagnates due to the buckling instability which leads to interwound structures. For DNA molecules the behavior is roughly the same, except that thermal fluctuations round off the transition. RIGHT: we have plotted the extension of a real DNA molecule which displays a richer behavior. At low force ($F = 0.2pN$) the rubber tube model with thermal fluctuations is correct. The molecule contracts if it is over- or under-wound. The rounding off due to fluctuations is so strong that the extension curve does not have a flat top. At intermediate force ($F = 1pN$), the buckling transition is observed as predicted for $n > 0$ at $n_{c,b}$ but disappears for $n < 0$. Here the appearance of denatured DNA occurs before the torque has reached the buckling instability threshold at that force. For still larger forces ($8pN$), the buckling instability is not seen on either side. Denatured DNA (for $n < 0$) and a new highly twisted phase, P-DNA (for $n > 0$), will prevent the torque from reaching its critical value for buckling.

4 The torsional buckling instability

Twisting DNA leads to a torsional buckling instability analogous to that observed on telephone cords or rubber tubes. This instability leads to the formation of interwound structures known as plectonemes. Of course, a DNA molecule is also animated by very strong thermal fluctuations which play an important role. However it is instructive to first consider the purely mechanical (zero temperature) instability of a rubber tube of length l and torsional constant κ. If we firmly hold one end of the tube while simultaneously rotating and pulling on the second end with a force F, we observe the following phenomenon (see Fig.3, LEFT): when the twist constraint is small, the associated torque Γ increases linearly with the twist angle θ, $\Gamma = \kappa\theta/l$ and the tube remains straight. As the tube is further twisted, a critical twist angle $\theta_{c,b}$ and torque $\Gamma_{c,b}$ are reached and the tube ceases to be straight: it locally buckles and forms a small loop of radius $R_{c,b}$. The torsional energy thus gained is $2\pi\Gamma_{c,b}$, whereas the energy cost (due to bending and work against F) is (see Eq.1): $\pi B/R + 2\pi RF$ (which is minimized for a loop of radius $R_{c,b} = \sqrt{B/2F}$).

The critical torque for the formation of plectonemes is controlled by the balance between energy gain and cost, i.e. by the stretching force: $\Gamma_{c,b} = \sqrt{2BF}$. As we twist the tube further, we increase the length of the plectonemes but, the torque in the tube remains basically fixed at its critical value $\Gamma_{c,b}$.

For DNA, the picture is pretty much the same [24]. The thermal fluctuations which will be most important near the mechanical instability at $\theta_{c,b}$ will tend to round it off. Hence as one is coiling a DNA molecule under fixed force F, one observes the following behavior (see Fig.3): at low degree of supercoiling $|\sigma|$ the molecule's extension varies little. Beyond a critical value σ_c (which depends on the force), the molecule shortens continuously as it is twisted further. An accurate theoretical treatment of this behavior has been given by Bouchiat and Mézard [16].

The torsional buckling instability just described treats the DNA molecule as a continuous elastic tube. It ignores the underlying double-helical structure of the molecule, and its relevance is therefore limited to very low forces ($F < 0.4$pN) or low degrees of supercoiling ($-0.015 < \sigma < 0.037$). For higher forces and degrees of supercoiling, the buildup of torque in the molecule can be large enough to actually modify its internal structure. This is evidenced by breaking of the $\sigma \to -\sigma$ symmetry in the extension vs. supercoiling curves. It is also obvious in the force vs. extension curves of coiled molecules. As a critical force is reached (*ipso facto* a critical torque), the molecule undergoes a transition from a contracted state (plectonemic B-DNA) to a highly extended one. As we shall see below, this state is characterized by the coexistence of B-DNA with denatured DNA (dDNA, for $\sigma < 0$) and with a new phase called P-DNA (for $\sigma > 0$). In 10 mM phosphate buffer (PB), the critical force F_{c-} required to induce the formation of localized regions of denatured DNA is $F_{c-} \sim 0.5$ pN. The critical degree of unwinding beyond which DNA can be induced to denature is $\sigma_{c-} = -0.015$ and the associated critical torque is $\Gamma_{c-} \sim 8$ pN nm. For overwound molecules the relevant values are: $F_{c+} = 3$ pN, $\sigma_{c+} = 0.037$ and $\Gamma_{c+} \sim 20$ pN $\cdot$ nm. Notice that $\Gamma_{c-}/\Gamma_{c+} \sim \sqrt{F_{c-}/F_{c+}}$, as our simple calculation above showed.

These torque-induced transitions are reminiscent of the B $\to$ S transition observed in overstretched DNA, although there are some major differences. First, they occur at forces two orders of magnitude smaller than the 70 pN necessary for the generation of S-DNA. Second, whereas B-DNA is completely transformed to S-DNA upon increase of the force, this is not the case here. Due to the topological constraint of a fixed linking number, the proportion of the new phase in B-DNA is determined by the degree of coiling σ and the intrinsic twist of the new phase (this is a rather unusual statistical mechanics situation). We shall now present our evidence for the existence of these twist-induced phases.

5 Unwinding DNA leads to denaturation

Let us first consider negative supercoiling ($n < 0$). It is known [23,25] that for values of $\sigma < -0.07$, unstretched DNA undergoes localized denaturation. When stretched, similar local denaturation is observed at smaller values of $|\sigma|$, due to the increased torsional stress resulting from the inhibition of writhe (see Figs.3 left and 4(A) and [15,7]). The denaturation is detected mechanically in the F vs. l curves by a sharp increase in the extension of the molecule at a force $F_{c-} \sim 0.5pN$ (see Fig.4). It is also seen by the breaking

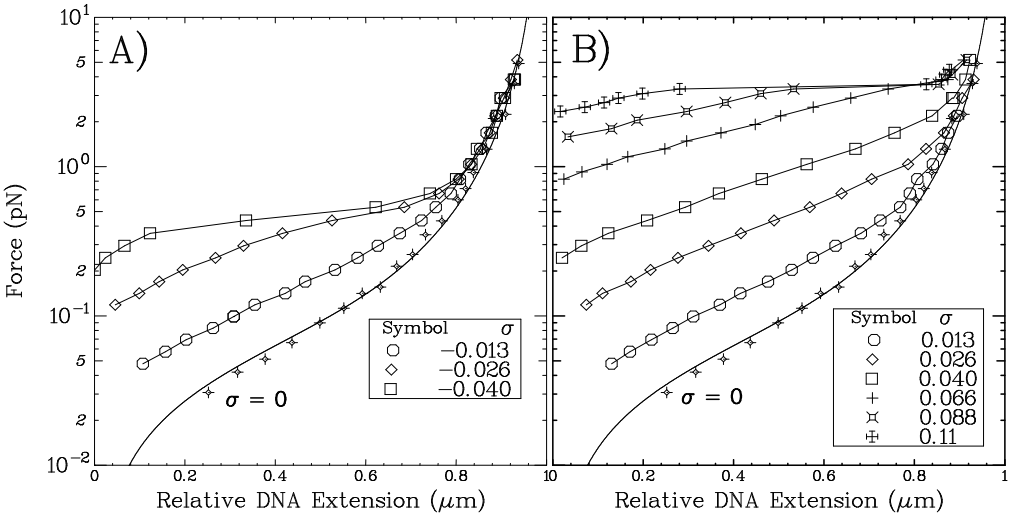

Fig. 4. Force versus extension curves for negatively (A) and positively (B) supercoiled DNA in 10 mM PB. A small range of supercoiling is represented here. The solid curves serve as visual guides. At low forces ($F < 0.3$ pN) the curves are the same for positive and negative supercoiling, whereas at $F_c^- \sim 0.5$ pN the negatively supercoiled molecule undergoes an abrupt transition to an extended state which behaves like a molecule with $\sigma = 0$. The positively supercoiled DNA undergoes a similar abrupt transition to an extended state when $\sigma > 0.037$ and $F = F_c^+ \sim 3$ pN.

of the ($\sigma \to -\sigma$) symmetry in the l vs. σ curves (see Fig.3 right). If, as suggested by these results, the twisted molecule separates into a pure B-DNA phase, with a critical degree of supercoiling, $\sigma_{c-} = -0.015$, and denatured regions, with $\sigma_d \sim -1$, then every extra turn applied to the molecule should increase the fraction of dDNA by 10.5 base-pairs (bp) [9].

Physical evidence for this coexistence of the two phases of DNA can be deduced from the force versus extension curves. With the hypothesis of a separation between the two phases one may write :

- Free energy balance : $\mathcal{F}(F,l) = \beta\mathcal{F}_B + \delta\mathcal{F}_d$ where β (or δ) is the proportion of B (or d) DNA. The mixing entropy is negligible if the interfacial energy between the two phases (which can be neglected in comparison to bulk energies) is greater than k_BT, as expected from the high cooperativity of the transition.
- Conservation of the number of bases : $\beta + \delta = 1$.
- Conservation of the linking number : $\beta\sigma_B + \delta\sigma_d = \sigma$.

Assuming that the portion of B-DNA is torsionally relaxed (we neglect its value: $\sigma_B = \sigma_{c-} \sim -0.015$) and that dDNA is equivalent to two parallel strands ($\sigma_d = -1$), one has : $\delta\sigma_d \approx \sigma$. Using these results and differentiating $\mathcal{F}(F,l)$ with respect to F leads to a linear relation between the extension of the molecule, $l(F,\sigma)$ ($= -\partial\mathcal{F}/\partial F$) and the degree of supercoiling σ :

$$l(F,\sigma) = (1 - \sigma/\sigma_d)\; l_B(F) + (\sigma/\sigma_d)\; l_d(F) \tag{5}$$

where $l_B(F) = l(F,0)$ and $l_d(F)$ are respectively the extension (at a given force F) of the pure B-DNA and dDNA structures. Plotting the extension versus σ at constant force F, we indeed observe a linear relation as expected for $-1 < \sigma < 0$ (data not shown).

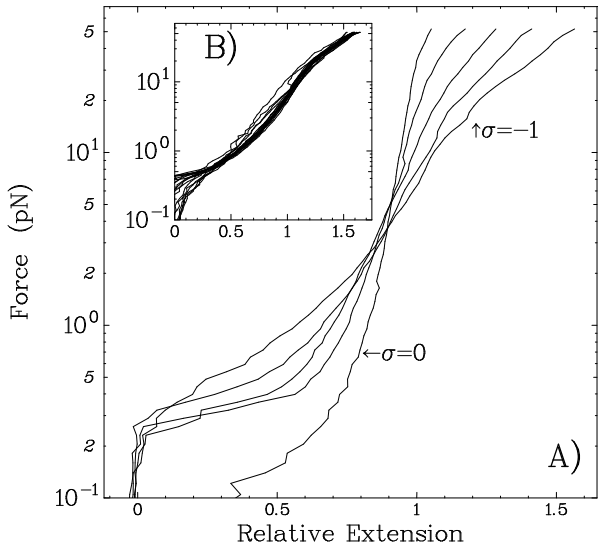

Fig. 5. Experimental evidence for the coexistence of B-DNA and denatured DNA at negative supercoiling in 10 mM PB. (A) Force (F) vs. extension curves for a single DNA molecule obtained at different degrees of supercoiling ($-1 < \sigma < 0$) above the transition to dDNA. As can be seen in (B), all the data collapse to a single curve $l_d(F) = l(F,0) + \frac{\sigma_d}{\sigma}(l(F,\sigma) - l(F,0))$, the force vs. extension curve for dDNA.

Moreover, the force versus extension curves obtained at different values of $-1 < \sigma < 0$ can all be collapsed onto a single $l_d(F)$ curve (independent of σ) in agreement with Eq.5 (see Fig.5 B).

For a stretched molecule, the threshold for denaturation at $\sigma_{c-} \sim -0.015$ is a measure of the denaturation energy E_{denat} provided that the torsional stiffness C of DNA is known. For $C = 75nm$ [17], $E_{denat} \sim 1k_BT$ per bp in 10 mM PB [15], a value corresponding to denaturation of Adenine/Thymine (AT) rich regions of DNA.

To further corroborate the physical picture previously described we have performed two kinds of experiments:

- A biological experiment where we have hybridized a DNA strand homologous to the AT rich region of our DNA [26];
- A chemical experiment where we have chemically modified the bases which were not involved in Waston-Crick pase pairing [8]. This was done by incubating an undertwisted and stretched DNA molecule with glyoxal, a reagent specific for unpaired bases.

Both experiments confirm the presence of denatured regions in stretched underwound DNA in the proportions described in Eq.5, i.e. about $-\sigma$.

6 Overtwisting DNA leads to P-DNA

Let us now consider positive supercoiling ($n > 0$). The elastic behavior of a stretched, overwound DNA reveals the existence of a sharp transition at $F_{c+} \sim 3$ pN (see Fig.6A). By analogy with negative supercoiling, we suggest that stretched, overwound DNA undergoes a phase separation between a fraction of pure B-DNA and a fraction with a new structure, which we term P-DNA. As shown previously, see Eq(5), the coexistence of two phases

implies a linear dependence between the extension of the molecule, $l(F,\sigma)$, and σ (see Fig.6B). This linearity is indeed observed up to $\sigma \approx +3$, where the extension goes to zero for forces < 25pN. The natural twist of the new P-DNA phase, σ_p, is thus $+3$ ($Lk = 4Lk_0$), which corresponds to ~ 2.6 base-pairs per turn. Using this value of σ_p in Eq.(1) (σ_p replacing σ_d), we find that the experimental force versus extension curves for $0.037 < \sigma < 3$, do indeed collapse to a single curve $l_p(F)$, the extension at given force, F, of the pure P-DNA phase (see Fig.6).

Molecular modeling has been used to investigate possible structures for this highly twisted DNA using the JUMNA program [27,28] to minimize the energy of a DNA molecule at $T = 0$ and under twist constraints ranging from $-6 < \sigma < +4$. As σ increases, the phosphate backbones move to the center of the structure and the bases are expelled.

The fact that extreme twisting leads to base-pair disruption can be understood by noting that the distance between successive phosphates within one phosphodiester strand cannot exceed roughly 7.5Å. For a rise of 3Å, the maximum length of the phosphate-phosphate (P-P) vector projected into the plane perpendicular to the helical axis is therefore roughly 6.9Å. For a helix radius of roughly 10Å (as in B-DNA), simple geometry gives the angle subtended by the projected P-P vector (that is, the maximum twist) as roughly 40°. To increase this angle, it is necessary to decrease the radius of the helix (50° at 8Å, 70° at 6Å and, finally, 180° at 3.45Å) - which implies bringing the backbones to the center of the helix, and, consequently, pushing the bases out.

It is remarked that at intermediate forces ($3pN < F < 25pN$), the measured length of strongly overwound DNA ($\sigma \to \sigma_p \sim 3$) decreases to zero (Fig.6A). We propose that this shortening is due to the formation of plectonemic conformations of P-DNA stabilized by interactions between their exposed unpaired bases, a possibility suggested by the numerical simulations. When the stretching force, F, exceeds about $25pN$, the molecule extends by destroying these plectonemes (as discussed in [29,30]). The hysteresis observed upon increasing the force could well be due to sporadic and cooperative base unpairing in these plectonemic structures. A simple theoretical model incorporating plectonemes in P-DNA nicely fits our measurements. As a function of force, we expect the P-DNA phase to have a certain fraction q, in plectonemic form stabilized by non-specific interactions between the exposed bases with an average energy per unit length: $-\epsilon$. The rest, i.e. a fraction $1-q$, behaves like a worm like chain (WLC) polymer [1,2] of length $l_{p,0}$, persistence length ξ_p and an energy per unit length: $g_{WLC}(F\xi_p/k_BT)$ [1,2]. The free energy per unit length $\mathcal{F}$ of the chain will thus be:

$$\mathcal{F} = -\epsilon q + (1-q)g_{WLC} + (k_BT/\xi_p)\{q\log q + (1-q)\log(1-q)\} \qquad (6)$$

The last term in Eq.6 is the mixing entropy. The elasticity curve, obtained by minimization of $\mathcal{F}$ with respect to q, is the continuous curve in Fig.6 top, which fits the collapsed data over more than an order of magnitude in force. The parameters of the fit imply that P-DNA has a relaxed length $l_{p,0} = 1.75$ (i.e. 75% longer than B-DNA) which is consistent with the molecular modeling, and a persistence length $\xi_p = 19nm$ ($\xi_B = 53nm$ for B-DNA [1,2]). The average plectonemic energy per unit length $\epsilon = 0.5$ pN $= 0.12\ k_BT$/nm.

Finally, we have also performed chemical experiments to show that the bases in P-DNA are really exposed (data not shown). We followed a protocol similar to the one used

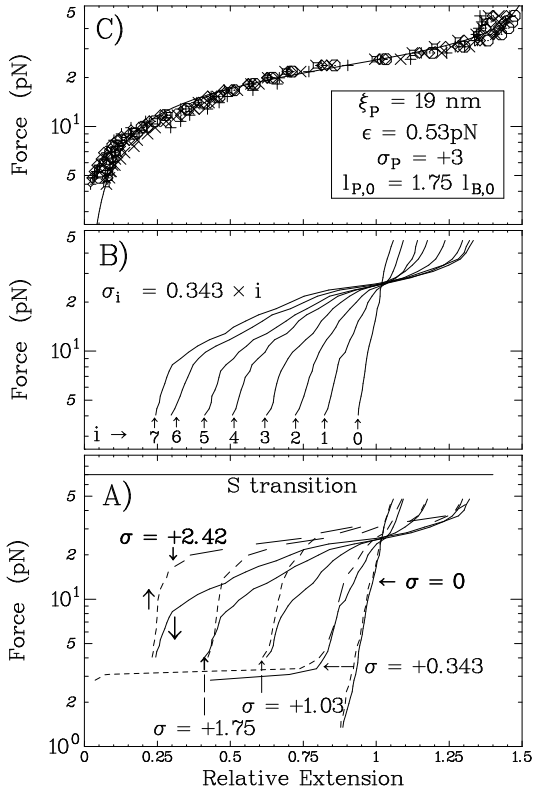

Fig. 6. Mechanical characterization of P-DNA. A) Elasticity curves showing two sharp transitions at 3 pN and 25 pN. The first transition (not shown for all curves) is associated with the disappearance of plectonemes in B-DNA and the formation of P-DNA. The second transition, showing hysteresis, is attributed to the disappearance of plectonemes in the P-DNA sub-phase. Note that due to the possibility of stabilizing interactions between exposed bases, these plectonemes should be more stable than in B-DNA. The existence of such plectonemic structures explains the shortening of the molecule at relatively low forces $3pN < F < 10pN$. At higher forces, these curves show that P-DNA is actually longer than B-DNA. B) Complete set of decreasing force scan curves as in A) with $\sigma_i = i \times 0.343$. C) Rescaling of the curves in B), following Eq. 5, enables all the curves shown in (B) to be collapsed to a single curve $l_p(F)$ which describes the extension vs. force behavior of a pure P-DNA. The full line is a fit to the model for P-DNA (Eq. 6) with $l_{p,0} = 1.75$, $\xi_p = 19$ nm and $\epsilon = 0.12 k_B T/\text{nm}$.

previously to demonstrate the existence of localized denatured regions in underwound DNA by reacting the exposed bases with glyoxal. As a result we were able to show that the proportion of P-DNA in the stretched overwound DNA is indeed about $(\sigma - \sigma_{c+})/\sigma_p$ [8].

7 Conclusions

Mechanical experiments on single DNA molecules are found to offer a simple way to generate and measure changes in DNA structure. These experiments show how the interplay of coiling and stretching can lead the molecule to partition itself between various phases such as plectonemic coils or denaturation bubbles at the entropic force-scale ($F \sim 0.5pN$),

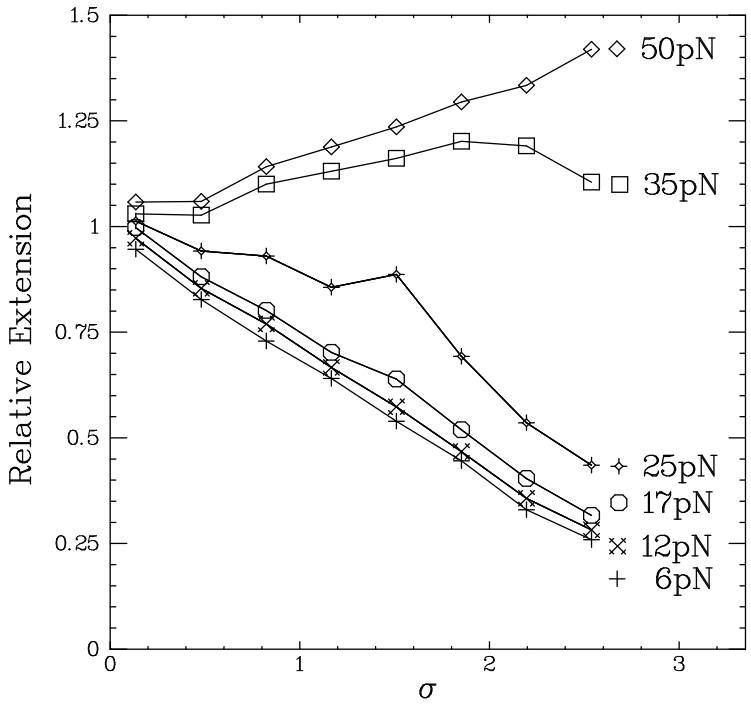

Fig. 7. Molecule extension versus σ at various forces. For $3 < f < 25pN$ the molecule gradually shrinks as σ increases until it reaches a zero extension at $\sigma \sim 3$. On the contrary, for forces $F > 30pN$, the molecule's extension increases regularly.

or hypertwisted P-DNA and overstretched S-DNA at higher forces (3 and 75 pN, respectively). Such data can be viewed as an example of how mechanical constraints provoke structural transitions in DNA. In this connection, X-ray crystallography, molecular modeling and cryo-electron microscopy support the view that DNA-protein interactions are concomitant with strong deformations of the double-helix. It is therefore interesting to compare the results of such studies with the structures generated by applying direct mechanical constraints to DNA. As an example, cryo-electron microscopy indicates that DNA coated with the recA protein (which guides the homologous recombination of sister chromatids) assumes a form characterized by 70% overextension [31]. Such data converges nicely with the mechanical stabilization of the overstretched S-DNA phase and molecular modeling of DNA at high stretching forces. Micromanipulation work has yet to specify the helical pitch of the S-DNA phase, and it will be interesting to compare this number with future X-ray data.

As another example, X-ray fiber diffraction has shown that the genome of the Pf1 virus (a circular, single-stranded DNA) is packaged in a structure [32] which is very similar to P-DNA. In this hypertwisted genome the sugar-phosphate backbone is on the inside of the molecule and the base-pairs on the outside. The structure is stabilized by the mechanical constraints applied by specialized packaging proteins. The helical pitch of the structure observed by X-ray crystallography is very close to the experimentally measured winding of P-DNA and to the structures obtained by molecular modeling.

Finally, cryo-electron microscopy has shown that the plectonemic structures which are stabilized at low forces in supercoiled DNA are important for the activity of topoisomerases, the enzymes whose role *in vivo* is to regulate DNA supercoiling. Topoisomerase II,

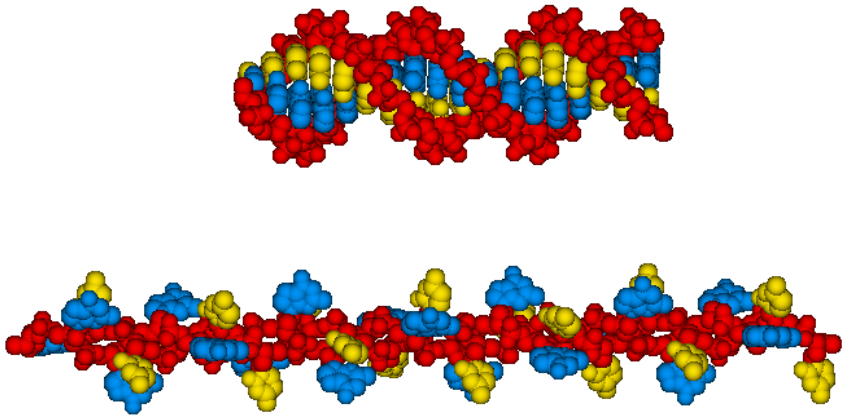

Fig. 8. Structure of P-DNA deduced from numerical energy minimization of a molecular model of DNA at $T = 0$. Space filling models of a $(d\mathrm{G})_{18}.(d\mathrm{C})_{18}$ fragment in B-DNA (top) and P-DNA (bottom) conformations. The backbones are colored red and the bases blue (guanine) and yellow (cytosine). These models were created with the JUMNA program [27,28], by imposing twisting constraints on helically symmetric DNA's with regular repeating base sequences.

responsible for resolving DNA knots and entanglements by passing one strand through another, has been seen by electron microscopy to preferentially interact with DNA crossovers. We have therefore begun to use our mechanical control of plectonemic supercoils to study in real-time the activity of topoisomerase enzymes on DNA. It is our hope that the mechanical control of DNA structures will help advance the study of DNA-protein interactions from the perspective of an underlying mechanism for protein action, i.e. deformation induced metastable states of DNA which can facilitate complexation and eventually play a role in specific recognition.

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