Micro-mechanical measurement of the torsional modulus of DNA

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The torsional modulus C of DNA is determined from the difference between the work of stretching a single overwound molecule and the work done stretching one underwound by the same number of turns. The value obtained $C/k_BT = 86 \pm 10$ nm is within the range (75 ± 25 nm) estimated by more indirect methods.

Key words *DNA elasticity, supercoiling, DNA torsional rigidity.*

DNA supercoiling plays a fundamental role in the cell. In prokaryotes, plasmids and genomic DNA is underwound, a property apparently required for proper initiation of replication. In eukaryotes'nuclei, DNA is highly compacted by successive stages of coiling. First, it is organized in nucleosomes by winding twice around the histone core, forming a bead on a string structure of nucleosomes known as chromatin, which is further compacted by winding into a solenoidal structure about 34 nm in diameter. DNA supercoiling is generated in processes such as transcription and replication, where it is relaxed by the specific action of a large class of enzymes: the topoisomerases. Finally, DNA supercoiling is implicated in gene regulation, where it mediates DNA unwinding, a necessary step for transcriptional activation and recombinational repair.

For the torsionally constrained molecules studied here, topological considerations provide the conceptual framework we shall use to analyze our data. 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 double strand, its writhe (Wr) and its twist (Tw): $Lk = Wr + Tw$. Wr is a measure of the coiling of the DNA's axis about itself, like a twisted cord forming interwound structures in order to relieve its 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, $Lk = Lk_0 = Tw_0$ (=number of helical turns) [1]. One defines the relative change in linking number, or the degree of supercoiling, $\sigma = (Lk - Lk_0)/Lk_0 = \Delta Lk/Lk_0$. The value of σ for most isolated plasmids is roughly -0.06. At constant 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 the effective torque applied to the molecule.

The energy scale for macromolecules is set by the thermal energy : $k_BT = 4.10^{-21}J$ (or $RT = 0.6Kcal/mol$). As the length scale of biomolecules is of the order of $1nm$, the force scale is on the order of the picoNewton $1pN = 10^{-12}N$. To produce and measure such forces on a DNA molecule we use a single molecule manipulation technique. Briefly, it consists of stretching a single $\lambda-$DNA molecule ($\sim$ 50 Kbps $\sim 16.2\mu$m) 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 one added turn on the molecule, we have simply $\Delta Lk = n$, where n is the number of turns the magnet rotates. The tethered bead ($\sim 2.8\mu m$ in diameter) exhibits a Brownian motion which amplitude gives access to the force applied on the molecule: the stronger the force, the smaller the fluctuations. This system allowed us to apply and measure forces from few femtoNewtons to $\sim 100pN$ (see [2]). In the range of forces studied here, $F < 3$ pN, the DNA molecule is not overstretched [3,4] and responds as a random coiled polymer [5].

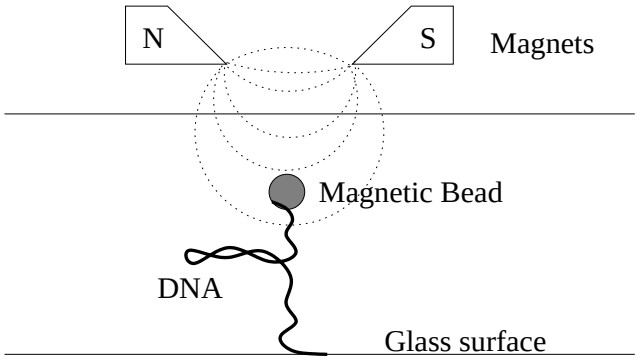

FIG. 1. Schematic representation of the experiment. A DIG/biotine end-labelled $\lambda-$DNA molecule ($\sim 16\mu$m long) is attached at one end to a glass surface by DIG/anti-DIG bonds and at the other to a 2.8μm magnetic bead via streptavidine/biotin links. By varying the distance between the sample and permanent magnets the stretching force is controlled while rotating those induces DNA supercoiling. The sample is placed on an inverted microscope and viewed with a 63× objective. The force is measured by a real time video analysis of the bead's Brownian fluctuations: the greater the force, the more restricted its fluctuations.

The formation of plectonemes during supercoiling results from a balance between the bending energy cost of

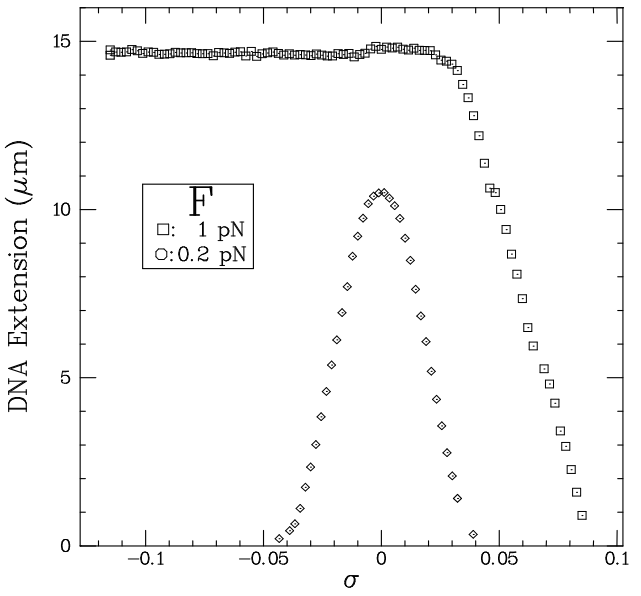

FIG. 2. Extension vs. supercoiling curves obtained for two different stretching forces in 10 mM PB. In the low-force regime $F < 0.5$ pN, the DNA molecule responds in a symmetric manner to positive or negative supercoiling by forming plectonemes. These plectonemes grow with $|\sigma|$, reducing the molecule's extension. At higher forces $0.5\ \text{pN} < F < 3$ pN, the extension of negatively supercoiled DNA is relatively insensitive to changes in the molecule's linking number. Positively supercoiled DNA, on the other hand, contracts as $|\sigma|$ and the plectonemic length grow. At still higher forces, $F > 3$ pN (not shown here), the extensions of both positively and negatively supercoiled DNA are relatively independent of changes in the linking number.

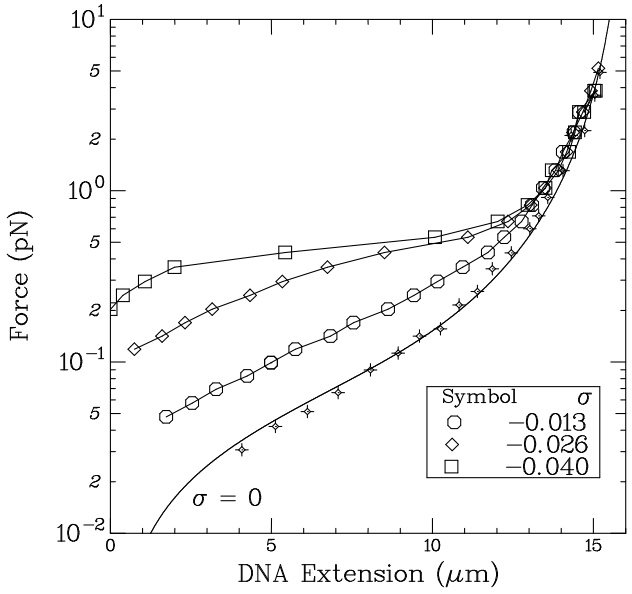

FIG. 3. Force vs. extension curves for underwound DNA in 10 mM PB. The $\sigma = 0$ curve was fitted by a WLC with a persistence length of 48 nm. The solid curves serve as guides for the eye. Notice the abrupt transition at $F_c \sim 0.5$ pN to an extended state which behaves like a molecule with $\sigma = 0$.

these tertiary structures and the torsional energy gained by their presence [6,7]. The bending and torsional moduli of DNA (A and C) [8] which set this balance are therefore important parameters to determine. The bending modulus is well known from various measurements of the DNA's persistence length $\xi_p = A/k_BT \sim 50 \pm 5$ nm in 10 mM phosphate buffer at pH=8.0 (PB) [5,9]. However the value of the torsional modulus of DNA is known with much less precision: $50 < C/k_BT < 100$ nm. It is estimated indirectly from the rapid motions of various spectroscopic probes bound to DNA (luminescence depolarization) or from the determination of the equilibrium distribution of twist in plasmids (topoisomer analysis) [8].

Here we would like to present a novel mechanical method for the determination of C. It consists in measuring the difference in work done while stretching a single DNA molecule wound either positively or negatively by the same number of turns.

Fig.[2] shows the molecular extension as a function of supercoiling for various forces. At a low force ($F \sim 0.2$ pN) the elastic behavior of DNA is symmetric under $\sigma \to -\sigma$. Like a twisted cord the molecule reduces its torque by writhing and thus "contracting", as each added turn (positive or negative) is relaxed through the formation of plectonemes. Pulling on the molecule removes the writhe and thus increases the twist and the torque on the molecule. For underwound molecules (in 10 mM PB) above a certain critical force ($F_c \sim 0.5$pN) and its associated critical torque ($\Gamma_c \sim 9$pN.nm, see below) writhing becomes energetically unfavorable. The molecule elongates, see Fig[3] as plectonemes (which used to absorb twist) are converted locally into melted (denatured) regions of DNA. For positive supercoilings ($\sigma > 0.037$), a similar transition is observed [2,10,11] at still higher forces ($F_{c+} \sim 3$pN), but we will not address this regime here.

In the following we shall use the force extension measurements on DNA supercoiled by $\pm n$ turns, i.e. with the same $|\sigma|$, to estimate the torsional constant, C, the critical torque at denaturation Γ_c and the energy of denaturation per base pair (bp), ϵ_d.

Consider the case of a DNA of crystallographic length l_0 at an initial extension $l_{A^+} (< l_0)$. Let us coil the molecule by $n > 0$ turns to state A^+, (Fig.[4]), requiring a torsional energy T_{A^+} ($F < 0.2$ pN) and then extend it to state B^+ ($F = 4$pN), so as to pull out its plectonemes and eliminate its writhe. Alternatively state B^+ could be reached by first stretching the initially uncoiled DNA and then twisting it. In that case its torsional energy T_{B^+} is purely twist and by energy conservation we must have:

$$T_{A^+} + \Delta W_{AB^+} = T_{B^+} = \frac{C}{2l_0}(2\pi n)^2 \qquad (1)$$

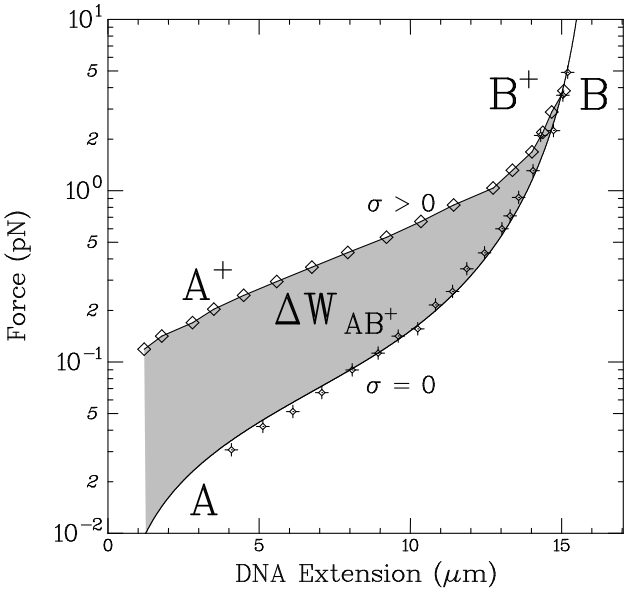

FIG. 4. The extra work performed while stretching an overwound DNA. The molecule is overwound from point A to point A^+ and then stretched along the $\sigma > 0$ curve to point B^+. The extra work performed while stretching is the shaded area between the $\sigma > 0$ and the $\sigma = 0$ curves.

Here ΔW_{AB+} is the extra work performed in stretching a coiled molecule from A^+ to B^+, the shaded area in Fig.[4]. For the sake of simplicity we neglect the correction to the bare torsional constant C_0 due to the thermal fluctuations [12,13]. We shall see later that this approximation ($C \approx C_0$) is justified. Consider now the case in which DNA is underwound by $-n$ turns to state A^- and then stretched to state B^-. By the same reasoning as above we may write:

$$T_{A-} + \Delta W_{AB-} = T_{B-} \tag{2}$$

Since when underwound the molecule partially denatures as it is pulled from A^- to B^-, the torsional energy T_{B-} will consist of twist energy and energy of denaturation. We can nevertheless estimate T_{B-} by considering the alternative pathway for reaching B^- by first stretching the molecule and then twisting it. In this case as the molecule is underwound, the torque Γ initially raises as in a twisted rod:

$$\Gamma = \frac{C}{l_0} 2\pi n \tag{3}$$

When Γ reaches a critical value Γ_c after $-n_c$ turns, the molecule starts to denature. Any further increase in n enlarges the denaturation region, without affecting the torque in the molecule which stabilizes at $\Gamma = \Gamma_c$. The energy of denaturation is thus simply, see Fig.[5]:

$$E_d = 2\pi(n - n_c)\Gamma_c \tag{4}$$

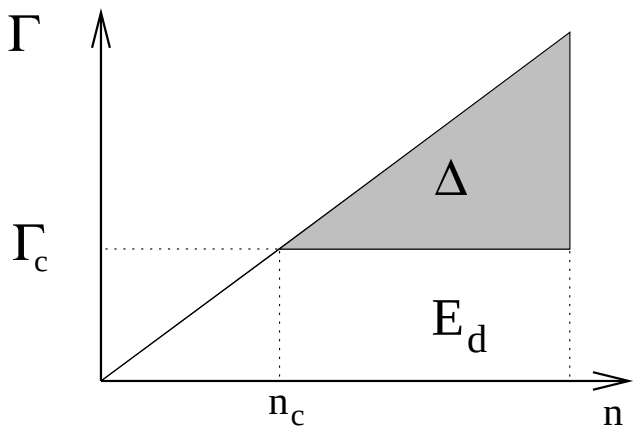

FIG. 5. Dependence of the torque on the twist (number of turns). In a DNA molecule as in a twisted rod the torque increases linearly with the twist angle (number of turns). If the molecule melts because of torsional yield as expected when underwound, the torque stabilizes at a value Γ_c as it does in a rod which undergoes a torsional buckling instability. The difference Δ in the work of over and under-twisting is the shaded area shown here and in Fig.6.

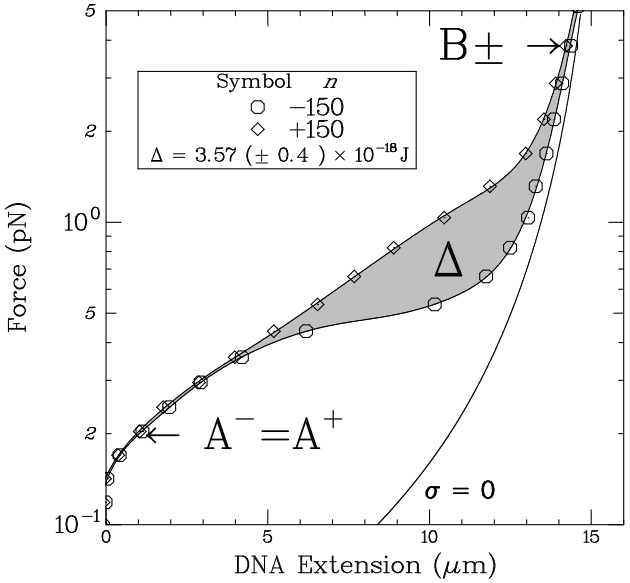

FIG. 6. Difference in the work of stretching over and underwound DNA. ∘: DNA unwound by $n = -150$ turns. ⋄: DNA overwound by $n = 150$ turns. The solid curves are polynomial fits to the force-extension data. The bottom curve is the theoretical (worm-like chain) fit to the data obtained for this molecule at $\sigma = 0$: $l_0 \sim 15.7\mu$m and $\xi_p = A/k_BT \sim 48$ nm [Bustamante et al., 1994]. The shaded surface between the $\sigma > 0$ and $\sigma < 0$ curves represents the work difference Δ. In both cases, point A^+ (respectively, A^-) is reached by overwinding (underwinding) the DNA which is initially at low extension (point A, not shown). Point B^+ (B^-) is reached by stretching the molecule along the appropriate $\sigma > 0$ ($\sigma < 0$) curve.

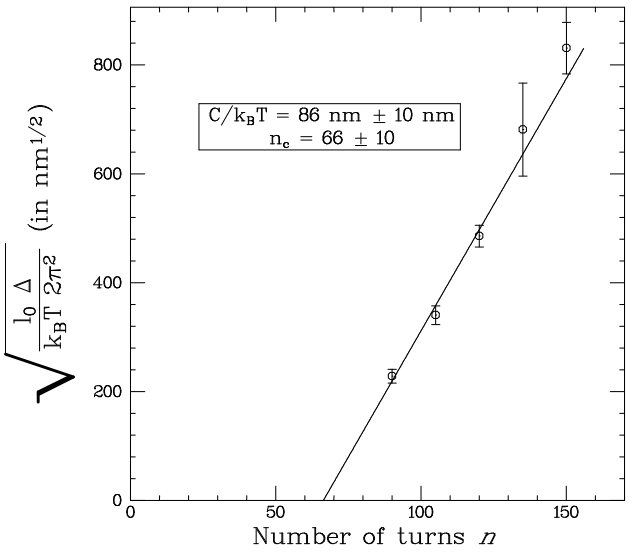

FIG. 7. Plot of the square root of the work difference Δ vs. the number of turns n the molecule is over or underwound. The straight line is a best fit through the experimental points. From its slope we extract the value $C/k_BT = 86 \pm 10$nm and from its intercept with the n-axis, $n_c = 66$ turns, we infer $\Gamma_c = 2\pi n_c C/l_0 = 9$ pN nm.

The torsional energy in state B^- is thus:

$$T_{B^-} = \frac{C}{2l_0}(2\pi n_c)^2 + E_d \qquad (5)$$

Since at low force the elastic behavior of a DNA molecule is symmetric under $n \to -n$: $T_{A^+} = T_{A^-}$, subtracting Eq.[2] from Eq.[1] yields:

$$\Delta \equiv \Delta W_{AB^+} - \Delta W_{AB^-} = T_{B^+} - T_{B^-} = \frac{2\pi^2 C}{l_0}(n - n_c)^2$$

Δ is the measured difference between the work performed while stretching an overwound molecule and the work done while pulling on an underwound one, see shaded area in Figs.[5,6]. Plotting the value of $\sqrt{\Delta}$ vs. n, one obtains a straight line (see Fig.[7]), from which slope one can determine the value of the torsional constant: $C/k_BT = 86 \pm 10$ nm. The intercept of that line with the n-axis yields $n_c = 66$ turns, from which one can estimate the critical torque $\Gamma_c = 9$ pN nm and denaturation energy per bp $\epsilon_d = E_d/10.5(n - n_c) = 1.35 k_BT$. Although the error bar on the measurement of C is still rather large, this method can be improved to yield a more precise value of C. It is nevertheless at least consistent with the current very imprecise estimate of $C/k_BT = 75 \pm 25$ nm.

We may now estimate the validity of our approximation neglecting the correction to C_0 due to the thermal fluctuations [12]. At high force these renormalize C as:

$$\frac{1}{C} = \frac{1}{C_0} + \frac{k_BT}{4A\sqrt{AF}} \qquad (6)$$

The last term on the right implies a correction of only 5% to the value of C at $F = 4$pN smaller than our experimental uncertainty. It is interesting to notice that the value of C determined here is in very good agreement with the one obtained from the measurement of the molecular extension versus σ at constant force [13], a totally independent measurement based on the model of a Rod Like Chain polymer.

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