

$K_2C_{59}N$ salt, which is isoelectronic with the K_3C_{60} superconducting phase.

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- The XRD measurements of as-prepared solid $(C_{59}N)_2$ reveal at ambient temperature a mostly amorphous solid with a minority hexagonal ($a = 9.97$ Å, $c = 16.18$ Å) crystalline phase (K. Prassides *et al.*, in preparation).
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The Elasticity of a Single Supercoiled DNA Molecule

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Single linear DNA molecules were bound at multiple sites at one extremity to a treated glass cover slip and at the other to a magnetic bead. The DNA was therefore torsionally constrained. A magnetic field was used to rotate the beads and thus to coil and pull the DNA. The stretching force was determined by analysis of the Brownian fluctuations of the bead. Here, the elastic behavior of individual λ DNA molecules over- and underwound by up to 500 turns was studied. A sharp transition was discovered from a low to a high extension state at a force of ~ 0.45 piconewtons for underwound molecules and at a force of ~ 3 piconewtons for overwound ones. These transitions, probably reflecting the formation of alternative structures in stretched coiled DNA molecules, might be relevant for DNA transcription and replication.

The supercoiling of DNA—that is, the twisting and bending of the double helical axis—is an extensively studied aspect of DNA topology (1, 2). It affects both structural transitions and interactions between DNA and other molecular complexes. For example, a locally underwound DNA strand is necessary for transcriptional activation (3) and recombinational repair (4). Supercoiled DNA is also a key structural factor in chromosomal organization, in which the winding of the molecule around histone proteins is necessary for DNA compaction (5). More specifically, the entropic tension generated in supercoiled DNA in anaphase during chromosomal condensation is released by the action of a specific enzyme (6), topoisomerase II, thus allowing the disentanglement and segregation of the chromosomes necessary before cell division.

Most previous studies of DNA supercoiling were done on solutions of circular supercoiled DNA molecules with the use

of dynamic light-scattering methods (7), fluorescence depolarization (8), and topoisomer analysis during the circularization of small DNA molecules (9–12). Among the studies on single molecules are the analysis of static electron microscopy images of small plasmids (13–15) and Monte-Carlo (MC) or molecular dynamics simulations (16–19). Most of these experiments have been interpreted on the basis

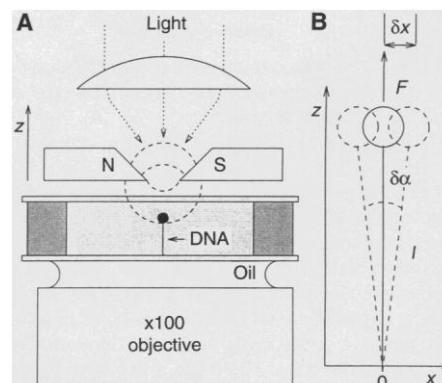
of models of DNA that assume that the twisting and bending contributions to the free energy of the molecule are harmonic and isotropic (20–22), a good approximation for unstretched DNA (8–12).

For closed circular DNA and for linear DNA molecules whose ends are not free to rotate (6), topological models provide the conceptual framework (2, 13) 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 DNA (Lk)—is a topological constant, the sum of two geometrical characteristics of the shape of the DNA, writhe (Wr) and twist (Tw), where $Lk = Wr + Tw$ (2). Wr is a measure of the coiling of the DNA axis, like a twisted cord forming interwound structures (plectonemes). Tw reflects the helical winding of the DNA strands around each other (13). For an unconstrained (relaxed) linear DNA molecule (assuming the absence of any spontaneous local curvature), $Lk = Lk_0 = Tw_0$ (= number of helical turns). One defines the relative change in linking number or degree of supercoiling, σ , as

$$(Lk - Lk_0)/Lk_0 \equiv \Delta Lk/Lk_0$$

The value of σ for most circular molecules

Fig. 1. (A) Experimental setup: The DNA molecule was bound to a glass cover slip at one end by DIG-anti-DIG links and at the other end to a paramagnetic bead by biotin-streptavidin links (26, 27). A force was applied on the bead with Co-Sm magnets equipped with a polar piece to focus the field in a 2-mm gap located just above the sample. The magnetic device can spin about the optical axis, causing the paramagnetic beads to rotate. We controlled the magnitude of the force by displacing the magnets vertically. To determine this force, we measured the Brownian fluctuations and the coordinates of the tethered bead. The samples were observed on a Diaphot-200 Nikon microscope with a 100 \times immersion oil objective. The x and y coordinates were directly measured on the image. The value of the z coordinate was obtained with an optical sensor to measure the position of the objective, which yielded the best image determined by a polynomial fit to the bead's profile. The Brownian motion of the tethered bead was measured in real time with a special tracking algorithm inspired by previous work (41). We obtained video data using a square pixel XC77CE Sony camera connected to a Cyclope frame grabber (timed on the pixel clock of the camera) installed in a 486 (33 MHz) personal computer (42). N and S represent the two poles of a magnet. **(B)** Schematic of a bead undergoing angular fluctuations ($\delta\alpha$) in the xOz plane transverse to the direction of stretching. The transverse restoring force is $F_{\perp} = F\delta\alpha = (F/l)\delta x$.



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isolated from cells and virions lies between -0.05 and -0.07 (6). Our experiments were done in 10 mM phosphate buffer on λ DNA molecules at values of $\sigma \leq 0.102$, which constitute conditions typical of many previous studies. On the basis of our common experience with objects like telephone cords, one expects different elastic behaviors at high and low forces. A highly extended supercoiled DNA (or telephone cord) will tend to be twisted rather than writhed. As one lowers the stretching force, the molecule (or cord) will start to writhe and form plectonemes.

The elastic properties of single torsionally relaxed DNA molecules were first studied by Smith *et al.* (23). It was later shown that these measurements matched the predictions of the entropic theory of a wormlike chain polymer (24, 25). Here, we report measurements of the entropic elasticity properties of a single supercoiled linear

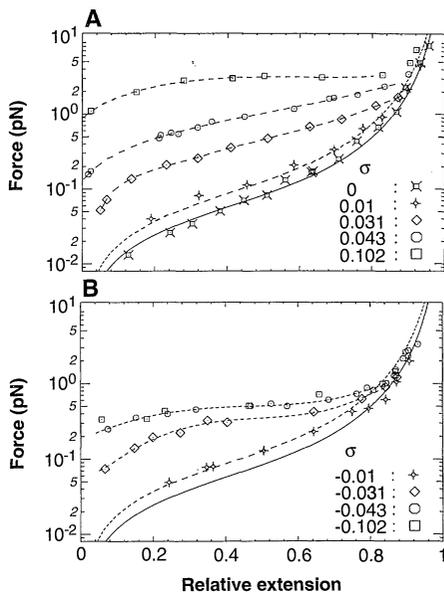


Fig. 2. Force versus relative extension (43) curves for a single torsionally constrained molecule at various degrees of supercoiling (σ). (A) $\sigma = 0, 0.01, 0.031, 0.043,$ and 0.102 . (B) $\sigma = -0.01, -0.031, -0.043,$ and -0.102 . The full line is the theoretical prediction for a wormlike chain with a persistence length of 53 nm (25). The dashed lines are polynomial fits, as a guide to the eye, except for $\sigma = \pm 0.01$, where a wormlike chain with a persistence length of 35 nm was fitted. Notice that at low forces ($F < 0.3$ pN) the curves for positive and negative supercoiling are similar. However, at forces greater than ~ 0.45 pN, there is a significant difference between the two curves as the underwound molecule undergoes a transition to an extended state whose elastic behavior is similar to that of the $\sigma = 0$ torsionally relaxed one. Notice also the similar sharp transition for $\sigma = 0.102$ at $F \sim 3$ pN. For short relative extensions ($< 10\%$), the force measurements are unreliable because of interactions with the surface and poor Brownian statistics.

DNA molecule in solution.

In our experiments (Fig. 1), we made use of two important features. First, the extremities of the DNA were constrained in order to twist the molecule. To prevent the DNA from swiveling about its anchoring points, we multiply labeled the end segments of the molecule with biotin at one end and with digoxigenin (DIG) at the other (26). The DIG-labeled end was bound by means of DIG antibodies to a glass cover slip, and the biotinylated extremity was bound by means of streptavidin to a small superparamagnetic bead (27). We then used small magnets to pull on the DNA-tethered beads. Because these beads were observed to turn synchronously with the rotation of the magnets (23, 28), we could coil the molecule in a controllable and reversible fashion.

Second, we used the Brownian fluctuations of the DNA-tethered bead to measure the vertical stretching force, F , on the molecule. The bead's fluctuations $\langle \delta x^2 \rangle$ transverse to the direction of stretching are associated with the rigidity k_x by the equipartition theorem

$$F/l = k_x = k_B T / \langle \delta x^2 \rangle$$

where T is the temperature, k_B is the Boltzmann constant, and l is the extension of the tethering molecule, which is obtained from the displacement of the bead under the mi-

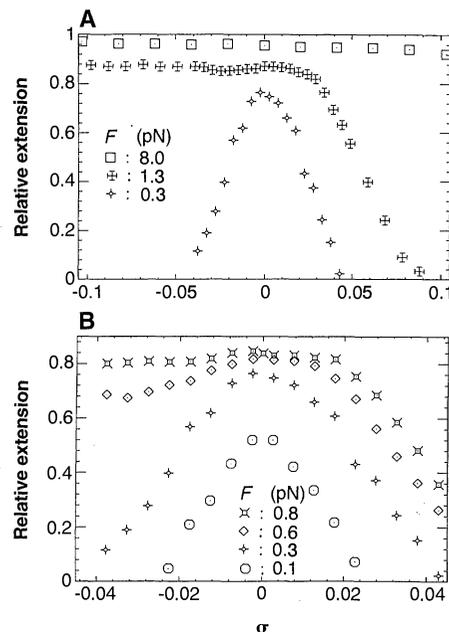


Fig. 3. Relative extension versus degree of supercoiling σ at various forces. (A) $F = 8$ pN, 1.3 pN, and 0.3 pN. (B) $F = 0.8$ pN, 0.6 pN, and 0.3 pN [as in (A)] and $F = 0.1$ pN. As in Fig. 2, one notices the symmetric behavior under $\sigma \rightarrow -\sigma$ at smaller forces and the transition to an extended state at greater forces, first at negative supercoillings (above 0.45 pN) and then at positive supercoillings (above 3 pN).

croscope (29). The advantage of this method is that we can directly measure forces in the range of 6 fN to 20 pN (30).

To ensure that we were pulling on a single DNA molecule, we used k_x to construct the force versus extension curves of our candidate tethered beads and compared them with published experimental (23) and theoretical results (25) (Fig. 2A). The contour length of our DNA molecule between its anchoring points was deduced from these curves (31) as described by Bustamante *et al.* (25). By rotating the magnets by n integer turns, the magnetic field configuration and the force F on the tethered bead are unchanged. However, if the molecule is not free to rotate (is not nicked or cannot swivel about its anchoring point), the DNA linking number is changed by $\Delta Lk = n$ (16). Two complementary experiments can then be performed. We can obtain force versus extension curves at fixed values of σ by varying the distance between the magnets and the sample (Fig. 2, A and B). We can also obtain extension versus σ curves at fixed force by rotating the magnets at a fixed distance (Fig. 3, A and B).

These experiments show that positively and negatively supercoiled DNA behave similarly at low forces ($F < 0.3$ pN; Fig. 3B). Although slightly coiled molecules ($\sigma = 0.01$) show an increased rigidity compared to the relaxed ($\sigma = 0$) case, their elastic behavior can still be reasonably fitted by a wormlike chain model, albeit with a shorter persistence length [$\xi(\sigma = 0.01) \sim 35$ nm (32)]. These results are in agreement with theoretical expectations (33, 34) and previous experiments (8) performed at $F = 0$ and interpreted on the basis of a harmonic theory of DNA elasticity (20–22). We were unable to extend the low-force measurements to high degrees of supercoiling because upon winding the bead recoils to the surface of the cover slip. Indeed, below a certain threshold force $F_c(\sigma)$, which increases with $|\sigma|$, supercoiled molecules maintain a very small extension. For example, a force of 0.1 pN is necessary to begin stretching DNA overwound to $\sigma \sim 0.025$ (Fig. 3B). This force most likely corresponds to the traction (or energy per unit length) necessary to begin unraveling the plectonemic structures, presumed to exist at low forces (34).

Another critical force F_c^- becomes apparent when one subjects DNA to higher degrees of supercoiling and stronger forces (Fig. 2B). Above values for $F_c^- \sim 0.45$ pN, our experimental apparatus becomes sensitive to the intrinsic chirality of the molecule, as positively supercoiled DNA becomes more difficult to stretch to the same extent as negatively supercoiled DNA.

This is true even for values of σ as low as 0.01 (Fig. 2B). This critical force F_c^- also heralds the beginning of a sharp transition in negatively supercoiled DNA, which appears to be independent of the degree of supercoiling. At this force, the underwound molecule yields and undergoes a transition from a low extension form to a more extended one. That transition, predicted by Marko and Siggia (33, 34), has been associated with the concentration of Lk deficit in regions of non-B-DNA (35), such as denaturation bubbles, cruciforms, and Z-DNA.

The behavior of telephone cords suggests that as one stretches a coiled molecule one should indeed observe a sharp transition from a low extension pleconemic state dominated by writhe to an extended state dominated by twist. The study of underwound plasmids (at $F = 0$) has revealed the existence of alternative DNA structures at values of σ more negative than -0.05 (12, 36). Because the ratio of change in writhe to change in twist in these plasmids is $\sim 4:1$ (14), the relative change in twist at which these alternative structures have been observed is ~ -0.01 . This is also the value of σ at which we begin to observe (at F_c^-) the transition to an extended twist-dominated state (Fig. 2B). For forces higher than F_c^- , the elastic behavior of negatively coiled DNA converges toward that of the torsionally relaxed case of $\sigma = 0$.

At still higher forces, one observes a similar kind of sharp transition in positively supercoiled DNA. Above a critical force $F_c^+ \sim 3$ pN, overwound molecules also yield and undergo a transition from a low extension state to a more extended one (Figs. 2A and 3A). Analogous to the transition of underwound DNA, the excess Lk might be concentrated in bubbles of alternative overtwisted DNA forms (such as D-DNA) (35, 37). The rest of the molecule would then be in a less twisted state. Finally, at force values above that for F_c^+ , in the range of σ explored here, the DNA extension is not very sensitive to the sign and degree of supercoiling (Fig. 3A).

These results are in good agreement with the predictions of Marko and Siggia (33, 34) for a molecule with a torsional constant $C = 75$ nm (38). We hope that they will stimulate MC simulations of supercoiled stretched DNA molecules, an extension of Vologodskii's work (16), in order to address the existence and characterization of the transitions observed in supercoiled molecules. Further experiments in different solutions (such as those with higher ionic strength and multivalent cations) will allow us to test how sensitive our results are to various conditions.

These experiments demonstrate that one can perform controlled and reproduc-

ible measurements on single supercoiled DNA molecules (39) with a sensitivity to supercoiling variations as small as 0.005 (Fig. 3). Our results could be relevant to situations where supercoiled DNA is under stressed conditions mimicking those in the cell (for example, in the presence of binding proteins or crowded environments, such as during chromosomal condensation). In particular, the behavior of topology-modifying enzymes, such as topoisomerases and gyrases, could be studied under various stresses in an in vitro assay, modeling some of the conditions existing during cell division. Our results should also be relevant to DNA transcription. As RNA polymerases move along DNA, they increase the degree of supercoiling in front of the complex and decrease it behind (3). Recent experiments by Yin *et al.* (40) have shown that a single RNA polymerase can exert a force on the molecule as large as 14 pN. If the polymerase is solidly bound to some cellular component, our results suggest that such a force could cause extensive melting in supercoiled DNA.

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26. One batch (25 μ g) of λ DNA (Boehringer) was randomly labeled [every ~ 400 base pairs (bp)] with photobiotin (Pierce) and another with photodigoxigenin (Boehringer). The labeled DNA was purified and cut with restriction enzyme Nru I (New England Biolabs). The cohesive-left (4600 bp) and cohesive-right (6700 bp) fragments were isolated on a gel (0.6% agarose

- and $1 \times$ TAE). They were then purified (GeneCleanII system, Bio101), precipitated, and resuspended in 10 μ l of buffer (10 mM tris and 1 mM EDTA). The cohesive-left biotin-labeled fragments were annealed at 37°C for 24 hours to λ DNA in 10 mM MgCl₂ and then ligated for 1 hour at 37°C with 5 units of T4 DNA ligase (Boehringer). The cohesive-right DIG-labeled fragments were then added to the mixture and similarly annealed and ligated.
27. The DNA construct was incubated with streptavidin-coated paramagnetic beads (Dyna, M280) in phosphate-buffered saline (PBS) buffer for 10 min for binding. The DNA-bead assembly was then incubated in wells coated with a polyclonal antibody to digoxigenin (anti-DIG; Boehringer). The wells were formed from glass cover slips and coated with polystyrene (or coated with silane), to which a small polyvinyl chloride ring was glued with paraffin. Anti-DIG [2.5 μ g in 100 μ l of PBS or carbonate buffer (0.1 M, pH 9)] was incubated in each well overnight at 4°C. The anti-DIG was then rinsed thoroughly in phosphate buffer (10 mM, pH 8) and 0.1% Tween. Sonicated fish sperm DNA (molecular biology grade; Boehringer) in phosphate buffer was added and incubated for 1 hour at 37°C to prevent nonspecific binding. The wells were finally washed in phosphate buffer and Tween before addition of the DNA-magnetic bead assembly. The samples thus prepared and sealed to prevent contamination are stable for more than a week if stored at 4°C. Single DNA molecules can be studied for a few days.
28. Superparamagnetic beads are made of many small ferromagnetic domains. Their susceptibility is anisotropic, and therefore their response to a magnetic field is similar to that of a compass needle.
29. We thank C. Bouchiat for proving that relation to be valid even at small extensions where $l \sim \Delta x$ (personal communication).
30. The rigidity of the molecule can be determined from the longitudinal fluctuations of the bead:

$$\delta l / \delta z_l = k_z = k_B T / \langle \delta z^2 \rangle$$
31. Our DNA construct is $\sim 20\%$ longer than native λ DNA. The binding of the molecule to the bead and to the surface may not occur on the full length of the functionalized extremities. We thus observed tethered molecules able to be coiled with maximal extensions between ~ 17 μ m and ~ 17.9 μ m.
32. A shorter persistence length corresponds to a smaller bending stiffness and results in a higher entropic rigidity.
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39. The study of the braiding of a bundle of DNA molecules is in progress. See A. Simon and F. Heslot, *Proceedings, Instrumentation Physique en Biologie et en Médecine* (Technique & Documentation Lavoisier, 93, 1995).
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42. We checked the overall acquisition chain by measuring the drag on the bead-DNA system (from the cutoff frequency in the spectrum of the Brownian motion) and comparing it with the expectations from Stokes law. Agreement within 4% was observed.
43. The relative extension is defined as the measured extension of the molecule divided by its maximal extension.
44. We thank A. Berneman, C. Bouchiat, A. Chiffaudel, L. Jullien, J. Marko, X. Michalet, E. Siggia, J.-L. Sikorav, and S. Smith for useful discussions, correspondence, and making available unpublished data. We also thank one of the referees for incisive and helpful comments. We are grateful to Boehringer-Mannheim for a generous gift of photo-digoxigenin.

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