LETTERS

DNA overwinds when stretched

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DNA is often modelled as an isotropic rod[1–4], but its chiral structure suggests the possible importance of anisotropic mechanical properties, including coupling between twisting and stretching degrees of freedom. Simple physical intuition predicts that DNA should unwind under tension, as it is pulled towards a denatured structure[4–8]. We used rotor bead tracking to directly measure twist–stretch coupling in single DNA molecules. Here we show that for small distortions, contrary to intuition, DNA overwinds under tension, reaching a maximum twist at a tension of ~30 pN. As tension is increased above this critical value, the DNA begins to unwind. The observed twist–stretch coupling predicts that DNA should also lengthen when overwound under constant tension, an effect that we quantitatively confirm. We present a simple model that explains these unusual mechanical properties, and also suggests a possible origin for the anomalously large torsional rigidity of DNA. Our results have implications for the action of DNA-binding proteins that must stretch and twist DNA to compensate for variability in the lengths of their binding sites[9–11]. The requisite coupled DNA distortions are favoured by the intrinsic mechanical properties of the double helix reported here.

Many cellular proteins bend or wrap DNA upon binding, loop DNA to make contact with two non-adjacent binding sites, or twist DNA during translocation along the double helix[12]. The energetics of these distortions are governed by the mechanical properties of DNA, which have been investigated using a variety of bulk[1] and single-molecule techniques[2,3]. For small deformations, DNA in physiological buffer is modelled as an isotropic rod with bending rigidity $B = 230 \pm 20\,\mathrm{pN\,nm^2}$, twist rigidity $C = 460 \pm 20\,\mathrm{pN\,nm^2}$, and stretch modulus $S = 1{,}100 \pm 200\,\mathrm{pN}$ (refs 3, 13–16; and Z.B, J.G., N.R.C. and C.B, manuscript in preparation).

A fourth mechanical parameter is allowed in the linear theory of a deformable rod[5,6,17]: the twist–stretch coupling g, which specifies how the twist of the helix changes when the molecule is stretched. At forces sufficient to suppress bending fluctuations, the energy of a stretched and twisted DNA molecule may be written as[5,6,17]:

$$E_{\mathrm{DNA}} = \frac{1}{2}\frac{C}{L}\theta^2 + g\theta\frac{x}{L} + \frac{1}{2}\frac{S}{L}x^2$$

where L is the contour length at zero force, x is the distance that the DNA is stretched beyond its contour length L, and θ is the angle through which the DNA is twisted from its unperturbed equilibrium value.

Interpolation between the B-form helix and denatured or overstretched forms of DNA suggests that g should be positive[5,6], so that DNA unwinds as it is stretched (in an analogous process to the unwinding of DNA at elevated temperatures[18]). Previous fits[5–7] to experimental single-molecule data[4,8,14] have indeed yielded positive g values ($g = 200 \pm 100\,\mathrm{pN\,nm}$). However, an analysis of the distribution of base pair step parameters in atomic structures of DNA:protein complexes showed a weak positive correlation between twist and rise, implying a negative g value[9]. All-atom simulations have likewise suggested that twist and rise may be positively correlated for small distortions[10,19]. Finally, in contrast to the overstretching transition[8,13,14] (in which DNA unwinds as it extends), the B–A transition involves a slight unwinding coupled to compression of the DNA helix[20]. Conclusive determination of the sign and magnitude of g requires direct measurement in isolated DNA molecules.

To measure the twist–stretch coupling of DNA, we used the rotor bead tracking technique[13,15,21], in which a submicrometre 'rotor' bead is attached to the middle of a stretched DNA molecule, immediately below a free swivel consisting of an engineered single strand nick (Fig. 1a, b). Tension is applied to the molecule using magnetic tweezers[2,4]. Changes in the rotor bead angle reflect changes in the twist of the lower DNA segment.

Under fixed tension, the rotor bead fluctuated around a mean angle as a result of thermal noise (Fig. 1c). As the molecule was stretched by increasing the magnetic force, the mean angle of the rotor bead increased. A ~1% stretching of DNA led to an increase in the twist of ~0.1% (Fig. 1d). Analysis of this data yielded a twist–stretch coupling constant $g = -90 \pm 20\,\mathrm{pN\,nm}$ ($N = 4$ molecules), opposite in sign to most previous estimates[5–7] but consistent with twist–rise correlations in crystal structures[9] and molecular simulations[10,19]. Our measurement of g was robust to alterations in the length and sequence of the torsionally constrained DNA segment (Fig. 1d).

For small deformations, we have shown that DNA overwinds when stretched. However, in the limit of high forces, DNA must eventually unwind as the backbone is pulled straight. To test for sign reversal of g at elevated tensions, we monitored the twist of DNA molecules while gradually increasing the magnetic force. We found that the twist of DNA increases until the tension reaches a critical value $F_c \approx 30\,\mathrm{pN}$, beyond which the DNA begins to unwind (Fig. 1e).

The negative twist–stretch coupling g observed at lower tensions implies that DNA should lengthen when overwound (Fig. 2a, b). To predict the expected magnitude of this effect, we write the total energy of the DNA/magnetic bead system as

$$E_{\mathrm{T}} = \frac{1}{2}\frac{C}{L}\theta^2 + g\theta\frac{x}{L} + \frac{1}{2}\frac{S}{L}x^2 - xF$$

and minimize it with respect to the stretching distance x, holding the twist θ and force F constant ($(\partial E_{\mathrm{T}}/\partial x)_{\theta,F} = 0$). This analysis yields the DNA extension, x^*, that minimizes the total energy given some imposed twist value[5,6]:

$$x^* = \frac{L}{S}\left(F - \frac{g\theta}{L}\right) \Rightarrow \frac{\partial x^*}{\partial \theta} = -\frac{g}{S}$$

Given our measured $g = -90 \pm 20\,\mathrm{pN\,nm}$, we expect the DNA molecule to lengthen by $\Delta x = 0.5 \pm 0.1\,\mathrm{nm}$ for each rotation imposed in the overwinding direction.

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To measure changes in extension upon imposed overwinding, we employed the magnetic tweezers assay introduced in refs 4 and 22, in which a single constrained DNA molecule is stretched between a coverslip and a magnetic bead (Fig. 2a, b). The twist in the molecule can be controlled via rotation of the magnets, and extension is monitored by measuring the focal depth of the bead using image analysis. Overwinding caused the DNA to extend by 0.5 nm per turn, in quantitative agreement with our prediction (Fig. 2c, d).

What could be the physical origin of the negative twist–stretch coupling of DNA? A model helix with a fixed backbone length and fixed radius must necessarily unwind as it is stretched. However, if the radius of the helix is allowed to shrink as the helix is stretched, then unwinding is no longer guaranteed. In simulations of DNA stretching using all-atom potentials[10], a reduction in the radius of the double helix has been seen concurrent with stretching and overwinding. We constructed a simple 'toy' model in which the radius of the helix was allowed to vary as the molecule was stretched, and asked whether this model could capture the twist–stretch coupling and other mechanical properties of DNA.

The model consists of an elastic rod with a stiff helical 'wire' (analogous to the sugar-phosphate backbone) affixed to the outside surface (Fig. 3a). The inner rod is constructed from a material with a Poisson's ratio $\nu = 0.5$, so that it conserves volume under stress[23]. As this system is stretched, the inner rod decreases in diameter. When the model is stretched without constraining twist, changes in helicity arise from the tendency of the stiff outer 'wire' to resist changes in contour length (Fig. 3b). The inner rod alone has stretch modulus $S_r = \pi R_r^2 Y_r$, bending rigidity $B_r = \pi R_r^4 Y_r/4$ and torsional rigidity $C_r = B_r/(1+\nu)$, where Y_r is the Young's modulus of the rod material and R_r is the rod's radius[24,25].

For small deformations, the complete toy model has the following effective mechanical parameters (see Supplementary Information):

$$B_{\mathrm{eff}} \approx B_r$$

$$S_{\mathrm{eff}} = S_r + S_h \mathrm{cosec}\alpha(\sin^2\alpha - \nu\cos^2\alpha)^2 \approx S_r$$

$$C_{\mathrm{eff}} = C_r + R_r^2 S_h \sin\alpha\cos^2\alpha$$

$$g = R_r(\sin^2\alpha - \nu\cos^2\alpha)S_h$$

where S_h is the stretch modulus of the outer wire, and $\alpha = \arctan(3.4\,\mathrm{nm}/2\pi R_r)$ is the helix angle (Fig. 3a). Thus, the presence of the outer wire does not change the bending rigidity or stretch modulus appreciably, but it does stiffen the molecule to torsion and also generates a non-zero twist–stretch coupling, g. With the three free parameters fitted to $R_r = 0.924$ nm (close to the crystallographic radius of DNA), $S_h = 965$ pN, and $Y_r = 0.393$ GPa (similar to estimated Y values for DNA and within the range of measured Y values for bulk polymeric materials[14,24,26]), we obtain the correct experimentally measured values for the mechanical parameters of DNA: $B_{\mathrm{eff}} = 225\,\mathrm{pN\,nm^2}$, $C_{\mathrm{eff}} = 460\,\mathrm{pN\,nm^2}$, $g = -90$ pN nm and $S_{\mathrm{eff}} = 1{,}081$ pN.

Although construction of this toy DNA model was motivated by the discovery of negative twist–stretch coupling, it also provides a possible explanation for the anomalously large torsional rigidity of DNA. For an isotropic rod, the torsional rigidity C must be smaller than the bending rigidity B (see equation for C_r above) unless the rod

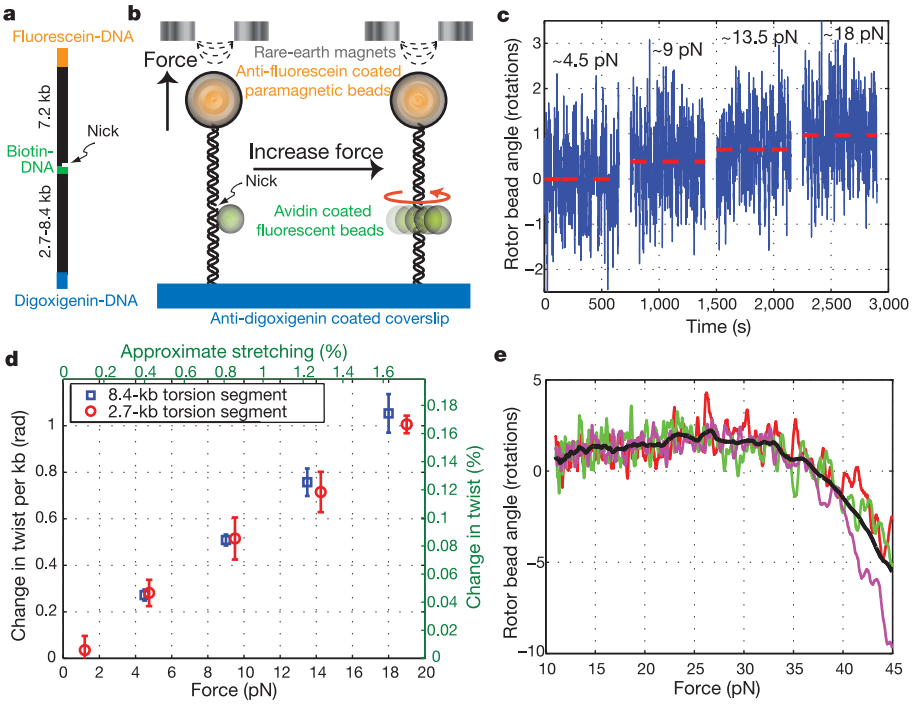

Figure 1 | DNA overwinds when stretched. **a**, The molecular construct for rotor bead tracking experiments contains three distinct attachment sites and a site-specific nick, which acts as a swivel[13,15,21]. **b**, Molecule/bead assemblies were constructed in parallel in a flow chamber, and assayed with an inverted microscope equipped with permanent magnets[21]. Each molecule was stretched between the glass coverslip and a magnetic bead, while a fluorescent avidin-coated rotor bead was attached to the central biotinylated patch. Tension in the DNA was controlled by raising or lowering the magnets, and changes in twist were observed by tracking the rotation of the fluorescent bead. **c**, When the DNA molecule is held at a fixed force, the rotor bead angle (blue trace) fluctuates around a mean (red dashed lines). As tension is increased, the mean rotor bead angle increases, reflecting overwinding of the DNA. **d**, The overwinding scales linearly with applied tension and with the length of the torque-bearing DNA segment. Plotted data (mean ± s.e.m.) correspond to an 8.4-kb segment (blue squares) and a 2.7-kb segment (red circles). **e**, Rotor bead angle versus force during experiments in which the DNA tension was gradually increased by lowering the magnets (8.4-kb segment). Three different experiments are shown in colour; they were averaged and smoothed to obtain the solid black trace. The DNA overwinds until the tension reaches ~30 pN; as the tension is increased above this critical value, the molecule begins to unwind. Equivalent results have also been obtained with DNA constructs containing the 2.7-kb torque-bearing segment (not shown).

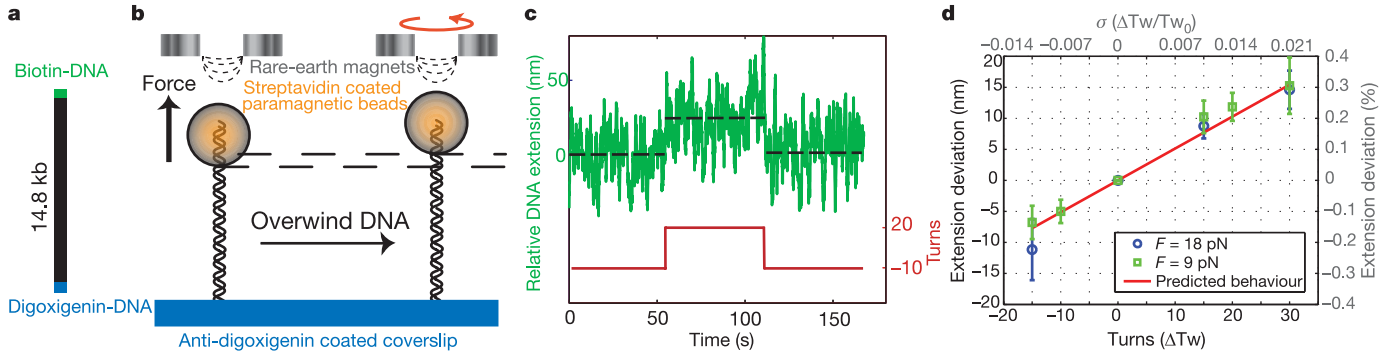

Figure 2 | DNA extends when overwound under constant tension. **a**, **b**, Rotating magnets[4] were used to introduce torque into a single 14.8-kb DNA tether. **c**, Raw data trace demonstrating that overwinding the DNA molecule at constant tension (9 pN) caused the DNA to extend. **d**, Relative extension as a function of the number of excess turns at 9 pN (green squares) and 18 pN (blue circles). Each data point shows the mean ± s.e.m. for a minimum of three molecules. The red line is the predicted behaviour based upon our prior determination of the twist–stretch coupling, $g = -90$ pN nm. Alternative axes show the percentage increase in length induced by a fractional increase in twist, σ.

is constructed from a material with a negative Poisson's ratio (having the unlikely property that the radius increases as the rod is stretched). It has therefore long been puzzling that most measurements of C have been larger than B for DNA[27]. However, unlike the isotropic rod model, our toy model displays the correct values of B and C without resorting to exotic material properties. The outer helix stiffens the system to torsion because any twisting of the model DNA requires stretching or compression of the outer helical 'wire'.

In the toy model, negative twist–stretch coupling occurs only for shallow helix angles below a critical angle $\alpha_c = \arctan\left(\sqrt{\nu}\right) \approx 0.62$ rad. The geometry of B-form DNA lies just within the regime of negative coupling $\alpha = \arctan(3.4\,\text{nm}/2\pi R_r) \approx 0.53\,\text{rad} < \alpha_c$. Although the model is only intended to capture the behaviour of DNA for small distortions, it does predict a sign reversal of twist–stretch coupling upon stretching, reminiscent of the behaviour we observe at high tensions (Fig. 1e).

The mechanical properties of DNA have been studied at the single-molecule level for over a decade; why is it that the surprising twist–stretch coupling has not been observed experimentally in the past? One study[7] obtained the incorrect sign for the coupling by analysing relatively noisy magnetic-tweezers data[4] that were generated to study DNA buckling, an effect with a much larger signal than the twist–stretch coupling. In the final phases of preparing this manuscript, we learned that Croquette and co-workers had performed a new set of magnetic-tweezers measurements, focusing on changes in extension of DNA under small changes in twist. They find that overwinding causes DNA to extend[28], in agreement with the results presented in Fig. 2.

As discussed in ref. 10, the anomalous twist–stretch coupling of DNA has important implications for plasticity in site recognition by DNA-binding proteins. Consider the effect of a base-pair insertion or deletion in the binding site for a protein. Even if the change does not directly involve a DNA:protein contact, the protein must overcome a geometric mismatch in both extension and twist in order to bind the DNA. And yet, proteins are able to recognize binding sites with variable sequence lengths; this can be achieved by simultaneously stretching and overwinding (or compressing and underwinding) the DNA. An extreme example occurs in the 146-base-pair (bp) nucleosome core particle structure[11]. Equivalent binding sites in the two halves of the nucleosome are occupied by 13 bp of DNA in one case and only 12 bp in the other. For the 12-bp sequence to fit into its binding site, it must be stretched to the same length as the 13-bp sequence, and also overwound to give the same total twist as the 13-bp sequence. This large distortion is outside the range of negative twist–stretch coupling we have observed for random sequences, but single-basepair insertions or deletions in longer sequences will lead to more modest distortions. In addition, larger effects might be facilitated by sequence-dependent mechanics: coupling between twist and rise may be particularly strong for certain base-pair steps[9], and thus certain sequences may be optimized for coupled twisting and stretching. This hypothesis may be tested by future single-molecule studies of the sequence dependence of twist–stretch coupling.

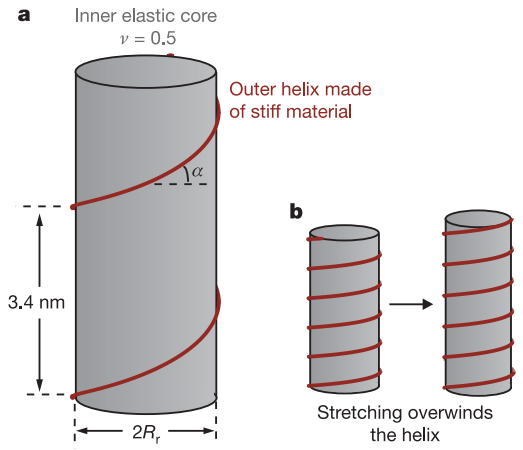

Figure 3 | Toy mechanical model of DNA. **a**, We model DNA as an elastic rod (grey) wrapped helically by a stiff wire (red). The inner core of radius R_r is assumed to have a Poisson's ratio $\nu = 0.5$. The outer wire is affixed to the inner rod helically with a pitch of 3.4 nm, and contributes to the overall mechanical properties because it resists stretching and compression. The outer helix increases the torsional rigidity and yields a twist–stretch coupling that depends upon the helix angle, α. **b**, Stretching generates an overwinding of the helix because the inner rod decreases in diameter as it is stretched. The outer helix is then able to wrap a larger number of times over the length of the molecule. In this figure, a shallow helix angle was used in order to exaggerate the overwinding effect seen with DNA.

METHODS

The rotor bead tracking experiments were performed in an inverted epifluorescence microscope (Zeiss Axiovert 100A) equipped with permanent magnets mounted on a motorized translation stage, as described previously[15,21]. The applied force was estimated to within 20% by bright-field imaging of the transverse fluctuations of the magnetic bead[4,15]. The DNA construct was prepared by serial ligation as described previously[13], with the torque-bearing DNA segment replaced by the 8.4-kilobase (kb) *Bgl*II-*Sal*I fragment of pSV8[13] or (where noted) the 2.7-kb *Bam*HI-*Sal*I fragment of pUC18. Each molecule was stretched between a glass coverslip coated with anti-digoxygenin (Roche) and a 2.8-μm magnetic bead (Dynal M-280) coated with anti-fluorescein (Molecular Probes), while a fluorescent avidin-coated rotor bead (Spherotech VFP-0552-5, nominal diameter 0.46 μm) was attached to the central biotinylated patch.

Movies of the fluorescent rotor beads were recorded at 100 Hz on an electron-multiplying CCD camera (Andor iXon DU-860E-CS0-#BV). Experiments were performed at room temperature (23 ± 2 °C) in 40 mM Tris HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.2% sodium azide, 0.2% Tween-20, 50 μg ml^{-1} BSA. Replacing EDTA with 10 mM $MgCl_2$ led to similar degrees of overwinding with tension.

The effect of imposed twist (Fig. 2) was studied in a purpose-built microscope (S. Hong, D. Humphries, M. D. Stone, C.B. and N.R.C., manuscript in preparation) equipped with high-powered magnets and a piezoelectric objective positioner (Physikintrumente). Changes in extension of the DNA were measured by tracking the focal depth of the streptavidin-coated magnetic bead (Dynal M-280 or MyOne) using image analysis of ring patterns[4,29]. The effect of drift was minimized by alternating between successive twist values (as in Fig. 2c). The DNA tether for these experiments was the 14.8-kb *Bam*HI-*Sal*I fragment from pPIA2-6[30].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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