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New Measurements of DNA Twist Elasticity

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The symmetries of the DNA double helix require a new term in its linear response to stress: the coupling between twist and stretch. Recent experiments with torsionally-constrained single molecules give the first direct measurement of this new material parameter. We extract its value from a recent experiment. We also present a very simple microscopic theory predicting a value comparable to the one observed. Finally we sketch the effect of constrained twist on *entropic* elasticity of DNA arising from the connection between Link, Twist, and Writhe.

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1. Introduction

The idea of studying the response of DNA to mechanical stress is as old as the discovery of the double helix structure itself. While many elements of DNA function require detailed understanding of specific chemical bonds (for example the binding of small ligands), still others are quite nonspecific and reflect overall mechanical properties. Moreover, since the helix repeat distance of $\ell_0 \approx 3.4\,\mathrm{nm}$ involves dozens of atoms, it is reasonable to hope that this length-scale regime would be long enough so that the cooperative response of many atoms would justify the use of a continuum, classical theory, yet short enough that the spatial structure of DNA matters. Since moreover various important biological processes involve length scales comparable to ℓ_0 (notably the winding of DNA onto histones), the details of this elasticity theory are important for DNA function.

Recently, techniques of micromanipulation via optical tweezers and magnetic beads have yielded reliable numerical values for the bend stiffness from the phenomenon of thermally-induced entropic elasticity (Smith et al., 1992 Bustamante et al., 1994; Vologodskii, 1994; Marko and Siggia, 1995), as well as the direct measurement of another elastic constant, the stretch modulus, by exploring the force range 10–50pN (Cluzel et al., 1996; Smith et al., 1996; Wang et al., 1997). Significantly, the relation between bending stiffness, stretch modulus, and the diameter of DNA turned out to be roughly as predicted from the classical theory of beam elasticity (Smith et al., 1996), supporting the expectations mentioned above.

Still missing, however, has been any direct physical measurement of the elastic constants reflecting the *chiral* (*i.e.* helical) character of DNA. Recent experiments with torsionally constrained DNA have permitted the determination of one such constant, the coupling between twist and stretch (Strick et al., 1995; Marko, 1997; Kamien et al., 1997). This coupling may be relevant for the binding of the protein RecA to DNA, which stretches and untwists the DNA (Stasiak et al., 1982). We will explain why this term is needed, extract its value from the experiment, and compare it to a the prediction of a simple microscopic model to see that its magnitude is in line with the expectations of classical elasticity theory. Finally we will briefly sketch how to understand another phenomenon visible in the data, the effect of constrained link on entropic elasticity (Bouchiat and Mézard, 1998) (Moroz and Nelson, 1997).

2. Experiment

DNA differs from simpler polymers in that it can resist twisting, but it is not easy to measure this effect directly due to the difficulty of applying external torques to a single molecule. The first single-molecule stretching experiments constrained only the locations of the two ends of the DNA strand. The unique feature of the experiment of Strick *et al.* was the added ability to constrain the *orientation* of each end of the molecule.

We will study Fig. 3 of (Strick et al., 1995). In this experiment, a constant force of 8pN was applied to the molecule and the end-to-end length $z_{\rm tot}$ monitored as the terminal end was rotated through $\Delta{\rm Lk}$ turns from its relaxed state (which has ${\rm Lk}_0$ turns). In this way the helix could be over- or undertwisted by as much as $\pm 10\%$. Over this range of imposed linkage $z_{\rm tot}$ was found to be a linear function of σ:

$$\varepsilon = {\rm const.} - 0.15\sigma \quad \text{where} \quad \sigma \equiv \Delta{\rm Lk}/{\rm Lk}_0 \quad \text{and} \quad \varepsilon \equiv (z_{\rm tot}/z_{\rm tot,0}) - 1 \ . \tag{2.1}$$

Thus σ is the fractional excess link and ε is the extension relative to the relaxed state. Eqn. (2.1) is the experimentally observed twist-stretch coupling.

The existence of a linear term in (2.1) is direct evidence of the chiral character of the molecule, and its sign is as expected on geometrical grounds: untwisting the molecule tends to lengthen it. Still geometry alone cannot explain this result. Consider the outer sugar-phosphate backbones of the DNA. Suppose that the twist-stretch phenomenon were due to the straightening of these helical backbones while they maintained constant length, 0.6 nm per phosphate, and constant distance 0.9 nm from the center of the molecule. Then since each basepair step is $h = 0.34\,{\rm nm}$ high, the circumferential length per step is $\ell_c =$

$\sqrt{.6^2 - .34^2}$ nm. The corresponding twist angle per step is given by $\theta = (\ell_c/2)/.9\mathrm{nm} = 32°$, roughly as observed. Supposing now an extension by $\Delta h/h = \varepsilon$, we find an untwisting by $\sigma = \delta\theta/\theta = \mathrm{const.} - \varepsilon/2.0$, quite different from what is observed, eqn. (2.1). We must seek an explanation of the experimental result not in terms of a geometrical ball-and-stick model but in the context of an elastic response theory.

3. Simple Model

We will begin by neglecting bend fluctuations (see below). A straight rod under tension and torque will stretch and twist. We can describe it by the reduced elastic free energy

$$\frac{F(\sigma,\varepsilon)}{k_{\mathrm{B}}TL} = \frac{\omega_0^2}{2}\left[C\sigma^2 + B\varepsilon^2 + 2D\varepsilon\sigma\right] - \tilde{f}\varepsilon \ . \tag{3.1}$$

Here C is the twist persistence length, $B \approx 1100\,\mathrm{pN}/\omega_0^2 k_B T \approx 78\,\mathrm{nm}$ is the stretch modulus (Wang et al., 1997), and D is the desired twist-stretch coupling. L is the relaxed total length, $\omega_0 = 2\pi/\ell_0 = 1.85/\mathrm{nm}$, and the reduced force $\tilde{f} = 8\,\mathrm{pN}/k_B T \approx 1.95/\mathrm{nm}$ in the experiment under consideration. For a circular beam made of isotropic material the cross-term D is absent, since twisting is odd under spatial inversion while stretching is even. For a helical beam, however, we must expect to find this term.

We now minimize F with respect to ε at fixed force with an imposed constraint on the overtwist σ to find

$$\varepsilon = \varepsilon_{\sigma=0} - (D/B)\sigma \ . \tag{3.2}$$

Comparing to (2.1), we obtain the desired result: $D = 12\,\mathrm{nm}$.

4. Bend Fluctuations

We have discussed the term linear in overtwist σ in (2.1). For the highest-force curve at 8 pN this is the dominant effect. At lower forces, however, it is quickly overwhelmed by an effect *symmetric* under $\sigma \to -\sigma$, which we have so far neglected. This effect is due to the *coupling between applied overtwist and thermal bend fluctuations.* We now sketch a simple approximate analysis of this effect. For the full analysis see (Bouchiat and Mézard, 1998) (Moroz and Nelson, 1997).

Since in this section we want to study *nonchiral, low-force* effects, we will revert to a model of a cylindrical rod of fixed length L with bend and twist elasticity. We will consider small deviations from the unstressed state of the rod, which we take to run along the $\mathbf{z}$ axis. Initially we paint a straight stripe on the outside of the unstressed rod. To describe the deformed rod, let us introduce, at each point, an orthonormal triad $\{\mathbf{E}_i(s)\}$, where $\mathbf{E}_1$ is the tangent to the curve determined by the rod centerline, $\mathbf{E}_2$ is the normal vector from the centerline to the stripe, $\mathbf{E}_3$ is $\mathbf{E}_1 \times \mathbf{E}_2$, and s is arclength. Let $\eta \equiv \omega_0\sigma$ be the imposed excess helix density, and define the convenient reference frame

$$\mathbf{e}_1(s) \equiv \mathbf{x}\cos(\eta s) + \mathbf{y}\sin(\eta s) \; ; \quad \mathbf{e}_2(s) \equiv -\mathbf{x}\sin(\eta s) + \mathbf{y}\cos(\eta s) \; ; \quad \mathbf{e}_3 \equiv \mathbf{z} \; .$$

We can now describe the deformed rod by three small variables: the projection $\mathbf{t}_\perp \equiv t_1\mathbf{e}_1 + t_2\mathbf{e}_2$ of $\mathbf{E}_3$ to the xy plane and the angle φ between $\mathbf{x}$ and the projection of $\mathbf{E}_1$ to the xy plane. We propose to expand the elastic energy to quadratic order in these and thus find the thermal fluctuations in harmonic approximation. In terms of $t_1,\ t_2$, and φ we find

$$\begin{aligned}
\mathbf{E}_1 &= (1 - \tfrac{1}{2}{t_1}^2 - \tfrac{1}{2}\varphi^2)\mathbf{e}_1 + \varphi\mathbf{e}_2 - (t_1 + t_2\varphi)\mathbf{z} \\
\mathbf{E}_2 &= -(\varphi + t_1 t_2)\mathbf{e}_1 + (1 - \tfrac{1}{2}{t_2}^2 - \tfrac{1}{2}\varphi^2)\mathbf{e}_2 + (-t_2 + t_1\varphi)\mathbf{z} \\
\mathbf{E}_3 &= (1 - \tfrac{1}{2}{t_1}^2 - \tfrac{1}{2}{t_2}^2)\mathbf{z} + t_1\mathbf{e}_1 + t_2\mathbf{e}_2 \; ,
\end{aligned}$$

plus terms cubic and higher in $t_1,\ t_2$, and φ. We may now differentiate with respect to arc-length to get the body-fixed angular velocities $\Omega_1 \equiv \mathbf{E}_3 \cdot \dot{\mathbf{E}}_2 = -\tau_2 + \varphi\tau_1$, $\Omega_2 \equiv \mathbf{E}_1 \cdot \dot{\mathbf{E}}_3 = \tau_1 + \varphi\tau_2$, $\Omega_3 \equiv \mathbf{E}_2 \cdot \dot{\mathbf{E}}_1 = \eta + \dot{\varphi} - \frac{1}{2}\eta({t_1}^2 + {t_2}^2) + \dot{t}_1 t_2$, where we abbreviated $\tau_1 \equiv \dot{t}_1 - \eta t_2$, $\tau_2 \equiv \dot{t}_2 + \eta t_1$. Note that the formula for the Twist, Ω_3, just amounts to a simple rederivation of Fuller's formula (Fuller, 1978) for the Writhe of a nearly-straight curve.

Our formulas become compact if we introduce the complex variable $\mathcal{T} \equiv (\mathbf{x}+\mathrm{i}\mathbf{y})\cdot\mathbf{t}_\perp$. We then have ${t_1}^2+{t_2}^2 = |\mathcal{T}|^2$ and ${\tau_1}^2+{\tau_2}^2 = |\dot{\mathcal{T}}|^2$. Finally we expand in Fourier modes: $\mathcal{T} = \sum_q \alpha_q \mathrm{e}^{\mathrm{i}qs}$ and similarly with φ. We may impose periodic boundary conditions without affecting the intensive properties of a long rod, and so in the summation q is an integer multiple of $2\pi/L$. Substituting into the elastic energy $E/k_\mathrm{B}T = \frac{1}{2}\int \mathrm{d}s\,\left[A({\Omega_1}^2+{\Omega_2}^2)+C{\Omega_3}^2\right]$ yields the harmonic elastic energy

$$E/k_\mathrm{B}T = \tfrac{1}{2}\sum_q \left[Aq^2 - C\eta q + \widetilde{f}\right] |\alpha_q|^2 + \tfrac{1}{2}C\sum_q q^2|\varphi_q|^2 \ . \qquad (4.1)$$

We have introduced the applied stretching force $\widetilde{f} \equiv f/k_\mathrm{B}T$. Eqn. (4.1) is the natural generalization of the familiar worm-like chain at high force: indeed setting $C = 0$ we recover the harmonic energy formula found in (Marko and Siggia, 1995). There is no need for any short-distance (large-q) cutoff in eqn. (4.1), since in one dimension the Aq^2 term already suppresses these modes.

The physics of this formula is simple: twist fluctuations decouple from bend fluctuations, but the imposition of nonzero *net* overtwist η creates a new crossterm (the term $-C\eta q$ in (4.1)) which affects the long-scale *bend* fluctuations. Indeed completing the square in the first term of (4.1) shows that the effect of η is to *reduce the effective tension* from f to $f - \frac{k_\mathrm{B}T}{4A}(C\eta)^2$. This effective reduction is what makes the relative extension plummet as the overtwist $\eta \equiv \omega_0\sigma$ is increased at fixed f. This effect, combined with the intrinsic twist-stretch effect from the previous section, explains qualitatively all the phenomena in the region of the experiment where linear elasticity is valid. A more precise version of this calculation also affords a direct determination of the value of the twist stiffness C (Bouchiat and Mézard, 1998) (Moroz and Nelson, 1997). The high-force regime studied here is free from some of the difficulties of the Monte Carlo approach (Vologodskii et al., 1979; Marko and Vologodskii, 1997; Bouchiat and Mézard, 1998); in particular, there is no need for any artificial short-length cutoff.

5. Microscopic Model

The elastic theory in §3 was very general, but it gave no indication of the expected magnitudes of the various couplings. To gain further confidence in our result, we have *estimated* the expected twist-stretch coupling based on the measured values of the other elastic constants and geometrical information about DNA (Kamien et al., 1997; Kamien et al., 1998). We used a simple, intuitive microscopic picture of DNA as a helical rod to show how twist-stretch coupling can arise and get its general scaling with the geometric parameters. The model shows that the value of D deduced above from experiment is reasonable.

6. Conclusion

We have pointed out a strong twist-stretch coupling in torsionally-constrained DNA stretching experiments, evaluated it, argued that it reflects intrinsic elasticity of the DNA duplex, and shown that the value we obtained is consistent with elementary considerations from classical elasticity theory. We also showed how the interplay between twist and writhe communicates a constraint on link into the entropic elasticity of DNA, as seen in experiment.

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