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Twist–stretch coupling and phase transition during DNA supercoiling†

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As a single DNA molecule is positively supercoiled under constant tension, its extension initially increases due to a negative twist–stretch coupling. The subsequent attainment of an extension maximum has previously been assumed to be indicative of the onset of a phase transition from B- to scP-DNA. Here we show that an extension maximum in fact does not coincide with the onset of a phase transition. This transition is evidenced by a direct observation of a torque plateau using an angular optical trap. Instead we find that the shape of the extension curve can be well explained with a theory that incorporates both DNA twist–stretch coupling and bending fluctuations. This theory also provides a more accurate method of determining the value of the twist–stretch coupling modulus, which has possibly been underestimated in previous studies that did not take into consideration the bending fluctuations. Our study demonstrates the importance of torque detection in the correct identification of phase transitions as well as the contribution of the twist–stretch coupling and bending fluctuations to DNA extension.

Introduction

During various cellular processes DNA molecules often experience moderate stress and torque. These perturbations may be generated by motor enzymes and provide a mechanism for the regulation of DNA replication, DNA repair, transcription, and DNA recombination.[1–5] Therefore, understanding tensile and torsional responses of DNA is essential to understanding how mechanical perturbations may regulate cellular activities.

A single DNA molecule can be extended under force and rotated under torque, as has been investigated using optical and magnetic tweezers and micropipettes during the past two decades.[6–13] In particular, some of these studies revealed that the tensile and torsional responses of DNA are coupled. Initial analyses suggested that DNA undertwists when extended, indicating a positive twist–stretch coupling coefficient.[14,15] More recent studies by Gore *et al.*[16] and Lionnet *et al.*[17] using magnetic tweezers, as well as our own work using angular optical trapping[12] provide compelling evidence to the contrary: DNA overtwists when extended and, conversely, DNA extends when overtwisted. However, there remains some ambiguity in identifying the signatures of twist–stretch coupling in the extension curves when the DNA was overtwisted under constant force. While Lionnet *et al.*[17] interpreted a peak in this curve to be indicative of the onset of a phase transition from B- to supercoiled P- (scP-) DNA, our earlier work indicated that the peak of the curve and the phase transition did not coincide at the particular force examined.[12] In general, there was a lack of understanding of the nature of the extension peak location and its relation to both twist–stretch coupling and phase transitions.

The goals of this work are to differentiate between the signatures of twist–stretch coupling and those of phase transitions, and to provide an explanation for the existence of the maximum in the extension signal. To this end, we carried out DNA torsional experiments using an angular optical trap in conjunction with nanofabricated quartz cylinders so that the torque, angle, force, and extension of a DNA molecule were simultaneously measured during DNA supercoiling, using previously described methods.[12,13] An important advantage of this approach is the direct detection of the torque signal, allowing unambiguous identification of the onset of the phase transition where torque plateaus.

Experimental

Materials

The DNA template used in this study was constructed using previously described protocols.[13] In brief, a 4218-base pair (bp) piece of DNA was ligated at one end to a short (60-bp) oligonucleotide labeled with multiple digoxygenin (dig) tags and at the other end to an oligonucleotide of the same length labeled with multiple biotin tags.

Experimental setup

The experimental configurations and procedures were similar to those described previously.[12,13] In brief, prior to a measurement, DNA molecules were torsionally constrained at one end to streptavidin-coated nanofabricated quartz cylinders[12] and at the other end to an anti-dig coated coverslip. All experiments were performed in phosphate-buffered saline (157 mM Na^+, 4 mM K^+, 12 mM PO_4^{3-}, 140 mM Cl^-, pH = 7.4) at 23 ± 1 °C. The experiment began with a torsion-free DNA molecule which was held under constant tension. The DNA was first slightly undertwisted and then overwound *via* a steady rotation of the input laser polarization

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at 5 Hz, so as to explore a range of DNA supercoiling. During this time, torque, angular orientation, position, and force of the cylinder as well as the location of the coverglass were simultaneously recorded. The torque exerted on the DNA was measured from the torque exerted on the cylinder by the optical trap after subtracting the viscous drag torque of the rotating cylinder.

Results and discussion

Fig. 1 shows representative single traces of extension and the corresponding torque as a function of number of turns added to the DNA at three different applied forces (1.9, 7.7, and 9.6 pN). The number of turns was also converted to the degree of supercoiling σ, defined as the number of turns added to dsDNA divided by the number of naturally occurring helical turns in the given dsDNA. The DNA extension is also shown as the relative extension, defined as the extension normalized to the contour length of DNA template in its B-form.

Some overall features of these data are summarized below. At the beginning of each trace ($\sigma \sim -0.02$) DNA was in its B-form. As the DNA was twisted, torque increased linearly while extension remained approximately constant. At lower forces (<6 pN) this continued until the DNA buckled to form a plectoneme, evidenced by a sudden decrease in extension and a concurrent plateau in torque, whose value was force-dependent.[13] Under this low force range, the signatures for the onset of the buckling transition may be recognized in either extension or torque. At higher forces we used ($6 \text{ pN} < F < 10 \text{ pN}$), as the molecule was overtwisted, DNA underwent a scP-DNA transition instead, as previously observed by Allemand *et al.*[10] The onset of this transition was only evidenced by a sudden torque plateau around 40 pN nm.[11,12] These phase transitions may be thought of as first-order phase transitions since two separate phases may coexist and can be transformed from one to another by simply changing the twist in the DNA.[18] Because there were no concurrent distinct features in the extension signal, the ability to monitor torque signal was essential in unambiguously locating the onset of B- to scP-DNA transition. In addition, the buckling torque showed a strong force-dependence as previously observed,[13] whereas the scP transition torque showed little force-dependence.

Several features relevant to twist–stretch coupling were also immediately evident. First, near $\sigma = 0$, the extension curve had a small but positive slope, which implied a negative twist–stretch coupling coefficient, *i.e.* DNA was extended when overtwisted. This observation was in accordance with previous studies.[12,16,17] Second, upon overtwisting, extension reached a maximum (indicated by an arrow in Fig. 1) at σ_{z_max}, as has also been previously observed.[12,17] In addition, as the force increased, σ_{z_max} increased while the magnitude in the curvature of the extension at σ_{z_max} gradually decreased. Third, the location of the extension maximum σ_{z_max} clearly did not coincide with the onset of the phase transition (indicated by a dashed line) where torque began to plateau. This was the case for the range of forces we examined. Therefore the location of the maximum is not indicative of an onset of a phase transition, contrary to what has been previously reported with the magnetic tweezers experiments, where the lack of torque signal might have complicated the interpretation of the results.[17]

To understand the nature of the extension signal for the B-form DNA, we performed a detailed analysis of both the extension and torque signals to gain insights in the twist–stretch coupling coefficient and the location of the extension maximum. We followed the analysis developed by Marko.[14] This theory also takes into account the contribution from bending fluctuations to DNA extension, using a

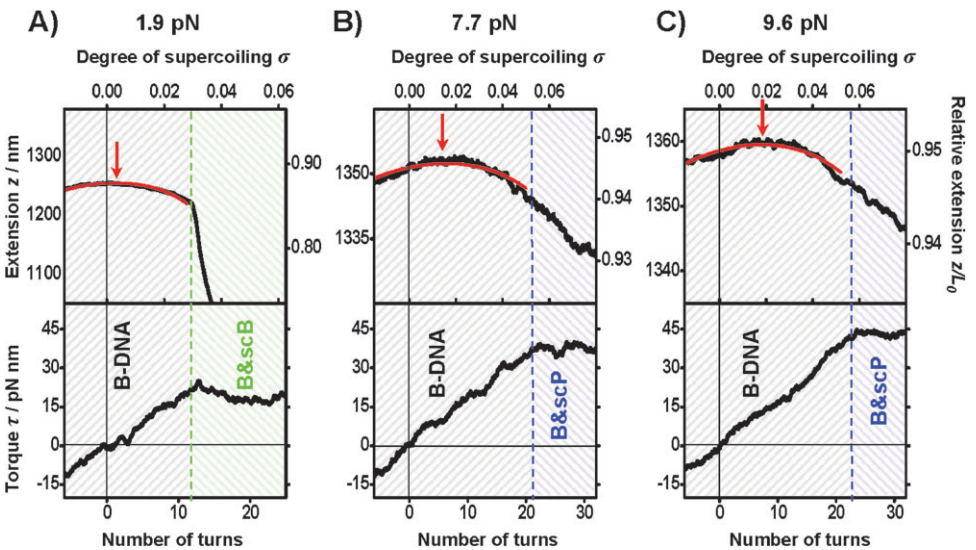


Fig. 1 Examples of extension and torque *versus* turn number during DNA supercoiling. DNA molecules of 4.2 kbp in length were wound at 5 Hz under constant forces: (A) 1.9 pN; (B) 7.7 pN; (C) 9.6 pN. Data were collected at 2 kHz and averaged with a sliding box window of 2.0 s for torque and 0.2 s for extension. The torque signal had more Brownian noise relative to signal and was subjected to more filtering. Red curves are fits to eqn (2) for B-DNA. Extension maxima are indicated with red arrows. A plateau in the torque reflects a phase transition and the onset of each transition is indicated by a dashed line.

treatment similar to that of Moroz and Nelson.[15] For a DNA molecule held under constant degree of supercoiling σ and force F, the free energy G includes contributions from bending, stretching, twisting, and twist–stretch coupling, and can be expressed as:

$$\frac{G}{k_BTL_0} = \frac{1}{L_p}\sqrt{\frac{L_pF}{k_BT} - \frac{1}{4}\left(C_0\omega_0\sigma + \frac{gF}{K_0}\right)^2} + \frac{C_0}{2}\omega_0^2\sigma^2 - \frac{F}{k_BT} - \frac{k_BT}{2K_0}\left(\frac{F}{k_BT} - g\omega_0\sigma\right)^2, \quad (1)$$

where L_p is the bending persistence length, K_0 the stretch modulus, C_0 the twist persistence length, g the twist–stretch coupling modulus (unitless), k_BT the thermal energy, L_0 the contour length, and $\omega_0 = 2\pi/3.57\ \mathrm{nm}^{-1}$ the natural twist rate. This theory predicts DNA extension z as a function of σ and applied force F:

$$\frac{z}{L_0} = -\left.\frac{\partial(G/L_0)}{\partial F}\right|_\sigma = 1 + \frac{F}{K_0} - \frac{k_BTg\omega_0\sigma}{K_0} - \sqrt{\frac{k_BT}{4L_pF}}\frac{1 - \frac{gC_0\sigma\omega_0 + g^2F/K_0}{2L_pK_0}k_BT}{\sqrt{1 - \frac{k_BT}{4L_pF}\left(C_0\omega_0\sigma - \frac{gF}{K_0}\right)^2}}. \quad (2)$$

We used eqn (1) to obtain a prediction of torque τ as a function of σ and applied force F:

$$\tau = \frac{1}{\omega_0}\left.\frac{\partial(G/L_0)}{\partial\sigma}\right|_F = k_BT\omega_0\sigma\left(C_0 - \frac{k_BTg^2}{K_0}\right) + \frac{k_BTgF}{K_0} - \frac{\frac{k_BTC_0}{4L_p}\left(C_0\omega_0\sigma + \frac{gF}{K_0}\right)}{\sqrt{\frac{L_pF}{k_BT} - \frac{1}{4}\left(C_0\omega_0\sigma + \frac{gF}{K_0}\right)^2}}. \quad (3)$$

Thus, eqn (2) and (3) form a complete set of relations that fully describe force, extension, torque, and twist for a B-form DNA.

Below, we will focus on the analysis of the extension signal. Eqn (2) contains several terms and its last term is due to the contribution of DNA bending fluctuations in the presence of the twist–stretch coupling. We found that eqn (2) predicts the existence of a maximum in the extension curve, reflecting an interplay between twist–stretch coupling and bending fluctuations. In the absence of any twist–stretch coupling, the maximum is centrally located, *i.e.* if $g = 0$, $\sigma_{z\_max} = 0$. Twist–stretch coupling shifts the location of the maximum away from the center (ESI†). If $g > 0$, then $\sigma_{z\_max} < 0$; and if $g < 0$, then $\sigma_{z\_max} > 0$. The absolute value of σ_{z_max} increases with an increase in force.

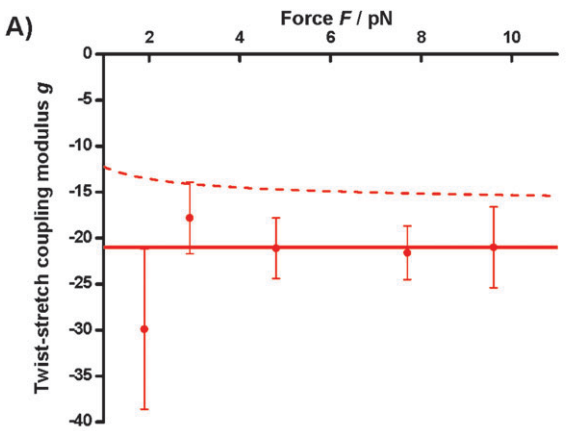
Notice that if bending fluctuations are neglected, eqn (2) is then simplified to:

$$\frac{z}{L_0} = 1 + \frac{F}{K_0} - \frac{k_BTg\omega_0\sigma}{K_0}. \quad (4)$$

In contrast to eqn (2), eqn (4) predicts a linear relation between extension and the degree of supercoiling: if $g > 0$, then slope < 0; and if $g < 0$, then slope > 0. It does not predict a maximum in the extension curve.

We performed a fit of eqn (2) to our extension data for the B-form DNA with g as the only fit parameter. Other parameters were taken from previous measurements obtained under similar experimental conditions: $K_0 = 1200$ pN,[6,8] $C_0 = 100$ nm,[11,13] and $L_p = 43$ nm.[6,8,13] Our data were well fit by eqn (2) and some examples are shown in Fig. 1 (red). In particular, the experimental observations that $\sigma_{z\_max} > 0$ and increases with increasing force were indicative of a negative value for the twist–stretch coupling modulus g. Fig. 2A shows g values obtained from the fits under various forces. Over the force range examined, g was essentially independent of the force: $g = -21 \pm 1$ (mean $\pm$ standard error of the mean (SEM), $N = 41$). The g value is sensitive to the value used for C. For example, a $\pm 10\%$ uncertainty in C will result in a $\pm 15\%$ uncertainty in g.

If eqn (4) is used to obtain a g value instead, the magnitude of g is underestimated by $\sim 20\%$ over the range of forces



B)

	g (unitless)	Bending fluctuations
This work	-21±1 (mean±SEM, N=41)	Considered
Lionnet et al. (2006)	-16±7 (mean±SD, N>36)	Neglected
Gore et al. (2006)	-22±5 (mean±SEM, N=4)	Neglected, but the effect of this approximation is canceled by another approximation (extension calculated as proportional to force)

Fig. 2 (A) Measurement of the twist–stretch coupling modulus g. For each force, the twist–stretch coupling modulus was determined using eqn (2) from 7–11 traces of data. The mean of the modulus g is shown as the solid horizontal line. For comparison, the magnitude of g would have been underestimated by $\sim 20\%$ if eqn (3) were to be used instead (dashed line). (B) Summary of the values of the twist–stretch coupling modulus obtained in recent single-molecule experiments. Considerations pertaining to bending fluctuations are specifically indicated.

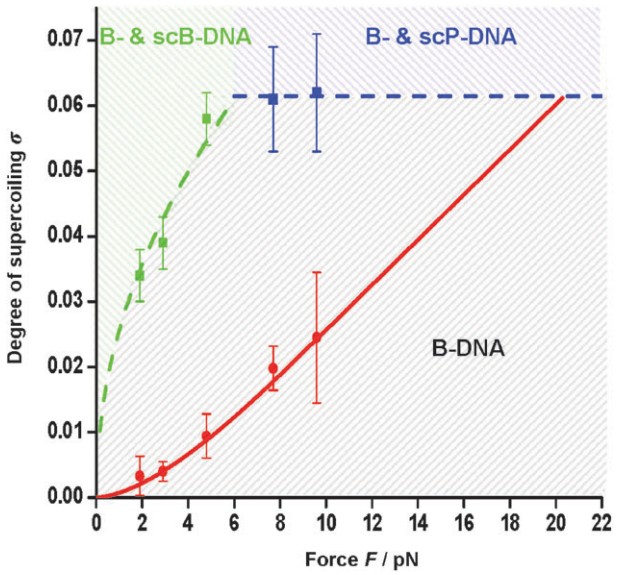


Fig. 3 The degree of supercoiling at extension maximum σ_{z_max} and at the onsets of phase transitions. The measured values of σ_{z_max} (red circles) are plotted together with σ_{z_max} calculated using the mean value of g from Fig. 2 (red line). For comparison, also shown are the measured degree of supercoiling at the buckling transition and its fit using a Marko theory[20] (green), as well as the measured degree of supercoiling at the onset to the scP transition with a constant fit (blue).

examined (dashed line in Fig. 2A). This difference does not vanish even with an increase in force, since the geometric coupling between bending and writhe fluctuations leads to a decrease in extension.[14] Interestingly, Lionnet *et al.*[17] used eqn (4) to fit their extension data and obtained $g = -16 \pm 7$ (mean $\pm$ SD, $N > 36$), whose magnitude is $\sim$20% lower than our measured value. On the other hand, Gore *et al.*[16] made a measurement of twist angle as a function of extension change when the DNA was not torsionally constrained and obtained $g = -22 \pm 5$ (mean $\pm$ SEM, $N = 4$), more in accord with our measured value. In the analysis by Gore *et al.*,[16] the bending fluctuations were also neglected resulting in a similar linear approximation. Thus one might expect that the magnitude of g was similarly underestimated. Careful analysis using eqn (2) and (4) reveals that this effect was almost completely canceled by another linear approximation made to convert force to extension change. Taking together all these results (summarized in Fig. 2B), the twist–stretch coupling modulus g should be about -21.

The extension maximum, however, can only be explained using eqn (2) and not eqn (4). Fig. 3 plots σ_{z_max} obtained as a function of force. For comparison, we also plotted the critical σ_c values for phase transitions to plectoneme (scB-) or scP-DNA. As this Figure indicates, when DNA is positively supercoiled under moderate forces (2 pN $< F <$ 20 pN), the DNA extension reaches a maximum long before DNA buckling or a transition to scP-DNA. At even higher forces ($F >$ 20 pN), the scP-DNA transition is reached before the extension reaches a maximum. This Figure clearly indicates that σ_{z_max} and σ_c do not coincide, except at $\sim$20 pN. Therefore, the maximum in the extension in general is not indicative of a phase transition, in contrast to the interpretation of Lionnet *et al.*[17]

Twist–stretch coupling should also alter the torque signal. We found that consideration of the twist–stretch coupling as in eqn (3) will lower the expected torque value at most by $\sim$1 pN nm (ESI†). This is below the uncertainty of our experimental determination of torque.

The analysis described in this work may not be valid at forces significantly higher than those used in the current work. In the absence of torsional constraints, eqn (3) predicts a monotonic increase in overtwisting angle with an increase in force. However, Gore *et al.*[16] found that the twist angle starts to decrease with force above $\sim$30 pN.

It is also worth mentioning that we do not consider sequence-dependent effects on twist–stretch coupling or phase transitions here. Prior simulation work[17,19] suggests that DNA sequence may modulate the twist–stretch coupling. Future experiments with more refined measurements may help verify this prediction.

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