Torsional Mechanics of DNA Are Regulated by Small-Molecule Intercalation

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Whether the bend and twist mechanics of DNA molecules are coupled is unclear. Here, we report the direct measurement of the resistive torque of single DNA molecules to study the effect of ethidium bromide (EtBr) intercalation and pulling force on DNA twist mechanics. DNA molecules were overwound and unwound using recently developed magnetic tweezers where the molecular resistive torque was obtained from Brownian angular fluctuations. The effect of EtBr intercalation on the twist stiffness was found to be significantly different from the effect on the bend persistence length. The twist stiffness of DNA was dramatically reduced at low intercalator concentration (<10 nM); however, it did not decrease further when the intercalator concentration was increased by 3 orders of magnitude. We also determined the dependence of EtBr intercalation on the torque applied to DNA. We propose a model for the elasticity of DNA base pairs with intercalated EtBr molecules to explain the abrupt decrease of twist stiffness at low EtBr concentration. These results indicate that the bend and twist stiffnesses of DNA are independent and can be differently affected by small-molecule binding.

Introduction

Mechanical properties of DNA have important implications in gene regulation, chromosomal packaging, gene transcription, and replication. These fundamental biological processes often apply a twist to the DNA molecule.[1,2] DNA has torsional rigidity and develops a resistive torque when it is twisted.[3] Therefore, to understand the mechanics of such processes, it is critical to know the torque required to twist DNA under different conditions. Binding of small molecules to DNA is known to affect the bending persistence length of DNA.[4–10] However, it is unknown how the presence of small molecules and drugs affects the twist stiffness of DNA.

We recently developed a single-molecule technique that allows for the introduction of turns into a single DNA molecule and subsequent measurement of the resistive torque of the molecule under tension. This approach is particularly well-suited to probe the torque properties of nucleic acids under low pulling forces. We previously used this technique to measure the torque of bare DNA molecules.[3] The torque, τ, required to twist an extended DNA molecule increases linearly as turns are introduced into the molecule, with a slope proportional to the twist stiffness of the molecule.

Single-molecule studies have shown that the mechanical properties of DNA are modulated by the presence of ethidium bromide (EtBr).[4–6,11–13] EtBr is representative of a class of small molecules that intercalate between DNA base pairs, including anticancer and antibiotic drugs in clinical use.[14–18] EtBr is extensively used as a dye that fluoresces in the presence of UV light when inserted between base pairs.[19–21] The intercalation of EtBr both increases the distance between base pairs by ~0.3 nm and unwinds the double helix by 26°.[20,22] The bending persistence length of DNA can decrease or increase in the presence of intercalators, depending on the intercalator concentration and externally applied pulling forces.[4–7] At low pulling forces, for a large range of EtBr concentrations, the persistence length has been reported to decrease by less than 20% with respect to the value of bare DNA.[5,12] However, under the same conditions, Lipfert et al.[12] indirectly observed a significant reduction in DNA twist stiffness for saturating concentrations of EtBr.

The effect of EtBr on DNA twist stiffness has also been measured in bulk experiments from the topoisomer distribution of circular DNA in the presence of EtBr. Some experiments reported lower supercoiling free energies in the presence of EtBr, whereas others indicated that DNA twist stiffness is unaffected by low EtBr concentrations.[23,24]

In this article, we report the direct measurement of the torque response of DNA molecules subjected to torsional forces under low pulling tension in the presence of EtBr at concentrations between 3.4 nM and 10 μM. We found that the torque response of DNA is dramatically affected by EtBr. The intercalator changes the twist stiffness of DNA in a way that is different from the change it induces on DNA bend persistence length. The twist stiffness abruptly decreases at low EtBr concentration. We propose a model to explain the effects of EtBr on the twist stiffness of DNA.

Experimental Methods

Preparation of DNA Molecules. DNA molecules were functionalized with moieties at both ends for specific attachment. The plasmid vector pFOS1 (9.7 kb, New England Biolabs, Ipswich, MA, USA) was used for the EtBr and naked DNA

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experiments. The plasmid vector pMXB10 (7.8 kb, New England Biolabs) was also used for the naked DNA experiments. The molecules were linearized with the restriction enzyme Xba I and functionalized using two DNA segments of 900 bp generated by polymerase chain reaction (PCR) in the presence of either biotin dUTP or digoxigening dUTP. The two segments were cut with Nhe I to produce XBA I compatible sticky ends that were finally ligated to the vectors in the presence of the restriction enzymes to enrich the desired product.[25]

Preparation of Capillary Tubes. Capillary tubes, 2 × 0.2 mm i.d. (Vitrocom, Mountain Lake, NJ, USA), previously cleaned in piranha solution, were incubated for 8 h in anti-digoxigenin solution (phosphate-buffered saline complemented with 0.2 mg/mL polyclonal antidigoxigenin, Roche, Indianapolis, IN, USA) and 12 h in standard buffer (10 mM phosphate buffer with 0.1% Tween-20) complemented with 10 mg/mL acetylated BSA (Sigma-Aldrich, St Louis, MO, USA), 0.1% Tween-20, 10 mM EDTA, and 3 mM NaN$_3$. Both incubations were performed at 37 °C, and the capillary tubes were stored at 4 °C.

Synthesis of Magnetic Nanorods. Nanorods are prepared by electrodeposition into the 200-nm-diameter pores of an aluminum oxide template membrane (Whatman, Springfield Mill, Kent, U.K.), similarly to previously published protocols.[26,27] The nanorods formed by filling the pores of the membrane with the deposited material. Segments were deposited by changing the electrolytic solution. The template was finally etched to generate the nanorods. We produced nanorods with a 1.7-μm platinum segment and a 0.1-μm nickel segment. Nanorods were functionalized by incubating them for 30 min in standard buffer complemented with 0.1 mg/mL Neutravidin tetramethyl-rhodamine conjugate (Invitrogen, Eugene, OR, USA). Shaking during the incubation prevented aggregation of the nanorods.

Magnetic Tweezers to Measure Torque. We used a recently introduced magnetic tweezers configuration that allows for measurements of molecule torque in addition to molecule extension (Figure 1A). Briefly, the linearized DNA plasmid was attached at its digoxigening end to the interior surface of a glass capillary previously functionalized with antidigoxigening antibodies, while the biotin end was attached to a probe consisting of a streptavidin-functionalized Pt/Ni nanorod and a 1-μm superparamagnetic bead (Figure 1A). This assembly was manipulated using the magnetic field generated by a 6-mm-diameter cylindrical magnet placed ~2 mm above the glass capillary. A constant pulling force was applied to the molecule by a gradient generated by the magnetic field that pulled the probe away from the glass substrate. The pulling force was modified by changing the distance between the magnet and the capillary. The pulling force was measured from the fluctuations of the probe in the xy plane of focus, $F = k_BTz/\langle\delta x^2\rangle$, where z is the extension of the molecule. Turns were introduced into the molecule by rotating the probe. Torque measurements were obtained from the angular fluctuations of the probe. Torque at u number of turns was calculated as $\tau(u) = k[\langle\theta_u(t)\rangle - \langle\theta_0(t)\rangle]$, where k is the stiffness of the magnetic angular trap and $\theta_0(t)$ and $\theta_u(t)$ are the angular distributions of the probe before and after turns had been introduced into the molecule, respectively. The stiffness k was obtained using the principle of equipartition of energy, as $k = k_BT/\langle\delta\theta^2\rangle$. Molecular extension was measured from the diffraction pattern of the bead using a previously established method.[28,29] The z coordinate of the center of the bead was obtained by finding the best match of the bead profile in a calibration profile set.
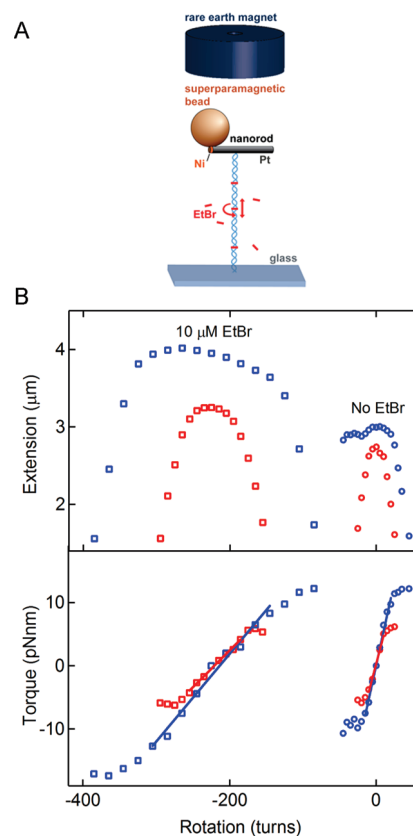

Figure 1. Effects of ethidium bromide (EtBr) on DNA torsional properties. (A) Schematic representation of the experiment. A 10 kbp DNA molecule is attached to a nanorod-bead probe at one end and to the glass surface at the other end. The nanorod is made of Pt with a short Ni segment that magnetically attaches to a superparamagnetic bead. The nanorod-bead probe is pulled vertically by the magnetic field generated by a cylindrical magnet. EtBr intercalates between DNA base pairs, locally stretching and unwinding the DNA molecule (arrows). Twists are introduced in the molecule by rotating the nanorod-bead probe, and the resistive torque is measured from the Brownian angular fluctuations of the nanorod. (B) Extension and torque measurements on (○) bare DNA and (□) DNA in the presence of 10 μM EtBr at (blue) 0.8 and (red) 0.3 pN pulling force. The intercalator unwinds the molecule shifting the DNA extension and torque curves. The effective twist stiffness of the molecule is calculated from the slope of the central region of the torque curves. The effective twist stiffness is ~3 times lower in the presence of the intercalator (Table 1).

TABLE 1: DNA Stiffness in the Presence of EtBr

pulling force	effective twist stiffness[a] (nm)		bend persistence length (nm)
	0.3 pN	0.8 pN	
bare DNA	65 ± 2.0[b]	74 ± 2.8[b]	53.2
DNA with EtBr (10 μM)	22 ± 0.8[b]	25 ± 1.2[b]	54.7
ratio	2.9	3.1	0.97

[a] Effective twist stiffness obtained from the slope of a straight line fitted to the central region of the torque curves (at least seven points, ±fitting error), the contour length of the bare molecule (3.6 μm), and the contour length in the presence of the intercalator (4.5 μm). [b] Value ± standard deviation.

Measurements of Torque. The single-molecule experiments were conducted in standard buffer. The experiments requiring EtBr intercalation were performed in standard buffer complemented with EtBr (Sigma-Aldrich). DNA molecules (50 ng/mL) were incubated in the capillary tube for 10 min to allow digoxigenin-labeled DNA ends to interact with the antidigoxi-

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genin-coated glass surface. Unbound DNA was washed away by flowing standard buffer through the capillary. Finally, the superparamagnetic beads (Dynabeads MyOne, Tosylactivated, Invitrogen, Carlsbad, CA, USA) and functionalized nanorods were flowed into the capillary. The surface of the beads was not functionalized. The beads and nanorods self-assembled in the absence of an external magnetic field, by the attraction between the ferromagnetic Ni segment and the superparamagnetic bead. The probes were lifted by placing the capillary tube under the cylindrical magnet that was held by a linear stage (460P-XYZ, Newport, Irvine, CA, USA).

Samples were observed by brightfield microscopy using an inverted microscope (Nikon Eclipse, TE2000-E) equipped with a 100× oil-immersion objective. Video images were collected with a CCD digital camera (Hamamatsu, ORCA-ER) connected to a PC. Video images were analyzed in real time at 21 Hz using customized software written in MATLAB (The Math-Works, Inc., Natick, MA).

Results and Discussion

We performed measurements on single DNA molecules to study the effects of EtBr on DNA twist properties. We used a magnetic tweezers configuration that allows the extension and resistive torque of the DNA molecule to be obtained[3] (Figure 1A). Figure 1B shows representative curves for DNA with 10 μM EtBr at pulling forces of 0.3 and 0.8 pN, as well as curves for bare DNA under the same conditions. The extension and torque curves were significantly affected at both forces by the intercalator. In contrast, the bend persistence length has been reported to be unaffected[5,30] or only moderately affected[12] by 10 μM EtBr. To confirm this result for our experimental conditions, we measured the bend persistence length of the DNA molecules under the same conditions in which the torque measurements were conducted (Figure S1, Supporting Information). In agreement with previous measurements, we obtained a bend persistence length of 53.2 nm for bare DNA and 54.7 nm for DNA in the presence of the intercalator.

The torque measurements (Figure 1B) showed that, for pulling forces of 0.3 and 0.8 pN, the presence of the intercalator reduced the slope of the torque as a function of the number of turns. We obtained an effective twist stiffness (C_{eff}) by fitting straight lines to the central region of the curves (Figure 1B) and using the formula $\tau = C_{eff}k_BT2\pi u/D$,[31] where u is the number of turns introduced into the DNA molecule, D is the DNA contour length, k_B is the Boltzmann constant, and T is the temperature. Table 1 lists the values of C_{eff} obtained in the various cases. At both forces, the C_{eff} value of DNA in the presence of the intercalator EtBr was approximately 3 times lower than the value of C_{eff} for bare DNA.

The extension of the molecule with EtBr (Figure 1B) was longer than that of naked DNA at both pulling forces, reflecting the change in the molecule contour length. Whereas the extension curves with and without EtBr at 0.3 pN were qualitatively similar, the extension curves at 0.8 pN were significantly different. Under a pulling force of 0.3 pN, the formation of plectonemes following the application of either overwinding or unwinding turns produced extension curves that were symmetric for both bare and EtBr-conjugated DNA. In contrast, under a pulling force of 0.8 pN, the introduction of unwinding turns in bare DNA eventually started to separate the two strands of the molecule, and the extension of the molecule did not change significantly. Overwinding turns still formed plectonemes at this force, which produced an asymmetric extension curve. In contrast, the extension curve at 0.8 pN of
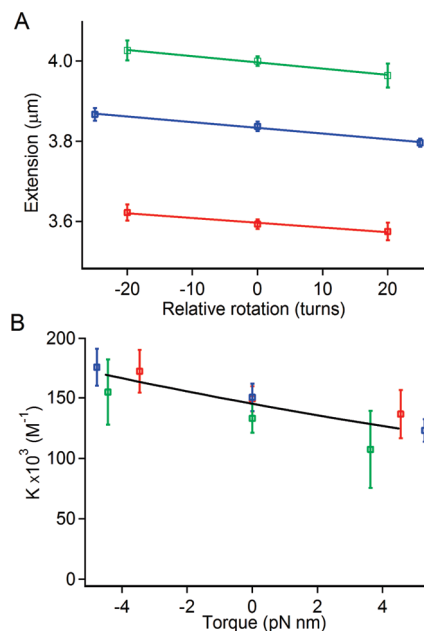

Figure 2. Dependence of EtBr intercalation on torque. (A) DNA molecule extension in the presence of EtBr (green, 10; blue, 4.4; and red, 1.7 μM) as a function of relative rotation. Relative rotations were calculated as the number of rotations minus the number of rotations at the center of the extension curves. (B) Binding constant (K) as a function of torque. K was obtained from the number of EtBr molecules bound to DNA, which is related to the molecule extension change shown in part A. Measurements were fit with an exponential function of the form $K = K_0 \exp(-\tau\Delta\theta/k_BT)$ (black line).

the molecule with the intercalator showed shortening in the presence of either overwinding or unwinding turns. Similar extension curves in the presence of EtBr were obtained recently.[12,13]

The extension curve at 0.8 pN with intercalator was asymmetric even if DNA shortened at both overwinding and unwinding conditions (Figure 1B). The extension in the central region of the curve increased as unwinding turns were introduced into the molecule. This can be explained considering that unwinding turns in the presence of sufficient pulling force favor EtBr intercalation, which increases the DNA contour length. Figure 2A shows extension measurements in the region close to zero turns at three EtBr concentrations. The extension slope was similar in each case and about 1.5 nm/turn, which corresponds to ~4.5 new EtBr molecules intercalated per turn.

The binding constant of EtBr has been shown to depend on pulling force.[6] Here, we used a similar approach to study the dependence of the binding constant of EtBr on torque. The fraction of sites occupied by EtBr, v, can be related to the molecule extension by

$$v = \frac{(D_{EtBr} - D_{bare})/d_{EtBr}}{D_{bare}/d_{bp}} \quad (1)$$

where D_{EtBr} and D_{bare} are the molecule contour lengths in the presence and absence, respectively, of the intercalator; d_{EtBr} is the extension increment due to the intercalation of one EtBr molecule; and d_{bp} is the length of one DNA base pair. The binding constant, K, is related to v by the neighbor-exclusion model obtained by McGhee and von Hippel[32]

$$K = \frac{v(1 - nv + v)^{n-1}}{L(1 - nv)^n} \qquad (2)$$

where n is the exclusion parameter and L is the EtBr concentration. We used $n = 2.5$[6,22] and assumed that n is not affected by low torques (≤ 5 pN·nm). We based this assumption on recent measurements that showed that pulling forces up to 20 pN do not significantly change n.[6] Figure 2B shows K as a function of torque for the experimental measurements shown in Figure 2A.

The torque acting on the DNA affects the free energy of the species involved in the intercalation reaction. In particular, the free energy of the transition state is modified; therefore, the reaction rate is altered according to the Arrhenius equation. The observed dependence of K on torque can be fitted with an exponential function of the form $K = K_0 \exp(-\tau\Delta\theta/k_BT)$, where $\tau\Delta\theta$ corresponds to the difference in the work done by the imposed torque on the DNA before and after EtBr intercalation. We obtained $K_0 = 145 \times 10^3$ M^{-1}, which is in agreement with previous measurements obtained under similar conditions.[6,12,33]

We further investigated the effects of lower EtBr concentrations on DNA twist stiffness. We obtained torque versus number of turns curves for DNA at 0.8 pN pulling force with EtBr concentrations between 3.4 nM and 10 μM (Figure 3). The red square symbols in Figure 3B represent the twist stiffness obtained from the slope of the torque curves shown in Figure 3A. The twist stiffness suffered an abrupt decrease at low EtBr concentration and then remained relatively constant. These values of DNA twist stiffness had to be corrected as explained below.

Because the binding constant K is affected by torque (Figure 2B), the number of EtBr molecules bound to DNA is not constant when torque is not constant. Because binding of additional EtBr molecules unwinds the DNA molecule, the number of externally introduced turns does not represent the twist imposed on the molecule. When u turns are externally introduced into the molecule and EtBr unwinds DNA by 26°, torque and C_{eff} are related according to the equation

$$\tau = \frac{C_{eff}k_BT2\pi\left(u + \dfrac{26N(v - v_0)}{360}\right)}{D_{EtBr}} \qquad (3)$$

where N is the number of binding sites in the molecule and v_0 is the fraction of occupied sites at $u = 0$. Note that $v - v_0$ will be negative when u is positive and positive when u is negative. The neighbor-exclusion model (eq 2) and the obtained dependence of the binding constant on torque can be combined to relate v to the applied τ value through the equation

$$\frac{v}{L} = K_0 e^{-\tau\Delta\theta/k_BT} \frac{(1 - nv)^n}{(1 - nv + v)^{n-1}} \qquad (4)$$

We found that v can be well approximated as a linear function of τ (Figure S2, Supporting Information); specifically, v can be expressed as $q\tau + v_0$ with a different q for each L. Substituting into eq 3, we obtained a linear relation between τ and u as

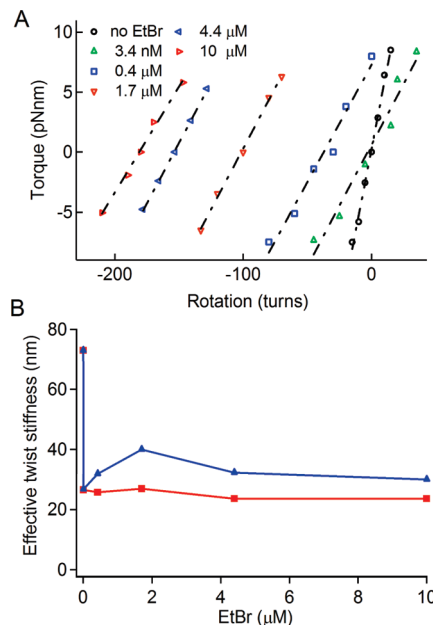$$\tau = \frac{C_{eff}k_BT2\pi u}{D_{EtBr} - \dfrac{52C_{eff}k_BT\pi q}{360}} \qquad (5)$$

Figure 3. Dependence of DNA effective twist stiffness on EtBr concentration. (A) Torque measurements of DNA at several EtBr concentrations at 0.8 pN pulling force. Measurements in the absence of the intercalator are shown for comparison (black circles). The slopes of the curves in the presence of EtBr are significantly lower than in the absence of EtBr. (B) Twist stiffness of DNA with intercalator molecules. Red squares represent the effective twist stiffness of DNA obtained from the slope of a straight line fitted to the torque measurements. Blue triangles show the effective twist stiffness corrected for the twist introduced by EtBr molecules that intercalate when torque is applied to the molecule (see text).

Using this relation to fit the torque versus turns data (Figure 3A), we obtained values of C_{eff} that were corrected for the unwinding effect of EtBr intercalation (blue line in Figure 3B). The twist stiffness dropped from 74 nm in the absence of EtBr to about 25 nm at 3.4 nM EtBr. Then, the twist stiffness increased by almost a factor of 2 to 40 nm at 2 μM EtBr. Finally, twist stiffness was constant, at about 30 nm, for higher EtBr concentrations.

Topoisomer distribution experiments, performed in the absence of pulling force, indicated that, in certain cases, low concentrations of EtBr have no effect on twist stiffnesss.[23,24] However, these experiments extracted the effect of EtBr from the change in supercoiling free energy. Changes in twist stiffness could have been masked by other changes induced by EtBr binding, such as bend stiffness changes. There is a possibility that the pulling force used in our experiments could influence the effect of EtBr on twist stiffness. However, any change is expected to be small because pulling forces below 2 pN do not significantly alter the secondary structure of DNA. These low forces do not fully extend DNA, meaning that the molecule end-to-end distance is less than its contour length, and the main effect of the low force is to reduce the Brownian fluctuations of the DNA. Indeed, measurements of the binding constant of EtBr at high stretching forces showed that binding of EtBr is basically unaffected at pulling forces of <10 pN.[6]

The observed twist stiffness of a DNA molecule in the presence of EtBr arises from the specific elasticity of DNA base pairs and DNA base pairs intercalated by EtBr molecules (Figure 3A). The number of EtBr molecules bound to a DNA molecule (e) can be estimated using the neighbor-exclusion model (eq 2), with $e = vN$. Using the binding constant determined above, we obtained $e = 5, 535, 1402, 2140,$ and 2675, for $L = 3.4$

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nM, 0.4 μM, 1.7 μM, 4.4 μM, and 10 μM, respectively. Therefore, $e \approx 5$ is sufficient to produce an abrupt reduction of the twist stiffness of the molecule. The effect of a few intercalators can be compared with the effect of a nick base pair on the torsional properties of DNA. In a nick base pair, the molecule can swivel around a single covalent bond that poses no resistance to rotation. For this reason, a DNA molecule with a nick base pair does not exert resistive torque when it is twisted. A nick base pair has twist stiffness basically equal to zero and dramatically modifies the twist stiffness of the whole molecule.

We modeled the DNA molecule in the twist experiment as several torsional springs in series. EtBr binding changes the local twist properties of the DNA and produces a segment of different elasticity than bare DNA. Therefore, we assumed that two types of springs alternate along the DNA molecule. In this model, the resistive torque exerted by each spring is the same, and the total rotation of the molecule is the sum of the rotations of each spring. Each individual torsional spring exerts a torque that depends on its rotation angle, θ_s. We considered a relation between these two quantities of the form

$$\tau_s = a\left(\frac{\theta_s}{D_s}\right) + b\left(\frac{\theta_s}{D_s}\right)^2 + c\left(\frac{\theta_s}{D_s}\right)^3 \quad (6)$$

where D_s is the contour length of the segment. Equation 6 represents a nonlinear elastic model for DNA torsion. The twist stiffness of DNA is dramatically altered because EtBr perturbs the base-pair molecular structure. We found that a nonlinear model was necessary to describe the elasticity of regions affected by EtBr (see below). Instead, for DNA without EtBr, a linear elastic model was sufficient, which can be represented with eq 6 using $a = 74k_BT$ nm and $b = c = 0$. Therefore, for a DNA molecule where these two types of torsional springs are present in series, the following expression relates the torque (τ), the total rotation of the molecule (θ), and the rotation of a region affected by an EtBr molecule (θ_e)

$$\tau = \frac{74k_BT(\theta - e\theta_e)}{D_b} = a\left(\frac{\theta_e}{m}\right) + b\left(\frac{\theta_e}{m}\right)^2 + c\left(\frac{\theta_e}{m}\right)^3 \quad (7)$$

where D_b is the sum of the lengths of bare DNA segments in the molecule (DNA segments not affected by intercalators); a, b, and c are the parameters that describe the elasticity of DNA affected by intercalators; and m is the DNA length for which the twist stiffness is affected by a intercalated molecule. Note that, in this model, the order of the springs along the DNA molecule is irrelevant. The modeling results presented below consider $m = 2.5l_{bp} = 0.8$ nm. Similar results were obtained with values in the range of 0.33 nm $< m <$ 3.3 nm.

We tried different linear and nonlinear models for the torsional spring of the regions affected by EtBr intercalation. Figure 4A shows three representative models for the elasticity of these regions. The dash-dotted and dashed lines are linear models ($b = c = 0$), with $a = 30k_BT$ nm and $a = 15k_BT$ nm, respectively. The solid line is a nonlinear model with $a = 40k_BT$ nm, $b = -195k_BT$ nm^2, and $c = 195k_BT$ nm^3. Equation 7 was used to calculate τ as a function of θ for several EtBr concentrations. We obtained the torsional stiffness at each concentration (Figure 4B) from the slopes of straight lines fitted to the resulting curves.
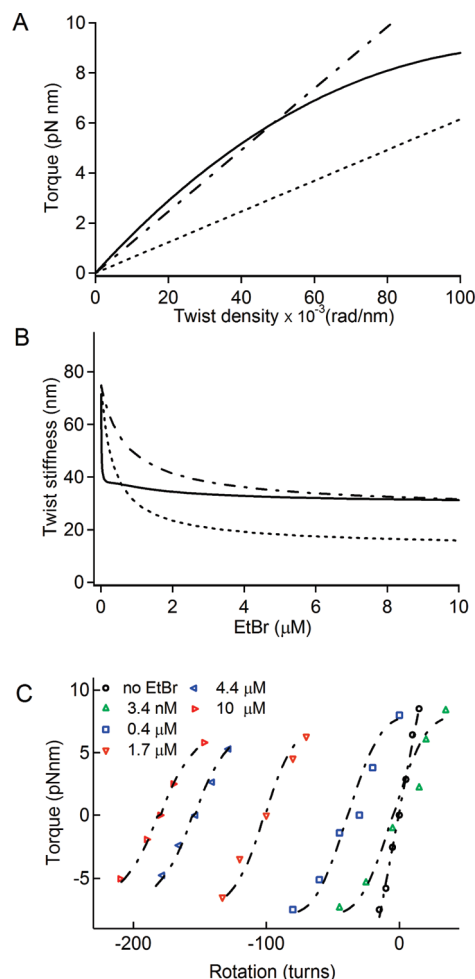

Figure 4. Model of intercalator effects on DNA twist stiffness. (A) Three models for the torsional properties of DNA affected by EtBr. The dash-dotted and dashed lines are linear elasticity models with twist stiffness values of 30 and 15 nm, respectively. The solid line is a nonlinear elasticity model. (B) Twist stiffness of DNA as a function of EtBr concentration obtained from the slope of straight lines fitted to the torque versus rotation curves produced by the theoretical models. Dash-dotted and dashed lines show the results when the corresponding linear models shown in A are used to model the elasticity of the EtBr regions. The dash-dotted line converges to a twist stiffness of 30 nm, but fails to explain the abrupt reduction of the twist stiffness observed in the experimental results. The dashed line has a faster stiffness decrease; however, it converges to a stiffness of 15 nm, significantly lower than the experimental value. The solid line is the result when the nonlinear behavior shown in A is used. In this case, the model shows an abrupt decrease in twist stiffness at low EtBr concentrations, and twist stiffness remains above 30 nm for higher EtBr concentrations, in agreement with the experimental data. In the three cases, regions of DNA without EtBr were modeled with linear elasticity and a twist stiffness of 74 nm. (C) Torque measurements fitted using the model that considers nonlinear elasticity in the EtBr regions.

We looked for a model that satisfies two trends observed in the experimental data: the torsional stiffness exhibits an abrupt decrease at low EtBr concentration and remains above 30 nm for higher intercalator concentrations. We first used the linear model represented by the dash-dotted line in Figure 4A to model the elasticity of the regions affected by the intercalator. This produced a torsional stiffness curve that converged to 30 nm. However, the model failed to decrease fast enough to explain the experimental data (dash-dotted line in Figure 4B). Linear models with twist stiffness below 30 nm do not explain the experimental measurements because the twist stiffness of DNA converges to values below 30 nm at high EtBr concentrations,

which is not consistent with the experimental data (dashed line in Figure 4B). Therefore, we turned to nonlinear elasticity to explain the observed behavior of the DNA torsional stiffness. Using the nonlinear model described in Figure 4A for the elasticity of the regions affected by EtBr produced the desired behavior (solid line in Figure 4B). The values of the constants *a*, *b*, and *c* were obtained by fitting the experimental data shown in Figure 4C with a nonlinear model consisting of two equations (eq 7). Fitting was performed in MATLAB (version 7; The MathWorks, Inc., Natick, MA) using an unconstrained nonlinear optimization function to minimize the sum of the squared errors.

The torque versus turns curves generated by this model are not linear (Figure 4C). Curves have lower slopes at higher numbers of turns than at zero turns. We were not able to confirm or reject the deviation from linearity in the torque versus turns curves because of experimental noise. However, this model fits the experimental data (Figure 4C), and the apparent stiffness of the obtained curves explains the observed DNA stiffness over a wide range of concentrations, from 3 nM to 10 μM EtBr (Figure 4B).

The nonlinear elasticity of DNA regions affected by EtBr intercalation can be rationalized considering that the interaction of an intercalated EtBr molecule with adjacent DNA base pairs can be affected by the angle between the bases. EtBr intercalates between base pairs by stacking its planar aromatic rings between the aromatic rings of two consecutive DNA base pairs. To maximize the interaction, the EtBr rings align with the DNA base pairs.[22] If the twist angle between bases changes, the alignment between the EtBr rings and the bases decreases, possibly reducing the interaction energy and the torque required to further twist the bases. This explanation is consistent with the nonlinear model shown in Figure 4A, where the torque required to twist DNA decreases as the twist angle increases. Furthermore, when there are few intercalated EtBr molecules, each one is twisted by a relatively large amount. This pushes the torsional springs to behave as a very soft material (see the nonlinear model in Figure 4A at more than 60×10^{-3} rad/nm), explaining the abrupt decrease in DNA twist stiffness at low *e*. As the number of intercalated EtBr molecules increases, the torsional springs are less twisted and therefore stiffer, explaining the convergence of the model with nonlinear torsional springs to 30 nm at high EtBr concentrations (Figure 4B).

There are marked differences between the extension curves at 0.8 pN in the presence and absence of intercalators (Figure 1A). As mentioned previously, the molecule shortens in the presence of EtBr at 0.8 pN with negative unwinding turns. Under the same conditions, bare DNA melts with its extension remaining constant. The shortening of the molecule can be associated with plectoneme formation. DNA resilience to melting is a consequence of intercalation.[15,22] In our experiments, DNA containing intercalators did not denature when subjected to unwinding torques >15 pN·nm (Figure 1B). Naked DNA, instead, denatured at −10 pN·nm. DNA base-pair stabilization by intercalator binding can also be detected from the increase in the melting temperature of the double-stranded DNA in bulk experiments.[14,15] More stable base pairs are able to withstand higher unwinding torques without melting. We were not able to systematically study the torque required to melt DNA in the presence of EtBr because our magnetic tweezer system did not allow the measurement of torque at higher pulling forces. Therefore, we will further study this behavior in future work.

Conclusions

We have used a single-molecule technique to measure the torque and extension of bare DNA molecules and DNA in the presence of EtBr intercalators. Our results show that the mechanical response of DNA under torsion is significantly affected by EtBr (Figure 1B). We found that the effective twist stiffnesses, buckling torques, and shapes of the extension curves were different when the intercalator was present. Low EtBr concentrations (<10 nM) reduced the twist stiffness of DNA by a factor of 3. Increasing the EtBr concentration by 3 orders of magnitude did not further reduce the twist stiffness (Figure 3B). In contrast, previous measurements showed that the bend persistence length does not decrease by more than 20% under similar experimental conditions.[5,12] This result indicates that the bend and twist stiffness are independent and can be differently affected by small-molecule binding. Therefore, the bend persistence length is not sufficient to characterize the effects of small-molecule binding on the mechanical properties of DNA, and twist stiffness needs to be considered as well. We found evidence that EtBr perturbs the DNA structure significantly and that only a nonlinear twist model is sufficient to explain the observed dependence of the twist stiffness on EtBr concentration.

The results of this work show that DNA bend and twist mechanics are each dominated by molecular forces that can be decoupled by intercalator molecules, such as EtBr. Comparing the EtBr results with future measurements of other small molecules will increase our understanding of the binding mechanism of these molecules and, ultimately, their physiological effects.

Supporting Information Available: Extension measurements of bare DNA and DNA in the presence of EtBr (Figure S1) and fraction of DNA sites occupied by EtBr (Figure S2). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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