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Magnetic Tweezers Measurement of Single Molecule Torque

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Abstract

Torsional stress in linear biopolymers such as DNA and chromatin has important consequences for nanoscale biological processes. We have developed a new method to directly measure torque on single molecules. Using a cylindrical magnet, we manipulate a novel probe consisting of a nanorod with a 0.1 μm ferromagnetic segment coupled to a magnetic bead. We achieve controlled introduction of turns into the molecule and precise measurement of torque and molecule extension as a function of the number of turns at low pulling force. We show torque measurement of single DNA molecules and demonstrate for the first time measurements of single chromatin fibers.

Graphical abstract

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Supporting Information Available: Movies and further description of methods and materials used in the new magnetic tweezers configuration. This material is available free of charge via the Internet at <http://pubs.acs.org>.

DNA and other linear biopolymers accumulate torsional stress under the action of a rotary force or torque. This property has important biological implications, for example, torsional stress affects the action of enzymes that act on DNA.^{1–3} Fundamental biological processes such as DNA replication and transcription involve biological nanomachines that exert torque on double-stranded (ds) DNA and chromatin.^{4,5} Thus, in order to reveal the mechanisms and energetics of these processes, it is imperative to have tools to measure the torque required to twist linear biopolymers, such as DNA and chromatin. Understanding how biological processes and linear biopolymers are affected by torsional stress is important in the design of chemotherapeutic drugs,⁶ and applications involving DNA biological machines.⁷ Moreover, the ability to measure the torque of single molecules is important in understanding molecular structure, such as the chromatin structure,⁸ and, in general, an essential tool to study the torsional properties of nanoscale linear biopolymers.

The dependence of torque on twist for DNA has been measured with optical angular trapping methods,^{9–11} and has been analyzed from viscous drag forces.¹² However, like other biopolymers, the structure of DNA is sensitive to pulling force, and these techniques employ pulling forces greater than 1 pN, sufficient for melting duplex DNA when negative twists are introduced.¹³ DNA and chromatin in vivo are usually free from stretching forces and hence it is of fundamental interest to study these fibers using a device that can measure torque at low pulling force. Twisting a single molecule at low pulling force can be achieved using magnetic tweezers. This technique has been extensively used to study naked DNA,^{13,14} chromatin,^{8,15–17} and enzymes that act on DNA.^{2,18–22} In a common magnetic tweezers configuration, two magnets are used to pull and rotate the probe used to manipulate the molecule.¹³ This configuration, however, does not allow measuring single molecule torques (~ 10 pN·nm, ref 9) because, as we show below, they do not produce a measurable change in the angular orientation of the probe. To allow measurement of torque at low pulling forces (0.1–1.5 pN), we have developed a new magnetic tweezers configuration (Figure 1). We use a probe consisting of a 2 μm long and 0.2 μm diameter Ni-Pt nanorod with a 0.1 μm Ni segment, produced by electrochemical template synthesis,²³ coupled to a 1 μm diameter superparamagnetic bead. This probe is manipulated using a cylindrical magnet. The new device allows direct and precise torque measurement of single molecules. We use the new method to measure torque in single DNA molecules and for the first time present results for single chromatin fibers. Our bare DNA measurements match existing measurements at pulling forces >1 pN and agree with theoretical predictions at lower pulling forces. The results for chromatin show that the torsional rigidity of chromatin fibers is lower than that for naked DNA at the same pulling force.

In order to study the torsional properties of DNA and other linear biopolymers at low pulling force in a single molecule experiment, there are four main requirements. First, we must be able to introduce turns into the molecule. This is accomplished by first linking one end of the molecule to the nanorod and the other end to the glass substrate, and then rotating the nanorod-bead probe. The probe is rotated by exposing the probe to a rotating magnetic field. Second, we must be able to measure torque at any configuration (i.e., number of turns). This is achieved in the following way. Under equilibrium conditions the magnetic field holds the probe at a given angular orientation but sufficiently weakly to allow thermal fluctuations. Torque can be calculated from the change in the angular distribution of the probe before and

after introducing turns into the molecule. This requirement necessitates that the probe is confined in a weak trap, so that the orientational change can be observed. Third, we need to measure the extension of the molecule perpendicular to the surface. This is measured from analysis of the diffraction pattern from the bead attached to the nanorod.²⁴ Fourth, we must apply and measure a small vertical force. This force is applied to the molecule through the probe and is the result of a vertical gradient in the magnetic field. This force keeps the molecule extended and allows us to explore the influence of applied force on torque and on molecule extension. The vertical force is measured from the fluctuations of the probe in the x - y plane.¹³ Below we discuss how these four requirements are achieved in our approach. The methodologies used to achieve the first and second requirements are new, and we discuss them in detail; the third and fourth requirements are implemented using established approaches and are hence briefly summarized.

Controlled Introduction of Turns into the Molecule

We introduce twist or turns into the molecule by rotating the nanorod-bead probe attached to it. To obtain the configuration shown in Figure 1a, the axis of the nanorod must be horizontal under the vertical magnetic field. This particular configuration of the nanorod is accomplished by limiting the length of the ferromagnetic Ni segment of the nanorod to $\sim 0.1 \mu\text{m}$, approximately half of the nanorod diameter, which makes the magnetic dipole of the Ni segment perpendicular to the long axis of the nanorod²⁵ (see Figure S1 of the Supporting Information). The magnetic attraction between the ferromagnetic Ni segment and the magnetic dipole that it induces in the superparamagnetic bead results in self-assembly. In the assembled probe, the Ni segment attaches the nanorod to the bead in such a way that the bead dipole $\vec{m}$ (arrow with color gradient in Figure 1a) is perpendicular to the nanorod axis. The vertical magnetic field created by the cylindrical magnet $\vec{B}$ applies a torque $\vec{m} \times \vec{B}$ to the probe which vertically orients $\vec{m}$. The dipole $\vec{m}$ remains aligned with the vertical field during fluctuations of the probe horizontal angle (θ in Figure 1a) and $\vec{m} \times \vec{B} \approx 0$ at any horizontal orientation of the probe. However, fluctuations of the angle θ are constrained by a horizontal force $\vec{F}_h$ applied to the magnetic center of the probe, due to a horizontal gradient of the magnetic field (see Figure S2 of the Supporting Information). The torque $\vec{L}_h \times \vec{F}_h$, where $\vec{L}_h$ is the horizontal component of the distance between the DNA point of attachment to the probe and the magnetic center of the probe, traps the fluctuations of the angle θ and orients the nanorod in the direction of the force $\vec{F}_h$.

We use this alignment of the nanorod with $\vec{F}_h$ to rotate the probe. The force $\vec{F}_h$ points outward, away from the axis of symmetry of the cylindrical magnet and is symmetric around this axis (Figure 1b). $\vec{F}_h$ is zero at the point where the axis of the cylindrical magnet intersects the plane of the glass substrate, and it increases as one moves radially away from this point. Positioning the probe at more than $50 \mu\text{m}$ from that point produces a horizontal force $\vec{F}_h > 0.1 \text{ pN}$. The probe is rotated by moving the sample stage in such a way that the probe moves around the projected center of the cylindrical magnet in a path $\approx 50 \mu\text{m}$ away from this center (see Figure S3 and movie S1 of the Supporting Information). Due to the

radial nature of $\vec{F}_h$, this motion rotates the force $\vec{F}_h$ applied to the probe which allows for controlled rotation of the probe and thus introduces turns into the DNA molecule. Precise movement of the probe is facilitated by a motorized stage (H117 Proscan, Prior Scientific, Rockland, MA). After the molecule is rotated to a specific configuration, the probe is returned to the initial position with 2 pixel precision (88 nm) using a separate immobilized bead adhered to the glass surface as a reference.

Torque Measurement

We measure torque from the horizontal angular fluctuations of the probe attached to the molecule, before and after introducing turns into the molecule. The probe angle θ is the result of the torque of the angular trap from the magnetic field and the resistive torque from the twisted molecule. Measurement of molecular torque requires confinement of the probe in a weak angular trap such that the change in the in angular distribution due to the resistive torque is large enough to be measured. The torque $\vec{L}_h \times \vec{F}_h$ from the magnetic field traps the fluctuations of the horizontal angle of the probe (θ). The angular trap stiffness (k_θ) is $\approx |\vec{L}_h||\vec{F}_h|$, and therefore, is determined by the point of attachment of DNA to the nanorod and by the position of the probe in the magnetic field (see Figure S4 of the Supporting Information). We use probes where $\vec{L}_h$ is between 0.3 and 1 μm , and we position the probes at $<50 \mu\text{m}$ (typically 40 μm) from the projected center of the cylindrical magnet, such that $|\vec{F}_h| < 0.1 \text{ pN}$. The horizontal angular trap generated by the torque $\vec{L}_h \times \vec{F}_h$ has, in these conditions, k_θ as low as 30 pN·nm. In the magnetic tweezers configuration with two magnets,¹³ however, the horizontal magnetic field generated by the magnets tightly constrains the horizontal angular movements of the probe. In experiments where we use the two-magnet configuration, the stiffness of the angular trap is at least 80 times higher than the stiffness obtained with the nanorod-bead probe manipulated with the cylindrical magnet (see Figure S5 of the Supporting Information). We conclude that the two-magnet setup cannot be used to measure the torque of single DNA molecules, since its high trap stiffness does not allow resolution of torque differences lower than 40 pN·nm. This is important since DNA molecules buckle at 17 pN·nm at a pulling force of 1 pN.⁹ The low trap stiffness of the nanorod-bead probe and the cylindrical magnet configuration allows a torque resolution of $<1 \text{ pN·nm}$, essential for torque measurements on DNA.

The precision of the torque measurements is enhanced if the angular fluctuations of the nanorod out of the horizontal plane (vertical angular fluctuations) are minimized.

Minimizing these fluctuations ensures that the horizontal distance $\vec{L}_h$ is constant, which conserves the torque $\vec{L}_h \times \vec{F}_h$ and also conserves the shape of the nanorod image, which allows tracking the angle of the nanorod with precision (Figure 2 and movie S2 of the Supporting Information). Vertical angular fluctuations of the nanorod are strongly constrained by the alignment of the probe dipole with the vertical magnetic field. Measurements of the angle between the nanorod and the horizontal plane show that the vertical angle fluctuates within ($\pm 6^\circ$) (Figure S7 and movie S3 of the Supporting Information).

Figure 3 shows measurements of the horizontal angular fluctuations of a nanorod-bead probe. The torque at n number of turns can be obtained from two angular histograms. First, the angular histogram $P_0(\theta)$ of the nanorod-bead probe is obtained before introducing turns into the molecule ($n = 0$). $P_0(\theta)$ describes the angular probability distribution of the probe in the absence of resistive torque from the molecule. Then, after introducing n turns into the molecule, the histogram $P_n(\theta)$ is obtained at the same (x,y) position as $P_0(\theta)$. The resistive torque of the molecule can be estimated by approximating the angular trap of the field as a harmonic potential well. Thus, the trap stiffness k_θ multiplied by the change in the average angle between $P_n(\theta)$ and $P_0(\theta)$ gives the torque at n turns. Torque can also be obtained from $P_n(\theta)$ and $P_0(\theta)$ without assuming the harmonic approximation (see Supporting Information). The difference between torque values obtained with and without the harmonic approximation is less than 1.5 pN·nm and typically less than 0.5 pN·nm (data not shown). Torque measurements rely on the fact that the angular trap from the magnetic field is not changing after turns are introduced into the molecule. We test this by calculating k_θ at different number of turns. No significant change in k_θ is observed associated with the change in $\langle \theta \rangle$ or with the change in the z position of the probe (Figure 3 and Table S1 of the Supporting Information). The observed stability of k_θ is a consequence of the horizontal force being constant for z changes in the scale of the experiment and the nanorod being highly constrained to the horizontal plane. Multiple torque measurements in two different DNA molecules give a standard deviation of 0.8 pN·nm or less (see Figure S8 of the Supporting Information).

Molecule Extension

Molecule extension is measured from the diffraction pattern of the bead using a established methodology.²⁴ The z -coordinate of the center of the bead is obtained by finding the best match of the bead profile in a calibration profile set (see Supporting Information). This method allows us to measure molecule extension with <50 nm precision.

Pulling Force

The vertical gradient of the magnetic field produces a vertical pulling force. Changing the distance between the cylindrical magnet and the glass substrate in a 1 mm range allows adjustment of this force between 0.1 and 1.5 pN. The probes are tethered to DNA or chromatin (see below), and with an adaptation of the method first developed for measuring pulling forces in beads,¹³ the vertical pulling force is computed from the fluctuations of the x - y position of the point along the nanorod where the DNA is attached (see Supporting Information).

Probe Assembly

The experiment is performed inside a capillary tube over an inverted microscope as shown in Figure 1c. One end of a linear DNA fragment (10 kb) is functionalized with several digoxigenin moieties and the other end with several biotin moieties (see Supporting Information). The same DNA fragment is used for chromatin reconstitution and naked DNA experiments. Functionalized DNA or chromatin (50 ng/mL) is incubated for 10 min in an antidigoxigenin coated capillary tube. Nanorods are functionalized by coating

nonspecifically with neutravidin during a 30 min incubation (see Figure S9 of the Supporting Information), mixed with the superparamagnetic beads, and introduced into a capillary containing functionalized DNA or chromatin. Nanorods and beads self-assemble and settle on the bottom of the glass capillary and are lifted by placing the capillary tube under the cylindrical magnet. In the resulting constructs, DNA or chromatin fibers are torsionally constrained because they are attached to the nanorod through multiple biotin/neutravidin linkages and to the glass substrate through multiple digoxigenin/antidigoxigenin linkages.

Measurements

We use the nanorod-bead probes and the cylindrical magnet configuration to track the motions of single 10 kb DNA molecules (Figure 4). The nanorod-bead probes allow simultaneous tracking of horizontal angular fluctuations (torque), molecule extension, and pulling force. Our measurements for DNA extension and resistive torque as a function of turns agree with and extend previously published observations.^{9,11,12} Positive rotations (to overwind DNA) and negative rotations (to unwind DNA) are introduced into the DNA at pulling forces between 0.3 and 1.4 pN. For pulling forces of 0.6 and 1.4 pN, the torque is a linear function for a low number of rotations. The linear behavior is interrupted at ≈ -20 turns, corresponding to a ratio between the turns introduced in the molecule and the molecule intrinsic number of helix turns of ≈ -0.02 . As more negative turns are introduced, melting of the duplex DNA occurs,¹³ and the torque is constant at -10 pN-nm. A similar value (-9.6 pN-nm) was obtained by Bryant et al. at pulling forces of 15 and 45 pN.¹² At 0.3 pN and higher pulling forces, the linear behavior of the torque curve is also interrupted at positive turns. The exact number of rotations at which this happened is force-dependent and coincides with the buckling transition where plectonemic DNA started to form. The torque remains flat after DNA buckling (postbuckling torque), which has been observed at pulling forces above 1 pN,^{9,10,12} and is consistent with a phase transition between extended and plectonemic DNA.²⁶ The inset in Figure 4 shows the postbuckling torque in our measurements (circles), the values obtained in previous measurements at forces above 1 pN (ref 9) (black crosses), and a theoretical prediction of DNA postbuckling torque²⁶ (dashed line). The postbuckling torque in our measurements matches previous experimental results at ~ 1 pN and agree with the theoretical curve in the low pulling force regime (< 1 pN), where previous experimental measurements are not available.

The resistive torque of chromatin fibers has not been measured experimentally. We obtain turn-vs-extension and turn-vs-torque curves for chromatin at 0.3 pN vertical pulling force, which is sufficiently low to prevent DNA melting when it is twisted (parts a and b of Figure 5). The chromatin fibers are reconstituted using recombinant *Xenopus* histones and the same 10 kb DNA fragment as for the bare DNA experiments (see Supporting Information). The experiment is performed at low ionic conditions (10 mM phosphate buffer). The extension curves are significantly flatter than those obtained for the naked DNA at the same vertical pulling force (0.3 pN). They were also highly variable from molecule to molecule, which has been previously observed and correlated with the number of nucleosomes per fiber.² Using this correlation, we estimate that the shortest ($0.8 \mu\text{m}$ long) and longest ($1.7 \mu\text{m}$) fibers in Figure 5a differ by ~ 19 nucleosomes. Despite this, each molecule is able to

accommodate $>+40$ turns and the torque remain below 7 pN·nm before significant shortening occurs (Figure 5a). This contrasts with naked DNA, which, at the same pulling force, can only accommodate ~ 20 turns before shortening over a change in torque of 7 pN·nm (Figure 4). This shows that extended chromatin fibers have a significantly lower torsional rigidity than the bare DNA in which they are reconstituted.

The lower torsional rigidity of the chromatin fibers compared to bare DNA can be explained by a model in which the nucleosomes that form the fiber fluctuate between three configurations, each containing a different number of DNA supercoils.⁸ This model predicts a low torsional rigidity, which is the consequence of the low energy difference between these configurations of the nucleosomes and large number of combinations of nucleosome configurations that give rise to the same net twist. The lower torsional rigidity measured for the chromatin fibers compared to bare DNA means that the fibers can accumulate turns with a lower corresponding torque than bare DNA. This may have important biological implications since DNA transactions introduce turns in the molecule and are affected by torque.^{1,4,5} Our result indicates that the effects of such coupling should be weaker in the presence of chromatin.

Chromatin fibers have previously been reported to display hysteresis in single molecule twist-extension experiments.¹⁶ We find that such a twist-extension hysteresis is also coupled with torque hysteresis (Figure 5b). Hysteresis presumably indicates the formation of torsionally induced structural states that do not relax on the time scale of the experiments. The study of torque behavior for these fibers has potential to reveal valuable information about these states.

In summary, we have described a new method for measuring torque of single biomolecules. A magnetic nanorod-bead probe manipulated with a vertical magnetic field is the key component for measuring resistive torque with pN·nm resolution. In parallel with torque, molecule extension and pulling forces can be precisely measured. For the first time, torque at a physiologically relevant low pulling force (<1 pN) has been measured in a single molecule experiment. Our method is simple and does not require extensive calibration or feedback systems. The use of magnetic probes produced by self-assembled magnetic nanorods and superparamagnetic beads creates new possibilities for magnetic manipulation of single molecules. Future studies will be able to characterize in great detail the structural landscape of chromatin fibers and energetic requirements faced by enzymes that add or remove DNA twist.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Device for single molecule torque measurement. (a) The magnetic field created by a cylindrical magnet is used to manipulate a novel probe attached to the molecule under study. The probe consists of a Ni-Pt nanorod coupled to a 1 μm superparamagnetic bead. One end of the molecule is attached to the glass substrate and the other end to the nanorod at a distance L_h from the Ni segment. The magnetic field and the probe dipole (arrow with color gradient) align vertically. A horizontal force $\vec{F}_h$, due to a gradient in the magnetic field, produces a torque $\vec{L}_h \times \vec{F}_h$ which weakly traps the horizontal angular fluctuations of the probe (fluctuations of the angle θ), allowing controlled twisting of the molecule and detection of the torque applied to the molecule (see text). (b) Top view of the device. The horizontal force $\vec{F}_h$ is symmetric around the axis of the cylindrical magnet (red arrows). The torque $\vec{L}_h \times \vec{F}_h$ orients the nanorod in the direction of the force $\vec{F}_h$. Controlled rotation of the probe is obtained by moving the glass capillary in such a way that the probe follows a path around the projected center of the cylindrical magnet. (c) Single molecule manipulation takes place inside a capillary tube over an inverted optical microscope. The sample is illuminated through a 1 mm diameter hole in the axis of the cylindrical magnet.

Figure 2.

(a) Bright-field image of a nanorod-bead probe (bar = 1 μm). The bead is attached at one end of the nanorod. The self-assembly of nanorods with beads driven by the nanorod ferromagnetic Ni segment produces a uniform population of nanorod-bead probes in this configuration (see Figure S6 of the Supporting Information). (b) Binary image of nanorod-bead probe. The asymmetry of the probe allows precise angular measurement. The dashed line is the computed orientation of the probe based on the center of the bead and the center of the rod.

Figure 3. Measurements of the horizontal angle (θ) of a nanorod-bead probe tethered to a DNA molecule with different number of turns. Blue, red, and green curves are consecutive angle measurements at 0, -40, and +45 turns, respectively. Approximating the angular trap as a harmonic potential, torque at n turns is obtained from the angular trap stiffness (k_θ) times the change in the equilibrium angle $\langle \theta \rangle$ at n turns. Trap stiffness ($k_\theta = k_B T / \langle \delta \theta^2 \rangle$) are 34.5, 35.5, and 35.1 pN·nm at 0, -40, +45 turns, respectively. The angle measurements shown in this figure are used to obtain the torque values (-10.4 and 13.7 pN·nm) of the curve at a pulling force 0.6 pN shown in Figure 4.

Figure 4.

Single-molecule torque measurement of DNA at low pulling force. Extension and torque measurement of a 10 kb naked DNA molecule at pulling forces of 0.3, 0.6, and 1.4 pN. Extension and torque are symmetric for 0.3 pN and asymmetric for 0.6 and 1.4 pN. Introduction of sufficient positive rotations (overwinding DNA) generated plectonemes, indicated by linear shortening and constant torque. Negative rotations (unwinding DNA) at 0.6 and 1.4 pN pulling forces failed to shorten the DNA due to DNA melting, and resulted in a constant torque of ~ -10 pN·nm. Negative twisting at the lower pulling lower force of 0.3 pN allowed DNA shortening, indicative of negative plectoneme formation with a torque of 4–5 pN·nm. **Inset:** The torque at which DNA buckles to form plectonemes at positive rotations (postbuckling torque) as a function of pulling force. Black crosses are Forth et al. measurements⁹ and the dashed line represents a theoretical model proposed by Marko.²⁶ Circles are the postbuckling torques from curves at 0.3, 0.6, and 1.4 pN. Our measurements match previous measurement at ~ 1 pN and agree with the theoretical prediction at low pulling forces (<1 pN). The parameters used in the model (dashed line) are 50, 100, and 28 nm for the bend persistent length, twist persistent length, and twist stiffness of the plectonemic state.

Figure 5.

Extension and torque measurements of chromatin fibers. (a) Chromatin was generated from the same DNA used for bare DNA experiments described in Figure 4. The turn-vs-extension and turn-vs-torque curves at 0.3 pN pulling force are shown for three molecules. The extension curves display high variability, likely due to different number of nucleosomes for each fiber. In each case, however, the chromatin fibers can absorb ~ 40 turns before buckling with ~ 7 pN $\cdot$ nm torque. (b) Extension and torque hysteresis were observed in the chromatin fibers. Two consecutive loops are shown for a single molecule. The sequence is blue to the right, red to the left, green to the right, and black to the left. Hysteresis is observed at $>+50$ turns: Backward (to the left) paths show larger extension and lower torque.