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A method to estimate the elastic energy stored in braided DNA molecules using hydrodynamic equations

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Abstract

We present a single-molecule method for measuring the torque exerted by braided DNA molecules undergoing spontaneous unbraiding while attached to a paramagnetic dumbbell in the absence of external manipulation. A magnetic tweezers setup is employed to braid pairs of lambda DNA molecules covalently bound to a surface. Upon removing the magnetic field, the braided DNA molecules undergo spontaneous unbraiding, efficiently transforming the stored elastic energy into enough mechanical energy to rotate the tethered dumbbells for periods as long as 30 minutes. Using hydrodynamic equations we estimate the torque exerted on the dumbbells by the DNA braids, yielding values ranging from 47 to 166 pN nm.

Keywords

braided DNA; torque; single-molecule methods; magnetic tweezers; DNA motor

1. INTRODUCTION

In nature, DNA molecules often occupy spaces that are several orders of magnitude smaller than their total length. For instance, the chromosomal DNA in prokaryotic cells must be tightly packed into a nucleoid that takes up a small fraction of the internal cellular space. Unlike eukaryotic cells and certain Archaea, bacteria do not have histones to help pack the DNA into smaller structures. Instead, the compaction of their prokaryotic DNA relies largely on the formation of supercoils [1,2]. Researchers have found that several proteins work in the compaction of prokaryotic DNA and in the maintenance of supercoiled structures (e.g. HU, H-NS, integration host factor (IHF), DNA topoisomerase I, DNA gyrase) [3–6]. Viral capsids are another structure that contains tightly packed DNA. A capsid has a diameter of around 100 nm, while viral DNA can measure several micrometers in length. It has been demonstrated that a motor protein driven by ATP forces the DNA into each capsid during the virus assembly [7,8]. However, when the virus binds the bacterial membrane during viral infection, the high pressure in the capsid is enough to drive the injection of the viral DNA into the target cell [9]. The packing of DNA inside the capsid must be highly ordered so that tangling does not occur during the rapid injection. These

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processes of DNA compaction and packing, as well as numerous other important cellular processes, including DNA replication, all depend on the application of forces to DNA. The implementation of single-molecule manipulation techniques has contributed enormously towards improving the understanding of DNA physics by allowing precise measurement of such forces acting on single DNA molecules [10–16]. Acquiring knowledge about the forces experienced and exerted by DNA molecules not only allows researchers to better understand biomolecular processes such as DNA compaction, but also to design novel DNA-based nanodevices that use energy stored in the biopolymer to perform work. Therefore it is of interest to develop methods to estimate the magnitude of forces such as the torque stored on braided DNA molecules.

DNA molecules braided around each other can serve as a model for DNA supercoiling and compaction. Therefore, studying mechanical properties of DNA braids can provide a better understanding of how supercoiled DNA responds to applied forces and how efficiently DNA can use the stored energy to perform work. In this work, we take advantage of the unique physical properties of DNA to assess how efficiently the energy stored in DNA braids can be transformed into mechanical energy to perform work. Previous studies with braided DNA have focused on the braiding process, using external manipulation to create and maintain the braids while assessing their physical and mechanical properties [17,18], or on the relaxation of the braids by topoisomerases [19,20]. Instead, we have focused on the process of spontaneous unbraiding, removing all external manipulation and letting the DNA braids do the work. This allows a direct estimation of the magnitude of the torque that can be exerted by the compacted DNA. To this end, we employ magnetic tweezers to create DNA braids, followed by removal of the magnetic manipulation system to allow observation of the spontaneous unbraiding in the absence of external forces. Most of the previous DNA braiding studies have provided valuable information by using nicked DNA molecules attached to single microspheres [17,18,20,21]. Here, in order to assess the behavior of native DNA molecules, we use non-nicked lambda DNA molecules. Also, since it is difficult to discern the rotation of a single microsphere around its own axis, we use a paramagnetic dumbbell to study the braiding and unbraiding of pairs of DNA molecules. Applying previously established hydrodynamic models allows us to measure the torque exerted by braided DNA molecules undergoing spontaneous unbraiding.

2. MATERIALS AND METHODS

2.1 Materials

The lambda DNA construct employed in our work, prepared as previously described elsewhere [22], is schematically illustrated in Fig. 1A. Custom DNA sequences were synthesized by Integrated DNA Technologies (Coralville, IA). Lambda DNA and all enzymes were purchased from New England Biolabs (Ipswich, MA). The (3-aminopropyl)triethoxysilane (APTES), acetone and all reagents used to prepare buffers were purchased from Sigma-Aldrich (St. Louis, MO). Methoxy poly(ethylene glycol) succinimidyl valerate (mPEG-SVA) and Biotin-PEG-SC are from Laysan Bio (Arab, AL). Neutravidin was purchased from Thermo Fisher Scientific (Rockford, IL). The superparamagnetic microspheres used are 2.8- μm carboxylic acid Dynabeads M-270 from Invitrogen (Carlsbad, CA). Fab fragments of antidigoxigenin from sheep were obtained from Roche Applied Science (Indianapolis, IN). Neodymium iron boron magnets were purchased from Indigo Instruments (Waterloo, ON).

2.2 Magnetic Tweezers Setup Overview

Our setup consists of lambda DNA molecules immobilized on the bottom surface of a flow cell by one end and attached to 2.8- μm paramagnetic microspheres by the other end (Fig.

1A–B). The DNA molecules are anchored by only one strand to both the surface and the sphere, thereby allowing free rotation about single chemical bonds. Thus, if a single DNA molecule is attached to a microsphere it cannot be overtwisted or undertwisted. During experiments, the flowcell is placed on the stage of an Olympus IX71 inverted microscope. A permanent magnet placed above the flowcell is rotated to manipulate and braid the DNA molecules (Fig. 1C). Video data is obtained through a digital camera (Philips SPC 900NC, 1.3 Mpixel) and recorded on a computer. A particle tracking program (MaxTRAQ, Innovision Systems, Columbiaville, MI) is then used to determine the position of microspheres as a function of time.

2.3 Microsphere Modification

Carboxy-modified superparamagnetic microspheres are coated with antidigoxigenin Fab fragments using previously described procedures [22]. The amount of antibody on the spheres was found to be critical for the single-molecule experiments, since excessive antidigoxigenin caused problematic non-specific binding which resulted in extremely sticky spheres, hindering the manipulation of tethered DNA molecules.

2.4 Coverslip Surface Modification

Clean glass coverslips are covalently modified with amino groups by incubation in 2% v/v APTES in acetone for 5 minutes, followed by rinsing in excess water. The modified coverslips are cured by baking at 110 °C for 30 minutes. After letting them cool down, the dry coverslips are treated with a mixture of MPEG-SVA and Biotin-PEG-NHS in 100 mM sodium bicarbonate buffer, pH 8.3. This reaction is allowed to continue for at least 3 hours, followed by rinsing with nanopure water and air-drying. Prior to each experiment, a biotinylated coverslip is treated with 100 μ L of 200 μ g/mL neutravidin for 30 minutes, followed by rinsing with nanopure water and thoroughly drying with air.

2.5 Flow Cell Preparation

Our flow cells, illustrated in Fig. 1B, are built based on the design used by the research group of van Oijen [23]. The preparation procedure is described in detail elsewhere [22]. Briefly, a glass coverslip that has been modified as described above serves as the bottom of the flow cell, while a glass microscope slide is used for the top. Our adaptation of the original flow cell design makes use of five channels on each cell, allowing us to perform more than one experiment on each coverslip surface. 1.5-mm holes are drilled on the microscope slides to serve as inlets and outlets for the flow cell channels. The channels are formed by using double-sided adhesive tape as a spacer between the coverslip and the slide. Each flow cell is assembled once the neutravidin-treated coverslip has been prepared, working fast to avoid degradation and contamination of the surface. Each channel is then filled with 50 pM DNA construct and incubated for 30 minutes to allow binding of the biotinylated end of the DNA molecules to the neutravidin. Subsequently, the channels are filled with a solution of antidigoxigenin-coated microspheres and incubated for at least 3 hours. Experiments are carried out in TE buffer (10 mM Tris-HCl, 1 mM EDTA) at pH 8.0.

3. EXPERIMENTAL

3.1 DNA Manipulation

Our manipulation system, illustrated in Fig. 1C, consists of a neodymium magnet attached to the inner ring of a ball bearing (single-row deep groove, I.D. 1.8 cm, O.D. 3.6 cm). The outer ring of the ball bearing is kept at a fixed position while the rotation of the inner ring of the ball bearing is precisely controlled using a stepper motor [22]. Low rotational rates are employed to ensure low Reynolds numbers. As a result of the translational motion of the magnet, each microsphere is forced to describe a circular motion and to rotate about its own

axis. Since the magnet is not centered directly above each channel of the flow cell, the DNA molecules are stretched at an angle with respect to the normal to the surface. Forcing the microspheres to undergo a translational motion helps not only to identify regions with numerous tethers, but also to promptly detect the occurrence of non-specific interactions with the surface. The magnitude of the stretching force can be adjusted by changing the distance between the magnet and the flow cell.

The magnitude of the force is estimated by applying the equipartition principle and assuming the model of an inverted pendulum in which the microsphere is the bob and the DNA molecule is the string: $\langle F \rangle = k_B T L / \langle \delta x^2 \rangle$, where k_B is the Boltzmann constant, $\langle \delta x^2 \rangle$ is the root mean square displacement of the microsphere, T is the absolute temperature and L is the length of the DNA molecule [24]. The variance of the displacement of a sphere in the transversal to the DNA length axis is determined by tracking the position of the center of the DNA-tethered microspheres undergoing Brownian motion while the magnet remains in a fixed position. Using this procedure we measured stretching forces in the range of 0.5 – 1.0 pN.

3.2 Braiding DNA

Microspheres tethered to the surface by single DNA molecules display large and circular trajectories upon rotation. In contrast, microspheres attached to multiple DNA molecules display elliptical orbits with small diameters (Fig. 1D). When the latter undergo rotation, the diameter of their trajectory decreases as a function of time until reaching the point of attachment of the tethers on the surface (Fig. 2A). This indicates that more than one DNA molecules are attached to the same microspheres and that they are being braided forming a coiled structure, which causes the apparent tether length to decrease. During this braiding process we observe two compaction stages, as shown in Fig. 2A. In stage I, the radius of the trajectories of the microspheres decreases steadily until arriving at a minimum when the spheres reached the surface. In stage II, the microspheres can no longer describe a circular orbit due to their proximity to the surface, but they still rotate about their own axes, indicating that the DNA molecules are still undergoing further coiling. Upon changing the direction of rotation after compaction, we observe the mirror image of this behavior, demonstrating that the braiding process is reversible in both stages of compaction. As shown in Fig. 2B, this reversible behavior is reproducible regardless of the direction of braiding. Since each DNA molecule is attached to the microsphere by a single chemical bond, rotating spheres attached to single DNA molecules do not twist the polymer. Therefore single tethers do not undergo compaction and the tether length does not change as a function of the number of rotations (Fig. 2A). As a consequence, we can readily distinguish single tethers from multiple ones during a DNA braiding experiment.

In order to determine how many DNA molecules were bound to each sphere in the multiple-tether cases, we performed experiments in which we labeled the DNA molecules with YOYO-1 dye (Invitrogen, Carlsbad, CA) at a ratio of one dye molecule per each 5 bp. This allowed us to directly observe and image the DNA molecules by total internal reflection fluorescence microscopy, using a 100× oil immersion objective and a cw solid state laser with 473 nm emission. Under the preparation conditions described above (most importantly the DNA concentration and the amount of antidigoxigenin on the microspheres) we only observed single and double tethers (Fig. 3). We did not find any case in which there were more than two DNA molecules bound to the same microsphere. YOYO-1 labeling was only used for these experiments to determine how many DNA tethers were bound to the spheres. No labeling was used on the other experiments in this work, and the braiding process was followed as described above, using brightfield microscopy to track the position of the microsphere.

Stone et al. [20] used optical tweezers to characterize a DNA braiding system consisting of 41-kb DNA constructs. They defined the braiding density, σ_{br} , as the number of turns introduced into the braid divided by the number of helical repeats in each DNA molecule, or $\sigma_{br} = n_{turns}/(bp/10.5)$. They too observed the shortening of the braids as the braiding density increased. For braids held under a tension of 1 pN, they observed a buckling transition at braiding densities around 0.075, which indicated the formation of plectonemes. In order to avoid such structural transition, the braiding density in our experiments was never higher than 0.01.

3.3 Spontaneous DNA Unbraiding

In order to determine whether the elastic energy stored in DNA braids can be used to perform mechanical work, we braid pairs of DNA molecules and subsequently remove the magnetic manipulation system. This method allows the DNA braids to work by themselves, using only the elastic energy stored to make the tethered dumbbell rotate as unbraiding occurs. We can then use hydrodynamic equations to directly estimate the torque experienced by the dumbbell. Note that there are no external forces acting on the dumbbell during the spontaneous unbraiding process. The magnetic manipulation system is removed after braiding the DNA, and there is no flow or other forces present that could contribute to the dumbbell spontaneously gyrating. Therefore, the measured torque corresponds to the torque exerted by the DNA braids.

Because we use bright field microscopy to monitor the DNA-tethered microspheres, we cannot distinguish rotational movement of a single sphere around its own axis. Thus we use pairs of microspheres bound together forming a “dumbbell” (Fig. 4A). The dumbbells are formed spontaneously via magnetic attraction of nearby paramagnetic spheres in the presence of the magnet. After being bound for some time, the non-specific interactions between the antidigoxigenin-coated microspheres cause them to stick together even in the absence of the magnetic field. Thus no additional preparation steps are required to create the dumbbells aside from increasing the concentration of spheres in the flow cell.

3.4 Torque Calculations

The torque acting on a particle rotating in a fluid is given by $\tau = \omega \zeta_{rot}$, where ω is the angular frequency of the particle and ζ_{rot} is the rotational frictional drag it experiences. For our calculations, we applied two hydrodynamic models to determine the torque exerted by the braided DNA molecules on the tethered dumbbell. The first model employed applies the hydrodynamic equation derived by Majumdar and O'Neill to determine the torque τ experienced by a dumbbell, consisting of two identical spheres of radius r in contact, rotating about its center with angular frequency ω [25]:

$$\tau = -16\pi\eta\omega r^3(1.8704) \quad (\text{Eq. 1})$$

where η is the viscosity of the solution. In the second model applied, the rotational motion of the two spheres that form a dumbbell is treated assuming the shape of a prolate ellipsoid rotating about its minor axis. Under this assumption, we can estimate the rotational frictional drag of the ellipsoid using Perrin's expression [26]:

$$\zeta_{rot} = (8/3)\pi\eta d^3 / [\ln(2d/r) - (1/2)] = 1.9 \times 10^{-19} \text{ kg m}^2 \text{ s}^{-1} \quad (\text{Eq. 2})$$

where d is the diameter of the spheres. Given the approximation required by this model, it is expected to yield less accurate yet similar values as the first model described.

4. RESULTS AND DISCUSSION

4.1 Can the energy stored in DNA braids be used to perform mechanical work?

Pairs of DNA molecules bound to paramagnetic dumbbells are braided by rotating the magnet in one direction before reaching compaction stage II. Upon subsequent removal of the magnetic field, the dumbbells start to rotate in the opposite direction, indicating the spontaneous unbraiding of the DNA molecules (Fig. 4B; movie available as Supporting Material). Thus the elastic energy stored in the braids is transformed into enough mechanical energy to gyrate the dumbbell. The initial unwinding frequencies observed range from 0.1 s^{-1} to 0.7 s^{-1} , and decrease exponentially as the unbraiding progresses. Upon re-braiding the unwound DNA molecules, we are able to observe the spontaneous unbraiding process again, indicating that the process is reversible. We noticed that the number of spontaneous rotations performed by the DNA molecules often corresponds to the same number of rotations induced in the braiding process, which indicates that the transformation of elastic energy into mechanical work is highly efficient. The cases in which the number of spontaneous rotations was significantly less than the number of induced rotations were those in which non-specific interactions of the spheres with the surface interfered with the unwinding process. In some cases, interactions with the surface caused the unbraiding process to halt. It is easy to discern when interactions with the surface occur and affect the spontaneous unbraiding, since they cause pauses and irregularities in the rotation of the dumbbell. Only the cases in which the spontaneous unbraiding process was unaffected by non-specific binding were considered for the results presented on this paper.

4.2 Torque Measurement

By applying the equation derived by Majumdar and O'Neill (Eq. 1), we were able to determine the magnitude of the torque experienced by the DNA-tethered rotating dumbbells. Fig. 4C shows the estimated torque values as a function of time during the process of spontaneous unbraiding for two examples of DNA braided in opposite directions. Rotating the magnet clockwise generates DNA braids equivalent to (+) supercoils, conventionally called left-handed (L-) braids [19,21]. Conversely, rotation in the counterclockwise direction generates right-handed (R-) braids which can be considered equivalent to (-) supercoils. For the shown case of left-handed braiding, torsional force was applied to the dumbbell by rotating the magnet 52 times in the clockwise direction, with subsequent removal of the magnet before reaching the second stage of compaction. The initial angular frequency of the spontaneous unbraiding was 0.7 rad s^{-1} , which gives a frictional torque of 166 pN nm . In the case of an R-braid, 45 rotations were applied before the magnet was removed, and spontaneous braiding occurred with initial angular frequency of 0.4 rad s^{-1} and torque of 95 pN nm . As shown in Fig. 4C for both R- and L-braiding, the torque exerted by the DNA braids decreases exponentially as a function of time during the unwinding process. A total of 12 cases of spontaneous unbraiding were studied, six for each braiding direction. The values determined for the magnitude of the torque stored in the studied braids ranged from 47 to 166 pN nm . Under our experimental conditions we did not observe significant differences between the torque exerted by right-handed and left-handed DNA braids undergoing spontaneous unwinding. This is in agreement with previous work by Strick et al., Charvin et al. and Stone et al. under experimental conditions similar to ours [17,19,20]. In contrast, studies performed at high salt concentration and braiding density by Charvin et al. showed that right-handed braids required more twists than left-handed ones to reach a buckling transition. This indicated that the right-handed braids are more stable, which may be explained in terms of the chirality of the braid relative to that of the DNA molecule: braiding two DNA molecules with the same chirality as DNA is expected to form a more compact and stable structure [18].

Approximating the dumbbell as a prolate ellipsoid rotating around its minor axis and then using Perrin's equation gives slightly lower torque values compared to those obtained using the equation of Majumdar and O'Neill (Fig. 4 inset). The latter is expected to describe our system more accurately since it was derived specifically for the case of a dumbbell rotating in a fluid.

Since the motion of the rotor is affected by rotational Brownian motion, we estimate that the main source of error in our experiments is the root-mean-square drift of the angular frequency, $\langle \delta\phi \rangle^{1/2} = \sqrt{2D_{rot}t}$, where the rotational diffusion coefficient of the ellipsoid is given by $D_{rot} = k_B T / \zeta_{rot} = 0.02 \text{ rad}^2 \text{ s}^{-1}$. In the left-handed braiding experiment described above, which displayed the fastest spontaneous unbraiding and largest torque value out of all our experiments, initially it took 9.3 s to complete a rotation. The root-mean-square drift during that time interval is 0.6 rad, giving a relative error per turn of $0.6/2\pi = \pm 0.1$. The slowest spontaneous unbraiding process observed was for one of the right-handed braids, in which it took 31.6 s to complete one rotation initially. For this case, the root-mean-square drift is 1.1 rad and the relative error per turn is ± 0.2 . This being the maximum error observed indicates that our torque measurements near the beginning of the unbraiding process are almost unaffected by the rotational Brownian motion. However, the torque measurements are expected to be less precise at the end of the unbraiding process because then the dumbbells, besides rotating, can undergo a small translational circular motion.

The distribution of torque values observed is likely to be partially caused by varying degrees of separation between the points of attachment of the DNA molecules to each sphere and to the surface, since we did not control this parameter. In addition, since the magnetic manipulation system is removed during the unbraiding process, there is no external force keeping the dumbbells from reaching the surface. In order to determine if the vertical position of the dumbbells could cause them to be dragged through the surface, we used a red LED light source to observe the diffraction patterns on the microspheres. The diameter of the rings on the diffraction patterns changes depending on the focal plane in which the sphere is located, so it can be related to the distance from the sphere to the surface [27]. During the spontaneous unbraiding experiments, when the magnet (i.e. the stretching force) was removed, the position of the dumbbell in the z axis changed, as was evidenced by the change in the diffraction patterns of the spheres. The diffraction patterns observed around the dumbbells in the absence of the magnetic force were identical to the patterns around spheres that were nonspecifically bound to the surface, indicating that the dumbbell was also very close to the surface. This means that we cannot discard the drag of the sphere through the surface as the unbraiding process occurs. Therefore, the torque values estimated here should be considered a lower limit.

5. CONCLUSIONS

Typical single-molecule force studies of DNA twisting, braiding and unwinding utilize methods that constrict the polymer at both ends. In the presented method, the magnetic manipulation system is removed in order to allow braided DNA molecules to employ the stored energy in the form of torque to perform mechanical work. The work done by the DNA braids makes the tethered dumbbell behave like a clockwork rotor. This setup allows the direct estimation of the torque exerted by the braided DNA molecules on the tethered dumbbell.

The braiding process in both directions revealed that the pairs of DNA molecules undergo at least two reversible stages of compaction. The energy stored in the DNA braids during compaction was efficiently transformed into enough mechanical work to gyrate the tethered dumbbells for periods of up to 30 minutes, until the braids were completely unwound. The

magnitude of the torque stored in the DNA braids ranged from 47 to 166 pN nm and should be considered a lower limit due to surface drag.

The work presented here shows methodology that is useful for the direct measurement of forces exerted by supercoiled DNA molecules. In addition, the characterization of this rotor driven by the unwinding of braided DNA molecules opens up possibilities for the development of novel DNA nanomachines.

Research Highlights

- Unwinding of single DNA braids attached to a dumbbell without external manipulation.
- Torque stored in DNA braids is efficiently transformed into mechanical work.
- Use of hydrodynamic equations to directly estimate the torque stored in DNA braids.
- Estimated torque values ranged from 47 to 166 pN nm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Experimental setup. (A) Schematic of the immobilization of λ -DNA on a coverslip surface and attachment of its free end to a paramagnetic microsphere. (B) Schematic of the multichannel flow cell used in our experiments. (C) Magnetic tweezers system. (D) Rotating microspheres tethered to the surface via single DNA molecules describe large circular trajectories (i), while multiple DNA molecules bound to a same sphere cause it to display small irregular trajectories (ii, iii).

Fig. 2. Mechanical braiding of DNA molecules using magnetic tweezers. (A) When a microsphere is tethered to the surface by a single DNA molecule (*top*), the diameter of its rotation trajectory does not change, while for multiple tethers (*bottom*) it decreases as a function of the number of rotations induced. (B) DNA braiding was reversible in both the clockwise and counterclockwise braiding directions.

Fig. 3. Fluorescence imaging of immobilized DNA molecules being stretched by attached paramagnetic microspheres. The left image shows a sphere tethered by a single DNA molecule, while the right image shows a sphere tethered by two DNA molecules.

Fig. 4.

Spontaneous unwinding of braided DNA molecules. (A) In order to observe the unwinding of braided DNA molecules, we followed the rotation of dumbbells in which the DNA molecules were bound to only one of the spheres. (B) Frames from a video showing a tethered dumbbell rotating as the braided DNA spontaneously unwinds. (C) Torque values determined using the equation derived by Majumdar and O'Neill. The torque stored in the DNA braids decreases exponentially as the unwinding progresses. Inset shows the torque values estimated using Perrin's equation.