Rotational Drag on DNA: A Single Molecule Experiment

Philippe Thomen, Ulrich Bockelmann, and François Heslot*

Laboratoire de Physique de la Matière Condensée, Ecole Normale Supérieure, 24 rue Lhomond, 75005 Paris, France

(Received 10 December 2001; published 3 June 2002)

Within a single-molecule configuration, we have studied rotational drag on double stranded linear DNA by measuring the force during mechanical opening and closing of the double helix at different rates. The molecule is cranked at one end by the effect of unzipping and is free to rotate at the other end. In this configuration the rotational friction torque τ on double-stranded DNA leads to an additional contribution to the opening force. It is shown that the effect of rotational drag increases with the length of the molecule, is approximately proportional to the angular velocity of cranking, and we estimate that the torque τ is of the order of $1k_BT$ for 10 000 base pairs of DNA cranked at 2000 turns per second.

DOI: 10.1103/PhysRevLett.88.248102 PACS numbers: 87.14.Gg, 87.15.He, 87.15.La, 87.80.Cc

Torsional friction drag on DNA has been the subject of theoretical works but no direct experimental investigation. In a very early paper [1], Levinthal and Crane examined DNA replication and analyzed the coupled DNA rotation. They proposed a simple estimate of the friction torque. Friction torque has also been invoked in the kinetics of *in vitro* thermal DNA denaturation and renaturation [2] because DNA melting is expected to be coupled to a rotation of the double helix. Also, in the context of enzymes translocating on circular DNA, many *in vitro* experiments have been modeled with the involvement of frictional torque of DNA as a way to allow a significant difference in the helicity of the DNA upstream and downstream of the enzyme (see references in [3]). Nelson developed a model [3] to evaluate the frictional torque and proposed that the torque may actually be orders of magnitude higher than estimated by Levinthal and Crane. The argument is that DNA does not rotate as a "speedometer cable" as assumed by earlier models. Instead the presence of natural bends in the DNA should induce a sideways motion of the molecule when rotating, an effect expected to be associated with much higher friction. In this regard, an experimental determination of torsional friction of DNA is of interest and we report in this Letter such a measurement.

Unzipping of a DNA molecule is obtained by attaching the two single strands of one end of the molecule on two different solid supports that are progressively separated [4,5] while the other end of the molecule is free to rotate. The experiment is performed under a microscope equipped with an optical trapping interferometer as a force measuring device [5]. Force measurement is performed *in vitro* on a molecular construction [6] anchored between a glass microscope slide and a silica bead (0.5 μm radius) trapped in the optical trap. The opened molecule is λ DNA, comprising 48 502 base pairs. After suitable calibration, the force is determined from the measurement of the bead position with sub-nm resolution. The bead is held in the trap while the sample is displaced laterally with a piezoelectric translation stage at a controlled velocity. This leads to the opening of the molecule.

We now briefly recall the main features of the force-displacement curve when unzipping occurs at low-displacement velocities (below 1 μm/s). Initially when the sample is displaced laterally, the force starts to rise to a sizable value when the displacement becomes of the order of the length of the linker arms. The molecule begins to open at $\approx$14 pN: unzipping (or opening) then occurs with, on average, about 1000 base pairs (1 kbp) per μm of displacement. With 3 bases/nm for double-stranded DNA (ds-DNA), a stage displacement of d micrometers thus opens an average length $d/3$ micrometers of ds-DNA. A rotation of the double helix is associated with this DNA unzipping: the rate of rotation generated at the opening fork is related to the pitch of the double helix, i.e., approximately one turn for ten bases opened, in the B form. At displacement velocity below typically 1 μm/s, the unzipping force $F^0_{\mathrm{unzip}}(d)$ vs displacement d presents reproducible features that are governed by the sequence being opened [4,5]. Those features are connected to the GC content along the molecule, and the typical amplitudes of the force variations are of the order of 10% of the average force value. It is possible to zip (or close) an opened molecule by inverting the stage motion. Upon reopening a molecule after a cycle of opening and closing, the measurement is still similar to the initial measurement. In this low velocity regime, the measurements are also rather reproducible from one molecule to the other, and the opening and closing signal does not depend significantly on velocity [5]. In Fig. 1, we present an example of force measurements $F^v_{\mathrm{unzip}}(d)$ and $F^v_{\mathrm{zip}}(d)$ vs displacement d, on a given molecule, and done with displacement velocities v varying between 1 and 4 μm/s. When the velocity is increased in this range, more differences occur between $F^v_{\mathrm{unzip}}(d)$ and $F^v_{\mathrm{zip}}(d)$ at localized regions of displacement, i.e., those differences are sequence dependent [5].

In Fig. 2 we present a measurement done on a given molecule, where a number of openings and closings have been performed at different velocities from $v = 4$ μm/s to $v = 20$ μm/s. Starting from an unopened molecule the

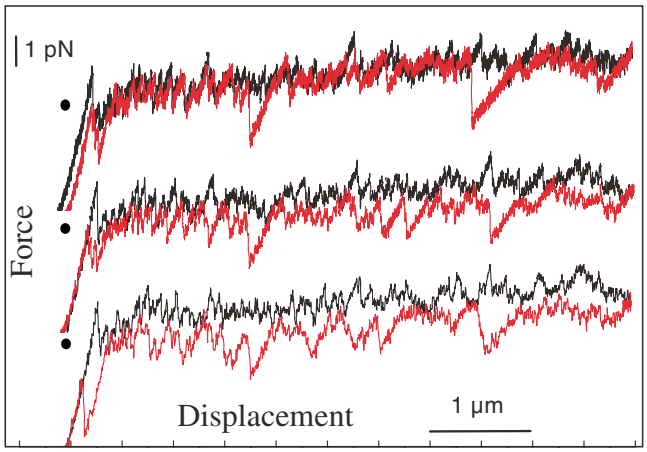

FIG. 1 (color). Three repeated openings (black curves) and closings (red curves) of the same molecule for three displacement velocities: 1 μm/s (top), 2 μm/s (middle), and 4 μm/s (bottom). The curves corresponding to different velocities have been shifted vertically for clarity. The dots indicate the same force level.

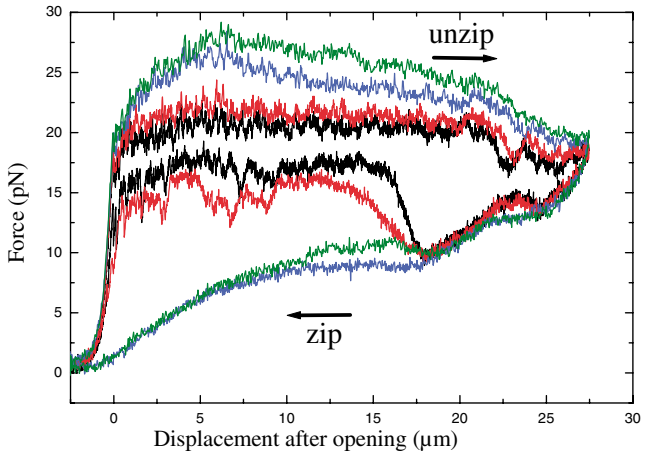

FIG. 2 (color). Force signal recorded upon opening and closing of the same molecule, at different displacement velocities [4 μm/s (black), 8 μm/s (red), 16 μm/s (blue), and 20 μm/s (green)]. The arrows indicate the direction of displacement.

stage is displaced with a velocity v. When the displacement has reached a given value (here 27 μm after opening) the motion is reversed (velocity, v); when the stage has reached the position it had before opening, this reverse motion is stopped for a few seconds or more to allow for an eventual completion of reannealing. Another cycle of opening and closing is then engaged. We have checked that the order of velocities at which the experiments are performed is not relevant. The force measurements on a given molecule are quite reproducible (data not shown). The opening force $F^{v}_{\mathrm{unzip}}(d)$ [closing force $F^{v}_{\mathrm{zip}}(d)$] at velocity v occurs at a higher (lower) force, the higher the velocity. The signal thus presents hysteresis, an effect that increases with velocity. When stopping the displacement during opening, the force decreases toward the value expected for low velocity displacement. The relaxation times are of the order of 15 to 60 ms (data not shown). The zipping curves of Fig. 2 display an approximately common large decrease in force as d is decreased from 27 to 18 μm, with very little local fluctuations. This effect is attributed to a slow reannealing of the central AT-rich region of λ DNA. When d decreases further, the force rises back for the 4 and 8 μm/s curves and the local fluctuations reappear, an indication that reannealing occurs approximately at the average speed imposed by the displacement. In this region there is, however, different behavior for the zipping curve at higher velocity: the force vs displacement curves decrease continuously to zero. We propose that this decrease corresponds to a regime where the velocity of reannealing of the molecule is less than the velocity imposed by the stage, i.e., the recombination of the molecule is not able to catch up with the displacement rate. Even if the motion is reversed before the AT-rich region has been opened, we observe the same kind of continuous decrease in the zipping curve for $v \geq 16$ μm/s (data not shown). This shows that the continuous decrease in force observed for $d < 15$ μm is not just a consequence of slow reannealing of the AT-rich region. We also observe that after at most one second, when the molecular construction is completely unloaded, it is possible to reopen the molecule with the same force level as before, indicating that reannealing has occurred.

An important characteristic of the type of measurement as presented, for example, in Fig. 2 is the fact that the amplitude of the hysteresis cycle is strongly molecule dependent, as is discussed below. We write $F^{v}_{\mathrm{unzip}}(d) = F^{0}_{unzip}(d) + \Delta F^{v}_{\mathrm{unzip}}(d)$ [respectively, $F^{v}_{\mathrm{zip}}(d) = F^{0}_{\mathrm{zip}}(d) + \Delta F^{v}_{\mathrm{zip}}(d)$]. Performing measurements on many different molecules, we find that, when a small $\Delta F^{v}_{\mathrm{unzip}}(d)$ is observed in the first few micrometers of the opening, the construct typically breaks after a rather small displacement. This strongly suggests the presence of a nick (a break in only one of the strands of the double-stranded DNA): a nick will (i) lead to a breakage when the opening fork is close to it and (ii) prevent the transmission of a significant mechanical torsion along the DNA [7]. When unzipping, the unopened part of the molecule is cranked positively at the opening fork and the rotational friction is responsible for an increase in the opening force while reversing the motion, rotational friction leads to a decrease in the measured force. The rotational friction is expected to be dependent on both the angular velocity ω of the cranking motion of the ds-DNA and the effective rotating length L_{eff} of the molecule. The total length of the λ DNA molecule is 16.4 μm but the presence of randomly distributed nicks may give a shorter L_{eff}, unknown, and be different from one molecule to another. Moreover, L_{eff} decreases during opening because the opening is coupled to a decrease in the length of the double-stranded DNA. We note $L^{\mathrm{max}}_{\mathrm{eff}}$, the effective rotating length at the beginning of the opening.

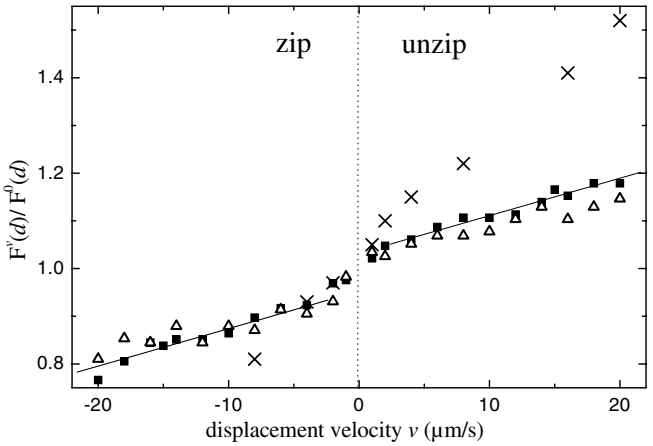

FIG. 3. The normalized force $F^{v}(d)/F^{0}(d)$ as a function of displacement velocity v, for three molecules of different lengths. The positive (negative) velocities correspond to unzipping (zipping). The crosses are extracted from the experiment of Fig. 2: $d \simeq 8.4$ μm and $L_{\mathrm{eff}}^{\max} > 9$ μm because the opening without breaking was performed over this length [we estimate later that $L_{\mathrm{eff}}^{\max} \approx 12$ μm (see Fig. 4)]. For negative velocities, the number of data points is limited because of the reannealing delay occurring for high velocities (discussed in the text). Squares: $L_{\mathrm{eff}}^{\max} \simeq 3.9$ μm (rupture event) and $d \simeq 2.7$ μm. Triangles: unknown effective length and $d \simeq 2.6$ μm. The lines are linear fits of the data corresponding to the squares, for $|v| > 4$ μm/s. The difference between the slopes for different molecules is attributed to different values of L_{eff}.

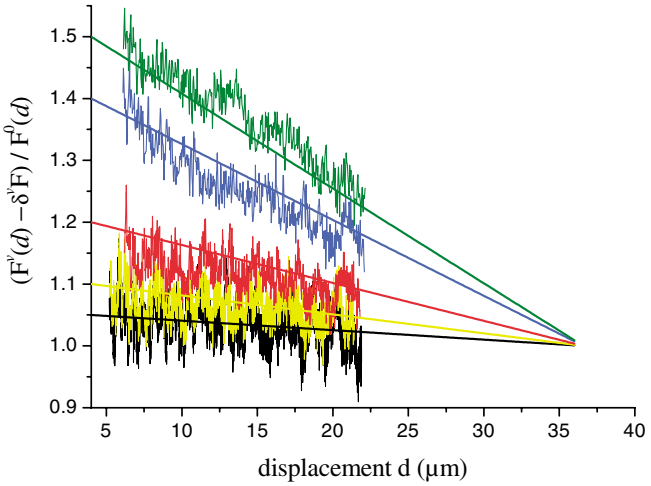

FIG. 4 (color). A part of a plot of $[F^{v}(d) - \delta F]/F^{0}(d)$ from Fig. 2; δF is the offset near $v = 0$ discussed above. This shows the effect of velocity and length. The corresponding velocities are black, 2 μm/s; yellow, 4 μm/s; red, 8 μm/s; blue, 16 μm/s; green, 20 μm/s. The linear fit curves are obtained using the following simple expression: $[F^{v}(d) - \delta F]/F^{0}(d) = 1 + a \cdot v \cdot L_{\mathrm{eff}} = 1 + a \cdot v \cdot (L_{\mathrm{eff}}^{\max} - d/3)$, with $L_{\mathrm{eff}}^{\max} = 12.2$ μm and $a = 2.3 \times 10^{-3}$ s $\cdot$ μm^{-2}.

We focus now on the role of the displacement velocity v. In Fig. 3, plots of $\Delta F_{\mathrm{unzip}}^{v}(d)$ and $\Delta F_{\mathrm{zip}}^{v}(d)$ vs velocity v are presented for three different molecules, where d (and therefore L_{eff}) is fixed for each molecule. The dependence appears to be linear with v for opening and closing. The slopes of the curves vary from one molecule to the other and is inferred to be an effect of the length L_{eff}. ΔF vs v presents a small but rapid variation at low velocity (about a few μm/s), taking the aspect of an offset. This offset at about $v = 0$ doesn't seem to depend on the effective length of the molecule; thus it is not related to rotational friction. We rather interpret this effect as being related to the sequence-dependent local hysteresis that was presented in Fig. 1.

For a given molecule, L_{eff} decreases during the opening of the double helix and we expect to see the effect on the force level. In order to avoid confusion with variations in force occurring because of the effect of the sequence, we have chosen in Fig. 4 to extract data from Fig. 2 for a limited region 6 μm $< d <$ 22 μm (corresponding to the opening between base pair index 6000 and 22 000). In this region, extending over 16 μm displacement, the sequence-induced variations in the opening force are relatively small. As presented in Fig. 4, the force is observed to decrease approximately linearly with displacement, an effect that we attribute to the progressively decreasing length of the molecule that has to rotate. We infer that in the initial stage of opening of the molecule (see Fig. 2, for $d < 6$ μm) the progressive rise in force observed for $v \geq 16$ μm/s may be attributed to a transient related to the beginning of the opening: it is possible that this is associated with the minimum time necessary for the propagation of the torsional stress down to the free end of the molecule.

Let us now discuss the various possible frictional effects and the torsional torque τ that the molecule undergoes in our experiments. During the process of unzipping DNA, one measures the force F which is applied to one of the two single strands of the DNA; the same force is applied to the other single strand. Thus, with respect to the opening fork and the local central axis of the molecule and if one assumes as a rough approximation that the bending stiffness of the single strands gives a negligible effect, a torque $M = 2F \cdot R$ is applied, where R is approximately the crystallographic radius of double-stranded DNA, taken to be 1 nm. In this present unzipping configuration, the stage is moved laterally at a velocity v while the trap is fixed. Thus, with respect to the laboratory coordinates, the liquid in the sample moves at a velocity v and the opening fork is predicted to be approximately in the middle of the two attachment points of the molecule and moves with a velocity $\frac{v}{2}$. Thus the unopened part of the molecule is pulled at the opening fork at a velocity $\frac{v}{2}$. It is then expected that the corresponding part of the DNA is both twisted, as described earlier, and partially stretched during the experiment. During the process of opening or closing DNA, the friction forces which may be relevant are the rotational friction of the rotating double-stranded DNA, the friction force f_{bead} applied directly to the bead because of a motion of the fluid, and the translational friction force f_{transl} applied to the molecule to be opened because of the motion of the fluid. The

force f_{bead} is estimated using the Stokes law: with a bead radius $a = 0.5\ \mu$m and a flow velocity $U = 10\ \mu$m/s, $f_{\text{bead}} = 0.1$ pN [8]. For the DNA, we take a longitudinal friction coefficient of about 8×10^{-9} Nm/s as determined in [9] for an extended λ DNA. Taking a flow velocity $U = 10\ \mu$m/s, we obtain $f_{\parallel} \approx 0.08$ pN. Those translation friction drag effects are thus negligible as compared to the change in force presented in Fig. 2. The measurements are dependent on the effective length of the molecule without nick. We can estimate the maximum observed torsional torque from Fig. 2, for $d \simeq 6\ \mu$m, at $v = 20\ \mu$m/s; one obtains $\Delta F^{20}_{\text{unzip}}(6) \simeq 9$ pN. This corresponds to $\tau_{\max} = 2\Delta F^{20}_{\text{unzip}}(6)R \simeq 5k_B$T. The naive model of a rigid rod following Levinthal and Crane [1] gives $\tau = \mu_{\text{spin}}\omega L_{\text{eff}}$, where $\mu_{\text{spin}} = 4\pi\eta R_H^2$ [10], η is the viscosity of water, and R_H is the hydrodynamic radius of DNA taken to be 1.05 nm as estimated in [11]. For $\omega \simeq 12\,000$ rad/s (corresponding to a displacement velocity of 20 μm/s), and $L_{\text{eff}} = 12\ \mu$m (the estimated value of $L^{\max}_{\text{eff}}$, given by the fit of Fig. 4), the equation gives $\tau \simeq 0.4k_B$T.

For reannealing to occur at the same average speed as the displacement velocity, the friction torque τ should be below the sequence-dependent denaturation torque τ_0, otherwise a denaturation bubble would form. This suggests that the denaturation torque sets an upper limit to the rate of reannealing for a given L_{eff}. In Fig. 2, this rate limit is reached for $8\ \mu\text{m/s} < v < 16\ \mu$m/s; the corresponding $\Delta F^{v}_{\text{zip}}(d)$ near $d = 12\ \mu$m gives an estimate of the denaturation torque: $2k_BT \lesssim \tau_0 \lesssim 5k_BT$. Strick *et al.* [12] made a single-molecule measurement of τ_0 (for an AT-rich region of λ DNA, $\tau_0 \simeq 1.3k_BT$ in unsalted phosphate buffer). The denaturation torque analysis thus gives a reasonable order of magnitude.

Nelson reported theoretical torque estimations for a naturally bent semiflexible rod [3]. In this case, the value of the viscous drag torque is then much larger as compared to the Levinthal-Crane estimation and our measurements; Eq. (3) of Ref. [3] gives $\tau \approx 15\,000k_BT$, for $\omega = 12\,000$ rad/s, and $L_{\text{eff}} = 12\ \mu$m. The results of our experiments are therefore closer to the naive model of a rigid rod. In our experiment, a flow $U = v/2$ changes the configuration of the molecule. However, it has been reported both experimentally [13] and through modeling [14] that a tethered DNA in a uniform flow is not free draining, up to a ratio r between the extension and length of the molecule of 0.8. Adapting those results for our configuration to λ DNA, we get $r \simeq 0.4$ for $U = v/2 = 10\ \mu$m/s. From this, one expects that, since hydrodynamic coupling is such that non-free-draining conditions occur for translation, there should be no extreme effects of the flow on rotational friction. Nevertheless unzipping experiments with a double trap would be of interest to evaluate by comparison the possible influence discussed above. Our present results indicate that the rotational drag undergone by DNA in our configuration is about $1k_B$T per 10 kbp DNA at 2000 turns per second, i.e., about 10 times the value expected for a straight rigid rod.

This work has been funded by CNRS and universités Paris VI and Paris VII.

*Electronic address: heslot@lpmc.ens.fr

[1] C. Levinthal and H. Crane, Proc. Natl. Acad. Sci. U.S.A. **42**, 436 (1956).
[2] C. H. Lee and J. G. Wetmur, Biopolymers **11**, 1485 (1972).
[3] P. Nelson, Proc. Natl. Acad. Sci. U.S.A. **96**, 14 342 (1999).
[4] (a) B. Essevaz-Roulet, U. Bockelmann, and F. Heslot, Proc. Natl. Acad. Sci. U.S.A. **94**, 11 935 (1997); (b) U. Bockelmann, B. Essevaz-Roulet, and F. Heslot, Phys. Rev. Lett. **79**, 4489 (1997); (c) U. Bockelmann, B. Essevaz-Roulet, and F. Heslot, Phys. Rev. E **58**, 2386 (1998).
[5] U. Bockelmann *et al.,* Biophys. J. **82**, 1537 (2002).
[6] The molecular construction is described in [5]. On one extremity of λ DNA, the two single strands are connected to 7 kbp linker arms, each terminated by different types of ligands (digoxygenin and biotin). The molecular construction is anchored between a glass slide and a bead coated, respectively, with antibodies against digoxygenin and with streptavidin. The experiment is performed in PBS Buffer (10 mM phosphate, pH 7, 150 mM NaCl).
[7] J. F. Léger *et al.,* Phys. Rev. Lett. **83**, 1066 (1999).
[8] The distance between the bead and the surface of the sample is typically 2 to 4 μm. The influence of the solid surface is small and we take a viscosity $\eta \simeq 10^{-3}$ pN $\cdot$ s $\cdot\ \mu\text{m}^{-2}$.
[9] J. C. Meiners and S. Quake, Phys. Rev. Lett. **84**, 5014 (2000).
[10] H. Lamb, *Hydrodynamics* (Dover, New York, 1945), 6th ed.
[11] B. S. Fujimoto, J. M. Miller, N. S. Ribeiro, and J. M. Schurr, Biophys. J. **67**, 304 (1994).
[12] T. R. Strick, D. Bensimon, and V. Croquette, Genetica **106**, 57 (1999).
[13] T. T. Perkins, DE. Smith, R. G. Larson, and S. Chu, Science **268**, 83 (1995).
[14] (a) B. H. Zimm, Macromolecules **31**, 6089 (1998); (b) J. F. Marko and E. D. Siggia, Macromolecules **28**, 8759 (1995).