Mechanically Controlled DNA Extrusion from a Palindromic Sequence by Single Molecule Micromanipulation

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A magnetic tweezers setup is used to control both the stretching force and the relative linking number ΔLk of a palindromic DNA molecule. We show here, in absence of divalent ions, that twisting negatively the molecule while stretching it at $\sim$1 pN induces the formation of a cruciform DNA structure. Furthermore, once the cruciform DNA structure is formed, the extrusion of several kilo-base pairs of palindromic DNA sequence is directly and reversibly controlled by varying ΔLk. Indeed the branch point behaves as a nanomechanical gear that links rotation with translation, a feature related to the helicity of DNA. We obtain experimentally a very good linear relationship between the extension of the molecule and ΔLk. We use then this experiment to obtain a precise measurement of the pitch of B-DNA in solution : 3.61 ± 0.03 nm/turn.

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Cruciform DNA structures may be encountered *in vivo*, for example, at sites of inverted DNA repeats, or during homologous recombination events where they are termed ''Holliday junctions'' and perform strand exchange between two homologous DNA molecules [1,2]. Early models [3–5] and previous bulk experiments on plasmids [6–9] suggest that, due to the helicity of DNA, the formation of cruciform DNA and subsequent branch point migration (or strand exchange) may be controlled by mechanical torsion on a palindromic DNA sequence (i.e., on a sequence with a two-fold symmetry about its center). In the present work we use a single molecule micromanipulation technique to explore directly these properties.

We use a magnetic tweezers setup [10,11] to micromechanically control a $\sim$12 kb long palindromic DNA molecule [12]. The molecule is tethered between a glass surface and a paramagnetic bead. Multiple attachments at the extremities of the molecule ensure that the molecule is rotationally constrained. A pair of magnets, motor controlled above the sample, imposes the force and the rotation applied to the bead. Using video microscopy and image analysis of each video frame, the vertical and lateral positions of the tethered bead with respect to the surface of the sample is determined in real time. The extension of the molecule is deduced from the measurement of the average vertical position of the bead. The lateral Brownian fluctuations of the bead is analyzed to deduce, using the equipartition theorem, the vertical force exerted on the molecule [10].

Figure 1 shows an example of measurement of the extension of a palindromic DNA molecule as a function of the relative linking number ΔLk [13] for two levels of force. At low force (typically $<$0.5 pN), positive or negative values of ΔLk induce the formation of plectonemes, resulting in a symmetric reduction of the extension of the molecule around the torsionally relaxed state $\Delta\text{Lk} = 0$ (Fig. 1, circles). At higher forces ($\simeq$1–2 pN), the formation of plectonemes for negative values of ΔLk is prevented by the creation of denaturation bubbles [14] (Fig. 1, squares, arrow 1).

With the palindromic substrate used here, for sufficiently negative values of ΔLk (typically -50 to -400 turns, depending on the conditions, see Ref. [15]) an abrupt and irreversible shortening of the extension occurs (Fig. 1,

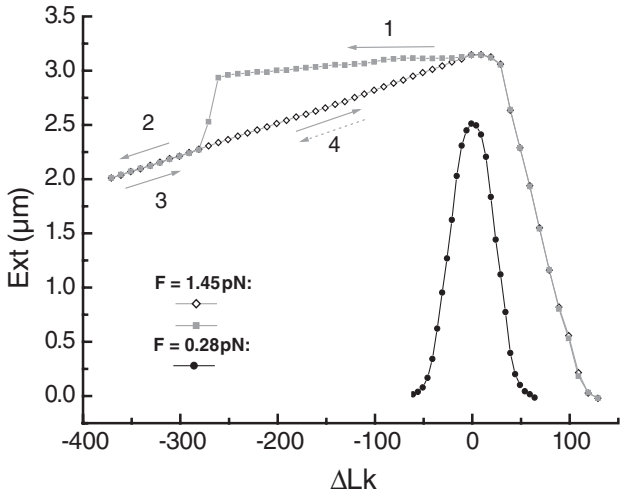

FIG. 1. DNA extrusion from of a palindromic molecule. Example of measurement of the extension (Ext) vs the relative linking number (ΔLk) of a palindromic DNA molecule, for two levels of force, 0.3 pN and 1.5 pN, respectively. The curves are obtained by averaging the extension every 10 turns for a period of 1 s to 4 min depending on the force level (arrows indicate the direction of rotations). Buffer conditions: 25 mM Tris-Acetate pH 8, 0.5 mM EDTA, 0.1% BSA, 0.01% NaN_3, $T = 27\,^{\circ}$C. The slope of the curve segments 2, 3, and 4 is $\sim$3.1 nm/turn.

squares, $\Delta Lk = -270$ turns); after which, as long as ΔLk stays negative, the system can be displaced reversibly back and forth along a well-defined slope (Fig. 1, open diamonds), showing that it is at a different equilibrium, and that the initial state before the transition was metastable.

Several arguments indicate that this transition corresponds to the formation of a cruciform DNA structure and that the slope corresponds to subsequent strand exchange (see Fig. 2): (i) The order of magnitude of the slope is in accordance with a rotationally induced strand exchange where each new turn added to the molecule produces the exchange of one turn of DNA through the branch point, varying the extension accordingly (see detailed analysis later). (ii) Once the shortening jump has occurred, the initial extension of the relaxed state is progressively recovered by decreasing ΔLk down to 0, as expected upon disappearance of the cruciform structure; also, the only way to return to the initial configuration and form again denaturation bubbles by uncoiling, is to reach the value $\Delta Lk = 0$. (iii) As expected for the formation of a cruciform structure and consistent with previous experiments on plasmids [16], a lower degree of uncoiling is needed for the transition to occur if the temperature is raised (see Ref. [15]). (iv) Consistent with previous experiments [16–18], magnesium ions dramatically hinder the transition and strongly affect the kinetics of the migration [19].

In the present conditions, without magnesium ions, the strand exchange is found to occur faster than the instrumental rotational speed limit of 6 tr/ sec (data not shown). Once the Holliday junction is formed, the local value of the slope $d\mathrm{Ext}/d\Delta Lk$ appears (at a given force) to be constant within our instrumental resolution: no sequence-specific (hence ΔLk-dependant) change of slope was detected.

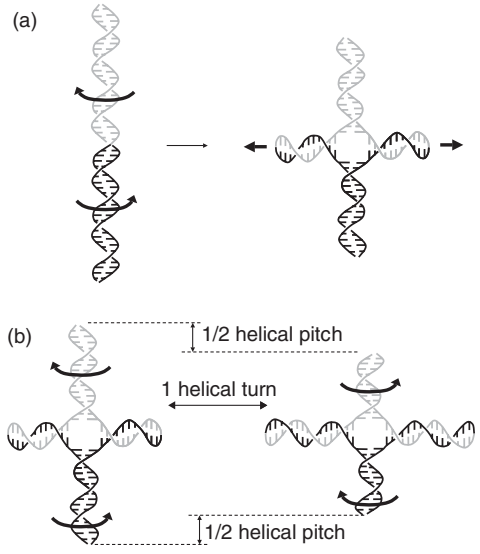

FIG. 2. Coupling between torsion and extrusion (arrows) for a palindromic DNA sequence. The gray and black double strands are related by a two-fold symmetry. (a) Cruciform DNA formation. (b) Strand exchange or branch point migration.

The cruciform DNA structure behaves like a nanomechanical gear that can be rotationally driven and that smoothly and reversibly converts linking number changes into vertical displacement. In the following, we use this remarkable feature to measure the helical pitch of DNA in solution.

The slope [slope(F)] of a migration curve depends on the vertical force F applied (Fig. 3). Because of the thermal agitation, the conformation of the molecule in space is fluctuating: in the force range studied, the molecule is not straight and the extension measured corresponds to the contour length $\mathcal{L}$ of the molecule scaled by a factor smaller than 1 (the relative extension λ): $\mathrm{Ext} = \lambda\mathcal{L}$, where λ depends on the force; the slope corresponds then to the pitch p of DNA scaled by the relative extension λ of the molecule:

$$\mathrm{slope} = d\mathrm{Ext}/d\Delta Lk = \lambda d\mathcal{L}/d\Delta Lk = \lambda p. \quad (1)$$

The curves obtained at different levels of force with an individual molecule form a spindle of lines that appear to converge toward a common intersection point. This intersection corresponds to the value of ΔLk for which the length of DNA in the vertical arms is zero. This point is determined by fitting each curve with a straight line while imposing a common intersection as a free parameter. Experimentally, this intersection point may not be reached for two reasons: (i) because of the labeling method for the molecules' extremities [12], there may not be an exactly equal length of DNA available for the strand exchange on each vertical arm; (ii) since the bead can freely rotate only about the direction of its paramagnetic momentum, which is imposed by the magnetic field, the anchoring position of the DNA may not be at the lowest position of the bead surface [20]; this leads to a steric hindrance between the bead itself and the capillary surface at short extensions.

In spite of the remarks above, the extrapolated intersection point gives, first, the total number of helical turns (Lk_{tot}) initially present within the molecule between the proximal biotin and digoxygenin attachments (because of the labeling method for the extremities of the molecules [12], this value may differ slightly between molecules). Secondly, it gives the exact zero extension reference which is then used here in order to improve the precision of both the extension and the force measurements.

The relative extension of DNA as a function of force $\lambda(F)$ is well described by the wormlike-chain (WLC) model [21] with only one parameter: the persistence length L_P of DNA [22]. Therefore, from the relation $\mathrm{Ext}(F) = \mathcal{L}\lambda(F)$, one can deduce the contour length $\mathcal{L}$ of a molecule by fitting its force-extension curve with the WLC model, and with $\mathcal{L}$ and L_P as free parameters. Since the migration spindle gives also the total number Lk_{tot} of helical turns present inside the molecule, we have here all the elements to determine the helical pitch of DNA in solution $p = \mathcal{L}/Lk_{tot}$. Equivalently, the pitch p can also be determined directly by fitting the curve $\mathrm{slope}(F) = p\lambda(F)$ with the

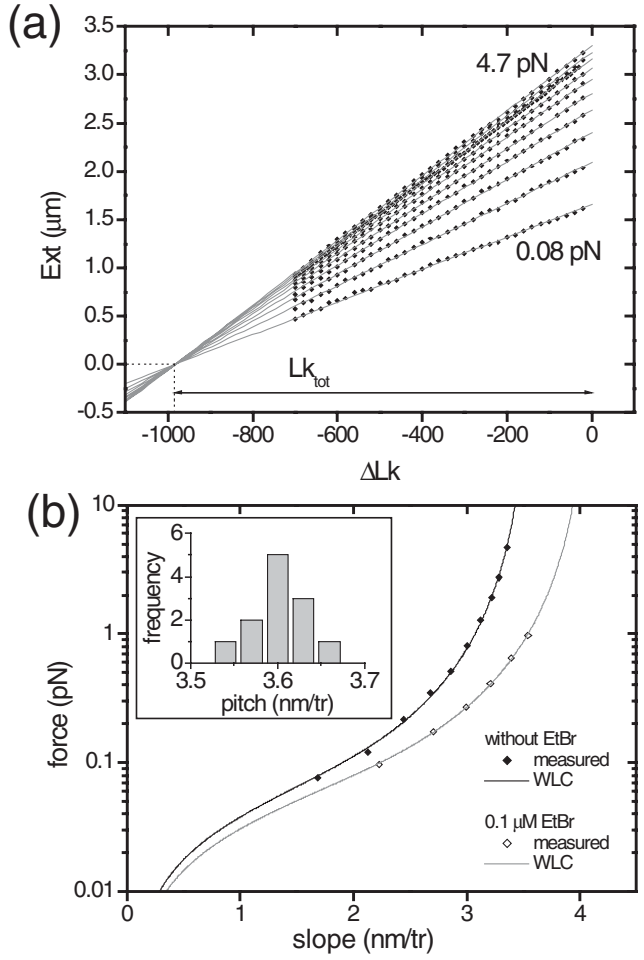

FIG. 3. Helical pitch measurement. (a) Migration curves (Ext vs ΔLk) at forces from 0.08 pN to 4.7 pN (buffer as in Fig. 1, $T = 37\,^{\circ}\mathrm{C}$). Each curve is obtained by averaging together a forward and reverse extrusion. The same DNA molecule is used for all data points in this figure. Identical results are found on other molecules. gray lines: linear fits with a common intercept imposed. (b) Force vs slope (slope $= d\mathrm{Ext}/d\Delta\mathrm{Lk}$). Filled diamonds: results from the linear fits of the spindle in (a). Open diamonds: same experiment on another molecule in the presence of 0.1 μM of ethidium bromide (EtBr). Solid lines: fits using the WLC model (see text). Results: black line: $p = 3.59 \pm 0.01$ nm/turn, $L_P = 50 \pm 3$ nm; gray line: $p^{0.1\mathrm{EB}} = 4.12 \pm 0.05$ nm/turn, $L_P^{0.1\mathrm{EB}} = 52 \pm 5$ nm. Inset: pitch distribution obtained from 12 different DNA molecules in the absence of EtBr.

WLC model, and with p and L_P as free parameters. In the following we apply this second method. We use for the WLC model, the analytical approximation obtained by Bouchiat *et al.* [24] which approaches the exact solution to 0.01%.

The black solid line in Fig. 3(b) corresponds to the fit of the relation slope(F) using the data [Fig. 3(b), filled diamonds] obtained from the spindle in Fig. 3(a). We obtain for this particular experiment $p = 3.59$ nm/turn and $L_P = 50$ nm. By averaging the results of identical experiments on 12 different molecules [see inset Fig. 3(b) and Table I], we obtain $p = 3.61 \pm 0.03(SD)$ nm for the pitch of DNA in solution and $L_P = 50 \pm 2(SD)$ nm for the persistence length.

As a control that we measure the helical pitch of DNA, and to illustrate the sensitivity of our measurement, we have performed similar experiments in the presence of ethidium bromide, known to change the helicity of DNA upon intercalation [25] [Fig. 3(b), open diamonds]. Accordingly we found a significant increase of the pitch of the molecule. Also, in accordance with previous measurements [20], we found no significant variation of the persistence length (Table I).

Does the force exerted on the structure alter significantly the pitch measured? Indeed, stretching on two opposite arms of a cruciform DNA structure while imposing a fixed linking number may displace the equilibrium position of the branch point toward a lengthening of the tethered arms and also, consequently, toward their uncoiling. This effect may result in an artificial increase of the pitch measured. However, theoretical arguments and control experiments show that within our instrumental resolution this effect is insignificant (see Ref. [15]).

Finally, we have tested the effect of changing the temperature, salt, and pH conditions (Table I). In the range of conditions explored, and consistent with previous measurements [26], we found slight variations of the persistence length, but we detected no significant effect on the pitch of DNA.

It is to be noted that the value of the pitch of DNA *in solution* found in textbooks [27,28] is only indirectly inferred by taking an helical periodicity of 10.5 bp/turn [29–31] and assuming a rise of 0.34 nm/bp identical to that found in crystals [32] (even though effects of crystal packing might induce a change of the rise between the liquid environment and the crystal). On the other hand, a value of $\sim$3.3 nm/bp for the rise has been found [33] by transient electric birefringence on small DNA fragments, but it involves a hydrodynamic model. Other measurements in liquid are, for example, the direct measurement by AFM [34] of the pitch (with low resolution), or the measurement of the average rise of DNA (contour length measurement by electron microscopy [35] on a DNA with a known number of bases); those, however, both involve the adsorption of DNA on a surface, hence possibly artifacts on the conformation of DNA. Our measurement in solution (3.61 ± 0.03 nm) (i) is significantly more precise than previous estimates, and (ii) confirms the 3.6 nm value usually assumed for the pitch of DNA in solution.

In conclusion, the present experiment is the first direct demonstration that branch migration can be controlled over a considerable molecular distance, by the relative linking number. The process in the absence of divalent ions appears to be fast (>6 turns/sec) and reversible. Such molecular configuration with a Holliday junction appears to be a prototype of a nanomechanical device that converts rotation to translation.

TABLE I. Helical pitch of DNA(p) and persistence length (L_P).

condition	p (nm) ($\pm SD$)	L_P (nm) ($\pm SD$)	number of molecules
standard[a]	3.61 ± 0.03	50 ± 2	12
std[a]+0.2 M KGlu[b]	3.59 ± 0.03	42 ± 2	3
std[a] but pH 7	3.59 ± 0.05	45 ± 3	6
std[a] but 27 °C	3.64 ± 0.06	46 ± 3	2
std[a]+0.1 μM EtBr[b]	4.12 ± 0.05	52 ± 5	3

[a]25 mM Tris-Acetate pH 8, 0.5 mM EDTA, 0.1% BSA, 0.01% NaN_3, 37 °C.
[b]KGlu: potassium glutamate, EtBr: ethidium bromide.

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[12] The palindromic DNA molecule is obtained by head-to-head ligation of two identical 5.92 kb DNA duplexes, each prepared with the same protocol as for the ''A fragment'' described in Ref. [11] except that one is labeled with digoxygenin-modified nucleotides (dig) and the other with biotin-modified nucleotides (biot). The resulting DNA fragments are labeled at one extremity only (multiple ligands spanned over approximatively 1 kb), and the other extremity has a ligatable 4 bases overhang (HindIII cut). The ligation results in palindromic dimers where a fraction is tetherable (incorporating both dig and biot fragments). The DNA sequence on each side of the center of the palindrome is a cloned fragment of λ-phage DNA encompassing the region 23 130 bp (HindIII site) to 18 560 bp (KpnI site). Because of the labeling method, the exact tether length (free DNA encompassed between the bead and the surface) is not precisely known.
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