Molecular Stick-Slip Motion Revealed by Opening DNA with Piconewton Forces

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We have pulled apart the two strands of a DNA double helix. The forces measured during this process show a sequence specific variation on the piconewton scale. Opening two helical molecules with the same sequence from opposite sides gives two signatures which are not simply related by symmetry. In a theoretical model, this is explained as a molecular stick-slip motion which does not involve instabilities and is determined by the sequence. [S0031-9007(97)04560-2]

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Stick-slip motion is a characteristic nonuniform mechanical movement, where in a first phase of relatively small displacement (stick) strain energy is accumulated which in a second phase (slip) transforms into kinetic energy. Common manifestations of stick-slip dynamics occur in macroscopic solid friction [1,2]. Earthquakes are a well known example, where sudden seismic motions correspond to slip phases [3,4]. In this paper it is shown that a qualitatively similar dynamics exists at the molecular level: The mechanical opening of a DNA double helix involves a series of pronounced stick-slip cycles. In contrast to macroscopic stick slip, the results of this single molecule experiment can be understood as an entirely deterministic equilibrium process.

During the last years, force measurements have been performed on DNA molecules to study, e.g., the extension of single-stranded (ss) [5] and double-stranded (ds) [6–8] DNA and the rotational elasticity of ds-DNA [9]. In this Letter, we report on the first measurements of the interaction force between the two complementary strands of the DNA double helix and present a comparison to detailed theoretical studies. After a brief introduction to the experimental configuration, we confront the experimental and theoretical results and show that good agreement is obtained. In the second part we briefly describe the theoretical approach which will allow us to clearly expose the new physics. More details about the biological and chemical steps of sample preparation, the technique of the piconewton force measurement, the theoretical description, as well as a comprehensive statistical analysis of the data, will be presented elsewhere [10,11].

The principle of our force measurements on the opening of DNA is presented in Fig. 1. The two strands on one end of a ds-DNA molecule (phage λ, contour length 16.2 μm, known sequence of 48 502 base pairs) are separately attached to a glass microscope slide (via a λ ds-DNA linker arm) and a microscopic polystyrene bead. The tip of a glass microneedle is attached to the bead and serves as the force lever [10,12]. The experiments are performed in a buffered solution with nearly physiological salt concentrations (10 mM phosphate, 150 mM NaCl, pH7). Keeping the base of the lever fixed, the DNA helix is forced open by a lateral displacement x_0 of the microscope slide (using a piezo translation stage). The bead and lever are imaged by an inverted optical microscope. A change in the lever deflection x below 0.1 μm can be resolved by a numerical analysis of the microscope image. For the lever of stiffness $k_{\mathrm{lev}} = 1.7$ pN/μm used in the experiment this corresponds to a force resolution better than 0.2 pN [10].

Two measured and a calculated force versus displacement curves are presented in Fig. 2. Sample displacement x_0 controls the extension and opening of the molecular construction. At small displacements in the measurements (below 8 μm in Fig. 2), we essentially extend the linker arm ds-DNA against the entropic force. When the force approaches 12 pN, the DNA molecule starts to open at base index 1. Further increasing the displacement, the double helix is consecutively pulled open and the force signal shows variations between 11 and 14 pN. These variations arise from the base sequence, and the gross features simply reflect the *G-C* versus *A-T* content of the DNA under study [10]. The two elementary pairings in DNA are the *G-C* and *A-T* pairs of Watson and Crick. As a *G-C* base pair involves three hydrogen bonds and an *A-T* pair involves two, it is expected that regions with higher content of *G-C* pairs open at higher force than *A-T* rich regions. This is indeed observed; e.g., the dip around 30 μm corresponds to an extended region of low *G-C* content.

It is possible to perform several cycles of opening and closing on the same molecule. In this paper we focus on the curves measured upon opening (during closing sometimes important drops occur in the measured force which we tentatively attribute to a transient delay of the

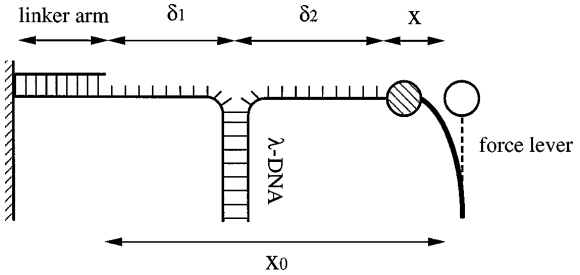

FIG. 1. Schematic view of the force measurement on the opening of a DNA double helix.

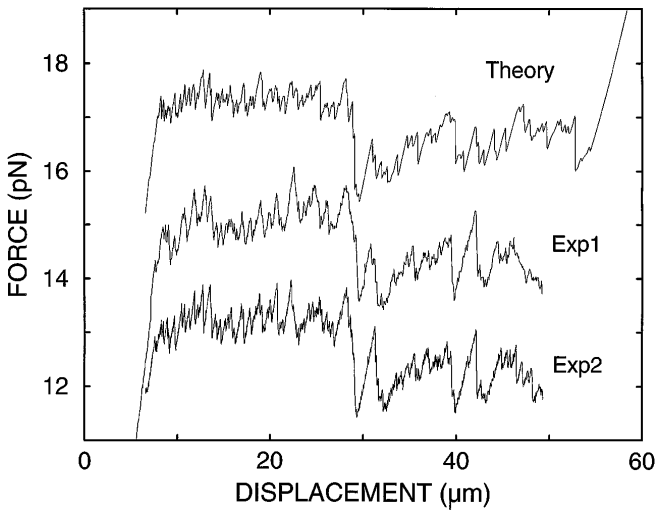

FIG. 2. Force curves for the opening of a λ-phage DNA. Two measurements performed on the same molecule with a displacement velocity of 40 nm/s (bottom) and 200 nm/s (middle, shifted by $+2$ pN) and a calculation (top, shifted by $+4$ pN) are compared. The measurements are stopped before complete opening is achieved. The calculated force rises for a displacement x_0 above 55 μm since we assume that the two strands of the double helix do not separate after complete opening (e.g., are linked by a minihairpin loop). The experimental curves are horizontally shifted to remove the offset caused by the linker arm.

recombination of the single strands). We have performed a series of measurements with different velocities of translation either on the same molecule (opening and closing several times) or on different molecules of the same base sequence. The two experimental curves of Fig. 2 are measured with velocities of 40 nm/s (bottom) and 200 nm/s (middle). The top curve shows the result of a calculation based on equilibrium statistical mechanics. The similarity between the three curves suggests that the measurements are performed close to equilibrium. We have calculated the cross correlation between the different experimental and theoretical force curves. Maximum correlation [13] is systematically obtained at the smallest displacement velocities, both between the measured curves and between experiment and theory.

Let us now turn to the experimental manifestation of molecular stick-slip motion. In Fig. 3, a measured force curve ($E1$) is plotted for a restricted range of displacement which corresponds to opening with a base index increasing from about 14 000 and 24 000. On this scale a series of sawteeth is apparent. The sawtooth shape is a characteristic feature of stick-slip processes in solid friction. Let us suppose that the opening blocks at a certain position. Then a linear increase Δx_0 in the sample displacement leads to a linear rise ΔF of the force, determined by the harmonic average of the stiffness of the single strands $k_{\rm ss}$ (local stiffness of the two single strands in series at the opening force) and the lever stiffness $k_{\rm lev}$. When we take $k_{\rm ss}$ from published experimental data [5], this simple analysis is consistent with the measured slopes of the sawteeth. This already suggests that the rise of the observed sawteeth corresponds to a "blocking" of the opening fork (position in the sequence where the double helix opens) and the drop to a fast progression.

We have designed a second molecular construction which allows us to open the λ-phage DNA from the other end; i.e., the opening starts at base index 48 502 rather than 1. In this construction the DNA to be opened is reversed (in the presentation of Fig. 1 the top ends of the center DNA are exchanged with the bottom ends), while the force measurement itself remains unchanged [10]. In Fig. 3, force curves measured upon opening the original construction ($E1$) and the inverted construction ($E2$) are presented. $E2$ is plotted with the horizontal (displacement) axis reversed. In this representation a given value on the horizontal axis corresponds on the average to a given region on the sequence. While, as mentioned above, the gross variations in the force curves are given by the G-C content of the sequence, $E1$ and $E2$ are different at the local scale of Fig. 3. Sawtooth structures appear in both measured curves but they are reversed in $E2$ with respect to $E1$. For a given sequence, the force curve thus clearly depends on the direction of the mechanical opening. Between the theoretical curves $T1$ and $T2$ similar characteristic differences appear as between the measurements $E1$ and $E2$, and a reasonable agreement between experiment and theory is obtained, even on the fine scale of Fig. 3.

Our theoretical description of the mechanical opening of the double helix is based on equilibrium statistical mechanics. Four energies are of central importance: the potential energy $E_{\rm DNA}$ of the partially opened double helix,

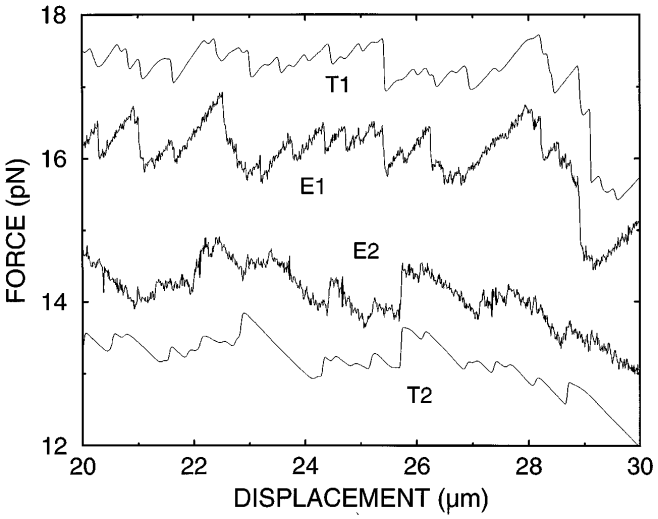

FIG. 3. Experimental ($E1, E2$) and theoretical ($T1, T2$) force curves showing the characteristic dependence of the force signal on the direction of the opening. Both measurements are performed with a displacement velocity of 20 nm/s. A displacement x_0 of 29 μm corresponds to the position where the molecules are forced open to the base pair of index $j = 24\,000$. $T1$, $E1$, and $E2$ are vertically shifted by $+4$, $+3$, and $+1$ pN, respectively.

the elastic energies of the two liberated single strands $E_{\rm ext}$, the elastic energy of the force lever $E_{\rm lev}$, and the thermal energy kT.

Regarding $E_{\rm DNA}$, the double helix structure is stabilized by a stacking energy and a pairing energy which depends on the sequence. To date no microscopic theory is available for these energies in an aqueous medium. Melting experiments on short oligonucleotides of various *G-C* content have been compared to thermodynamical models including interactions between second neighbor base pairs, which has allowed us to estimate the different binding energies of the base pairs occurring in DNA [14]. Melting is driven by fluctuations and therefore strongly depends on the conformations available to the single stranded parts. Our opening experiment is performed at lower temperature, and the single strands are under tension. Therefore fluctuations should be less important. For the energy corresponding to the opening of a *G-C* (*A-T*) pair we use $E_{\rm pair} = E_{GC}$ ($E_{\rm pair} = E_{AT}$), where E_{GC} and E_{AT} are fitting parameters. The potential energy $E_{\rm DNA}(j)$ of a DNA molecule with j separated base pairs is the sum over the $E_{\rm pair}$ values of the opened sequence.

Regarding $E_{\rm ext}$, force versus extension curves of a DNA single strand have been measured by Smith *et al.* [5] and fitted to an expression containing entropic and stretching contributions within a modified freely jointed chain (FJC) model. The expression contains three parameters: a single strand contour length $L_{\rm ss}$, a Kuhn length b, and a stretch modulus S. We obtain the elastic energy $E_{\rm ext}(j, \delta)$ of a stretched single strand with j bases by integrating the expression over the length δ of the strand. We take $b = 1.5$ nm and $S = 800$ pN (both from [5]) and a value of $L_{\rm ss} = 30\ \mu$m (27 μm in [5]) in combination with $E_{GC} = 2.9kT$ and $E_{AT} = 1.3kT$ [15]. The extension of the ds-linker arm can be neglected because it is relatively stiff. Taking its local stiffness at the opening force from the literature [6–8], we obtain a variation of its extension below 0.1 μm for the force variation during the opening. Moreover, measurements with longer ds-linker arms (two λ-DNA in series) show no sizable effect on the force curves.

The elastic energy of the lever $E_{\rm lev}$ is given by $E_{\rm lev} = 1/2 k_{\rm lev} x^2$. The lever deflection x arises from the difference between the sample displacement x_0 and the lengths of the single strands attached to the microscope slide (δ_1) and to the bead (δ_2): $x = x_0 - \delta_1 - \delta_2$ (see Fig. 1).

The thermal average of an observable A is calculated numerically according to

$$\langle A \rangle = \sum_{j,\delta_1,\delta_2} A e^{-E_{\rm tot}(j,\delta_1,\delta_2)/kT} \Big/ \sum_{j,\delta_1,\delta_2} e^{-E_{\rm tot}(j,\delta_1,\delta_2)/kT}.$$

The total energy is given by

$$E_{\rm tot} = E_{\rm DNA}(j) + E_{\rm ext}(j, \delta_1) + E_{\rm ext}(j, \delta_2) + E_{\rm lev}.$$

This description includes the thermal variations in the number of opened base pairs (j) and in the lengths δ_1 and δ_2 of the two single strands. The relevant dependence of $E_{\rm tot}$ on δ_1 and δ_2 at given j is well described by a quadratic development around the minimum of $E_{\rm tot}$. This allows us to analytically integrate over δ_1 and δ_2. The remaining, discrete summation over j is done numerically.

In Fig. 4 theoretical results on the mechanical opening and the relation between the force signal and the base sequence are compiled. The calculated force signal (bottom curve) locally exhibits a sawtoothlike shape. Concomitant with that, the average number of opened base pairs $\langle j \rangle$ (middle curve) increases in a staircase-like manner. The slowly rising part of a sawtooth in the deflection force corresponds to a quasiplateau in $\langle j \rangle$, the rapidly decreasing part in the force to a step in $\langle j \rangle$. The opening fork "blocks" at certain *G-C*-rich positions on the sequence (average *G-C* content on the right), strain builds up, and the lever deflection and the single strand extension increase until the accumulated elastic energy allows us to advance the opening fork again. A small additional increase in x_0 then suffices to open a sizable number of base pairs. This molecular stick slip does not involve instabilities; each point of the curves corresponds to an equilibrium position. This is a fundamental difference with respect to macroscopic stick-slip dynamics. In the molecular case it is possible to

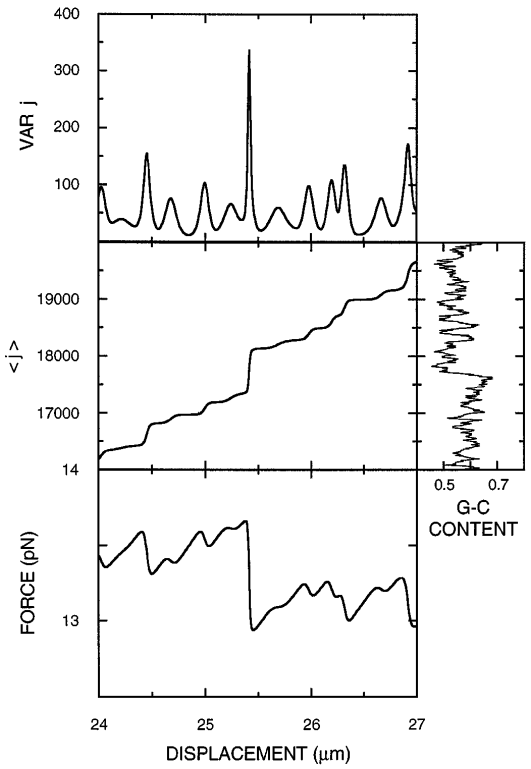

FIG. 4. Theoretical results on the molecular stick-slip motion. From bottom to top, the deflection force $k_{\rm lev}\langle x \rangle$, the number of opened base pairs $\langle j \rangle$, and the variance of j [var$j$ = $(\langle j^2 \rangle - \langle j \rangle^2)^{1/2}$] are presented. On the right the *G-C* content of the sequence is plotted (sliding average over 100 base pairs). Since common axes are used for the displacement x_0 and $\langle j \rangle$, the figure allows one to directly relate features in the *G-C* content to the deflection force, $\langle j \rangle$ and var$j$ (and vice versa).

recombine the double helix reversibly. The stick and slip events are induced by variations in the base sequence.

The variance of j, presented at the top of Fig. 4, can be interpreted as the resolution in base pairs of the force measurement, which strongly varies along the sequence. The double helix opens and recombines locally with an amplitude of breathing between 20 and 350 base pairs in this case. Pronounced maxima occur at the positions where the opening fork rapidly advances and the lever deflection drops. In this case the resolution is limited by the stick-slip process. In the stick zones (plateaus in $\langle j \rangle$) the variance of j is small. From this analysis follows that the force signal along the opening is not simply given by the *G-C* content but results from a complex interplay of sequence, elasticity, and thermal motion.

The molecular stick-slip motion was not predicted in earlier theoretical studies of the force signal expected upon mechanically separating the two strands of a DNA double helix [16,17]. This is mainly because only a simple sinusoidal modulation of the binding energy at the scale of one base pair was assumed. Our theoretical description includes a complex sequence, the elasticity of the strands and the thermal fluctuations. The intermediate scale variations in the *G-C* content are important as they induce the stick and slip events, in the way shown in Fig. 4.

Let us briefly comment on the remaining differences between the measured curves and between the measured and calculated curves. First, we notice that mechanical vibrations can occasionally induce small features in the measurements, which probably is the main limiting factor to the reproducibility at the smallest displacement velocities. Second, in particular at higher velocity we cannot be sure that the equilibrium value of the deflection force is always entirely reached during the course of the measurement. Third, regarding the theoretical description, there are additional degrees of freedom associated with the conformations of the free end of the ds-DNA to be opened and to the transversal motion and/or configurations of the strained single strands (e.g., transient formation of hairpin loops) as well as dissipations associated with the dynamics of the opening (rotation of the helix, viscous friction of the lever, etc.) which are neglected for the sake of simplicity.

In conclusion, complex stick-slip motion has been observed in a system where the molecular sequence is fully known. The present force measurement on single biomolecules allows for a detailed comparison with theory. Mechanical strand separation represents a new physical method for direct analysis of paired nucleotide sequences and may help to obtain a more quantitative understanding of the interaction energies which stabilize the DNA double helix.

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