Anharmonic Torsional Stiffness of DNA Revealed under Small External Torques

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DNA supercoiling plays an important role in a variety of cellular processes. The torsional stress related to supercoiling may also be involved in gene regulation through the local structure and dynamics of the double helix. To check this possibility, steady torsional stress was applied in the course of all-atom molecular dynamics simulations of two DNA fragments with different base pair sequences. For one fragment, the torsional stiffness significantly varied with small twisting. The effect is traced to sequence-specific asymmetry of local torsional fluctuations, and it should be small in long random DNA due to compensation. In contrast, the stiffness of special short sequences can change significantly, which gives a simple possibility of gene regulation via probabilities of strong fluctuations. These results have important implications for the role of DNA twisting in complexes with transcription factors.

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The double helical DNA in living cells is subjected to a constitutive unwinding torque created by special enzymes. This forces DNA to fold in a supercoiled state similarly to a flexible rod with bending and twisting elasticity. The supercoiling has long been known to play an important role in a variety of cellular processes [1]. Its magnitude changes regularly during the cell cycle and in response to environmental conditions, which is accompanied by activation or suppression of certain genes [2]. In *E. coli*, relaxation of the superhelical stress simultaneously alters activity of 306 genes (7% of the genome), with 106 genes activated and the others deactivated [3]. The genes concerned are functionally diverse, widely dispersed throughout the chromosome, and the effect is dose dependent. These and many similar observations suggest that the DNA supercoiling is used as a universal transcriptional regulator [2], but the corresponding physical mechanisms are not clear.

Detailed studies indicate that the promoter sensitivity to supercoiling stems from the recognition of promoter elements by RNA polymerase, and that it does not require DNA melting or transitions to alternative forms [4]. The supercoiling torque is distributed between twisting and writhing so that the untwisting of the double helix is estimated as 1%–2% [5], which is below the thermal noise and too small for reliable recognition. However, the action of the torsional stress can be conveyed through a property rather than the structure of the double helix. The behavior of the supercoiled DNA is governed by the interplay between the local bending and twisting fluctuations. If the bending flexibility or the torsional stiffness of the double helix varies with forced untwisting, parameters of thermal fluctuations could be noticeably affected already for short DNA stretches involved in the recognition. This idea is appealing and it is supported by some earlier data for long DNA [6–8]. Local torsional fluctuations are likely to be involved in regulation directly. In bacterial promoters, the optimal linker between the −10 and −35 elements involves 16 base pair steps (bps), but in promoters sensitive to supercoiling it is usually one step shorter or longer [4,9]. One step corresponds to rotation by 34.5°, which approximately equals the root-mean square width of torsional fluctuations for the linker. Very strong torsional fluctuations of short DNA stretches are necessary for activation of some animal promoters [10].

Local effects of the torsional stress are difficult to reveal experimentally, but they can be probed by all-atom molecular dynamics (MD) simulations. New methods were recently developed to apply steady forces and torques to short stretches of DNA [11]. In contrast with twisting by periodic boundary constraints and potential restraints used earlier [12–14], the steady stress emulates local conditions of a short fragment in a long supercoiled DNA, which makes possible evaluation of elastic parameters under very low torsional load corresponding to physiological conditions. This method captures linear elastic responses as well as the twist-stretch coupling effect under small torques corresponding to physiological degree of supercoiling [11]. Here we present the results of the first computational study of the elastic parameters of DNA in such conditions.

Dynamics of two tetradecamer DNA with AT- and GC-alternating sequences, respectively, were simulated in explicit aqueous solution using earlier described protocols [11]. For each duplex, nine 164 ns trajectories of all-atom dynamics were computed with fixed torque values in the range ± 20 pN · nm, which gives about 3 μs of simulations in total. Three additional trajectories were computed for the GC-alternating fragment for verification. Below we consider only evaluation of the torsional stiffness. Other methods and protocols are described elsewhere [15]. In the harmonic approximation the torsional free energy of a DNA fragment of length L subjected to external torque τ is

$$U(\Phi) = kT\frac{l_t}{2L}(\Phi - \Phi_\tau)^2, \quad (1)$$

where Φ is the overall winding angle, Φ_τ is its equilibrium value, l_t is the torsional persistence length, and kT is the Boltzmann factor. The equilibrium winding varies with the torque as

$$\Phi_\tau - \Phi_0 = \frac{\tau L}{kT l_t}. \quad (2)$$

In the course of MD simulations one measures the probability distribution P_Φ for the winding angle of one helical turn which, in the limit of infinite sampling, has a canonical form

$$P_\Phi \sim \exp\left[-\frac{l_t}{2L}(\Phi - \Phi_\tau)^2\right]. \quad (3)$$

The equilibrium winding is estimated as the time average $\langle\Phi\rangle_t$, and the torsional persistence length l_t is extracted from the time variance $\Delta_t^2\Phi$. The potential of mean force (PMF) corresponding to any Gaussian distribution is quadratic, but if the harmonic approximation is truly valid, l_t must be constant with different τ.

The top panel of Fig. 1 shows variations of Φ_τ corresponding to Eq. (2). All measurements were taken for the central 12 bp stretches, with the two terminal steps ignored, which gives about one helical turn. The amplitude of the forced winding is $\pm 2\%$, i.e., about 0.7° per base pair. The straight lines shown have the slopes corresponding to l_t obtained under zero torque. In the range of torques ± 10 pN · nm the points are compatible with a linear elastic response (harmonic elasticity). Beyond this range the profile remains roughly linear for the AT-alternating sequence, but for the GC-alternating duplex evident deviations from harmonicity are found. These deviations are reproducible and quite strong. If the l_t value were evaluated by Eq. (2) using Φ_τ for $\tau = \pm 20$ pN · nm, it would be about 200 nm.

The measured torsion persistence length changes with the applied torque as shown in the bottom panel of Fig. 1. The GC-alternating sequence exhibits strong anharmonicity, with the twist increase of 1.4° per bps accompanied by 30% growth in l_t. For the AT-alternating sequence, the l_t profile is nearly flat with a small decreasing trend. This trend becomes more visible with stronger twisting (not shown). The bending stiffness varies somewhat beyond the estimated statistical errors, but without regular trends.

Figure 2 shows the probability distributions P_Φ for the GC-alternating sequence for three representative values of τ. All of the distributions are close to the analytical Gaussians defined by Eq. (3) with different l_t. Since the width of the bells changes, the neat shapes of the computed distributions are not due to the harmonicity of the torsional potential. These Gaussian shapes result from the central limit theorem of the probability theory whatever the underlying potential. As seen in Fig. 3, the single-step twist fluctuations at GpC (guanine-phosphate-cytosine) and CpG steps produce wide and skewed non-Gaussian distributions strongly different from that predicted by Eq. (1)

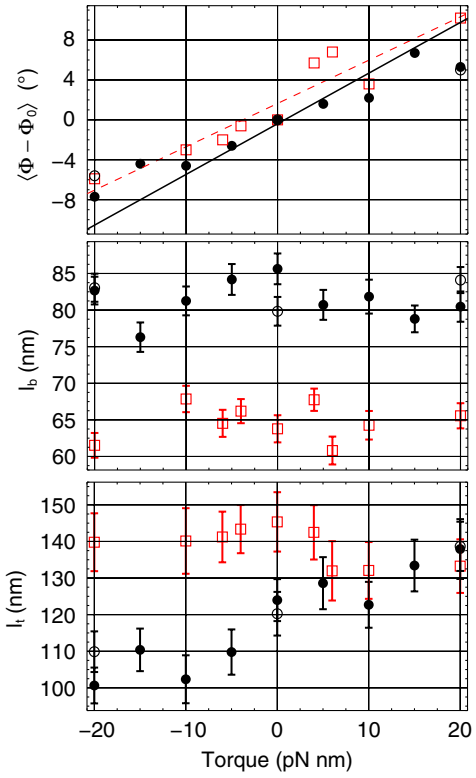

FIG. 1 (color online). Representative torque dependences obtained by all-atom MD simulations. The results are shown for the overall twisting (top panel), the bending persistence length (l_b, middle panel), and the torsional persistence length (bottom panel) of the AT-alternating ($\Phi_0 \approx 363.1°$, red squares) and GC-alternating ($\Phi_0 \approx 381.8°$, black circles) sequences. The open circles feature the verification tests. The straight lines on the top panel correspond to Eq. (2) with $l_t = 124$ nm (solid black line) and $l_t = 145$ nm (dashed red line). The error bars show statistical errors evaluated by the method of block averages [15]. In the top panel the symbol size corresponds to maximal errors.

(see also Ref. [15]). With the temperature around 300 K, the local DNA dynamics goes far beyond the area where the harmonic approximation is valid. However, the torsional fluctuations of four consecutive bps already give an almost ideal Gaussian. It can be formally described by Eqs. (1) and (3), but the shape of this bell does not correspond to the harmonic approximation of the local free energy. The Gaussian profile of fluctuations in long DNA is linked with the single-step distributions by a linear growth of the variance with the chain length. Consequently, not just the apex zones of the skewed distributions in Fig. 3, but their entire shapes, contribute. Therefore, the anharmonicity is significant, but hidden. In addition, the twist fluctuations at consecutive steps are anticorrelated and partially cancel out.

The asymmetry of the single-step PMFs is the probable cause of the variable torsional stiffness of the GC-alternating fragment. In the first approximation, the l_t value is proportional to the second derivative of the PMF in the

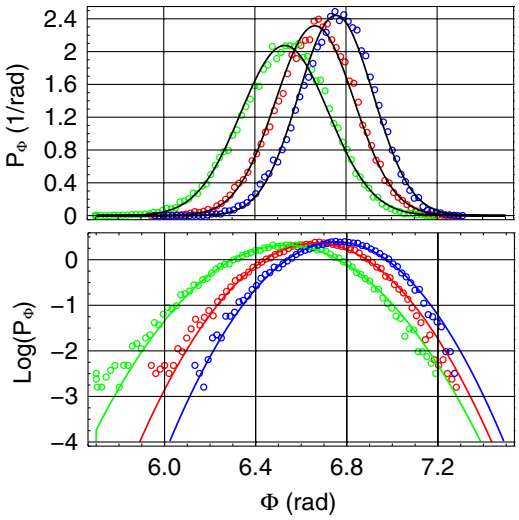

FIG. 2 (color online). The normalized probability density P_Φ obtained with different applied torques. From left to right, the MD results are shown for $\tau = -20$, 0, and $+20$ pN · nm by green, red, and blue points, respectively. The solid lines exhibit analytical distributions [Eq. (3)] corresponding to the measured values of l_t and Φ_τ. The upper and lower panels display the same data in linear and semilogarithmic coordinates, respectively.

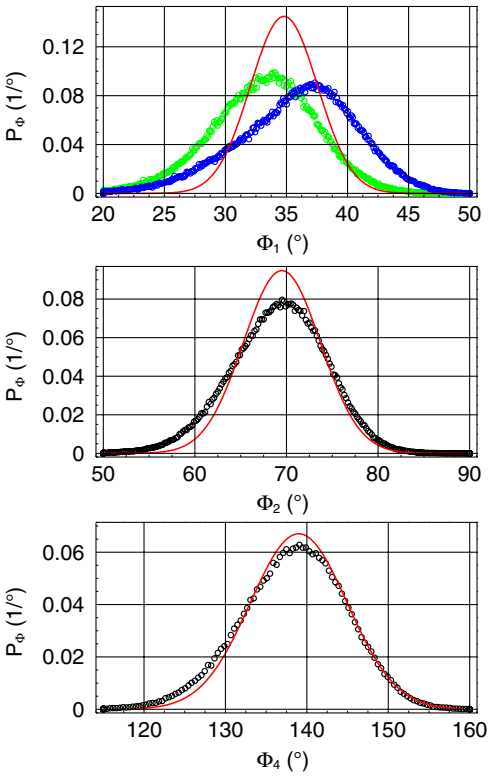

FIG. 3 (color online). The probability density P_Φ for GC-alternating fragments of one, two, and four bps (from top to bottom) obtained with $\tau = 0$. The solid red lines exhibit the analytical distributions [Eq. (3)] corresponding to the measured values of l_t and Φ_0. In the top panel, the distributions for GpC and CpG steps are shown in green (left) and blue (right), respectively.

energy minimum [see Eq. (1)]. For an asymmetric PMF a decrease in l_t may be expected when the external torque pushes towards the even slope of the energy profile. In the GC-alternating sequence both single-step distributions are left-skewed (see Fig. 3), so the right-hand slope of the PMF is steeper than the opposite one, which explains the sign of the trend in l_t observed in Fig. 1. The nearly flat l_t profile for the AT-alternating fragment can also be rationalized because in this case a strong positive skewness of TpA steps is partially compensated by a negative skewness of ApT steps [15]. Preliminary analysis of other sequences reveals that the strong negative skewness of the CpG single-step distributions is exceptional [15]. The homopolymer ApA and GpG steps are nearly symmetrical whereas the single-step distributions for AG- and AC-alternating DNA indicate that they would behave similarly to the AT-alternating fragment. These conclusions should be verified in more intensive computations, but we expect that for random DNA the macroscopic torsional stiffness should be nearly constant because among the steps with skewed distributions positive and negative skewness are equally represented. In contrast, for short sequence motives anharmonic effects of both signs are possible. They can be very significant because biological systems operate with much larger torques than we use here. For instance, the binding sites of the phage 434 repressor contain a variable 4 bp spacer that does not interact with the protein and supposedly participates in gene control via the sequence-dependent elasticity [16]. In the complexed state, this spacer is always overtwisted by about 30° [17], that is 10 times the amplitude of twisting in Fig. 1.

The experimental bending rigidity of free DNA is characterized by $l_b \approx 50$ nm [18]. The measured l_t values vary between 36 and 109 nm depending upon the specific methods and conditions [19]. Observations of sequence effects are rare [20], and there are a few reports on the influence of supercoiling [6–8]. If we assume that MD overestimates the stiffness of DNA uniformly, then the convergent estimate of l_t is around 90 nm, close to its value in single molecule experiments [21,22]. The bias can be due to the neutralizing salt condition in MD or other factors [15]. The nearly quantitative agreement between MD and experiment is remarkable because none of the parameters used in simulations was adjusted to reproduce the DNA elasticity. We hope, therefore, that the detailed microscopic picture provided by MD captures the qualitative physical trends dictated by the atom-level mechanics of the double helix. Our results argue that, under normal temperature, the local DNA elasticity is strongly anharmonic. Extrapolation from the apparent harmonic behavior of macroscopic DNA is not justified despite good agreement with atomistic simulations for chain lengths beyond one helical turn [23,24]. In addition, these computational observations shed new light upon some earlier controversial issues.

According to Fig. 1, with the helical twist slightly shifted from the equilibrium value the sequence dependence of the DNA elasticity can be significantly changed and enhanced. The measured torsional stiffnesses are similar without applied torque, but diverge with untwisting. The deformability of DNA is long considered as a possible governing factor in the sequence-specific site recognition [16], but this mechanism requires strong sequence dependence of elastic parameters compared to that observed in experiments with free DNA [20]. As we see, the properties of the relaxed DNA cannot be simply transferred to supercoiled and/or protein bound DNA states. Additional studies are necessary to check whether or not the elastic properties of the specific binding sites change under torsional stress. Its magnitude may be very large in some protein complexes [17].

Another debated issue concerns the mechanisms of gene regulation via DNA supercoiling [2,3]. Many such observations are readily rationalized if we assume that the sensitive promoters are regulated via the torsional stiffness. Even a slight shift in its value has a dramatic effect on the probabilities of strong twisting fluctuations. Many transcription factors are designed to bind the double helix at two sites separated by a spacer of several base pair steps. They can work as sensors of torsional fluctuations in DNA. A strong twisting fluctuation may be necessary for binding such a factor or for recognition by other proteins of a permanently bound torsional sensor. Figure 2 shows that, for fluctuations observable during 164 ns, physiological modulations of the torsional stress would change the corresponding probabilities by several times. For less frequent larger fluctuations the effect would be much stronger. One can extrapolate the pattern in Fig. 2 to events observable in the millisecond time range, and this leads to essentially all-or-nothing switching.

The external torque shifts the distributions in Fig. 2 by changing symmetrically the energies of opposite fluctuations. If the shape of the distributions does not change, each pair of curves should give a single intercept between the corresponding two apexes. However, if the shifting is accompanied by widening, one more intercept should appear in the range of large twisting opposite of the torque direction. For instance, the negative torque shifts the distribution in Fig. 2 to the left, but the simultaneous widening raises its right wing and, with very large overtwisting, the left curve should go above the other two. It is seen in Fig. 2 that the vertical difference between the three plots indeed exhibits a reducing trend with large Φ. This effect is somewhat paradoxical and it qualitatively contradicts the behavior of simple models where the torsional energy depends upon a single variable. Our attempts to reproduce it in discrete wormlike chains with anharmonic torsion potentials were unsuccessful. However, such behavior is possible, in principle, due to coupling between different degrees of freedom, and it requires further studies.

To conclude, it appears that small external torques can significantly alter the torsional stiffness of the double helical DNA. The effect is sequence-dependent, and, under variable degrees of supercoiling, different stretches of the double helix can become locally softer or stiffer. This can represent a versatile mechanism of gene regulation via the probabilities of strong twisting fluctuations.

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