Internal Dynamics of Superhelical DNA

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We present the first data on the temporal kinetics of monomer mean square displacements in DNA circles with defined degrees of superhelicity. The segmental dynamics of specifically labeled DNA plasmids with superhelical densities between 0 and -0.016 was assessed by fluorescence correlation spectroscopy. Introduction of superhelicity leads to progressively faster dynamics in the long time regime corresponding to the coil diffusion as observed previously by Langowski *et al.* [Biopolymers **34**, 639 (1994)], but also in the short time range corresponding to the segmental motion within the DNA coil.

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The conformational dynamics of polymers is determined by general polymer features such as bending rigidity, entropic elasticity, and hydrodynamic interactions between polymer segments. Circularized DNA is unique among other polymers in possessing an additional major property: superhelicity, characterized by linking number difference ΔLk which can be pictured as an amount of whole twist turns introduced into relaxed DNA prior to circularization. A corresponding intensive parameter, superhelical density, is defined as $\sigma = \Delta Lk/Lk_0$, where Lk_0 is the number of helical repeats of a relaxed double-helix. Superhelicity has a major effect on the equilibrium conformations and mechanical properties of DNA, and should greatly influence DNA dynamics. The equilibrium and stress-response behavior of superhelical DNA has been studied by a variety of experimental methods [1–4] and is well understood both in the framework of simulations and analytical theory [5–7].

However, the effect of superhelicity on the dynamics of DNA circles is known in much less detail. Most of the experimental progress was achieved by Langowski and coworkers using dynamic light scattering (DLS) which supplied data on the kinetics of rotational and translational diffusion of whole DNA circles, as well as on the dynamics of the dominant mode of their internal fluctuations [8–10]. The internal dynamics of DNA in this approach is characterized by a single kinetic parameter, the ''internal diffusion coefficient'' D_i, and by the relative amplitude of internal fluctuations. These parameters were measured for a DNA with different superhelicities [8–10]. With the analytical theory still in development [11–14], much of the current understanding of the dynamics of superhelical DNA comes from computer simulations [5,10,15] whose detailed predictions are verified on the more coarse-grained data from DLS experiments.

Here we report the first data on the stochastic segmental dynamics of superhelical DNA obtained with an experimental approach based on the fluorescence correlation spectroscopy (FCS) [16,17]. Whereas DLS measures the dynamics of the longest collective modes of DNA coil fluctuations, FCS applied to specifically labeled DNA reveals the dynamics of a single labeled position (essentially a ''monomer'') in a DNA polymer [18,19]. Here we present data on the temporal dependence of monomer mean square displacements (MSD) $\langle r^2(t)\rangle$ for 2686 bp long DNA circles with defined superhelicities ΔLk in 0 to -4 range ($-0.016 < \sigma \leq 0$).

In the FCS approach, DNA circles labeled specifically with a single fluorophore each move freely in solution illuminated with a focused laser beam. The stochastic motion of labeled DNA segments in the nonuniform excitation field results in the fluctuations $\delta I(t)$ in fluorescence intensity $I(t)$. Therefore the autocorrelation function $G(t) = \langle \delta I(0)\delta I(t)\rangle$ of emission fluctuations reflects the dynamics of base pair motion. For independent point sources of fluorescence randomly moving in a Gaussian beam, $G(t)$ is directly related to MSD of the fluorophore [20]:

$$\frac{G(t)}{G_0} = \left(1 + \frac{2}{3}\frac{\langle r^2(t)\rangle}{w_{xy}^2}\right)^{-1}\left(1 + \frac{2}{3}\frac{\langle r^2(t)\rangle}{\omega^2 w_{xy}^2}\right)^{-1/2}, \quad (1)$$

where $\omega = w_{xy}/w_z$ is the aspect ratio of the sampling volume, w_{xy} and w_z define the dimensions of the sampling volume in lateral and transversal dimensions, respectively, and G_0 is the amplitude of the correlation function determined from the behavior of $G(t)$ at short time scales. Then with calibrated w_{xy} and ω, the Eq. (1) can be used to convert the collected $G(t)$ into segmental MSD in a wide range of time and length scales [18].

We use pUC18 plasmids tagged specifically through the covalent attachment of prelabeled triple-helix forming oligonucleotides (TFO) according to the procedure developed in [21] with minor adjustments [20]. Topoisomers with defined superhelicities were prepared and gel separated by standard methods [22,23] modified to avoid exposure of DNA samples to UV and intercalating dyes [20]. Typically, we were able to obtain topoisomers from 6 bands in quantities sufficient for experiments: the first two bands correspond to relaxed and nicked circles and

both are characterized by $\Delta Lk = 0$, other bands have $\Delta Lk = -1$, -2, -3, and -4, respectively. The concentration of labeled DNA in the sample was in the 0.1 to 2 nM range corresponding to the dilute regime of polymer solutions (<400 nM for 2700 bp plasmids).

In Fig. 1 we present typical correlation functions obtained from topoisomers. The correlation function for nicked circles is very close to that of the relaxed circles and is not shown in the graph. With each increase in $|\Delta Lk|$, the correlation functions decay progressively faster, which implies faster DNA dynamics. Further insight into the dynamics of topoisomers can be obtained by converting the correlation functions $G(t)$ into segmental MSD by means of Eq. (1). We evaluate G_0 by averaging the $G(t)$ data in the 1 to 2 μs range, where all the correlation functions reach their plateau levels.

The extracted temporal dependences of segmental MSD are presented in Fig. 2. The data cover about 5 decades in time and reveal both the internal dynamics of DNA circles and their overall translational diffusion. Similar to data on linear DNA [18], the MSD curves for topoisomers can be split into three different regimes: (1) long time regime ($\gtrsim$2 ms) in which the segmental motion just tracks the diffusion of the DNA coil with a simple kinetic law $\langle r^2 \rangle = 6Dt$, (2) the intermediate regime (around 100 μs depending on ΔLk) which is characterized by subdiffusive segmental motion within the polymer coil, and (3) the short time regime ($\lesssim$20 μs depending on ΔLk) corresponding to displacements smaller or of the order of DNA persistence length L_p with the dynamics controlled by DNA stiffness [24].

There is a clear acceleration of segmental motion at all time scales. For MSD range $\sim$0.01 μm^2 the acceleration in dynamics is especially large: the characteristic times to reach a certain displacement differ about tenfold for $\Delta Lk = 0$ and $\Delta Lk = -4$. Overall, there appears to be a transition in the dynamic behavior which starts with $\Delta Lk = -2$ ($|\sigma| \approx 0.008$) and is already finished at $\Delta Lk = -3$ ($|\sigma| \approx 0.012$). The transition in dynamics is probably related to the transition in DNA conformation: at similar superhelicities the DNA state was observed to change from a random coil into a supercoiled plectoneme ($|\sigma| \approx 0.016$ corresponds to the supercoiled state already) [2]. The transition is a result of a trade off between the coil entropy and its bending and twisting energies [6]. Although the value $|\sigma| \approx 0.008$ for the observed transition in DNA dynamics is smaller than the theoretical prediction for the structural transition ($|\sigma| \approx 0.023$), it is close to 1 k_BT in introduced elastic energy per twisting persistence length: the superhelicity scale at which a DNA circle becomes strongly affected by twist [6].

We quantify the diffusion kinetics at long times by fitting segmental MSD with $\langle r^2 \rangle = 6Dt + 2a^2$, where a has a meaning of the amplitude of segmental motion within a coil equal to the root mean square distance between the labeled segment and the polymer center-of-mass. If all DNA base pairs behave in a statistically similar manner, a should give the polymer gyration radius R_g. The resulting diffusion coefficients and segmental amplitudes are presented as functions of ΔLk in Fig. 3(a). The diffusion coefficients are very close (differences do not exceed 18%) to those measured in DLS experiments [10]. The increase in D with growing superhelicity is also consistent with the findings of Ref. [10] and sedimentation studies [25]. The changes in the diffusion coefficients with increasing $|\Delta Lk|$ observed in our measurements appear to be larger than those found in DLS and in sedimentation studies. However, the discrepancies remain within the experimental errors, which in our measurements stem mostly from the small uncertainties ($\sim$2%–3%) in w_{xy}.

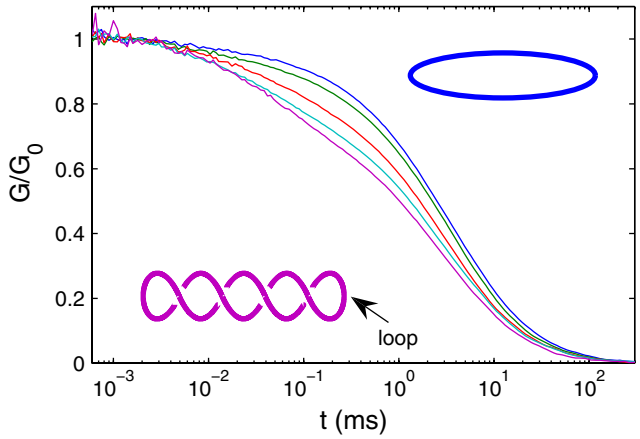

FIG. 1 (color online). FCS correlation functions for pUC18 topoisomers with ΔLk (*right to left*): 0 (blue), -1 (green), -2 (red), -3 (cyan), and -4 (magenta). To facilitate comparison all of the correlation functions were normalized to unity in the short time range ($\sim$1 μs). Insets show schematically the relaxed (top right) and plectonemic (bottom left) DNA conformations. The arrow points to a loop region in the plectoneme.

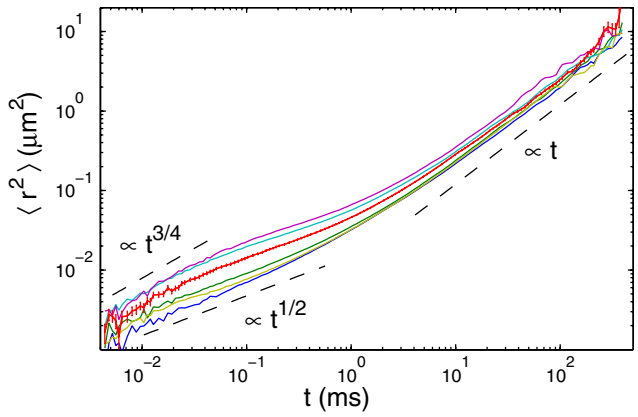

FIG. 2 (color online). Temporal dependences of segmental MSD for pUC18 topoisomers with ΔLk (*bottom to top*): 0—relaxed circles (blue), 0—nicked circles (yellow), -1 (green), -2 (red), -3 (cyan), and -4 (magenta). Typical error bars are shown for $\Delta Lk = -2$. Dashed lines are guides to the relevant power law dependences.

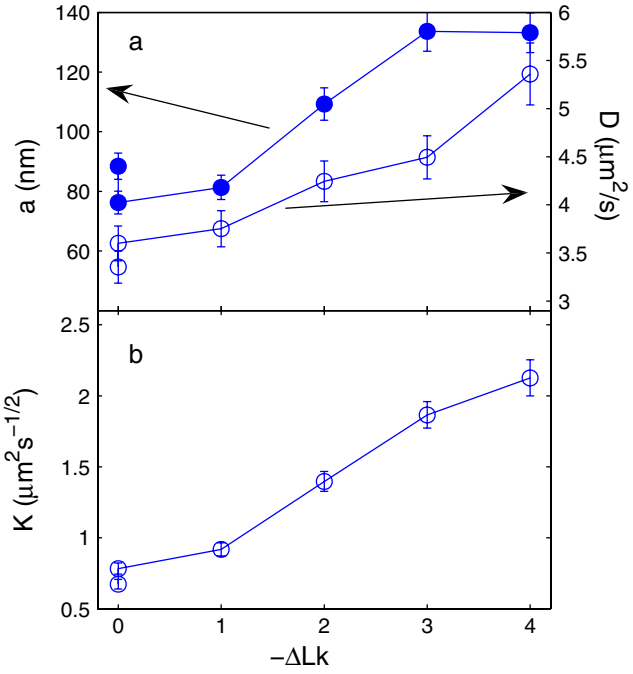

FIG. 3 (color online). Parameters characterizing segmental dynamics vs ΔLk: (a) full symbols, left axis—the amplitude of segmental motion a; open symbols, right axis—the coil diffusion coefficient D; (b) the kinetic parameter K of segmental dynamics in the intermediate regime.

The amplitude of the segmental motion a grows with the increasing superhelicity from ~80 nm for relaxed and nicked circles to ~130 nm for $\Delta Lk = -4$. As mentioned above, in general, one expects $a = R_g$. For a relaxed DNA circle of 2686 bp (~0.91 μm) in length and typical persistence length $L_p = 50$ nm, the estimate [12] of the gyration radius gives $R_g \approx 80$ nm, which is indeed close to the measured values of a for $\Delta Lk = 0$. It is more difficult to interpret the increase of a to 130 nm for $\Delta Lk = -4$. A minor increase in R_g (to ~90 nm at most) could be expected as DNA folds into a supercoiled plectoneme whose persistence length is about twice that of linear DNA. However, this effect should be more than offset by the loose structure of the plectoneme and its possible branching [2,5,6]. Thus we find it more likely that R_g of the circular DNA does not change significantly with supercoiling. However, due to the sequence specific effects, the labeled position in supercoiled DNA tends to localize near or in the loop regions of the supercoiled state. Since a is associated with the root mean square distance between the label and polymer center-of-mass we can expect then $a > R_g$. Some of the sequence specific effects which could lead to such localization of the label are known: the DNA sequences with intrinsic bends favor the loop regions of the plectonemes [9,26]. Indeed, in pUC18 plasmids there are two such naturally curved segments: one is close to the fluorescent label (at ~100 nm distance along the chain) and the second is directly opposite to the label position [9,27]. These two segments should bias the formation of a plectonemic loop to the vicinity of the label.

In the intermediate regime the exponent n characterizing the kinetics $\langle r^2 \rangle \propto t^n$ is close to that observed in linear DNA: $n \approx 0.5$ [18]. The exponent has some tendency to decrease with increasing $|\Delta Lk|$: from $n \approx 0.52$ for relaxed circles to $n \approx 0.45$ for $\Delta Lk = -4$. The intermediate range is overall rather short, just slightly over a decade in time, and shifts towards larger times with increasing $|\Delta Lk|$: from 0.01–0.15 ms range for $\Delta Lk = 0$ to 0.1–1 ms range for $\Delta Lk = -4$. In order to quantify the data we fit all the experimental curves in the intermediate regime with $\langle r^2(t) \rangle = K\sqrt{t}$ and present the parameter K in Fig. 3(b). As clear already from MSD dependences in Fig. 2, K grows monotonically with superhelicity with the major changes happening at $\Delta Lk = -2$.

The short time regime corresponds to the smallest measured displacements and therefore to the noisiest part of the data. Similarly to the intermediate regime it shifts to larger time and length scales with increasing $|\Delta Lk|$: from $\lesssim$ 10 μs temporal range and MSD $\lesssim 2 \times 10^{-3}$ μm^2 for $\Delta Lk = 0$, where it is barely detectable, to 5–50 μs temporal range and MSD up to $\sim 2 \times 10^{-2}$ μm^2 for $\Delta Lk = -4$. At least for topoisomers with $|\Delta Lk| \geq 2$, for which the short time regime can be observed relatively well, the kinetic law is close to $\langle r^2 \rangle = K_1 t^{3/4}$ expected for the dynamics of stiff polymers below their persistence length [24]. Interestingly, although the measured kinetics in this regime is subdiffusive, forcing the $\langle r^2 \rangle = 6D_i t$ gives D_i close to that obtained with DLS [10].

Our major and most surprising finding is that the internal dynamics of DNA accelerates with increasing superhelicity at all time scales. A qualitative interpretation of our data can be attempted in the framework of classical theories of polymer dynamics. The fact that DNA diffusion at long time scales accelerates with the increase in superhelicity can be explained by DNA folding into more compact, plectonemic structures at larger $|\Delta Lk|$. The intermediate regime with $\langle r^2 \rangle \propto t^{1/2}$ may reflect the dynamics of Rouse modes of polymer fluctuations [28]. With the formation of a plectoneme the rigidity of a DNA coil approximately doubles, which may lead to a moderate (by a factor $<\sqrt{2}$, see, e.g., Eq. 3 in Ref. [18]) speeding up of dynamics in this regime. The shift of the labeled position towards the loop regions of the plectoneme would also contribute to the acceleration in the observed dynamics: the shape of the Rouse modes is such that the MSD of the polymer ends is twice as large as the monomer MSD averaged over all monomers. The short time regime depends directly on the polymer rigidity, so it can be expected to become more pronounced when DNA folds into a more rigid conformation, as indeed observed at larger $|\Delta Lk|$. By itself this should not lead to faster dynamics in this regime, but rather to a minor (by a factor of $\sim 2^{1/4}$) slowing down of motion [24]. However, the shift of the labeled monomer to the loop regions of the plectoneme could lead to an accel-

eration of dynamics (up to a factor of 4, considering the shape of the normal modes of a stiff polymer [29]).

The factors cited here are the upper bounds in changes in dynamics. A more rigorous consideration of local friction, of plectoneme flexibility and of the label location is likely to make the changes significantly smaller. We thus do not believe that general theories of polymer dynamics are adequate to interpret our data. Additional effects specific to supercoiled conformation should be taken into account, such as the interplay between the motion of the DNA helices, plectoneme configurational and longitudinal fluctuations, their coupling to electrostatic interactions [30] as well as coupling between bend and twist deformations of DNA segments [31].

Intriguingly, DLS measurements [8,10] demonstrate an increase in the 'internal diffusion coefficient' with increasing superhelicity: this effect is qualitatively similar to our observations, although its magnitude appears to be much weaker in DLS experiments as compared to FCS. At the same time DLS reveals an opposing trend of decrease in the amplitude of the internal fluctuations with increasing $|\Delta Lk|$. Further experiments and simulations will be needed to understand how DLS and FCS data can be reconciled and how they complement one another. The locality of the probe may give the FCS approach larger sensitivity to changes in DNA dynamics as compared to the DLS technique. We note that the acceleration of internal dynamics in supercoiled DNA was also observed in an independent FCS study on different DNA molecules [32].

To summarize, we present here the first measurements of monomer dynamics in DNA toposisomers with defined degrees of superhelicity. The MSD dependence on time reveals two different regimes of motion within the DNA coil and, for large displacements, the motion together with the DNA center-of-mass. With increasing superhelicity the dynamics accelerates at all time scales. The major changes occur for superhelicity $\sigma \approx 8 \times 10^{-3}$ which we associate with the structural transition of the DNA ring from a random coil to a plectonemic state. The acceleration of DNA dynamics with increased supercoiling may have implications for processes in cells, such as regulation of transcription and on DNA recombination, which are dependent on a timely juxtaposition of two different DNA sites.

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