

Tightening of DNA knots by supercoiling facilitates their unknotting by type II DNA topoisomerases

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Using numerical simulations, we compare properties of knotted DNA molecules that are either torsionally relaxed or supercoiled. We observe that DNA supercoiling tightens knotted portions of DNA molecules and accentuates the difference in curvature between knotted and unknotted regions. The increased curvature of knotted regions is expected to make them preferential substrates of type IIA topoisomerases because various earlier experiments have concluded that type IIA DNA topoisomerases preferentially interact with highly curved DNA regions. The supercoiling-induced tightening of DNA knots observed here shows that torsional tension in DNA may serve to expose DNA knots to the unknotting action of type IIA topoisomerases, and thus explains how these topoisomerases could maintain a low knotting equilibrium in vivo, even for long DNA molecules.

Brownian dynamics | DNA topology | DNA structure | DNA configurations

During normal cell growth, various DNA transactions are facilitated by topoisomerase-mediated passage of one DNA segment through another. These strand passages can inadvertently lead to the formation of DNA knots (1–3), which are highly deleterious for living cells if not removed efficiently (4, 5). Type IIA DNA topoisomerases can use the free energy of ATP hydrolysis (6) to decrease the knotting level significantly below the equilibrium value for DNA rings subject to random passages between colliding segments (7). This topoisomerase (topo) IIA ability has been often assumed to be a physiologically relevant mechanism ensuring efficient DNA unknotting and decatenation (7–10), but doubts on this relevance have been raised as well (11). Particularly problematic is the fact that the original study establishing that type IIA DNA topoisomerases actively unknot DNA also revealed that this ability decreases sharply with increasing DNA length. Although based on only two plasmid sizes, that study showed the generality of this length dependence for several topoisomerases (7) and, therefore, active unknotting would be doomed to be ineffective for long DNA molecules, where random passages lead to very frequent knotting (12). While trying to understand why earlier experimental studies predicted ineffectiveness of type IIA DNA topoisomerases in unknotting of long DNA molecules, we recognized that these studies were performed using torsionally relaxed DNA (7), whereas naturally occurring DNA is frequently under torsional tension (13), the best-known examples of this state being negatively supercoiled bacterial plasmids. To investigate the potential effects of torsional constraints on the activity of type IIA topoisomerases, we have performed Brownian dynamics simulations to examine how negative supercoiling affects the structure of knotted DNA molecules and whether DNA supercoiling can cause knotted regions to be recognized specifically and then unknotted by topoisomerases. We observed that DNA supercoiling results in the tightening of knots, which leads directly to an increase in curvature of the knotted regions, in turn rendering these more likely to be bound and unknotted by type IIA topoisomerases.

Results

Brownian dynamics simulations of DNA molecules have proved themselves capable of predicting and/or reproducing the types of behaviors observed using direct-visualization methods. Even situations as complex as DNA stretching in extensional flow fields (14) or the diffusion of knotted regions in stretched DNA molecules (15) have been reproduced reliably by this simulation technique. Here we employ Brownian dynamics simulations to study the properties of negatively supercoiled DNA molecules that form right- or left-handed trefoil knots. To our knowledge, the interplay of supercoiling and knotting has not previously been addressed by Brownian dynamics simulations. Although our DNA model is coarse-grained, it is suitable to observe the phenomena occurring on a length scale that is larger than 30-bp stretches of DNA. In this study, we modeled knotted supercoiled plasmids having 1.5, 3, and 6 kb, where the largest one approaches the 7-kb size of knotted plasmids studied by Rybenkov et al. (7).

Effects of Supercoiling and Knotting on Electrophoretic Mobility. We decided to test first whether our simulations can explain the results of previous experiments showing that negatively supercoiled, left-handed DNA trefoil knots migrate slightly faster during gel electrophoresis than the corresponding negatively supercoiled right-handed trefoils (16). Although a detailed simulation of gel electrophoresis of DNA requires currently unavailable information concerning factors such as gel structure and gel–DNA interactions, a number of experiments and numerical simulations have established that the electrophoretic migration speed of DNA molecules with the same molecular weight increases linearly with their average crossing number (17, 18). By considering equilibrium configurations of the DNA molecules being modeled, we could thus test whether the supercoiled DNA trefoils of the simulation behave in a manner consistent with earlier experimental data. Indeed, we observed (Fig. 1) that, for 1.5-kb molecules, the left-handed forms have higher average crossing numbers, and are therefore expected to migrate more rapidly during gel electrophoresis than the right-handed forms. We explain this difference by the differential absorption of left-handed writhe generated by negative supercoiling by knotted portions of molecules that have positive or negative writhe. The observed correspondence between simulation and experiment for knotted DNA molecules encouraged a deeper investigation of how DNA supercoiling affects the overall structure of the knotted portions of these molecules by Brownian dynamics.

Supercoiling and Knot Size. The particular focus of our interest is the size of the knotted domain, which in the case of torsionally

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Methods

DNA Simulation. The Brownian dynamics method is based on the model proposed originally by Allison (30) and later refined by several groups (31, 32). The version used in this study is close to that of Vologodskii (15). The DNA chain is modeled as a discrete, worm-like chain of N segments, each of length $l_0 = 10$ nm (30 bp). A local reference frame is attached to each segment, whose displacement and rotation obeys standard Brownian dynamics equations with the following properties: time increment $\Delta t = 600$ ps, diffusion constant $D = 0.098 \times 10^{-9}$ m²/s, rotational diffusion constant $D_{rot} = 3.3 \times 10^7$ s⁻¹. The force field F used to simulate the properties of DNA is derived from four potentials. (i) A stretching potential $E_s = h(l_i - l_0)^2/2$, where l_i is the current length of the i th segment and $h = 100k_b T/l_0^2$ is the stretching constant. This potential ensures that the molecule maintains an approximately constant length. (ii) A bending potential $E_b = k_b T \alpha \theta_i^2/2$, where θ_i is the bending angle between segments i and $i + 1$, and α is the bending rigidity constant, which is set to $\alpha = 4.81$ for a DNA molecule with a persistence length of 50 nm. (iii) A torsion potential $E_t = C \tau_i^2 l_0/2$, where τ_i is the twist angle between segments i and $i + 1$, and C is the torsional rigidity constant, which is set to $C = 300 \times 10^{-21}$ J nm. (iv) An electrostatic Debye-Hückel interaction calculated for $n = 5$ charges placed on each segment and given by $E_e = k_b T H (l_i/n)^2 \exp(-kr_{ij})/r_{ij}$, where r_{ij} is the distance between charges i and j , and $H = 6.45$ nm⁻¹ (for a solution of 20 mM NaCl) is a constant calculated by Stigter (33) to mimic a cylinder-cylinder interaction. In total, 10^9 iterations were performed for each specific linking difference ΔLk_e .

The topological state of the molecule is monitored by measuring its writhe Wr and its twist deviation from the relaxed state ΔTw , which is the sum over all twist angles τ_i , and by definition the sum $Wr + \Delta Tw$ is an integer. For an unknotted plasmid, the previous sum gives the difference in linking number $\Delta Lk = Wr + \Delta Tw$ that describes the deviation from the torsionally relaxed state. This relation is only valid because the writhe of the relaxed unknotted plasmid, the intrinsic Wr_0 , is zero. In contrast, a knot has generally a nonzero Wr_0 because of the coiled path it has to follow. In consequence, one has to modify the previous formula to describe the effective difference in linking number $\Delta Lk_e = (Wr - Wr_0) + \Delta Tw$ ²⁰. When introducing torsional rigidity in the relaxed form of the molecule, the state with minimal elastic energy should have on average $Wr = Wr_0$ and $\Delta Tw = 0$. However, the complication is that Wr_0 for a trefoil is noninteger, which again by definition means that

$\Delta Tw \neq 0$. For example, for a trefoil with $Wr_0 = 3.4$, there will be a minimum difference in twist $\Delta Tw = 0.4$. The problem is even worse if one wants to compare the two chiral forms of a knot for which $Wr_0^L = -Wr_0^R$. To be able to compare these two forms, we therefore compensate the noninteger part of Wr_0 by correcting each twist angle τ_i by adding/subtracting $2 \times \pi \times 0.4/N$ to/from it. Starting from such torsionally relaxed but covalently closed DNA molecules, we then decrease the linking number of the molecules progressively to characterize their equilibrium behavior.

Knot Detection. To detect the knotted region, we adapted a method proposed by Marcone et al. (19). We measure the Alexander polynomial $A(s)$ of subchains of increasing length by starting with subchains of five segments. To close the subchain, three points (p_1, p_2, p_3) are added to each subchain: Points p_1 and p_2 are situated far (1 μ m) from the center of mass of the subchain (p_{CM}), on the lines connecting p_{CM} to each of the subchain's ends; p_3 is also far from p_{CM} (2 μ m), equidistant from p_1 and p_2 , and in the plane formed by p_{CM}, p_1 , and p_2 . If upon closure the subchain indicates the same knotting as the entire molecule, its two ends are pulled away from p_{CM} by using the Brownian dynamics algorithm. Torsional rigidity is suppressed during pulling to allow for the relaxation of supercoils. Once each end has been pulled away by 100 nm from p_{CM} , $A(s)$ is recalculated. If it still indicates the correct knotting, the subchain length is considered as the minimal length of the knotted portion. This complex procedure is in particular necessary for long chains, where the closure procedure sometimes passes through supercoiled branches, and indicates a false knotting.

Bending Angles. The bending angles characterizing the curvature of the knotted and unknotted portions are calculated as the bending between the two end segments of four-segment subchains. This procedure is similar to that used by Vologodskii et al. (25) in their model of the active bending mechanism.

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