# Monte Carlo Implementation of Supercoiled Double-Stranded DNA

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ABSTRACT Metropolis Monte Carlo simulation is used to investigate the elasticity of torsionally stressed double-stranded DNA, in which twist and supercoiling are incorporated as a natural result of base-stacking interaction and backbone bending constrained by hydrogen bonds formed between DNA complementary nucleotide bases. Three evident regimes are found in extension versus torsion and force versus extension plots: a low-force regime in which over- and underwound molecules behave similarly under stretching; an intermediate-force regime in which chirality appears for negatively and positively supercoiled DNA and extension of underwound molecule is insensitive to the supercoiling degree of the polymer; and a large-force regime in which plectonemic DNA is fully converted to extended DNA and supercoiled DNA behaves quite like a torsionless molecule. The striking coincidence between theoretic calculations and recent experimental measurement of torsionally stretched DNA (Strick et al., *Science.* 271:1835, 1996; *Biophys. J.* 74:2016, 1998) strongly suggests that the interplay between base-stacking interaction and permanent hydrogen-bond constraint takes an important role in understanding the novel properties of elasticity of supercoiled DNA polymer.

## INTRODUCTION

Recent years have witnessed a remarkably intense experimental and theoretical activity in searching for the elasticity of a single supercoiled DNA molecule (see, e.g. Strick et al., 1996, 1998; Fain et al., 1997; Vologodskii and Marko, 1997; Moroz and Nelson, 1997; Bouchiat and Mezárd, 1998; Zhou et al., 1999). Within a cell, native double-stranded DNA (dsDNA) often exists as a twisted and heavily coiled, closed circle. Differing amount of supercoiling, in addition to affecting the packing of DNA within cells, influences the activities of proteins that participate in processes—such as DNA replication and transcription—that require the untwisting of dsDNA (Wu et al., 1988). It is believed that changes in supercoiling can also promote changes in DNA secondary structure that influences the binding of proteins and other ligands (Morse and Simpson, 1988).

In recent experiments (Strick et al., 1996, 1998) on single torsionally constrained DNA molecules it was found that the supercoiling remarkably influences the mechanical property of DNA molecules. When applied with relatively low stretching force, a supercoiled molecule can reduce its torque by writhing, forming structures known as plectonemes. Therefore, the distance between two ends of the polymer decreases with increasing supercoiling. However, above a certain critical force $f_c$, this dependence of extension on supercoiling disappears. More strikingly, the value of $f_c$ is significantly different for positively and negatively supercoiled DNA, i.e. $f_c \sim 0.8$ pN for underwound molecule and $f_c \sim 4.5$ pN for overwound ones. On the theoretical side, harmonic twist elasticity and bending energy according to the wormlike chain model have been used to understand supercoiling of DNA polymer (Fain et al., 1997; Vologodskii and Marko, 1997; Moroz and Nelson, 1997; Bouchiat and Mezárd, 1998), and some qualitative mechanic features of plectonemic structures of supercoiled DNA polymer have been described by the harmonic twist elasticity. However, because of the chiral symmetry of harmonic twist elasticity, the asymmetry of elastic behaviors of supercoiled DNA cannot be understood by this model, and, especially, the three obvious mechanic regimes observed in experiment of supercoiling DNA (Strick et al., 1996, 1998) still need better understanding. To understand the supercoiling property and the highly extensibility of DNA, we have developed a more realistic model in which the double-stranded nature of DNA is taken into account explicitly (Zhou et al., 1999). The supercoiling property of highly extended DNA was investigated analytically. Here, we aim at performing a thorough and systematic investigation into the property of supercoiled DNA by using Monte Carlo simulations based on this model.

As we have known, the bending energy of DNA polymer is mainly associated with the covalent bonding between neighboring atoms of DNA backbone (Nossel and Lecar, 1991). In our previous work (Zhou et al., 1999), van der Waals interactions between adjacent basepairs was introduced, and this helps to explain the highly cooperative extensibility of overstretched DNA (Cluzel et al., 1996; Smith et al., 1996). It has also been shown that the short-range base-stacking interaction takes a significant role in determining the elastic property of DNA. Lennard–Jones type potential between adjacent basepairs can be written as

$$U(\theta) = \begin{cases} \epsilon\left[\left(\dfrac{\cos\theta_0}{\cos\theta}\right)^{12} - 2\left(\dfrac{\cos\theta_0}{\cos\theta}\right)^{6}\right], & \text{for} \quad \theta > 0, \\ \epsilon[\cos^{12}\theta_0 - 2\cos^{6}\theta_0], & \text{for} \quad \theta \le 0, \end{cases} \tag{1}$$

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(see also Fig. 1). The folding angle $\theta$ of the sugar-phosphate backbones around DNA central axis is associated with the steric distance $r$ of adjacent basepairs by $r = r_0 \cos\theta$, where $r_0$ is the backbone arc length between adjacent bases. The asymmetric potential related to positive and negative folding angle $\theta$ in Fig. 1 ensures a native DNA to take a right-handed double-helix configuration with its equilibrium folding angle $\theta_{eq} \sim \theta_0$. This double-helix structure is anticipated to be very stable because $\epsilon$ ($\sim 14\ k_BT$) is much higher than thermal energy $k_BT$ according to the results of quantum chemical calculations (Saenger, 1984).

In the case in which DNA polymer is torsionally constrained, the basepair folding angle will deviate from the equilibrium position $\theta_{eq}$. However, if the stretching force is very small, the folding angle may deviate from $\theta_{eq}$ only slightly. This is because, as we can infer from Fig. 1, the base-stacking potential is very sharp around $\theta_0$, and a relatively large force is needed to make $\theta$ deviate considerably from its equilibrium value. It is reasonable for us to anticipate that a supercoiled DNA under low stretching force will convert its excess or deficit linking number into positive or negative writhing of its central axis. Because the central axis is symmetric with respect to positive or negative writhing, the elastic response of DNA at this force regime will certainly be symmetric with a positive or negative degree of supercoiling. Only when the stretching force becomes large enough will the chirality of supercoiled DNA appear. In this regime, it becomes more and more difficult for the central axis to writhe to absorb the linking number, and an increasing portion of the linking number will be converted to a twisting number of the backbones, which will certainly change the twisting manner of dsDNA. Because Eq. 1 shows that, for dsDNA, untwisting is much easier than overtwisting, chiral behavior is anticipated to emerge. This opinion is consistent with the experimental result of Strick et al. (1996).

In this paper, we investigate the mechanical properties of supercoiled DNA by numerical Monte Carlo method. Base-stacking van der Waals interactions between adjacent basepairs are incorporated by introducing the new degree of freedom, namely the folding angle $\theta$. A fundamental difference from the previous approaches (see, e.g., Vologodskii and Marko, 1997), which try to include the twist degrees of freedom by adding extra terms to the free energy, is that the twist and supercoiling are treated as the *cooperative* result of base stacking and backbone bending constrained from permanent basepairs. The striking coincidence between theoretic calculations and experimental data of supercoiling DNA (Stick et al., 1996, 1998) indeed confirms this treatment.

## MODEL AND METHOD OF CALCULATION

In the simulation, the double-stranded DNA molecule is modeled as a chain of discrete cylinders, or two discrete wormlike chains constrained by basepairs of fixed length $2R$ (Fig. 2). The conformation of DNA molecule of $N$ straight cylinder segments is specified by the space positions of vertices of its central axis, $\mathbf{r}_i = (x(i), y(i), z(i))$ in three-dimensional Cartesian coordinate system, and the folding angle of the sugar-phosphate backbones around the central axis, $\theta_i$, $i = 1, 2, \ldots, N$. Each segment is

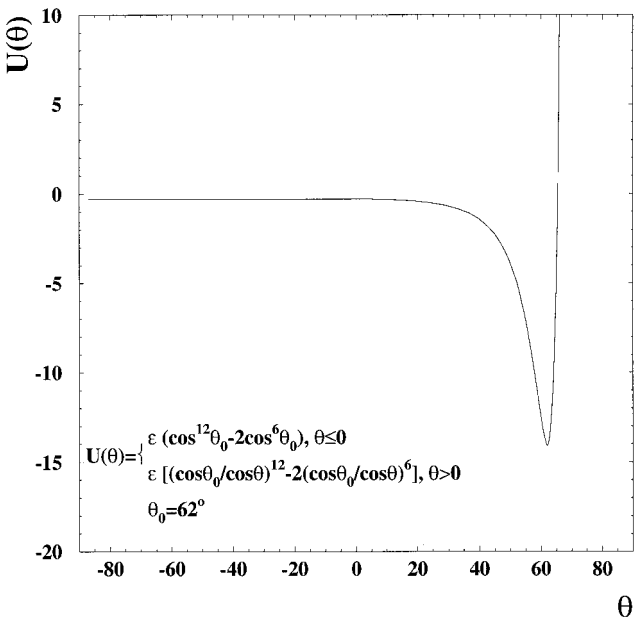


FIGURE 1 The van der Waals interaction potential versus folding angle of sugar-phosphate backbones around DNA molecule axis.

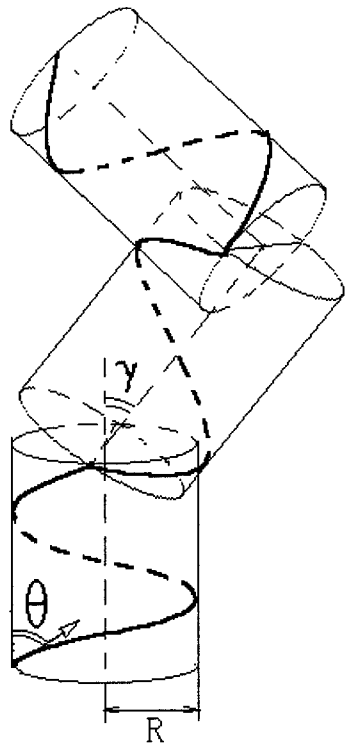


FIGURE 2 The configuration of discrete DNA chain in our model.

assigned the same amount of basepairs, $n_{bp}$, so that the length of the $i$th segment satisfies

$$\Delta s_i = |\mathbf{r}_i - \mathbf{r}_{i-1}| = 0.34 n_{bp} \frac{\cos\theta_i}{\langle\cos\theta\rangle_0}, \quad (2)$$

where $\langle\cdots\rangle_0$ means the thermal average for a relaxed DNA molecule. Moreover, bearing in mind the experimental fact that there are ~10.5 basepairs for each turn of a native double helix DNA and the average distance between the adjacent basepairs is about $d_0 = 0.34$ nm, we have set the basepair length as $2R = (10.5 d_0/\pi)\langle\tan\theta\rangle_0$ in our model.

Metropolis Monte Carlo method (Metropolis et al., 1953) is used to simulate the equilibrium evolution procedure of torsionally stretched dsDNA molecules. At each step of the simulation procedure, a trial conformation of the chain is generated by a movement from the previous one. The starting configuration is chosen arbitrarily (except that some topology and bound conditions should be satisfied, see below) and the averaged results of equilibrium ensemble are independent of the initial choice after numerous movements. The probability of acceptance of the movement depends on the difference in energy between the trial and the current conformations, according to the Boltzmann weight. When a trial movement is rejected, the current conformation should be counted once more. This procedure is repeated numerous times to obtain an ensemble of conformations that, in principle, is representative of the equilibrium distribution of DNA conformation.

## The DNA model

As we have known, double strand DNA is formed by winding two polynucleotide backbones right-handedly around a common central axis. Between the backbones, nucleotide basepairs are formed with the formation of hydrogen bonds between complementary bases. In our continuous model (Zhou et al., 1999), the embeddings of two backbones are defined by $\mathbf{r}_1(s)$ and $\mathbf{r}_2(s')$. The ribbon structure of DNA is enforced by having $\mathbf{r}_2(s')$ separated from $\mathbf{r}_1(s)$ by a distance $2R$, i.e. $\mathbf{r}_2(s') = \mathbf{r}_1(s) + 2R\mathbf{b}(s)$ where the hydrogen-bond-director unit vector $\mathbf{b}(s)$ points from $\mathbf{r}_1(s)$ to $\mathbf{r}_2(s')$. As the result of the wormlike backbones, the bending energy of two backbones can be written as

$$E_B = \frac{\kappa}{2}\int_0^L \left[\left(\frac{d\mathbf{r}_1^2}{ds^2}\right)^2 ds + \left(\frac{d\mathbf{r}_2^2}{ds'^2}\right)^2 ds'\right]. \quad (3)$$

The formation of basepairs leads to rigid constraints between the two backbones and, at the same time, they hinder considerably the bending freedom of DNA central axis because of the strong steric effect. In the assumption of permanent hydrogen bonds (Everaers et al., 1995; Liverpool et al., 1998; Zhou et al, 1999), $|s' - s| = 0$. The relative sliding of backbones is prohibited, and the basepair orientation lies perpendicular to the tangent vectors $\mathbf{t}_1 = d\mathbf{r}_1/ds$ and $\mathbf{t}_2 = d\mathbf{r}_2/ds$ of the two backbones and that of the central axis, $\mathbf{t}$: $\mathbf{b}\cdot\mathbf{t}_1 = \mathbf{b}\cdot\mathbf{t}_2 = \mathbf{b}\cdot\mathbf{t} = 0$. By defining the folding angle as half of the rotation angle from $\mathbf{t}_2(s)$ to $\mathbf{t}_1(s)$, i.e., the intersection angle between tangent vector of backbones $\mathbf{t}_{1(2)}$ and DNA central axis $\mathbf{t}$, we have

$$\begin{cases}\mathbf{t}_1 = \cos\theta\mathbf{t} + \sin\theta\mathbf{b}\times\mathbf{t}\\ \mathbf{t}_2 = \cos\theta\mathbf{t} - \sin\theta\mathbf{b}\times\mathbf{t}.\end{cases} \quad (4)$$

Therefore, the bending energy of the two backbones can be rewritten as

$$E_B = \int_0^L \left[\kappa\left(\frac{d\mathbf{t}}{ds}\right)^2 + \kappa\left(\frac{d\theta}{ds}\right)^2 + \kappa\frac{\sin^4\theta}{R^2}\right] ds, \quad (5)$$

where $ds$ denotes arc-length element of the backbones, $L$ the total contour length of each backbone, and $\kappa$ is the persistence length of one DNA backbone. Bearing in mind that the pairing and stacking enthalpy of the bases significantly increase bending stiffness of the polymer axis, the experimental value of persistent length of dsDNA polymer is considerably larger than that of a DNA single strand (see, e.g. Smith et al., 1996). To incorporate the steric effect, and also considering the typical experiment value of persistent length of dsDNA $p = 53$ nm, the simplest way is to substitute $k$ in the first term of Eq. 5 with a phenomenological parameter $\kappa^* = 53.0/2\langle\cos\theta\rangle_0$ nm $k_BT$ (Zhou et al., 1999), hereafter this is assumed.

Taking into account Eqs. 1 and 5, the total energy of dsDNA molecule with $N$ segments in our discrete computational model is expressed as

$$E = \alpha\sum_{i=1}^{N-1}\gamma_i^2 + \alpha'\sum_{i=1}^{N-1}(\theta_{i+1} - \theta_i)^2 + \frac{\kappa}{R^2}\sum_{i=1}^{N}\Delta s_i \sin^3\theta_i\tan\theta_i + \sum_{j=1}^{N_{bp}} U(\theta_j) - fz(N), \quad (6)$$

where $\gamma_i$ is the bending angle between the $(i-1)$th and the $i$th segments (Fig. 2), $N_{bp}$ the total number of basepairs of DNA polymer, and $z(N)$ is the total extension of the DNA central axis along the direction of the external force $f$ (assumed in the $z$-direction).

Because the Kuhn statistical length of dsDNA polymer is associated with its bending stiffness (the Kuhn length is twice the persistence length of dsDNA polymer according to the wormlike chain model), one can decide bending rigidity parameter $\alpha$ of the discrete chain accordingly. Suppose that we take the $N$ discrete segments to simulate the behaviors of a dsDNA polymer of $n$ Kuhn statistical length, the length of $m$ $(= N/n)$ segments should correspond to one Kuhn statistical length. Therefore, for any chosen value $m$, we can decide the bending rigidity parameter $\alpha$ in the way (see Appendix)

$$m = \frac{1 + \langle\cos\gamma\rangle}{1 - \langle\cos\gamma\rangle}, \quad (7)$$

where

$$\langle\cos\gamma\rangle = \frac{\int_0^\pi \cos\gamma\exp(-\alpha\gamma^2)\sin\gamma\, d\gamma}{\int_0^\pi \exp(-\alpha\gamma^2)\sin\gamma\, d\gamma}. \quad (8)$$

In principle, the discrete DNA model becomes continuous only when $m$ approaches infinity. The CPU time needed for a simulation, however, increases approximately as $N^2 = (nm)^2$. So it is necessary to choose a value of $m$ that is large enough to ensure reliable results but small enough to keep the computational time within reasonable bounds. Our calculation and also previous work (Vologodskii et al., 1992) showed that simulated properties do not depend on $m$ if it exceeds 8. Therefore, $m = 8$ was used in the current calculation, for which the bending constant $\alpha = 1.895 k_BT$. Furthermore, we have chosen $N = 160$ in consideration of the feasible computer time. Because Kuhn statistical length of dsDNA is taken as 106 nm, the B-form length of the polymer in our simulation corresponds to

$L_B$ = 2120 nm or 6234 basepairs. The constant $\alpha'$ in the second term of Eq. 6 should be associated with stiffness of the DNA single strand. As an crude approximation, we have taken here $\alpha' = \alpha = 1.895 k_B T$. Our unpublished data show that the amount of the second term of Eq. 6 is quite small compared with the other four terms, and the result of simulation is not sensitive to $\alpha'$.

The fourth term in Eq. 6 accounts for van der Waals interactions between adjacent basepairs (see Eq. 1). $\theta_0$ (= 62°) is related to the equilibrium distance between a DNA dimer. The base-stacking intensity $\epsilon$ is generally influenced by the composition and sequence of nucleotide chains. For example, the solubility experiments in biphasic systems show that stacking interactions between purine and pyrimidine bases follow the trend

$$\text{purine–purine} > \text{pyrimidine–purine} > \text{pyrimidine–pyrimidine.}$$

Because we do not distinguish the specific base sequence of purine and pyrimidine in our DNA model, we take the statistic average of stack energies as $\epsilon = 14 k_B T$, according to the result of quantum chemical calculations (Saenger, 1984).

To simulate the extension of the stretched DNA chain, we fixed one of its ends at the original point in the three-dimensional Cartesian system and applied a force $f$ directed along the $z$ axis to the second end, which corresponds to the fifth term of Eq. 6.

## Calculation of link number

The number of times the two strands of DNA double helix are interwound, i.e., the link number $Lk$, is a topologic invariant quantity for closed DNA molecule and also for linear DNA polymer in the case in which the orientations of two extremities of the linear polymer are fixed and any part of the polymer is forbidden to go around the extremities of the polymer. An unstressed B-DNA molecule has one right-handed twist per 3.4 nm along its length, i.e., $Lk_0 = L_B/3.4$. Under some twist stress, the link number of a DNA polymer may be different from its torsionally relaxed value. In all cases, when $\Delta Lk = Lk - Lk_0 \neq 0$, the DNA polymer is called supercoiled (Vologodskii and Cozzarelli, 1994). The relative difference in link number

$$\sigma = \frac{Lk - Lk_0}{Lk_0} \tag{9}$$

signifies the degree of supercoiling that is independent of the length of DNA polymer. The native DNA of organisms living at physiological environment are found always slightly underwound, and its supercoiling degree is between −0.03 and −0.09 (Bauer, 1978; Vologodskii and Cozzarelli, 1994), which is believed to be significantly relevant in some fundamental biological processes (Wu et al., 1988; Morse and Simpson, 1988).

In addition to directly counting the number of times the two strands are interwound, the link number of the closed DNA circle can be conveniently calculated by White's theorem (White 1969)

$$Lk = Tw + Wr. \tag{10}$$

The twist, $Tw$, is the number of times basepairs twist around the central axis and does not depend upon the configuration of molecule axis. The writhe, $Wr$, of the molecule is a simple function of only the molecule axis vector $\mathbf{r}(s)$ (White, 1969; Fuller, 1971)

$$Wr = \frac{1}{4\pi} \int\int \mathrm{d}s\, \mathrm{d}s' \, \frac{\partial_s \mathbf{r}(s) \times \partial_{s'} \mathbf{r}(s') \cdot [\mathbf{r}(s) - \mathbf{r}(s')]}{|\mathbf{r}(s) - \mathbf{r}(s')|^3}. \tag{11}$$

$Wr$ is scale invariant and dimensionless and changes sign under reflection or inversion of $\mathbf{r}$, reflecting the cross product in the formula above. Therefore, $Wr = 0$ if $\mathbf{r}(s)$ is planar or otherwise reflection symmetric.

To control and measure experimentally the supercoiling degree of linear DNA polymer, Strick et al. (1996, 1998) attached one end of a DNA molecule to a glass cover slip by DIG-anti-DIG links and the other end to a paramagnetic bead by biotin-streptavidin links. Bearing in mind that the diameter of the magnetic bead (≈4.5 $\mu$m) is far beyond that of a polymer, the anchoring points can be considered as on impenetrable walls, and ~16-$\mu$m-long DNA (Strick et al., 1996), in fact, is prohibited from passing around the ends of the polymer. A magnetic field pointing in the plane of the microscope slide was applied to fix the orientation of the bead. Therefore, by rotating the magnets and counting the time of turns, the link number $Lk$ of the linear DNA molecule can be controlled and measured experimentally.

In Monte Carlo calculations, we restrict the DNA chain by two impenetrable parallel walls crossing the chain ends, which is to simulate the above-mentioned experimental equipment of the magnet bead and the microscope slide (see also the treatment in Vologodskii and Marko, 1997). The walls are always parallel to the $xy$ plane in our Cartesian coordinate system and thus perpendicular to the direction of the force applied to the chain ends.

One way to calculate the link number $Lk$ of DNA molecule in our Monte Carlo simulation is to use White's formula Eq. 10. However, the writhe $Wr$ is defined only for closed chain. To solve the problem, we add three long flat ribbons to the two ends of the DNA chain in each conformation during the simulation procedure. The axes of these ribbons are kept in the same planar and consist of a closed circle together with the linear DNA chain. Because there is not any twist in the added three flat ribbons, it is not difficult to verify from Fig. 3 that the number of times two strands interwind $Lk_l$ in Fig. 3 *a* is equal to the link number of new closed polymer $Lk_c$ in Fig. 3 *d*. Therefore, we only calculate $Lk$ of the closed chain in our simulations according to Eqs. 10 and 11.

Quite similar to the model by Tan and Harvey (1989) in which the twist of each basepair of DNA chain is explicitly specified, the folding angle of backbones in each segment has been given in our model. So the twist can be directly calculated by

$$Tw = \frac{1}{2\pi R} \sum_{i=1}^{N} \Delta s_i \tan \theta_i. \tag{12}$$

The writhe $Wr$ of the new DNA circle can be calculated according to Eq. 11.

## Simulation procedure

For any given force, equilibrium sets of conformations of DNA chain are constructed using the Metropolis MC procedure (Metropolis et al., 1953). Three kinds of movements have been considered in our simulations (see Fig. 4).

In the first type of movement, a random chosen segment is undertwisted or overtwisted by an angle $\lambda_1$. In other words, the folding angle $\theta_i$ of the chosen segment is modified into a new value $\theta_i' = \theta_i + \lambda_1$. When $\theta_i'$ is beyond the setting interval $[-\theta_m, \theta_m]$ from one side, it will re-enter the interval from the opposite side according to the periodicity assumption. Although the geometric limit of folding angle of DNA backbone is $\theta_m = \pi/2$, we set $\theta_m = 85°$ here to avoid the possible divergency in numerical calculation of potential of Eq. 1. It should be mentioned that this movement modifies not only the folding angle of the chosen segment but also the coordinates of all the behind vertices $\mathbf{r}_j$, $j = i, \cdots, N$ along the length, because, when the folding angle $\theta_i$ is changed, we have also changed the length of the segment $\Delta s_i$ according to Eq. 2. So we should translate all those segments behind this one to make the chain match up (Fig. 4 *a*).

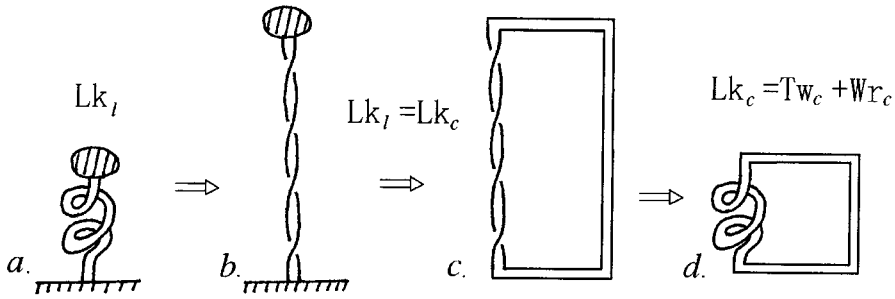


FIGURE 3 The schematic diagram to calculate link number in our simulations. (*a*) For a linear supercoiled DNA chain with one end attached to a microscope slide and the other attached to a magnetic bead, when the orientation of the bead is fixed and the DNA chain is forbidden to go round the bead, the number of times for two strands to intertwine, and the linking number of the linear DNA ($Lk_l$), is a topological constant. (*b*) The DNA double helix is stretched to a fully extended form while the orientation of bead remains unchanged. The link number of the linear DNA chain is equal to the twist number, i.e., $Lk_l = Tw_l$. (*c*) Three long, flat ribbons are connected to the two ends of the linear twisted DNA of (*b*). The link number of the new double helix circle is equal to that of the linear DNA chain, i.e., $Lk_c = Tw_c = Tw_l = Lk_l$ because the writhe of the rectangle loop is 0. (*d*) The DNA circle in (*c*) can be deformed into a new circle, one part of which has the same steric structure as the linear supercoiled DNA chain in (*a*). So, by adding three straight ribbons, the link number of linear double-helix DNA can be obtained by calculating the link number of the new DNA circle, i.e., $Lk_l = Lk_c = Tw + Wr$.

In the second type of movement, an interval subchain containing an arbitrary amount of segments will be rotated by an angle of $\lambda_2$ around the straight line connecting the vertices bounding the subchain (Fig. 4 *b*). The third type of movement involves a rotation of the subchain between chosen vertices and the free end by an angle of $\lambda_3$, around an axis with arbitrary orientation (Fig. 4 *c*). All three types of movements satisfy the basic requirement of the Metropolis procedure of microscopic reversibility, i.e., the probability of trial conformation *B* when current conformation is *A* must be equal to the probability of trial conformation *A* when current conformation is *B*.

All three types of movements change the configurations of the DNA chain. However, from the viewpoint of energy, their functions are quite different. Although the first type of movement is concerned mainly with modifying twist and stacking energy, the second one changes only the bending energy and the third modifies both bending energy and extension of the DNA chain. Each of them is performed with a probability of ⅓. The value of $\lambda_1$, $\lambda_2$, $\lambda_3$ are uniformly distributed over interval $(-\lambda_1^0, \lambda_1^0)$, $(-\lambda_2^0, \lambda_2^0)$, and $(-\lambda_3^0, \lambda_3^0)$, respectively, and $\lambda_1^0$, $\lambda_2^0$, and $\lambda_3^0$ are chosen to guarantee that about half of the trial moves of each type are accepted.

The starting conformation of DNA chain is unknotted. But the configurations after numerous steps of movements may become knotted, which violates the topologic invariance of chain and is incorporeal. Especially, both ends of molecule are anchored in the experiment and knots never occur. To avoid knotted configuration, we should check the knot status for each trial conformation. The most effective way to clarify the knot categories of the DNA circle is to calculate its Jones polynomial (Jones, 1985), which is a strictly topological invariant for knot categories. But the computational calculation of the Jones polynomial is quite prolix at this moment. In our case, where it is only necessary to distinguish between unknot and knot categories, the classic Alexander polynomial (Alexander, 1928; Conway, 1969) is enough to meet this requirement, although it is for weaker topological invariants and does not distinguish mirror images. For trivial knots, the Alexander polynomial $\Delta(t) = 1$; and $\Delta(t)$ is usually not equal to 1 for knotted chains. (Although there are nontrivial knots whose Alexander polynomials equal unity, this case is very rare. One example of a nontrivial knot with $\Delta(t) = 1$ can be found in Vologodskii et al. 1974.) Convenient algorithms for computer calculation of the Alexander polynomial had been well built (see, e.g., Vologodskii et al. 1974; Harris and Harvey, 1999). We only calculate the value of $\Delta(-1)$ in our simulation. In a case where the trial movement knots the chain, the energy of trial conformation is set to be infinite, i.e., it will be rejected.

Another interaction considered in our simulation is the steric effect of a polymer chain. Because the segment has finite volume, other segments cannot come into its own space region. This interaction evidently swells the polymer (Doi and Edwards, 1986). To incorporate this exclude-volume effect into our simulation, for each trial conformation, we calculate the distance between any point on the axis of a segment and any point on the axis of another nonadjacent segment and check whether this distance is less than the DNA diameter 2*R*. If the minimum distance for any two chosen segments is less than 2*R*, the energy of trial conformation is set infinite and the movement is rejected.

During the evolution of the DNA chain, the supercoiling degree $\sigma$ may distribute around all the possible values. To avoid the waste of computation events, we bound the supercoiling $\sigma$ of the DNA chain inside the experimental region (Strick et al., 1996, 1998), i.e., $-0.12 \geq \sigma \geq 0.12$. When the torsion degree of trial conformation is beyond the chosen range, we simply neglect the movement and reproduce a new trial movement.

## RESULT OF MONTE CARLO SIMULATION

To obtain an equilibrium ensemble of DNA evolution, $10^7$ elementary displacements are produced for each chosen applied force *f*. The relative extension *x* and supercoiling degree $\sigma$ of each accepted conformation of the DNA chain are calculated. When the trial movement is rejected, the current conformation is counted up twice (see Metropolis et al., 1953).

To see the dependence of mechanics property of DNA upon supercoiling degree, the whole sample is partitioned into 15 subsamples according to the value of the supercoiling degree $\sigma$. For each subsample, we calculate the averaged extension,

$$\langle x_j \rangle = \frac{1}{N_j} \sum_{i=1}^{N_j} \frac{z_i(N)}{L_B}, \quad j = 1, \ldots, 15, \tag{13}$$

and the averaged torsion

$$\langle \sigma \rangle = \frac{1}{N_j} \sum_{i=1}^{N_j} \sigma_i, \quad j = 1, \ldots, 15, \tag{14}$$

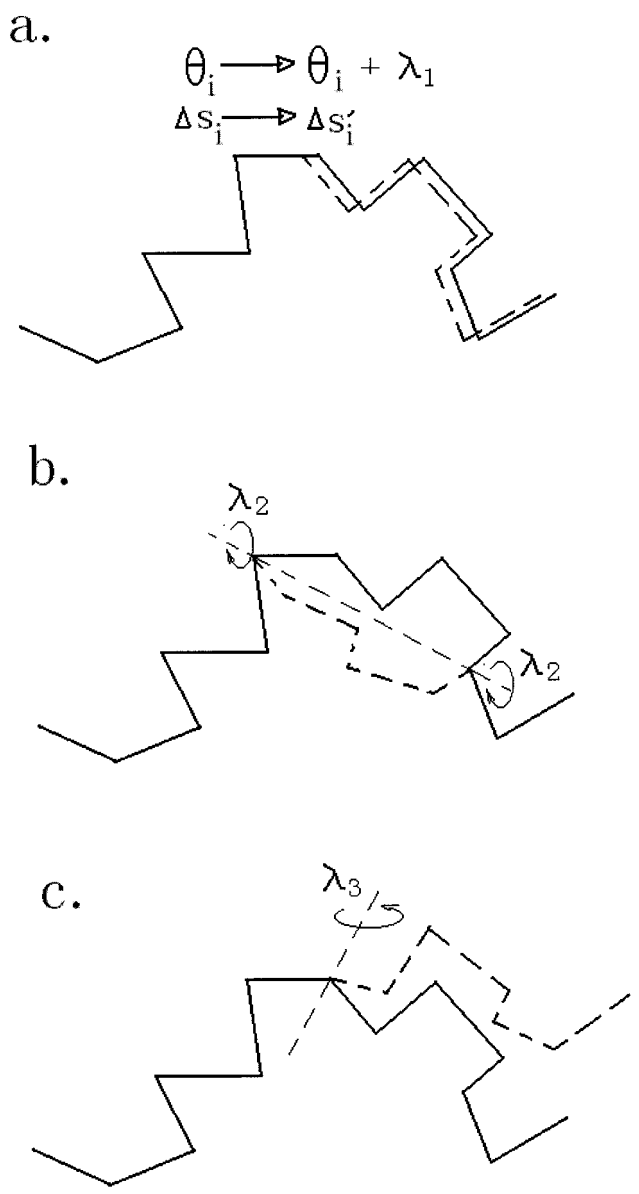


FIGURE 4 Trial motions of the DNA chain during Monte Carlo simulations. The current conformation of the DNA central axis is shown by solid lines and the trial conformation by dashed lines. (*a*) The folding angle in *i*th segment $\theta_i$ is changed into $\theta_i + \lambda_1$. All segments between *i*th vertex and the free end are translated by the distance of $|\Delta s_i - \Delta s_i'|$. (*b*) A portion of the chain is rotated by an angle of $\lambda_2$ around the axis connecting the two ends of rotated chain. (*c*) The segments from a randomly chosen vertex to the free end are rotated by an angle $\lambda_3$ around an arbitrary orientation axis that passes the chosen vertex.

where $N_j$ is the number of movements supercoiling that belong to *j*th subsample.

We display the force versus relation extension for all positive and negative supercoiling in Fig. 5, *a* and *c*, respectively. As a comparison, the experimental data (Strick et al., 1998) are shown in Fig. 5, *b* and *d*. In Fig. 6 is shown the averaged extension as a function of supercoiling degree for three typical applied forces. At low force, the extension in our Monte Carlo simulation saturates at a value greater than zero because of the impenetrable walls, which astrict the vertical coordinate of the free end always higher than that of any other points of the DNA chain. The same effect of the impenetrable walls was found in earlier works (see Fig. 9 of Vologodskii and Marko, 1997). For conciseness, we did not show the relative extension points of less than 0.15 in Figs. 5 and 6.

Despite quantitative difference between Monte Carlo results and experimental data, the qualitative coincidence is striking. Especially, three evident regimes exist in both experimental data and our Monte Carlo simulations:

1. At a low force, the elastic behavior of DNA is symmetrical under positive or negative supercoiling. This is understandable, because the DNA torsion is the cooperative result of hydrogen-bond constrained bending of DNA backbones and the base-stacking interaction in our model. At very low force, the contribution from applied force and the thermodynamic fluctuation perturbate the folding angle $\theta$ of the basepair to derive very little from the equilibrium position $\theta_0$. Therefore, the DNA elasticity is achiral at this region (see Introduction). For a fixed applied force, the increasing torsion stress tends to produce a plectonemic state, which shortens the distance between the two ends; and therefore, the relative extension of linear DNA polymer. These features can also be understood by the traditional approaches with harmonic twist and bending elasticity (Vologodskii and Marko, 1997; Bouchiat and Mezárd, 1998).
2. At intermediate force, the folding angle of basepairs is pulled slightly further away from equilibrium value $\theta_0$ where van der Waals potential is not symmetric around $\theta_0$. So the chiral nature of elasticity of the DNA molecule appears. In the negative supercoiling region, i.e., $\theta < \theta_0$, the contribution of applied force dominates that of potential force because of the low plateaus of $U(\theta)$. So, the extension is insensitive to negative supercoiling degree. In contrast, the positive supercoiling still tends to contract the molecule.
3. At higher force, the contribution of the applied force to the energy dominates that of van der Waals potential in both over- and underwound DNA. The extension of DNA accesses to its B-form length. Therefore, the plectonemic DNA is fully converted to extended DNA, the writhe is essentially entirely converted to twist and the force–extension behavior reverts to that of untwisted ($\sigma = 0$) DNA as expected from a torsionless worm-like chain model (Smith et al., 1992; Marko and Siggia, 1995; Zhou et al., 1999). Because of the effect of the impenetrable wall, however, the extension of the DNA molecule in our calculation is slightly higher than in experimental data.

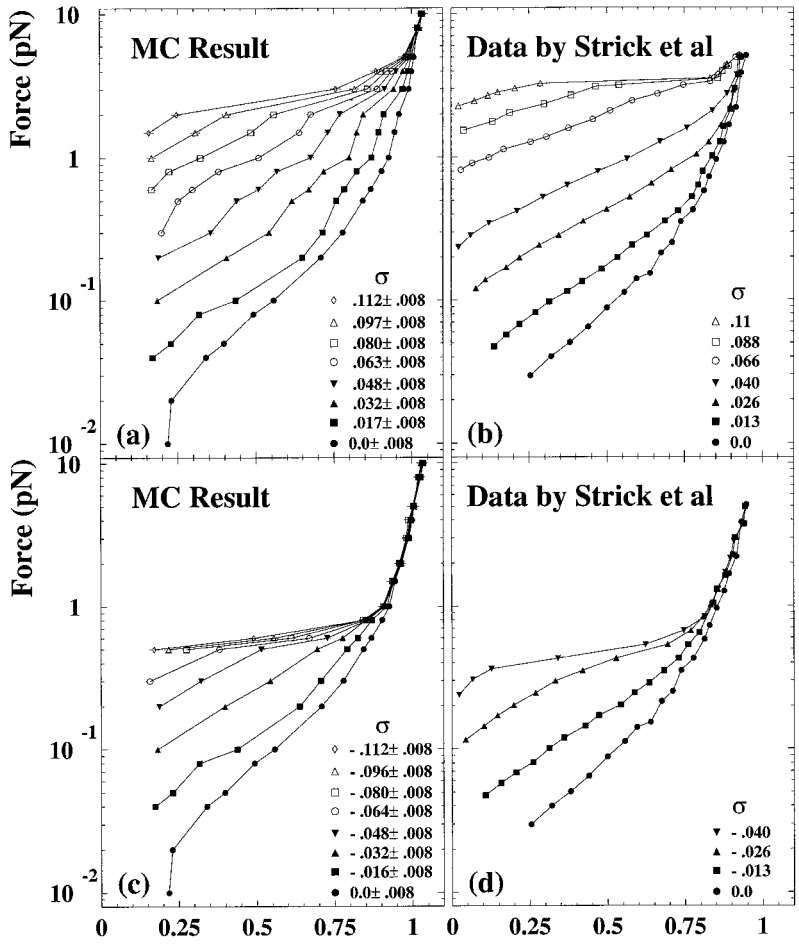


FIGURE 5 Force versus relative extension curves for negatively (*a, b*) and positively (*c, d*) supercoiling DNA molecule. (*a*) and (*c*) are the results of our Monte Carlo simulation, and the horizontal bars of points denote the statistic error of relative extension in our simulations. (*b*) and (*d*) are the experimental data (Strick et al., 1998). The solid curves serve as guides for the eye.

In conclusion, the elasticity of supercoiled double-stranded DNA is investigated by Monte Carlo simulations. Instead of introducing an extra twist energy term, twist and supercoiling are led into as a natural result of cooperative interplay between base-stacking interaction and sugar-phosphate backbones bending constrained by permanent hydrogen bonds. Without any adjustable parameter, the theoretic results on the correlations among DNA extension, supercoiling degree, and applied force agree qualitatively with recent experimental data by Strick et al (1996, 1998).

It should be mentioned that there is an upper limit of supercoiling degree for extended DNA in the current model, i.e., $\sigma_{max} \sim 0.14$, which corresponds to $\theta = 90°$ of folding angle. In recent experiments, Allemand et al. (1998) twisted the plasmid up to the range of $-5 < \sigma < 3$. They found that, at this "unrealistically high" supercoiling, the curves of force versus extension for different $\sigma$ split again at higher stretch force ($>3$ pN). As argued by Allemand et al. (1998), in the extremely under- and overwound torsion stress, two new DNA forms, denatured-DNA and P-DNA with exposed bases, will appear. In fact, if the deviation of the angle that specifies DNA twist from its equilibrium value exceeds some threshold, the corresponding torsional stress causes local distraction of the regular double-helix structure (Vologodskii and Cozzarelli, 1994). So, the emergence of these two striking forms is essentially associated with the broken processes of some basepairs under super-highly torsional stress. In this case, the permanent hydrogen constraint will be violated, and the configuration of base-stacking interactions vary considerably. We hope, with incorporation of these effects at high supercoiling degree, our model should reproduce the novel elastic behavior of DNA. This part of work is in progress.

## APPENDIX: KUHN STATISTICAL LENGTH OF DISCRETE CHAIN

Let us consider a discrete chain of $N$ segments with each of length $l_0$, the end-to-end vector of which is written as

$$\mathbf{R} \equiv l_0 \sum_{i=1}^{N} \mathbf{t}_i, \tag{A1}$$

where $\mathbf{t}_i = (\mathbf{R}_i - \mathbf{R}_{i-1})/|\mathbf{R}_i - \mathbf{R}_{i-1}|$.

For chains with bending stiffness, e.g., the DNA model described in Eq. 6, $\langle \mathbf{t}_{i+k} \cdot \mathbf{t}_i \rangle$ does not vanish for $k \neq 0$. $\mathbf{t}_{i+k}$ can be expressed relative to ($i$ +

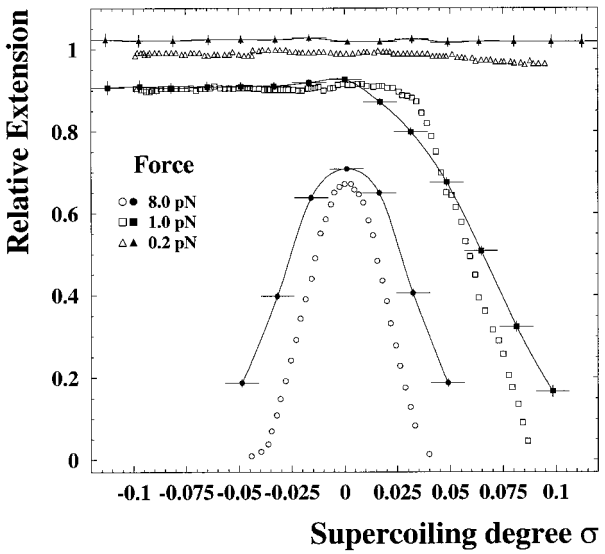


FIGURE 6 Relative extension versus supercoiling degree of DNA polymer for three typical stretch forces. Open symbols denote the experimental data (Strick et al., 1998) and solid symbols the results of our Monte Carlo simulation. The vertical bars of the solid symbols signify the statistic error of the simulations, and the horizontal ones denote the bin width that we partition the phase space of supercoiling degree. The solid lines connect the solid points to guide the eye.

$k-1$)th segment as

$$\mathbf{t}_{i+k} = \cos\gamma_{i+k-1}\mathbf{t}_{i+k-1} + \sin\gamma_{i+k-1}\mathbf{n}_{i+k-1}, \qquad (A2)$$

where $\gamma_{i+k-1}$ is the bending angle between (i + k − 1)th and ($i + k$)th segments as defined in Eq. 6, and $\mathbf{n}_{i+k-1}$ is the unit vector coplanar with $\mathbf{t}_{i+k}$ and $\mathbf{t}_{i+k-1}$ but perpendicular to the latter. If the average of $\mathbf{t}_{i+k}$ is taken with the rest of the chain (i.e., $\mathbf{t}_i$, $\mathbf{t}_{i+1}, \cdots, \mathbf{t}_{i+k-1}$) fixed, one obtains

$$\langle \mathbf{t}_{i+k}\rangle_{t_i,t_{i+1},\ldots,t_{i+k-1}\text{fixed}} = \langle\cos\gamma_{i+k-1}\rangle\mathbf{t}_{i+k-1}, \qquad (A3)$$

because $\langle \mathbf{n}_{i+k-1}\rangle_{t_i,t_{i+1},\ldots,t_{i+k-1}\text{fixed}} = 0$ according to Eq. 6. Multiplying both sides of Eq. A3 by $\mathbf{t}_i$ and taking the average over $\mathbf{t}_i$, $\mathbf{t}_{i+1}, \cdots, \mathbf{t}_{i+k-1}$, one has

$$\langle \mathbf{t}_{i+k}\cdot\mathbf{t}_i\rangle = \langle\cos\gamma\rangle\langle\mathbf{t}_{i+k-1}\cdot\mathbf{t}_i\rangle, \qquad (A4)$$

where $\langle\cos\gamma\rangle$ is not specific to segments and is given by Eq. 8. This recursion equation, with the initial condition $\mathbf{t}^2 = 1$, is solved by

$$\langle \mathbf{t}_{i+k}\cdot\mathbf{t}_i\rangle = \langle\cos\gamma\rangle^k. \qquad (A5)$$

Thus, for large $N$, $\langle\mathbf{R}^2\rangle$ is given by

$$\langle\mathbf{R}^2\rangle = l_0^2\sum_{i=1}^{N}\sum_{j=1}^{N}\langle\mathbf{t}_i\cdot\mathbf{t}_j\rangle$$

$$= l_0^2\left(N + 2\sum_{i+1}^{N-1}\sum_{k=1}^{N-i}\langle\mathbf{t}_i\cdot\mathbf{t}_{i+k}\rangle\right)$$

$$\simeq Nl_0^2\frac{1+\langle\cos\gamma\rangle}{1-\langle\cos\gamma\rangle}.$$

Therefore, the Kuhn statistical length of the discrete chain can be written as

$$b \equiv \frac{\langle\mathbf{R}^2\rangle}{R_{max}} = l_0\frac{1+\langle\cos\gamma\rangle}{1-\langle\cos\gamma\rangle}, \qquad (A6)$$

where $R_{max}$ is the maximum length of the end-to-end vector.

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