

Conformational response of supercoiled DNA to confinement in a nanochannel

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Monte Carlo simulations were done to study the conformation of supercoiled DNA confined in a nanochannel. The molecule has a superhelical density of around -0.05 and is bathed in a monovalent salt solution with an ionic strength of 2, 10, or 150 mM. The cross-sectional diameter of the circular shaped nanochannel was varied in the range of 10 to 80 nm. The conformational properties were characterized by the writhing number and the distribution in the distance between the two opposing strands of the superhelix. With increasing confinement, as set by a smaller tube diameter and/or decreased screening of the Coulomb interaction, the supercoil becomes more tightly interwound and long-range structural features such as branching and the formation of hairpins are progressively suppressed. Analysis of the energetics shows a concurrent increase in electrostatic energy and energy of interaction of the supercoil with the wall, but the elastic twisting energy decreases. Confinement in a nanochannel or otherwise hence results in a decrease in the absolute value of the twist exerted on the duplex. The bending energy remains approximately constant, which means that there are no significant deflections from the wall. The simulation results are interpreted with theory based on the wormlike chain model, including the effects of the wall, charge, elasticity, and configurational entropy. It was found that the theory is reasonably successful in predicting the structural response to the confinement at the local level of the diameter and pitch of the supercoil.

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I. INTRODUCTION

Closed circular DNA usually exists in a supercoiled configuration, in which the duplex is wound around another part of the same molecule to form a higher order helix. Supercoiling is utilized in many biological functions, examples include replication and transcription, formation of protein complexes, and altered primary structures such as cruciforms.¹ The three-dimensional tertiary structure is determined by topological and geometrical properties such as degree of interwinding and number of interwound branches. The topological constraint sets the physical extent of the molecule and determines its excluded volume. Supercoiling has accordingly been reported to be a major compaction mode for DNA in a liquid crystal.²⁻⁴ In order to be accommodated in a congested state, such as in a nanochannel or in a strong nematic field, supercoiled DNA has to decrease its physical extent by a change in tertiary structure. It has been argued that segments of the duplex start to ripple with a wavelength (deflection length) less than the bending persistence length.⁵ The rippling is similar to the undulation of a linear wormlike polymer confined in a narrow nanochannel.⁶⁻⁸ The structure of pUC18 plasmid (2686 base pairs) dispersed in salt solutions has been measured with small angle neutron scattering.^{3,4} With increasing DNA concentration, prior and covering the transition to the liquid crystalline phase, the supercoil was seen to decrease its ex-

cluded volume by becoming more tightly interwound with a change in elastic twisting rather than bending energy.

Besides theoretical analysis based on statistical thermodynamics, information about the tertiary structure can be obtained from computer simulation. Monte Carlo computer simulations have shown the effects of ionic strength and/or protein binding on the structure of the supercoil.⁹⁻¹¹ Most of the previous work was concerned with the properties of single molecules and did not consider the effects of an external potential. An exception is the Monte Carlo simulation study by Fujimoto and Schurr,¹² which showed that the confinement of a supercoiled DNA molecule to a plane greatly changes its tertiary structure. We have employed a similar approach to explore how the restriction of the configurational degrees of freedom by a cylindrical potential, which mimics confinement in a nanochannel, alters certain structural properties of the supercoil. Our primary aim was to explore the extent to which supercoiled DNA becomes more tightly interwound and/or rippled when it is compacted in a congested state. For this purpose, we have carried out Monte Carlo computer simulations and interpreted the emerging structure and energetics with the wormlike chain model including the effects of the hard wall, charge, elasticity, and configurational entropy.

II. WORMLIKE CHAIN MODEL

The gist of the wormlike chain model is that the conformation of the supercoiled DNA molecule is a regular superhelix. On top of this structure, fluctuations are considered.

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Furthermore, the effects of the end loops of the supercoil are neglected. In its original form, the theory includes a superposition of three contributions to the molecular free energy.^{13–15} These three contributions are the elastic bending and twisting energies of the duplex, the electrostatic interaction energy of the two opposing strands of the superhelix, and an entropic term coming from the restriction in fluctuations in plectonemic radius and pitch due to the topological constraint. For a supercoil confined in a nanochannel, we also need to consider the interaction energy of the duplex with the wall.

The elastic energy is given by the sum of the bending and twisting contributions,

$$\frac{F_{\text{elas}}}{Lk_B T} = L_b \kappa^2 / 2 + L_t \Omega^2 / 2, \quad (1)$$

where the persistence lengths associated with bending and twisting of the duplex are denoted by L_b and L_t , respectively. Note that we express all (free) energies per unit contour length of the duplex L and thermal energy $k_B T$. The bending and twisting persistence lengths are the corresponding elastic rigidity constants in units $k_B T$. With the plectonemic radius r_{sc} and pitch p_{sc} , the local curvature $\kappa = r_{\text{sc}} / (r_{\text{sc}}^2 + p_{\text{sc}}^2)$. The twist Ω exerted on the duplex and the writhe Wr are related to the linking number deficit ΔLk according to

$$\Omega = 2\pi(\Delta Lk - \text{Wr})/L. \quad (2)$$

Wr is the number of signed crossings of the duplex averaged over all possible views and characterizes the tertiary path.¹⁶ In the case of a right-handed plectoneme without end loops, the writhing number is related to the plectonemic radius and pitch according to¹⁷

$$\text{Wr} = -\frac{L p_{\text{sc}}}{2\pi(p_{\text{sc}}^2 + r_{\text{sc}}^2)}. \quad (3)$$

For a charged chain, the bending persistence length is modified by the electrostatic interaction. In the Odijk–Skolnick–Fixman model, it is given by the sum of the bare and electrostatic parts,^{18,19}

$$L_b = L_b^{\circ} + L_b^e. \quad (4)$$

The electrostatic persistence length has been derived in the Debye–Hückel approximation by considering the electrostatic energy cost of bending a wormlike chain and takes the following form:

$$L_b^e = v_{\text{eff}}^2 l_B / (4\lambda_D^2). \quad (5)$$

For the electrostatic interaction energy of the two opposing strands of the superhelix, we use the approximate expression proposed by Ubbink and Odijk,¹⁴

$$\frac{F_{\text{elec}}}{Lk_B T} = \frac{1}{2} v_{\text{eff}}^2 l_B \left(\frac{\pi}{\lambda_D r_{\text{sc}}} \right)^{1/2} \exp(2\lambda_D^2 d_r^2 - 2\lambda_D r_{\text{sc}}) \times (1 + 0.207/\mu + 0.054/\mu^2), \quad \mu = p_{\text{sc}}^2 / 4r_{\text{sc}}^2. \quad (6)$$

Note that the electrostatic interaction energy is renormalized for fluctuation in r_{sc} with amplitude d_r . The third contribution to the molecular free energy is of entropic origin. Besides radial displacements, a given point on the supercoil

exhibits longitudinal displacements of the order of πp_{sc} . For a superposition of these two fluctuation modes, the free energy reads,^{13,14,20}

$$\frac{F_{\text{conf}}}{Lk_B T} = \frac{c_r}{L_b^{1/3} d_r^{2/3}} + \frac{c_p}{L_b^{1/3} (\pi p_{\text{sc}})^{2/3}}. \quad (7)$$

In the case of a supercoil confined in a nanochannel, we need to consider the energy of interaction of the duplex with the wall. We use a hard wall potential with interaction energy,

$$\frac{F_{\text{cyl}}}{Lk_B T} = k_{\text{cyl}} r_{\text{sc}}^{10} (1 + 45d_r^2/r_{\text{sc}}^2), \quad (8)$$

and k_{cyl} being a constant to be discussed below. Note that the interaction energy with the wall has been renormalized for fluctuation in the radius of the supercoil up to and including second order with a second moment d_r . The central axis of the superhelix always coincides with the symmetry axis of the potential and in the wormlike chain model we do not consider lateral displacements of the whole molecule. Furthermore, there is no confinement in the longitudinal direction along the tube of infinite length.

The sum of the molecular free energy and the interaction energy with the wall,

$$F_{\text{tot}} = F_{\text{elas}} + F_{\text{elec}} + F_{\text{conf}} + F_{\text{cyl}} \quad (9)$$

is then minimized with respect to the plectonemic radius r_{sc} , the fluctuation in radius d_r , and the plectonemic pitch p_{sc} . This was done by numerical means with a multidimensional, nonlinear Nelder Mead search algorithm in MATLAB (Natick, MA). The resulting values of the structural parameters, including Wr following from Eq. (3), and the various energy contributions will be compared to the corresponding results obtained from Monte Carlo computer simulation. For a free unconstrained supercoil without hard wall potential, the predictions of the wormlike chain model have been reported to be in excellent agreement with experimental data for the plectonemic radius and pitch as a function of the superhelical density as well as ionic strength of the supporting medium.^{14,15}

III. SIMULATION PROTOCOL

In the Monte Carlo simulation, the DNA molecule was modeled as a closed circular polygonal space curve consisting of 150 vertices.²¹ The bonds connecting the vertices are elastic with a stretching constant $k_s = 17 k_B T$, which results in an average bond length $\langle l \rangle = 3.1$ nm and a contour length of 465 nm (1.3 kbp). The bonds cannot bend nor twist. The twisting and bending flexibility is concentrated at the connection points, i.e., the vertices. The linking number deficit $\Delta Lk = -6$, which corresponds with a superhelical density $\sigma = -0.044$ for DNA in the B-form with a helical pitch of 3.4 nm. With the commonly used bare bending persistence length $L_b^{\circ} = 50$ nm, the corresponding elasticity constant $k_b = L_b^{\circ} / \langle l \rangle = 16 k_B T$. The elasticity constant for twisting of the duplex is not precisely known. For weakly strained circular DNA, the experimental values of L_t , as obtained from fluorescence depolarization anisotropy measurements, are around

50 nm (see Ref. 22 and references therein). Single molecule manipulation experiments of linear DNA have given larger values for L_t in the range of 75–110 nm. Our simulations were done with $L_t=75$ nm ($k_t=1.5 k_b$), a value that is often used in computational and theoretical work. We have checked, however, that simulations with $k_t=k_b$ gave qualitatively the same results. The vertices interact through the sum of a hard sphere (diameter $\sigma_D=2.4$ nm) and an isotropic screened Coulomb potential,

$$V(r) = \left(\frac{\sigma_D}{r} \right)^{12} + l_B \alpha^2 \frac{\exp(-\lambda_D r)}{r}. \quad (10)$$

Note that this potential includes the short-range electrostatic repulsion and there is no need to modify the bending persistence length. The strength and range of the electrostatic interaction are determined by the effective number of charges per vertex $\alpha = \nu_{\text{eff}} \langle l \rangle$ and the Debye screening length λ_D , respectively. We obtained $\alpha=6.0, 8.4,$ and 30.5 by numerically solving the nonlinear Poisson–Boltzmann equation for a rod-like polyelectrolyte of 2.4 nm diameter and dispersed in 2, 10, and 150 mM of a monovalent salt with screening lengths $\lambda_D=6.8, 3.0,$ and 0.8 nm, respectively.¹⁵

In order to simulate the effect of confinement, we have applied a cylindrical external potential,

$$V_{\text{cyl}} = \lambda k_{\text{cyl}} \sigma_D r_{\text{cyl}}^{10}. \quad (11)$$

This potential is a good approximation of hard wall repulsion with minimal computational difficulties. The value of k_{cyl} is determined by the tube diameter D_{tube} and was chosen so that the energy of a contact per vertex with the wall equals a unit $k_B T$. Our computer code for the model Hamiltonian, including the interaction with the wall, was programmed using FORTRAN 90 and it was executed on a cluster of 1.5 GHz Itanium 2 processors at Singapore's National Grid Computation Platform. For each ionic strength, we started with the simulation of the free supercoil with $\lambda=0$. The initial conformation was a regular ring with zero Wr . Subsequently, a series of simulations was done with progressively smaller values of D_{tube} ranging from 80 to 10 nm. For each tube diameter, the parameter λ was incremented from 1×10^{-5} to 1 in ten logarithmically spaced steps. This procedure mimics the transfer of the DNA molecule from solution into the tube.¹² In the presence of the confining potential, the initial conformation was always taken to be the equilibrated conformation pertaining to the previous run. We have applied small random displacements on each vertex as well as displacements that transfer the whole molecule in the transverse direction of the confining potential. On both types of moves, an acceptance ratio of 50% was imposed. The simulation was considered to have reached equilibrium when the radii of gyration R_g of the DNA molecule pertaining to two consecutive runs with a total of at least 2×10^6 cycles agreed within 95% confidence level. For a free supercoil in 10 mM monovalent salt, the convergence of Wr and R_g is illustrated in Fig. 1. For the other ionic strengths, similar convergence is observed. The writhing number Wr was obtained by the evaluation of the Gauss double integral.²³ A typical run for one tube diameter and one ionic strength took about 9 days.

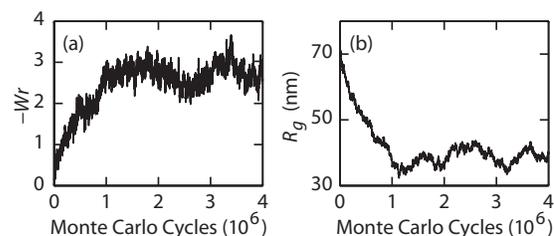


FIG. 1. (a) Writhe vs the number of Monte Carlo cycles for a free supercoil immersed in 10 mM monovalent salt. (b) As in panel (a), but for the radius of gyration. Convergence is achieved after about 1×10^6 cycles.

One should bear in mind that there are important differences between the models employed in the theory and simulation. In the wormlike chain model, the conformation is assumed to be a superhelix and end loop effects are neglected. The model employed in the simulation is more realistic; there is no imposed helical structure (the interwound structure follows naturally), end loops are included, and the supercoil can branch and form hairpins. Furthermore, the simulation includes moves of the whole molecule in the transverse direction of the confining potential, which are not considered in the wormlike chain model. Despite these differences, we thought that it is of interest to explore the extent to which the wormlike chain model is capable to predict the simulation results because it might provide an efficient and fast way to evaluate the effects of a confining potential on the structure of the supercoil.

IV. RESULTS AND DISCUSSION

Typical snapshots of well-equilibrated structures are displayed in Fig. 2. In all cases, the DNA molecule takes a locally interwound conformation. At lower ionic strength and/or smaller tube diameters, the supercoil is linearly packed inside the tube. At higher ionic strength and/or wider tubes, the structure is less regular in the sense that the supercoil might branch or form a hairpin. In a relatively narrow tube, the formation of branch points or hairpins requires sharp bending of one or two DNA duplexes, respectively, and carries a relatively large bending energy on the order of a few times $k_B T$. Confinement in the tube also suppresses branching and hairpin formation for entropic reasons because of the restriction in the degrees of freedom pertaining to the configuration of the DNA molecule. Here, we will not pursue the effects of confinement on the long-range structure, but focus on the local plectonemic structure in terms of the writhe Wr and diameter of the supercoil $D_{\text{sc}}=2r_{\text{sc}}$.

We obtained the writhing number Wr by the evaluation of the Gauss double integral.²³ For the interwound section of the supercoil, Wr has also been obtained with the wormlike chain model by numerical minimization of the total energy (Eq. (9)) and the results are displayed in Fig. 3. Overall, there is excellent agreement between the simulation and theoretical results. The minor deviations observed at high ionic strength for the larger tube diameters are related to branching and hairpin formation, which are not captured by the wormlike chain model. With increasing D_{tube} , Wr first increases after which it levels off at a constant value determined by the ionic strength (note that $-Wr$ decreases with increasing

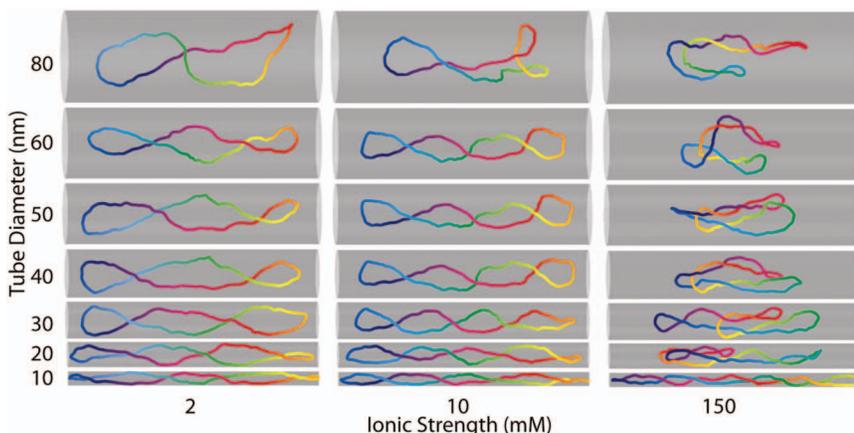


FIG. 2. (Color) Snapshots of equilibrated conformations taken after 2×10^6 Monte Carlo cycles.

D_{tube}). We have checked that the limiting values of Wr for large tube diameters agree with the values for the free DNA molecule without confining potential. The range of the transition also depends on the salt concentration, in the sense that Wr takes a constant value at smaller tube diameters with increasing ionic strength. As we will see shortly, the transition from a constant to a decreasing value of Wr is caused by progressive squeezing of the supercoil once the tube diameter becomes less than the diameter of the free supercoil. Notice that the limiting values of Wr for small tube diameters do not become smaller than the linking number deficit $\Delta Lk = -6$. As shown by the decrease in Wr , the supercoil becomes more tightly interwound with increasing ionic strength and/or increasing confinement by a smaller tube diameter.

To illustrate the squeezing of the supercoil by the confining potential, we have obtained the distance distribution function of the vertices. The results for the lowest employed ionic strength of 2 mM are shown in Fig. 4. In the case of an ionic strength of 10 and 150 mM similar results are obtained (not shown). In the calculation of the distribution function, we have excluded the ten nearest neighbors at each side of the vertex of reference. In this way, correlations over a length scale on the order of the bending persistence length are excluded. The position of the maximum then gives the most probable diameter of the supercoil D_{sc} .²⁴ The results for D_{sc} are shown in Fig. 5, together with the predictions of the wormlike chain model as obtained from the minimization of

the total energy (Eq. (9)). Again, there is good agreement, but the simulation results for an ionic strength of 150 mM fall a bit below the mark set by the theory. Notice that the differences between the theoretical and simulation results are much smaller than the widths of the distribution function. Furthermore, the widths agree with the fluctuation bandwidth d_r following from the theory (results not shown).

With increasing tube diameter, the intervertex distribution function broadens. Concurrently, the position of the maximum D_{sc} first increases, after which it levels off at a constant value depending on the ionic strength. For the highest employed ionic strength, the small increase in D_{sc} , as predicted by the theory, is not clearly seen in the simulation results due to limited statistical accuracy. As in the case of Wr , we have checked that the limiting values of D_{sc} for large tube diameters agree with those of the free DNA molecule. The confining potential has no significant effect on D_{sc} as long as D_{tube} is much larger than the diameter of the free molecule. For smaller tube diameters, the supercoil is squeezed as shown by the decrease in D_{sc} with decreasing values of D_{tube} . The transition from a constant to a decreasing value of D_{sc} occurs in the same range of tube diameters as for the transition in the writhe. Notice that D_{sc} already starts to decrease when the value of D_{tube} is about twice the value of the plectonemic diameter pertaining to the free su-

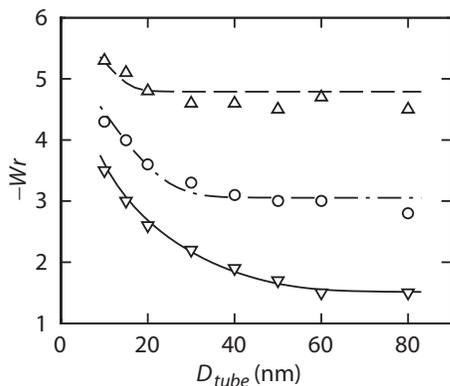


FIG. 3. Writhe vs tube diameter. The symbols and curves are simulation and theoretical results, respectively. The ionic strengths are 150 (dashed, Δ), 10 (dashed dotted, \circ), and 2 (solid, ∇) mM.

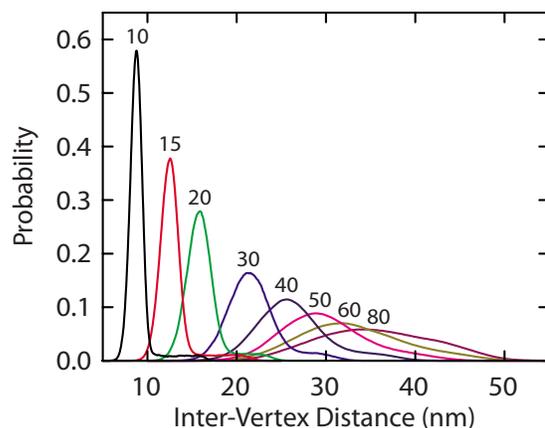


FIG. 4. (Color online) Intervertex distribution function. The tube diameters pertaining to the different curves are indicated in the figure (in units of nanometer). Correlations over a distance scale less than the bending persistence length (20 vertices) are excluded.

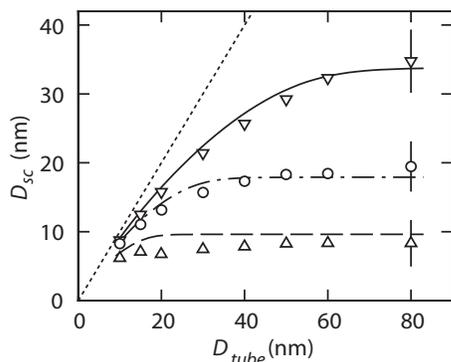


FIG. 5. Diameter of the supercoil vs tube diameter. The symbols and curves are simulation and theoretical results, respectively. The ionic strengths are 150 (dashed, Δ), 10 (dashed dotted, \circ), and 2 (solid, ∇) mM. The bars denote the standard deviation of the distance distribution function. The dotted line demarcates $D_{sc}=D_{tube}$.

percoil. This is due to the cutoff of the spectrum of fluctuation in the radial direction imposed by the confining potential.

It is interesting to compare our results with directly measured experimental data. Hammermann *et al.*²⁴ investigated the diameter of pUC18 plasmid with a superhelical density $\sigma=-0.05$ and dispersed in monovalent salt solutions with small angle neutron scattering. Our limiting values of D_{sc} for large tube diameters and, in particular, the dependence on the ionic strength, are in excellent agreement. For the same plasmid, but with $\sigma=-0.03$, and by using the same methodology, the dependencies of D_{sc} and Wr on the density of DNA have been reported by Zakharova *et al.*³ In 50 mM NaCl, the average value of D_{sc} was seen to decrease from 20 to 10 nm once the spacing between the molecules is decreased from 39 to 13 nm. This decrease in D_{sc} with increasing DNA density is in fair agreement with the results shown in Fig. 5. Zakharova *et al.* also reported a decrease in Wr with increasing DNA concentration. This observation is in qualitative agreement with the present results because both the simulation and theory show that the supercoil becomes more tightly interwound with increasing confinement. With increasing DNA density, the experimental values of Wr become however smaller than the linking number deficit ΔLk . Such behavior is not observed in our simulations, neither predicted by the wormlike chain model. This discrepancy is probably related to complications in the analysis of the scattering data arising from intermolecular interference. More experimental work in order to elucidate the conformation of supercoiled DNA in a crowded and congested state is in progress.

The energetics of the confinement, as obtained from the simulation as well as the wormlike chain model, is shown in Fig. 6. The stretching energy of the vertices is not shown because it was found constant, irrespective of the value of the tube diameter. All energies are per unit contour length and represent relative changes from the energies pertaining to the free supercoil. The theoretical elastic bending and twisting energy contributions are obtained from the corresponding terms in Eq. (1) and the values of the structural parameters following from the minimization of the total energy (Eq. (9)). In a similar way, the electrostatic energy and the energy of interaction with the wall are obtained from

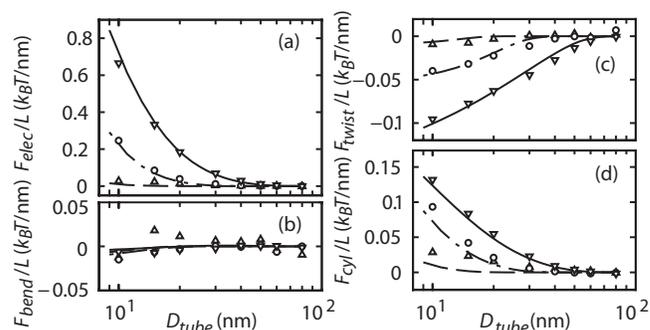


FIG. 6. (a) Electrostatic energy per unit contour length vs tube diameter. (b) As in (a), but for the bending energy. (c) As in (a), but for the twisting energy. (d) As in (a), but for the interaction energy with the wall. The symbols and curves are simulation and theoretical results, respectively. Note that the energies represent changes from the respective values without the confining tube. The ionic strengths are 150 (dashed, Δ), 10 (dashed dotted, \circ), and 2 (solid, ∇) mM.

Eqs. (6) and (8), respectively. The relative changes and their dependencies on the ionic strength are overall well predicted by the wormlike chain model. At high ionic strength, deviations in the bending energy and the interaction energy with the wall are observed, but again, this is related to branching and hairpin formation. With increasing confinement, the electrostatic energy increases due to the reduced interduplex distance. With increased ionic strength, the increase in electrostatic energy becomes less pronounced, in accordance with increased screening of Coulomb interaction. The elastic bending energy is almost constant, which shows that sections of the DNA molecule do not start to ripple with increasing confinement. The elastic twisting energy decreases with decreasing tube diameter because according to White's equation $\Delta Lk=Wr+\Delta Tw$ the sum of the writhe Wr and total excess twist $\Delta Tw=L\Omega/2\pi$ exerted on the duplex is conserved.¹⁶ With increasing confinement, more segments of the duplex are in close contact with the wall and the corresponding interaction energy increases. The changes in elastic twisting and wall interaction energies also depend on the ionic strength and become vanishingly small at high salt concentration.

V. CONCLUSIONS

We have analyzed the structure and energetics of a supercoiled DNA molecule confined in a cylindrical potential, both with theory based on statistical thermodynamics as well as Monte Carlo computer simulation. The value of the superhelical density around -0.05 is typical for plasmids in the cytoplasm of bacteria. Simulations were done for a molecule of 1.3 kbp, which is large enough to exhibit long-range structural phenomena, and yet, small enough to achieve significant results within a reasonable time span. With increasing confinement, as set by a smaller tube diameter, the supercoil becomes more tightly interwound and long-range structural features such as branching and the formation of hairpins are progressively suppressed. The electrostatic energy and the energy of interaction of the supercoil with the wall increase, but the elastic twisting energy decreases. Confinement in a nanochannel or otherwise, such as in a strong nematic field, hence results in a decrease in the absolute value of the twist

exerted on the duplex, which might have biological implications such as for the transcription, replication, and repair of the genome. The bending energy remains constant, which indicates that the duplex does not ripple. In agreement with earlier work, the supercoil also becomes more tightly interwound with increasing salt concentration.^{9,10,24} Despite that the wormlike chain model has some obvious deficiencies, it is reasonably successful in predicting the structural response to the confinement at the local level of the plectonemic diameter and pitch. The wormlike chain model can hence be confidently used for the analysis of experimental results involving the compaction of supercoiled DNA in a crowded and congested state such as in a liquid crystal or a gene delivery system.^{2-4,25} Lithographic methods to produce nanochannels in cheap polymer based biochips are progressively becoming available.^{26,27} In our laboratories, the cross-sectional diameter of such nanochannels is now being pushed below 80 nm and the study of topologically constrained supercoiled DNA confined inside these nanochannels is in progress.

It is interesting to compare the conformational responses of an unconstrained linear wormlike polymer and a topologically constrained supercoiled polymer in a cylindrical nanochannel. If the diameter of the tube is smaller than the persistence length (for DNA hence less than 50 nm), the linear polymer will undulate inside the channel and will only bend when it bounces off the wall. The deflection length scales as $\lambda_{\text{def}} \sim D_{\text{tube}}^{2/3} L_b^{1/3}$ and becomes progressively shorter than the bending persistence length with smaller tube diameter.⁶ For the supercoiled molecule, sections of the duplex are expected to undulate if the deflection length is shorter than the superhelical pitch, i.e., for $\lambda_{\text{def}} < p_{\text{sc}}$.⁵ However, in the present contribution we have seen that with increasing confinement the supercoil becomes more tightly interwound with a concurrent decrease in superhelical diameter and pitch. With a physiological value of the superhelical density around -0.05 , the duplex does not ripple and remains interwound because the pitch is shorter than and stays put with the deflection length.

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