Bending and Base-Stacking Interactions in Double-Stranded DNA

Zhou Haijun,[1,2,*] Zhang Yang,[1] and Ou-Yang Zhong-can[1,3]
[1]*Institute of Theoretical Physics, Academia Sinica, P.O. Box 2735, Beijing 100080, China*
[2]*State Key Laboratory of Scientific and Engineering Computing, Beijing 100080, China*
[3]*Center for Advanced Study, Tsinghua University, Beijing 100084, China*
(Received 13 October 1998)

An elastic model for double-stranded polymers is constructed to study the recently observed DNA entropic elasticity, cooperative extensibility, and supercoiling property. With the introduction of a new structural parameter (the folding angle φ), bending deformations of sugar-phosphate backbones, steric effects of nucleotide base pairs, and base-stacking interactions are considered. The comprehensive agreements between theory and experiments both on torsionally relaxed DNA and on negatively supercoiled DNA strongly indicate that base-stacking interactions, although short-ranged in nature, dominate the elasticity of DNA and, hence, are of vital biological significance. [S0031-9007(99)09139-5]

PACS numbers: 87.15.By, 36.20.Ey, 61.25.Hq, 87.10.+e

Recent *in vitro* experiments done on single double-stranded DNA (dsDNA) molecules or DNA-protein complexes reveal that DNA double helix has nontrivial elastic properties [1–7]. At low external forces, it can be viewed as a simple wormlike chain [8]; at moderate forces, it becomes a rod with a large stretch modulus. But, if pulled with a large force of about 70 pN, the molecule as a whole can suddenly be driven to an almost fully stretched state with a contour length 1.7 times its native value [3,4]. More strikingly, if a dsDNA at the same time has a slight deficit in linking number, i.e., negatively supercoiled, a pulling force as small as 0.3 pN can distort the native structure of DNA considerably [5,6,9].

On the theoretical side, to understand these novel properties of DNA is of current interest, and many models are suggested and some valuable insights attained [8,10–20]. For example, to interpret the extensibility of DNA, some authors suggest one-dimensional two-state models with [3,12,13,19] or without [14] nearest-neighbor interactions; and, to explain the supercoiling property of DNA, wormlike rod chain models, with [12,16] or without [10,11,15] bend-twist and/or stretch-twist coupling, are investigated. Nevertheless, a unified description still seems to be lacking, and the underlying mechanism which should account for DNA cooperative extensibility and novel supercoiling properties is still elusive. Here, we show that it is possible to understand all of these experimental observations from a unified viewpoint [21].

A simple elastic model is proposed by taking into account the structural properties of realistic dsDNA. Bending energy of the sugar-phosphate backbones, base-stacking interaction between adjacent nucleotide base pairs, as well as their steric effects on DNA axial bending rigidity are considered. We have introduced a new structural parameter, the folding angle φ. Model calculations on the elastic properties of torsionally relaxed and negatively supercoiled DNAs are in quantitative agreement with all of the known experimental observations [1,3–6]. The model indicates that base-stacking interaction is the main factor determining the high extensibility and unwinding instability of DNA. We suggest that the present model, after some revisions, will also be able to account for the elasticity of positively supercoiled DNAs [5,7].

In the model, the two inextensible backbones of DNA [22] are characterized by the same bending rigidity $\kappa = k_B T \ell_p$, where $\ell_p \simeq 1.5$ nm is their bending persistence length (BPL). Their position vectors are $\mathbf{r}_i = \int^s \mathbf{t}_i(s')\, ds'$, where $\mathbf{t}_i$ $(i = 1, 2)$ is the unit tangential vector of the ith backbone, and s is its arclength. The nucleotide base pairs between the backbones [22] are viewed as rigid planar structures with finite area and volume. First, we consider bending energy of the backbones alone, and each base pair connecting the two backbones is replaced for the moment by a thin rigid rod of length $2R$, with a unit vector $\mathbf{b}$ pointing along it from $\mathbf{r}_1$ to $\mathbf{r}_2$, i.e., $\mathbf{r}_2(s) - \mathbf{r}_1(s) = 2R\mathbf{b}(s)$. Relative sliding of the backbones is prohibited; the base pair planes are assumed to lie perpendicular to the DNA central axis and $\mathbf{b} \cdot \mathbf{t}_1 = \mathbf{b} \cdot \mathbf{t}_2 \equiv 0$ [23]. The central axis of dsDNA can be defined as $\mathbf{r}(s) = \mathbf{r}_1(s) + R\mathbf{b}(s)$, and its tangential vector is denoted by $\mathbf{t}$, with $\mathbf{t} \cdot \mathbf{b} = 0$. Since both $\mathbf{t}_1$ and $\mathbf{t}_2$ lie on the same plane perpendicular to $\mathbf{b}$, we obtain that $\mathbf{t}_1 = \mathbf{t}\cos\varphi + \mathbf{n}\sin\varphi$ and $\mathbf{t}_2 = \mathbf{t}\cos\varphi - \mathbf{n}\sin\varphi$, where $\mathbf{n} = \mathbf{b} \times \mathbf{t}$ and φ is half the rotational angle from $\mathbf{t}_2$ to $\mathbf{t}_1$ ($\mathbf{b}$ being the rotational axis). We call φ the folding angle, it is in the range between $-\pi/2$ and $+\pi/2$ ($\varphi > 0$ for right-handed rotations and <0 for left-handed ones). It is not difficult to verify that

$$d\mathbf{b}/ds = (\mathbf{t}_2 - \mathbf{t}_1)/2R = -\mathbf{n}\sin\varphi/R\,, \qquad (1)$$

(here and after, ds always denotes the arclength element of the *backbones*). With Eq. (1) and the definition of $\mathbf{r}$, we know that

$$d\mathbf{r}/ds = (\mathbf{t}_1 + \mathbf{t}_2)/2 = \mathbf{t}\cos\varphi\,. \qquad (2)$$

Then total bending energy of the backbones, $E_b = \int(\kappa/2)[(d\mathbf{t}_1/ds)^2 + (d\mathbf{t}_2/ds)^2]\, ds$ [23], can be rewritten,

 0031-9007/99/82(22)/4560(4)$15.00

with the help of Eqs. (1) and (2), as

$$E_b = \int_0^L \left[\kappa \left(\frac{d\mathbf{t}}{ds} \right)^2 + \kappa \left(\frac{d\varphi}{ds} \right)^2 + \frac{\kappa}{R^2} \sin^4 \varphi \right] ds \,. \tag{3}$$

Here, L is the total contour length of each backbone. This expression proves to be very useful. The second and the third terms in Eq. (3) are deformation energy caused by folding of the backbones with respect to the central axis, and the first term, $\kappa(d\mathbf{t}/ds)^2$, is the bending energy of the DNA central axis contributed by the backbone bending rigidity κ. So far, base pairs are viewed as thin rods and their contribution to the bending rigidity of DNA chain is not considered. Because of steric effects caused by finite volume and area, base pairs will certainly increase the bending rigidity of the DNA chain [24]. The simplest way to consider such effects is to replace κ in the first term of Eq. (3) with a phenomenological parameter κ^*, with $\kappa^* > \kappa$. Hereafter this is assumed.

Besides steric effects, nucleotide base pairs contribute also base-stacking energy. This energy mainly originates from noncovalent van der Waals interactions between adjacent base pairs [22]. Base-Stacking interaction is short-ranged and is characterized by an attraction potential proportional to $1/r^6$ and a strong repulsion potential proportional to $1/r^{12}$ (here, r is the axial distance between adjacent base pairs). In our continuous model, the line density of such a Lennard–Jones-type potential can be written as

$$\rho(\varphi) = \begin{cases} \frac{\epsilon}{r_0} \left[\left(\frac{\cos\varphi_0}{\cos\varphi} \right)^{12} - 2 \left(\frac{\cos\varphi_0}{\cos\varphi} \right)^6 \right] & \text{for } (\varphi \geq 0) \,, \\ \frac{\epsilon}{r_0} \left[\cos^{12}\varphi_0 - 2\cos^6\varphi_0 \right] & \text{for } (\varphi < 0) \,, \end{cases} \tag{4}$$

and the total base-stacking energy is $E_{\mathrm{LJ}} = \int_0^L \rho \, ds$. In Eq. (4), r_0 is the backbone arclength between adjacent bases; φ_0 is a parameter related to the equilibrium distance between a DNA dimer; ϵ is the base-stacking intensity which is generally base-sequence specific. Here we focus on macroscopic properties of DNA and just consider ϵ in the average sense and take it as a constant, with $\epsilon \simeq 14.0 k_B T$ as indicated by quantum chemical calculations [22]. The asymmetric base-stacking potential [Eq. (4)] ensures a relaxed DNA to take on a right-handed double-helix configuration with its folding angle $\varphi \sim \varphi_0$. However, if adjacent base pairs are pulled apart slightly from the equilibrium distance by external forces or thermal stretching fluctuations, the base-stacking interaction intensity quickly decreases because of its short-range nature. In other words, the base-stacking potential can endure only a limited pulling force. We believe this to be closely related to the observed DNA highly cooperative extensibility. It may also account for the novel elasticity of negatively supercoiled dsDNA, since negative supercoiling actually leads to an effective pulling force. This insight, which is developed in more detail in the following, seems to be confirmed by experiments [25].

We first discuss the elastic response of the model DNA when a pulling force $\mathbf{F} = f\mathbf{z}_0$ along direction $\mathbf{z}_0$ is applied at its end. The total energy functional is then $E = E_b + E_{\mathrm{LJ}} - \int_0^L f \cos\varphi \mathbf{t} \cdot \mathbf{z}_0 \, ds$. And the Green function $G(\mathbf{t}, \varphi; \mathbf{t}', \varphi'; s)$ [8], which determines the probability distribution of $\mathbf{t}$ and φ along DNA chain, is governed by

$$\frac{\partial G}{\partial s} = \left[\frac{\partial^2}{4\ell_p^* \partial \mathbf{t}^2} + \frac{\partial^2}{4\ell_p \partial \varphi^2} - \frac{f}{k_B T} \cos\varphi \mathbf{t} \cdot \mathbf{z}_0 - V(\varphi) \right] G \,, \tag{5}$$

where $\ell_p^* = \kappa^*/k_B T$ and $V(\varphi) = \rho(\varphi)/k_B T + \ell_p \sin^4\varphi / R^2$. The spectrum of the above Green equation is discrete and, hence, for long chains, the average extension can be obtained either by differentiation of the ground-state eigenvalue, g, of Eq. (5) with respect to f:

$$\langle Z \rangle = \int_0^L \langle \cos\varphi \mathbf{t} \cdot \mathbf{z}_0 \rangle \, ds = L k_B T \partial g / \partial f \,, \tag{6}$$

or by a direct integration with the normalized ground-state eigenfunction, $\Phi(\mathbf{t}, \varphi)$, of Eq. (5):

$$\langle Z \rangle = L \int |\Phi|^2 \mathbf{t} \cdot \mathbf{z}_0 \cos\varphi \, d\mathbf{t} \, d\varphi \,. \tag{7}$$

Both g and $\Phi(\mathbf{t}, \varphi)$ can be obtained numerically through standard diagonalization methods and identical results are obtained by Eqs. (6) and (7). The resulting force vs extension relation in the whole relevant force range is shown in Figs. 1 and 2. Our theoretical curves are obtained with just

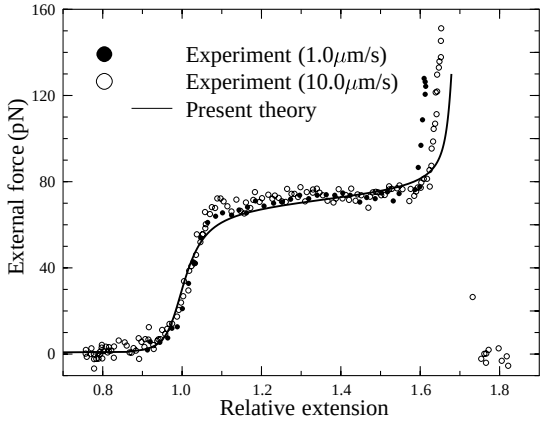

FIG. 1. Force-extension relation of DNA. Experimental data is from Fig. 2A of [3] (symbols). Theoretical curve is obtained by the following considerations: (i) $\ell_p = 1.5$ nm [4] and $\epsilon = 14.0 k_B T$ [22]; (ii) $\ell_p^* = 53.0/2\langle\cos\varphi\rangle_{f=0}$ nm [29], $r_0 = 0.34/\langle\cos\varphi\rangle_{f=0}$ nm and $R = (0.34 \times 10.5/2\pi)\langle\tan\varphi\rangle_{f=0}$ nm [30]; (iii) adjust the value of φ_0 to fit the data. For each φ_0, the value of $\langle\cos\varphi\rangle_{f=0}$ is obtained self-consistently. The present curve is drawn with $\varphi_0 = 62.0°$ (in close consistence with the structural property of DNA [22]), and $\langle\cos\varphi\rangle_{f=0}$ is determined to be 0.573840. DNA extension is scaled with its **B**-form contour length $L\langle\cos\varphi\rangle_{f=0}$.

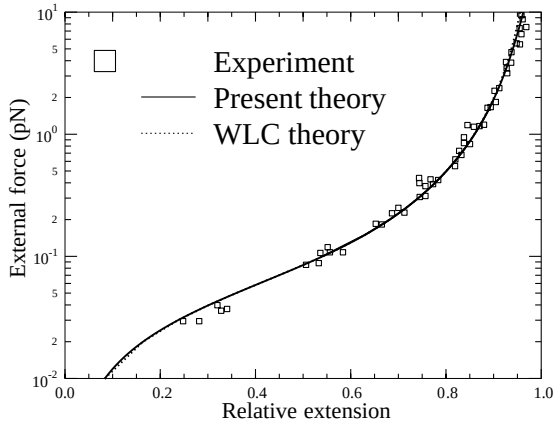

FIG. 2. Low-force elastic behavior of DNA. Here, experimental data are from Fig. 5B of [1], the dotted curve is obtained for a wormlike chain with BPL 53.0 nm, and the parameters for the solid curve are the same as those in Fig. 1.

one adjustable parameter (see caption of Fig. 1); the agreement with experiments is strikingly excellent. According to our theory, the onset of cooperative extension of DNA axial length at forces about 70 pN is mainly caused by the yielding of the short-range base-stacking interaction [26].

Below the onset of cooperative elongation, DNA seems to be very stiff and calculations show that at $f = 50$ pN the total extension of DNA is only 4.1% longer than its **B**-form contour length, in close accordance with the value of 4.6% reported by Smith *et al.* [4]. This is related to the fact that the base-stacking intensity ϵ is very strong [26]. At low forces ($f < 10$ pN), because the fluctuation of the folding angle φ is extremely small, it can just be neglected and DNA elasticity is caused by thermal fluctuations of the axial direction $\mathbf{t}$ (entropic elasticity). It is easy to prove that the now well-known entropic elasticity (wormlike chain) model [8] with contour length $L\langle\cos\varphi\rangle_{f=0}$ and persistence length $2\ell_p^*\langle\cos\varphi\rangle_{f=0}$ is just an excellent approximation of the present theory (here $\langle\cos\varphi\rangle_{f=0}$ is the average of $\cos\varphi$ at zero force). This point is demonstrated clearly in Fig. 2.

Now we continue to study the supercoiling property of the model DNA. Mathematically, a supercoiled dsDNA is characterized by its fixed value of linking number Lk. It measures the total topological turns one DNA backbone winds around the other or around the central axis, and can be expressed as the sum of the twisting number, $Tw(\mathbf{r}_1, \mathbf{r})$, of backbone $\mathbf{r}_1$ around the central axis $\mathbf{r}$ and the writhing number $Wr(\mathbf{r})$ of the central axis; i.e., $Lk = Tw + Wr$ [27,28]. According to Eq. (1), $Tw(\mathbf{r}_1, \mathbf{r}) = \int \mathbf{t} \times (-\mathbf{b}) \cdot d(-\mathbf{b}) = \int \sin\varphi \, ds/R$ [27,28]. For a linear DNA chain, the writhing number of its central axis can be expressed as [28]

$$Wr(\mathbf{r}) = \int \frac{\mathbf{z}_0 \times \mathbf{t} \cdot d(\mathbf{z}_0 + \mathbf{t})/ds}{1 + \mathbf{z}_0 \cdot \mathbf{t}} \, ds \,. \qquad (8)$$

The elasticity of such a supercoiled DNA chain is determined by the following energy functional: $E = E_b + E_{\mathrm{LJ}} - f \int \cos\varphi \mathbf{t} \cdot \mathbf{z}_0 \, ds - \Gamma k_B T L k$, where $\Gamma k_B T$ is torque associated with the topological constraint. However, the writhing number expression given by Eq. (8) is correct only for $\mathbf{t} \cdot \mathbf{z}_0 \neq -1$, i.e., for chains whose tangential vector $\mathbf{t}$ never points to $-\mathbf{z}_0$ [28]. This condition is satisfied actually only for a highly extended chain whose $\mathbf{t}$ fluctuates slightly around $\mathbf{z}_0$. In this case, Eq. (8) leads to $Wr(\mathbf{r}) \simeq (1/2) \int (t_x dt_y/ds - t_y dt_x/ds) \, ds$, where t_x and t_y are, respectively, the x and y component of $\mathbf{t}$. This approximation is used hereafter. If we are to use Eq. (8) in the general case, a cutoff procedure seems necessary to avoid divergent results [15].

The Green equation for this case is written as

$$\frac{\partial G}{\partial s} = \left[\frac{\partial^2}{4\ell_p^* \partial \mathbf{t}^2} + \frac{\partial^2}{4\ell_p \partial \varphi^2} + \frac{f \cos\varphi}{k_B T} \mathbf{t} \cdot \mathbf{z}_0 - V(\varphi) + \frac{\Gamma}{R} \sin\varphi + \frac{\Gamma^2}{16\ell_p} (t_x^2 + t_y^2) \right] G = 0 \,, \qquad (9)$$

and the force-extension and torque-linking number relations can then be determined through the ground-state eigenvalue and eigenfunction of Eq. (9). Finally, the relation between extension and linking number is obtained by elimination of torque Γ from these two relations.

The numerically calculated relations between extension and supercoiling degree σ at various fixed forces are shown in Fig. 3 and compared with the experiment of Strick *et al.* [5]. Here σ is defined by $\sigma = (\langle Lk\rangle - \langle Lk\rangle_{\Gamma=0})/\langle Lk\rangle_{\Gamma=0}$, where $\langle Lk\rangle_{\Gamma=0} = \int_0^L ds \langle \sin\varphi\rangle_{\Gamma=0}/R$ is the linking number for a torsionally relaxed DNA. The parameters for the theoretical curves in Fig. 3 are the same as those of Figs. 1 and 2; no adjustment has ever been made to fit the data. For negatively supercoiled DNA, the theory is in quantitative accordance with experiment (left half of Fig. 3).

For $\sigma < 0$, both theory and experiment give three distinct regions of DNA elasticity: (i) For forces >1.3 pN,

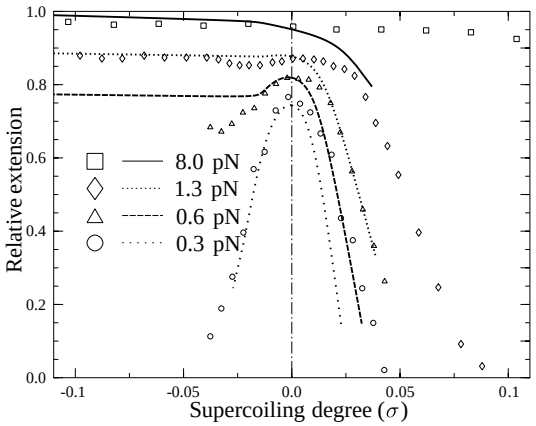

FIG. 3. Extension vs supercoiling relations at fixed pulling forces. The parameters for the curves are the same as Fig. 1 and experimental data is from Fig. 3 of [5] (symbols).

DNA extension does not shrink with the increase of negative supercoiling; on the contrary, it may even slightly increase as $|\sigma|$ increases. (ii) For $1.3 \geq f > 0.3$ pN, there exists a critical negative supercoiling degree σ_c. Extension of DNA shrinks as σ decreases from 0 to σ_c, then it remains approximately constant as σ further decreases. $\sigma_c \simeq -0.02$ at 0.6 pN. (iii) For $f \leq 0.3$ pN, DNA extension shrinks constantly with the increase of $|\sigma|$. In this case, no evident difference between the behaviors of negatively and positively supercoiled DNAs is observed; i.e., DNA can be regarded as achiral [15].

Thus, the complex elastic property of a negatively supercoiled DNA as well as that of an overstretched DNA can be satisfactorily understood by the same framework. In this context, although DNA double helix is quite good at enduring external forces it is much weaker at enduring torques: while a force $\sim$70 pN is needed for a torsionally relaxed DNA to trigger cooperative changes of configuration [3,4], 0.6 pN is just sufficient for a negatively supercoiled DNA with σ as small as -2%. This "shortcoming" of DNA might have been well noticed and captured by various proteins. For example, it seems that RecA protein stretches DNA by exerting a torque on the molecule.

However, as shown in the right half of Fig. 3, for positively supercoiled DNA the agreement between theory and experiment is poor. It is possible that positive supercoiling leads to strong radial as well as axial compressions on DNA base pair planes as to make them shrink considerably or even corrupt. In support of this point, a recent experiment of Allemand *et al.* [7] indicates that positively supercoiled DNA can take on very surprising configurations with exposed bases. Therefore, it seems necessary for us to take into account the possible deformability of DNA base pairs in our theory to understand the elasticity of positively supercoiled DNA. We plan to perform such an effort.

We are grateful to Professor L.-S. Liu and Professor B.-L. Hao. Z. H. is indebted to Professor L.-S. Liu for technical help on the computer, to J.-Z. Lou for informing him of a website of Fortran programs, and to D. Thirumalai, J. Yan, Q.-H. Liu, and K. Kroy.

*Email address: zhouhj@itp.ac.cn

[1] S. B. Smith, L. Finzi, and C. Bustamante, Science **258**, 1122 (1992).
[2] S. C. West, Annu. Rev. Biochem. **61**, 603 (1992).
[3] P. Cluzel *et al.*, Science **271**, 792 (1996).
[4] S. B. Smith, Y. Cui, and C. Bustamante, Science **271**, 795 (1996).
[5] T. R. Strick *et al.*, Science **271**, 1835 (1996).
[6] T. R. Strick, V. Croquette, and D. Bensimon, Proc. Natl. Acad. Sci. U.S.A. **95**, 10 579 (1998).
[7] J. F. Allemand *et al.*, Proc. Natl. Acad. Sci. U.S.A. **95**, 14 152 (1998).
[8] J. F. Marko and E. D. Siggia, Macromolecules **28**, 8759 (1995).
[9] This is biologically significant. We know that RNA polymerases moving along DNA can exert pulling forces of 20 to 30 pN [M. D. Wang *et al.*, Science **282**, 902 (1998)]; such large forces certainly can lead to severe deformations in DNA's local structure since *in vivo* DNAs usually have a linking number deficit of -6%.
[10] J. F. Marko and E. D. Siggia, Phys. Rev. E **52**, 2912 (1995).
[11] B. Fain, J. Rudnick, and S. Östlund, Phys. Rev. E **55**, 7364 (1997).
[12] J. F. Marko, Europhys. Lett. **38**, 183 (1997); Phys. Rev. E **57**, 2134 (1998).
[13] R. Cizeau and J.-L. Viovy, Biopolymers **42**, 383 (1997).
[14] M. Rief, J. M. Fernandez, and H. E. Gaub, Phys. Rev. Lett. **81**, 4764 (1998).
[15] C. Bouchiat and M. Mézard, Phys. Rev. Lett. **80**, 1556 (1998).
[16] R. D. Kamien, T. C. Lubensky, P. Nelson, and C. S. O'Hern, Europhys. Lett. **38**, 237 (1997).
[17] B.-Y. Ha and D. Thirumalai, J. Chem. Phys. **106**, 4243 (1997).
[18] K. Kroy and E. Frey, Phys. Rev. Lett. **77**, 306 (1996).
[19] A. Ahsan, J. Rudnick, and R. Bruinsma, Biophys. J. **74**, 132 (1998).
[20] H. Zhou and Z.-C. Ou-Yang, J. Chem. Phys. **110**, 1247 (1999); Phys. Rev. E **58**, 4816 (1998).
[21] An earlier treatment can be found in the e-print archive [H. Zhou and Z.-C. Ou-Yang, cond-mat/9810132].
[22] W. Saenger, *Principles of Nucleic Acid Structure* (Springer-Verlag, New York, 1984).
[23] T. B. Liverpool, R. Golestanian, and K. Kremer, Phys. Rev. Lett. **80**, 405 (1998).
[24] Experimentally, the BPL of dsDNA is about 53 nm, quite larger than that of a DNA single strand [4].
[25] In this work, we have considered neither the possible tilting of the base pairs relative to the central axis [3] nor the possible nicks existed in DNA backbones [4]. It seems that although these effects can also be important in understanding DNA elasticity, they are not the dominating factors.
[26] Indeed, our unpublished data shows that during cooperative elongation, the height, width, and slope of the force plateau are sensitive only to ϵ and φ_0, the parameters characterizing the base-stacking potential Eq. (4).
[27] J. H. White, Am. J. Math. **91**, 693 (1969).
[28] F. B. Fuller, Proc. Natl. Acad. Sci. U.S.A. **68**, 3557 (1978).
[29] Because at low forces dsDNA can be viewed as a wormlike chain with BPL of 53 nm [8].
[30] These expressions for r_0 and R follow from the facts that, for relaxed DNA, the axial distance between adjacent base pairs is 0.34 nm, and there are 10.5 base pairs in one period of the double helix [22].