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# DNA Extension under the Action of an External Force

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ABSTRACT: We computed the extension–force dependence for the wormlike model of a polymer chain. The dependence that we obtained differs essentially from the corresponding well-known dependence for the freely jointed chain when the extension exceeds half of the chain contour length. We used the computed results to analyze the measurements of extension of individual DNA molecules under the action of force made recently (Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122). We took into account the electrostatic interaction between DNA segments for a very low salt concentration. The theoretical dependencies obtained are in very good agreement with the experimental data.

## I. Introduction

The bases of polymer elasticity theory were created 60 years ago when Guth and Mark[1] and Kuhn[2] remarkably explained the unusual elasticity of polymer materials. The simplest model of the phenomenon is in textbooks (see ref 3 for example). This model describes the elasticity of an individual polymer chain. Although the elastic properties of the isolated molecule are much simpler for the theoretical analysis, it is very difficult to study them experimentally. Until recently, nobody has done such an investigation. Such an impressive experiment was done first in 1992 by Bustamante and co-workers.[4] They measured the extension–force relation of individual DNA molecules 94 000 base pairs in length and analyzed their data in terms of the existing theory. They found that the behavior of DNA chains under stress follows the theory well when the extension does not exceed half of the molecular contour length. Much greater force was required for high extensions than the theory predicted. The investigators assumed that the intrinsic curvature of DNA caused the observed discrepancy.[4] However, the theory of polymer elasticity used may not have been good enough for high extensions of DNA.

The existing elasticity theory of polymer molecules is based on the simplest freely jointed model of polymer chains.[3] Because the force applied is transmitted along the chain, it is the same for all chain segments. Therefore the analysis of an extension–force relation for the model is simply reduced to the analysis of the orientation of one segment under the action of forces applied to its ends, $F$. The energy of segment orientation is equal to $-Fb\cos\phi$, where $b$ is the segment length and $\phi$ is the angle between the force and segment directions. It means that the probability of a particular value of $\phi$ is proportional to $\exp(Fb\cos\phi/kT)$, where $T$ is the temperature and $k$ is the Boltzmann constant. Thus, a lower force is required to align longer segments. The extension of the model chain, $\langle x\rangle$, normalized to the total contour length, $L$, is independent of the segment length and of the number of segments in the chain if plotted as $\langle x\rangle/L$ vs $Fb/kT$. This normalized extension–force dependence is the Langevin function. To analyze the properties of actual polymers, one should substitute $b$ by the Kuhn statistical length of a particular polymer.[3]

However, there is no reason to expect that the freely jointed model would work well for limiting extensions of DNA. It is certainly preferable to use the wormlike chain model,[5] which provides a much better description of DNA conformational properties. Although it is difficult to calculate the extension–force dependence for the wormlike chain analytically, the dependence can be obtained by computer simulation. We performed these calculations. We took into account the electrostatic interaction between DNA segments for a very low salt concentration. The results obtained were used to analyze the experimental data of Smith et al.[4]

## II. Models and Methods of Computations

A polymer chain composed of $n$ Kuhn statistical segments was modeled as a chain consisting of $mn$ rigid straight segments of equal size. The elastic energy of the chain, $E_b$, was computed as

$$E_b = kT\alpha \sum_{i=1}^{mn^{-1}} \theta_i^2 \qquad (1)$$

where the summation is done over all of the joints between the rigid segments, $\alpha$ is the bending rigidity constant, and $\theta_i$ is the angular displacement of segment $i$ relative to segment $i+1$. The bending constant $\alpha$ is defined so that the Kuhn statistical length corresponds to $m$ rigid segments.[6] This model is transformed into the wormlike chain model as $m$ approaches infinity.

To simulate the extension of the model chain, we fixed one of its ends at point 0 and applied a force, $F$, directed along the $x$ axis, to the second end. The total energy of a particular conformation, $E$, was:

$$E = E_b - xF \qquad (2)$$

where $x$ is the $x$-coordinate of the second end of the chain.

Equilibrium sets of the chain conformations for different $F$ values were constructed by the Metropolis procedure.[7] There were two types of chain displacement used in the procedure. In the first type, a subchain containing an arbitrary number of adjacent segments is rotated by a randomly chosen angle, $\phi^1$, around the straight line connecting the vertices bounding the subchain. In the second type, a subchain containing an arbitrary number of segments and including the free end of the chain is rotated by a randomly chosen angle, $\phi^2$, around a randomly oriented line passing through the internal end of the subchain. The values of $\phi^k$ were uniformly distributed over intervals, $(-\phi_0^k, \phi_0^k)$, chosen so that about half of the proposed moves of each type were accepted.

Most of the calculations were done for model chains consisting of 10 Kuhn statistical segments, but when checked, results were the same for 5 and 50 segments. For accuracy, up to $10^8$ elementary displacements were

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produced for any particular values of $F$ and $m$. The average value of $x$, $\langle x \rangle$, was calculated as the average through a particular set.

For the very low salt concentration, $10^{-4}$ M NaCl, we introduced the electrostatic interaction to the model in terms of the Debye–Hückel approximation. Although in general this approximation is not accurate enough to describe the electrostatic contribution to DNA bending rigidity,[8,9] at $10^{-4}$ M NaCl the approximation is satisfactory. The term $E_{el}$ which we added to the chain energy was

$$E_{el} = \sum_i \sum_{j>i} \int \int \frac{\upsilon^2 \exp(-\kappa r)\, dr_i\, dr_j}{D r_{ij}} \tag{3}$$

where $\upsilon$ is the effective linear charge density of DNA, $\kappa$ is the Debye–Hückel screening parameter, $D$ is the dielectric constant of water, and $r_i$ is a point of chain segment $i$, $r_{ij} = r_i - r_j$. The sum here is over all couples of chain segments, and the integrals should be taken along the segments $i$ and $j$. For [NaCl] = $10^{-4}$ M, the $\upsilon$ value should be very close to that predicted by condensation theory, 0.14 e/Å.[9,10] The bending rigidity constant corresponded to $b = 100$ nm. Changing this constant up to 30% did not alter the results significantly because the electrostatic interaction provides the main contribution to the bending rigidity at this salt concentration. Most of the simulated results for this model were obtained for $m = 100$ and chain length = 2000 nm. A total of $10^5$ elementary displacements were produced for each point.

## III. Results and Discussion

We computed the extension–force relation for the discrete wormlike chain model. This discrete model is transformed into the wormlike chain as the number of chain segments per Kuhn statistical length, $m$, approaches infinity. The calculations showed that the relative extension of the chain, $\langle x \rangle / L$, does not depend on chain length if it exceeds 5 Kuhn statistical segments (within the accuracy of the calculations). So for such chain lengths the extension–force relation is described by a universal function in coordinates ($Fb/kT$, $\langle x \rangle/L$) for any value of $m$. The functions depend neither on $L$ nor on $b$ values. The calculated functions for $m$ = 1, 10, 20, 50, 100, and 200 are shown in Figure 1. One can see that for $\langle x \rangle / L < 0.98$, the limiting behavior is realized with high accuracy for $m$ = 100. The limiting function can be approximated by the equation

$$\log(Fb/kT) = -0.1097t^3 + 0.4509t^2 + 1.373t + 0.02 - 0.038/(t + 0.018) \tag{4}$$

where

$$t = -\log(1 - \langle x \rangle / L) \qquad 0.1 \le (\langle x \rangle / L) \le 0.99$$

The limiting function obtained differs essentially from the corresponding function for the freely jointed chain model ($m = 1$) if the extension exceeds half of the chain contour length (Figure 1). The discrete model used in the simulation helps us to understand why it is more difficult to extend the wormlike chain in comparison to the freely jointed chain. Although the angle between two neighboring segments in the model used in our simulations is randomly distributed, its values are small; i.e., the segments are nearly parallel. Therefore, the force aligns groups of adjacent segments, which correspond to Kuhn statistical length, rather than each individual segment as for the freely jointed chain. The chain rigidity helps to align the segments of the discrete wormlike chain. The situation is different for the case of limiting extensions. For limiting extensions, the force should provide a more narrow angle distribution between the direction of the force and the direction of individual segments, and correspondingly between neighboring segments, than the bending rigidity provides. In this case, the bending rigidity does not help to align the segments and subsequently the force has to align each straight segment independently. Much greater force is required to do it.

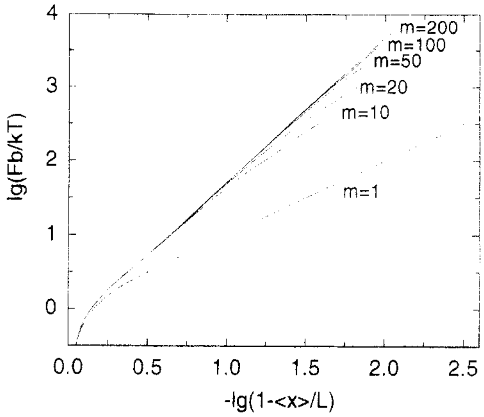

**Figure 1.** Computed extension–force relation for the wormlike chain. The discrete model used is transformed into the wormlike chain as the number of the chain segments per Kuhn statistical length, $m$ approaches infinity. The calculated dependencies for different $m$ values are shown by lines. To show the relation in a universal form, the force $F$ is normalized by multiplying by the Kuhn statstical length, $b$, and dividing by the temperature factor, $kT$. The extension $\langle x \rangle$ is normalized on the total chain length, $L$. The dependencies shown do not depend on the chain length if it exceeds 10 persistence lengths and on $b$ value. The statistical error is close to the line thickness. The curve for $m$ = 1 corresponds to the extension–force dependence for the freely jointed chain model.

We have only one adjustable parameter, the Kuhn statistical length, to fit the experimental data to the calculated extension–force dependence. In the logarithmic coordinates which were used, changing this parameter does not deform an experimental curve, but only moves it up or down. Thus, it is easy to choose a $b$ value to arrive at the best fit between the experimental and theoretical data. Figure 2 shows the experimental results of Smith et al.[4] for DNA extension under the action of force for three different NaCl concentrations in solution plotted together with the calculated function. One can see from the figure that the experimental data fit very well to the calculated dependence for $10^{-2}$ and $10^{-3}$ M NaCl. The values of $b$ found from the comparison are in agreement with the results of other methods.[11]

There is a discrepancy between the experimental data and the calculated function at the lowest salt concentration studied, $10^{-4}$ M NaCl (Figure 2C). The simplest explanation for this discrepancy is that the calculated dependence does not take into account the excluded volume effect provided by the electrostatic repulsion between DNA segments. Although the excluded volume effect is also important for NaCl concentrations of $10^{-2}$ and $10^{-3}$ M, it can be neglected at first approximation for the highly extended conformations considered. However, the effect increases dramatically at $10^{-4}$ M NaCl, and DNA conformational properties do not fit the wormlike chain model even for such extended conformations.

At very low salt concentration, $10^{-4}$ M NaCl, one cannot separate the local effect of electrostatic interaction (increasing DNA bending rigidity) from the excluded volume effect (an interaction of segments far apart along the DNA contour). The successive analysis requires one to take into account the electrostatic interaction of all charges without dividing it into the two distinct categories.

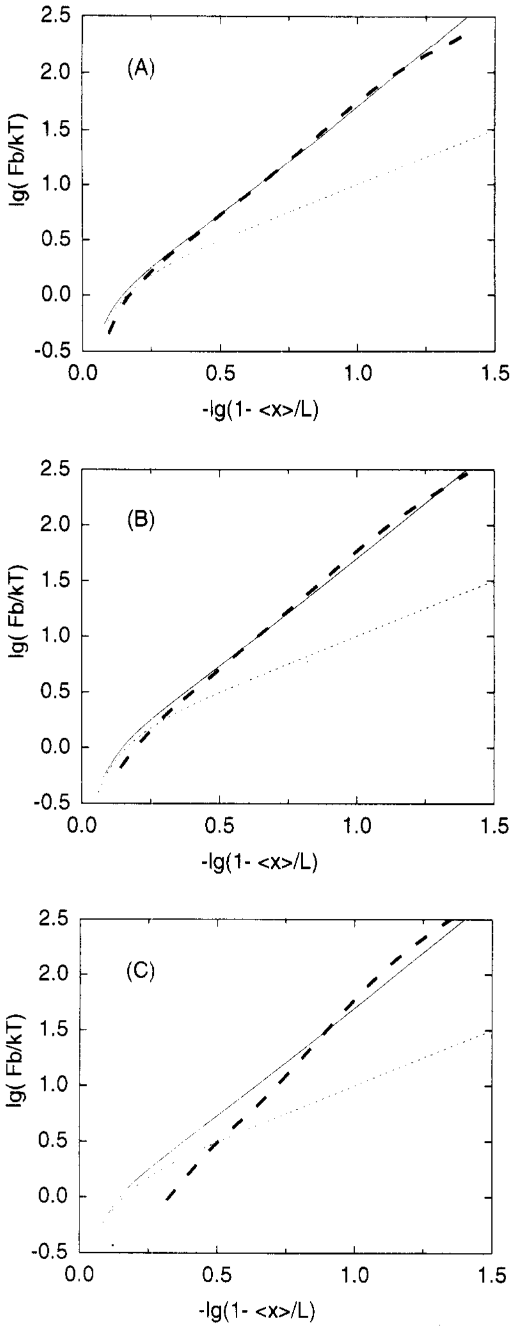

**Figure 2.** Comparison of the experimental data for the extension–force dependence for DNA and the calculated dependence for the wormlike chain. The data of Smith et al.[4] are plotted as the bold dashed curves which give the best approximation of the experimental points. These data were fitted to the theoretical curve (thin solid line) by choosing $b$ values for DNA independently for each concentration of NaCl: $10^{-2}$ (A), $10^{-3}$ (B), and $10^{-4}$ (C) M. The best fitting values of $b$ are equal to 120, 180, and 300 nm. The thin dashed line corresponds to the theoretical dependence for the freely jointed model.

We introduced the electrostatic interaction to the model in terms of the Debye–Hückel approximation. It has been shown that the approximation is accurate enough at a NaCl concentration of $10^{-4}$ M.[9]

We found that, despite this long-range interaction between chain segments, the chain length effect on the extension–force dependence is negligible if $\langle x \rangle / L > 0.6$ and $L > 1000$ nm. This allowed us to consider rather short chains in the majority of the calculations and to obtain reasonable statistical error. Unlike the extension–force dependence for the wormlike chain model, we cannot present the results in universal form because they depend on the relative values of three parameters: $\alpha$, $\nu$, and $\kappa$. Since the values of all parameters are known from independent data, there was no adjustable parameter to fit these simulated results to the experimental data. Therefore we consider the agreement between the simulation and the experimental results (Figure 3) as a strong argument that only the electrostatic interaction is responsible for the specific extension–force behavior of DNA at very low salt.

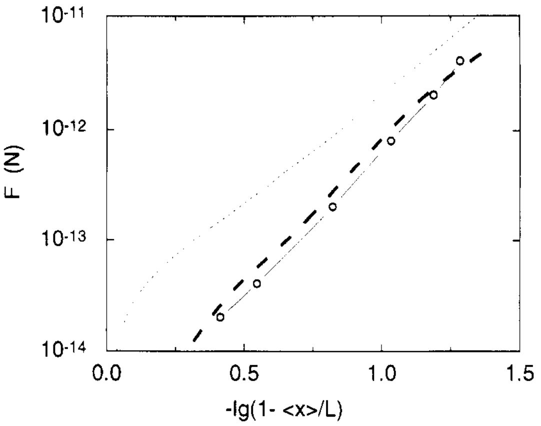

**Figure 3.** Extension–force dependence for DNA at $10^{-4}$ M NaCl. The bold dashed line represents the experimental data of Smith et al.[4] The open circles correspond to the calculated dependence for the discrete model, with internal bending rigidity and electrostatic interaction between chain segments in terms of the Debye–Hückel approximation (eq 3). The nonelectrostatic internal rigidity of the model chain corresponds to a Kuhn statistical length value of 100 nm. The extension–force dependence for an uncharged wormlike chain with such rigidity is shown by thin dashed line. The statistical error is close to the size of the circles.

The remarkable agreement between the experimental data of Smith et al.[4] and the simulated extension–force relations was reached only when the basic well-known properties of DNA, the bending rigidity and the electrostatic interaction between double helix segments, were taken into account. We may conclude that for regular sequences intrinsic DNA curvature does not contribute significantly to the elastic properties of DNA.

The extension–force dependence for the wormlike chain model obtained in this work and the comparison of the computational results with the experimental data show that the approach developed by Bustamante and coworkers[4] is a sensitive method to measure DNA persistence length under various experimental conditions. The experimental data may be analyzed effectively in terms of the simple wormlike chain model if the concentration of monovalent salt is higher than $10^{-3}$ M.

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## References and Notes

(1) Guth, E.; Mark, H. *Monatsh Chem.* **1934**, *63*, 93.
(2) Kuhn, W. *Kolloid-Z.* **1934**, *68*, 2.
(3) Bueche, F. *Physical Properties of Polymers*; Interscience: New York, 1962.
(4) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122.
(5) Kratky, O.; Porod, G. *Recl. Trav. Chim. Pays-Bas* **1949**, *68*, 1106.
(6) Frank-Kamenetskii, M. D.; Lukashin, A. V.; Anshelevich, V. V.; Vologodskii, A. V. *J. Biomol. Struct. Dyn.* **1985**, *2*, 1005.
(7) Metropolis, N.; Rosenbluth, A. W.; Rosenbluth, M. N.; Teller, A. H.; Teller, E. *J. Chem. Phys.* **1953**, *21*, 1087.
(8) Le Bret, M. *J. Chem. Phys.* **1982**, *76*, 6243.
(9) Stigter, D. *Macromolecules* **1985**, *18*, 1619.
(10) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.
(11) Hagerman, P. J. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 265.