COMMUNICATIONS IN COMPUTATIONAL PHYSICS
Vol. **6**, No. 3, pp. 577-585

Commun. Comput. Phys.
September 2009

A Solution to a Single Molecular Experiment Problem

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Received 18 July 2008; Accepted (in revised version) 13 November 2008

Communicated by Wei Cai

Available online 6 February 2009

Abstract. Recently Hari Shroff and his collaborators [Nano Letters 5 (2005)] developed a nanoscopic force sensor, but the force which they measured in their single molecular experiment was much lower than the theoretical critical value. In order to fix this problem, we investigate the micromechanics of dsDNA based on the worm-like chain model and flexible hinge model by using Monte Carlo algorithm. The simulation results not only address Hari Shroff's experiment difficulty reasonably, but also provide strong support for flexible hinge mechanism put forward recently by Yan and Marko [Phys. Rev. Lett. 93 (2004)].

AMS subject classifications: 65Z05, 92-08, 65Y99, 68W99

Key words: Monte Carlo algorithm, worm-like chain (WLC) model, flexible hinge model, single molecular experiment, micromechanics of double-stranded DNA (dsDNA).

1 Introduction

Double-stranded DNA (dsDNA) mechanics is essential to understand the DNA organization in cell, since the DNA is tightly folded in cell. The current understanding of DNA mechanics is based on the single molecule stretching experiments for DNA larger than one micrometer ($1\mu m$) or the cyclization experiments for DNA larger than 230 base pairs (bp). Based on the force extension curve and the cyclization probability measurement of long DNA, DNA is understood to be semi-flexible, WLC model, in agreement with these

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experiments with the choice of one parameter, persistence length. It has been widely accepted by biophysicists.

But compared with DNA packaged in cell, the DNA is less bent in experiments mentioned above. Recently there have been more evidences that challenge the worm-like chain model when local bending is too sharp. Cloutier and Widom reported that DNA with length around 100 bp has looping probability several orders of magnitude larger than the prediction of traditional WLC model at temperature 30 degree Celsius [2, 3]; their results do not conflict with earlier experiments because this regime of sharp DNA bending was not investigated by experiments. Later Du and coworkers found that the looping probability of DNA about 100bp is consistent with prediction of the WLC model with 47 nm persistence length at lower temperature 21 degree Celsius [4].

Two experiments above trigger the interests of the theoretical biophysicists. The excited flexible hinge model was proposed to explain the unusual bending elasticity found in Cloutier and Widom's experiment. Traditionally, in physiological conditions, the stiffness of double stranded DNA is considered to be a result of double helix structure, which is made robust by base-pairing and the stacking interactions. By comparison, the covalently bonded sugar-phosphates are completely flexible, characterized by a persistence length of about 1nm. It suggests a mechanism for generations of localized regions of extreme flexibility along the double helix: local disruption of bases interaction, called flexible hinge, may give rise to regions where the double helix can be bent easily. Such disruptions might occur by thermal fluctuations which open bases in localized regions of double helix and explain Cloutier and Widom's experiment successfully. Because the excitation energy to form a hinge defect should be dependent on the temperature, the model does not contradict the results of Du and coworkers in reference [4].

Some other experiments are challenging WLC model further, all of them indicate that the sharp bending of DNA is much easier than WLC model. It is found that spontaneous large-angle bending is more prevalent than predicted by the WLC model [5]. Shroff and coworkers found that short DNA could be bent by force much smaller than the theoretical value predicted by the WLC model [6]. Especially in another recent work by Du and Vologodskii, they also reported that the disruption of base pairs was found in the minicircle with its length shorter than $70nm$ [7]. By comparison, the flexible hinge is noharmonic sharp bending, and the semi-flexible WLC model based on linear elasticity of a continuous material or on harmonic bending of base steps, so it can not solve the problem presented by the experiments mentioned above.

The common features of the recent experiments are that the DNA is sharply bent which challenges the traditional worm like chain model. Motivated by these experiments, we construct two simple models: (I) dsDNA is connected by hard spring; (II) dsDNA is connected by single stranded DNA (ssDNA) and the flexible hinge model is introduced to dsDNA. For model (I), the Euler instability of dsDNA is to be investigated. For the model (II), a comparison will be made between the worm like chain model and the flexible hinge model to identify the reasons that caused the difficulty mentioned in Shroff's work and show some insights into the sharp bending in other experiments.

2 Model and algorithm

2.1 Worm like chain model and flexible hinge model for dsDNA

In our simulation, we consider the dsDNA molecule consisting of N straight segments of equilibrium length b. Each segment contains three base pairs. The conformation is described by the orientations of the segments $\hat{t}_i$, where $i=1,\cdots,N$ and the conformational energy is carried by the bending of the vertices connecting the adjacent segments. The energy of this model is a summation of the vertex energy, *i.e.*,

$$E=\sum_{i=1}^{N}E_i(\hat{t}_i,\hat{t}_{i+1}), \tag{2.1}$$

where E_i is the bending energy of the i-th vertex, which depends on the two adjacent tangent vectors. For the WLC model of dsDNA, the bending energy at one vertex can be expressed as,

$$E_i(\hat{t}_i,\hat{t}_{i+1})=\frac{\alpha}{2}\sum_{i=1}^{N}(\hat{t}_i-\hat{t}_{i+1})^2=\frac{\alpha}{2}\sum_{i=1}^{N}(2-2\hat{t}_i\cdot\hat{t}_{i+1})=\alpha\sum_{i=1}^{N}(1-\cos\theta_i), \tag{2.2}$$

where θ_i is the angle between the orientations of two adjacent segments at a vertex, α is dimensionless quantity describing the bending rigidity of dsDNA. In the continuum limit $b\to 0$, the bending elastic constants of the discrete and continuum models are related by $\alpha b=l_p$, wherein l_p is the persistence length, charactering the bending rigidity of the dsDNA.

For the flexible hinge model, the net bending energy at one of the segment-segment vertexes can be written as [8],

$$\begin{aligned}E_i(\hat{t}_i,\hat{t}_{i+1})&=-\ln\left[\exp^{-\frac{\alpha}{2}(\hat{t}_i-\hat{t}_{i+1})^2}+\exp^{-\mu}\exp^{-\frac{\alpha'}{2}(\hat{t}_i-\hat{t}_{i+1})^2}\right]\\&=\frac{\alpha}{2}(\hat{t}_i-\hat{t}_{i+1})^2-\ln\left[1+\exp^{-\mu}\exp^{-\frac{\alpha-\alpha'}{2}(\hat{t}_i-\hat{t}_{i+1})^2}\right],\end{aligned} \tag{2.3}$$

where μ represents the energy cost of generating a flexible hinge, controls the probability that a flexible hinge appears at any particular location and ranges from $8k_BT$ to $11k_BT$. The α' represents the bending rigidity of the dsDNA at one vertex where the flexible hinge generates. The bending energy above is defined in unite of k_BT. The simulation is based on a choice of $b=1nm$, $l_p=50nm$.

For small bends, the elastic energy $\frac{\alpha}{2}(\hat{t}_i-\hat{t}_{i+1})^2$ dominates. However, with the bends increasing, the tight bends $\frac{\alpha'}{2}(\hat{t}_i-\hat{t}_{i+1})^2$, characterized by a small bending rigidity, takes over the former. Their crossover occurs at the vertex, where the critical bending angle can be obtained from the following equation,

$$\frac{\alpha}{2}(\hat{t}_i-\hat{t}_{i+1})^2-\frac{\alpha'}{2}(\hat{t}_i-\hat{t}_{i+1})^2\approx\mu. \tag{2.4}$$

If the bending angle is more than the critical bending angle, the flexible hinge defects happen. From Eqs. (2.2) and (2.4), the critical bending can be decided by

$$(1-\cos\theta_c)(\alpha-\alpha')\approx\mu,$$

which depends on the flexible hinge defect generation energy and bending rigidity.

2.2 The modified freely joined chain model for ssDNA

In the model (II), one part is the dsDNA and the other part is ssDNA. The bending rigidity of dsDNA is far bigger than the one of ssDNA, so the single strand DNA will be stretched. In order to learn about the qualitative information from the stretching ssDNA, the modified freely joint chain model in Smith et al.'s work is applied to model the stretching ssDNA, which incorporates the Kuhn segments that can stretch as well as align under force is then tested [9],

$$x(f)=L_{ss}\left[\coth\left(\frac{fb}{k_BT}\right)-\frac{k_BT}{fb}\right]\left(1+\frac{f}{S}\right), \tag{2.5}$$

where L_{ss} is the contour length, b is a Kuhn length and S is the stretch modulus of ssDNA. The work of [9] is to fit the experimental data, $S=800pN$. In our simulation, the force that ssDNA is undergoing is only a few Pico Newton, so f/S in the right-hand side of Eq. (2.5) can be neglected. The remaining part of Eq. (2.5),

$$x(f)=L_{ss}\left[\coth\left(\frac{fb}{k_BT}\right)-\frac{k_BT}{fb}\right],$$

is Langevin function, from which the force that the ssDNA is undergoing can be derived as

$$f=\frac{k_BT}{b}\frac{x}{L_{ss}}\left[3-\left(\frac{x}{L_{ss}}\right)^2\right]\left[1-\left(\frac{x}{L_{ss}}\right)^2\right]^{-1}. \tag{2.6}$$

The stretching energy of ssDNA can be integrated from Eq. (2.6):

$$E_{strech}=\frac{k_BT}{2L_{ss}b}x^2-\frac{k_BT}{L_{ss}b}\ln(1-x^2). \tag{2.7}$$

2.3 The Euler instability of dsDNA

In the elementary theory of bending, the stress and deflections in beams are directly proportional to axial compressive force, $f=EIn^2\pi^2/L^2$. When the axial compressive force approaches a limiting value, the beam becomes unstable, even the smallest lateral force will produce considerable lateral deflection. This limiting force is called the critical force [10]:

$$f_{critical}=\frac{EI\pi^2}{L^2},$$

where E is the modulus of elasticity, I is the area moment of inertia. EI represents the flexural rigidity of the beam, for dsDNA molecular, which can be generalized as $EI = l_p k_B T$, where l_p is the persistence length of dsDNA molecular. So the critical force of dsDNA is

$$f_{cri-DNA} = \frac{l_p k_B T \pi^2}{L^2}. \tag{2.8}$$

In Section 3.1, we will investigate the critical force of dsDNA based on the WLC model.

2.4 Algorithm

In the simulation, we take the Metropolis-Monte Carlo procedure, which is used for the statistical sampling of chain conformations. In the process of the procedure, chain parts are displaced consecutively. The initial conformation can be chosen arbitrarily. In order to generate the new trial conformations, every position in the conformation fluctuates around its equilibrium position; the fluctuation is controlled by random number. Whether the new trial conformation is accepted or not, all the conformations must be added to the conformations series. The Metropolis-Monte Carlo criteria is applied to decide whether the trial conformation is accepted or not.

3 Results

3.1 The critical force of dsDNA

We model the linear dsDNA as worm-like chain and connect its two ends by a hard spring, which can avoid strong fluctuations of the end-to-end distance of dsDNA. By changing the initial length of the hard spring, we can measure the force that the hard spring is undergoing. While the initial length of the hard spring approaching the length of dsDNA, a transition shown in Fig. 1 occurs. At this transition point, the force, which the dsDNA is undergoing, is the critical force of linear dsDNA.

3.2 The comparison between the theoretical critical force and the force measured from the ssDNA with the dsDNA section described by WLC model

In Fig. 2, the red squares are the theoretical critical force calculated from Eq. (2.8), the green ones are the force, measured from the ssDNA section with the double-stranded DNA section modeled as WLC. The dsDNA section covalently connects with ssDNA analogue, and the ssDNA analogue consists of 12 base pairs (about 8.52nm), which is the same as that of Hari Shroff's experiment. There is a crossover point between the two lines; on the right side of the crossover point, the length of dsDNA is much bigger than the one of the ssDNA analogue. At the same time, the critical force of dsDNA decreases gradually with the length of dsDNA increasing, so the dsDNA can be bent easily. Once the dsDNA is bent, the forces, measured from ssDNA, are bigger than the theoretical

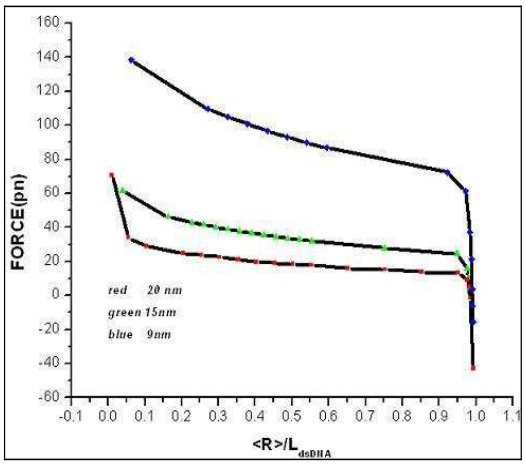

Figure 1: Force that dsDNA is undergoing changes with the scaled end-to-end distance (the initial length of hard spring). The red, green and blue squares represent the dsDNA with length 20nm, 15nm and 9nm, respectively.

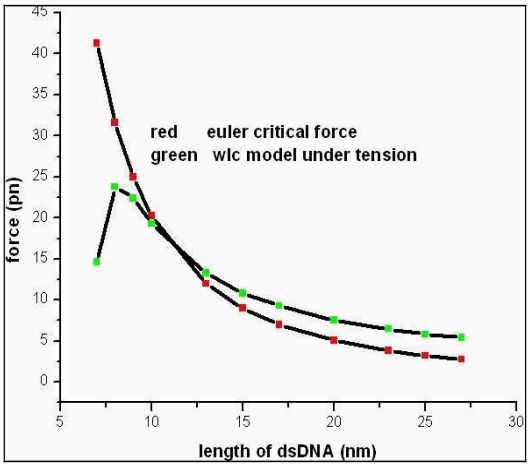

Figure 2: The comparison between the theoretical critical force and the force measured from the ssDNA section. The red squares and the green ones are the theoretical critical force and the force that the ssDNA section is undergoing with the dsDNA section described by WLC model.

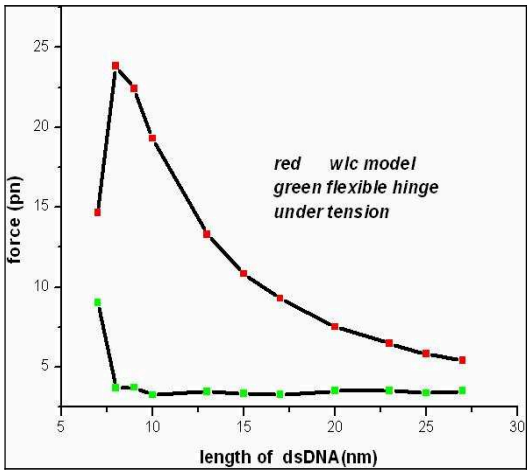

Figure 3: The comparison between the forces that the ssDNA section is undergoing. For the red squares and green squares, the dsDNA is described by the WLC model and flexible hinge model, respectively.

ones. On the left side of the crossover point, with the length of dsDNA section decreasing, on one hand, the critical force is increasing, and on the other hand, the length of dsDNA is approaching to the one of ssDNA section so that the ssDNA can not be stretched critically. Consequently, the force measured from ssDNA decreases gradually. When the length of dsDNA is less than the one of ssDNA, the ssDNA can not be stretched, then the forces fall down. At this point, the force is only decided by the entropy elasticity (discussed in Section 3.3).

3.3 The comparison between the force that the ssDNA section is undergoing with the dsDNA section described by WLC model and flexible hinge model, respectively

In Fig. 3, the red squares are the force that the ssDNA section is undergoing with the dsDNA described by WLC model, and the green squares are the force, measured from the ssDNA section with the dsDNA described by flexible hinge model. Under the flexible hinge model, the force, measured from the ssDNA section, is around 3pN, which is lower than the ones measured from the ssDNA section with the double-stranded DNA analogue described by the WLC model. Shroff's experiment result is about 6pN, and the experiment error is 5pN, so the experiment result ranges from 1 to 11pN. Our simulation result falls into this range and the discrepancy between the WLC model and the flexible hinge model highlights the key role of the flexible hinge in Shroff's experiment.

While the length of dsDNA section decreases to $7nm$, for the red squares, the force falls down to less than $15pN$, while for the green squares, the force goes up to about $10pN$. The entropy elasticity of ssDNA can show some insights into these transitions. The entropic spring constant of an ideal chain is $3k_BT/(Nb^2)$, the entropy force is

$$\overrightarrow{f} = \frac{3k_BT}{Nb^2}\overrightarrow{R},$$

where $\overrightarrow{R}$ is the end-to-end distance of the ideal chain. It is easier to stretch the ssDNA with numbers of the monomers N, and the monomers size b at lower temperature. For an ssDNA with 12 base pairs in length and $0.71nm$ per base pair, its entropic spring constant is about $2.03pN/nm$. The end to end distance of single-stranded DNA is less than $7nm$, so the entropy force that the ssDNA is undergoing is less than $14.21pN$.

Fig. 4 is the direct conformations, in which the length of ssDNA is $12bp$. The length of dsDNA in Figs. 4-1, 4-3 and in Figs. 4-2, 4-4 is $9nm$ and $27nm$, respectively. The dsDNA in Figs. 4-1, 4-3 and in Figs. 4-2, 4-4 is described by the WLC model and the flexible hinge model, respectively. In all of the conformations, it is obvious that the dsDNA part is bent and the ssDNA part is stretched. Especially in the direct conformations 4-2 and 4-4, the flexible hinge appears. The flexible hinge enhances the flexibility and dsDNA can be bent easily. Effects of flexible hinge mechanism in short dsDNA is then confirmed again.

4 Discussion and conclusion

Except the flexible hinge model, mismatch of base pairs in DNA and melting of base pairs at ends of DNA are two possible ways to explain the problems raised in Shroff et al.'s experiment. Schallhorn et al. [11] reported that the mismatch of base pairs can enhance the flexibility of dsDNA, but as far we know from the Shroff's work, no mismatch of base pairs is introduced into the dsDNA. As for the melting of base pairs at ends, there are no chances to happen in the experiments mentioned above except Shroff et al.'s work;

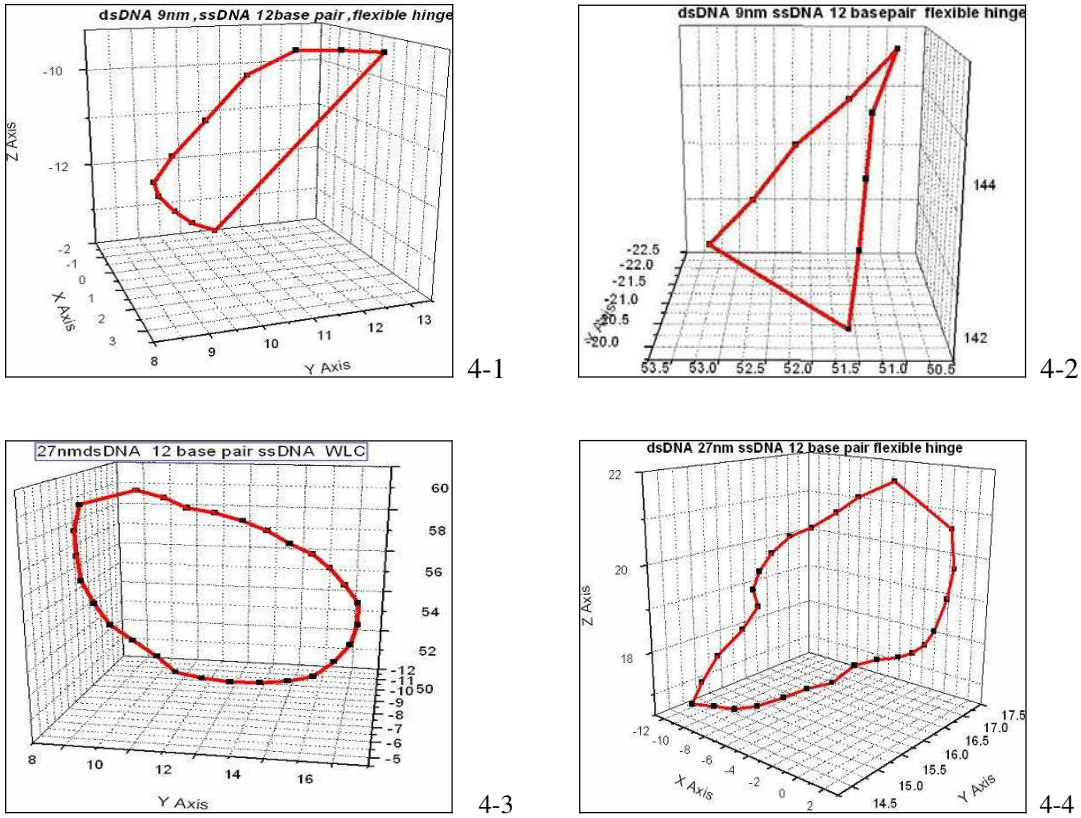

Figure 4: The direct conformations consist of dsDNA and ssDNA. In all conformations, the ssDNA is modeled modified freely joint chain. In conformations 4-1, 4-3 and 4-2, 4-4, the dsDNA part is described by the worm like chain model and the flexible hinge model, respectively.

most of the works investigated the circular DNA or a part in the middle of linear DNA. In order to highlight the common features, the flexible hinge model seems to be the right model to explain the difficulty occurred in [6]. According to our simulation results, the discrepancy between the Euler critical force and the force that dsDNA with the flexible hinge is in good agreement with the experiment results in [6]. For Shroff et al.'s work, the melting of the base pairs at the ends of DNA still deserves further investigation, which is one of our ongoing projects.

Acknowledgments

This research was supported by the Young Investigator Award received by Yan in 2006 and the Foundation for the Visiting PhD Candidate of the Chinese Academy of Science received by Liu in 2006. Liu is also supported by the Innovation Foundation for Graduate Students of Guizhou University (2007022).

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