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DNA Conformational Variations Induced by Stretching 3′5′-Termini Studied by Molecular Dynamics Simulations *

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Investigating the interaction between protein and stretched DNA molecules has become a new way to study the protein DNA interaction. The conformations from different stretching methods give us a further understanding of the interaction between protein and DNA. We study the conformational variations of a 22-mer DNA caused by stretching both 3′- and 5′-termini by molecular dynamics simulations. It requires 250 kJ/mol to stretch the DNA molecule by 3′5′-termini for 3.5 nm and the force plateau is at 123.8 pN. The stretching 3′5′-termini leads to large values of the angle opening and the dihedral propeller between bases in one base pair, the double helix untwists from 34° to 20° and the successive base pairs rolls to the side of the DNA major groove. The distances between successive base pairs increases from 3.2 Å to 5.6 Å.

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Studying the structures of DNA molecules stretched in different ways can improve the understanding of protein recognition and interaction with DNA molecules. Recently, with the development of single molecule manipulation, the mechanical properties of DNA have been found to play an important role in DNA-protein interactions.[1–3] For example, changes in DNA tension have been proved to greatly influence the interaction between DNA and DNA gyrase.[1] The mechanical properties of DNA are also apparently influenced the interaction between DNA and restriction endonucleases.[2,3]

Stretching of single DNA molecules in different ways has been extensively studied since 1996. Experimentally, Leger *et al.*[4] found that the qualitatively different structural transitions of DNA molecules depend on whether the DNA winding is allowed to relax, or kept fixed during the stretching process. Prentiss et al[5] found that DNA structures by stretching 3′-termini differed from the structure made by stretching 5′-termini via observing the force hysteresis with glyoxal appearing in the solvent. Very recently, Gaub *et al.*[6] found that the DNA molecules are more easily ruptured by stretching 5′-termini than by stretching 3′-termini. Theoretically, Lavery *et al.*[7,8] studied the stretching of DNA molecules in different ways with molecular mechanics calculations, suggesting that the S-form has a ladder structure where the base pairing is retained, but the nucleobases are tilted with respect to the axis of the helix and base stacking is interstrand, rather than intrastrand.[9] Using base level models, Lei *et al.*[10,11] have studied the stretching and unzipping process of DNA molecules. Recently, molecular dynamic (MD) simulation has become a useful tool to describe a single molecule at the atomic level.[12–14] By engaging the MD simulation, the stability of the ladder S-DNA structure has been questioned by some simulations of DNA stretching.[15,16] In 2010, we studied the conformational changes of double-stranded DNA in stretching 3′-termini and 5′-termini, and found that stretching 5′-termini would favor the activity of enzyme DNase I, while stretching 3′-termini could restrict the activity of enzyme DNase I.[17] However, the conformational variations of a DNA molecule by stretching 3′5′-termini have not been studied and its effects on protein DNA interaction have not been discussed.

In this Letter, we present a study of the DNA structure changes induced by stretching the opposing 3′5′-termini (3′5′-terminus is the geometrical center of an atom group, which contains two atoms: O3′ in one strand and O5′ in the complementary strand at one end of the DNA) of a 22-mer (22 base pairs) DNA molecule for 3.5 nm. It requires 250 kJ/mol to stretch the DNA molecule by 3′5′-termini for 3.5 nm and the force plateau is at 123.8 pN. Stretching 3′5′-termini led to the increment of the angle opening and the dihedral propeller between bases in one base pair, the double helix untwists about 15° and the successive base pairs rolls to the side of the DNA major groove. The distances between successive base pairs increase about 2.5 Å. Compared with the previous study, we give a comprehensive understanding of the conformational variations under different stretching methods, which is important for understanding the interactions between protein and DNA molecules and may be valuable for the design of new nano devices using DNA molecules.

All of the MD simulations were performed via Gromacs-3.3.1[18] with force field AMBER-94.[19] Starting conformations for the DNA double helix were generated by the *nucgen* program in Amber8,[20] and the B-DNA[21] was used as the native conformation. The DNA segment with sequence of $(GTCTGAATTCTAATGTAGTATA)_2$, was immersed in a periodic box of TIP3P water[22] with a

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size of 4.5 nm × 4.5 nm × 18.0 nm. A total of forty-two Na^+ ions were added into the box to neutralize the negative charges of the DNA molecule. Because there are 42 O-P-O atom groups in the 22-mer (two O-P-O atom groups missing at the terminal) and there is almost 1e negative charge on each group. A cut-off of 1 nm was applied for the Lennard–Jones interaction and the real space portion of electrostatic interaction, while the PME method[23,24] was used to calculate the reciprocal space portion of electrostatic interaction.

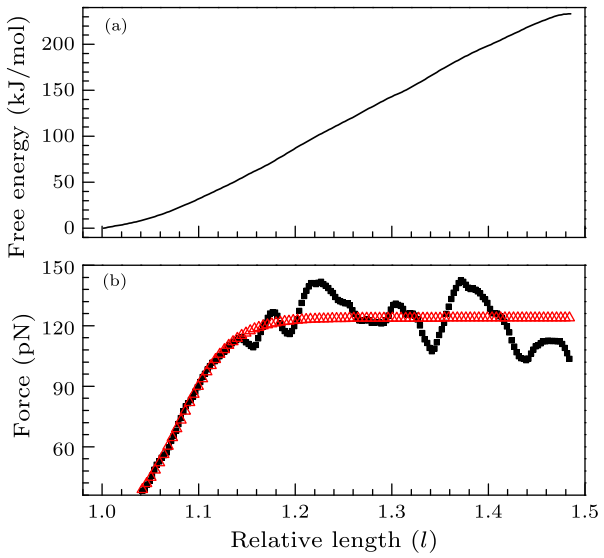

Fig. 1. (a) Free energy versus relative extension l as calculated by weighted histogram analysis of the umbrella sampling simulations of stretching 3′5′-termini. (b) Force versus relative extension l. Forces have been obtained as the derivation of the free energy profile stretching 3′5′-termini. The empty triangle is the fitting curve of force and the plateau is 123.8 pN.

In the preparation of the structure for the umbrella sampling simulations, stretching the B-DNA duplex under constant pressure and temperature conditions (300 K and 1 bar) were performed by connecting a harmonic spring, with original length of zero, to one 3′5′-terminus of the B-DNA duplex while the other end (3′5′-terminus) of the DNA was fixed. The force constant of the spring was 833 pN/nm. The other end of the spring moved away from the DNA with a constant velocity of 0.5 nm/ns, and consequently the DNA was stretched. The structures (namely, the starting structures) were stored for umbrella sampling simulation when the distance between the two 3′5′-termini of the DNA reached a length L. $L = 7.4\,\text{nm} + n \times 0.05\,\text{nm}$, $n = 0, 1, 2, \cdots, 69$, with a maximal value of 10.85 nm.

For each starting structure, we performed an 8-ns umbrella sampling simulation with the distance L restrained during storage and the data of the last 4 ns was collected for analysis. The force constant of the spring for umbrella sampling was also 833 pN/nm. These structures of the DNA molecule from each simulation were classified into different clusters. The criterion of the classification was that the rms deviation of the structures in a cluster was within 2 Å. For each cluster, a central structure was calculated by averaging all the structures in this cluster. The two base pairs at each end of the averaged structures were ignored, and only 18 base-pairs in the middle were analyzed by the program curves.[25] The forces were calculated as the derivation of the free energy profiles, while the free energy profiles were reconstructed with the weighted histogram analysis method, as implemented in the WHAM[26,27] program by Alan Grossfield.

The free energy with respect to the DNA relative extension l is presented in Fig. 1(a). The free energy required for stretching the 22-mer DNA to the relative extension $l = 1.45$ is about 250 kJ/mol. In Fig. 1(b) we present the force derived from the free energy profile. We employ the Boltzman function equation

$$F = \frac{A_1 - A_2}{1 + e^{(l-l_0)/dl}} + A_2$$

to fit the force profiles. For stretching 3′5′-termini, the fitted parameters are $l_0 = 1.08$, $A_1 = 18.0$, $A_2 = 123.8$. The plateau in the fitting line is at 123.8 pN and there is nonzero force at the beginning of stretching. We believe that the force of about 10 pN at the initial extension comes from straightening of the DNA molecules since the DNA molecules usually curve in the native state.[28] As shown in Fig. 1(b), we find two parts in our simulation of stretching processes: the force increasing region and the force plateau region with consistent force. The transition point of the force occurred at about the relative extension $l = 1.2$. Compared with our previous simulation results of stretching 3′-termini and 5′-termini,[17] the free energy and force plateau have the same shape in the three different stretching methods. However, stretching 3′5′-terimini is more difficult than the other methods for it needs more energy and the force plateau in stretching 3′5′-termini is higher than both stretcing 3′-termini and 5′-termini.

For understanding the variations of DNA conformation in stretching 3′5′-termini, we present a systemic study of the variations of the DNA transformation in base rotation and translation. The parameters in Fig. 2 describe the variations of the base rotation.[25] As the DNA is stretched, the fluctuations of all the parameters become larger and the obvious variations of these parameters appear after the relative extension $l = 1.2$.

During the stretching, the averaged angle between the base pairs and the z axis, inclination, changes from about 90° to about 45°, the angle between the two bases in one base pair, opening, changed from 5° to 20° in the major groove and the dihedral propeller varied from −12° to 25°. At the same time, the twist angle between successive base pairs decreases from 34° to 20° and the successive base pairs rolls to major groove (from 0° to 8°). The other parameters are fluctuated around the initial values with increasing fluctuations as the stretching processes. The opening of base pair and the large propeller angle are the most obvious differences between stretching 3′5′-termini and stretch-

ing 3′-termini or 5′-termini comparing with our previous results.[17]

As seen in Fig. 3, the fluctuations of the $Y_{\rm disp}$, the shear, the slide and the shift become larger when the relative extension increases. The deviation of stagger and stretch from 0 Å to −2.4 Å shows that stretching 3′5′-termini causes the two bases in one base pair moving out of the original base pair plane and inserted to the opposite strand. This tendency of conformational transformation is not obvious in both stretching 3′-termini and stretching 5′-termini.[17] The distance between successive base pairs (rise) increases from 3.2 Å to 5.6 Å. As the variations of rotation parameters (Fig. 2), the fluctuation and variation of the base translation occur from $l = 1.2$ where the force plateau is initiated.

Comparing the results of stretching 3′5′-termini with our previous study of stretching 3′-termini and 5′-termini,[17] we find that stretching 3′5′-termini bring very large values of the angle opening and the dihedral propeller. Furthermore, we find that the stretching 3′5′-termini induces larger variations of stretch, stagger and the free energy than both stretching 3′-termini and 5′-termini did. From the variations of these parameters, we can deduce that the base pairs are disturbed and have much larger conformational variations by stretching 3′5′-termini compared with other two methods. We find that some effects of stretching 3′5′-termini on DNA conformation are similar to the effects of stretching 5′-termini for the similar profiles in the inclination, twist and roll respect to the relative extension. Especially, the successive base pairs rolls to the side of DNA major groove, indicating that the DNA molecule opens the minor groove under this stretching methods.

Suck *et al.*[29] have found that the enzyme DNase I bends the DNA to the major groove at the binding site. Our findings that stretching 3′5′-termini makes successive base pairs roll to the major groove would favor the activity of enzyme DNase I. Smith and coworkers[2,3] have found that the reaction rate of DNase I with the stretched DNA is slowed down by stretching the both 3′-termini of the DNA molecule, which is a favor with our previous findings.[17] However, we have not found any experiment to study the effects of stretching 3′5′-termini till now. We hope that there will be more experiments on this topic to examine our results, and these studies will help us to understand the protein-DNA interaction even in designing new nano devices using the DNA molecules.

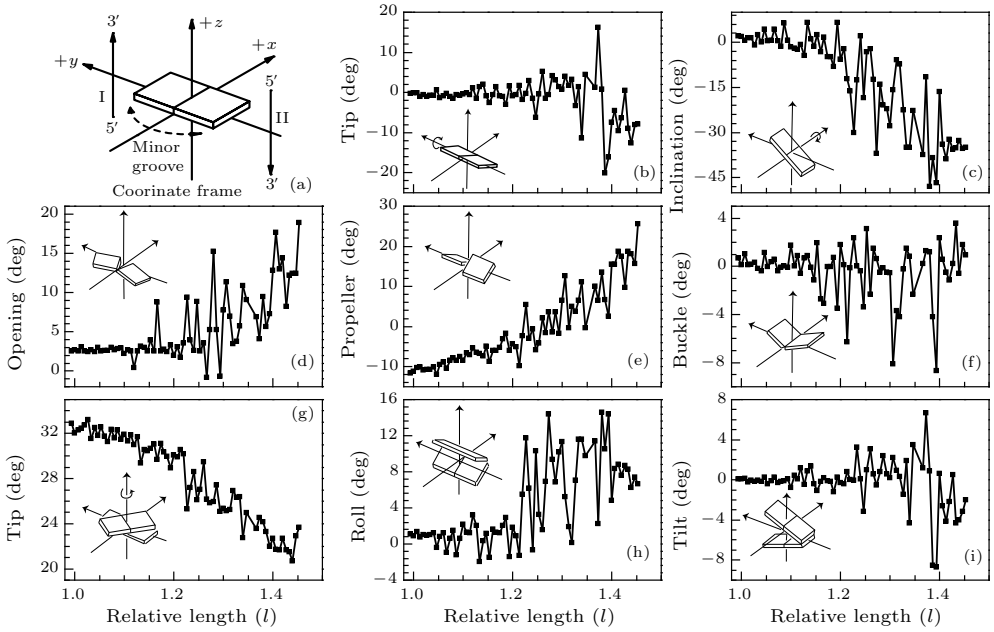

Fig. 2. Base rotation parameters versus relative extension l. (a) The standard coordinate frame; (b) tip is the angle of a base pair rotating around y axis; (c) inclination is the angle of a base pair rotating around the x axis; (d) opening is the angle of two bases in a base pair rotating in opposite directions about the z axis; (e) propeller is the dihedral angle of two bases in a base pair about the y axis; (f) Buckle is the dihedral angle of two bases in a base pair about the x axis; (g) twist is the angle between two successive base pairs about the z axis; (h) roll is the dihedral angle of two successive base-pairs about the y axis; (i) tilt is the dihedral angle of two successive base-pairs about the x axis.

In the experiments of Leger *et al.*,[4] they found that there is a second stretching force plateau at 110 pN when the DNA is kept without relax. In our simulation, the DNA molecules are allowed to relax during the stretching process, but the forces are comparable with the force of DNA stretching without relax. The discrepancy between the results from the simulations and the experiments may mainly result from the high stretching velocity in the simulation (the velocity is 6.25 mm/s in our simulation and about 2 μm/s in experiments). Notably, Lindahl *et al.*[18] showed that the much larger forces are needed when stretching at high velocities. We hope that this simulation can be renewed in the future after both the simulation method and the computer capacity are considerably improved.

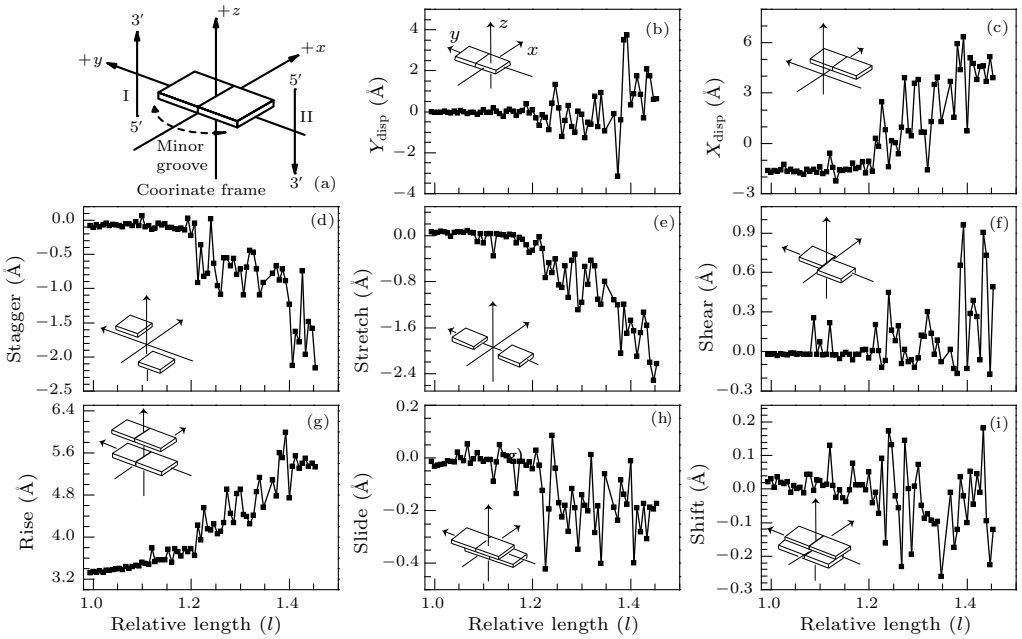

Fig. 3. Base translation parameters versus relative extension l. (a) The standard coordinate frame; (b) $Y_{\rm disp}$ is the translation of a base pair moved along the y axis in its mean plan; (c) $X_{\rm disp}$ is the translation of a base pair moving along the x axis in its mean plan; (d) stagger is the relative displacement of one base compared to the other one in a base pair along the z axis; (e) stretch is the relative displacement of one base compared to the other one in a base pair along the y axis; (f) shear is the relative displacement of one base compared to the other one in a base pair along the x axis; (g) rise is the relative displacement of one base pair compared the other one along the z axis; (h) slide is the relative displacement of one base compared to the other one in a base pair along the y axis; (i) shift is the relative displacement of one base compared to the other one in a base pair along the x axis.

In summary, the conformational variations for stretching 3′5′-termini of a 22-mer DNA have been studied by molecular dynamics simulations. Stretching 3′5′-termini by 3.5 nm required about 250 kJ/mol and the force plateau was at about 123.8 pN. The stretching 3′5′-termini leads to the large values of the angle opening and the dihedral propeller between the two bases in one base pair, the untwisting of the double helix changes from 34° to 20° and the successive base pairs rolls to the side of DNA major groove. The distances between successive base pairs increases from 3.2 Å to 5.6 Å. This study together with our previous simulation[17] give an integrated picture of the DNA conformational transformation under different stretching methods. This study may improve the understanding of the protein-DNA interaction especially the deformed DNA molecules and may be valuable to design new nano devices using DNA molecules.

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