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Mechanical behavior of double-stranded DNA at atomistic scale

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Abstract
Besides its key role in many of the major processes of cell biology, including DNA replication, DNA repair, DNA recombination, etc., DNA molecule can serve as a promising material in nanostructures. enhance the understanding of different overstretched DNA structures. In silico tensile tests were performed using all atom molecular dynamics method to study nanomechanical behavior of DNA duplex. The DNA molecule is immersed in the Monte Carlo equilibrated periodic TIP3P water bath and is elongated with various pulling speeds. The double-stranded DNA is stretched from opposite end of each strand under shear mode. Force-extension curves were obtained and atomistic structures of DNA dodecamer were investigated. The results of this study show that the rupture force of short DNA duplex is a nonlinear function of the pulling velocity. At larger extensions correspond to the first structural transition phenomenon, the average number of H-bonds is observed to be almost constant. Presented results provide a fundamental molecular understanding of the DNA stretching mechanism.

Keywords: double-stranded DNA, nanomechanics, atomistic molecular dynamics

Introduction

Molecular dynamics (MD) is one of the most common computational techniques employed to obtain mechanical properties of biomolecules (see reviews in Refs. [1] and [2]). Deoxyribonucleic acid (DNA) is a biomolecule that contains genetic information on the development and functioning of all living organisms. In order to control or predict the DNA functions under environmental stimuli, a detailed understanding of mechanical behavior of DNA molecule is necessary. The accuracy of an MD simulation of DNA depends critically on an appropriate choice of the force field parameters used in the potential energy function. These empirical parameters are derived from high-level quantum mechanical calculations on tractable molecular fragments. The simulation of a highly charged molecule, such as DNA, also requires particularly careful treatment of long-range electrostatic interactions. Typically, simulations are restricted to timescales of nanosecond. However, the method can be tailored to provide very detailed information about the key aspects of the biological processes.

MacKerrel and Lee [3] studied the stretching of a $d(ACTG)_3$ dodecamer in explicit surrounding solvent using potential of mean force calculations. Similar to Kornard and Bolonick [4], they observed an overstretching transition state and an elongation of about 60 Å (i.e., approximately 1.6 times its initial B-form length) before the complete melting take place.

Stretching a 22-mer double-stranded DNA (dsDNA) by applying force on the both 3' and 5' terminal atoms of one end (non-shear stretching mode), in NVT ensemble, resulted a novel structure in which all base pair H-bonds were broken and a remarkable base stacking was developed with the bases strongly tilted, forming a zipper-like stack on the major groove side [5]. Another non-shear stretching study on a dsDNA dodecamer also revealed that DNA can undergo 20-25% extension without any base pair breakup [6].

A combination of MD and statistical physics was used by Harris et al. [7] to calculate the configurationally entropy change in an overstretched d(CGCAAAAAAGCG) duplex. The results revealed that the DNA would unbind at extensions of ~18 Å due to the increased flexibility of highly elongated structures. A similar research on dsDNA oligomers with $d(ACTG)_3$, poly(dA), and poly(dC) sequences showed that a sequence-dependent structural transition is occurred at 25% extension and the newly formed conformation is stable towards further extension up to 50-60% [8]. Most interestingly, the calculations performed by Piana [8] showed a Na^+ counterion localized within the denaturized bubble, suggesting that open conformations can be stabilized by interactions with the solvent.

By employing an anisotropic pressure-control method and periodic boundary conditions, force-extension dependences of effectively infinite dsDNA molecules were obtained [9]. A coexistence of stretched and relaxed domains was observed during the overstretching transition. The MD simulations also revealed that strain softening may occur in the process of stretching torsionally constrained DNA.

MD simulations enabled Li and Gisler [10] to probe the effect of defects on the force-extension curve and structure of a 30-mer dsDNA at atomistic level. They observed that in the presence of a base mismatch or a nick, force-extension curves are very similar to the ones of the defect-free duplex. Nevertheless, the obtained structures at high extensions differed significantly from each other in the two cases.

A series of simulations on 30-bp [11] and 22-bp [12] dsDNA have been carried out to examine the behavior of DNA under different stretching pathways. The results demonstrate that overstretching DNA by applying the force to either both 3' termini or both 5' termini produces some clear variations in the DNA conformation which lead to higher stability of 3' stretched structure in

comparison with the 5' stretched structure, at large extensions. Qi and Li [13] completed their previous work [12] by studying the conformational variations of a DNA molecule by stretching 3′5′-termini. More recently, Garai et al. [14] reported a structural polymorphism of the S-DNA when a canonical B-DNA is overstretched under different pulling pathways.

MD simulations and quantum mechanical calculations for $d(AT)_{15}$ and $d(GC)_{15}$ dsDNAs indicated that it is possible to use the AMBER force field for a description of stretched DNA with the same confidence as for canonical DNA structures [15]. In addition, the MD simulations showed that the force-induced melting pathway for $d(AT)_{15}$ and $d(GC)_{15}$ sequences is different and is influenced by the availability of noncanonical hydrogen-bond interactions that can assist the disassociation of the DNA base pairs.

Many scientists worked on different aspects of DNA structure and function, and still, there are many secrets to unlock and keys to find as our hereditary material is being used in ever-increasing fields, from traditional medicine and food enhancement to computer chips and biomarkers. In this article, all atom MD simulations are performed to study behavior of dsDNA dodecamer under mechanical stretching. The results presented here may improve our existing knowledge about DNA mechanical properties.

Methods

MD simulations were carried out on 12 base pairs $d(ACTG)_3$ oligomers, which were constructed in a canonical B-DNA conformation. Model building were performed using *tleap* and simulations are carried out by *sander* and *pmemd* programs of the AMBER 12 suite of programs [16]. Starting coordinates were built with the NAB program distributed with the AMBER, using Arnott B-DNA fiber diffraction data [17]. In all simulations, chemical bonds involving hydrogen atoms were restrained using SHAKE algorithm. Programs VMD [18] and Chimera [19] were used for graphical visualization of the MD trajectories. Two different types of simulations were performed: Explicit solvent and steered MD.

To neutralize the negative charge on the phosphate groups of the constructed oligomer, firstly, 22 Na^+ ions were added at the 22 points of highest negative potential on a 1-angstrom resolution charge grid created around the DNA molecule (there are 22 O-P-O atom groups in the 22-mer [two O-P-O atom groups missing at the terminal] and almost 1e negative charge resides on each group). The system of DNA and counterions were then immersed in the Monte Carlo equilibrated periodic TIP3P water bath [20].

The potential which atoms of the DNA molecules experience is mathematically described by the parm99 force field with the parmbsc0 modifications [21]. Typically, a cutoff of 9 Å or 15 Å were used for the vdW interactions and the real space part of the electrostatic interactions. The PME method was used to describe the reciprocal space part of electrostatic interactions.

Prior to start pulling process, minimization, heating, and equilibration was carried out in the vicinity of 1 ns according to a standard multistage protocol [22]. The equilibration of the system consists of the following consecutive steps: I) 10 ps MD (T = 100 K) of water, II) minimize water, III) minimize all system, IV) 10 ps MD (T = 100 K) of system with restrains in DNA (k = 100 kcal/(mol.Å^2)), V) as IV but T = 300 K, vi) 25 ps MD (T = 300 K) of system with restrains in DNA (k = 50 kcal/(mol.Å^2)), VII) as VI but k = 25 kcal/(mol.Å^2), VIII) as VII but k = 10 kcal/(mol.Å^2) , IX) as VIII but k = 5 kcal/(mol.Å^2) , X) as IX but k = 0.2 kcal/(mol.Å^2) . These calculations should be performed with the nucleic acid atoms fixed or harmonically constrained to allow for the solvent to relax around the nucleic acid. Once the solvent has adequately equilibrated around the solute, the entire system, including the nucleic acid, should be energy minimized. Pulling simulation was conducted in the isothermic-isobaric ensemble, typically using an integration time step of 2 fs, else otherwise stated. The constant pressure control was executed by isotropic position scaling [23] with reference pressure of 1 atm and a relaxation time of 1 ps. Constant temperature was maintained at 300 K using Langevin thermostat with a collision frequency of 1 ps^{-1}.

Steered MD simulations [24] are used in this work to compute the forces exerted on DNA during mechanical manipulation, when DNA is elongated. This technique is widely used in computational biological science whenever a forced transition in a molecule must be observed. This approach has the advantage that it corresponds closely to micromanipulation through AFM or optical tweezers.

The DNA double helix contains two anti-parallel strands (whose orientation is conventionally defined as 5′→3′ on the basis of the sugar linkages of each nucleotide), pulling of DNA is carried out using the two 5′ termini.

Results and Discussions

Figure 1 shows variations of temperature, kinetic and potential energies, pressure, volume, and density of the explicitly solvated system, as a function of simulation time during 5' stretching process with velocity of 0.3 m/s. Discounting potential energy, the profiles of the other system properties depicted in Figure 1 fluctuate around individual constant values. Although Figure 1d show that the pressure fluctuates wildly during the simulation, the mean pressure stabilized around the desired value of 1 atm (The negative pressures correspond to a force acting to decrease the box size and the positive pressures to a force acting to increase the box size). It is expected that as the length of DNA is increased during the pulling simulation, potential energy of the system is increased too. However, Figure 1c reveals that no considerable increase in the total potential energy is occurred as the simulation proceeds (The energy increases almost with a slight slope of 6 kcal/(mol.ns) up to reach to its maximum mean value, when the rupture happens). This can be due to the fact that the whole system size is much bigger than the DNA molecule size. Convergence is indicated by the lack of significant change in the average value of the presented properties of the system as the simulation time increases.

Figure 2 shows the pulling force as a function of DNA

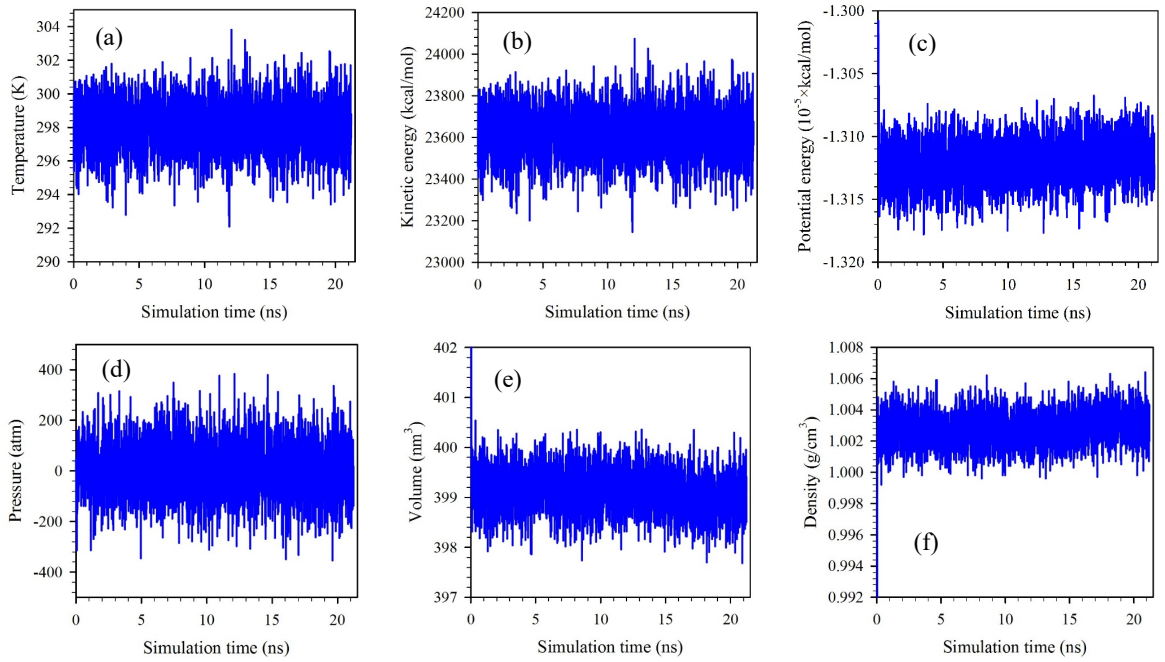

Figure 1. Variations of (a) temperature, (b) kinetic energy, (c) potential energy, (d) pressure, (e) volume, and (f) density of the explicitly solvated system versus simulation time, during stretching process.

extension obtained with stretching velocities v =10, 3, 1, and 0.3 m/s for 5' pulling of d(ACTG)$_3$ duplex. It is to be noted that pulling simulations for v = 10 m/s and v = 3 m/s has been performed with an integration time step dt = 1 fs and for v = 1 m/s and v = 0.3 m/s with dt = 2 fs. The locally-weighted scatterplot smoothing approach [25] has been used to reduce the variability and noise of the presented data. Tricube weight function to weight the data with a local polynomial of degree 2 and a sampling proportion of 0.1 for the presented data is employed. All force profiles show that as the dodecamer is slightly displaced from the canonical length, it firstly seems to behave as an elastic spring (The tensile force increases almost linearly with respect to extension of the DNA). But after this region the molecule exhibit an unusual behavior. That is "strain softening"; i.e., the force suddenly decreases from its local maximum. It is noted that there were still nonzero forces at the beginning of the stretching. This is because the DNA molecules usually curve in the native state [26], while the initial state of the DNA molecule for our simulations was assumed to be straight. It is assumed that the forces of about 10 pN at the initial state results from the requirement for straightening the DNA molecule [12].

With the highest velocity v = 10 m/s, the force increases almost linearly up to ~135 pN due to an extension of x = 1.2 nm. After this elastic region, a strain softening effect which is followed by a force plateau (structural transition state) is observed and the helix can be further extended up to about 2.9 nm at a constant mean force of 130 pN. We name the force of onset of this structural transition, "yield force" (F_y), in what follows in this text. After this transition, the force begins again to grow up and the molecule maintains an ability to resist additional strain with an increase in load. This response is usually called "strain hardening". At $x \approx 3.2$ nm, when $F \approx 200$ pN, another strain softening effect is again observed. The force then increases sharply with a nonlinear manner up to 630 pN, where an unexpected second transition happens in interval of (5.1,5.5) nm. After this short force plateau, the force again increases abruptly until reaches to its maximum, around 780 pN and subsequently the two strands of DNA commence completely unbind. This corresponds to a DNA extension of about 5.7 nm. When the DNA is stretched, it should become thermodynamically unstable and unbind as soon as the work performed by the external force becomes equal to the free energy holding the two strands together plus the work performed by the friction force (at such pulling speeds studied here, the force is not static, the simulations are in the friction regime and are not in the thermally active regime).

When the stretching velocity is decreased from 10 m/s to 3 m/s, the slope of elastic region of the force-extension curve remains unchanged. After the elastic region, a strain softening effect again is observed and the helix can be further extended up to about 3.6 nm at an almost constant average force of 110 pN. For extensions that exceed 3.6 nm, the force required to further stretch the duplex increases linearly until it reaches to a maximum value of around 440 pN at $x \approx 5$ nm. At this point a second transition is observed that can be considered as "melting transition", and then the force falls down abruptly.

For pulling velocity of 1 m/s, the yield force of DNA decreases to 100 pN, and the corresponding yield strain takes place again at an extension of about 1.5 nm. Consequently, if dsDNA is considered as a rod with effective radius of 1 nm, the Young's modulus decreases from ~100 MPa to ~80 MPa by the reduction of pulling velocity from 3 to 1 m/s. After the elastic region, a strain softening effect which is accompanied with a relatively short transition state at an average force of about 80 pN is observed. During this transition, the force initially decreases up to an extension of 2.5 nm, and then begins to grow gradually up until x reaches ~3.3 nm. Difference

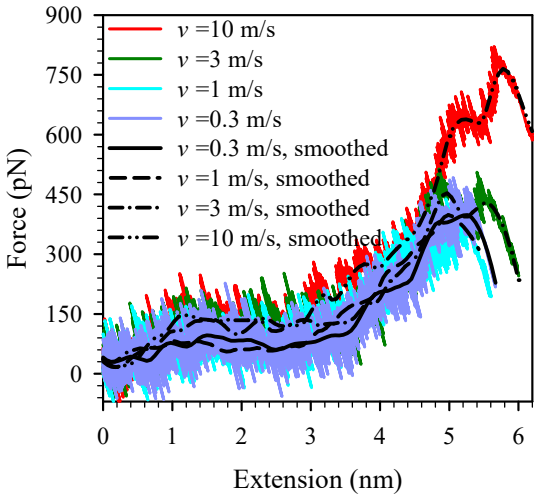

Figure 2. Pulling force of the d(ACTG)$_3$ duplex as a function of extension for different stretching velocities.

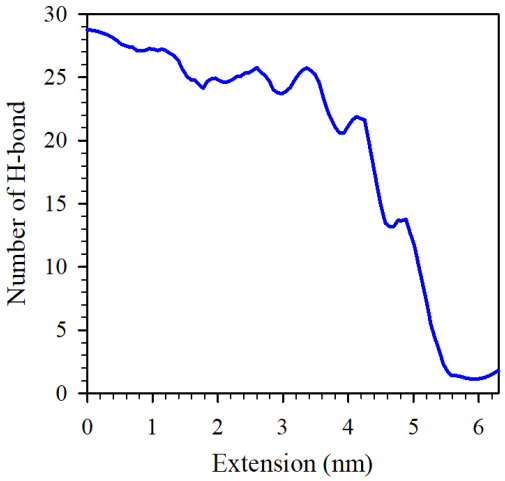

Figure 3. Variations of H-bonds between the two complementary strands upon pulling with velocity of 1 m/s versus the extension (smoothed curve).

between the minimum and maximum forces, in this transition region, does not exceed than 50 pN. At strains in the vicinity of 0.77, the force increases steeply up to ~300 pN (with slope of about 210 pN/nm), where an unexpected second transition with a length of ~5 Å occurs. After this step, at x = 4.8 nm, the force again increases abruptly until reaches to its maximum, around 360 pN at $x \approx$ 5.2 nm and then the two strands commence completely unbind. The simulations with the pulling velocity of 0.3 m/s result in a force- extension curve close to one obtained for v = 1 m/s, except that a) the forces near the end of the first transition are rather smaller, and b) the second transition occurs at a lower force of ~200 pN with a distinguishable slope. In consistent with the experimental results [27-30], the results of this study show that the rupture force of short DNA duplex is a nonlinear function of the pulling velocity.

Although the pulling rates provided by the current experimental setups are not high enough to reach the overstretching regime for dsDNA molecules shorter than 30 bp, the presented results can be used for interpretation of the experimental results which may be obtained in future under higher pulling velocities. In addition, since the force-extension profiles of long length DNAs obtained from the pulling experiments are qualitatively similar to those of short DNAs obtained in silico under high pulling rates, the presented results may be also used to interpret the experimental results for the long DNA molecules.

A good measure of the overall stability and structural integrity of DNA during stretching is the fraction of native WC H-bonds present as a function of relative stretch. Figure 3 shows the evolution of the number of H-bonds between paired complementary bases of the d(ACTG)$_3$ duplex, under 5'5' pulling at 1 m/s velocity, with increasing extension. The geometrical criterion is used to define a H-bond, when the donor-acceptor distance is less than 3.5 Å and the angle is less than 30°.

For small x < 1.4 nm corresponding to the initial, strong increase in the force-extension curve, the number of H-bonds remains almost constant and only one H-bond is disrupted. In short interval of (1.3,1.8) nm the average number of H-bonds suddenly decreases by ~3. That is in vicinity of where the strain softening initiates (see Figure 2). At larger extensions correspond to the first structural transition phenomenon, the average number of H-bonds is observed to be almost constant. After 3.3 nm any further extension leads to the loss of H-bonds with a rate of around 11 H-bonds per nm, until unbinding takes place. The final stretched conformation, at unbinding boundary, has only ~20% of its H-bonds remaining. As the H-bonds in the duplex are almost entirely localized between the complementary nucleobases, the reduction of number of H-bonds indicates the loss of base pairing with increasing force. A more precisely look at Figure 3 indicates that near x = 4.2 nm, where the short plateau in the force-extension curve initiates, the decreasing trend of number of H-bonds, temporarily, is replaced by a small increase.

Visual inspection of the MD trajectories provides a better understand of the presented numerical result. Figure 4 shows DNA configurations at several key stages of the elongation process. It is seen that neglecting end effects, for extensions lesser than 1.4 nm (i.e., before the structural transition take place) configuration of the specimen does not change significantly from the normal B-DNA. However, as the simulation proceeds, the stretching wave begins to propagate from the two ends of molecule. Despite the WC hydrogen bonding between bases A_1 and T_{24} is weaker than that is formed by G_{12}:C_{13}, Figure 4 illustrates that A_1:T_{24} bp is considerably more stable than G_{12}:C_{13}. The main reason for this unexpected manner of the boundary bps can be stronger hydrophobic interaction between A_1 (with two rings) and C_2 bases toward C_{13} (with one ring) and A_{14} bases. The slight change in original conformation of the molecule would imply its elastic behavior up to beginnings of the force plateau region.

During the structural transition (1.4 < x < 3.4 nm with v = 1 m/s) elongation is mainly due to unwinding the double helix which is accompanied with inclination of bps (see Figure 4c-e). The native WC H-bonds between a given base pair lie along the plane formed by the base pair, so as the base pair becomes more inclined, so do the H-bonds. As the bases become more inclined, a greater

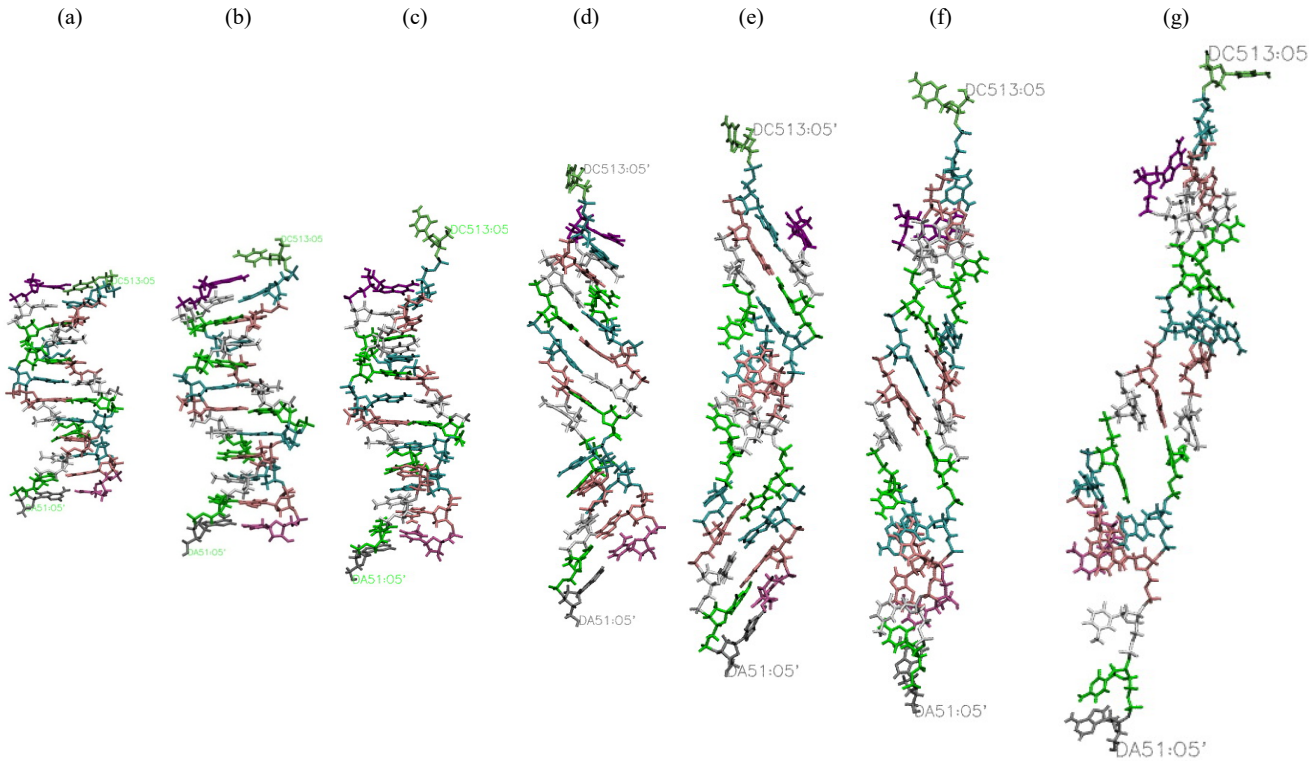

Figure 4. Snapshots of molecular configuration at different characteristic stages of extension: (a) $x = 0.0$ nm (after the equilibration step), (b) $x = 0.7$ nm, (c) $x = 1.4$ nm (at the beginning of the structural transition), (d) $x = 2.4$ nm, (e) $x = 3.4$ nm (at the end of the structural transition), (f) $x = 4.2$ nm, and (g) $x = 5.2$ nm (near the rupture point). The pictures refer to pulling simulation with $v = 1$ m/s.

portion of the stretching force acts upon the H-bonds, increasing the probability they will rupture.

The snapshots shown here also illustrate with entrance of the DNA to the transition region a cavity is formed in the middle part of the molecule where one or more of the H-bonds between complementary bases break spontaneously. Along with increase in the DNA length, number of the cavities and also their size increase. The velocity of this phenomenon increases especially after the structural transition stage. The kinetics associated with the growth and motion of the breathing (unpaired regions) in partially melted DNA is expected to be very slow, a lot slower than the structural rearrangements that take place before the structural transition. Therefore, although ssDNA might be the most stable structure in the transition state, the new phase forms slowly on the timescale of the unbinding simulations. Consequently, larger forces are required to unbind the molecule than would be expected from equilibrium thermodynamics (which assumes the experiment is performed infinitely slowly). The effect is more pronounced at faster pulling rates, so larger forces are required, as has been observed experimentally. This is because more irreversible work must be done against molecular friction if the experiment takes place further away from the equilibrium regime. Figure 4f and Figure 4g indicates that for extensions larger than 3.4 nm (i.e., beyond the structural transition) the duplex become almost entirely unwound and the elongation is mostly due to the creepage of the two strands on each other.

Conclusions

Along with the rapid growth of computer power, introducing more realistic force fields, and improvement in simulation programs, in recent years, MD is being turned into a potent method for studies of bio-systems at the atomic level. Atomistic MD simulations were employed to investigate the mechanical behavior of dsDNA molecule subjected to external tensile loadings. Additional simulations were conducted to study the effect of pulling velocity on the mechanical properties of dsDNA at high stretching rates. In consistent with the experimental results obtained under low stretching rates, the results of this study show that the rupture force of short DNA duplex is a nonlinear function of the pulling velocity. Since the force-extension profiles of long length DNAs obtained from the pulling experiments are qualitatively similar to those of short DNAs obtained in silico under high pulling rates, the presented results may be also used to interpret the experimental results for the long DNA molecules. Structural snapshots at the atomistic level adopted by B-DNA under the influence of external force obtained providing further insight into the structural aspects. At larger extensions correspond to the first structural transition phenomenon, the average number of H-bonds is observed to be almost constant. This discovery of DNA's structure and mechanical behavior opens new doors for scientists, becoming the key to molecular biology and modern biotechnology.

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