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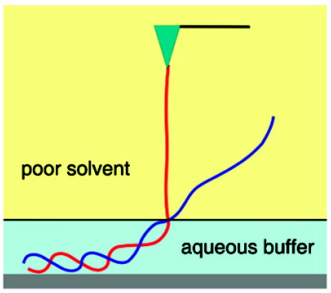
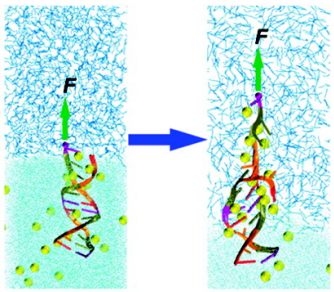


AFM pulling



MD simulation

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Double-Stranded DNA Dissociates into Single Strands When Dragged into a Poor Solvent

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Abstract: DNA displays a richness of biologically relevant supramolecular structures, which depend on both sequence and ambient conditions. The effect of dragging double-stranded DNA (dsDNA) from water into poor solvent on the double-stranded structure is still unclear because of condensation. Here, we employed single molecule techniques based on atomic force microscopy and molecular dynamics (MD) simulations to investigate the change in structure and mechanics of DNA during the ambient change. We found that the two strands are split apart when the dsDNA is pulled at one strand from water into a poor solvent. The findings were corroborated by MD simulations where dsDNA was dragged from water into poor solvent, revealing details of the strand separation at the water/poor solvent interface. Because the structure of DNA is of high polarity, all poor solvents show a relatively low polarity. We speculate that the principle of spontaneous unwinding/splitting of dsDNA by providing a low-polarity (in other word, hydrophobic) micro-environment is exploited as one of the catalysis mechanisms of helicases.

Introduction

DNA typically forms a double helix in the physiological environment. The double helix structure is essential for the long-term stability of information storage. Moreover, the double helix structure provides the redundancy of information, enabling the repair of damaged segments in one chain of double-stranded DNA (dsDNA).

To read DNA sequences, the strands of the double helix need to be separated. It is well-known that the mechanical stability of dsDNA strongly depends on the ambient conditions.[1,2] It is also known that water molecules have been shown to play an important role in the internal conformation of DNA strands. But, it is difficult to discern effects of water molecules in aqueous media.[3] DNA is soluble in some nonaqueous solvents (such as formamide) and becomes denatured into single-stranded DNA (ssDNA), just as it is treated with a high concentration of denaturant aqueous solution.[4] Besides these, more organic solvents are poor solvents for DNA. Recent studies revealed that DNA condenses into toroids in poor solvents.[5−7] Other detailed information on DNA in poor solvents is still unclear.

An interesting question is whether the poor solvent[8] environment has an impact on base pairing in the dsDNA, in particular if such a change of ambient conditions leads to strand separation. If so, one could envisage certain kinds of DNA-splitting proteins acting as molecular machines, which separate DNA strands by altering the local micro-environment from DNA-philic to DNA-phobic. This is reasonable since it is presumed that changes in the environment are a common mechanism for many enzymes.

Here, we investigated the impact of the environment change on double-strand stability by two complementary single molecule techniques: atomic force microscope (AFM)-based single molecule force spectroscopy (SMFS) and molecular dynamics (MD) simulations. We prepared a dsDNA sample at a hydrophilic surface and exchanged the aqueous buffer with a poor solvent. The mechanical properties of DNA were measured by stretching the DNA with the cantilever of an atomic force microscope and recording the force−distance relation. Likewise, the change in molecular organization and its inherent dynamics were followed and analyzed in MD simulations at an interface of water/poor solvent.

Throughout the years, AFM-based SMFS has evolved into a powerful tool to investigate molecular mechanics at the single-

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molecule level. New insights could be attained ranging from biophysics to material sciences.[9−14] For example, the mechanical behavior of dsDNA has been directly investigated by SMFS at the single-molecule level, revealing the plateaus in the force-extension curve (force curve) that are now known to be a fingerprint for the force-induced melting of dsDNA.[15−18] Further studies showed apparent differences between the mechanical properties of ssDNA and dsDNA at the single-molecule level.[2,15,16] Thus, SMFS can be exploited to detect the status of DNA in terms of the molecule being single-stranded or double-stranded. One of the key features of AFM is that the observed object can be examined in different environments. Therefore, in the current study, AFM offers a unique option to mimic the process of ambient change as it may occur under the physiological action of helicases: dsDNA can be dragged into a poor solvent, and the change of the structure can be determined.

To interpret the experiments in structural terms, MD simulations were carried out. In these simulations, one strand of dsDNA was dragged from water to poor solvent. The results from both experimental and theoretical studies provide new insight into DNA separating mechanisms.

Experimental Procedures

Materials and Sample Preparation. λ-BstE II digest DNA was purchased from Sigma.[2] For the preparation of dsDNA samples, the DNA was used as received and diluted with 1×SSC buffer (150 mM NaCl, 15 mM sodium citrate, pH 7.0 aqueous solution) to a final concentration of 1.5 μg/mL. The dsDNA was allowed to adsorb physically onto an amino-functionalized glass slide (Quantifoil Micro Tools GmbH) or a freshly evaporated gold surface for 10 min, followed by rinsing with the SSC buffer thoroughly to remove excess dsDNA from the sample.[13,16] The customized ssDNA sample was purchased from IBA GmbH. The ssDNA sample (0.1 mM) was diluted 20 000 times in PBS buffer to a concentration of 5 nM. The sample preparation of ssDNA is similar to that of dsDNA. All the other chemical reagents were purchased from Sigma or Fluka and were analytically pure.

Force Measurements. The dsDNA sample was mounted in the AFM instrument (MFP-1D, Asylum Research). Prior to the measurements, a drop of liquid was introduced between the V-shaped Si_3N_4 AFM cantilever (Veeco Instruments Inc.) and the sample. Then, during the AFM manipulation, the data were collected at the same time and converted to force-extension curves later. The spring constant of the AFM cantilevers was measured by a thermo-excitation method,[19] ranging from 10 to 30 pN/nm. The stretching velocity applied in this study was 2.0 μm/s if not mentioned otherwise. The details of the AFM instrumentation can be found elsewhere.[20,21]

MD Simulation on Pulling dsDNA. The simulation system was composed of ∼37 000 atoms (∼60 Å × 60 Å × 140 Å) in simulation A, including 7368 water molecules, 551 octane molecules, and a 12-bps double-stranded B-form DNA (ACCGGTACCGGT); 24 sodium and 2 chloride ions were added to neutralize the 22 charges of B-form DNA (the aqueous phase is actually a ∼ 0.01 M salt solution). In simulation B, the system included an additional 551 octane molecules, amounting to ∼52 000 atoms (∼60 Å × 60 Å × 210 Å). Each simulation was performed in the NPT ensemble (1 atm and 310 K) using the program NAMD[22] and the CHARMM27 force field,[23] with an integration time step of 1 fs and periodic boundary conditions; van der Waals (vdW) energies were calculated using a smooth (10−12 Å) cutoff; and the particle-mesh Ewald method[24] was employed for full electrostatics. After energy minimization and a 1 ns equilibration of the simulation system, the dsDNA was pulled vertically from water to octane solvent by attaching a harmonic spring (with a force constant of 2 kcal/mol Å^2) to the terminal O3′ atom on one strand of the dsDNA and pulling the other end of the spring at a constant speed of 5 Å/ns (in simulation A) or 2 Å/ns (in simulation B), perpendicular to the interface between water and octane. During the pulling process, the center of mass of all octane was constrained to avoid shifting of the octane layer.

Results and Discussion

In a typical SMFS experiment, the cantilever tip picked up a molecule, which was pinned by nonspecific absorption on the surface.[16] While retracting the cantilever from the surface, the sample was set under force until the applied force was large enough to break the attachment. From such a force curve, the required force for a given end-to-end distance could be extracted. These data serve as an intrinsic fingerprint for the entropic and enthalpic properties of the molecule and, in general, are in very good agreement with simulations or ab initio calculations.[25,26]

Structure of dsDNA in Water and Poor Solvent. To confirm that DNA exhibits a double-stranded structure in common aqueous environments, force measurements were initially carried out in 1×SSC buffer. dsDNA absorbed on the amino-modified glass slide was picked up by the cantilever tip and retracted from the surface (see Figure 1A). At a force of approximately 65 pN, the dsDNA underwent a structural transition resulting in an extended plateau at constant force. At a length of about 600 nm, one strand began to dissociate, which resulted in an additional lengthening of the sample. Finally, the backbone of the ssDNA was stretched, resulting in a steep increase in the force upon further extension. At a force of roughly 350 pN, the one end of the DNA detached from the surface, which resulted in a sharp drop of the force. The two characteristic plateaus in the force curves correspond to the force-induced dsDNA melting upon stretching, which agrees well with previous observations.[2,15−18]

In the following experiment, we repeated the manipulation with an identical sample slide but changed the liquid environment from the SSC buffer to a poor solvent, diethylbenzene (DEBenzene). By picking up the dsDNA at one strand with the cantilever tip, the DNA can be stretched in the poor solvent. In nearly all force curves obtained in such experiments, we found that the characteristic plateaus, which we had measured in an

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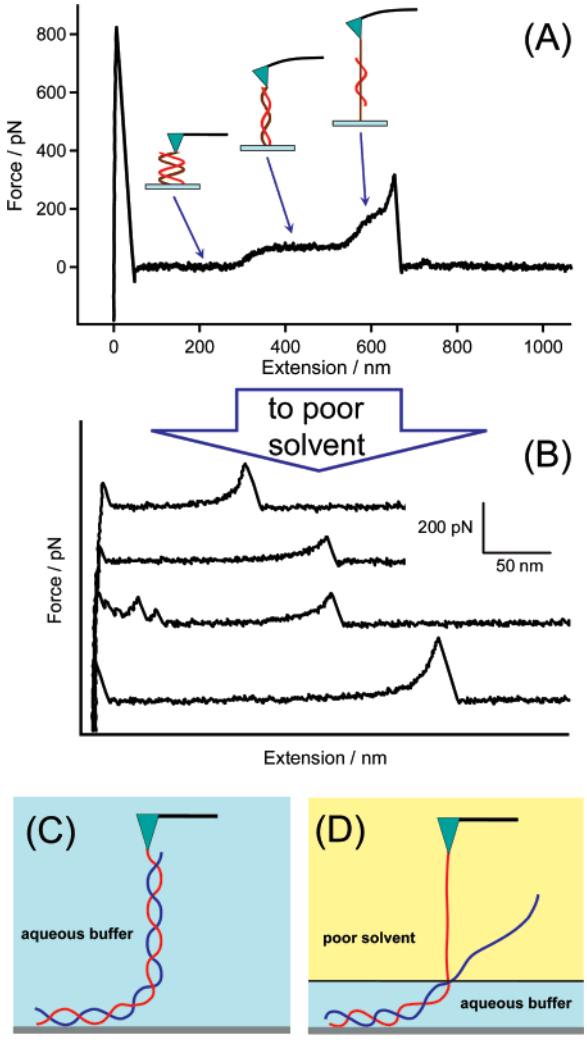

Figure 1. Pulling dsDNA in aqueous buffer and poor solvent. We prepared one dsDNA sample on an amino-modified glass for two sets of experiments that only differed in the applied liquid environment around dsDNA. In panel A, the environment is SSC buffer, while in panel B, it is DEBenzene. Also shown are schematic presentations of dsDNA being stretched in (C) aqueous micro-environments and (D) from aqueous (adsorbed water film) to poor solvent environments.

aqueous environment, were lacking as shown in Figure 1B. The force was found to rise monotonically with extension, corresponding to the increasing restoring force during elastic elongation,[27] as would be expected for ssDNA. In the following discussion, we will confirm that the measured force curves can be ascribed to ssDNA and not to novel behavior of dsDNA in a poor solvent environment.

Since DEBenzene is capable of aromatic stacking interactions with the base pairs of DNA, one might argue that the observed effect is not due to the general poor solvent property (i.e., low polarity) but rather to specific interactions with the solvent (i.e., the solvent competing for base pair stacking). To test this hypothesis, another poor solvent for DNA, 1-propanol, was used. 1-Propanol is not capable of aromatic stacking interactions but possesses a hydroxyl group potentially being able to form hydrogen bonds. In this regard, the solvent is more similar to water but has a much lower polarity. The obtained results in 1-propanol resemble those in DEBenzene. Thus, the observed

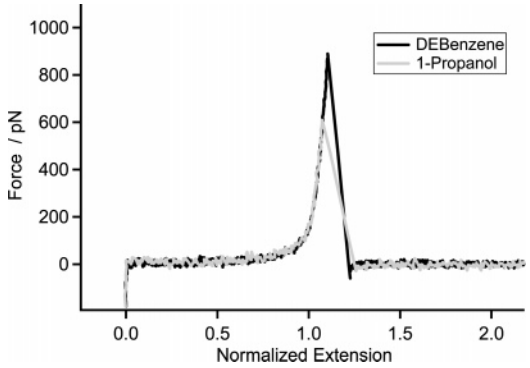

Figure 2. Normalized force curves obtained from a dsDNA sample in 1-propanol (gray line) and DEBenzene (black line).

effect in DEBenzene is not caused by aromatic stacking or by the inability of DEBenzene to form hydrogen bonds but indeed by the general poor solvent effect (low polarity) of the medium. Figure 2 compares the normalized force curves obtained in different poor solvents, demonstrating their similarities.

Interactions between the amino-modified surface and the adsorbed polymers might lead to unexpected effects.[28] Therefore, we additionally performed an experiment with the dsDNA sample on a freshly evaporated gold substrate in 1-propanol, holding the other parameter (i.e., the liquid medium) constant. However, we observed no evident difference between the results obtained from amino-modified and the results obtained from gold substrates.

The measured force curves imply that we are observing ssDNA. To strengthen this conclusion, we compared our force curves to a theoretical prediction of the stretching behavior of ssDNA. Following the pioneering works by Flory and Bueche,[29,30] researchers have successfully developed several physical models to describe the behavior of single macromolecules upon stretching.[9,10,31] To compare our experimental data to the predictions, we employed the freely rotating chain (FRC) model combined with the molecular mechanical parameters of ssDNA obtained from an advanced ab initio quantum mechanical (QM) calculation (QM-FRC model).[25,26] In the QM-FRC model, the relationship between the extension of the macromolecule (R_z) and the stretching force (F) can be written in a good approximation as

$$R_z/L_0 = (L[F]/L_0)[1 - k_BT/(2bF)] \quad (1)$$

where $L[F]$ is the force dependent contour length, L_0 is the contour length at zero force, b is the length of the rotating unit, k_B is Boltzmann's constant, and T is temperature.[25,26]

The molecule calculated in QM is set to be in a vacuum condition. The interactions between the common organic solvent molecules and the contacted molecules are vdW interactions in general, which are the weakest intermolecular interactions. Accordingly, the solute molecules' behavior should be close to that of the solute molecule calculated in a vacuum.

Figure 3A shows the theoretical force curve of ssDNA according to the QM-FRC model and also the normalized

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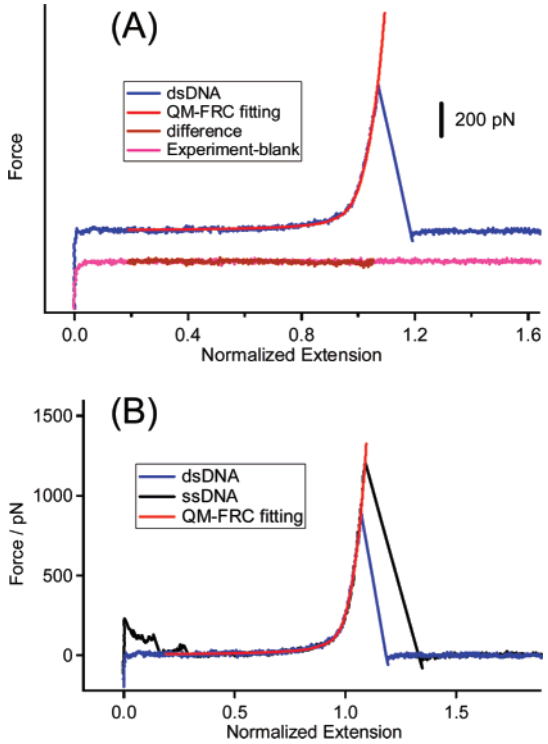

Figure 3. (A) Force curve according to the QM-FRC model (red line) and the normalized experimental force curve obtained from a dsDNA sample in poor solvent (blue line). The difference (brown line) between experimental force curve and fitting curve is very small and should be due to the noise of the experimental curve. The experimental blank curve (magenta line) is plotted to show the high quality of the fitting. For clarity, the force curves are offset. (B) Normalized force curves of denatured dsDNA (blue line) and ssDNA (black line), both obtained on an amino-modified substrate in 1-propanol. The QM-FRC fitting curve of ssDNA is also shown as a reference (red line).

experimental force curve obtained from a dsDNA sample in poor solvent. The perfect superposition of the two curves for the entire force range corroborates our assumption that the observed force curves in organic poor solvents refer to ssDNA.

As a further corroboration, we measured the force curves of DNA in a denaturing environment. Guanidine chlorate (Gua) aqueous solutions are often used as denaturants for proteins and dsDNA. Each Gua molecule has three amino groups, which allow it to form multiple H-bonds with DNA. We used an 8 M Gua aqueous solution, in which dsDNA was denatured into ssDNA. We found that the force signal of dsDNA under 8 M Gua was similar to that under poor solvents.

Although we have demonstrated that dsDNA would be denatured into ssDNA in poor solvents by several supporting results, a direct comparison with the experimental data of ssDNA will be more convincing. Therefore, force measurements were conducted in 1-propanol with ssDNA on an amino-modified glass slide. To avoid complicated structures (such as loops and hairpins) within the ssDNA chain, we used a customized oligomer ssDNA sample containing 176 bases with a random sequence of 1:1 thymine (T)/cytosine (C). Figure 3B shows the normalized force curves of dsDNA and ssDNA both obtained on an amino-modified substrate in 1-propanol. The perfect superposition of the force curves provides direct evidence that dsDNA is denatured into ssDNA in poor solvents.

It is worth noting that many steps in dsDNA isolation and preparation are carried out in organic poor solvents that seem to leave the dsDNA structure intact. We rationalize this as follows. The dsDNA is denatured in procedures conducted in poor solvent. However, the ssDNA strands are sterically held together by random coiling of the denatured single strands. When the DNA is brought into aqueous buffer again, it will reverse the denaturation process quickly due to the spatial closeness of both strands. This indicates that the unwinding caused by poor solvents is completely reversible once the DNA is again in water.

Because of the hydrophilicity of the amino-modified slide, a thin water film of up to several 10 nm remains on the surface[32] and keeps the adsorbed dsDNA in an aqueous environment. This water film and the integrity of the dsDNA at the surface were convincingly demonstrated by STM imaging of DNA on mica where the conductivity of the water film and its modulation by DNA were employed for contrast formation.[33] The interaction with the surface was found to slightly destabilize the dsDNA locally but left the double-stranded structure intact. For the case of 50% relative humidity and 25 °C, the thickness of the water film can be several nanometers,[32] which is larger than the diameter of dsDNA. The adsorbed water film is expected to remain intact when a water nonmiscible organic solvent (such as DEBenzene) is layered onto the sample surface. Therefore, dsDNA is actually dragged from water to the poor solvent in the force measurement (see Figure 1D). Note that the stretching velocity is 2.0 μm/s, such that the time for one base pair of dsDNA to go across the water/DEBenzene interface should be very short. The fact that we only obtained force curves corresponding to ssDNA in DEBenzene implies that the denaturation process is very fast. Because of the limited time resolution of SMFS, we resorted to MD simulations to investigate the kinetics of the structure transition at the water/poor solvent interface.

MD Simulation Pulling dsDNA from Water to Poor Solvent. MD simulations pulling dsDNA from water into a poor solvent (octane) demonstrated initiation of dsDNA strand separation. A first simulation (simulation A) pulling the dsDNA at a speed of 5 Å/ns lasted about 19 ns (see Figure 4A); a second simulation (simulation B) pulling the dsDNA at a speed of 2 Å/ns lasted about 60 ns (see Figure 4B). In both simulations, the dsDNA moved smoothly through water first, as seen in molecular graphics and revealed from the relatively stable and small forces needed to pull on the terminal O3′ atom of the DNA (see upper diagrams in Figure 5 and the movie in the Supporting Information). At about 5 ns in simulation A, or at about 21 ns in simulation B, as the O3′ atom crosses the interface between water and octane, the forces begin to increase, suggesting a resistance of DNA to the poor solvent environment.

In simulation A, as one DNA strand was forced into the poor solvent (at about 5 ns), the other strand seems to lag behind. This can be clearly discerned in Figure 5 from the gradually increasing separation between the terminal O3′ and the terminal O5′ atoms on the two strands. During this process, the helical twists of the DNA become reduced. However, a transition to strand separation of the dsDNA did not happen until about 17 ns (see Figure 4A). At this moment, the terminal end of the lagging DNA strand swiftly shifted away from that of the

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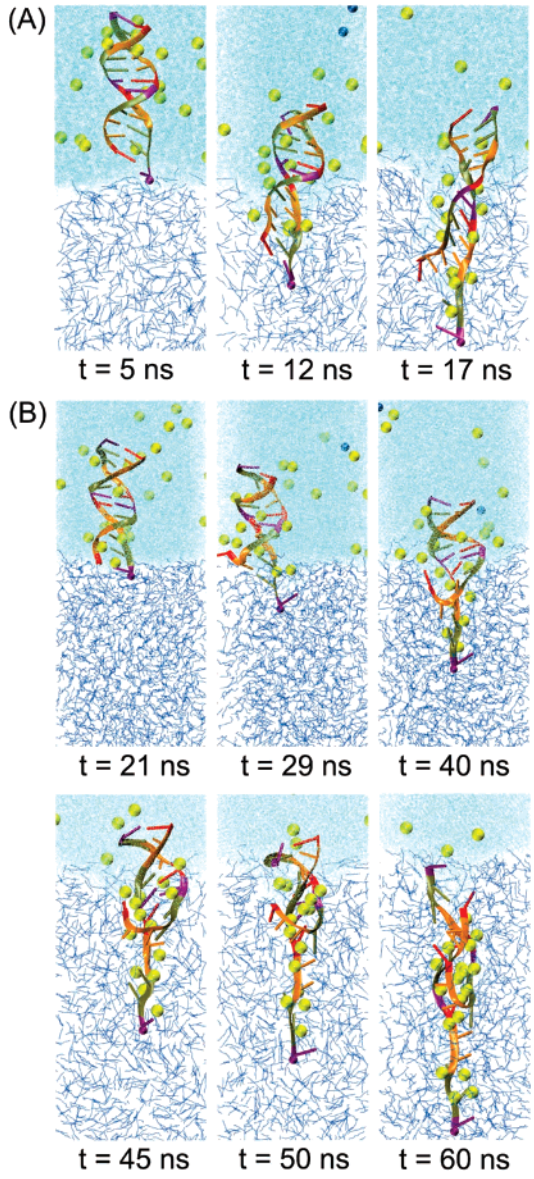

Figure 4. MD simulation pulling dsDNA from water to octane. The dsDNA is shown in cartoon presentation and is colored according to base type (A, red; T, purple; C, orange; and G, tan); water is shown in cyan (solvent presentation), while octane is shown blue; sodium and chloride ions are presented as yellow and blue vdW spheres, respectively. A harmonic spring (with a force constant of 2 kcal/mol Å^2) was attached to a terminal O3′ atom (shown as a purple vdW sphere) on one strand of the dsDNA and was pulled down (along the *z*-axis) at a constant speed of 5 Å/ns (A) or 2 Å/ns (B). In simulation A, there are three snapshots taken at times $t = 5$, 12, and 17 ns, with strand separation of the dsDNA occurring at about $t =$ 17 ns. In simulation B, there are six snapshots taken at times $t = 21$, 29, 40, 45, 50, and 60 ns, with strand separation of the dsDNA starting at about $t = 21$ ns.

leading strand, with the terminal base pair separated. This can also be recognized in the middle and lower diagrams in Figure 5A, as the trace of the O5′ atom shows a sudden kink, and the separation between the two terminal atoms jumped from below 20 Å to over 30 Å. The DNA pulling was stopped at about 19 ns as the terminal end of the leading DNA strand approached the other boundary of octane.

In simulation B, conducted at a slower pulling speed, once one DNA strand was forced into the poor solvent (at about 21 ns), a transition to strand separation of the dsDNA started (see Figure 4B). This is also revealed from the first kink appearing

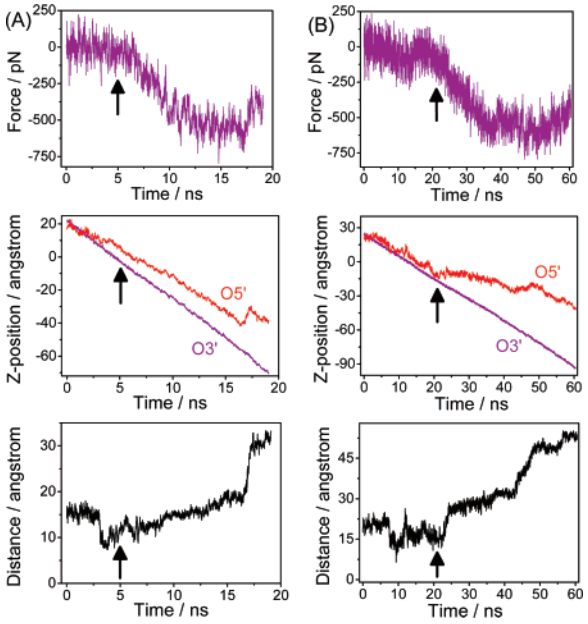

Figure 5. Measurements from the MD simulation pulling dsDNA from water to octane at a constant speed of 5 Å/ns (A) and 2 Å/ns (B). For both simulations (A and B), the upper images show the force applied to the terminal O3′ atom (seen as a purple vdW sphere in Figure 4) vs simulation time; the middle images show the positions of the two DNA termini along the pulling direction (*z*-axis) vs simulation time, purple representing the terminal O3′ atom on one strand, and red representing the terminal O5′ atom on the other strand; the lower images show the separation between terminal O3′ and terminal O5′ atoms vs simulation time. In simulation A, the water−octane boundary crossing of the O3′ atom at about $t = 5$ ns is indicated by an arrow; the kinks seen in the curves in the middle and lower diagrams, at about $t = 17$ ns, correspond to the initiation of dsDNA strand separation. In simulation B, the water−octane boundary crossing of the O3′ atom at about $t = 21$ ns is indicated by an arrow; the kinks seen in the curves in the middle and lower diagrams, at about $t = 21$ ns, correspond to the initiation of dsDNA strand separation.

in the middle and lower diagrams of Figure 5B. Comparison between simulation A and simulation B suggests that once the pulling speed is slow enough, the strand separation can actually occur right at the interface where the dsDNA is forced to cross from water into octane. Continued pulling in simulation B demonstrated further separations of the dsDNA, up to about five base pairs, near the boundary (see Figure 4B), which can also be recognized from the drastic separations of over 50 Å between the O3′ and the O5′ atoms in Figure 5B. On the basis of the simulation, therefore, one may presume that a complete strand separation would occur at the interface as the dsDNA is pulled slowly enough (as in the case of the AFM measurements described previously in this paper, where a stretching velocity of 2×10^{-5} Å/ns is used typically) from water to poor solvent.

We note that due to limited computation time (simulation B required several weeks), the MD simulation pulling dsDNA from water to octane, even at the lower speed of 2 Å/ns, was still conducted relatively quickly (within tens of nanoseconds) by applying very large forces (hundreds of pN), which may not replicate exactly what happens in experiment. However, the observations and measurements from the MD simulation clearly show the tendency of strand separation of dsDNA, supporting the suggestion that dsDNA would be forced to separate its strands as it is pulled to cross the interface from water to poor solvent. Besides, it is unlikely that the strand separation in octane is mainly due to friction. The reason is that pulling DNA through

water does not separate the strands and that the viscosity of octane is actually smaller ($\sim 5 \times 10^{-4}$ Pa s at room temperature) than that of water ($\sim 9 \times 10^{-4}$ Pa s), such that a frictional effect would more likely separate strands in water than in octane, while the simulations do not show such behavior.

Furthermore, the MD simulations may answer the following two questions: salt being insoluble in organic solvents, when dsDNA is dragged from water to poor ambient solvent, will the counterions condense around the DNA chains, or will they not pass the water/poor solvent interface? How does octane interact with DNA specifically? As one can see from Figure 4 with regard to the first question, the counterions tend to condense around the DNA chains. This is energetically more favorable since it will neutralize the charges from the phosphate groups of DNA. With regard to the second question, the simulations reveal that octane forms a wide cavity around the DNA, leaving space for some water and ions to become close to DNA, but not contacting it much. Interestingly, when placing the DNA directly into octane and equilibrating the system, the DNA elongates and untwists within tens of picoseconds, changing from B-DNA to S-DNA. A movie showing this behavior is provided in the Supporting Information.

Preliminary Energy Analysis of dsDNA Unwinding in Poor Solvent. It has been reported that DNA can develop different behaviors in good solvents: in glycerol and ethylene glycol, DNA maintains the double-stranded structure, whereas in dimethyl sulfoxide (DMSO) and formamide, dsDNA is denatured into ssDNA.[4] However, in the present study, we find that DNA tends to be denatured in all poor solvents, such as DEBenzene, 1-propanol, and octane. One remarkable contrast between the good and the poor solvent for DNA is that all the good solvents have very strong polarities, while all the poor solvents have much weaker polarities. This is reasonable since the structure of DNA is also of strong polarity. In a previous study, we have found that water has a relatively low affinity for DNA.[26] It is reasonable that when the affinity between DNA and good solvent is much stronger than that between DNA and water, DNA is likely to be denatured. For good solvents that have an affinity close to water, the double-stranded structure would be maintained. Another important contribution to the stabilization of the dsDNA structure comes from solvophobic interactions (for water, these are hydrophobic interactions). It is well-known that water, glycerol, and ethylene glycol all have strong solvophobic interactions.[34] Therefore, DNA can maintain the supramolecular structure in these three good solvents.

The interaction between poor solvent and DNA is mainly of the vdW type. The corresponding very weak affinity should not interfere with the binding forces within the DNA helix, such as arise in H-bonds and in aromatic stacking. However, solvophobic interactions are absent due to the nature of the poor solvent of DNA. In the past decade, the unzipping force (i.e., the overall binding force) of dsDNA in aqueous buffer was detected to be as low as 9−20 pN.[16] However, the hydrophobic effect may generate a higher force.[35,36] It is expected that the double helix will be unstable if the hydrophobic (solvophobic) interactions are absent. In such a case, the thermal fluctuation at room temperature ($1\ k_BT$) could be strong enough to destroy the supramolecular structure of dsDNA.

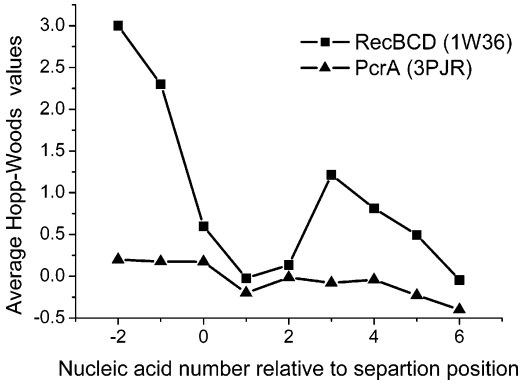

Figure 6. Average hydrophilicity values (Hopp−Woods index) of the helicase (PcrA, PDB ID: 3PJR and RecBCD, PDB ID: 1W36) pocket as a function of the DNA nucleic acid number relative to the separation position. The structural data are captured when the helicases are hosting DNA. The lower averaged Hopp−Woods values are indicative of a relative hydrophobic ambient close to the corresponding nucleic acid position.

Potential Implications for Helicases. Our experimental and MD simulation results show that the only precondition for separating two strands of DNA is to pull DNA from water into a poor solvent environment. For the situations when a nonliquid environment is provided, it is reasonable to assume that relatively low polarity and high hydrophobicity of the environment are equivalent. Such an environmental change can potentially furbish a mechanism for an enzymatic protein to access the base pairs by changing the local micro-environment of DNA. To corroborate that a change of hydrophobicity of the environment also takes place in helicases, we analyzed the structural data of two DNA−helicase complexes. To this end, the environment of the nucleic acids was investigated while the dsDNA was separated by the helicase. The average hydrophobicity values (using the Hopp−Woods index) for the surrounding amino acids (within a distance of 0.8 nm between helicase and DNA) were calculated and plotted against the numbered nucleic acids (see Figure 6). At the position where the dsDNA is separated (i.e., zero of the abscissa in Figure 6), the local environment of the helicase changes to relative hydrophobic values. Thus, the helicases provide a relatively hydrophobic micro-environment for the dsDNA. Therefore, it is very possible for dsDNA to unwind into a ssDNA segment at the binding site spontaneously at physiological temperature.

Conclusion

In summary, by means of AFM-based single molecule force measurements we discovered that at room temperature, dsDNA is denatured into ssDNA in organic poor solvents where the solvophobic effect is absent. The interpretation of the measurement data is confirmed by QM-FRC model fitting, a direct comparison with ssDNA experimental data, and by MD simulations that drag dsDNA from water to poor solvent. The poor solvents of DNA are similar with regard to low polarity. The principle of spontaneous denaturation of dsDNA by locally changing the polarity or hydrophobicity of the micro-environment may be utilized by DNA helicases.

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Supporting Information Available: MD simulation movies. This material is available free of charge via the Internet at http://pubs.acs.org.

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