J|A|C|S
ARTICLES
Published on Web 04/29/2006

Weakly Bound Water Molecules Shorten Single-Stranded DNA

Shuxun Cui,*,†,‡ Christian Albrecht,† Ferdinand Kühner,† and Hermann E. Gaub†

Contribution from the Lehrstuhl für Angewandte Physik and Centre for Nanoscience, Ludwig-Maximilians Universität München, Amalienstrasse 54, 80799 München, Germany, and State Key Lab of Polymer Materials Engineering of China, Polymer Research Institute, Sichuan University, Chengdu 610065, P.R. China

Received December 4, 2005; E-mail: cuisx@scu.edu.cn

Abstract: In this paper, we measure the single chain elasticity of an oligomer single-stranded DNA (ssDNA) in both aqueous and nonaqueous, apolar liquid environments by AFM-based single molecule force spectroscopy. We find a marked deviation between the force-extension relations recorded for the two conditions. This difference is attributed to the additional energy required to break the H-bond-directed water bridges around the ssDNA chain in aqueous solutions, which are nonexistent in organic solvents. The results obtained in 8 M guanidine-HCl solution provide more evidence that water bridges around ssDNA originate the observed deviation. On the basis of the results obtained by an ab initio quantum mechanics calculation, a parameter-free freely rotating chain model is proposed. We find that this model is in perfect agreement with the experimental force-extension curve obtained in organic solvents, which further corroborates our assumption. On the basis of the experimental results, it is suggested that the weak H-bonding between ssDNA and water molecules may be a precondition for stable double-stranded DNA to exist in water.

Introduction

DNA is the key molecule of life. The discovery of the structure and function of DNA triggered a great boom in life science. The mechanical properties of DNA, especially elasticity, have a close correlation to its biological function, from replication to transcription to packaging in the capsids. In pioneering experiments the elasticity of DNA on the single-molecule level was investigated by several methods, including optical tweezers,[1] magnetic beads,[2] and atomic force microscope (AFM).[3] DNA keeps the double helix structure in the physiological environment most of the time, except when activated or separated by proteins. The structure of double-stranded DNA (dsDNA) is known to be polymorphic, depending on the environment, e.g. temperature,[4] ion strength[5] and the applied force.[1,6] Compared with dsDNA, single-stranded DNA (ssDNA) is much simpler and thus it is much easier to correlate the experimental data and its molecular structure. It is to be expected that the relationship between the ssDNA fine structure and the corresponding environment can be depicted, which will provide insights for dsDNA studies in future.

Previous investigations on the mechanical properties of DNA were mainly focused on aqueous surroundings. However, many steps in DNA isolation and preparation are carried out in apolar solvents. Also the microenvironment of the DNA in protein binding pockets may be rather apolar, and mechanics will definitely play an important role for the function of these enzymes, particularly when it comes to splitting the two halves of the double strand apart.

One should also keep in mind that water is a very complicated solvent. With hydrogen-bonding donor and acceptor, this polar solvent strongly influences the properties of solute molecules. It is clear that the physiological function of the DNA molecule crucially depends on the aqueous environment. Nevertheless, the increasing range of novel applications for DNA ,e.g. as template for metallic wires or as nanoscale building blocks for 3D molecular architecture, go far beyond the scope of biological function and may be most useful in other surroundings that may be apolar or even a vacuum. Constrained by the limits of characterization methods, however, it is often impossible to carry out mechanical experiments under vacuum conditions. The missing van der Waals buoyancy of the solvent typically results in a strong adhesion of the polymer to any surface and thus prevents a meaningful characterization of the mechanical properties of the polymer itself. An alternative choice is to carry out the experiments in organic solvents. The interactions between the apolar organic solvent molecules and the solute molecules are van de Waals interactions in general, which should be the weakest intermolecular interactions. Under this condition, it is to be expected that the solute molecules' behavior is close to that under the vacuum condition. In this paper, we directly

† Ludwig-Maximilians Universität München.
‡ Sichuan University.

(1) Smith, S.; Cui, Y.; Bustamante, C. *Science (Washington, D. C.)* **1996**, *271*, 795.
(2) Strick, T. R.; Allemand, J.-F.; Bensimon, D.; Bensimon, A.; Croquette, V. *Science (Washington, D. C.)* **1996**, *271*, 1835.
(3) Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Nat. Struct. Biol.* **1999**, *6*, 346.
(4) Blake, R. D.; Delcourt, S. G. *Nucleic Acids Res.* **1998**, *26*, 3323.
(5) Baumann, C. G.; Smith, S. B.; Bloomfield, V. A.; Bustamante, C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6185.
(6) Clausen-Schaumann, H.; Rief, M.; Tolksdorf, C.; Gaub, H. E. *Biophys. J.* **2000**, *78*, 1997.

10.1021/ja0582298 CCC: $33.50

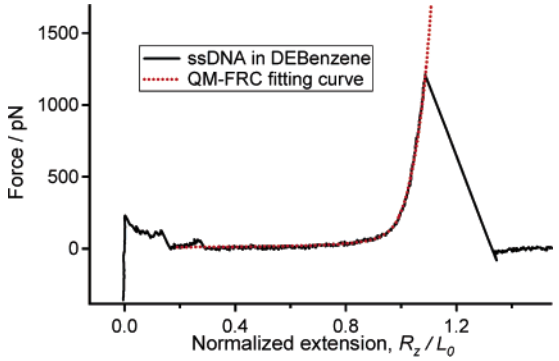

Figure 1. The normalized force curve of single-chain stretching of ssDNA obtained in DEBenzene. The curve can be fitted well by the QM-FRC model (dotted line) with no free parameter for the entire force range.

stretch an ssDNA oligomer molecule in organic solvents by means of AFM, attempting to detect the inherent elasticity of ssDNA.

Experimental Section

Materials and Chemicals. The ssDNA used here is a customized sample (IBA GmbH) that is an oligomer containing 176 bases with a random sequence of 1:1 thymine (T) and cytosine (C). The contour length of this molecule is approximately 100 nm. We only choose T and C in the oligomer sample to avoid complicated intramolecular structures such as hairpins and loops. We expect that this kind of oligomer ssDNA will present only elasticity upon stretching in organic solvent. All the other chemical reagents are purchased from Sigma or Fluka and are analytically pure.

Sample Preparation and Measurements. The ssDNA sample (0.1 mmol/L) is diluted 20 000 times in PBS buffer to a concentration of 5 nmol/L. To prepare the sample for measurements, ssDNA is allowed to adsorb onto an amino-functionalized glass slide (Quantifoil Micro Tools GmbH) for 10 min, followed by thoroughly rinsing with Milli-Q water. After that, the sample is mounted in the AFM (MFP-1D, Asylum research, CA). Prior to the measurements, a drop of liquid is introduced between the V-shaped Si_3N_4 AFM cantilever (Veeco Instruments Inc.) and the sample. Then during the AFM manipulation, the data is collected at the same time and transferred to force-extension curves later. The spring constant of the AFM cantilever is measured by a thermoexcitation method,[8] ranging from 10 to 30 pN/nm. The stretching velocity applied is 2.0 μm/s if not mentioned otherwise. The details of the AFM instrumentation can be found elsewhere.[9−11]

Results and Discussion

Diethylbenzene (DEBenzene) is a typical apolar organic solvent with a lower toxicity and slower volatilization speed than benzene. The typical force curve of ssDNA obtained in DEBenzene is shown in Figure 1. The force rises monotonically with extension, corresponding to the increasing restoring force during the elastic elongation. When the polymer bridge between the AFM tip and glass substrate ruptures, the force drops rapidly to zero.

Recently, the stretching response of the so-called freely rotating chain (FRC) model was investigated theoretically.[12] Later the FRC model was successfully employed to fit the force curves of macromolecules upon stretching.[13,14] In the force range relevant for this study here, the relationship between the extension of the macromolecule (R_z) and the stretching force (F) can be written in a good approximation as

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

where $L[F]$ is the contour length and b is the length of the rotating unit. For ssDNA, the length of the repeating unit is 0.59 nm.[1] Considering that the sugar ring and the phosphate group are stiff, we argue that the rotation within the two groups is hindered. In this case, the repeating unit is divided into two rotating units. The actual length of the two rotating units may differ slightly, but this does not affect the equivalent length of 0.59/2 nm each.

Note that the contour length $L[F]$ is force-dependent. This takes into account that at higher forces enthalpic backbone deformations add significantly to the purely entropic contributions of the FRC model. Bond angles as well as bond lengths are altered. These effects were recently investigated in great depth with an ab initio quantum mechanical (QM) calculation on the backbone elasticity. The parameter free numerical result was approximated in a polynomial expansion to provide the basis for a numerical fit of the measured force extension curves. Three polynomial coefficients were found to represent the numerical result with an accuracy of better than 1%[13] in the force range investigated here (see eq 2)

$$F = \sum_{n=1}^{\infty} \gamma_n (a[F]/a_0 - 1)^n$$
$$\gamma_1 = 8.44 \text{ nN}, \gamma_2 = 29.5 \text{ nN}, \gamma_5 = 19637 \text{ nN} \quad (2)$$

where a_0 is the length of the subunit at zero force for ssDNA, $a[F]$ is the length in the stretched status, γ_1 is the linear elastic modulus, and other coefficients are nonlinear corrections that become important at the higher force range.[13] The coefficients listed in eq 2 are the QM results for ssDNA. Since the changes of bond angle and bond length are already considered in the calculations on one subunit, eq 2 can be rewritten to describe the whole polymer chain:

$$F = \sum_{n=1}^{\infty} \gamma_n (L[F]/L_0 - 1)^n$$
$$\gamma_1 = 8.44 \text{ nN}, \gamma_2 = 29.5 \text{ nN}, \gamma_5 = 19637 \text{ nN} \quad (3)$$

In eq 1, the FRC model has one free parameter, $L[F]$, the contour length of the polymer. If we divide the extension by the contour length at zero force, L_0, the force curve is normalized and the FRC model has the form below:

$$R_z/L_0 = L[F]/L_0[1 - k_B T/(2bF)] \quad (4)$$

During the experimental elongation of ssDNA, the stretching force (F) is increased monotonically from zero to a value normally larger than 300 pN, until the attachment ruptures (see Figure 1). At the same time, $L[F]$, the contour length of the polymer, also increases gradually starting from L_0, due to the

(7) Cui, S.; Liu, C.; Wang, Z.; Zhang, X.; Strandman, S.; Tenhu, H. *Macromolecules* **2004**, *37*, 946.
(8) Florin, E.; Rief, M.; Lehmann, H.; Ludwig, M.; Dornmair, C.; Moy, V.; Gaub, H. E. *Biosens. Bioelectron.* **1995**, *10*, 895.
(9) Hugel, T.; Seitz, M. *Macromol. Rapid Commun.* **2001**, *22*, 989.
(10) Zhang, W.; Zhang, X. *Prog. Polym. Sci.* **2003**, *28*, 1271.
(11) Zhang, D. and Ortiz, C. *Macromolecules* **2005**, *38*, 2535.
(12) Livadaru, L.; Netz, R. R.; Kreuzer, H. J. *Macromolecules* **2003**, *36*, 3732.
(13) Hugel, T.; Rief, M.; Seitz, M.; Gaub, H. E.; Netz, R. R. *Phys. Rev. Lett.* **2005**, *94*, 048301.
(14) Neuert, G.; Hugel, T.; Netz, R. R.; Gaub, H. E. *Macromolecules* **2006**, *39*, 789.

elastic nature of polymers. In this way, the value of $L[F]/L_0$ increases with the increasing of F, starting from 1. Therefore, the value of $L[F]/L_0$ is a monotonic increasing function of F and vice versa. During the elongation of ssDNA, $L[F]/L_0$ is an ergodic value ranging from 1 to a number corresponding to the rupture of the attachment. Here, we utilize the strength of a typical covalent bond as the upper limit for the stretching force, e.g. 2000 pN.[15] Thus, the upper limit for $L[F]/L_0$ is about 1.12, according to eq 3. In the range from 1 to 1.12, any arbitrary value of $L[F]/L_0$ is reasonable and corresponds to a mapping value of F in the force curve, which can be calculated with eq 3. From this pair of values for $L[F]/L_0$ and F, the corresponding normalized extension of ssDNA, R_z/L_0, can be calculated with eq 4. One value pair of R_z/L_0 and F corresponds to one point in the fitting curve. In this way, the whole fitting curve can be generated when changing the value of $L[F]/L_0$ from 1 to 1.12 (see the dotted line in Figure 1).

Note that during the fitting process described above, the values of $a[F]$, a_0, $L[F]$, and L_0 are not used directly, which simplifies the calculations involved in model fitting. And the value of $2b$ is fixed to 0.59 nm. Therefore, there is no free parameter left in eq 4. We call eq 4 the QM-FRC model.

The ab initio calculations were carried out in a vacuum. It is therefore very interesting to see whether the QM-FRC model can fit our experimental data, which were obtained in organic solvent. We were pleased to find that the QM-FRC curve superposes with our normalized experimental data very well for the entire force range (see Figure 1). This positive result indicates that all the preconditions are tenable: (1) the ab initio results from the literature are precise enough, (2) the QM-FRC model is appropriate for ssDNA, (3) the equivalent length of the rotating unit in ssDNA is 0.59/2 nm, and (4) the ab initio calculation results obtained for vacuum conditions can be applied to the condition of organic solvents.

In aqueous environment, additional interactions with and among solvent molecules are expected to further complicate the situation. We therefore repeated the above experiment in different aqueous solutions. The force curves obtained in PBS and DEBenzene were normalized to the same extension and are shown together in Figure 2. It can be observed that, at the very low and very high force range, the two force curves superpose well. In the middle force range (about 35−250 pN), however, there is an obvious deviation. To find out the possible reason, we also used other kinds of aqueous solutions, such as 1 mol/L KCl and 0.1 mol/L Tris, in similar experiments. We found no evident difference among the force curves obtained in various aqueous solutions after normalization (see Figure 3).

Similarly, another organic solvent, 1-propanol, was utilized to compare with DEBenzene. Figure 4 clearly shows that the ssDNA elasticity is the same for the two organic solvents.

These two blocks of experimental results clearly point toward hydrogen-bonding mechanisms and transient water networks for a potential explanation.[16]

There are many functional groups containing H-bonding acceptors and donors in ssDNA chain. SsDNA is soluble in water and can form H-bonds with water molecules in various combinations. It is possible that one water molecule can form two H-bonds with one ssDNA molecule and create a water "bridge" around the ssDNA chain.[17] To meet the length requirement of the water "bridge", the ssDNA chain may be shortened in some extent. Upon stretching by external force, the ssDNA chain is lengthened and the length requirement is no longer met, which will consequently break the water bridges. The rearrangement of H-bonds around ssDNA chain will consume additional energy besides those contributions stored in the "pure" elastic behavior. In this way, the elongation of ssDNA in water will consume more energy than that consumed in organic solvents, as reflected by the deviation between the force curves shown in Figure 2.

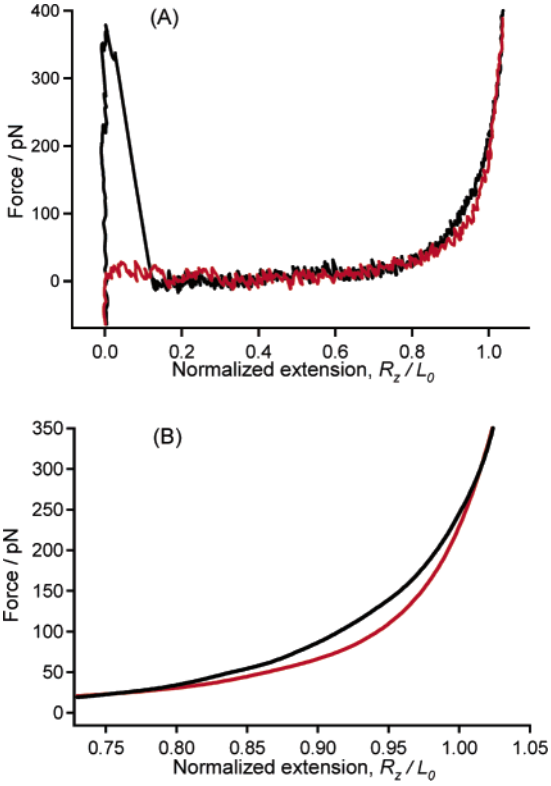

Figure 2. The comparison of normalized force curves obtained in aqueous solutions (black curve) and organic solvents (red curve). The orginal force curves are shown in A. The force curves shown in B are smoothed and enlarged to show a clearer deviation between them.

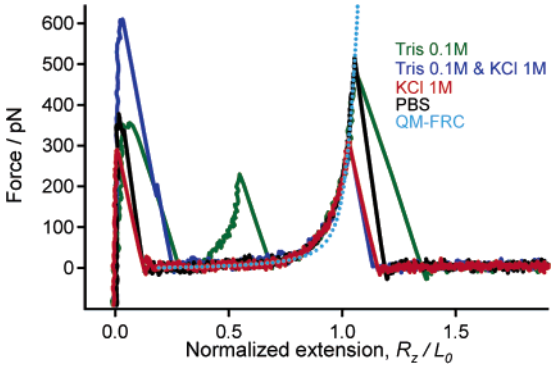

Figure 3. The normalized force curves obtained in various aqueous solutions. For clarity, only a few force curves are shown. There is an evident difference between the force curves and the QM-FRC model fitting curve in the middle force range.

(15) Grandbois, M.; Beyer, M.; Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Science (Washington, D. C.)* **1999**, *283*, 1727.
(16) Lum, K.; Chandler, D.; Weeks, J. D.*J. Phys. Chem. B* **1999**, *103*, 4570.
(17) Oesterhelt, F.; Rief, M.; Gaub, H. E. *New J. Phys.* **1999**, *1*, 6.1.

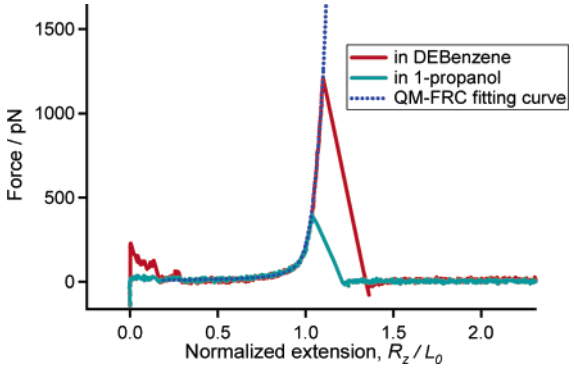

Figure 4. The normalized force curves obtained in DEBenzene and 1-propanol. The QM-FRC model can fit the force curves well.

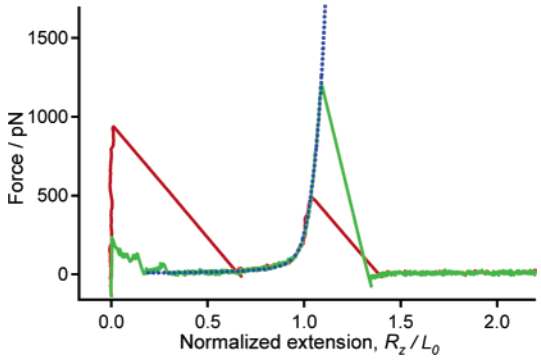

Figure 5. The comparison of force curves obtained in organic solvent (green curve) and 8 mol/L guanidine-HCl (red curve). The QM-FRC model can fit the force curves well.

Another possibility for the deviation between the force curves obtained in two environments may come from the different charge density along the ssDNA chain. The phosphate groups in the ssDNA chain are presumably associated with counterions in organic solvent, or they are protonated; either way the molecule is effectively neutralized. However, in the dilute polyelectrolyte (here is ssDNA) aqueous solution with no additional salt, the counterions are diffused into the solution. This situation is called "unsaturated condensation"; i.e., the counterions in the "cylindrical cell" are far from enough to screen the charges of the ssDNA chain. Upon addition of external salt, the cations in the "cylindrical cell" start to affect the charge screening.[7] In this study, we have used various solutions as the aqueous environment for the ssDNA force measurements. For instance, we used PBS buffer ranging from 0.1× to 5× and KCl solutions from 0.05 to 1 mol/L. The large range of salt concentration should result in different degrees of charge screening of the ssDNA chain. However, we did not observe an evident difference among the force curves obtained in the solutions (see Figure 3). This may indicate that in the high-force regime probed in our studies here the mechanical behavior of ssDNA is independent of the salt concentration. In other words, the charges along the ssDNA chain do not contribute much to the mechanical behavior of ssDNA. This finding is an indirect support for the hypothesis that the deviation shown in Figure 2 is caused by water bridge.

After smoothing, the deviation between the two force curves was estimated to be $0.58k_BT$ per repeating unit (see Figure 2B). It is helpful to note here a recent experimental progress by Omta et al. They reported that the presence of ions does not lead to an enhancement or a breakdown of the H-bond network in liquid water,[18] which can explain why we obtain similar results in various aqueous solutions. Comparing with other systems having water bridges,[17,19] we find that ssDNA has the lowest breaking energy among them. It is helpful to note that measured hydrogen-bond energies for water range between $0.4k_BT$ and $2.8k_BT$.[20] The weak competition influence of water may be an important factor for DNA to form a stable double helix in water. One could speculate that if water would be a stronger competitor, dsDNA would not exist.

Guanidine-HCl aqueous solutions are often used as denaturant for proteins. Here, we use an 8 mol/L guanidine-HCl solution as the buffer to measure the single chain elasticity of ssDNA. Interestingly, the force curve obtained in this buffer is similar to that obtained in organic solvents (see Figure 5). It seems that the ssDNA is "denatured" in the 8 mol/L guanidine solution. It is likely that the very high concentration of guanidine may form a shield around the ssDNA chain and isolates ssDNA from water molecules, so ssDNA behaves as in organic solvents. This finding validates our assumption that the difference between the two types of force curves originates from water bridges.

In this study, we also perform the ssDNA stretching under different pulling speed and find that there is no evident difference. This finding is consistent with the fact that the lifetime of the H-bonds between ssDNA and water as well the thermal fluctuation of the ssDNA chain is much shorter than the time scale in our experiments (0.2−1 ms), which implies that the single molecule experiments are carried out close to the thermodynamic equilibrium.[9,21,22]

Conclusions

In summary, we measured the single chain elasticity of an oligomer ssDNA in both aqueous and nonaqueous liquid environments by AFM. With the results from advanced ab initio calculations, we found out that the force curve obtained in organic solvents could be fitted well by the FRC model with no free parameter. However, there was an evident deviation between the results obtained under the two conditions, which was estimated to be $0.58k_BT$ per base in energy. Compared with others systems, this energy was rather low. The weak competition influence of water may be an important factor for DNA to form a stable double helix in water. This difference was attributed to the additional energy consumption needed to break the H-bond-directed water bridges around the ssDNA chain in aqueous solutions, which is nonexistent in organic solvents. This hypothesis was confirmed by the results obtained in 8 mol/L guanidine-HCl solution.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) through SFB 486 and the Fonds der Chemischen Industrie. Shuxun Cui appreciates the support of this work through the Alexander von Humboldt Fellowship. Helpful suggestions from Gregor Neuert are acknowledged.

JA0582298

(18) Omta, A. W.; Kropman, M. F.; Woutersen, S.; Bakker, H. J. *Science (Washington, D. C.)* **2003**, *301*, 347.
(19) Liu, C.; Cui, S.; Wang, Z.; Zhang, X. *J. Phys. Chem. B* **2005**, *109*, 14807.
(20) Williams, D. H. and Westwell, M. S. *Chem. Soc. Rev.* **1998**, *27*, 57.
(21) Evans, E. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 105.
(22) Zapotoczny, S.; Auletta, T.; de Jong, M.; Schönherr, H.; Huskens, J.; van Veggel, F.; Reinhoudt, D.; Vancso, G. *Langmuir* **2002**, *18*, 6988.