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Statistics of Time-Dependent Rupture of Single ds-DNA

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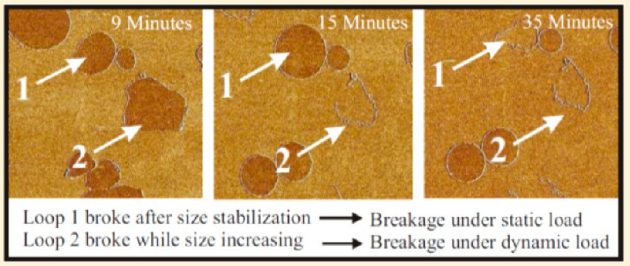
ABSTRACT: Double-stranded (ds-) DNA molecules were stretched and ruptured on molecularly modified graphite surfaces with a scanning force microscope (SFM) exerting a force parallel to the surface. The stretching force was either large enough to break the molecule immediately or compensated by the elastic restoring force of the DNA backbone, which stabilized the molecular length. However, the size-stabilized molecules broke gradually from longer molecules to shorter ones with time. The breakage of different lengths of stabilized molecules was recorded in order to study time-dependent mechanical properties of the molecules under constant forces. From these data, a relatively high rate constant, $k_0 = (2.2 \pm 0.1) \times 10^{-7}$ s^{-1}, was calculated. Moreover, we found a nonlinear stress−strain dependence of DNA on the surface which we attributed to DNA conformational transition. Assuming that the structural transition on the surface is similar to that in solution we estimated the forces needed to stretch the molecules and thereby verify the estimation of the activation energy barrier.



INTRODUCTION

The understanding of the rupture process of a macromolecule chain under an applied force is of fundamental importance, e.g., for single-molecule mechanics,[1] cell adhesion,[2] and mechanochemistry.[3,4] Experimental insight into the rupture process has been obtained by stretching and breaking of single polymer chains with micromanipulation techniques such as pulling with scanning force microscope (SFM) tips,[5,6] micropipets,[7] an optical trap,[8] and dragging with a receding meniscus.[9] In such experiments, the bond rupture strengths were acquired with typically dynamic force loads; i.e., the force was increasing with time until the bond broke, with the bond strengths under dynamic load depending on the force loading rate.[5,10,11] Under a static load, i.e., when a bond is stretched with a constant force, different bond strengths and rupture mechanisms are predicted by theory.[12,13] The specific experimental setups mentioned above limit the time under which a constant load can be maintained, and hence restrict the possibility to investigate relatively long-living bonds. To the best of our knowledge, the longest reported time for constant force holding in a force-clamp SFM is about 30 s.[14] In nature, however, many processes which involve breakage of a single covalent bond occur on a time scale of hours or even longer; e.g., polypeptide chains strained by the proteins degrade in vivo from minutes up to days.[15] Also, engineering materials are typically under stress on times exceeding seconds substantially. Direct conformational information on the target molecule is usually not accessible in such pulling experiments. Here we demonstrate the employment of a recently developed method,[16] which allows to expose plasmid ds-DNA molecules on a surface to a constant strain for a time on the order of an hour. Stretching of the DNA molecules was achieved through a SFM tip interacting with an ultrathin film of an organic liquid coating the surface.

EXPERIMENTAL METHODS

The force stretching the molecules on the surface, in the experimental setup we use, has been reported to depend on experimental details such as the SFM tip type, scan speed, scan size, the coating liquid, and temperature.[16] Here we chose experimental conditions under which the loops remained stretched for about an hour, and maintained these experimental conditions throughout the experiments. The experiments were performed in a temperature (20 ± 1 °C) controlled room. A submonolayer of dodecylamine ($C_{12}H_{25}NH_2$) in chloroform was spin-coated at 40 rps from a dilute chloroform solution (20 mg/L) onto the basal plane of a freshly cleaved piece of HOPG (ZYH grade, GE Advanced Ceramics, USA). Subsequently, a droplet (20 μL) of plasmid ds-DNA (pUC19, 2686 base pairs (bp), MoBitec GmbH, and Sk13.3, 1650 bp, kindly provided by J. Hecht, MPI for Molecular Genetics, Berlin) in aqueous solution (2.0 μg/mL) was applied to the surface and spun off after 5 s. Thereafter, the samples were imaged with an SFM (Nanoscope 3a, Veeco, USA) in tapping mode, using commercial silicon cantilevers (Olympus, Japan) with a typical resonant frequency of 300 kHz and a typical spring constant of 42 N/m.

RESULTS

Figure 1 exhibits SFM images of pUC19 plasmid DNA, on the same area (3 μm × 3 μm), at different times. The first image displays almost unstrained single DNA molecules on the surface. As imaging continued, the irregularly shaped loops grew into circles with the larger loops growing faster than the smaller ones. The loops continued to grow until the force

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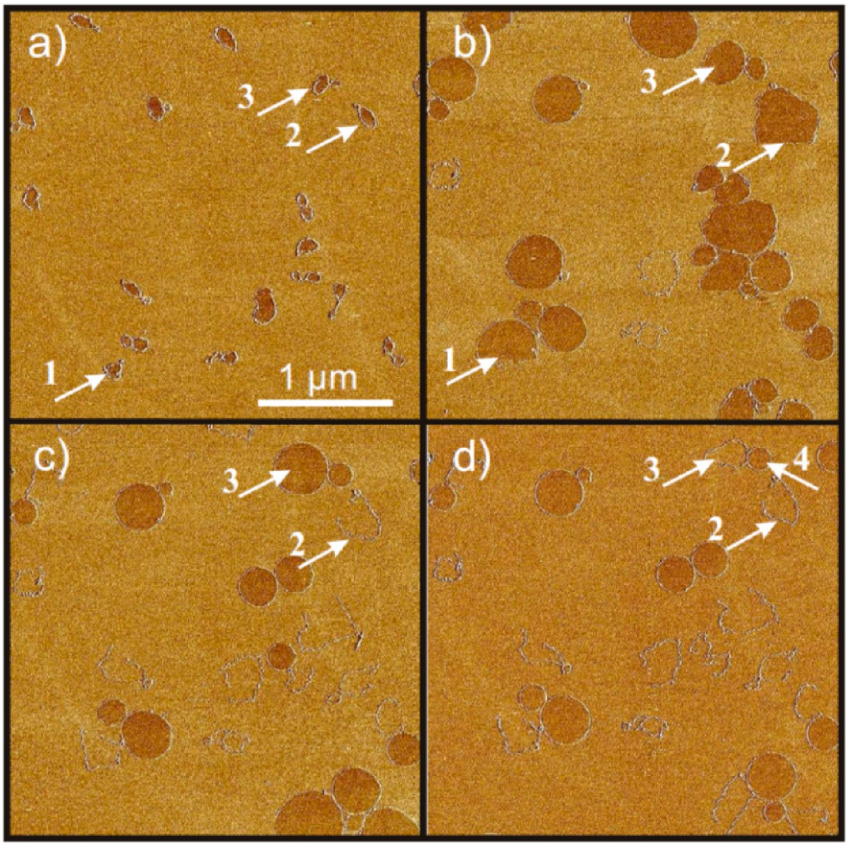

Figure 1. A few selected SFM phase images from a sequence of images of ds-DNA on HOPG precoated with dodecylamine in chloroform taken at the same scanning area as a function of time: (a) 3 min, (b) 9 min, (c) 15 min, and (d) 35 min after the experiment started. Arrows 1, 2, and 3 mark three loops, which break as one goes from (b) through (d). Loops 1 and 2 break during growth in size which present the breakage in a dynamic force load; loop 3 breaks after its size stabilization which introduces the breakage under a constant force. The images were scanned from top to bottom. Phase contrast between inside and outside of the DNA loops implies different SFM tip−surface interactions indicating different surface pressures inside and outside of the loops, giving rise to tangential forces stretching the molecules.[16].

stretching the molecules became either balanced by the restoring force of the DNA backbone, or the force became large enough to break the largest loops such as molecules indicated by the arrows 1 and 2. We will refer in the following to such breakage as "dynamic". The smaller loops that did not break stabilized their size after 15 min of scanning (Figure 1c). Breakages of these loops could be still observed up to 20 min after the size stabilization such as the molecule indicated by the arrow 3. We will refer in the following to such breakage as "static". Dynamic breakage occurred mostly when the tip was scanning over the molecules; examples are indicated by the arrows 1 and 2 in Figure 1b. This can be attributed to growth of the force during SFM tip scanning inside the DNA loops.[16] These loops increased their sizes up to 2.1 times of their original B-form lengths until they broke. Broken molecules relaxed into less stretched conformations with the length 30% longer than that of expected for B-form DNA. In contrast, static breakage happened typically after size stabilization of the loop and mostly when the tip was tapping outside the loop; an example is indicated by the arrow 3 in Figure 1c,d. This can be attributed to a constant force maintained inside the loop, also when the tip was scanning outside of the loop. In some cases, the molecules formed a few topological loops on the surface. In case if one of such loop broke, connected loops remained visually unaffected as such indicated by the arrow 4 in Figure 1d. Therefore, we included such topological loops into the statistics.

The experiment was repeated seven times and the size of each loop was traced and recorded at different time after the experiment started. We analyzed the loop size growth and breakages with the cumulative distribution functions of the loop sizes in order to avoid binning of the data. The results from different experiments were accumulated in one graph (Figure 2).

180 DNA loops with size varying from 150 to 1100 nm in seven independent experiments have been identified on the images taken within the first 3 min of the blowing experiment. The length of the longest non-supercoiled DNA was 1100 nm, slightly longer than the expected B-form length of 913 nm. Loops with size smaller than 913 nm were formed by supercoiled DNA molecules. During the next 12 min the 40 largest loops broke, while still increasing their sizes, i.e., in a dynamic load regime. The rest of the loops stabilized in their size. Breakage events of size stabilized loops still could be observed up to 24 min thereafter (Figure 2). The average surviving time in the static load regime for loops which broke at around 1780 nm and loops breaking at lengths from 1000 and 1075 nm is listed in Table 1. In order to exclude the uncertainty of coiled loops, we chose a shorter plasmid DNA sample with 1650 bp (sk13.3). The surviving time of sk13.3 in the static load regime for noncoiled loops is also listed in Table 1.

The tangential force has been previously attributed to the surface pressure developed by the SFM tip within the area enclosed by the loops. Thereby the force was argued to grow

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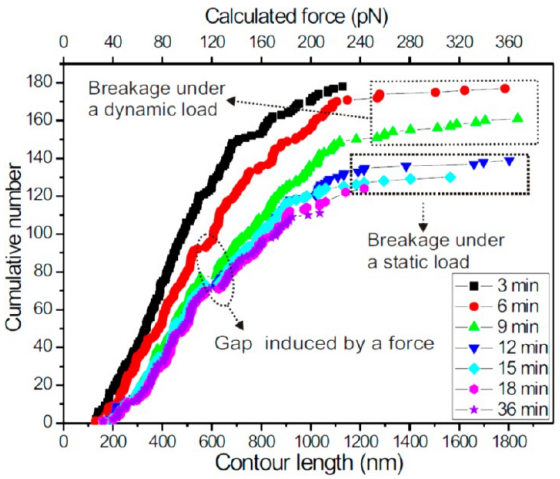

Figure 2. Time development of the cumulative distribution function of loop sizes of pUC19 ds-DNA. The data are taken from seven independent experiments. The dynamic load regime characterized by loops increasing in size is followed by a static regime.

Table 1. List of Average Time Period t for Breaking DNA Loops of Lengths l[a]

plasmid DNA	l (nm)	n_{bp}	t (s)
pUC 19	1787 ± 78	2686	337 ± 230
Sk13.3	1102 ± 69	1650	762 ± 320
pUC 19 (coiled)	1000 to 1075	1563	876 ± 144

[a]Error bars are the standard deviations and the number of base pairs n_{bp}, 13, 9, and 6 single breakage events were analyzed for pUC19, Sk13.3, and pUC19 (coiled), respectively. "Coiled" stands for the DNA loops formed topologically, i.e., by self-crossing of DNA.

linearly with the length of the loop.[16] The phase contrast in tapping mode reflects the difference between surfaces covered with different liquid films.[16] Therefore, a homogeneous phase contrast between inside and outside of the loops (Figure 1) on the images complies with the proposed surface pressure difference. Furthermore, our results imply that the force grows indeed with the length of the loops, since large loops break faster. We cannot, however, verify directly that the force grows linearly with the length of the molecules, and we will have to assume it in the following.

THEORETICAL BASIS

We will adopt in the following the existing theories of polymer chain breakage under static load to our case. Let $U(x)$ be the (free) energy of a bond as a function of a reaction coordinate x with a minimum located at x_- corresponding to the equilibrium bond length and a barrier at x_+ separating the bounded from the unbounded state. For a single covalent bond under a load f the combined energy surface is given by $U_f(x) = U(x) - fx$. For small forces the location of the transition state $\Delta x = x_+ - x_-$ is only slightly perturbed and the activation rate $k(f)$ can be well described by the phenomenological Bell form:[17]

$$k(f) = k^0 \exp\left(\frac{f\Delta x}{k_B T}\right) \tag{1}$$

to which several different approximations for bond failure process are reduced in quasistatic regime.[18,19] In our case, this approximation is applicable since the molecules are kept under constant tension over long time intervals. The thermal activation rate of an unloaded bond k^0 increases exponentially upon application of a static force f. In eq 1, k_BT is the thermal energy with temperature T and k_B is the Boltzmann constant. Note that the mean time for breaking DNA loops and their corresponding standard deviations given in Table 1 are of the same order of magnitude, which justifies our assumption of having a single-exponent process underlying bond breakdown.

In our experiment, the two strands of the double helix of the ds-DNA are loaded in parallel and therefore the force acting on a single strand is half of the applied force f. We assume that, as soon as one strand breaks, the second one fails immediately under the double force load. Then, the thermal activation rate k of the whole loop is proportional to the number of weak bonds in the two strands of the double helix, which is proportional to the number of base pairs n_{bp} and to the number of weak links per base pair n_w, i.e.

$$k \propto 2n_{bp}n_w$$

yielding

$$k(f) = 2n_{bp}n_w k^0 \exp\left(\frac{f\Delta x}{k_B T}\right) \tag{2}$$

Considering two DNA molecules with different numbers of base pairs $n_{bp}^{1,2}$ the ratio of the activation rates is given by

$$\frac{k_1(f_1)}{k_2(f_2)} = \frac{t_1}{t_2} = \frac{n_{bp}^1}{n_{bp}^2} \exp\left(\frac{\Delta x}{2k_B T}\Delta f_{12}\right) \tag{3}$$

with $\Delta f_{12} = f_1 - f_2$. A combination of eqs 2 and 3 allows for the estimation of Δx and k^0, given the knowledge of the forces acting on the DNA molecules. Although we will discuss in the following the force calibration, we will first estimate k^0 without the explicit knowledge of the forces: combination of eqs 2 and 3 gives

$$k^0 = \frac{k_1(l_1)}{2n_{bp}^1 n_w}\left(\frac{k_1(l_1)n_{bp}^2}{k_2(l_2)n_{bp}^1}\right)^{-l_1/\Delta l_{12}} \tag{4}$$

Equation 4 does not require explicit knowledge of the forces acting on the molecules; rather it relies on the assumption of a linear relation between forces acting on the molecules and their lengths. Assuming that there is one weakest link in every phosphodiester group, we can set $n_w = 1$ in the following. Since eq 4 depends only linearly on n_w, variation of n_w in the reasonable range does not change our result dramatically. Substitution of the experimental data from Table 1 into eq 4 gives an intrinsic activation rate $k^0 = (2.2 \pm 0.1) \times 10^{-7}$ s^{-1}.

We will attempt in the following to explicitly calibrate the forces acting on the DNA molecules. Inspection of the cumulative length distribution curves reveals the formation of a gap around 550 nm after the first 6 min, absent at the beginning and persisting till the end of the experiment. Comparison of the loop sizes at the end of the experiment with their original size in the beginning of the experiment reveals that all loops above the gap have a stretching ratio larger than 1.5 while the loops below the gap have a stretching ratio smaller than 1.3. The gap implies a strongly nonlinear dependence of the DNA restoring force on its length, e.g., a structural transition. The gap position remains in the same size region in the curves after 6 min in Figure 2, which indicates that the transition does not depend on the force loading rate in our experiments.

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ds-DNA is an extensible molecule, with stretching behavior in solution that has been amply studied with the aid of single-molecule manipulation methods. The force−extension curves recorded in such experiments reveal a highly cooperative structural transition from B-form to a stress-induced S-form of DNA. The transition occurs at around 110 pN for un-nicked ds-DNA and is independent of force loading rates in the typical experimentally accessible range.[7,8,20] The ds-DNA structural transition takes place in solution at a broad range of different pH values and ionic strengths.[21,22] The length of the ds-DNA increases from around 1.2 to 1.7 times its native B-form during the transition.

It is tempting to attribute the gap in the length distribution we observe on the surface to a structural transition similar to that in solution. Although the interaction with the surface (e.g., mica) may influence DNA structure,[23] we assume in the following that the transition we observe on the surface happens under the same load as in solution, i.e., 110 pN for un-nicked DNA, thus obtaining an internal force calibration. Consequently, the force acting on each loop was calculated from the plot in Figure 2, using a linear dependence of force on the length of the molecules: $F_1 = F_2(l_1/l_2)$ with l being lengths of the molecules and F forces acting on them. Solving eq 3 for Δx and inserting the values given in Table 1 (together with the estimated values of the forces), we obtain for both combinations of measured data (pUC19/SK13.3 and pUC19/pUC19 (1 crossing)) Δx = 0.020 nm. This value agrees well with the one derived for a siloxane bond (Δx = 0.021 nm).[24]

Knowledge of Δx together with the previously estimated force values allows us to estimate an intrinsic activation rate k^0 with the help of eq 2: $k^0 = (2.2 \pm 0.1) \times 10^{-7}$ s^{-1}, which is in good agreement with the estimation we made above. This supports the assumption that the gap in the length distribution is indeed due to the DNA structural transition. A rather unexpected result that DNA molecules on the surface tend to keep B-form, i.e., base pair stacking, is supported by the observation of length relaxation of broken molecules (Figure 1).

Our results can be extrapolated to other force application dynamics and thus compared to those of other groups. Stretching experiments utilizing a receding meniscus breaks λ-DNA (48502 bp) with a force of 476 pN in about 20 s.[9,25] We obtain 15 s from eq 2 with the same force and number of base pairs. When extrapolating to zero force we find an apparent intrinsic activation barrier height of 45 k_BT, assuming the Arrhenius prefactor is of the order of 10^{13} s^{-1} in vacuum. Note that this result is obtained from the knowledge of k^0, which did not require questionable force calibration, and that this value is the upper limit of the barrier height since the Arrhenius prefactor is smaller under the experimental conditions. This value is still much smaller than the average bond energy of a single C−C (∼140 k_BT), C−O (∼145 k_BT), and P−O (∼135 k_BT) bond,[26] which compose the DNA sugar−phosphate backbone. At first glance this result differs from our knowledge that the ds-DNA backbone is quite stable without external forces. However, similar results were reported; e.g., an unexpected small energy barrier ∼30 pN nm or 7.4 k_BT for single Si−O bond (average bond energy ∼182 k_BT) was obtained in stretching experiments with SFM tip in solution.[24] It has been argued that external forces can not only lower the energy barrier to enhance the thermal activation of bond breakage but also may act as a catalyst to increase the reaction rates.[3,4] These features may increase the dissociation rate dramatically. An example is the exponentially increased dissociation rate of a disulfide bond upon the application of an external force.[27] The phosphate groups in the DNA backbone can undergo hydrolysis process and the reaction rate increases significantly in the presence of a catalyst, e.g., from a rate constant of ≈2 × 10^{-13} s^{-1} for uncatalyzed hydrolysis to a value of 6 × 10^{-7} s^{-1} for alkaline-catalyzed hydrolysis.[28] We attribute the high dissociation rate (≈ 2 × 10^{-7} s^{-1}) and low activation energy as well as low rupture forces for DNA obtained in the present work to force-catalyzed chemical processes such as DNA hydrolysis. It is tricky to determine directly from the experimental data which chemical bonds are ruptured in the DNA backbone. It has been reported that in vacuum DNA is fractured by low-energy electrons (3−20 eV) with a major cleavage of C−O bond at the 3′- or 5′-position through a resonance process, namely dissociative electron attachment, which leads to the formation of stable anions and radical fragments.[29,30] The hydrolysis of phosphodiester group in neutral solution occurs at least 99% by C−O cleavage.[31] We speculate that in our case C−O bond may probably be the bond ruptured in the DNA backbone.

Therefore, we conclude that the rupture of a ds-DNA chain, which consists of homogeneous covalent bond in series, depends on the time period under an external force as well as the environment. The height of the activation barrier of the covalent bonds in the DNA backbone on a surface in the liquid environment differs markedly from the isolated situation in vacuum and depends sensitively on the experimental conditions. The estimated small activation barrier of 45 k_BT can be explained with force-catalyzed chemical reactions such as hydrolysis.[24,32] A relatively low rupture force ranged from 220 to 380 pN with a mean breaking time of 20 to 5 min, respectively, was obtained by using the DNA structural transition as a force calibration.

CONCLUSIONS

In summary, we have demonstrated a method that combined dynamic load and static load to study the time-dependent mechanical properties of single DNA chains, which extends the accessible pulling time from seconds in a conventional SFM vertical pulling experiment to more than half an hour in our case (and for less volatile precoatings[16] for a day or more). A relatively high intrinsic activation rate of (2.2 ± 0.1) × 10^{-7} s^{-1} is obtained and an activation barrier with an upper limit of 45 k_BT under the given environmental conditions is estimated. Furthermore, the conformational changes of DNA were traced in real time and a structural transition was observed during stretching. Using the internal force calibration given by the assumed transition from B-form to S-form DNA under force, we have shown that ds-DNA has a mean breaking time of 5 min under a static load of 380 pN, which increases to 15 min at about half of the force load.

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Notes

The authors declare no competing financial interest.

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