Demonstration that the shear force required to separate short double-stranded DNA does not increase significantly with sequence length for sequences longer than 25 base pairs

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We have measured the shear force for short double-stranded DNA sequences pulled by either the 3′3′ or 5′5′ ends and find that the shear force is independent of the pulling technique. For the 50% GC sequences examined, the force is a linear function of DNA length up to 20 base pairs (bp); however, we show that, as predicted by deGennes, the shear force approaches an asymptotic value in the limit where the number of base pairs approaches infinity, where the shear force for a 32 bp sequence is within 5% of the asymptotic value of 61.4 pN. Fits to deGennes' theory suggest that the shear force is distributed over fewer than 10 bp at the end of the sequence, with the rest of the sequence experiencing negligible shear force. The single base pair rupture force and the ratio of the backbone spring constant to the base pair spring constant determined from fits of the data to deGennes' theory are consistent with *ab initio* predictions.

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I. INTRODUCTION

Intermolecular forces in double-stranded DNA (dsDNA) are important since these forces dictate the energy requirements for processes such as transcription, replication, and recombination; consequently, the shearing of dsDNA has been studied by a number of researchers [1–10]. It has been shown that for short strands with lengths of 12–30 base pairs (bp) the force required to shear the DNA increases linearly with the length [1–7]. Pierre deGennes [11] proposed that the linear trend would not continue for longer strands, and that the shear force would saturate to a finite value in the limit where the number of base pairs approaches infinity.

If the DNA phosphate backbone were infinitely stiff, then applying a shear force to the DNA would be equivalent to pulling on all of the base pairs in the sequence in parallel. A force F applied to opposite ends of a dsDNA molecule consisting of N bp will be uniformly distributed across all of the base pairs resulting in a force of F/N per base pair. If the shear force required to separate DNA is equal to the force at which the stress across each base pair exceeds a critical force F_c, then the force required to separate N base pairs is NF_c. Thus, the shear force would increase linearly with length for all lengths.

In contrast, if the backbone is not infinitely stiff, then both the backbone and the base pairs will stretch when a shear force is applied, as shown schematically in Fig. 1. As one moves in from either end, the differential force across the base pairs approaches zero; consequently, the shear force is only distributed over a few base pairs near the ends of the sequence, shown in the figure by the length χ^{-1}. This length is given by $\sqrt{(Q/2R)}$, where Q is the spring constant characteristic of stretching the backbone, and R is the spring constant characteristic of stretching the hydrogen bonds between base pairs. Thus, theory suggests that the shearing force of dsDNA can yield valuable information about the force distribution across the phosphate backbone of a single strand of DNA versus the force distribution across the paired bases of complementary DNA [11].

II. MATERIALS AND METHODS

We use magnetic tweezers [12] to probe the shearing force of matched sequences of length 12–50 base pairs, for both 5′5′ and 3′3′ pulling by applying a constant force to the DNA and measuring the fraction of DNA molecules that separate at that force. The spread in magnetization of the beads leads to a standard deviation of approximately 5% in the force measurements.

The sequences used are shown in Table I. Each sequence is 50% GC. The sequence is chosen to minimize improper annealing. For example, a misannealed 28 bp sequence can only have a maximum of seven matched bases, which should not be stable at room temperature.

To reduce the bead-surface interactions, one end of the sequence to be sheared is attached to a 16.5 μm linker DNA molecule (λ-DNA, New England Biolabs). The linker DNA is tagged with a 3′ digoxigenin-labeled sequence, while the sequence to be sheared is biotinylated on either the 5′ or 3′ side. The linker DNA and the sheared sequence are separated by a 3 base pair gap. Likewise, the biotin and digoxigenin labels are spaced from the dsDNA by a three base pair gap. A schematic of the molecular constructs for 3′3′ and 5′5′ pulling is shown in Fig. 2.

The DNA linker is attached to a glass capillary coated with a polyclonal antidigoxygenin antibody. The biotinylated end of the sequence to be sheared is bound to a 4.5 μm streptavidin-coated magnetic bead (Dynabeads). The shearing experiments are done at room temperature in PBS buffer (pH 7.4, 10 mM phosphate, 137 mM NaCl, 2.3 mM KCl).

Magnetic tweezers allow us to apply a constant force in parallel. In order to determine the shear force, the constant force is increased incrementally by a force interval ΔF after

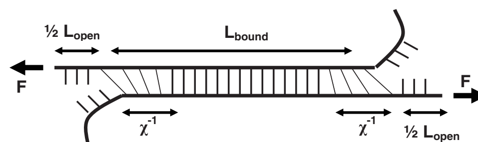

FIG. 1. Schematic of a dsDNA sequence under a shear stress.

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TABLE I. Base pair compositions of shearing sequences.

Length	Sequence ($5' \rightarrow 3'$)
12	gtcaccttagac
16	gtcaccgacattagac
20	gtcaccgatgtgcattagac
24	gtcaccgatgaacgtgcattagac
28	gtcaccgatgaaacagcgtgcattagac
32	gtcaccgatgaaactgtcagcgtcgattagac
50	gtcaccgatgaaactgtctacagactcagcatgcagagcgtcgattagac

which a constant force is applied for ΔT seconds. We measure only the molecules that shear during the time interval ΔT where the force is constant. For the results shown in Fig. 3, ΔF=2 pN and ΔT=1 sec. The measured shearing force for each molecule is the force on the bead at the magnet position where the bead is released from the surface. At least 50 individual molecules are measured for each sequence length and pulling technique to determine the most probable shearing force. The data is fit to a Gaussian distribution and the reported shearing force and error are the mean and standard deviation of the best fit, respectively.

AFM shearing experiments have been shown to be extremely dependent on force loading rates [4,6], where the loading rates are on the order of $40-10^4$ pN/s. We have measured the shear force for ΔT values from 1 to 3 seconds. We find no systematic variation in the shear force as a function of ΔT. This is reasonable since the time during which the force value changes in not ΔT, but the time required for the magnet to translate. The maximum velocity of the translator is 0.5 mm/sec, so for the data shown in Fig. 3 the force is changing for 200 ms and then remains constant for the remaining 1 sec. Thus, the loading rate is zero during the shear force measurement. To determine the effect of the nonzero loading rate during the time that the force is being incremented, we also studied the shear force for ΔF values varying from 2–10 pN, corresponding to magnet translations of 0.1–0.5 mm and found no systematic variation in shear force as a function of ΔF.

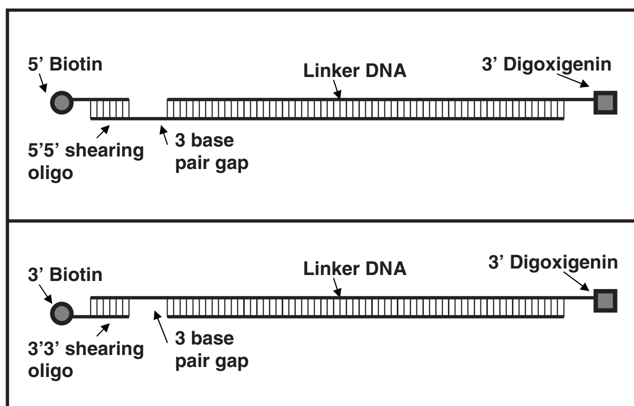

FIG. 2. Molecular constructs for $3'3'$ and $5'5'$ shearing. The short sequence (12–32 bp) is attached to a linker DNA molecule (48 kbp) with a three base pair gap separating the two sequences. The linker DNA attaches to an antidigoxigenin coated capillary via its digoxigenin labeled end. The sequence to be sheared is biotinylated and attached to a streptavidin coated magnetic bead. (Drawing is not to scale).

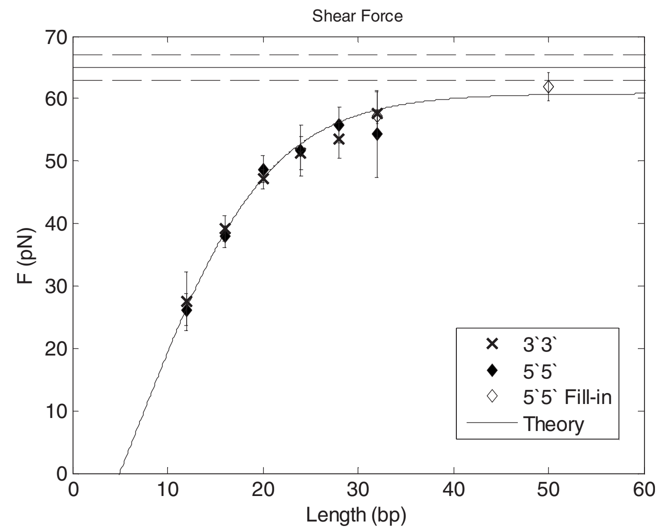

FIG. 3. The shearing force of sequences as a function of length when pulled either by the $3'3'$ or $5'5'$ ends. The solid line represents a best fit of Eq. (1) to the $5'5'$ data with an adjustment length of 6.8 bp and a single base shear force of 3.9 pN, with L_{open} =7 bp. The centre horizontal line represents the critical force and the upper and lower lines correspond to 10 and 90% of the overstretching transition, respectively.

III. RESULTS

Figure 3 shows the shearing force measured for sequences 12–32 bp long, pulled on both the $3'3'$ ends and the $5'5'$ ends. For sequences 12–20 bp in length, the shearing force increases linearly from approximately 30–55 pN. For longer sequences, 24–32 bp, the force as a function of length increases more slowly. The $3'3'$ data are shown by crosses and the $5'5'$ data by filled diamonds. The overlap between the $3'3'$ and $5'5'$ points is very good, suggesting that the pulling technique does not matter.

Saturation in the force required to separate the magnetic beads from the surface might be observed if the labels binding the DNA to the surface at the two ends fail at forces below the true shear force for the longer sequences. To demonstrate that this effect is not important for the data shown in Fig. 3, we attached the labels to two ends of the same strand of a dsDNA molecule; therefore, the DNA was pulled by the $3'5'$ labels at both ends and no shear force was exerted on the DNA. Under these conditions, forces in excess of 100 pN were required to separate the magnetic beads from the surface, showing that the rupture force for the labels exceeded the measured shear force shown in Fig. 3; therefore, the observed saturation is not due to a failure of the labels.

A second artifact that could cause the force at which the magnetic beads separate from the surface to saturate is imperfect annealing. Accurate joining of matched ssDNA strands becomes more difficult as the length of the strands

increases. Thus, the number of bound bases that must be sheared could increase more slowly than the number of bases in the sequence because some bases in the sequence were not bound. To demonstrate that the observed saturation is not due to imperfect annealing, we prepared a $5'5'$ construct by filling in a template using a DNA polymerase and free bases rather than by trying to anneal matching single strands. The shear forces for the filled sequences pulled from the $5'5'$ ends are shown by open diamonds in Fig. 3. The filled in sequence for 32 bp shows a similar shear force to the annealed sequences demonstrating that the force saturation is not due to improper annealing. The 61.5 pN shear for a 50 bp sequence prepared by filling in a template (with a polymerase) is approximately the same as the shear force for the 32 bp sequence prepared using exactly the same technique; therefore, the observed saturation in the force required to separate the magnetic beads from the surface is really due to a saturation in shear force as a function of sequence length and not to annealing failures in long sequences or to the rupturing involved in the bonds of the labels connecting to both surfaces.

Pierre deGennes predicted that in the continuum limit the shear force as a function of L_{bound}, the number of bound base pairs, is given by

$$F_c = 2f_1[\chi^{-1}\tanh(1/2\chi L_{\text{bound}}) + 1], \tag{1}$$

where f_1 is the force required to rupture a single base pair and $L_{\text{bound}}=L_{\text{sequence}}-L_{\text{open}}$ where L_{sequence} is the total number of base pairs in the sequence and L_{open} was equal to zero [11]. In the limit where $L_{\text{bound}}\to\infty$ this expression approaches a maximum asymptotic force, F_m,

$$F_c \to F_m = 2f_1(\chi^{-1}+1). \tag{2}$$

This theory ignored effects due to the finite temperature of the sample. It is known that short dsDNA molecules are not stable at room temperature and that for GC rich sequences approximately 6–10 base pairs are required for the molecule to be stable at room temperature with 10–15 base pairs required for AT rich sequences. Thus, we have allowed the number of bound base pairs to be smaller than the number of base pairs in the sequence by permitting $L_{\text{open}}>0$, where L_{open} is the number of base pairs at the ends that are open due to thermal energy as shown in Fig. 1. The solid line in Fig. 3 represents the best fit to Eq. (1), corresponding to $f_1=3.9$ pN, $L_{\text{open}}=7$ bp, and $\chi^{-1}=6.8$ bp. This value of χ corresponds to a ratio $Q/R=92.5$. This is consistent with predicted values of 77 [11] based on fits to previous data [3] and 18–32 [13] based on calculations of the spring constants for the base pairs and backbones. This theory is not expected to apply when the applied force distorts the bonds sufficiently that the change in energy as a function of displacement is no longer quadratic because the characterization of the force vs extension by a force-independent spring constant is no longer accurate.

Although the helicity of DNA was expected to play an important role in determining the shear force, we do not find strong evidence for it. We find that the same equation fits sequences where the number of bound base pairs is significantly less than is required for a helical turn, as well as sequences where several complete helical turns are present. The continuity of the result for sequences that are not helical and sequences that are strongly helical suggests that helicity does not play a significant role. In addition, pulling from the $3'3'$ ends gives the same shear force as pulling from the $5'5'$ ends, though in the first case the base pair tilt is decreased by the pulling and in the $5'5'$ case the base pair tilt is increased. The similarity in forces suggests that the base pair tilt does not play a significant role in the shearing of short DNA sequences.

As has been shown in previous work [6,14–17], long strands of dsDNA subjected to a shear force undergo a conformational change where the length increases by a factor of 1.7 before they shear. As a consequence, long natural DNA sequences that overstretch before they shear withstand shear forces much greater than the 61.4 pN asymptotic value measured here [14–17]. The horizontal lines in Fig. 3 show forces characteristic of the overstretching transition as measured for the complete lambda phage molecule. The center line shows the 65 ± 3 pN critical force. The lower line shows the 63 ± 3 pN force at which the extension of the molecule is 10% greater than its natural contour length of 16.5 μm. The highest line shows the 67 ± 3 pN force at which the molecule has reached 90% of its elongated state. It is reasonable to assume that molecules in this new state will be characterized by different potentials than molecules in the B form, so that the shear force as a function of base pair number that is measured for BDNA would not necessarily describe the shear force vs base pair number for the overstretched state. For the sequences we considered, the asymptotic value of the shear force corresponds to a force where some of the complete lambda phage molecules will already have begun to make the phase transition into the overstretched state. Our position resolution is not sufficient to determine whether the short sequences undergo an overstretching transition before they shear; however, earlier AFM work has shown that short DNA sequences have approximately the same overstretching force as lambda phage. [6]

IV. CONCLUSIONS

We have shown that the shear force as a function of sequence length from 12–50 bp increases linearly from 12–24 bp and then begins to saturate, approaching an asymptotic limit of 61.4 pN where the shear force for a 32 base pair sequence is within 5% of the asymptotic limit approached when the number of base pairs approaches infinity. This behavior is well described by a theory proposed by deGennes in 2001 [11], if one includes effects associated with the unbinding of a finite number of base pairs due to thermal energy. These results imply that when double-stranded DNA is subjected to a shear force, the shear stress is distributed over an adjustment length of approximately 7 bp at both ends of the sequence, implying that the effects of shear stress are extremely local covering less than a helical turn, while the remainder of the base pairs experience almost no shear force. The fits also give a single base shear force of 3–4 pN and a ratio of approximately 90 between the spring constant for the backbone and the spring constant for the

base pairs. These results are consistent with expectations from *ab inito* models of the spring constants for the hydrogen bonds and the backbone [13]. The fit to the theory covers sequences that are shorter than a helical turn as well as sequences that are much longer than a helical turn, providing further support for the idea that the helicity does not play a strong role in these experiments. Finally, we have made a comparison of the shearing of short DNA sequences pulled from either the $3'3'$ and $5'5'$ ends and find that the shear force is the same for both pulling techniques, suggesting that base pair tilt does not play a significant role in determining the shear force for these short sequences.

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