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Entropic boundary effects on the elasticity of short DNA molecules

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We have measured the entropic elasticity of double-stranded-DNA molecules ranging from 247 to 1298 bp in length using axial force-clamp optical tweezers. We show that entropic end effects and excluded-volume forces from surface attachments become significant for such short molecules. The effective persistence length of the shortest molecules decreases by a factor of 2 compared to the established value for long molecules, and excluded-volume forces extend the molecules to about one third of their nominal contour length. We interpret these results in the framework of an inextensible semiflexible rod model.

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Entropic springs are an important manifestation of thermally fluctuating mechanical systems and are relevant for understanding a wide range of phenomena that range from the structure of biopolymers to rubber elasticity [1,2]. Long double-stranded (ds)-DNA molecules have become the paradigm for entropic springs ever since Smith *et al.* [3] measured the elasticity of a single 32.8-μm-long ds-DNA molecule using magnetic tweezers. Their data were described well by the wormlike chain (WLC) model of Marko and Siggia [1]. The WLC model gives the entropic force of an inextensible polymer as

$$F_{WLC} = \left(\frac{k_B T}{l_p}\right)\left[\frac{1}{4(1-\varepsilon)^2} - \frac{1}{4} + \varepsilon\right], \qquad (1)$$

where l_p is the persistence length of the polymer and $\varepsilon = x/L$ is the relative extension of the molecule x with respect to its contour length L. It is important to note that the force depends only on the relative extension, but not on the absolute length of the molecule. The persistence length, then, is a measure of intrinsic entropic elasticity of the polymer in a fashion similar to the reciprocal of Young's modulus of a classical elastic material. In a polymer, however, the modes of thermal fluctuations that are supported by the molecule depend on the boundary conditions for the end as well as other constraints that may have been placed on it, such as excluded volumes. Therefore, in very short molecules, where modes that involve the ends contribute significantly [4], or in heavily constrained systems, the notion of an intrinsic entropic elasticity as described by a universal persistence length breaks down. This effect has been observed in stiff microtubules where the contour length is much shorter than the persistence length [5]. We believe that this also explains the significant decrease in the effective persistence length of the submicron DNA molecules of ~50% that we report in this Rapid Communication. Evidence for such an effect in DNA in a regime where the contour length is comparable or longer than the persistence length has come from two kinds of experiments. First, ring cyclization experiments with very short (~100 bp) ds-DNA fragments showed that the cyclization rates of the DNA are often significantly higher than expected [6], which points to enhanced flexibility. Some inconsistencies in these results, though, remain. They have been mainly attributed to the intricacies of the hybridization and ligation process [7], but it has also been suggested that the exact boundary conditions for the hybridization step, i.e., how much angular alignment of the overhanging ends is required, affect this particular process as well. This in turn may lead to the apparently contradictory results when an effective persistence length of the DNA is calculated from these experiments [8]. Second, a measurement of the elasticity of a surface-tethered 1870-bp-long ds-DNA molecule with an attached micron-sized polystyrene microsphere by Seol *et al.* [9] using optical tweezers yielded a persistence length of 42 nm, which is ~16% less than the commonly accepted value of ~50 nm for long molecules under these ionic conditions. Seol *et al.* attribute most of this effect to boundary conditions imposed on the system by the surface and the microsphere, but cannot rule out actual changes in the intrinsic bendability of the molecule on short length scales. While the geometric constraints in the tethered-particle motion experiments may seem highly artificial, they are unbiological only in the sense that transcriptionally active DNA is free to fluctuate only over hundreds of base pairs, not thousands. The attachments to surfaces and microspheres may mimic, for instance, attachment to adjacent histones. Excluded-volume constraints, from an impenetrable surface in the tethered-microsphere geometry, have been analyzed theoretically by Segall *et al.* [10] who predicted that the associated excluded-volume forces become increasingly stronger as the length of the molecules decreases. Under our experimental conditions, these forces are mostly associated with the impenetrability of the cover glass to the microsphere and less attributable to the exclusion of the DNA from these volumes. While the excluded-volume forces have been observed in measurements of the distribution of the tethered-microsphere positions [11], they have not been studied quantitatively. In fact, most analyses of tethered-particle experiments that use this geometry have conveniently ignored them altogether [12], even though the induced stretch can substantially affect processes such as protein-DNA complex formation, which is often studied in this kind of experiment [13]. In this Rapid Communication, we present measurements of the elasticity and excluded-volume forces of surface-tethered ds-DNA fragments that range from 247 to 1298 bp in length and show that the ef-

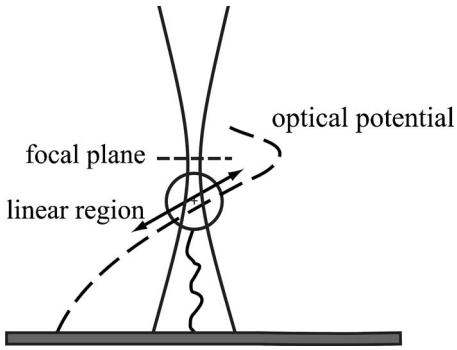

FIG. 1. Stretching DNA with axial constant-force optical tweezers. A short DNA molecule is attached to a cover glass at one end and linked to a microsphere at the other, and a laser beam is focused into the sample cell. The tethered microsphere is placed in the linear region of the optical potential below the focal plane, where the optical force is in good approximation independent of the axial position of the microsphere. The magnitude of the optical force can be changed by varying the intensity of the laser beam.

fective persistence length for the shortest of these molecules drops to as little as 28 nm, while excluded-volume effects stretch it to 34% of its nominal contour length.

Experimentally, we measured the force-extension relationships of four short ds-DNA fragments, which are 1298, 662, 390, and 247 bp in length, using axial optical force-clamp tweezers. The molecules were attached with one end to the bottom of a flow cell using digoxigenin-antibody binding and with the other end to a polystyrene microsphere with a diameter of 800 nm using biotin-streptavidin binding. Each of the functional groups is attached to one strand of the DNA backbone through a six-carbon linker. This essentially makes each attachment point a swivel joint that can pivot in all directions. Details of the sample preparation and characterization protocol are described by Chen *et al.* [14].

The force-extension relationship for the DNA molecules is measured by applying an optical force to the microsphere and then measuring the corresponding extension of the molecule. For this aim, the microsphere is placed in the approximately linear region of the axial optical potential, extending the DNA perpendicularly away from the cover glass, as shown in Fig. 1. The optical force that acts on the microsphere is then a combination of the gradient and the scattering forces, which remains constant to within 10% over a range of 330 nm. The details of the experimental setup, the characterization, and the calibration process for the optical potential are described by Chen *et al.* [14]. This axial optical force-clamp geometry has distinct advantages over conventional in-plane optical tweezers when the goal is to study the mechanics of submicron biomolecules in a quantitatively accurate fashion. The principal complication with in-plane stretching is that the angle between the DNA and the axis of the optical tweezers changes as the molecule is extended. This in turn may lead to a rotation of the microsphere. Since the microspheres are generally not perfectly spherical, measurement errors in the molecular extension result, which can be substantial when they are compared to the very small dimensions of the molecule itself. The axial geometry employed here does not suffer from these problems.

In order to obtain accurate elasticity measurements, we need to take entropic stretching forces that result from excluded-volume effects in the tethered-particle geometry properly into account. According to the theoretical and the computational analyses by Segall *et al.*, the motion of a microsphere of radius R in a tethered-particle experiment is increasingly constrained as the excursion number $N_R \equiv R/(Ll_p/3)^{1/2}$ rises. By modeling the tethered DNA fragment as a Gaussian chain, Segall *et al.* estimated the effective force resulting from the impenetrability of the cover class to the microsphere as

$$F_{eff} = \frac{k_B T}{\pi^{1/2}(Ll_p/3)^{1/2}} \left(\frac{1 - e^{-N_R^2}}{\mathrm{erf}(N_R)} \right). \tag{2}$$

To take the volume exclusion effects into account in our data analysis, we fit our data of the measurements of the force-extension relationships to a modified wormlike chain model, which incorporates the excluded-volume extension x_0 as an adjustable parameter in the fits and subsumes boundary-condition effects into an effective persistence length l_p^*. The excluded-volume force, which is calculated using the WLC model with the extension x_0 and the effective persistence length l_p^* instead of l_p, is added to the curve fitting equation to reflect the fact that the DNA molecule is stretched by both the optical and the excluded-volume forces. The modified fit equation is

$$F_{opt} = F_{WLC}(x_0 + x_{opt}, l_p^*, L) - F_{WLC}(x_0, l_p^*, L), \tag{3}$$

where F_{WLC} is the WLC model, as given by Eq. (1); F_{opt} is the optical force exerted by the laser beam, which is obtained from the calibration of the optical tweezers; x_0 is the extension under zero optical force; x_{opt} is the extension resulting from the optical force; and $F_{WLC}(x, l_p^*, L)$ is the force of an extended polymer in the wormlike chain model. In the measurement of the force-extension relationships, we applied optical forces of different magnitudes F_{opt} and measured the corresponding extensions x_{opt}. Then, we fitted the data to Eq. (3) to obtain x_0 and l_p^*. As shown in Fig. 2, this model describes the experimental data well.

Our main finding is that the effective persistence length drops dramatically as the contour length of the molecule decreases, as shown in Fig. 3, down to 27.9 nm for the 247 bp construct. This result is consistent with measurements of the elasticity of much longer DNA fragments ranging from 1870 to 7138 bp by Seol *et al.* [9], even though their analysis used a solution to the WLC model that does not include the excluded-volume effects between the microsphere and the cover glass. To compare our measurements to their data, we used their empirical interpolative formula

$$l_p^* = \left(\frac{l_{p\infty} T}{1 + a l_{p\infty}/L} \right) \tag{4}$$

with $l_{p\infty}$=51.51 nm and a fitted empirical parameter a =2.78 [9]. We note that the effective persistence lengths obtained from our measurements follow this interpolation formula well, even though we extend the range of the measurements by almost an order of magnitude. We can further compare our data to the finite wormlike chain model by Seol

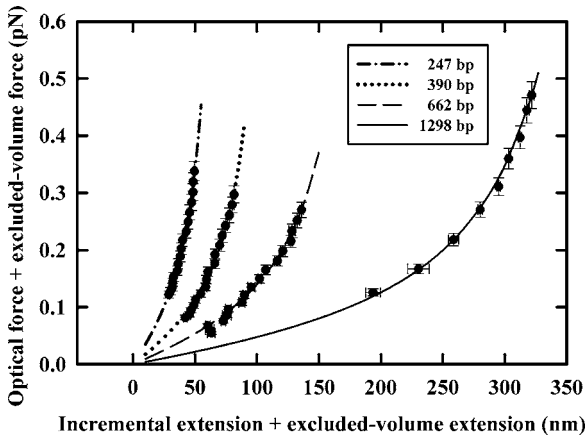

FIG. 2. Force-extension curves of four short ds-DNA constructs 1298, 662, 390, and 247 bp in length, respectively. The lines represent fits to a modified wormlike chain model with an effective persistence length l_p^* and an excluded-volume extension x_0 as adjustable parameters. The error bars represent the standard errors of means obtained from eight independent measurements. In each measurement, 400 frames were taken at a frame rate of 100 frames/s for each force point.

et al., which incorporates corrections for the finite chain length, the chain-end boundary conditions, and the microsphere rotational fluctuations. For our experimental geometry, half-constrained boundary conditions in which the tangent vector of the DNA at both ends is free to explore half of the orientational space in a hemispherical fashion are chosen. The reduced effective persistence length in this model is due to the additional freedom of the end tangent vector, which is more constrained when the DNA fragment is part of a longer chain. As shown in Fig. 3, this model predicts our observed

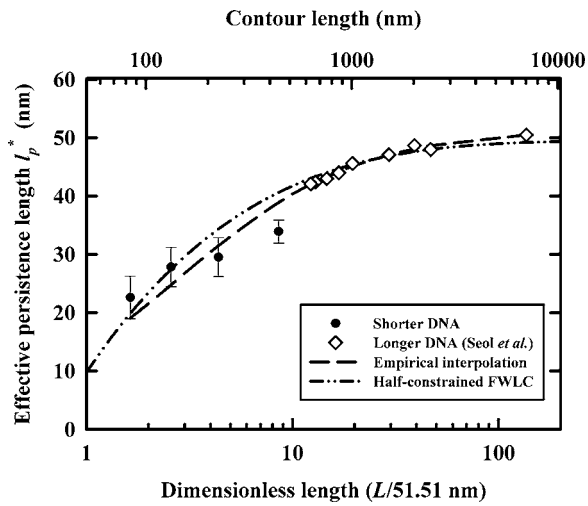

FIG. 3. The effective persistence lengths obtained by fitting a modified wormlike chain model to the four force-extension curves shown in Fig. 2, as a function of the contour length of the molecule. For context and comparison, we also show the persistence lengths measured on longer DNA molecules by Seol *et al.*, their empirical interpolation [Eq. (4)], and their theoretical predictions of the FWLC model of Seol *et al.* for the half-constrained boundary conditions.

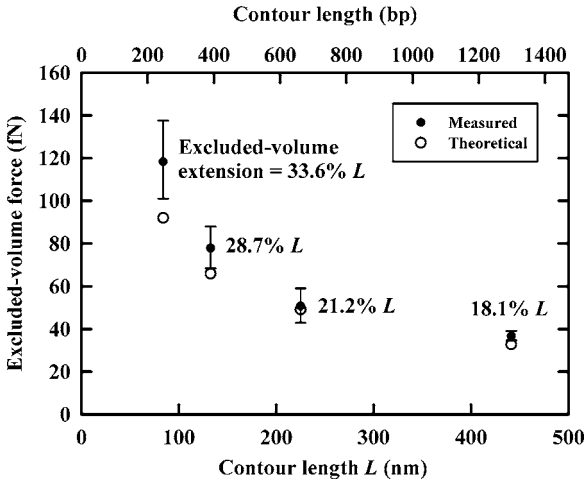

FIG. 4. Excluded-volume extensions and the corresponding excluded-volume forces for the four short DNA constructs shown in Fig. 2, as a function of contour length. The theoretical predictions for the excluded-volume force calculated using Eq. (2) of Segall *et al.* [10] are also plotted for comparison.

effective persistence lengths quite well, without any adjustable or empirical parameters. The small discrepancy for our longest DNA construct can likely be attributed to deviations of the optical potential from a perfectly linear shape for large extensions.

The other result from our measurements is the determination of the excluded-volume forces from the impenetrability of the cover glass to the microsphere. The corresponding forces and extensions of the DNA molecules are shown in Fig. 4. Comparing the excluded-volume forces to the values calculated using Eq. (2) from Segall *et al.* [10] with the measured effective persistence length l_p^*, we note a good agreement. This shows that the effective repulsive force from the interactions between the microsphere and the cover glass can be significant and stretch the DNA to as much as a third of its nominal contour length. This is particularly important for tethered-particle experiments, as forces that are comparable to the characteristic force scale of entropic forces in ds-DNA, $k_BT/l_{p\infty}=80$ fN, are thought to have a significant impact on the assembly of regulatory protein DNA [15,16], which is commonly probed in this kind of experiments [13].

In conclusion, we have measured the effective persistence length and the excluded-volume extension of surface-tethered submicron DNA molecules using constant-force axial optical tweezers. Because of the special geometry of this optical tweezing scheme, accurate measurements of the elasticity of short DNA molecules have become feasible, allowing us to manipulate molecules that are almost an order of magnitude shorter than what can be studied with conventional optical tweezers. The measurements reveal a significant reduction in the effective persistence length of the ds-DNA constructs with a decreasing contour length. We attribute this observation to entropic boundary effects that allow the orientation of the ends to explore a significant conformational space and not changes in the intrinsic bendability of the molecules. The effective persistence length as a function of contour length is well described by the empirical

interpolation by Seol *et al.* [9] and agrees with the theoretical predictions of the half-constrained FWLC model. The effect is quite dramatic: the effective persistence length of the 247-bp-long DNA molecule drops to nearly half of the established value for long DNA molecules. Thus, we conclude that when the WLC model by Marko and Siggia [1] is used to model the elasticity of very short DNA fragments in models for chromatin, for instance, or to interpret the experimental data in tethered-particle experiments with submicron DNA molecules, an effective persistence length that is appropriate for the boundary conditions in a given geometry can and should be used.

Moreover, we have shown that the excluded-volume constraints from the impenetrable surface in the tethered-microsphere geometry can result in significant entropic stretching forces when DNA molecules are short. We see a good quantitative agreement with the predictions by Segall *et al.* [10], which suggest that the excluded-volume effects for such short DNA molecules are dominated by volume exclusion effects between the microsphere and the cover glass. These effects can indeed be crucial for interpreting tethered-particle experiments, as the associated excluded-volume force can easily become larger than the characteristic force scale for entropic forces in ds-DNA, $f_c = k_B T / l_{p\infty} = 80$ fN.

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