Stretching Single Stranded DNA, a Model Polyelectrolyte

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The elastic properties of single stranded (ss)DNA, studied by pulling on an isolated molecule, are shown to agree with a recent model of ssDNA that takes into account base pairings and screened electrostatic repulsion of the phosphodiester backbone. By an appropriate physicochemical treatment, the pairing interactions were suppressed and ssDNA used as an experimental model for a generic polyelectrolyte. The elastic behavior of such an altered ssDNA deviates strongly from the behavior of an ideal polymer. This deviation is shown to result from the elasticity of the chain and its electrostatic self-avoiding interactions.

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New tools have recently been developed for the manipulation of single molecules such as DNA [1–3], protein fibers [4–6], and polymers [7,8]. Using these tools (optical or magnetic tweezers, flexible microscopic cantilevers, Stokes drag), single molecules can be stretched while their resulting extension is measured. The various theoretical models used to analyze the behavior of single molecules under tension [e.g., the freely jointed (FJC) or wormlike chain (WLC) models] are based on the elastic theory of an ideal (i.e., non-self-avoiding) polymer stretched in its entropic regime [9,10]. In the well-known case of double stranded (ds)DNA whose persistence length \( \xi \) is very large (\( \sim 50 \) nm), it can be shown that excluded volume interactions [of a typical scale determined by the Debye length, \( l_D \) of \( O(1) \) nm] do not become relevant until the molecules exceed \( 50 \mu m \) in length [11], an experimental regime rarely accessed. Thus, for all practical purposes the elastic properties of dsDNA resemble that of an ideal (noninteracting) polymer. This deviation is shown to result from the elasticity of the chain and its electrostatic self-avoiding interactions.

The elastic behavior of a self-avoiding chain, however, has not been addressed. Such a chain is a better model of polymers, in particular, flexible polyelectrolytes in which electrostatic self-avoiding interactions play an essential role. Examples include ssDNA and denatured proteins that are characterized by a persistence length \( \xi \sim l_D \). The purpose of this Letter is to use ssDNA as a model for a generic polyelectrolyte required suppression of these pairing interactions by a proper physicochemical treatment, detailed below.

The FJC model has long been used to describe the elasticity of flexible polymers [12]. However, in previous studies of ssDNA it was argued [13,14] that the FJC description of the molecule as a chain of \( N \) freely jointed segments of size \( b = 2\xi \) (the Kuhn length) had to be modified at high forces \( F \) to account for the intrinsic elasticity of the segments (of Young modulus \( Y \)). The free energy of this so-called modified freely jointed chain (mFJC) model is [14]

\[
\mathcal{E}_{m\text{FJC}} = Y \frac{N}{2} \sum_{i=1}^{N} (|\vec{r}_i - \vec{r}_{i-1}| - b)^2 - F z_{N},
\]

(1)

where \( \vec{r}_i = (x_i, y_i, z_i) \) is the position of node \( i \). Previous single molecule studies [15] suggested that this simple description is insufficient to explain the behavior of ssDNA at forces below 10 pN where the effects of sequence, ionic strength, presence of \( Mg^{2+} \) ions predominate. To account for these effects, it is necessary to add base-pairing interactions and electrostatic self-avoidance to the free energy of ssDNA [14,16,17], yielding:

\[
\mathcal{E}_{ss\text{DNA}} = \mathcal{E}_{m\text{FJC}} + \sum_{i=1}^{N_p} V_p + \epsilon_w \int ds_i ds_j \exp(-|\vec{r}_i - \vec{r}_j|/l_D),
\]

(2)

The second term on the right-hand side of Eq. (2) represents the base-pairing energy contribution: \( N_p \) is the number of (nested) paired nodes and \( V_p \) represents the average pairing energy over a Kuhn segment [14]. The last term in Eq. (2) represents the electrostatic repulsion between DNA segments in the Debye-Hückel approximation, where \( \nu \) is the effective DNA charge density, \( \epsilon_w = 80 \) is the dielectric constant of water, and integration is performed over the molecule’s curvilinear coordinates (with \( i < j \)). This term can be computed directly from the ionic concentrations with no free parameters [14].

The system defined in Eq. (2) was simulated by a Monte Carlo (MC) algorithm (described in Ref. [14]) to determine the mean extension \( l = \langle z_N \rangle \) versus \( F \). Although this model has been used to fit various disparate data [14], there has been no effort to test its general

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validity. In the following, we systematically compare its predictions with experiments on ssDNA under different solvent conditions, a large range in force, and by an appropriate chemical treatment without pairing interactions.

Experimental results.—The experimental configuration for the manipulation of single DNA molecules has been described previously [3,15]. Briefly, the sample consists of a ssDNA of extension \( l \) bound at one extremity to a small magnetic bead and at the other to a glass coverslip. Unless mentioned otherwise, the ssDNA used in these experiments is obtained by boiling an 11 kbps dsDNA charomid construct [15] labeled with biotin at one end and digoxigenin at the other. Permanent magnets placed above the sample pull on the bead with a force \((F < 100 \text{ pN})\) that decreases with their distance from the sample. Placement of the sample on an inverted microscope permits measurement of the distance \( l \) of the bead from the surface by calibration of the bead’s diffraction pattern. By simultaneously monitoring the bead’s transverse Brownian fluctuations \( \delta x \), we can obtain the force \([3,18]\) through the dissipation-fluctuation theorem:

\[
F = k_B T / \langle \delta x^2 \rangle.
\]

Figure 1 compares the force vs extension curves for the ssDNA construct in various ionic conditions. In contrast to dsDNA (grey line), ssDNA does not display universal elastic behavior. The latter is very sensitive to changes in salt concentration and presents hysteresis under high ionic conditions, Fig. 1(b). This complex behavior can best be explained by the formation of secondary structures (hairpins) at low forces. Indeed, it is well known that the energetics of hairpins depend on the pH, ionic strength (through the concentration of stabilizing cations), and on the sequence of the ssDNA [19,20]. To compute the elasticity data numerically, we performed a MC simulation of the model described above. We used known or estimated values of the parameters entering in the model:

1. The generally accepted ssDNA Kuhn length \( b = 1.6 \text{ nm} \) and Young modulus \( Y = 120k_BT/\text{nm}^2 \) [13].
2. The measured crystallographic length of the dsDNA molecule \( l_{ds} \) (to which data from various molecules have been normalized, \( l_{0,ds} = 1.69l_{ds} \) [13]). \( l_{ds} \) was estimated from the dsDNA force vs extension curves (for the charomid construct \( l_{0,ds} = 3.37 \mu\text{m} \)).
3. The effective DNA charge density and Debye length were calculated (without fitting) for each salt concentration as explained in [14] and the electrostatic interaction was then computed via Eq. (2). For example, in 10 mM phosphate buffer (PB): \( \nu = 1.28e/\text{nm} \) and \( l_D = 1.87 \text{ nm} \) (notice that \( l_D \approx b \)).

The only fit parameter was the pairing potential \( V_p \) which was best fitted by \( V_p = 4.6k_BT \) [14]. This value reflects the average base-pairing energy per Kuhn length (\( \sim 5 \) bases) in a hairpin, where not all bases are necessarily paired. As can be seen from Fig. 1, the results of the model fit our measurements very nicely over the entire force range and for various salt conditions. This good agreement suggests that the hairpin stabilization at increased salt concentrations is mostly due to a reduced electrostatic repulsion and not to an increase in the pairing energy (constant in the simulations).

By comparing the force-extension curves of DNA molecules with a different percentage of guanine and cytosine (GC) bases, we were able to probe the effect of the pairing interactions (modeled by the potential \( V_p \)) on the elasticity of ssDNA. Figure 2 compares the elasticity of a 50% GC rich ssDNA (charomid construct) with a 30% GC rich ssDNA (a construct based on a \( pX\Delta II \) plasmid [15]). The MC simulation of Eq. (2) fits
the data well with \( V_p = 4.6k_BT \) for the charomid and \( V_p = 3.8k_BT \) for the GC-poorer \( pX\Delta II \) construct. As expected, the higher the GC content, the higher the average pairing energy \( V_p \), the more stable are the hairpins and the higher the force required to stretch the ssDNA to a given length. This sensitivity to ionic conditions and nucleotide content disappears at forces large enough to unzip DNA [21] \( (F > 10 \text{ pN}) \). As hairpins are less likely, the elasticity of ssDNA is less sensitive to variations of sequence and buffers. Furthermore, recent analytical results [16,17] on the elastic behavior of ssDNA described as a mFJC model with nested positive interactions (hairpin structures) are in good agreement with our observations and simulations; see Fig. 1.

The ssDNA base-pairing interactions must be suppressed if one is to use it as an experimental model of a generic polyelectrolyte. To that goal, we have used two different approaches: (i) ssDNA was reacted with glyoxal to reduce the hydrogen bonding interactions between complementary bases; (ii) ssDNA elasticity was measured in a buffer containing 30% formamide (a DNA denaturing agent). The elastic behavior of this altered ssDNA differs significantly from the WLC model of an ideal polymer, used extensively to fit the elasticity of polyelectrolytes, such as proteins [6]. In fact, the molecule’s extension is observed to increase nearly logarithmically with force over at least three decades in force; see Figs. 3 and 4. Particularly striking is the agreement between our results and those obtained using an atomic force microscopy (AFM) cantilever to pull on a native ssDNA in a higher (though overlapping) force regime [22], where hairpins do not form [21]. As can be seen from Fig. 4, the behavior of ssDNA in the absence of base-pairing interactions remains totally at odds with the predictions of the WLC or mFJC models of polymer elasticity. Similar results have also been observed when stretching denatured proteins [23] and ssDNA in low salt [24], where hairpins are destabilized by the electrostatic repulsion. A MC simulation of Eq. (2) with \( V_p = 0 \) (as expected of a generic polyelectrolyte) fits our data up to 70 pN. Beyond that, the large deformation of the ssDNA backbone \( (l > 1.1l_{ss}) \) cannot be described by a simple elastic (quadratic) approximation but requires the introduction in Eq. (1) of a quartic term: 

\[
\frac{Y_2}{2} \sum_{i=1}^{N} (|\vec{r}_i - \vec{r}_{i-1}| - b)^4
\]

with an estimated value of \( Y_2 = 400k_BT/nm^4 \). This nonlinear elastic behavior may result from a structural transition in ssDNA as it is pulled above 70 pN [13].

In conclusion, we have observed that the elastic behavior of a polyelectrolyte, such as ssDNA, is more complex than the often studied WLC and mFJC models. Single stranded DNA is well described as a mFJC chain (of Kuhn length \( b = 1.6 \text{ nm} \) and Young modulus \( Y = 120k_BT/nm^2 \)) with sequence dependent attractive interactions and electrostatic repulsion. The latter can be calculated for various salt conditions directly from the Debye-Hückel approximation with no free parameters. The single fit parameter is the pairing energy \( V_p \) which is sufficient to account for the elasticity of ssDNA over a large range of forces \( (F < 70 \text{ pN}) \) and in different ionic conditions. When attractive interactions are suppressed \( (V_p = 0) \), the ssDNA extension is observed to increase almost logarithmically with the force, a behavior also observed in the stretching of denatured proteins. For a polyelectrolyte whose Kuhn length \( b \sim l_D \), MC simulations suggest that electrostatic self-avoidance interactions

![FIG. 2. Stretching of two different DNA molecules: a 50% GC-rich charomid (○) and a 30% GC-rich pXΔII (∗) in 10 mM Tris buffer, 5 mM Mg\(^{2+}\), 25 mM KCl (the calculated values of the Debye length and the DNA’s effective charge density are \( l_D = 1.54 \text{ nm} \) and \( \nu = 1.29e/nm \)). The continuous curves are results of MC simulations of Eq. (2) with \( V_p = 4.6k_BT \) for the charomid and \( V_p = 3.8k_BT \) for the pXΔII. The extension of the different ssDNA molecules was normalized to the crystallographic length of the dsDNA (whose WLC model is shown as a black line).](248102-3)

![FIG. 3. Extension \( l \) of a ssDNA single molecule in (a) 30% formamide/70% PB (1 mM) before (○) and after (+) addition of 2 mM Mg\(^{2+}\) and (b) 10 mM PB after treatment with glyoxal before (○) and after (+) addition of 2 mM Mg\(^{2+}\). In contrast with unmodified ssDNA, note the independence of the curves upon salt conditions and the absence of hysteresis.](248102-3)
(and nonlinear elastic effects when $F > 70 \text{ pN}$) account for these unexpected observations. This interpretation is supported by recent analytical calculations of the effect of self-avoidance on the elastic behavior of a model polymer [17].

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