Determination of the Elastic Properties of Short ssDNA Molecules by Mechanically Folding and Unfolding DNA Hairpins

Anna Alemany,[1] Felix Ritort[1,2]

[1] *Small Biosystems Lab, Departament de Física Fonamental, Universitat de Barcelona, C/ Martí i Franquès 1 08028 Barcelona, Spain*

[2] *CIBER-BBN de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, C/ Sinesio Delgado 4 28029 Madrid, Spain*

Received 12 May 2014; revised 30 July 2014; accepted 31 July 2014
Published online 4 August 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/bip.22533

ABSTRACT:

The characterization of elastic properties of biopolymers is crucial to understand many molecular reactions determined by conformational bending fluctuations of the polymer. Direct measurement of such elastic properties using single-molecule methods is usually hindered by the intrinsic tendency of such biopolymers to form high-order molecular structures. For example, single-stranded deoxyribonucleic acids (ssDNA) tend to form secondary structures such as local double helices that prevent the direct measurement of the ideal elastic response of the ssDNA. In this work, we show how to extract the ideal elastic response in the entropic regime of short ssDNA molecules by mechanically pulling two-state DNA hairpins of different contour lengths. This is achieved by measuring the force dependence of the molecular extension and stiffness on mechanically folding and unfolding the DNA hairpin. Both quantities are fit to the worm-like chain elastic model giving values for the persistence length and the interphosphate distance. This method can be used to unravel the elastic properties of short ssDNA and RNA sequences and, more generally, any biopolymer that can exhibit a cooperative two-state transition between mechanically folded and unfolded states (such as proteins). © 2014 Wiley Periodicals, Inc. Biopolymers 101: 1193–1199, 2014.

Keywords: *single-molecule experiments; dynamic-force spectroscopy; single-stranded deoxyribonucleic acid flexibility; elasticity polymer models*

This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of any preprints from the past two calendar years by emailing the Biopolymers editorial office at biopolymers@wiley.com.

INTRODUCTION

Single-molecule force experiments make it possible to grab a single molecule by its ends and apply mechanical forces in the range of piconewtons.[1] Changes in the molecular extension can be measured with nanometric precision and theories of entropic elasticity experimentally tested.

The elastic properties of biomolecules (such as proteins and nucleic acids) play a relevant role in several processes occurring at the molecular scale, like DNA packaging inside the cell nucleus, formation of the actin filament network inside the cytoplasm which is crucial for nutrient transport, or transcription and translation of nucleic acids performed by molecular motors that read and process the genetic information. Moreover, elastic properties determine the molecular structure, having important implications for molecular function.

Additional Supporting Information may be found in the online version of this article.

Correspondence to: Felix Ritort; e-mail: fritort@gmail.com

Contract grant sponsor: ERC Grant MagReps 267 862 (to A.A. and F.R.)
Contract grant sponsor: FP7 Grant Infernos 308850 (to A.A. and F.R.)
Contract grant sponsor: Icrea Academia 2013 (to A.A. and F.R.)

Nucleic acids are polymers made of four types of nucleotides, which are adenine (A), guanine (G), cytosine (C), and thymine (T) in the case of DNA or uracil (U) in the case of RNA. Two complementary DNA chains form a double helix through the basepairs that are formed between A and T or C and G. The elastic properties of the DNA double helix (hereafter referred to as double-stranded DNA, dsDNA) have been investigated using single-molecule force methods by pulling a long DNA molecule from its ends.[2–4] At forces below 65 pN, dsDNA behaves according to the worm-like chain (WLC) model with a persistence length approximately equal to 50 nm. In contrast, at higher forces dsDNA undergoes a highly cooperative transition known as overstretching, where it dramatically increases its extension with apparently no force resistance.[5]

In the case of single-stranded deoxyribonucleic acids (ssDNA), the elastic properties are more difficult to access. Even though at large forces the stretched molecule is well-fit by the ideal freely jointed chain or WLC models,[4,6,7] at low forces the molecule self-interacts and forms secondary structures through hydrogen bonds occurring between different nucleotides. This effect is revealed by the presence of a soft plateau in the force-extension curve at low forces which deviates from the ideal elastic behavior.[7,8] Available values to date of the persistence length of ssDNA at room temperature fall in the range 0.7–1 nm, depending on salt condition. Most studies have focused on long ssDNA molecules and the general question remains whether the elastic properties of long ssDNA can be extrapolated to short ssDNA (a few tens of bases), which is much more relevant for genomic regulation.

Recent attempts to investigate the ideal elastic properties of single-stranded nucleic acid molecules have addressed either the case of poly-U RNA molecules using force spectroscopy[9] and poly-T ssDNA using FRET experiments.[10] In the first case, only the elastic response of long molecules (a few thousands of bases) are accessible, whereas in the second case the extraction of the value of the radius of gyration of the ssDNA from FRET efficiency traces requires of several assumptions about the intrachain diffusive kinetics of the polymer. Both poly-U and poly-T molecules were chosen, as they hardly form any secondary structure so the ideal elastic response could be directly probed. However, results obtained with such type of molecules can lead to systematically deviated parameters in case of sequence-dependent elastic behavior. Therefore, the search for suitable methods that allow us to directly extract the persistence length of short polymers (such as ssDNA) that tend to form higher-order structures is still needed. In a previous work, we showed how it is possible to recover the elastic properties of short single-stranded nucleic acid molecules using Kramers theory for activated transitions.[11] In that case, the measurement of the force-extension response is not required,

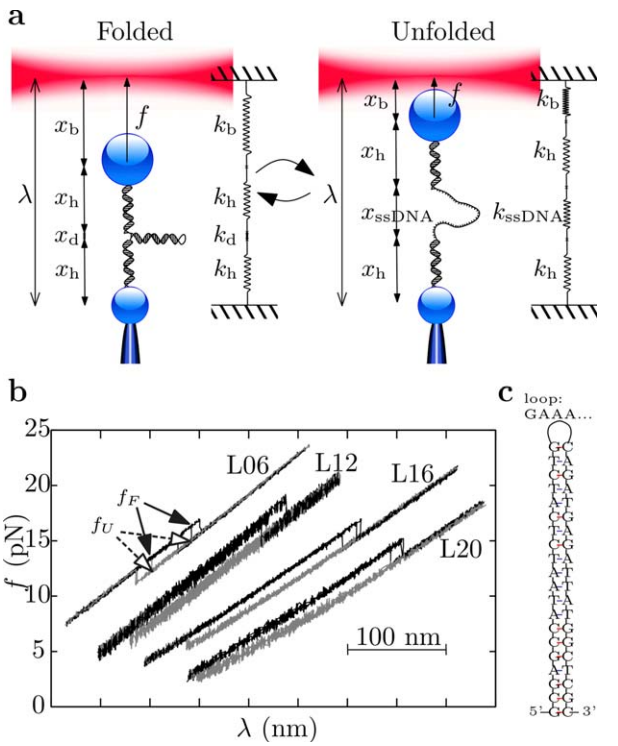

FIGURE 1 Dynamic-force spectroscopy experiments. (a) A DNA hairpin is inserted between two DNA handles, and each end of the molecular construct is tethered to micron-sized beads: one is immobilized in the tip of a micropipette and the other is captured in an optical trap. The system can be modeled by a sequence of serially connected springs. (b) Unfolding (black) and folding (gray) FDCs measured in pulling experiments performed at 60 nm/s for hairpins L06, L12, L16, and L20. Solid (dashed) arrows indicate forces $f_F(f_U)$ in the folded (unfolded)-force branch along unfolding and folding transitions. (c) Sequence of the hairpin stem (adenine = A, guanine = G, thymine = T, cytosine = C). All loops consist of a G followed by the corresponding number of A.

but the elastic parameters depend on the basepair free-energy contributions used to model the molecular free-energy landscape.[6,12]

Here, we introduce a new method useful to extract the ideal elastic response of short biopolymers that fold into compact structures at low forces. The basic idea is to measure and compare the elastic response of the polymer in the folded and unfolded states using dynamic-force spectroscopy experiments. This is achieved by extracting the force-dependent molecular extension absorbed/released upon folding/unfolding of the polymer and by determining the difference in elastic compliances of the polymer along the folded and unfolded force branches. The method is illustrated for the case of the elastic response of ssDNA using optical tweezers to pull short DNA hairpins that fold into well-known double helix secondary structures. Our results reveal that the value of the persistence length of short ssDNA molecules is approximately twice as

large as compared to the value commonly accepted for long ssDNA molecules in agreement with results recently reported by other groups and studies. The method can also be used to unravel the elastic properties of the polypeptide chain in proteins and, by suitably engineering molecular constructs, biopolymers in general.

METHODS

Optical tweezers is the name given by Arthur Ashkin to the discovery of the ability of a strongly focused laser light to exert attractive forces on micron-sized polystyrene microspheres toward the focus (commonly denoted as optical trap).[13] Optical tweezers make it possible to apply forces to biomolecules by tethering them between a fixed surface and a microsphere captured in the optical trap.

Here, we use DNA hairpins, which consist of a stable DNA double helix (hereafter referred to as stem) ended by a loop. In dynamic-force spectroscopy experiments, the hairpin is tethered between two identical 29-basepairs long dsDNA handles, and the free end of each handle is attached through different antibody–antigen bonds (digoxigenin-antidigoxigenin at one end; biotin–streptavidin at the other) to two micron-sized polystyrene microspheres.[14] One bead is immobilized by air suction in the tip of a micropipette, whereas the other is captured in an optical trap produced by a highly stable dual-beam optical tweezers apparatus (Figure 1a).

Our instrument allows us to manipulate the relative distance λ between the center of the optical trap and the tip of the micropipette.[6,15] In pulling experiments, the distance λ increases/decreases at a constant pulling speed along the unfolding/folding process (black/gray curves in Figure 1b). As a consequence, the force applied to the DNA hairpin also increases/decreases during the pulling cycle. At low forces, short DNA hairpins are in the folded state, where the stem forms a double helix; whereas at large forces they unfold in a stretched conformation, where the stem is found as ssDNA.[16,17] Transitions between both states are viewed in the force-distance curve (FDC) as a sudden jump in force. Forces at which such transitions take place change on repetition of the same experiment due to thermal fluctuations. Figure 1b shows different examples of FDC obtained from pulling experiments with different DNA hairpins (see below) at 60 nm/s. Two branches of force are observed: the upper branch shows the elastic response of the whole molecular construct when the hairpin is folded, whereas the lower branch shows it when the hairpin is unfolded.

The trap-pipette distance satisfies (Figure 1a):

$$\lambda = x_h + x_b + x_{DNA} \tag{1}$$

where x_h is the extension of the two handles, x_b is the position of the bead relative to the center of the optical trap, and x_{ssDNA} is the molecular extension of hairpin that depends on its state. If the hairpin is folded (Figure 1a, left), x_{DNA} is equal to the projection of the double helix diameter, x_d, along the force axis. If the hairpin is unfolded (Figure 1a, right), x_{DNA} equals the equilibrium end-to-end distance of the ssDNA at the given force, x_{ssDNA}.

If we divide a small change in the trap-pipette position, $\Delta\lambda$, by a small change in force, Δf, we get:

$$\frac{1}{k_{eff}(f)} = \frac{1}{k_h(f)} + \frac{1}{k_b(f)} + \frac{1}{k_{ssDNA}(f)} \tag{2}$$

where the stiffness of each element in the molecular construct k_i (i = h, b, or DNA) is equal to $\Delta f/\Delta x_i$. The stiffness of the DNA depends on whether the hairpin is folded, $k_{ssDNA} = k_d$, or unfolded, $k_{DNA} = k_{ssDNA}$. The effective stiffness of the system, $k_{eff} = \Delta f/\Delta\lambda$, equals the slope of experimental FDC and gets contributions from different serially connected springs, each one related to a different part of the experimental setup (Figure 1a).

Force-Jump Measurement

When the hairpin unfolds/folds, a sudden force-jump $\Delta f = f_F - f_U$ is observed at a fixed distance λ. f_F (f_U) corresponds to the force in the folded (unfolded) branch. From Eq. (1), we write:

$$x_h(f_F) + x_b(f_F) + x_d(f_F) = x_h(f_U) + x_b(f_U) + x_{ssDNA}(f_U) \tag{3}$$

as $\Delta\lambda = 0$ in the transition. If we divide $\Delta f = f_F - f_U$ by the effective stiffness of the system along the folded branch, k^F_{eff}, and we use that $\Delta f = k_i \Delta x_i$ (i = h, b, and d) it follows that:

$$\frac{\Delta f}{k^F_{eff}} = [x_h(f_F) - x_h(f_U)] + [x_b(f_F) - x_b(f_U)] + [x_d(f_F) - x_d(f_U)] \tag{4}$$

Inserting Eq. (3) into (4), we get:

$$x_{ssDNA}(f_U) = \frac{\Delta f}{k^F_{eff}} + x_d(f_U) \tag{5}$$

Therefore, we can obtain the force-dependent elastic response of ssDNA, $x_{ssDNA}(f)$, from unfolding and folding force-jump values measured in pulling experiments. A detailed derivation of Eq. (5) is provided in Supporting Information Section S1. The same method is also used in Ref. [18] to extract the elastic properties of a peptide chain, but the effect of the diameter of the folded molecule [the term $x_d(f_U)$ in Eq. (5)] was neglected. As we show below, this provides stiffer elastic properties for ssDNA. Here, the helix diameter is modeled as a single bond of length $d = 2.0$ nm (corresponding to the diameter of the DNA double helix) that is oriented in the presence of a force. The mathematical solution for this model is equivalent to the equilibrium orientation of a magnetic dipole in a magnetic field (see Supporting Information Section S2)[14,16]:

$$x_d(f) = d\left[\coth\left(\frac{fd}{k_B T}\right) - \frac{k_B T}{fd}\right] \tag{6}$$

where k_B is the Boltzmann constant and T is the absolute temperature taken equal to 298 K.

Measurements of Effective Stiffnesses

In the presence of hysteresis effects between experimental unfolding and folding FDC, the two force-branches (folded or unfolded) are measured for a large range of forces. Therefore, the respective force-dependent effective stiffnesses $k^F_{eff}(f)$ and $k^U_{eff}(f)$ can be measured from the slope of each force-branch at different forces (Figure 1b). From Eq. (2) applied to both branches at a force f, one gets (Supporting Information Section S3):

$$\frac{1}{k_{\mathrm{eff}}^{\mathrm{U}}(f)}-\frac{1}{k_{\mathrm{eff}}^{\mathrm{F}}(f)}=\frac{1}{k_{\mathrm{ssDNA}}(f)}-\frac{1}{k_{\mathrm{d}}(f)} \tag{7}$$

Therefore, the stiffness of ssDNA can be measured as a function of force from the slopes of the FDC. By taking the derivative of Eq. (6) with respect to force, we get the stiffness of the hairpin double helix:

$$\frac{1}{k_{\mathrm{d}}(f)}=\frac{\partial x_{\mathrm{d}}(f)}{\partial f}=\frac{d^2}{k_{\mathrm{B}}T}\left[\frac{-1}{\sinh^2\left(\frac{fd}{k_{\mathrm{B}}T}\right)}+\left(\frac{k_{\mathrm{B}}T}{fd}\right)^2\right] \tag{8}$$

where we take $d = 2.0$ nm.

RESULTS

Four different hairpins were pulled at 60 nm/s in a buffer containing 1*M* NaCl concentration (Figure 1b). All hairpins have identical stems of 20 basepairs and different loop sizes (Figure 1c). The name of each hairpin is L*X*, being *X* the number of bases in the loop (L06, L12, L16, and L20). The four hairpins show a two-state behavior: they are either folded or unfolded along the FDC and they show a sudden force-jump when they change conformation (Figure 1b). It can be seen in Figure 1b that the larger the loop size, the larger the hysteresis between unfolding and folding curves. Experiments with a minimum of three molecules were performed in each case and a minimum of 100 unfolding and folding trajectories were obtained for each molecule.

Method 1: Force-Jump Measurement

For each unfolding and folding transition, we determined $\Delta f = f_{\mathrm{F}} - f_{\mathrm{U}}$ and divided it by the corresponding value of $k_{\mathrm{eff}}^{\mathrm{F}}$. According to Eq. (5), we then added the extension of the hairpin double-helix diameter, computed using Eq. (6). This gives the elastic response of ssDNA, $x_{\mathrm{ssDNA}}(f)$, and can be fitted to the interpolation formula of the WLC model[2,3]:

$$f=\frac{k_{\mathrm{B}}T}{P}\left[\frac{1}{4(1-x_{\mathrm{ssDNA}}/L_{\mathrm{c}})^2}-\frac{1}{4}+\frac{x_{\mathrm{ssDNA}}}{L_{\mathrm{c}}}+\sum_{n=2}^{7}a_n\left(\frac{x_{\mathrm{ssDNA}}}{L_{\mathrm{c}}}\right)^n\right] \tag{9}$$

where P is the persistence length and L_{c} is the molecular contour length of the DNA hairpin. L_{c} is taken equal to the total number of bases of the hairpin multiplied by the interphosphate distance, d_{b}. The coefficients a_i $(i = 1\ldots, 7)$ can be taken equal to zero[2] or to the numerical values proposed in Ref. [3]. In Figure 2a, we show the two-dimensional (2D)-contour plot of the histogram of measured molecular extensions of ssDNA, $x_{\mathrm{ssDNA}}(f)$, versus the histogram of unfolding and folding forces obtained for the different molecules. It can be appreciated how hysteresis effects increase with the loop size as the sets of unfolding and folding forces separate from each other.

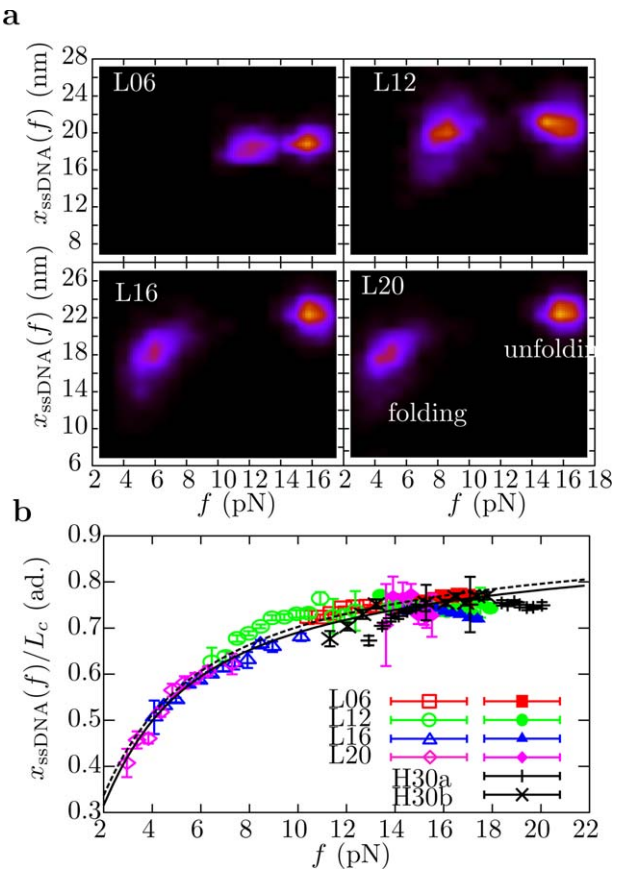

FIGURE 2 Elastic response of ssDNA by mechanically unfolding DNA hairpins. (a) 2D-contour plot of the histogram of x_{ssDNA} plotted against the histogram of unfolding and folding forces for the different DNA hairpins, L06, L12, L16, and L20. (b) Normalized elastic response of ssDNA. Solid (empty) symbols refer to values measured using data from unfolding(folding) curves for hairpins L*X*. The elastic response of hairpins H30a and H30b are represented using crosses (+, *x*). The black-solid line shows the fit to the WLC model using the interpolation formula proposed in Ref. [2] [$a_i = 0$ in Eq. (9)] and black-dashed line shows the fit to the WLC model using the extended version proposed in Ref. [3] ($a_i \neq 0$). Differences between both fits are small. Error bars are statistical errors from averaging over different molecules and force bins.

According to ideal elastic models for semiflexible polymers, the molecular extension at a given force is proportional to the contour length and hence $f = f(x_{\mathrm{ssDNA}}/L_{\mathrm{c}})$. For the four molecules (L06, L12, L16, and L20), we divide each extracted value of x_{ssDNA} (Eq. (5) and Figure 2a) by the appropriate value of the contour length $L_{\mathrm{c}} = (40 + X)d_{\mathrm{b}}$ and we average over different force bins. In Figure 2b, we merge all the data obtained from the four DNA hairpins. The pair of values P, d_{b} that best fit our experimental results to Eq. (9) are $P = 1.35 \pm 0.05$ nm, $d_{\mathrm{b}} = 0.58 \pm 0.02$ nm/base and $P = 1.10 \pm 0.05$ nm, $d_{\mathrm{b}} = 0.59 \pm 0.02$ nm/base, depending on whether we use the interpolation formula of Ref. [2] or the extension proposed in

Ref. [3], respectively. The elastic response x_{ssDNA}/L_c was also extracted from pulling experiments performed with two different DNA hairpins made of a 30-basepairs stem ended by a GAAA-tetraloop. Such hairpins, referred to as H30a and H30b, have randomized stem sequences unrelated to those of hairpins L*X*. It can be seen in Figure 2b that their elastic response is still well-described using the same elastic parameters obtained from the previous fit. If the presence of the hairpin diameter is neglected the values P, d_b that best fit our experimental data are $P = 1.45 \pm 0.05$ nm, $d_b = 0.53 \pm 0.02$ nm/base or $P = 1.20 \pm 0.05$ nm, $d_b = 0.54 \pm 0.02$ nm/base, again depending on whether we use the interpolation formula of Ref. [2] or the expansion proposed in Ref. [3], respectively. Therefore, persistence lengths are slightly overestimated whereas interphosphate distances tend to be shorter.

The quality of this method to extract the elastic properties of ssDNA has been tested in simulated pulling experiments (Supporting Information Section S4). We conclude that the force-jump method is very accurate to determine elastic properties of biopolymers. In addition, from simulations it can also be shown that stiffer elastic parameters (in particular, larger P and shorter d_b) are recovered by neglecting the contribution of the orientation of the DNA double helix when the hairpin is folded.

Method 2: Stiffness Measurement

The elastic response of ssDNA can also be obtained from the measurement of the effective stiffnesses of the molecular construct when the hairpin is folded and unfolded, as described by Eq. (7). To achieve this, we first averaged the different experimental FDC and computed the slopes of the FDC along the folded and unfolded force-branches. Results obtained for the different molecules are shown in Figure 3a. It can be seen that, as expected, $k_{eff}^F > k_{eff}^U$ and that the range of forces where both stiffnesses can be measured increases with hysteresis effects (i.e., with loop size). Then, we subtracted the inverse values of k_{eff}^U and k_{eff}^F at different forces and used Eq. (7) and Eq. (8) to extract values for $k_{ssDNA}(f)$. These have been fitted to the following expression provided by the WLC model:

$$k_{ssDNA} = \frac{k_B T}{P L_c}\left[\frac{1}{2(1-x_{ssDNA}/L_c)^3} + 1 + \sum_{n=2}^{7} n a_n \left(\frac{x_{ssDNA}}{L_c}\right)^{n-1}\right] \quad (10)$$

The values of P, d_b that best fit simultaneously all our experimental data are $P = 2.1 \pm 0.1$ nm, $d_b = 0.75 \pm 0.2$ nm/base, or $P = 1.00 \pm 0.05$ nm, $d_b = 0.56 \pm 0.02$ nm/base, depending on whether we use the interpolation formula of Ref. [2] or the extension proposed in Ref. [3], respectively. Despite the good agreement found for P and d_b between the extension-fitting and the stiffness-fitting methods (Figures 2b and 3b) when using the interpolation formula proposed by Bouchiat et al. in

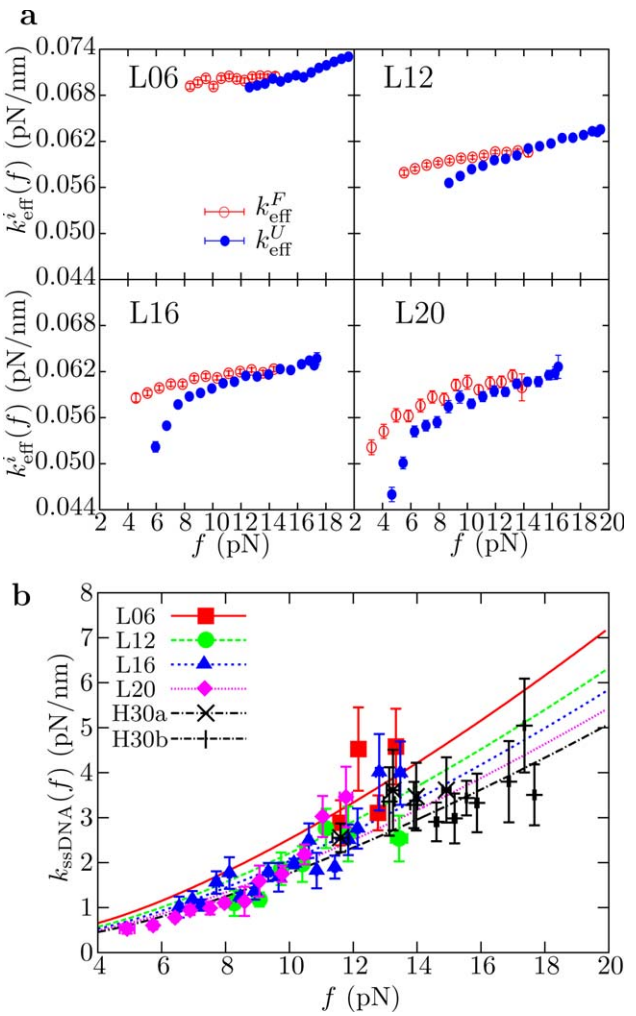

FIGURE 3 Force-dependence of the stiffness of ssDNA by mechanically unfolding DNA hairpins. (a) Effective stiffnesses of the whole molecular system when the hairpin is folded (k_{eff}^F, open-red circles) and unfolded (k_{eff}^U, closed-blue circles) measured for all DNA hairpins. (b) Experimental data and fit to the WLC model using the expression from Bouchiat et al.[3] (Eq. (10)) for molecules L06 (squares and solid line), L12 (circles and dashed line), L16 (triangles and dotted line), and L20 (diamonds and dashed-dotted line). Error bars are standard errors from averages taken over different molecules of a given type.

Ref. [3], a remarkable discrepancy is found when using the WLC expression proposed in Ref. [2] (Eq. (7) and Eq. (10) with $a_i = 0$). However, by setting d_b equal to 0.58 nm/base in the stiffness-fitting method, we obtain $P = 1.35 \pm 0.5$ nm, in very good agreement with the extension-fitting method. This analysis was repeated using results for hairpins H30a and H30b and a reasonable agreement is found between the experimental and the predicted $k_{ssDNA}(f)$ for such longer hairpins. The fit of the stiffness of ssDNA by neglecting the contribution of the hairpin diameter leads to lower values of the

interphosphate distance, whereas the persistence length in this case remains practically the same.

Again, the validity of method 2 to extract the elastic parameters of ssDNA has been tested on simulated pulling experiments (Supporting Information Section S4). From results, we conclude that the stiffness-fitting method is also robust to recover the elastic properties of biomolecules that can be mechanically unfolded/folded under the action of force.

DISCUSSION

Elastic properties of nucleic acids and proteins are determinant factors for the molecular structure and play an important role in genomic regulation. Single-molecule and dynamic-force spectroscopy experiments pave the way to unravel the mechanical properties of such biomolecules with unprecedented detail. The measurement of the ideal elastic properties of biopolymers at low forces is usually hindered by the formation of high-order structures, such as secondary and tertiary structures. Most studies to date have focused on the elastic response of long polymers whereas measurements in case of short polymers appear technically more challenging requiring methods such as FRET that nevertheless are strongly affected by intra-chain diffusive kinetics and secondary structure formation. In this regard, the development of methods that directly probe the ideal elastic response of short polymers capable of forming high-order structures is essential.

In this work, we show how we can extract the ideal elastic properties of short ssDNA molecules by mechanically unfolding short DNA hairpins of different contour lengths. When a force is applied to the ends of such hairpins, they unfold/refold in a two-state manner. From the measured unfolding and folding FDCs, it is possible to extract the elastic response of ssDNA. Two different methods are proposed: In the first method, the released/absorbed molecular extension on hairpin unfolding/folding is related to the end-to-end distance of ssDNA, $x_{\mathrm{ssDNA}}(f)$. In the second method, the slope of the experimentally measured FDCs is related to the rigidity of ssDNA, $k_{\mathrm{ssDNA}}(f)$, further characterizing its elastic response. For a given molecule, $x_{\mathrm{ssDNA}}(f)$ can only be measured in the range of forces at which the hairpin unfolds and folds, which is usually a small force interval (see hairpins L06 and L12 in Figures 2a and 2b). The same occurs with $k_{\mathrm{ssDNA}}(f)$ that should be measured along a force range where both the folded and unfolded states are kinetically stable. To increase the force interval explored in the two methods, we pulled different DNA hairpins with identical stem sequence and different loop sizes. The second method is particularly useful for biomolecules displaying strong hysteresis effects in pulling experiments (Figure 3a). However, error bars for the measured rigidities tend to be large due to experimental artifacts, such as tether misalignment and drift effects.

A remarkable property of both methods to extract elastic parameters is that neither of them requires a direct measurement of the absolute molecular extension, x_{m}. This becomes an advantage in optical tweezers setups that are only able to provide relative measurements of the molecular extension, that is, that take an undefined origin of coordinate.[7] In many optical tweezers setups, where video imaging can be used to measure bead-to-bead distances, it is often very difficult to extract the absolute value of the molecular extension in case of tethers that are misaligned due to geometrical constraints. In such cases, an extra free parameter x_0 related to the zero in molecular extension must be introduced in the theoretical force-extension curve $f = f(x_{\mathrm{m}} - x_0)$. The fit of the experimental data to such expressions can be inaccurate and lead to systematic errors in the derivation of elastic parameters for biomolecules under study.

The results obtained by the two methods were fit to theoretical expressions of the WLC model[2,3] to extract values for the interphosphate distance and the persistence length of ssDNA. Both methods were further tested on simulated pulling experiments, and results validated by the two approaches. By averaging over the best fits obtained with the two methods, we get for the interphosphate distance $d_{\mathrm{b}} = 0.58 \pm 0.02$ nm/base, whereas for the persistence length, we get $P = 1.3 \pm 0.2$ nm. Both numbers were obtained at room temperature (298 K) and standard 1*M* NaCl ionic conditions. The value found for d_{b} is in very good agreement with that generally reported in the literature ($d_{\mathrm{b}} = 0.59$ nm/base). In contrast, previous measurements of P for long ssDNA molecules obtained at 1*M* NaCl by fitting the WLC model to the force-extension curves predict lower values nearly half the ones reported here ($P = 0.76 \pm 0.05$ nm).[4,6,7]

Our results show that the elastic properties of ssDNA molecules of a few tens of basepairs differ from those reported for long ssDNA molecules (of a few kilo-bases). This fact has also been observed in previous works, for example, FRET studies of a series of oligodeoxythymidylates performed over a wide range of salt concentrations and chain lengths (between 10 and 70 nucleotides) have reported comparably large values for P (between 1.5 and 5 nm, depending on salt concentration).[10] These values are much larger than the ones reported for long ssDNA (in the range 0.7–1 nm depending on ionic conditions). The origin of such discrepancy is presently unknown and might be related to finite-size effects. Future studies should address this point by checking the systematic effect of sequence and finite contour length on the elastic properties of nucleic acids at different ranges of forces.

We conclude that it is possible to measure the ideal elastic properties of ssDNA between 1 and 20 pN, a force range where

the determination of the ideal elastic response for large ssDNA molecules is particularly difficult due to the formation of secondary structures. The methodology presented here can be applied to any molecule displaying two-states behavior under pulling experiments and exhibiting a cooperative transition mediating folded and unfolded states. In particular, it would be very interesting to apply this method to extract the elastic parameters of ssDNA, RNA, and polypeptides by varying ionic strength and temperature.

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Reviewing Editor: Nils G. Walter