Thermodynamics of DNA Interactions from Single Molecule Stretching Experiments

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ABSTRACT

On the basis of our analysis of detailed measurements of the dependence of the overstretching transition of double-stranded DNA (dsDNA) on temperature, pH, and ionic strength, we have demonstrated that a model of force-induced melting accurately describes the thermodynamics of DNA overstretching. Measurements of this transition allow us to determine the stability of dsDNA and obtain information similar to that obtained in thermal melting studies. This single-molecule technique has the advantage that it can be used to measure DNA stability at any temperature. We discuss the use of this technique to study the nucleic acid chaperone activity of the HIV-1 nucleocapsid protein.

1. Introduction

When single-molecule manipulation techniques were first developed, one of the earliest problems to be investigated was the determination of the mechanical properties of a double-stranded DNA (dsDNA) molecule as it is stretched to its B-form contour length.[1] However, it was only recently that high-resolution measurements of DNA stretching were made at the forces required to stretch DNA beyond its contour length.[2,3] These force−extension experiments provided the first accurate measurements of the elasticity of single DNA molecules at high forces.

The surprising result of these experiments was the observation of a structural transition, referred to as the overstretching transition, as the DNA molecule is stretched to even higher forces. At forces of ∼65 pN, a plateau is observed, and very little additional force is required to stretch dsDNA to extensions over 1.7 times the B-form contour length. A typical experimental force−extension curve for dsDNA is shown in Figure 1. The solid line on the left is a fit of the stretching data to the extensible wormlike chain (WLC) model for the elasticity of dsDNA.[4] This curve shows that a much greater force would be required to stretch the DNA beyond the B-form contour length if it remained in its helical form. The solid line on the right is data from the stretching of single-stranded DNA (ssDNA).[2]

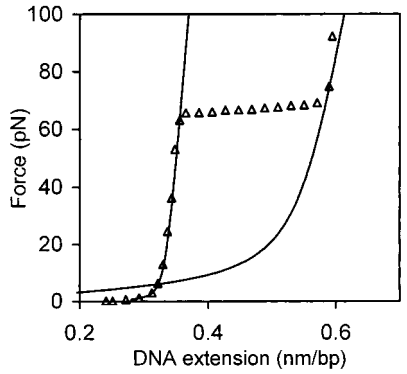

FIGURE 1. Typical DNA stretching curve in high-salt (250 mM NaCl) buffer (△). Lines representing the WLC model (eq 1) for the elasticity of dsDNA with $P_{ds} = 50$ nm and $K_{ds} = 1200$ pN (left) and the measured elasticity of ssDNA[2] (right) are also shown. The DNA stretching curve appears to be a transition from dsDNA to ssDNA.

Figure 1 suggests that the structural transition that occurs as dsDNA is stretched is a transition from dsDNA to ssDNA, breaking the base pairs holding the two strands together. However, it has been shown that the two DNA strands do not separate at the end of the overstretching transition.[5,6] This observation suggests that the base pairs do not break during the transition. Thus, it was proposed that during the overstretching transition the helical form of DNA is unwound but the base pairs are unbroken. This proposed double-stranded form of DNA, denoted S-DNA,[3] inspired several theoretical predictions of its structure;[7−9] however, these studies were unable to reproduce the experimental data.

In an attempt to resolve this issue, we carried out theoretical and experimental studies that lead to a new model describing the overstretching transition of dsDNA as a force-induced melting transition.[10,11] Detailed measurements of the dependence of this transition on temperature, pH, and ionic strength are consistent with a force-induced melting model to describe the thermodynamics of DNA overstretching. Measurements of this transition allow the determination of the free energy of the helix-coil transition of dsDNA under a variety of

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solution conditions. The information that is obtained in these single molecule studies is similar to that obtained in thermal melting studies, but the force-induced melting technique has several advantages, foremost of which is the ability to measure DNA stability isothermally at any temperature.

The capability to carry out melting studies at physiological temperatures is particularly important for measuring the effect of DNA binding proteins on DNA stability, since these effects often depend strongly on temperature, and many proteins melt at the temperatures required for thermal melting studies. In addition, proteins that cause DNA to aggregate at high concentrations make thermal melting studies difficult, but do not affect single-molecule force-induced melting studies. Here we describe the use of this new biophysical technique to study the effect of the nucleic acid chaperone protein HIV-1 nucleocapsid protein (NC) on DNA stability. We then use these results to derive a model for the nucleic acid chaperone activity of this protein, which is essential for HIV replication.

2. Force-Induced Melting Model for DNA Overstretching

In the force-induced melting model of DNA overstretching, DNA melts as the molecule is pulled through the overstretching transition. However, it is always thermodynamically favorable for the molecule to maintain a significant number of domain boundaries, even when a substantial fraction of the DNA base pairs is melted. In our model, it is these domain boundaries, which consist of short, helical base-paired sections distributed throughout the molecule, that hold the strands together at the end of the overstretching transition.[12,13] This implies that the majority of the DNA base pairs melt within internal domains rather than from the free ends, a conclusion that is in accordance with the one-dimensional nature of the DNA melting transition and with its sequence heterogeneity. These two factors are known to result in a large number of equilibrium boundaries within DNA throughout its thermal melting transition.[14,15]

A schematic illustration of the proposed structure of overstretched DNA is shown in Figure 2. The force-induced melting model consists of two stages. The first is an equilibrium melting transition in which domains of melted DNA are formed as the DNA is stretched. In high salt, this transition occurs over a force range of a few pN at about 65 pN under standard conditions, as shown in Figure 1. The second stage consists of nonequilibrium melting, in which the domain boundaries are removed and the two DNA strands separate in an irreversible process. This transition occurs over a wide range of forces from 68 pN to >150 pN. As expected for a nonequilibrium transition,[16] the observed stretching forces are rate-dependent.[5] This description applies only to DNA that is allowed to rotate as it is stretched. Significantly different DNA stretching curves are obtained when the DNA is torsionally constrained.[17,18] In fact, untwisting the DNA has been shown to lead to melting at very low forces.[19]

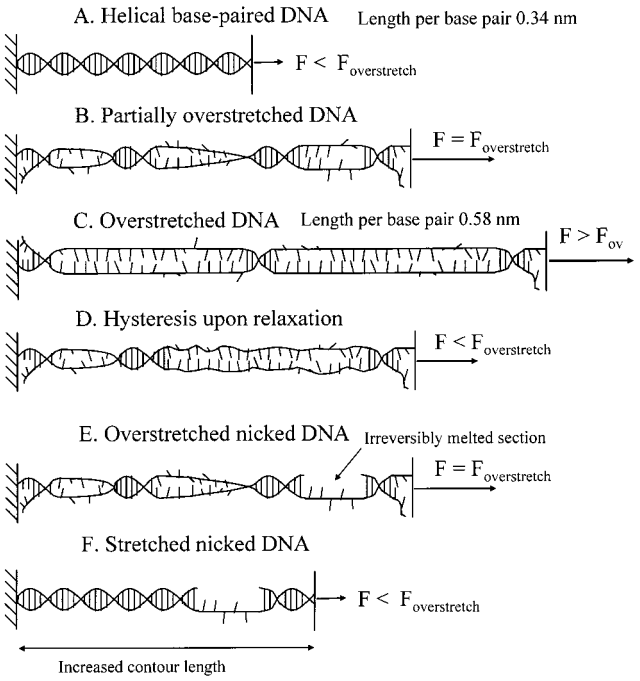

FIGURE 2. Schematic diagram of force-induced melting model for DNA overstretching. (A) When DNA is stretched at forces less than $F_{overstretch}$, the bases remain paired and the helical form is maintained. (B) During the overstretching transition, domains of melted DNA are separated by helical sections. (C) At the end of the transition, short, helical domain boundaries hold the largely melted strands together. (D) Under conditions that inhibit reannealing, when the molecule is relaxed to the same extension shown in B, the observed force is predicted to be less than $F_{overstretch}$. (E) A section of DNA may melt irreversibly during overstretching if the DNA backbone contains two nicks. (F) When a section is irreversibly melted during overstretching, subsequent stretches will reveal an increased contour length. An example of this is illustrated in Figure 3A.

This two-stage melting model is consistent with early observations that complete strand separation does not occur in thermal melting experiments until the DNA is heated to temperatures much greater than the midpoint of the melting transition.[20] The model also provides an elegant explanation for the hysteresis observed in conditions under which strand recombination is inhibited (low salt, high pH, and high temperature).[12,13] An example of this hysteresis is shown in Figure 3A. As the DNA molecule is stretched, most of the base pairs are broken. When the molecule is relaxed, the base pairs have to reanneal to form the correct sequence. Since under these conditions they do not reanneal on the time scale of the stretching experiment, a lower force is required when relaxing the DNA compared to its original stretching curve. Although we observed significant hysteresis upon relaxation of single DNA molecules under most conditions, the original stretching curve was usually recovered upon repeated stretching (data not shown). However, many of the DNA molecules that were stretched broke when overstretched or exhibited an increase in contour length upon subsequent stretching. This is presumably due to the presence of single-stranded breaks (nicks) in the backbone of the DNA molecule.

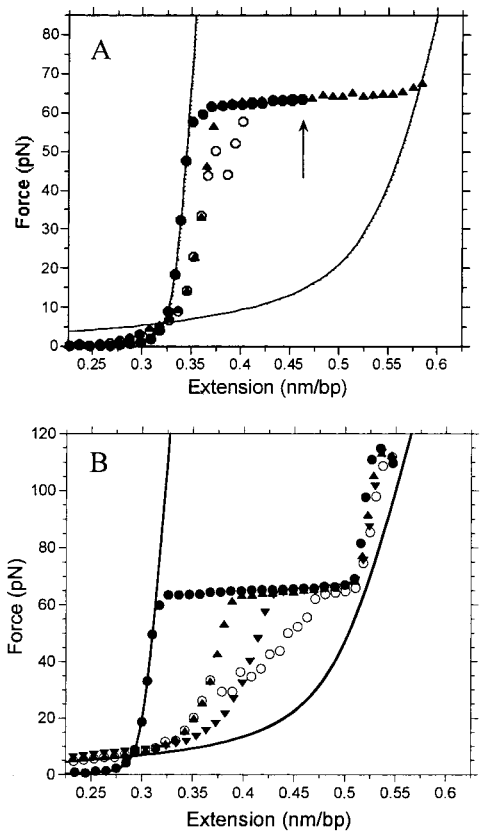

FIGURE 3. Hysteresis seen in DNA overstretching is consistent with force-induced melting. (A) A single dsDNA molecule that has been tethered and stretched to 0.46 nm/bp (arrow) in 250 mM buffer (●) displays partial ssDNA character during the relaxation curve (○) and subsequent stretch (▲). This is due to irreversible melting, as described in Figure 2E,F. (B) Multiple stretches to high extensions produce partially ssDNA. A dsDNA molecule that has been tethered in 250 mM buffer and stretched to near the maximum force achievable with this instrument (●) displays large hysteresis and partial ssDNA character during relaxation (○). Second (▲) and third (▼) stretches increase ssDNA character (from ref 23)

Figure 3A illustrates the consequences of the presence of nicks in the DNA molecule. When this particular molecule was stretched only partially through the overstretching transition, we observed significant hysteresis during the relaxation cycle. (This happens even when the DNA is not nicked.) When the same molecule was stretched again, its contour length had increased, and its elasticity had changed, indicating that a small section of one DNA strand had melted irreversibly. A model of how this is likely to occur is given in Figure 2E,F. This is strong evidence that melting of small sections of DNA occurs during the overstretching transition, at least for nicked DNA. Figure 3B illustrates the consequences of stretching a single DNA molecule to forces greater than the overstretching force. Here, a molecule is stretched to 120 pN, a large enough force to remove boundaries between melted domains. Nicks present in this strand allowed a long section of ssDNA to be melted away at these forces, as indicated by the long contour length observed in the second stretch.

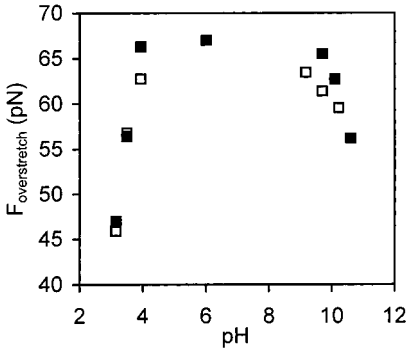

FIGURE 4. Measured (■) and predicted (□) overstretching force as a function of solution pH at 500 mM ionic strength. Predictions are made from measurements of DNA thermal melting using eq 1 and $\Delta S = 9.5$ cal/mol·K·bp. By assumption, theory and experiment are the same at pH 6.5. The measured and predicted points are not vertically aligned because of corrections due to the change in pH with temperature of the buffers used for thermal and force-induced melting measurements (from ref 12).

3. pH Dependence of DNA Overstretching

The hysteresis observed under conditions that inhibit strand recombination and the irreversible melting described in Figure 3 qualitatively support the model of DNA overstretching as force-induced melting. Additional evidence in favor of this model comes from its ability to quantitatively predict the dependence of the overstretching transition on changes in solution conditions.[11] The first prediction to be made by the model is that any solution conditions that destabilize DNA and, therefore, lower its melting temperature must also lower the overstretching force, which we define as the force required to stretch the molecule halfway through the overstretching transition. Since the melting temperature of DNA decreases dramatically at both low and high pH, we measured the overstretching force at both extremes of pH.[12] As shown in Figure 4, in both cases, we found that the overstretching force decreased.

In the force-induced melting model, the overstretching force is related to the melting temperature by the one-dimensional analogue of the Clausius−Clapeyron equation

$$\delta F_{\text{overstretch}} = \delta T_{\text{m}}(\Delta S/\Delta b) \qquad (1)$$

where $\delta F_{\text{overstretch}}$ is the change in overstretching force, δT_{m} is the change in melting temperature at high or low pH, Δb is the change in length of the DNA upon conversion from dsDNA to ssDNA, and ΔS is the change in entropy of the DNA upon melting. Assuming ΔS is independent of pH and temperature, the only fitting parameter is ΔS, for which we obtain a value of 9.5 cal/mol·K·bp. The measured overstretching force and that predicted by eq 1 using this value for ΔS are shown in Figure 4. Although the dependence of ΔS on pH is unknown, our data clearly show that the dependence of the overstretching force on pH is dominated by the change in melting temperature with pH. In addition, the value that we obtain for the

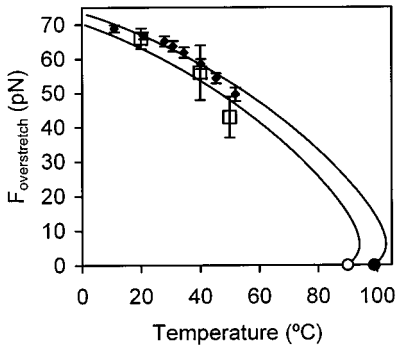

FIGURE 5. Measured overstretching force as a function of temperature using optical tweezers (◆) in 500 mM NaCl and AFM (□) in 150 mM NaCl. The solid lines are fits to the data using the appropriate melting temperature for the ionic strength used. The T_m is given by ● in 500 mM NaCl and by ○ in 150 mM NaCl (from ref 13).

change in entropy of DNA upon melting is in good agreement with room-temperature calorimetry measurements.[21]

4. Temperature Dependence of DNA Overstretching

In addition to the prediction that solution conditions that change the melting temperature of DNA will correspondingly change the overstretching force, the force-induced melting model also predicts that the overstretching force will decrease with increasing temperature. The first measurements of the temperature dependence of the overstretching force were made using atomic force microscopy (AFM).[5] These studies showed that the overstretching force decreased significantly at high temperature. However, the measurements were not accurate enough to determine the change in entropy of DNA upon melting. Using an optical tweezers instrument, we were able to measure accurately the dependence of the overstretching transition on temperature.[13] The results are shown as symbols in Figure 5, along with the data previously measured by AFM.

Predictions of the temperature dependence of $F_{overstretch}$ can be calculated directly from the force−extension curves of dsDNA and ssDNA shown in Figure 1. To do this, we assume that the total transition free energy can be written as the sum of a temperature-dependent melting free energy $\Delta G(T)$ and a force-dependent stretching free energy $\Delta\Phi(F)$. The overstretching transition occurs when the total transition free energy is zero. Thus, at the melting temperature, $\Delta G(T) = 0$, so no force is required to melt the DNA. As the temperature is decreased, $\Delta G(T)$ is positive, so a force must be applied to the DNA to lower $\Delta\Phi(F)$ until the total transition free energy is zero.

The parameter $\Delta\Phi(F)$ can be calculated directly from the theoretical stretching curves shown in Figure 1 using the relation

$$\Delta\Phi(F) = -\int_0^F [b_{ss}(F') - b_{ds}(F')]\mathrm{d}F \qquad (2)$$

where $b_{ss}(F)$ and $b_{ds}(F)$ are the lengths of ssDNA and dsDNA, respectively. Since this can be calculated directly

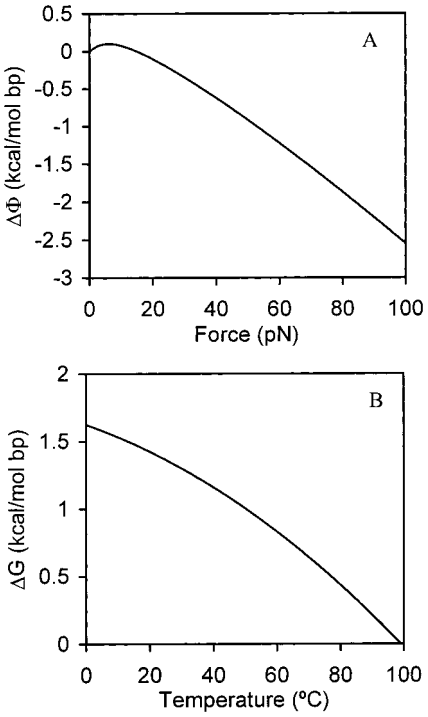

FIGURE 6. (A) Calculated difference in the stretching free energy between dsDNA and ssDNA as a function of applied force. (B) Melting free energy of dsDNA as a function of temperature calculated from the force-induced melting theory (from ref 13).

from stretching experiments and $\Delta G(T) + \Delta\Phi(F) = 0$ at $F_{overstretch}$, measurement of the force−extension curves of ssDNA and dsDNA provides a direct measurement of the stability of dsDNA ($\Delta G(T)$) at any temperature. Figure 6A shows the values of $\Delta\Phi(F)$ calculated from the theoretical stretching curves shown in Figure 1. $\Delta G(T)$ is then calculated from the measured $F_{overstretch}$ at various temperatures using the relation $\Delta G(T) + \Delta\Phi(F_{overstretch}) = 0$. $\Delta G(T)$ and is then fit to the relation[13]

$$\Delta G(T) \cong \Delta S(T_m)(T_m - T) - \frac{\Delta C_p(T - T_m)^2}{2\ T_m} \qquad (3)$$

where the fitting parameters are $\Delta S(T_m)$, the change in entropy of DNA upon melting at the melting temperature, and ΔC_p, the change in heat capacity of DNA upon melting. The resulting curve for $\Delta G(T)$ using eq 3 and $\Delta S(T_m = 99\ °C) = 24.7 \pm 1$ cal/mol·K·bp and $\Delta C_p = 60 \pm 10$ cal/mol·K·bp is shown in Figure 6B. This curve is then used to calculate $F_{overstretch}$ at any temperature using the relation $\Delta G(T) + \Delta\Phi(F_{overstretch}) = 0$, and the resulting predictions for $F_{overstretch}$ are shown as solid lines in Figure 5. The fit is very good, and the resulting values for $\Delta S(T_m)$ and ΔC_p are in excellent agreement with calorimetric measurements of these parameters.[13]

The results from the temperature and pH dependence of DNA overstretching support the model of DNA overstretching as a force-induced melting transition. The analysis described here demonstrates that force-induced melting experiments are a powerful technique for measuring thermodynamic parameters describing the stability of DNA over a wide range of temperature and solution

conditions. In addition to the properties normally measured in a DNA melting experiment, these experiments also show changes in the elasticity of DNA, which can be used to determine the nature of the interactions between DNA and its environment, including various binding ligands that may affect its stability.[22]

5. Ionic Strength Dependence of the DNA Overstretching Transition

We recently measured the dependence of the DNA overstretching transition on solution ionic strength in order to gain more information about the structure of overstretched DNA.[23] We find that the observed dependence of the overstretching force is consistent with the predictions of polyelectrolyte theory and the model presented in Figure 2. Under these conditions, the salt-dependent part of the helix-coil transition free energy is given by[24]

$$\Delta G_{\mathrm{el}} = k_{\mathrm{B}}T\left(\frac{1}{\xi_{\mathrm{ss}}} - \frac{1}{\xi_{\mathrm{ds}}}\right)\ln\left(\frac{I}{I_0}\right) \tag{4}$$

where ξ is the dimensionless linear charge density, with $\xi = l_{\mathrm{B}}/h$ and $l_{\mathrm{B}} = e^2/\epsilon k_{\mathrm{B}}T$. Here, h is the length per unit charge, ϵ is the dielectric constant of water, l_{B} is the Bjerrum length, and I is the solution ionic strength. From these relations, we find that the variation of the overstretching force with ionic strength is given by[11]

$$\frac{\partial f_{\mathrm{overstretch}}}{\partial \ln(I)} = \frac{k_{\mathrm{B}}T}{l_{\mathrm{B}}}\nu \tag{5}$$

where

$$\nu = \frac{h_{\mathrm{ss}} - h_{\mathrm{ds}}}{b_{\mathrm{ss}} - b_{\mathrm{ds}}} \tag{6}$$

is the ratio of the difference in length per unit charge and the change in length per base pair when DNA is overstretched. If the distance between the DNA strands is less than the Debye screening length, $r_{\mathrm{DH}} = (4\pi l_{\mathrm{B}}I)^{-(1/2)}$, then the two strands are equivalent to a single strand with twice the charge density, so $\nu = 0.5$. If the average distance between strands is greater than the Debye screening length, then $\nu = 1.7$. If one strand is stretched while the other is relaxed, then we have $\nu = 1.2$.[11] Thus, fits of our data to eq 5 allow us to determine the state of the two strands during the overstretching transition. The solid line in Figure 7 is a linear fit to the measured overstretching force as a function of log(I), which gives a value of $\nu = 0.49$ from eq 5. Therefore, our data support the idea that both DNA strands are stretched and remain very close to each other during the overstretching transition. Thus, we have developed a model that describes the structure and thermodynamics of overstretched DNA that is consistent with all available data on the dependence of DNA overstretching on solution conditions and temperature.

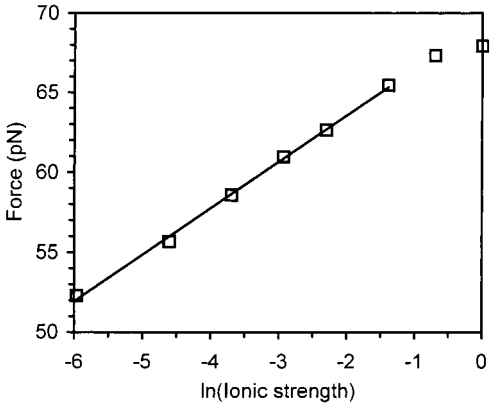

FIGURE 7. Measured overstretching force as a function of ionic strength. The error in force measurement is less than 2 pN.[23] The solid line is a linear fit to the data, which yields a value of $\nu = 0.49$ from eq 5 (from ref 23).

6. Measuring the Effect of DNA Binding Proteins on DNA Stability: Nucleic Acid Chaperone Activity of HIV-1 Nucleocapsid Protein

6.1 HIV-1 Nucleocapsid Protein (NC). Now that we have developed a model to describe the force-induced melting transition of dsDNA, we can use it to study the effect of DNA binding proteins on the stability and cooperativity of DNA melting. This will allow us to determine the effect of these binding proteins on both dsDNA and ssDNA and to elucidate the mechanism of interaction between the proteins and DNA in order to develop a more sophisticated understanding of how these proteins operate in biological systems.

The use of single-molecule force measurement techniques to study DNA−protein interactions has been reviewed.[25] However, the first application of the force-induced DNA melting model to the study of a DNA binding protein has been our recent work on the effect of HIV-1 nucleocapsid protein on DNA overstretching. The nucleocapsid protein of human immunodeficiency virus type 1 (HIV-1) is a small, highly basic nucleic acid binding protein that contains only 55 amino acids and two zinc finger motifs of the form $CX_2C\ X_4H\ X_4C$ or CCHC. NC possesses nucleic acid chaperone activity, by which it facilitates the rearrangement of nucleic acid molecules into their lowest energy conformations. To achieve such rearrangements, the base pairs of nucleic acid structures that are normally very stable must be broken, and other complementary structures must be formed.[26,27] Although it seems likely that NC acts by destabilizing the base pairs of nucleic acid structures, this does not explain the enhanced annealing of complementary structures observed in the presence of NC. Thus, although the chaperone activity of NC is of crucial importance in the life cycle of the retrovirus,[28,29] the mechanism by which it achieves this activity is not well-understood.

6.2. Effect of Wild-Type NC on DNA Overstretching. To investigate the capability of NC to alter the base pairing of nucleic acid structures, we used an optical tweezers instrument to measure the effect of NC on the force-

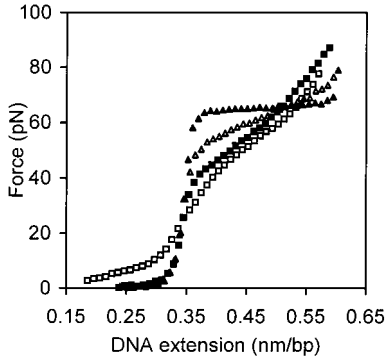

FIGURE 8. DNA overstretching with increasing amounts of NC bound or as the nucleotide to NC ratio (nt:NC) decreases from 50:1 (▲) to 15:1 (▲) to 8:1 (■) and, finally, to 5:1 (□) (from ref 22).

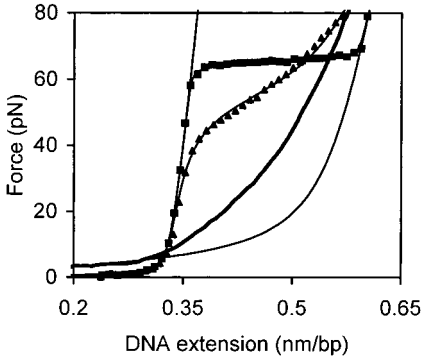

FIGURE 9. Representative fits of the data to the Bragg-Zimm melting model. The solid line on the left represents the elasticity of dsDNA according to the wormlike chain model.[4] The thin solid line on the right is stretching data for ssDNA in 150 mM ionic strength without NC.[2] The data for stretching dsDNA in the presence of NC at 150 mM ionic strength is shown (■) along with the fit to the melting model, indicated as a line between these data points. The thick solid line is our data from stretching ssDNA at 25 mM ionic strength in the presence of NC. The data for stretching dsDNA in 50 mM ionic strength with NC is also shown (▲), as well as a fit to that data, indicated as a line between the data points (from ref 22).

induced melting transition of single λ-DNA molecules. The results of DNA stretching experiments with NC are shown in Figure 8. Here, we stretched single DNA molecules in buffer of varying ionic strength, from which we estimated the binding of the positively charged NC protein to DNA.[30] The resulting stretching curves are shown as a function of the calculated number of bound nucleotides for each NC molecule (nt:NC) on the basis of the previously measured salt dependence of NC binding to DNA.[31] The results show a significant change in the slope of the overstretching transition as the amount of bound NC increases. This effect appears to saturate at a nt:NC ratio of 8, the ratio required to observe maximal nucleic acid chaperone activity.[26] Thus, the observed effect of NC on the overstretching transition is likely to be the same effect responsible for the chaperone activity.

To understand the effect of NC on DNA force-induced melting, we can fit our data using the standard description of DNA melting, which is the Zimm-Bragg model of the helix-coil transition.[32] To do this, we calculate the shape of the transition from the force−extension curves for dsDNA and ssDNA (Figure 1). The stretching curve for ssDNA is altered by NC, which binds preferentially to ssDNA (Figure 9, thick solid line). ssDNA appears to be much stiffer with NC bound.

In the Zimm-Bragg model, the fraction of base pairs in the helical state is given by

$$\Theta = \frac{1}{2} + \frac{s-1}{2[(s-1)^2 + 4s\sigma]^{1/2}} \qquad (7)$$

where s is the equilibrium constant for conversion of a base pair from single-stranded to double-stranded form, given by

$$s = \exp\left(\frac{\Delta G_{\text{total}} + \Delta\Phi(F)}{k_B T}\right) \qquad (8)$$

where σ is the cooperativity parameter. The free energy of the helix-coil transition as a function of force $\Delta\Phi(F)$ is obtained from force−extension curves[10] of Figures 1 and 9 by using eq 2. ΔG_{total} is obtained by directly calculating the area between the experimental stretching curves for dsDNA and ssDNA and represents the stability of DNA in the absence of force. The DNA extension as a function of force is then given by

$$b(F) = \Theta(F) b_{\text{ds}}(F) + (1 - \Theta(F)) b_{\text{ss}}(F) \qquad (9)$$

By fitting these relations to our experimental force−extension curves, we obtain an estimate of σ, which increases as the transition cooperativity decreases. Representative results of our fits are shown in Figure 9.

An examination of the area between the respective curves for dsDNA and ssDNA with and without NC reveals that the free energy of the helix-coil transition decreases significantly in the presence of NC. Under the conditions that generate the most binding of NC (25 mM Na^+), we find that $\Delta G_{\text{total}} \approx 2k_BT$, where T is 300 K. When NC is added, the free energy reduces to $\sim$1 k_BT, and the cooperativity parameter increases from $\sigma = 0.001$ to $\sigma = 0.25$.

These results help to explain the nucleic acid chaperone activity of NC. The fraction of melted base pairs ($1 - \Theta$) at room temperature can be obtained from eq 7. Without NC in 25 mM Na^+, $\Delta G_{\text{total}} = 2k_BT$ and $\sigma = 0.001$, so the fraction of melted base pairs is only 2×10^{-4}. Thus, two double-stranded structures that contain complementary base pairs and that would form a lower energy state if annealed have a very low probability of annealing without the aid of a nucleic acid chaperone because of this significant barrier to separation of the original base pairs. In contrast, when NC is added in a 25 mM Na^+ solution (nt:NC = 5:1), $\Delta G_{\text{total}} = 1k_BT$ and $\sigma = 0.25$, so the fraction of melted base pairs increases to 0.2. This indicates that, in the presence of NC, a double-stranded nucleic acid structure will always have about 20% of its base pairs melted. This provides an elegant explanation for the nucleic acid chaperone activity of NC. NC significantly destabilizes two nucleic acid structures that are normally very stable such that at any given time there exist melted domains with dissociated base pairs. When these

strands come into contact, they are able to rapidly sample various base-paired configurations until eventually the one with the lowest energy is found. Thus, NC facilitates the rearrangement of nucleic acid by lowering the energy barrier to creation of melted domains.

In addition to explaining the ability of NC to lower the barrier to melting nucleic acid secondary structure, these studies also shed light on another aspect of the chaperone activity of NC. We have shown that two complementary structures can anneal to form a lower energy structure by sampling different base paired structures. Normally, these double-stranded structures exhibit electrostatic repulsion. However, in the presence of highly positively charged ions, nucleic acids exhibit an attraction for one another. This is the basis of DNA condensation.[33] Since NC is a highly positively charged protein, it also induces condensation or aggregation of nucleic acids.[34] This is most likely the basis of NC's ability to induce rapid renaturation of nucleic acids. It is known that amounts of multivalent cations that are sufficient to cause DNA condensation increase the efficiency of renaturation of melted DNA.[35] We observe evidence of DNA aggregation forces in our stretching curves (Figure 8). When the nt: NC ratio is five, a finite force is required to stretch dsDNA to extensions less than the B-form contour length (0.34 nm). Thus, if we were not stretching the molecule, this attractive force would cause it to collapse. This suggests that there are two important aspects to the nucleic acid chaperone activity of NC. First, it induces an attraction between DNA molecules as a result of its high charge. Second, it significantly destabilizes the secondary structure of nucleic acids such that at any given time, a significant fraction of the base pairs are melted. Our DNA stretching curves allow us to observe both of these effects.

6.3 Effect of Deleting NC's Zinc Finger Structures. The zinc fingers are a key structural feature of NC.[36] Although the zinc fingers have long been known to be required for retroviral replication,[28,29,37] their specific role in nucleic acid chaperone activity has been less clear. In a recent study, Levin and co-workers found that a mutant form of NC in which both CCHC motifs had been changed to SSHS (SSHS NC) was significantly less active than wild-type NC in facilitating the annealing step in minus-strand transfer and in blocking nonspecific self-priming reactions.[38] In contrast, the zinc fingers are dispensable for tRNA primer annealing[39] and for plus-strand transfer.[38]

In an attempt to better understand the apparently conflicting data surrounding the importance of the zinc fingers for the nucleic acid chaperone activity of NC, we performed force-induced melting experiments of single molecules of λ-DNA in the presence of SSHS NC. In striking contrast to the data observed for wild-type NC, SSHS NC did not alter the cooperativity of the DNA overstretching transition or lower the helix-coil transition free energy.[22] In fact, SSHS NC appears to stabilize dsDNA. This, along with the finite DNA condensation force observed at low extensions, suggests that SSHS NC binds to DNA in a manner similar to multivalent cations and polycations. These measurements suggested that poly-lysine might act in a manner similar to SSHS NC. It was recently demonstrated that poly-lysine facilitated tRNA annealing.[39]

We have determined three important aspects of the nucleic acid chaperone activity of HIV-1 NC. First, NC facilitates annealing of complementary strands through electrostatic attraction on the basis of its high positive charge. Second, NC significantly destabilizes dsDNA as a result of its preferential binding to ssDNA. Third, NC greatly reduces the cooperativity of the helix-coil transition of double-stranded DNA structures. SSHS NC has only the first of these three properties. This indicates that the zinc fingers of NC are responsible for the preferential binding to ssDNA and for its capability to alter the helix-coil transition. These two properties are essential for the rearrangement of longer, more complex nucleic acid structures by NC, such as those required for minus strand transfer during retroviral DNA synthesis.

7. Conclusions

We have demonstrated that force-induced melting measurements of single molecules of dsDNA can be used as a sensitive probe to determine the stability of DNA under a wide range of solvent conditions. The results of these experiments complement those of thermal melting experiments. In addition, these experiments avoid complications due to poorly understood thermal effects and allow the measurement of DNA stability at any temperature. From our measurements, we were able to derive thermodynamic parameters that were in agreement with calorimetric measurements. The hypothesis of force-induced melting makes several qualitative and quantitative predictions that are confirmed by the experiments described here. The alternative hypothesis of a new form of dsDNA termed S-DNA does not make such predictions and is not needed to describe DNA overstretching.

This technique also allows us to measure the effect of DNA binding proteins on the stability of dsDNA. We have demonstrated its use in determining the effect of HIV-1 NC on DNA stability. We have used these results to develop a model that describes the nucleic acid chaperone activity of this protein. In the future, this technique could also be used as a probe to determine if a particular protein of unknown function exhibits nucleic acid chaperone activity. As an obvious extension, there are several systems that could be studied with the technique, such as single-stranded DNA binding proteins and DNA-drug interactions.

Recently, force-induced melting of single RNA hairpins was demonstrated.[40] The authors were able to determine the free energy of melting of specific sequences of RNA. The prospect of doing these same studies on specific RNA and DNA sequences opens up many new possibilities for using single molecule stretching for thermodynamic measurements. In this case, one could measure sequence-specific nucleic acid−protein interactions, such as the interaction of DNA with transcription factors.

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References

(1) Smith, S. B.; Finzi, L.; Bustamante, C. Direct Mechanical Measurements of the Elasticity of Single DNA Molecules by Using Magnetic Beads. *Science* **1992**, *258*, 1122−1126.
(2) Smith, S. B.; Cui, Y. J.; Bustamante, C. Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules. *Science* **1996**, *271*, 795−799.
(3) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J. L.; Chatenay, D.; Caron, F. DNA: An extensible molecule. *Science* **1996**, *271*, 792−794.
(4) Odijk, T. Stiff chains and filaments under tension. *Macromolecules* **1995**, *28*, 7016−7018.
(5) Clausen-Schaumann, H.; Rief, M.; Tolksdorf, C.; Gaub, H. E. Mechanical Stability of Single DNA Molecules. *Biophys. J.* **2000**, *78*, 1997−2007.
(6) Hegner, M.; Smith, S. B.; Bustamante, C. Polymerization and mechanical properties of single RecA-DNA filaments. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10109−10114.
(7) Konrad, M. W.; Bolonick, J. I. Molecular dynamics simulation of DNA stretching is consistent with the tension observed for extension and strand separation and predicts a novel ladder structure. *J. Am. Chem. Soc.* **1996**, *118*, 10989−10994.
(8) Kosikov, K. M.; Gorin, A. A.; Zhurkin, V. B.; Olson, W. K. DNA stretching and compression: Large-scale simulations of double helical structures [Review]. *J. Mol. Biol.* **1999**, *289*, 1301−1326.
(9) Lebrun, A.; Lavery, R. Modelling extreme stretching of DNA. *Nucleic Acids Res.* **1996**, *24*, 2260−2267.
(10) Rouzina, I.; Bloomfield, V. A. Force-induced melting of the DNA double helix − 1. Thermodynamic analysis. *Biophys. J.* **2001**, *80*, 882−893.
(11) Rouzina, I.; Bloomfield, V. A. Force-induced melting of the DNA double helix. 2. Effect of solution conditions. *Biophys. J.* **2001**, *80*, 894−900.
(12) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. The effect of pH on the overstretching transition of dsDNA: Evidence of force-induced DNA melting. *Biophys. J.* **2001**, *80*, 874−881.
(13) Williams, M. C.; Wenner, J. R.; Rouzina, I.; Bloomfield, V. A. Entropy and Heat Capacity of DNA Melting from Temperature Dependence of Single Molecule Stretching. *Biophys. J.* **2001**, *80*, 1932−1939.
(14) Frank-Kamenetskii, M. D.; Anshelevich, V. V.; Lukashin, A. V. Polyelectrolyte model of DNA. *<Translation> Soviet Physics − Uspekhi* **1987**, *151*, 595−618.
(15) Grosberg, A. Y.; Khokhlov, A. R. *Statistical Physics of Macromolecules*; American Institute of Physics: New York, 1994.
(16) Evans, E.; Ritchie, K. Dynamic Strength of Molecular Adhesion Bonds. *Biophys. J.* **1997**, *72*, 1541−1555.
(17) Allemand, J. F.; Bensiomon, D.; Lavery, R.; Croquette, V. Stretched and overwound DNA forms a Pauling-like structure with exposed bases. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14152−14157.
(18) Leger, J. F.; Romano, G.; Sarkar, A.; Robert, J.; Bourdieu, L.; Chatenay, D.; Marko, J. F. Structural Transitions of a Twisted and Stretched DNA Molecule. *Phys. Rev. Lett.* **1999**, *83*, 1066−1069.
(19) Strick, T.; Croquette, V.; Bensimon, D. Homologous pairing in stretched supercoiled DNA. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 10579−10583.
(20) Geiduschek, E. P. On the factors controlling the reversibility of DNA denaturation. *J. Mol. Biol.* **1962**, *4*, 467−487.
(21) Privalov, P. L.; Ptitsyn, O. B. Determination of Stability of the DNA Double Helix in an Aqueous Medium. *Biopolymers* **1969**, *8*, 559−571.
(22) Williams, M. C.; Rouzina, I.; Wenner, J. R.; Gorelick, R. J.; Musier-Forsyth, K.; Bloomfield, V. A. Mechanism for nucleic acid chaperone activity of HIV-1 nucleocapsid protein revealed by single molecule stretching. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6121−6126.
(23) Wenner, J. R.; Williams, M. C.; Rouzina, I.; Bloomfield, V. A. Salt Dependence of the Elasticity and Overstretching Transition of Single DNA Molecules. *Biophys. J.*, submitted.
(24) Bond, J. P.; Anderson, C. F.; Record, M. T. Conformational transitions of duplex and tripler nucleic acid helices − thermodynamic analysis of effects of salt concentration on stability using preferential interaction coefficients. *Biophys. J.* **1994**, *67*, 825−836.
(25) Bustamante, C.; Smith, S. B.; Liphardt, J.; Smith, D. Single-molecule studies of DNA mechanics. *Curr. Opin. Struct. Biol.* **2000**, *10*, 279−285.
(26) Rein, A.; Henderson, L. E.; Levin, J. G. Nucleic-acid-chaperone activity of retroviral nucleocapsid proteins: significance for viral replication. *Trends Biochem. Sci.* **1998**, *23*, 297−301.
(27) Tsuchihashi, Z.; Brown, P. O. DNA strand exchange and selective DNA annealing promoted by the human immunodeficiency virus type 1 nucleocapsid protein. *J. Virol.* **1994**, *68*, 5863−5870.
(28) Gorelick, R.; Gagliardi, T.; Bosche, W.; Wiltrout, T.; Coren, L.; Chabot, D.; Lifson, J.; Henderson, L.; Arthur, A. Strict conservation of the retroviral nucleocapsid protein zinc finger is strongly influenced by its role in viral infection processes: Characterization of HIV-1 particles containing mutant nucleocapsid zinc-coordinating sequences. *Virology* **1999**, *256*, 92−104.
(29) Dorfman, T.; Luban, J.; Goff, S. P.; Haseltine, W. A.; Gottlinger, H. G. Mapping of functionally important residues of a cysteine-histidine box in the human immunodeficiency virus type 1 nucleocapsid protein. *J. Virol.* **1993**, *67*, 6159−69.
(30) Rouzina, I.; Bloomfield, V. A. Competitive Electrostatic Binding Of Charged Ligands to Polyelectrolytes − Practical Approach Using the Non-Linear Poisson−Boltzmann Equation. *Biophys. Chem.* **1997**, *64*, 139−155.
(31) Vuilleumier, C.; Bombarda, E.; Morellet, N.; Gérard, D.; Roques, B. P.; Mély, Y. Nucleic Acid Sequence Discrimination by the HIV-1 Nucleocapsid Protein NCp7: A Fluorescence Study. *Biochemistry* **1999**, *38*, 16816−16825.
(32) Zimm, B. H.; Bragg, J. K. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* **1959**, *31*, 526−535.
(33) Bloomfield, V. A. DNA condensation by multivalent cations. *Biopolymers* **1997**, *44*, 269−282.
(34) Stoylov, S. P.; Vuilleumier, C.; Stoylova, E.; Rocquigny, H. D.; Roques, B. P.; Gérard, D.; Mély, Y. Ordered aggregation of ribonucleic acids by the human immunodeficiency virus type 1 nucleocapsid protein. *Biopolymers* **1997**, *41*, 301−312.
(35) Sikorav, J. L.; Church, G. M. Complementary recognition in condensed DNA: accelerated DNA renaturation. *J. Mol. Biol.* **1991**, *222*, 1085−1108.
(36) de Guzman, R. N.; Wu, Z. R.; Stalling, C. C.; Pappalardo, L.; Borer, P. N.; Summers, M. F. Structure of the HIV-1 Nucleocapsid Protein Bound to the SL3 −RNA Recognition Element. *Science* **1998**, *279*, 384−388.
(37) Berkowitz, R.; Fisher, J.; Goff, S. P. RNA packaging. *Curr. Top. Microbiol. Immunol.* **1996**, *214*, 177−218.
(38) Guo, J.; Wu, T.; Anderson, J.; Kane, B. F.; Johnson, D. G.; Gorelick, R. J.; Henderson, L. E.; Levin, J. G. Zinc Finger Structures in the Human Immunodeficiency Virus Type 1 Nucleocapsid Protein Facilitate Efficient Minus- and Plus-Strand Transfer. *J. Virol.* **2000**, *74*, 8980−8988.
(39) Hargittai, M. R. S.; Mangla, A. T.; Gorelick, R. J.; Musier-Forsyth, K. HIV-1 nucleocapsid protein zinc finger structures induce tRNALys,3 tertiary structural changes but are not critical for primer/template annealing. *J. Mol. Biol.* **2001**, *312*, 985−997.
(40) Liphardt, J.; Onoa, B.; Smith, S. B.; Tinoco, I., Jr.; Bustamante, C. Reversible unfolding of single RNA molecules by mechanical force. *Science* **2001**, *292*, 733−737.

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