Entropy and Heat Capacity of DNA Melting from Temperature Dependence of Single Molecule Stretching

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ABSTRACT When a single molecule of double-stranded DNA is stretched beyond its B-form contour length, the measured force shows a highly cooperative overstretching transition. We have measured the force at which this transition occurs as a function of temperature. To do this, single molecules of DNA were captured between two polystyrene beads in an optical tweezers apparatus. As the temperature of the solution surrounding a captured molecule was increased from 11°C to 52°C in 500 mM NaCl, the overstretching transition force decreased from 69 pN to 50 pN. This reduction is attributed to a decrease in the stability of the DNA double helix with increasing temperature. These results quantitatively agree with a model that asserts that DNA melting occurs during the overstretching transition. With this model, the data may be analyzed to obtain the change in the melting entropy ΔS of DNA with temperature. The observed nonlinear temperature dependence of ΔS is a result of the positive change in heat capacity of DNA upon melting, which we determine from our stretching measurements to be $\Delta C_p = 60 \pm 10$ cal/mol K bp, in agreement with calorimetric measurements.

INTRODUCTION

By stretching single molecules, a number of investigators have shown that DNA exhibits unusual elastic behavior (Cluzel et al., 1996; Rief et al., 1999; Smith et al., 1996). These experiments move one end of a DNA molecule while measuring the force on the opposite end by means of an optical trap or atomic force microscope (AFM) tip. The resulting force-extension curve is then used to describe molecular behavior under various solution conditions. At ~60–70 pN, the force-extension curve for double-stranded DNA (dsDNA) exhibits a plateau, indicating that the DNA can be elongated with very little additional force. This cooperative overstretching transition continues until the molecule is stretched to 1.7 times its B-form contour length, at which point the force increases rapidly. The slope of the increase in force after the overstretching transition has been shown to depend on the rate at which the DNA molecule is stretched (Clausen-Schaumann et al., 2000). At low pulling rates, the force-extension curve matches that of single-stranded DNA (ssDNA) in this force regime. At high pulling rates, the slope at this point increases, but at forces greater than 120 pN it again matches the ssDNA curve (Clausen-Schaumann et al., 2000; Hegner et al., 1999). This description applies only to the case in which one strand of DNA is allowed to rotate freely so that the DNA can untwist while being stretched. If the DNA strand is torsionally constrained, a much broader overstretching transition occurs at ~110 pN (Clausen-Schaumann et al., 2000; Leger et al., 1999).

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The overstretching transition has been attributed to a transition from B-form dsDNA to another form of dsDNA termed S-DNA (Cluzel et al., 1996). Several molecular modeling studies were published (Konrad and Bolonick, 1996; Kosikov et al., 1999; Lebrun and Lavery, 1996) based on the assumption that the DNA remains in double-stranded form. However, these models were unable to predict the correct overstretching transition force and width. Other models incorporating the idea of S-DNA were able to fit the observed overstretching data (Ahsan et al., 1998; Cizeau and Viovy, 1997; Haijun et al., 1999; Marko, 1998), but they did not predict the transition force.

Rouzina and Bloomfield (2001a) have developed a theory that predicts that DNA melting occurs during the overstretch transition. The proposed process consists of two stages. In the first stage, during the cooperative overstretching transition described above, an equilibrium melting process occurs until there are a small number of base pairs separating domains of melted DNA. In the second stage, the remaining base pairs are broken. However, removal of the bonds separating the melted domains represents an irreversible process, in which the two strands completely separate. Because the process is irreversible, the two strands are unable to sample bound and unbound states, so the entropy gained by the strands upon melting is not meaningful. Under these conditions it is the enthalpy of ~15 kT/bp rather than the free energy of ~2 kT/bp that determines the stretching force. Thus we would expect the force required to completely separate the strands to be much greater than the overstretching force. In addition, as with any non-equilibrium process, the strand separation force should be rate dependent (Evans and Ritchie, 1997).

In contrast to the S-DNA models, the melting theory quantitatively accounts for the observed overstretching force and the slope of the overstretching force as a function of extension. It also predicts that the overstretching force will decrease if conditions are changed to destabilize the

helix. Since destabilizing conditions are less likely to affect a transition between states that are both double-stranded, these predictions are a good way to distinguish between the force-induced melting and S-DNA models.

In a recent paper (Williams et al., 2001) we presented measurements of the overstretching force and the width of the overstretching transition as a function of pH. We showed that the overstretching force and thermal melting point exhibit similar trends as a function of pH and that the model of force-induced melting accurately describes the dependence of the overstretching force on pH. From these data we obtained a reasonable value for the DNA melting entropy at room temperature.

To further test the theory of Rouzina and Bloomfield, we now present measurements of the overstretching force as a function of temperature. Because increasing temperature destabilizes the DNA helix, this theory predicts that the overstretching force will decrease with increasing temperature. The rate of change with temperature is determined by the elongation of the DNA upon melting at a particular force as well as the entropy of melting. Thus, fits of the model to our data can be used to measure the entropy of melting. The melting entropy has been measured calorimetrically (Blake and Delcourt, 1998; Breslauer et al., 1986; Santalucia, 1998), but this measurement is necessarily made at the melting temperature of the DNA, which is generally much greater than room temperature. Our experiments provide an alternate measurement of the DNA melting entropy below the temperature range normally accessible to calorimetry experiments.

Our results from stretching DNA in high and low pH as well as the temperature dependence of the overstretching transition provide strong evidence that the overstretching transition is a force-induced melting transition, rather than a transition between two double-stranded forms of DNA. The strong sensitivity of the overstretching transition to temperature and to the DNA melting point are both clear indications that the overstretching transition involves a change in entropy, and the resulting estimate of the transition entropy is very close to that expected for melting. Furthermore, the temperature dependence of the entropy allows determination of the change in heat capacity of DNA upon melting, which is also in good agreement with calorimetric measurements.

MATERIALS AND METHODS

The dual-beam optical tweezers instrument used in this study consists of two counter-propagating 150-mW, 850-nm diode lasers (SDL, San Jose, CA) focused to a small spot inside a liquid flow cell with 1.0 NA Nikon water-immersion microscope objectives. The force measurement was calibrated by applying a known external force to a bead in the optical trap and measuring the resulting change in bead position using position-sensitive photodiode detectors (UDT Sensors, Hawthorne, CA). After trapping a bead, the liquid cell surrounding the bead was oscillated at a known frequency and amplitude. The amplitude of the observed oscillating force due to viscous drag on the bead (Mehta et al., 1998) was measured as a function of frequency, giving the detector signal as a function of applied force. The amplitude of this signal was linear in applied force, and this linear relation determined the calibration factor for the two detectors. The calibration was performed as a function of temperature, and errors in force measurement were determined from the reproducibility of the calibration at each temperature.

To control its temperature, the cell was placed between oxygen-free high-conductivity copper plates with small holes to allow the laser beams to enter the cell. The copper plates were placed in thermal contact with a thermoelectric cooler (Marlow Industries, Dallas, TX) that was used to heat and cool the cell. The temperature of the cell was monitored with a thermocouple located inside the cell (~3 mm from the pipette tip in the center of the cell) and connected to a temperature-compensated indicator with an accuracy of ±0.3°C (Omega Engineering, Stamford, CT.) The cell thickness is ~3 mm, so the pipette tip is located 1.5 mm from each objective. We verified that the temperature measurement did not change within an error of ±1°C when the cell was shifted 3 mm so that the thermocouple was in the same position as the pipette tip.

To tether single molecules of DNA, one 4.1-μm-diameter streptavidin-coated bead (Spherotech, Libertyville, IL) was trapped in the optical tweezers and then attached by suction to a pipette with a 1–2-μm tip. Another such bead was captured and held in the optical trap while a dilute solution of biotinylated DNA was run through the cell. Once a DNA molecule was attached to the trapped bead, the bead on the pipette was moved toward the trapped bead until the opposite end of the molecule was bound. The procedure is identical to that described in Fig. 3 of Bennink et al. (1999). The tethering buffer was 10 mM Hepes with 495 mM NaCl and 5 mM NaOH, pH 7.5. All of the experiments reported here were done in this buffer.

To assure that the beads remained connected by at least one strand of DNA at high temperatures, the DNA used was biotinylated on both ends of the same DNA strand, rather than on opposite strands. To do this, a 69-bp oligonucleotide with one end complementary to a 5′ 12-bp overhang of bacteriophage λ DNA was annealed to one end of the DNA molecule. A 30-bp oligo was annealed to the 3′ end of the 69-bp oligo to be used as a primer. The DNA was then incubated with biotin-11-dCTP (Sigma Chemical Co., St. Louis, MO), dATP, dTTP, dGTP, and Klenow *exo*$^-$ DNA polymerase (New England Biolabs, Beverly, MA). T4 DNA ligase (New England Biolabs) was added at 16°C to attach the oligos and repair single-strand nicks. The labeled strand has four biotin sites spaced evenly between nucleotides 30 and 69 on one end and six biotin sites in the last 12 base pairs at the other end of the strand.

When a single molecule was tethered between the two beads, force-extension measurements were made by measuring the force on the bead in the trap while moving the pipette a known distance. A schematic diagram of the experiment is shown in Fig. 1. The absolute extension of the molecule was estimated by measuring the distance between the centers of the two beads using an image captured with a CCD camera (Edmund Industrial Optics, Barrington, NJ). The change in position of the pipette was measured using a feedback-compensated piezoelectric translation stage that is accurate to 5 nm (Melles Griot, Irvine, CA). The position measurement was converted to a measurement of the molecular extension by correcting for the trap stiffness, which was 62 ± 3 pN/μm. For the measurements reported here, the pipette was moved in 500-nm steps, and after each step the force was measured 100 times and averaged, thus averaging out contributions of thermal motion to the force measurement. Each step took ~0.5 s. At forces below 70 pN, the force-extension curves did not change significantly when the pulling rate was varied by changing the step size from 10 to 500 nm.

RESULTS

A typical overstretching curve for DNA stretched in the tethering buffer at 21°C is shown in Fig. 2. The force (F)

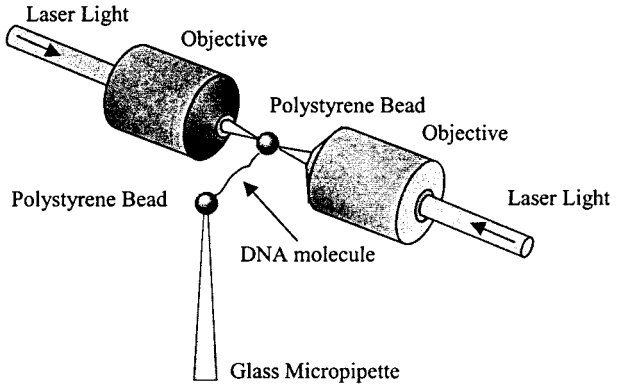

FIGURE 1 Schematic drawing (not to scale) of an optical tweezers experiment in which a single DNA molecule is stretched between two polystyrene beads. One bead is held on the end of a glass micropipette by suction, while another bead is held in an optical trap. Two counter-propagating laser beams focused to a common point form the optical trap.

versus extension per base pair (b) curve begins to rise as the DNA helix is extended to near its normal contour length of 0.34 nm/bp. As the force increases to 65 pN, the elasticity can be described by the extensible worm-like chain (WLC) model. The extension of the WLC in response to an applied force is described in the limit of high force ($FP_{ds}/kT > 1$) by (Odijk, 1995)

$$b_{ds}(F) = b_{ds}^{max}\left\{1 - \frac{1}{2}\left(\frac{k_B T}{F P_{ds}}\right)^{1/2} + \frac{F}{S_{ds}}\right\}, \qquad (1)$$

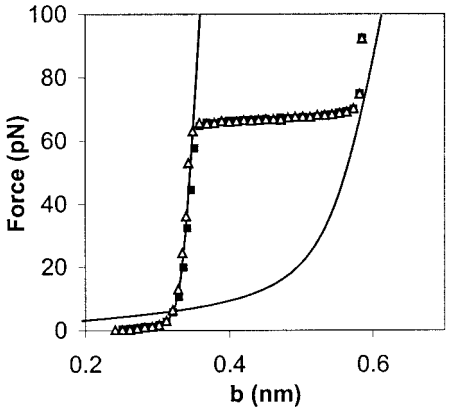

FIGURE 2 Typical room temperature force-extension (per base pair) curve for a single dsDNA molecule in 500 mM ionic strength buffer at pH 7.5. The data obtained while stretching the DNA (△) and the data obtained when the DNA is relaxed (■) are almost identical, so there is very little hysteresis under these conditions. The solid line on the left is the theoretical curve for an extensible worm-like chain (dsDNA) with a persistence length of 50 nm, a contour length of 0.34 nm/bp, and an elastic stretch modulus of 1300 pN. The solid line on the right is the curve for an extensible freely jointed chain (ssDNA) with a persistence length of 0.75 nm, a contour length of 0.56 nm/bp, and an elastic stretch modulus of 800 pN (Smith et al., 1996). The data are interpreted as a transition between dsDNA and ssDNA.

where b_{ds} is the extension of the molecule per base pair. For the fit shown in the figure, we obtain a contour length for dsDNA of $b_{ds}^{max} = 0.34$ nm/bp, a persistence length of $P_{ds} =$ 50 nm, and an elastic stretch modulus of $S_{ds} = 1300$ pN. These values are in good agreement with those that have been reported previously using other optical tweezers instruments (Baumann et al., 1997; Wang et al., 1997).

The data obtained from stretching dsDNA match this curve exactly until the force reaches 65 pN, at which point the force plateaus. The change in force over this plateau is a few pN as the DNA molecule is pulled to ~1.7 times its B-form contour length. We believe the plateau represents a transition from the double-stranded state, the left solid line in Fig. 2, to the single-stranded state represented by the right solid line. After the plateau, the force increases rapidly with a slope that depends on the rate at which the DNA is stretched. This part of the transition represents the removal of the last bonds connecting the DNA strands, as discussed in the Introduction.

The ssDNA force-extension curve is described by the extensible freely jointed chain (FJC) model, which is governed by the relation (Smith et al., 1992)

$$b_{ss}(F) = b_{ss}^{max}\left(\coth(2\tilde{F}) - \frac{1}{2\tilde{F}}\right)\left(1 + \frac{F}{S_{ss}}\right), \qquad (2)$$

where $\tilde{F} = FP_{ss}/k_B T$ is the reduced force, b_{ss}^{max} is the contour length per base in ssDNA, and P_{ss} is the persistence length of ssDNA. Experimental measurements of ssDNA stretching were made by Smith et al. (1996). The measured curve can be fit well to either the WLC or FJC models by varying the three parameters, with the latter model providing a slightly better fit. The actual flexibility of the ssDNA molecule is most likely between these two limiting regimes of polymer flexibility. For ssDNA stretching at pH 8 and 150 mM ionic strength the FJC fit values were $P_{ss} = 0.75$ nm, $b_{ss}^{max} = 0.56$ nm, and $S_{ss} = 800$ pN (Smith et al., 1996).

The stretching portions of the force-extension curves as a function of temperature are shown in Fig. 3. The overstretching force clearly decreases as temperature is increased, as expected for a force-induced melting transition. We confirmed that the sensitivity of the force measurement did not change with temperature by performing a calibration at the lowest and highest temperatures used. For temperatures below 45°C, all of the force-extension data matched the room-temperature WLC curve shown in Fig. 2 at forces less than 40 pN. For temperatures above 45°C, the DNA molecules exhibited partially single-stranded characteristics. We have observed this behavior in many cases, and it does not affect the overstretching force. Many of these molecules broke while being extended, which may be due to single-strand nicks in the DNA strand attached to the beads (despite the treatment with ligase) or a decrease in the biotin-streptavidin bond strength with increasing temperature (Hyre et al., 2000). Due to the difficulty of pulling a

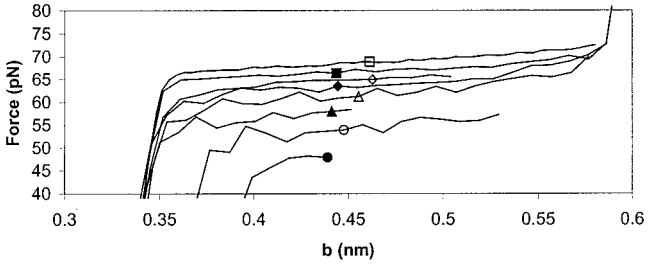

FIGURE 3 Temperature dependence of DNA stretching in 500 mM ionic strength Hepes buffer at pH 7.5. The data are shown as lines between data points separated by 500 nm. The data were obtained at 11°C (□), 21°C (■), 28°C (◇), 31°C (◆), 35°C (△), 40°C (▲), 45°C (○), and 52°C (●). As the temperature of the environment surrounding the DNA is increased, the overstretching force decreases, as expected for force-induced DNA melting. For the curves shown at the highest temperatures, the DNA strand was already partially single-stranded, so at low extensions the force does not match the other curves. The data obtained at less than 45°C exhibit the standard force-extension behavior shown by the data in Fig. 2 at forces below 40 pN. All of these curves were obtained when stretching the DNA.

DNA strand completely through the overstretching transition, especially at high temperatures, many of the curves shown in Fig. 3 show only part of the transition and do not extend to high forces.

The stretching and relaxation portions of two force-extension curves are shown in Fig. 4 as a function of temperature. When the DNA is stretched at 35°C, the relaxation curve follows the ssDNA curve from 66 pN to 48 pN before beginning to re-anneal and become double-stranded. The relaxation curve never matches that of dsDNA, so at this temperature part of the DNA molecule remains single-stranded. When stretched at 45°C, almost the entire length of DNA that was stretched remains single-stranded as it is relaxed. This shows that even when the DNA molecule is stretched only partially through the overstretching transition, at high temperatures the two strands do not re-anneal on the time scale of the experiment. This hysteresis can be explained by the slow recombination kinetics of DNA at high temperatures. In traditional melting, the recombination kinetics are strongly affected by the equilibrium concentration of the single strands in solution. In our single-molecule experiment this concentration is zero, but the local effective concentration of recombining strands depends on the length of the melted portions as well as their diffusion coefficient, which in turn may strongly depend on temperature. A quantitative understanding of this recombination process will require a separate study.

To analyze the stretching behavior quantitatively, we have taken all the curves shown in Fig. 3 and fit the transition region to a straight line. The line matching each transition was evaluated at its intersection with the WLC curves for dsDNA and ssDNA. We define the overstretching force as the force required to stretch a DNA molecule halfway through the overstretching transition, or half the length between the dsDNA curve and the ssDNA curve shown in Fig. 2. The position along the fit line corresponding to the transition midpoint was evaluated, and the force at this point, denoted $F_{\text{overstretch}}$, for each temperature is shown in Fig. 5. The maximum error in the overstretching force measurement, represented by the error bars in Fig. 5, is the root mean square of the instrumental force measurement error and any error due to extrapolation of the curves

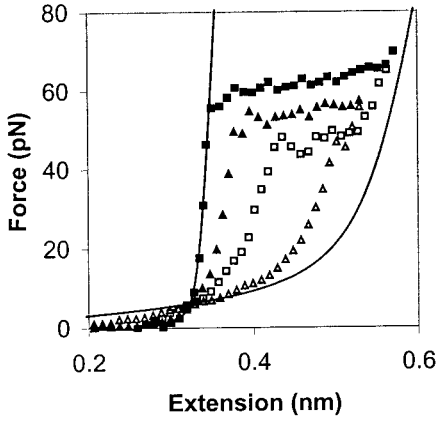

FIGURE 4 The hysteresis between the stretching and relaxation of DNA increases greatly at high temperature. When the DNA is stretched at 35°C (■), the relaxation force (□) drops from 66 to 48 pN before beginning to re-anneal and become double-stranded. When stretched at 45°C (▲), almost the entire length of DNA that was stretched remains single-stranded as it is relaxed (△). As shown in Fig. 2, there is very little hysteresis at room temperature. The worm-like chain fit for dsDNA (*left line*) and the FJC fit for ssDNA (*right line*) with the same parameters used in Fig. 2 are shown for comparison.

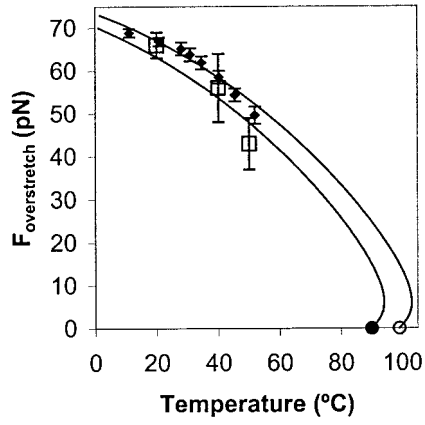

FIGURE 5 Measured overstretching force as a function of temperature at pH 7.5, 500 mM ionic strength (◆) as measured in this study using optical tweezers. Also shown are the data of Clausen-Schaumann et al. (□) measured in 150 mM ionic strength using AFM (Clausen-Schaumann et al., 2000). The solid lines are calculations from our model with $\Delta C_p = 60$ cal/K mol bp and $\Delta S(T_m) = 24.7$ cal/K mol bp using melting temperatures calculated using Eq. 14 for 150 mM ionic strength (●) and 500 mM ionic strength (○). The fit to the data represents a least-squares fit with the constraint that $F_{\text{overstretch}} = 0$ at $T = T_m$.

of Fig. 3. The measured width of the overstretching transition remained approximately constant at ~4 pN. Our results agree within experimental error with published data obtained at 150 mM ionic strength using atomic force microscopy (AFM), which is also shown in Fig. 5 (Clausen-Schaumann et al., 2000). The uncertainty in our measurement of the overstretching force is much lower, so our data can be used to obtain an accurate measurement of the melting entropy of dsDNA.

DISCUSSION

If the overstretching transition of dsDNA is force-induced melting, then the transition force $F_{\text{overstretch}}$ should be a sensitive function of the temperature. The stability of the double helix with respect to two single strands, $\Delta G(T)$, is a strong function of the temperature. This is because the entropy of the highly flexible single strands is significantly higher than that of the rigid double helix. Thus, increasing the temperature drastically reduces the duplex stability, and leads to its melting at 40–100°C depending on solution conditions and DNA composition. In contrast, the transition between any two double-helical forms of DNA should be practically temperature independent, because such transitions involve the restructuring of intra-strand bonds, which have a primarily enthalpic nature. Therefore, our experimental study of the temperature dependence of the overstretching transition should definitively distinguish between force-induced DNA melting and a B- to S-DNA transition.

The phase boundary between the helix and the coil in the (F,T) plane can be found from the condition of zero total transition free energy:

$$\Delta G_{\text{tot}}(F, T) = 0 \tag{3}$$

The slope of $F_{\text{overstretch}}(T)$ can be found by analogy to the Clausius-Clapeyron relation from the condition of zero total derivative of the transition free energy at the transition midpoint, $d\Delta G_{\text{tot}}(F,T)/dT = 0$:

$$\frac{\partial F_{\text{overstretch}}}{\partial T} = -\frac{\Delta S(F, T)}{\Delta b(F, T)}, \tag{4}$$

where ΔS is the entropy of the transition, and Δb is the change in the length of the molecule per base pair between the helix and coil states (Smith et al. 1996). Here we have used the relation $\Delta S(F,T) = -d\Delta G_{\text{tot}}(F,T)/dT$ and $\Delta b(F,T) = -d\Delta G_{\text{tot}}(F,T)/dF$ (Rouzina and Bloomfield, 2001a) for the transition entropy and elongation. Both quantities should be taken at the force and temperature $F_{\text{overstretch}}(T)$ of the transition. These quantities can be calculated from the WLC curves of Fig. 2. Thus, ΔS can be obtained by fitting the data in Fig. 5 to Eq. 4.

If ΔS is not a function of temperature, the data shown in Fig. 5 should be linear. However, if there is a significant change in DNA heat capacity upon melting, this leads to the following temperature dependence of the melting entropy:

$$\Delta S = \Delta S(T_{\text{m}}) + \Delta C_{\text{p}} \times \ln\left(\frac{T}{T_{\text{m}}}\right) \tag{5}$$

Here ΔC_{p} is the change in the DNA heat capacity per base pair upon melting, and T_{m} is the DNA melting temperature. Because $\Delta S(T_{\text{m}})$ can be measured by calorimetry, our data provide a measurement of ΔC_{p}. Although ΔC_{p} has long been assumed to be negligible (Blake and Delcourt, 1998; Breslauer et al., 1986; Grosberg and Khokhlov, 1994; Gruenwedel, 1975; Petruska and Goodman, 1995; Santalucia, 1998), a number of workers have recently demonstrated that it is finite and positive (Chalikian et al., 1999; Holbrook et al., 1999; Jelesarov et al., 1999; Mrevlishvili et al., 1996; Rouzina and Bloomfield, 1999). Mrevlishvili et al. (1996) measured $\Delta C_{\text{p}} = 46.2 \pm 15$ cal/(K mol bp) at $T_{\text{m}} = 77$°C. Holbrook et al. (1999) measured ΔC_{p} of short DNA duplexes to be 57 ± 29 cal/(K mol bp) at 66°C and 93 ± 7 cal/(K mol bp) averaged over a range of temperatures between 9°C and 39°C. Measurement of a series of synthetic polynucleotides by Chalikian et al. (1999) yielded an average value of $\Delta C_{\text{p}} = 64.6 \pm 21.4$ cal/(K mol bp). These measurements were later shown to agree with thermodynamic data on short DNA duplexes (Jelesarov et al., 1999).

We assume that the total transition free energy can be represented as a sum of the temperature-dependent transition free energy in the absence of force, $\Delta G(T)$, and a force-induced part, $\Delta\Phi(F)$, which is relatively independent of the temperature:

$$\Delta G_{\text{tot}}(F, T) = \Delta G(T) + \Delta\Phi(F). \tag{6}$$

It can be shown (Rouzina and Bloomfield, 2001a) that the changes in transition entropy induced by the force within our range of interest are minor compared with its total value.

$\Phi(F)$ is a one-dimensional thermodynamic potential analogous to the Gibbs free energy and is given by

$$\Phi(F) = -\int_0^F b(F')dF' \tag{7}$$

This free energy can be obtained by direct integration of the WLC or FJC curves shown in Fig. 2. The force-dependent free energy change from the helix to coil state is therefore

$$\Delta\Phi(F) = -\int_0^F [b_{\text{ss}}(F') - b_{\text{ds}}(F')]dF'. \tag{8}$$

In Fig. 6 *A* we present $\Delta\Phi(F)$ obtained numerically according to Eq. 8 using a FJC fit to the experimental stretching curve of ssDNA measured at room temperature and 150 mM ionic strength (Smith et al., 1996) and a fit of the extensible WLC model to our room-temperature dsDNA

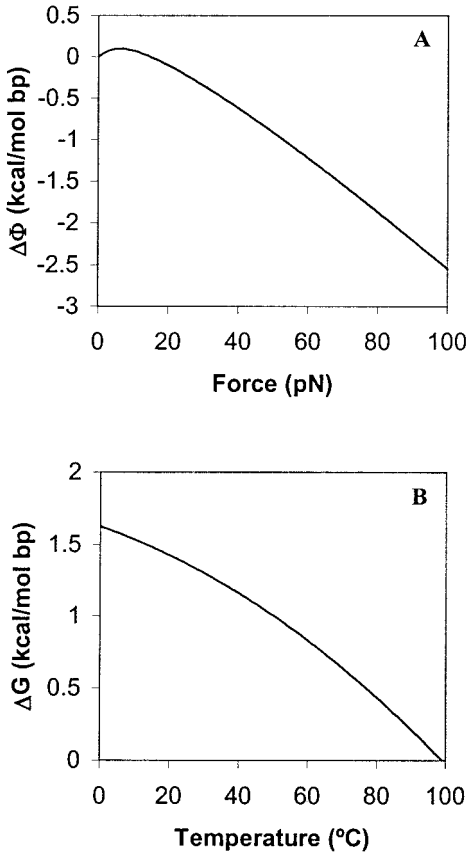

FIGURE 6 (*A*) Force-dependent part of the transition free energy $\Delta\Phi(F)$ between dsDNA and ssDNA as a function of applied force. The line is calculated by integrating the curves shown in Fig. 2 using Eq. 8. At low forces, the double-stranded state is energetically more favorable, but as the force is increased the single-stranded state has a lower energy. (*B*) Free energy of melting at zero force $\Delta G(T)$ of dsDNA calculated using Eq. 12. The overstretching transition occurs when $\Delta\Phi = -\Delta G$.

stretching curve measured in 500 mM ionic strength. There are no experimental data on ssDNA stretching at 500 mM ionic strength and the temperatures used in this study. According to fluorescence recovery after photobleaching (FRAP) experiments, the persistence length of ssDNA changes from ~10 Å to ~7 Å between 150 mM and 500 mM ionic strength based on the FJC model (Tinland et al., 1997). This should increase the work required to stretch ssDNA by only a few percent. It is known that the temperature usually decreases the persistence length of a polymer as $P_{ss}(T) \approx 1/T$ (Grosberg and Khokhlov, 1994), so the relative change in persistence length is small over the temperature range reported here. Thus, the temperature and ionic strength difference between the data used for our calculation and the conditions used in this study should have only a minor effect on $\Delta\Phi(F)$.

The free energy of melting is given by

$$\Delta G(T) = \Delta H(T) - T\Delta S(T), \tag{9}$$

where

$$\Delta H(T) = \Delta H(T_m) + \Delta C_p(T - T_m). \tag{10}$$

We expand Eq. 5 to obtain

$$\Delta S(T) \cong \Delta S(T_m) + \Delta C_p\left(\frac{T - T_m}{T_m} - \frac{(T - T_m)^2}{2T_m}\right). \tag{11}$$

Substituting Eqs. 10 and 11 into Eq. 9 gives

$$\Delta G(T) \cong \Delta S(T_m)(T_m - T) - \frac{\Delta C_p}{2} \times \frac{(T - T_m)^2}{T_m}. \tag{12}$$

In Fig. 6 *B* we present the free energy of melting as a function of temperature as calculated from Eq. 12 with the parameters from our fit obtained below. Combining Eqs. 3, 6, 8, and 12 gives the following quadratic equation:

$$\Delta\Phi(F_{overstretch}) + \Delta S(T_m)(T_m - T) - \frac{\Delta C_p}{2}\frac{(T - T_m)^2}{T_m} = 0 \tag{13}$$

Specifically, $\Delta\Phi(F_{overstretch})$ is obtained by numerical integration of Eq. 8. We then use the values from this calculation to numerically solve Eq. 13 for $T(F_{overstretch})$. Note that $\Delta\Phi(F_{overstretch}) = 0$ and therefore $F_{overstretch} = 0$ at $T = T_m$. We then vary ΔC_p and $\Delta S(T_m)$ and perform a least-squares fit to the data for each value of these parameters. For our calculation we used a theoretical value for the melting temperature of λ DNA in 500 mM ionic strength buffer (Blake and Delcourt, 1998):

$$T_m(°C) = 193.67 - (3.09 - x_{GC})[34.47 - 6.52\log(I)], \tag{14}$$

where I is the ionic strength and x_{GC} is the average GC base composition, which is 0.5 for λ DNA. The results of the calculation with $T_m = 99°C$ are shown as solid lines in Figs. 7 and 8. The curvature in the fit at low forces is due to the change in sign of $\Delta\Phi(F)$, as shown in Fig. 6 *A*. We obtain the best fit with a value of $\Delta S(T_m) = 24.7 \pm 1$ cal/mol K bp and $\Delta C_p = 60 \pm 10$ cal/mol K bp. This corresponds to a melting entropy at room temperature of 10.3 cal/mol K bp. These values are in agreement with the calorimetric measurements cited above. The data of Clausen-Schaumann et al. (2000), which was measured in 150 mM ionic strength using AFM, also fit well to our model (Fig. 5) by changing only the value of T_m as calculated using Eq. 13. Although the data points all lie between the reported error in our measurement of $\Delta S(T_m)$ and ΔC_p, there is no single value of ΔC_p that gives a perfect fit. This is most likely due to a variation of ΔC_p with temperature. Holbrook et al. (1999) report a significant increase in ΔC_p as temperature is decreased, which would give an increased curvature to the predictions of $F_{overstretch}$ as room temperature is approached from above in Figs. 5, 7, and 8. However, we do not have

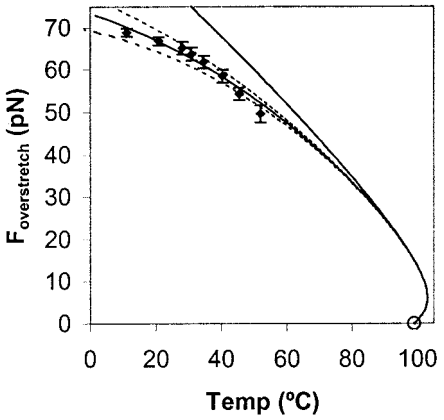

FIGURE 7 Predicted temperature dependence of the overstretching transition. The measured data (♦) are fit well by our model (*lower solid line*) with $\Delta S = 24.7$ cal/K mol bp and $\Delta C_p = 60$ cal/K mol bp. Also shown are fits with $\Delta C_p = 50$ cal/K mol bp (*upper dashed line*) and $\Delta C_p = 70$ cal/K mol bp (*lower dashed line*) to illustrate the error in our measurement of ΔC_p. Also shown is the predicted temperature dependence assuming $\Delta C_p = 0$ (*upper solid line*). The fit to the data represents a least-squares fit with the constraint that $F_{overstretch} = 0$ at $T = T_m$.

a model to explain this temperature dependence, so we have not included it in our calculations.

In Fig. 7 we also show the calculated overstretching force assuming $\Delta C_p = 0$ and using $\Delta S(T_m) = 25$ cal/mol K bp from calorimetric measurements (Blake and Delcourt, 1998; Breslauer et al., 1986; Santalucia, 1998). This clearly demonstrates that the large positive value of ΔC_p has a strong effect on the stability of DNA. In fact, DNA is 30% less stable at room temperature than would be predicted if ΔC_p were zero.

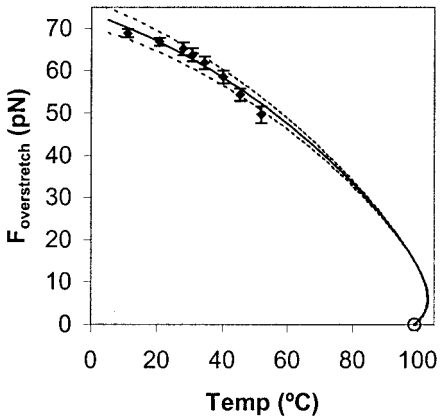

FIGURE 8 Predicted temperature dependence of the overstretching transition. The measured data (♦) are fit well by our model (*solid line*) assuming $\Delta S = 24.7$ cal/K mol bp and $\Delta C_p = 60$ cal/K mol bp. Also shown are fits with $\Delta S = 25.7$ cal/K mol bp (*upper dashed line*) and $\Delta S = 23.7$ cal/K mol bp (*lower dashed line*) to illustrate the error in our measurement of ΔS. The fit to the data represents a least-squares fit with the constraint that $F_{overstretch} = 0$ at $T = T_m$.

CONCLUSIONS

In this work we have measured the DNA overstretching transition force as a function of temperature. We have shown that destabilizing the DNA double helix by increasing its temperature causes a decrease in the overstretching force, as is expected for a force-induced melting transition. We have applied the force-induced melting theory of Rouzina and Bloomfield (2001b) to predict the change in overstretching force with temperature. We find excellent agreement between theory and experiment and obtain an accurate measurement of the change in heat capacity of DNA upon melting, ΔC_p. This measurement is in agreement with reported values obtained using calorimetry.

Our data support the interpretation of the overstretching transition of dsDNA as a force-induced melting transition. In addition to the agreement between the prediction of the temperature dependence of the overstretching transition and the measured data, we also see significant hysteresis at high temperatures, when the DNA double helix is destabilized. At high temperature, a DNA strand that was stretched only partially through the overstretching transition relaxed as almost completely single-stranded DNA, whereas at room temperature there was very little hysteresis.

The temperature dependence of the overstretching transition also supports our earlier work on the pH dependence of the overstretching transition (Williams et al., 2001). In that work we found that the overstretching force closely followed the melting temperature in its dependence on solution pH. Based on that data we were able to determine the melting entropy of DNA at room temperature. The value of 9.5 cal/mol K bp obtained from the pH dependence of the overstretching force is in good agreement with the measurement of 10.3 cal/mol K bp obtained in this work from the temperature dependence of the overstretching transition.

Our primary evidence for the force-induced melting model is the quantitative prediction of the temperature dependence of the overstretching transition from first principles, which yields reasonable values for the relevant calorimetric parameters. Our model is also supported by its ability to explain a large collection of independent experimental data. This includes the observed transition hysteresis, the pH dependence of the transition, the cooperative nature of the transition, and the ionic strength dependence of the transition. In contrast, although our prediction comes from first principles, the existence of the overstretched double-stranded S-DNA is mere speculation, because all of the modeling studies predict that dsDNA overstretching should happen much less cooperatively and at forces almost an order of magnitude higher.

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