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Effects of temperature on the mechanical properties of single stranded DNA

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We present the first measurements of the temperature dependent extension of single stranded DNA. At forces between 2 and 10 pN the extension increases with temperature. This increase in extension is consistent with the disruption of hairpins, and a simple theory that includes hairpin formation shows good agreement with the data at these low forces. In contrast, at forces above 10 pN and temperatures higher than 40 °C, the extension decreases rapidly with temperature in a manner not consistent with predictions.

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There has been great interest in the mechanical properties of DNA single molecules [1–7] as they may provide information about how DNA replication or transcription functions *in vivo* and *in vitro*; moreover, they provide an excellent opportunity to verify theoretical models of DNA behavior. Though it is known that small changes in temperature can have a tremendous impact on biological processes, single molecule studies of DNA have almost all been done at room temperature. Experiments have shown that at forces below 60 pN, the elastic properties of double stranded DNA (dsDNA) are well described by a wormlike chain model (WLC) [2,5,7]. Effects of temperature on the elasticity of dsDNA have been recently reported, where the measurements emphasized the overstretching transition that occurs at forces above 60 pN [8]. Those results demonstrate that at high forces, the force vs extension curves for dsDNA display significant temperature dependence in the temperature range between 33 °C and 45 °C [8]. In this work we demonstrate that the elastic properties of ssDNA also have significant temperature dependence.

We measured the extension versus force curves for λ phage ssDNA molecules tethered between one bead and the surface in phosphate buffer saline *p*H 7.4 (PBS) at several temperatures. The experimental procedure for measuring single DNA molecules in parallel shown in Fig. 1 has been described previously [6,9]. Briefly the DNA molecules are held between a magnetic bead and a flat surface (capillary). The force applied to the beads is determined by the separation between a permanent magnet and the flat surface. The force is obtained from a calibration curve at several distances and no difference in the force values is found upon changes in temperature between 10 °C and 50 °C. The extension of the molecules is the distance between the edge of the bead and the flat surface.

By maintaining a constant temperature, but changing the applied constant force in increments, the extension vs force curve for ssDNA can be obtained for forces between 1 and 17 pN. The temperature was increased to the next temperature and the curve was measured again. The data corresponds to the same molecule stretched by the same bead, to avoid any effects associated with the variation in magnetization of the beads in the sample. In this experiment the mechanical behavior of the single stranded DNA (ssDNA) molecule was followed as the force was increased and decreased at each temperature. Fig. 2(a) shows the changes in elasticity as a function of force for an individual ssDNA molecule, at temperatures between 25 °C and 45 °C. To compare with the isometric experiments, the extension vs force curves are shown by plotting force versus extension. Only the data for the increase in force is shown. The extension as a function of decreasing force frequently shows a slight hysteresis at low forces, but if the force vs extension curve is measured again the second increasing force curve usually follows the first one quite closely.

It can be seen in Fig. 2(a) that the extension increases with temperature at forces below 10 pN. Previous studies of the force vs extension curves for different sequences at room temperature suggested that at forces below 10 pN hairpin formation occurred in the ssDNA, but that at larger forces hairpins were disrupted [10]. Thus, the low force results above would be consistent with the effect of hairpins, if the hairpins were assumed to melt at a temperature of approximately 40 °C. Fluctuations in the length due to changes in hairpin formation might be expected, but were not observed possibly because our resolution of approximately 500 base pairs was insufficient.

Earlier work had suggested that glyoxal disrupts the formation of hairpins [10]. One way to explore the role of hairpins would be to stretch the ssDNA in the presence of glyoxal, where hairpins are not expected to form. Figure 2(b)

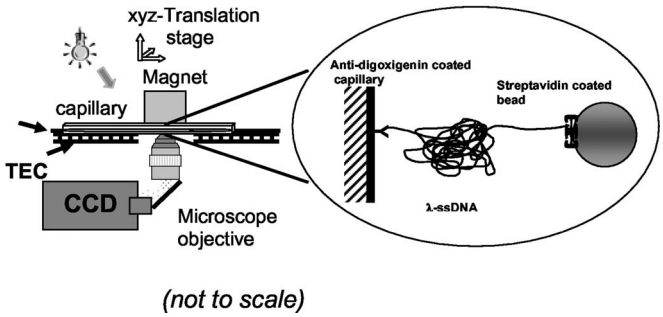

FIG. 1. Scheme of the experimental setup. The suspension containing the beads and ssDNA was incubated in a square microcell. The temperature can be controlled within 10 °C–80 °C using a thermoelectric cooler (TEC) mounted on the aluminum piece that holds the square capillary. A temperature sensor follows the temperature close to the capillary channel.

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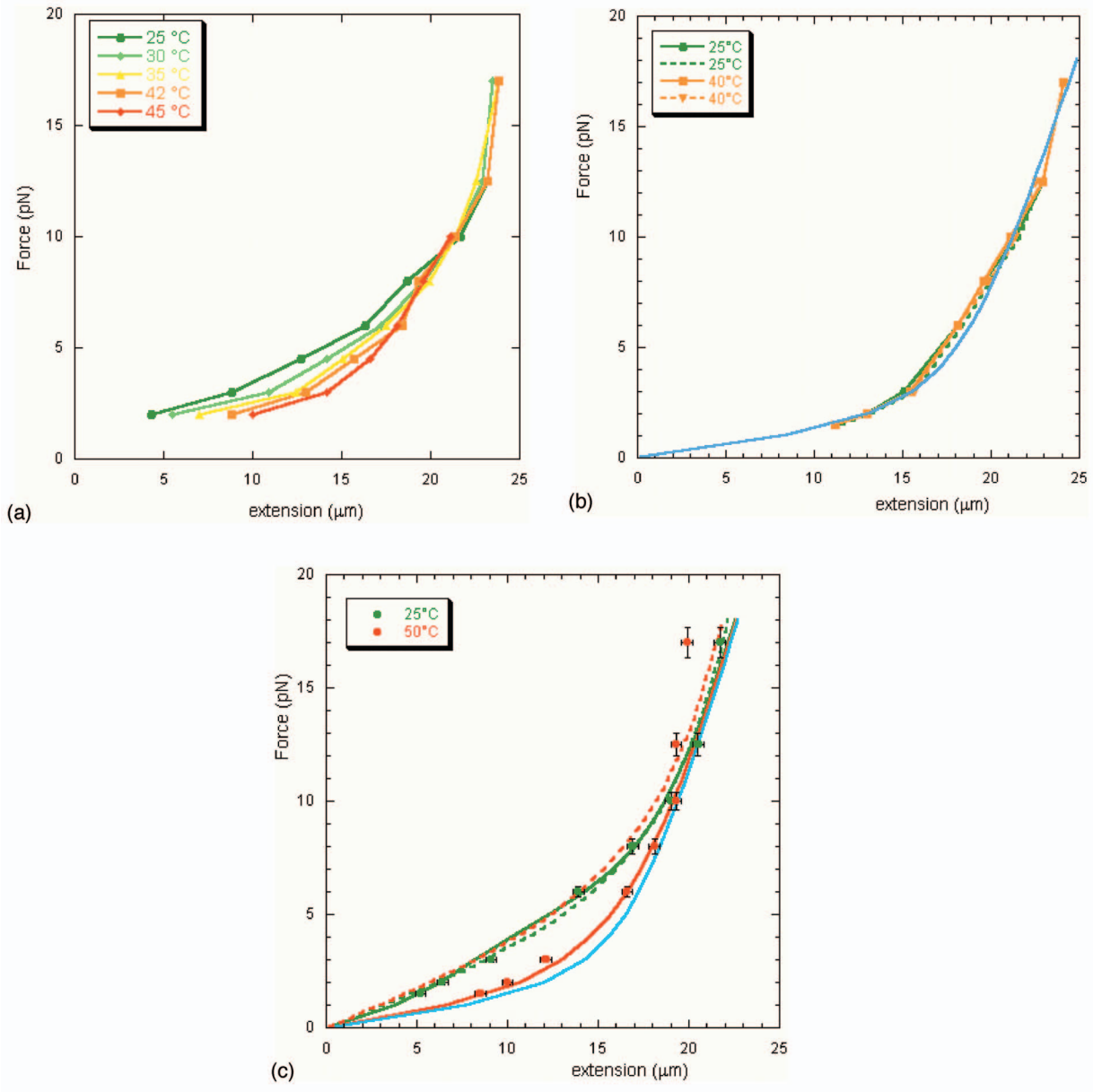

FIG. 2. (Color) Stretching curves of several ssDNA single molecules. (a) Extension curves starting at 25 °C (green), 30 °C, 35 °C, 42 °C, and 45 °C (left to right) as the force was increased in PBS. (b) Extension curves in glyoxal (green and orange) at 25 °C and 40 °C as the force was increased (solid lines) and then decreased (dashed lines) for the same bead at each temperature; the solid blue line was calculated using Eq. (1). (c) The temperature was increased from 25 °C to 50 °C: green and red circles, respectively; the dashed lines are the theoretical predictions using the mFJC model described by Eq. (1). The green and red solid lines correspond to the fits done with the theory including hairpins (see text). The error bars in (a) and (b) are not shown for clarity but are similar to those shown in (c).

shows the force vs extension curves for ssDNA stretched in the presence of 0.1 M glyoxal in PBS buffer. For each temperature, a curve is shown for the case where the force was increased with time, and a second dashed curve is shown for the case where the force was decreased with time. For temperatures between 25 °C and 40 °C, the force vs extension curves are all indistinguishable. The overlapping of the curves obtained at these two temperatures also shows that there is no significant variation in the magnetization of the beads as temperature is varied. The curves in glyoxal exhibit no hysteresis and are more extended at low forces than the curves in PBS whereas the high force extensions are the same in both PBS and glyoxal.

The stretching curve in glyoxal can be fit using the modified freely jointed chain (mFJC) expression for the force vs extension given below,

$$dx(F) = -\left\{L\left[-\coth\left(\frac{bF}{k_BT}\right)+\frac{k_BT}{bF}\right]\right\}\left(1+\frac{F}{S_{ss}}\right), \quad (1)$$

where k_B is Boltzmann's constant, b is the Kuhn length, L is the distance between base pairs, and S_{ss} is the modification of the freely jointed chain that allows for additional increases in length at high forces due to bond elasticity [11]. This describes the stretching curve for ssDNA in the absence of hairpins. The blue curve in Fig. 2(b) shows the predictions of

Eq. (1) using values $S_{ss}=50$ pN, $b=6.1$, and $L=0.39$ that were obtained by fitting the data shown in Fig. 2(b). These are significantly different from the typical values of $S_{ss}=300$ pN, $b=1.73$, and $L=0.53$ that are obtained by fitting the force vs extension curves measured at 25 °C in the same apparatus in the absence of glyoxal. The latter parameters are in good agreement with earlier results where the ssDNA was stretched at room temperature using optical tweezers [11].

Figure 2(c) shows the force vs extension data at 25 °C and 50 °C for another single molecule measured after equilibrating the sample at 25 °C initially and finally at 50 °C, green and red solid circles, respectively. The experimental curve at 25 °C can be fit to Eq. (1), and the result is shown by the dashed green line. Equation (1) predicts that the force vs extension curves for ssDNA molecules do not change significantly as a function of temperature in the temperature range between 15 °C and 50 °C, and much theoretical work incorporates a similar temperature insensitivity [12–21]. The red dashed line shows the predicted force vs extension curve at 50 °C using Eq. (1) and the parameters obtained from the fit of Eq. (1) to the force vs extension curve at 25 °C. At all nonzero forces, the theoretical extension calculated from the mFJC decreases slightly with increasing temperature. The predicted differences approach zero in the high and low force limits, with a maximum difference of approximately 10% at forces of approximately 8 pN. It can be noted that above 10 pN the extension decreases by 10% with increasing temperature, however, the decrease in length is much more dramatic than that predicted by the mFJC (1%) and in good agreement with recently reported results on conformational changes in ssDNA as a function of temperature [22].

If one assumes that the stretching curve in glyoxal shown in Fig. 2(c) by the blue solid line, represents the force vs extension curve in the absence of hairpins, then a simple model for the force vs extension curve in the presence of hairpins is obtained by multiplying the measured temperature insensitive curve obtained in glyoxal by a factor representing the fraction of the sequence that is in the coil form. If one ignores sequence dependence, except for including a factor α that describes what fraction of the total molecule is eligible to participate in hairpin formation, and one applies the Poland-Scheraga model [23] to describe the fraction in the single stranded state x_{ssDNA}, then

$$x_{\mathrm{ssDNA}} = \alpha + \frac{1}{2}(1-\alpha)\left(1 - \frac{s-1}{\sqrt{4s\sigma + (s-1)^2}}\right), \qquad (2)$$

where s and σ are the Bragg-Zimm parameters given by $s=\exp(-F_1\beta)$ and $\sigma\exp(-2F_2\beta)$, where F_1 is the free energy difference between the helix and coil states of the hairpins, F_2 is the free energy of a helix coil junction, and $\beta=1/(k_bT)$ where T is the temperature in Kelvin and k_b is Boltzmann's constant. The free energy difference F_1 can be calculated according to

$$F_1 = \Delta H - T\Delta S - 2g_u, \qquad (3)$$

where ΔH is the enthalpy difference, ΔS is the entropy difference, and g_u is the contribution to the free energy due to the stretching of ssDNA [20]. Given the experimentally determined values of g_u and ΔS, one can get good agreement with the experimentally determined force vs extension curves if $\alpha=0.4$, $F_2=5.5\times10^{-21}$ ($\sigma=0.088$) and the critical temperature is 40 °C, where $\Delta H=T_c\Delta S$. This critical temperature is well below the 91 °C melting temperature for an intact double stranded lambda phage with 48 502 completely matched base pairs. The hairpins that can form in a single strand of lambda phage do not contain long sequence matches so the melting temperature for the hairpins should be substantially lower. For example, the melting temperature for eight GC base pairs is below 40 °C. The predictions of Eq. (2) are in good agreement with the experimental results as shown by the solid green and red lines in Fig. 2(c) that represent the predictions of Eq. (2) given the parameters above. At 25 °C, the agreement between this simple theory and the experiment is quite good for all forces. At 50 °C, the agreement is quite good for forces below 10 pN, but the prediction and observation diverge at higher forces.

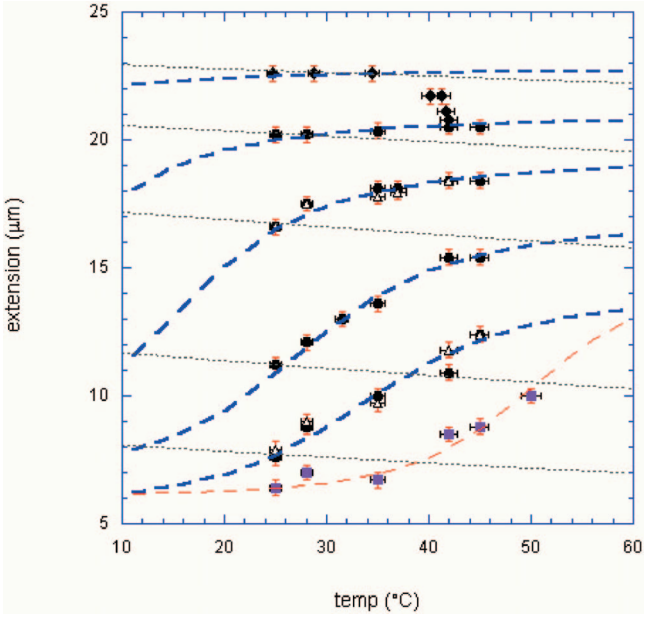

FIG. 3. (Color) Extension vs temperature for several beads at constant force. (a) Extension data at several forces and increasing temperature in PBS *p*H 7.4 from bottom to top: 2, 3, 6, 10, and 12.5 pN (black solid symbols); 2 and 6 pN and decreasing temperature (black empty triangles); 3 pN and increasing temperature in 0.75 M NaCl- phosphate buffer *p*H 7.4 (purple squares); theoretical predictions are shown with dotted gray lines for Eq. (1) and blue (PBS) and red (0.75 M NaCl) dashed lines correspond to the fits that include hairpins in the theoretical calculations.

In order to evaluate the effect of temperature at a given force, the behavior of several ssDNA molecules was followed at constant forces. This constant force measurement avoids any hysteretic effects that might occur when taking a series of force vs extension curves like those shown in Fig. 2. Figure 3 shows the extension of typical single ssDNA molecules that were stretched by a constant force as the temperature of the sample was changed in steps from 25 °C to 45 °C. Each curve was obtained for one individual molecule stretched by a single magnetic bead in order to avoid effects due to variations in the magnetization of the beads. At 2 and 6 pN, measurements for a given bead were

taken twice. The first extension vs temperature curve was taken as a function of increasing temperature from 25 °C to 45 °C (solid circles), and the second curve was taken as the temperature was decreased again (empty triangles). Thus the figure shows that the temperature dependence is reversible. Control experiments where molecules were stretched at constant force and a constant temperature of 25 °C show no change in length over times longer than those required to take the data shown in Fig. 3.

As can be seen in the figure, at forces less than 10 pN the extension for a given force increases monotonically with temperature. At 10 pN, the extension is insensitive to temperature. At 12.5 pN, the extension remains constant up to 35 °C and then at higher temperatures the extension decreases with increasing temperature. This decrease in extension at temperatures above 35 °C and forces above 10 pN is consistent with the temperature dependence of the force vs extension curves shown in Fig. 2(c).

Figure 3 also shows the theoretical extension as a function of temperature predicted by Eq. (1) (dotted gray lines) where the fit parameters were obtained from the typical force-extension data in PBS at 25 °C: $S_{ss}=300$ pN, $b=1.73$, and $L=0.53$. One can see from the figure that at all forces and temperatures, the extension is predicted to decrease with increasing temperature, with the smallest decrease occurring at the largest forces. This prediction is clearly not consistent with the experimental data. In contrast, the dashed blue lines corresponding to predictions of Eq. (2), show very good agreement where the values of F_1, F_2, and α were the same as those for the force vs extension curves shown by the dashed lines in Fig. 2(c).

Finally, earlier work also suggested that hairpin formation will be more stable in buffers at higher ionic strength [10], so increasing the ionic strength of the buffer should result in qualitatively similar data; however, at a given force the temperature required to begin pulling out hairpins will be larger. In PBS and at 2 pN the extension increases linearly as the temperature is increased above 25 °C whereas in a 0.75 M NaCl-phosphate buffer, the extension is independent of temperature up to 35 °C, above which increasing the temperature again results in an approximately linear increase in extension with temperature (purple squares). Again, a fit to Eq. (2) is shown, but in this different buffer reasonable agreement required that F_2 increase to 7×10^{-21} ($\sigma=0.03$) and the critical temperature increased to 58 °C.

We have demonstrated that the force vs extension curves of ssDNA obtained by thermally melting dsDNA have significant temperature dependence in the temperature range between 10 °C and 50 °C. At forces less than 10 pN, the measured extension increases with increasing temperature. At low forces, disrupting hairpin formation by adding glyoxal increases the extension and eliminates the temperature dependence suggesting that the observed temperature dependence at low forces is the result of hairpin formation in the ssDNA. A simple theory that includes hairpin formation shows very good agreement with these results. At forces above 10 pN, where hairpins are not expected to play any role at temperatures equal or above 25 °C, the measured total extension of the ssDNA molecules decreases with increasing temperature. This result is qualitatively consistent with existing theories, but the size of the decrease is an order of magnitude larger than predicted suggesting that at forces of about 13 pN other changes in the structure of ssDNA may also contribute to the changes in the elasticity of ssDNA. The demonstrated variation in elasticity as a function of temperature suggests that research that has assumed the elasticity of ssDNA is not very sensitive to temperature [14–20] may need to be reconsidered and more emphasis may need to be put on the propensity of ssDNA to form sequence dependent secondary structures.

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