Force-Induced Melting of the DNA Double Helix. 2. Effect of Solution Conditions*

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ABSTRACT In this paper, we consider the implications of the general theory developed in the accompanying paper, to interpret experiments on DNA overstretching that involve variables such as solution temperature, pH, and ionic strength. We find the DNA helix-coil phase boundary in the force-temperature space. At temperatures significantly below the regular (zero force) DNA melting temperature, the overstretching force, $f_{ov}(T)$, is predicted to decrease nearly linearly with temperature. We calculate the slope of this dependence as a function of entropy and heat-capacity changes upon DNA melting. Fitting of the experimental $f_{ov}(T)$ dependence allows determination of both of these quantities in very good agreement with their calorimetric values. At temperatures slightly above the regular DNA melting temperature, we predict stabilization of dsDNA by moderate forces, and destabilization by higher forces. Thus the DNA stretching curves, $f(b)$, should exhibit two rather than one overstretching transitions: from single stranded (ss) to double stranded (ds) and then back at the higher force. We also predict that any change in DNA solution conditions that affects its melting temperature should have a similar effect on DNA overstretching force. This result is used to calculate the dependence of DNA overstretching force on solution pH, f_{ov}(pH), from the known dependence of DNA melting temperature on pH. The calculated f_{ov}(pH) is in excellent agreement with its experimental determination (M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, *Biophys. J.*, accepted for publication). Finally, we quantitatively explain the measured dependence of DNA overstretching force on solution ionic strength for crosslinked and noncrosslinked DNA. The much stronger salt dependence of f_{ov} in noncrosslinked DNA results from its lower linear charge density in the melted state, compared to crosslinked or double-stranded overstretched S-DNA.

INTRODUCTION

In this paper, we consider the implications of the general theory developed in the previous paper (Rouzina and Bloomfield, 2001), which equates DNA overstretching with force-induced melting, to interpret experiments on DNA overstretching that involve variables such as solution temperature, pH, and ionic strength.

If DNA overstretching is equivalent to force-induced melting, it should be sensitive to temperature. We therefore begin by analyzing dsDNA stability as a function of both force f and temperature T. We find the helix-coil phase boundary corresponding to the force at the midpoint of the overstretching transition as a function of T, $f_{ov}(T)$, or, conversely, the temperature midpoint of the helix-coil transition as a function of applied force $T_m(f)$. At temperatures much lower than the regular (zero force) DNA melting temperature $T_m = T_m(f = 0)$, $f_{ov}(T)$ decreases almost linearly with f. However, at $T \geq T_m$, the dependence on $f_{ov}(T)$ becomes strongly nonlinear, and there are two, rather than one, critical forces $f_{ss-ds}(T)$ and $f_{ds-ss}(T)$. The DNA double helix is only stable under applied forces between these: $f_{ss-ds}(T) < f < f_{ds-ss}(T)$. This range of forces becomes narrower as T is raised, and the two forces finally converge at f_{cr} as T reaches a critical value T_{cr}. We calculate f_{cr} and T_{cr} and discuss their physical meaning.

We then use this general theory to calculate the $f_{ov}(T)$ curve for λ-DNA and for comparison with available data (Clausen-Schaumann et al., 2000; M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, submitted for publication). The major conclusion is that the temperature-dependence of the DNA overstretching force is in complete agreement with its interpretation as force-induced melting. The fit of the experimental $f_{ov}(T)$ curve not only yields a reasonable value for the DNA melting entropy, but also allows estimation of its temperature dependence, $\Delta S(T)$, which in turn yields a heat capacity of DNA melting, ΔC_p in good agreement with DNA thermal melting studies (Chalikian et al., 1999; Rouzina and Bloomfield, 1999a).

Finally, we show how changes in solution conditions that change the melting temperature, such as pH and ionic strength, will change the overstretching force in a predictable way, and compare theoretical predictions with available experimental data.

TEMPERATURE DEPENDENCE OF DNA OVERSTRETCHING

Phase diagram

The phase boundary $f_{ov}(T)$ between helical and coil states of DNA is determined by the condition $\Delta G(f, T) = 0$, or $\Delta\Phi(f) = -\Delta G^0(T)$. Here $\Delta\Phi(f)$ is the force-dependent contribution to the Gibbs free energy of DNA melting transition defined by Eq. 4 of Rouzina and Bloomfield (2001). The slope $\partial f_{ov}/\partial T$ can be calculated using the fact that, not only the transition free energy $\Delta G(f, T)$, but also its total derivative should vanish at the

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transition point

$$\frac{\partial \Delta G}{\partial T} + \frac{\partial \Delta G}{\partial f}\frac{\partial f}{\partial T} = 0. \quad (1)$$

Therefore,

$$\frac{\partial f_{ov}}{\partial T} = -\frac{(\partial \Delta G(f)/\partial T)_f}{(\partial \Delta G(f)/\partial f)_T} = -\frac{\Delta S(f, T)}{\Delta b(f, T)}. \quad (2)$$

Here we took into account that, in each state $\partial G/\partial T = -S(f, T)$, and $\partial G/\partial f = -b(f, T)$, according to Eq. 2 of Rouzina and Bloomfield (2001). Both derivatives should be taken at the force and the temperature at the midpoint of the transition.

To illustrate, we use, for $\Delta\Phi(f)$, the value obtained from experimental dsDNA and ssDNA stretching curves under standard conditions of room temperature T_r = 293 K and 150 mM NaCl. First, we take the transition free energy without applied force, $\Delta G^0(T)$, in the form of linear dependence described by Eq. 24 of Rouzina and Bloomfield (2001) with the values from thermal melting studies of T_m = 360 K and ΔS = 25 cal/mol-K (Blake and Delcourt, 1998; Rouzina and Bloomfield, 1999; Santalucia, 1998).

We assume that the dependences of ΔS on f and of $\Delta b(f)$ on T are negligible. The first of these assumptions is justified by the fact that, as was shown in Rouzina and Bloomfield (2001), $\partial\Delta\Phi(f)/\partial T = -\Delta S(f, T) \ll \Delta S$ at any reasonable force. The second assumption implies that flexibilities of ds and ssDNA change insignificantly with temperature. This assumption holds much better for the ds than for ssDNA in the range of experimental temperature variation. Direct measurement of the ssDNA flexibility at various temperatures would be needed to enable more accurate prediction of $f_{ov}(T)$. In any case, the effect of varying ssDNA flexibility with temperature on $f_{ov}(T)$ should be minor compared to the main effect described below.

The calculated $f_{ov}(T)$ dependence (*long dashed line* in Fig. 1), captures the main features of the effect. Its slope at $T \ll T_m$, is constant and equal to $\partial f_{ov}/\partial T = -\Delta S/\Delta b^{max} \approx -25$ cal/mol-K/0.22 nm = −0.8 pN/K, where $\Delta b^{max} \approx 0.22$ nm is the maximum difference between the stretched-out extension per base of ss and dsDNA. A similar fit of their experimental $f_{ov}(T)$ dependence was performed by Gaub et al. (Clausen-Schaumann et al., 2000). Our procedure is different in that the DNA melting temperature at zero force is required to equal the value determined from thermal melting studies. Also we take into account the variation of Δb with force, and the variation of ΔS with temperature, as described in the next subsection.

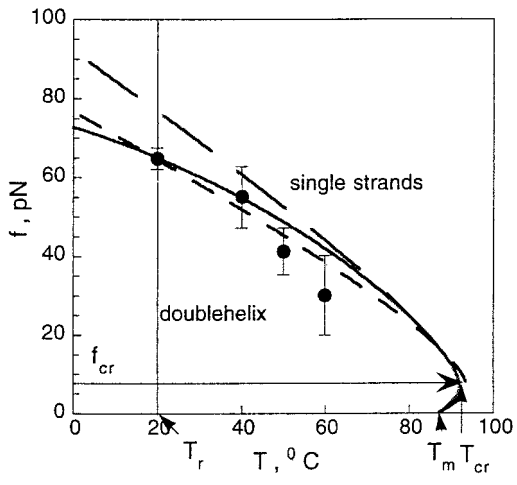

FIGURE 1 The helix-coil phase boundary in (f, T) plane. Circles with error bars are data points from Clausen-Schaumann et al. (2000). *Long dashed line*: $f_{ov}(T)$ calculated according to equation $\Delta\Phi(f) = -\Delta S \cdot (T - T_m)$ with experimental values T_m = 87°C and $\Delta S(T_m)$ = 25 cal/mol-K. *Short dashed line*: the same but with ΔS = 20 cal/mol-K. *Solid line*: calculation assuming nonlinear dependence of transition free energy on the temperature, Eq. 4, with the heat capacity of DNA melting per basepair of ΔC_p = 65 cal/mol-K and $\Delta S(T_m)$ = 25 cal/mol-K. In all calculations, $\Delta\Phi(f)$ was taken equal to its experimental room temperature value (Fig. 3 *A* of Rouzina and Bloomfield, 2001).

The slope of $f_{ov}(T)$ calculated assuming ΔS = 25 cal/mol-K (*long dashed line* in Fig. 1) apparently overestimates the experimental slope from the data of Gaub et al. (Clausen-Schaumann et al., 2000). The best fit to all data points is provided by ΔS = 20 cal/mol-K (*short dashed line* in Fig. 1), rather than ΔS = 25 cal/mol-K. This lower value of the melting entropy would explain why the DNA double helix is melted at room temperature by f_{ov} = 65 pN rather than about 80 pN as predicted by the long dashed line in Fig. 1. In other words, analysis of the stretching experiment suggests that dsDNA at room temperature is less stable than expected from the conventional estimate according to Eq. 24 with the calorimetric value of ΔS, as discussed in Rouzina and Bloomfield (2001).

Heat capacity effects

It is possible to resolve this contradiction between the measured and fitted values of the DNA melting entropy by taking into account its dependence on temperature (Landau and Lifshitz, 1988),

$$\Delta S = \Delta S(T_m) + \Delta C_p \cdot \ln\left(\frac{T}{T_m}\right). \quad (3)$$

Here, ΔC_p is the change of DNA heat capacity per basepair upon melting. For a long time, ΔC_p was considered negligible due to experimental difficulties in its determination. Only recently was it directly measured to be $\Delta C_p = 65 \pm 20$ cal/mol-K (Chalikian et al., 1999; Holbrook et al., 1999; Jelesarov et al., 1999), and its importance for DNA melting thermodynamics realized (Rouzina and Bloomfield, 1999a,b).

In calorimetric experiments, it is $\Delta S(T_m)$ which is measured. However, DNA melting by stretching can occur at much lower temperatures, which, according to Eq. 3, should have much lower transition entropy. Thus, at the room temperature T_r = 293 K and ΔC_p = 65 cal/mol-K, $\Delta S(T_r)$ = 11 cal/mol-K rather than 25 cal/mol-K.

The simplest way to calculate the dependence of f_{ov} on (T), taking into account the nonzero ΔC_p, is to solve the quadratic equation $\Delta\Phi(f) = -\Delta G^0(T)$ with

$$\Delta G^0(T) = \Delta S(T_m) \cdot (T_m - T) - \frac{\Delta C_p}{2} \cdot \frac{(T_m - T)^2}{T_m}. \quad (4)$$

This expression for $\Delta G^0(T)$ can be obtained as its expansion to the second order with respect to small parameter $(T - T_m)/T_m \ll 1$ using the standard relation $\Delta G = \Delta H - T\Delta S$, with $\Delta H = \Delta H(T_m) + \Delta C_p \cdot (T - T_m)$ and $\Delta S(T)$ given by Eq. 3 (Rouzina and Bloomfield, 1999). The transition free energy calculated according to Eq. 4 with ΔC_p = 65 cal/mol-K is presented in Fig. 2 in comparison with the behavior if $\Delta C_p = 0$. The double helix stability at room temperature in the former case is indeed smaller by $\sim 0.5 k_B T_r$ = 0.3 kcal/mol. In other words, the actual DNA stability at room temperature is about 30% smaller than conventionally thought, based on the linear approximation to its temperature dependence, Eq. 24 of Rouzina and Bloomfield (2001). We performed such $f_{ov}(T)$ calculation fixing $\Delta S(T_m) = 25 \pm 2$ cal/mol-K and adjusting ΔC_p; the best fit to experiment was obtained with $\Delta C_p = 65 \pm 15$ cal/mol-K, in very good agreement with the calorimetric determination. More detailed discussion of the fitting procedure applied to highly accurate data $f_{ov}(T)$ can be found in a recent paper from our laboratory (M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, submitted for publication). There, we arrive at the conclusion that the melting theory describes the $f_{ov}(T)$ dependence very

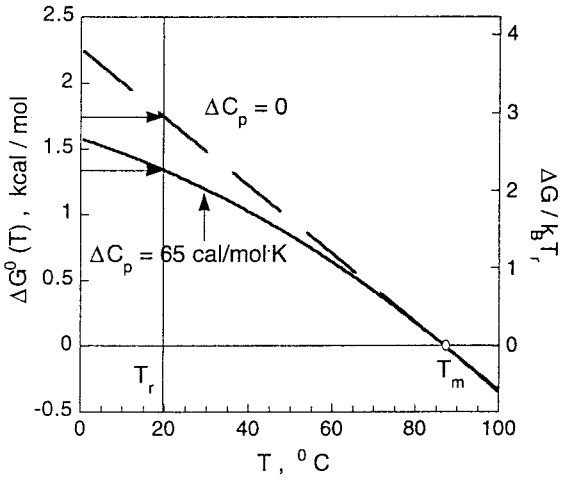

FIGURE 2 Temperature effect on $\Delta G^0(T)$, the free energy of DNA melting in the absence of applied force, calculated with (*solid curve*: Eq. 4), and without, (*dashed curve*: Eq. 24 of Rouzina and Bloomfield, 2001) heat capacity change. In the former case, we took $\Delta C_p = 65$ cal/mol-K; whereas $\Delta S(T_m) = 25$ cal/mol-K for both curves.

well with the best fit values $\Delta C_p = 60 \pm 10$ cal/mol-K and $\Delta S(T_m) = 24.5 \pm 1$ cal/mol-K, in perfect agreement with DNA thermal melting studies.

High-temperature behavior

So far, we have discussed only the low-temperature end of the $f_{ov}(T)$ curve, where the DNA duplex is rather stable and the overstretching force is high. Therefore, both dsDNA and ssDNA are almost fully stretched out, and $\Delta b(f)$ is almost saturated. This results in nearly linear $f_{ov}(T)$ dependence at $T \ll T_m$. However, when the temperature approaches T_m, dsDNA is brought to the verge of its stability, so that only a very small force is needed to melt it. The difference in extension per basepair between the ss and ds forms of DNA decreases with f until it becomes zero at some force f_{cr}, i.e., $\Delta b(f_{cr}) = 0$. At $f < f_{cr}$, Δb reverses sign (see Fig. 3 *B* of Rouzina and Bloomfield (2001)), which, in turn, causes reversal of the sign of the slope $\partial f_{ov}/\partial T$ (Fig. 1). The specific value of f_{cr} depends on the flexibility of ds and ssDNA at the given conditions. If both DNA forms are described as wormlike chains (WLC), f_{cr} should lie between the two characteristic stretching forces $k_BT/A_{ds} < f_{cr} \le k_BT/A_{ss}$. In our reference case of λ-DNA in 0.15 M salt, $f_{cr} \approx 7$ pN.

Because an applied force always preferentially stabilizes the longer molecular form, at $f > f_{cr}$, it drives equilibrium toward ssDNA, while at $f < f_{cr}$, it stabilizes dsDNA. At the same time, raising the temperature promotes the first transition and opposes the second. This is the physical meaning of the sign reversal of $\partial f_{ov}/\partial T$.

At $T > T_m$, when DNA is single-stranded without force, application of moderate force $f < f_{cr}$ should promote its transition to the double-stranded form. When the force is raised further, the reverse transition to ssDNA occurs. This behavior is illustrated in Fig. 3 *A*, which presents DNA stretching profiles $f(b)$ for several different temperatures near $T_m = 87$°C, calculated using Eq. 3 of Rouzina and Bloomfield (2001) for experimental $\Delta\Phi(f)$ and $\Delta G^0(T)$ given by Eq. 4. We see that the curve at $T = 88$°C is calculated to have two overstretching transitions: one at $f_{ss\to ds} \sim 3$ pN and the other at $f_{ds\to ss} \sim 12$ pN. Neither transition is very cooperative, because the extensions of ss and dsDNA are not very different at such forces, and strongly depend on the force. Raising T beyond T_m increases $f_{ss\to ds}$ and decreases $f_{ds\to ss}$, until they finally converge at the critical point (f_{cr}, T_{cr}). Beyond this point, no dsDNA can exist. T_{cr} can be interpreted as the maximum melting temperature of dsDNA, due to its additional stabilization by force. It can be found from the condition $\Delta G^0(T_{cr}) = -\Delta\Phi(f_{cr})$,

$$T_{cr} = T_m + \frac{\Delta\Phi(f_{cr})}{\Delta S}. \tag{5}$$

Reading the maximum value of $\Delta\Phi(f_{cr}) \approx 0.2k_BT_r$ from the solid curve in Fig. 3 *A* of Rouzina and Bloomfield (2001), and using $\Delta S = 25$ cal/mol-K, we arrive at a maximum melting temperature increase of about 5 K, as shown in Fig. 1 of this paper.

MELTING FORCE DEPENDENCE ON OTHER SOLUTION CONDITIONS

General theory

If DNA overstretching is due to force-induced melting, then any solution changes that affect double-helix stability should have an effect on the overstretching force. For an arbitrary parameter Y, the $f_m(Y)$ dependence can be described by analogy to Eq. 2,

$$\frac{\partial f}{\partial Y} = -\frac{(\partial\Delta G/\partial Y)_{T,f}}{(\partial\Delta G(T, f, Y)/\partial f)_{T,Y}}. \tag{6}$$

The derivatives $\partial\Delta G/\partial Y$ and $\partial\Delta G/\partial f$ should be taken at the transition point. If $\Delta G(f, T, Y)$ is known, then $f_m(Y)$ can be found explicitly from Eq. 6. This will be done below when Y is ionic strength.

For most solution variables, however, it is their effect on T_m rather than ΔG that is known. If the dependence of T_m on Y is available, it is possible to make an approximate prediction of $f_m(Y)$:

$$f_{ov}(Y) - f_{ov}(Y^0) = (T_m(Y) - T_m(Y^0)) \cdot \frac{\Delta S^*}{\Delta b}, \tag{7}$$

where $f_{ov}(Y^0)$ and $T_m(Y^0)$ are the melting force and melting temperature at some reference value Y^0. The coefficient of proportionality $\Delta S^*/\Delta b$, where ΔS^* is some average measure of the transition entropy, can be approximated as independent of T, f, and Y.

Then, in analogy to Eq. 1 one can write

$$\left(\frac{\partial\Delta G}{\partial Y}\right)_{T_m, f=0} - (\Delta S)_{T_m, f=0}\frac{\partial T_m}{\partial Y} = 0$$

and (8)

$$\left(\frac{\partial\Delta G}{\partial Y}\right)_{T,f} - (\Delta b)_{T,f} \cdot \frac{\partial f_m}{\partial Y} = 0.$$

Therefore

$$\frac{\partial f_m}{\partial Y} = \frac{\Delta S_{T_m}}{\Delta b} \cdot \frac{\partial T_m}{\partial Y} + \frac{1}{\Delta b} \cdot \frac{\partial[\Delta G(T, f) - \Delta G(T_m, f = 0)]}{\partial Y}. \tag{9}$$

The difference between the two transition energies in brackets can be approximated as $-\Delta S \cdot (T - T_m) - \Delta b \cdot f$. Therefore, if ΔS and Δb are not very sensitive to Y, we arrive at the result given by Eq. 7, with ΔS^* the transition entropy averaged between T and T_m.

pH Dependence

Under normal solution conditions, the range of interesting temperatures, $T_m < T < T_{cr}$, is too narrow and the temperatures are too high for direct

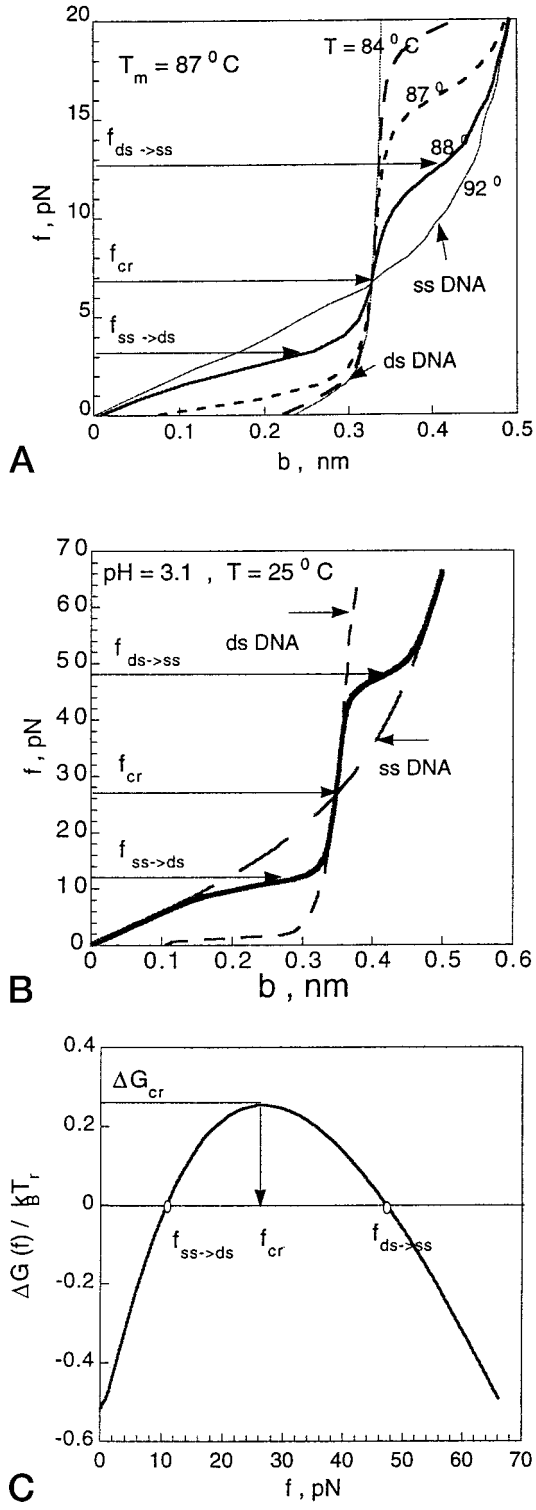

FIGURE 3 (*A*) Calculated DNA stretching curves at different temperatures around original melting temperature without force $T_m = 87°C$. The temperature corresponding to each curve is indicated. All curves at $T < T_m$ have one ds–ss transition. The curve corresponding to $T = 88°C$ just above T_m has two overstretching transitions, whereas the curve at $T = 92°C$ is single stranded at any force. At $T = 84°C < T_m$, there is a single overstretching transition from ds to ssDNA at ≈25 pN. (*B*) The same but for pH = 3.1 and temperature slightly above room temperature. The solid line is a stretching curve with two well-defined overstretching transitions. Long and short dashed lines are ss and ds stretching curves, respectively. (*C*) Total melting transition free energy at pH = 3.1, $\Delta G(f) = \delta\Phi(f) + \Delta G^0$, from experimental data of (M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, submitted for publication) and used for calculation of stretching curve in Fig. 3 *B*. The crossover force, f_{cr}, and maximum dsDNA stabilization $\Delta\Phi_{cr} = \Delta G(f_{cr}) - \Delta G(f = 0) = 0.72 k_B T_r$, are much larger under these conditions compared to neutral pH.

observation. However, if the double helix is destabilized by other solution conditions, so that T_m is lowered into an experimentally accessible range, one might be able to observe both predicted DNA stretching transitions at the same temperature $T \geq T_m$. This strategy was used in our study of the effect of pH on DNA overstretching force (Williams et al., 2001), where we made parallel measurements of f_{ov}(pH) and T_m(pH) in the range $3 < \text{pH} < 11$. f_{ov} remains unchanged at the 65-pN value typical of neutral pH in the interval $4 < \text{pH} < 9.5$, but drops abruptly at higher and lower pH. The overall f_{ov}(pH) dependence can be nicely predicted from T_m(pH) using Eq. 7 with $\Delta S^* = 10$ cal/mol-K. This value is close to the average transition entropy determined calorimetrically in Privalov et al. (1969), $\Delta S = 12$ cal/mol-K. It also agrees with the value of $\Delta S(T = 20°C) = 11$ cal/mol-K calculated according to Eq. 3, with $\Delta C_p = 65$ cal/mol-K. A similar value, $\Delta S(T = 20°C) \approx 10$ cal/mol-K, was estimated from the temperature dependence of overstretching force in M. C. Williams, J. R. Wenner, I. Rouzina, and V. A. Bloomfield, submitted for publication. This striking result argues strongly in favor of the melting nature of the overstretching transition. It also essentially rules out the existence of overstretched double-stranded S-DNA with intact hydrogen bonds, because strong destabilization of B-DNA duplex at low and high pH occurs specifically due to protonation and deprotonation at the sites of broken interbase hydrogen bonds only on single-stranded DNA.

At pH 3.1, dsDNA is on the verge of stability at room temperature, and a stretching force can easily shift the equilibrium between double- and single-stranded forms. Single-stranded DNA appears to become more flexible at low pH, because of charge neutralization by protonation. This increased flexibility of ssDNA, in turn, results in a higher f_{cr}, and stronger stabilization of dsDNA by the crossover force. Thus, the DNA stretching profile at low pH and T slightly above T_m should have two overstretching transitions. Such a stretching curve obtained with DNA parameters at pH 3.1 taken from Williams et al. (2001) is presented in Fig. 3 *B*. Here, $f(b)$ was calculated according to Eqs. 4, 25, and 26 of Rouzina and Bloomfield (2001) with the total transition free energy $\Delta G(f, \text{pH}, T) = \Delta G^0(T) + \Delta\Phi(f, \text{pH})$ obtained from experimental stretching curve at pH = 3.1, presented in Fig. 3 *C*.

Ionic strength dependence

The effect of ionic strength on stability of the DNA double helix is well known. The salt-dependent part of the helix-coil transition free energy, ΔG^{el}, can be adequately described by polyelectrolyte theory in low salt, ($I \ll I_0$, $I_0 \approx 1$ M) by (Bond et al., 1994; Frank-Kamenetskii et al., 1987)

$$\Delta G^{el} = k_B T \cdot \left(\frac{1}{\xi_{ss}} - \frac{1}{\xi_{ds}}\right) \ln(I/I_0), \qquad (10)$$

where ξ is the dimensionless linear charge density,

$$\xi = \frac{l_B}{h} \quad \text{where} \quad l_B = \frac{e^2}{\epsilon k_B T}. \qquad (11)$$

Here h is the length per unit charge e, ϵ is the dielectric constant of water, and l_B is the Bjerrum length. In water at room temperature, $\epsilon = 78$ and $l_B = 0.71$ nm.

Dependence of the overstretching force on solution ionic strength can be calculated by substituting $\ln I$ for the solution variable Y in Eq. 6:

$$\frac{\partial f_{ov}}{\partial \ln(I)} = -\frac{\partial \Delta G(f)/\partial \ln(I)}{\partial \Delta G(f)/\partial f} = \frac{\partial \Delta G^{el}/\partial \ln(I) + \partial \Delta \Phi/\partial \ln(I)}{\Delta b(f)}, \tag{12}$$

where, as usual, the free-energy derivatives should be taken at the transition point. The second equality in Eq. 12 was obtained by taking into account that the total transition free energy is a sum of three components: $\Delta G(f, T, I) = \Delta G^0(T) + \Delta\Phi + \Delta G^{el}$, of which only $\Delta\Phi(f)$ depends on the force, so that $\partial \Delta G(f)/\partial f \approx -\Delta b(f)$. Of the two terms in the numerator of Eq. 12, it is the first that is significant. It is shown in the Appendix that the second term, which takes into account the variation of dsDNA and ssDNA flexibility with salt, is negligible compared to the first term. Taking into account Eq. 10, we calculate the slope as

$$\frac{\partial f_{ov}}{\partial \ln(I)} = \frac{k_B T}{l_B} \cdot \nu, \tag{13}$$

where

$$\nu = \frac{h_{ss} - h_{ds}}{b_{ss} - b_{ds}} \tag{14}$$

is the ratio of the difference in the length per unit charge, $h_{ss} - h_{ds}$, and the difference in length per basepair projected on the direction of the force, $b_{ss} - b_{ds}$. At the high forces typical of the overstretching transition, stretching of both DNA forms is almost complete, so $b_{ss}/b_{ds} \approx 1.7$.

In the fully stretched double-helical state, the length per unit charge is always half the rise per basepair, $h_{ds} = \frac{1}{2} b_{ds}$. In the coil state, the length per unit charge depends on the details of the state, and is less well determined. As discussed in Rouzina and Bloomfield (2001), there are two possibilities: either both strands are under tension when melted, or one is relaxed. In the first case, the length per unit charge is determined by the proximity of the two melted strands to each other. When the tension is high, both melted strands are almost completely extended, and therefore are close to each other. Electrostatically, two such strands are equivalent to a single strand with double charge. In this case, $h_{ss} = \frac{1}{2} b_{ss}$. Then

$$\nu = \frac{1}{2}\frac{b_{ss} - b_{ds}}{b_{ss} - b_{ds}} = 0.5. \tag{15}$$

This approximation will be justified if the average distance between the strands is much smaller than the Debye screening length, $r_{DH} = 1/\sqrt{4\pi l_B I}$. This condition will always be satisfied for two intact strands under high enough tension. If this condition does not hold, then two melted strands are separate polyelectrolyte chains, and $h_{ss} = b_{ss}$, so

$$\nu = \frac{b_{ss} - b_{ds}/2}{b_{ss} - b_{ds}} = 1.7. \tag{16}$$

In the second case, when one strand is nicked or unattached while the other is under tension, the length per unit charge in the coiled state should be an average of its value in the stretched strand $h_{ss} \approx b_{ss}$ and its value in the melted relaxed single strand $h_{ss} \approx b_{ds}$. The latter relation follows from studies of the salt dependence of the thermal melting of DNA (Bond et al., 1994; Frank-Kamenetskii et al., 1987). The average h_{ss} value between the two strands then is $h_{ss} = (b_{ss} + b_{ds})/2$, and

$$\nu = \frac{(b_{ss} + b_{ds})/2 - b_{ds}/2}{b_{ss} - b_{ds}} = \frac{b_{ss}}{2(b_{ss} - b_{ds})} \approx 1.2. \tag{17}$$

Recently, Stigter (1998) considered this very problem, trying to distinguish between double-stranded S-DNA and melted single-strand models of overstretched DNA. He modeled melted DNA strands as distant, and S-DNA as a single rod with twice the linear charge density. Comparison of the calculated $\Delta G^{el}(I)$ for both models with that derived from the experimental $f_{ov}(I)$ behavior obtained in Smith et al. (1996) clearly favors the double-stranded model of overstretched DNA. The experimental (Smith et al., 1996) slope is $\partial f_{ov}/\partial \log(I) = 8.5$ pN (Fig. 4), equal to the value given by Eq. 13 with $\nu = 0.64$. This is close to the value $\nu \approx 0.5$ expected for a transition into a state with two close parallel strands.

However, in this experiment (Smith et al., 1996), λ-DNA was crosslinked with psoralen at every 20th base pair. The overstretching transition still occurred in this crosslinked DNA, but it was much broader than without crosslinking. This is easy to understand, taking into account that the size of the cooperatively melting unit was reduced from ~100 bp to ~20 bp as fixed by the crosslinking frequency. The melted state of two strands in such crosslinked DNA is, perforce, electrostatically similar to that of S-DNA.

We now have additional experimental information, summarized in Fig. 4, on the salt dependence of the overstretching transition in noncrosslinked DNA (C. G. Baumann, S. B. Smith, V. A. Bloomfield, and C. Bustamante, manuscript in preparation; Clausen-Schaumann et al., 2000; Williams, private communication). At high salt, $I \geq 0.2$ M, f_0 saturates at the same value 65–67 pN. But the overstretching force decays much faster with salt in the noncrosslinked DNA. Thus, the slope at lower salt (C. G. Baumann, S. B. Smith, V. A. Bloomfield, and C. Bustamante, manuscript in preparation), $\partial f_{ov}/\partial \log(I) = 14.4$ pN, is almost twice as large. It corresponds, according to Eq. 13, to $\nu \approx 1.1$. This result agrees with a picture in which about 30% of the DNA length has two strands under tension, with only one strand under tension along the rest of the length. The particular state of melted DNA under tension depends on the DNA sequence and location of single-strand nicks. Thus, in Fig. 4, there are slightly different data on the salt dependence of f_{ov} from a recent atomic force microscopy study on a λ-DNA digest (Clausen-Schaumann et al., 2000). It is incontestable, regardless of these details, that the noncrosslinked form of overstretched DNA has a significantly lower linear charge density than the crosslinked form. It is very difficult to rationalize the strong effect of crosslinking on DNA overstretching in lower salt if it were a transition to a double-stranded S-DNA form.

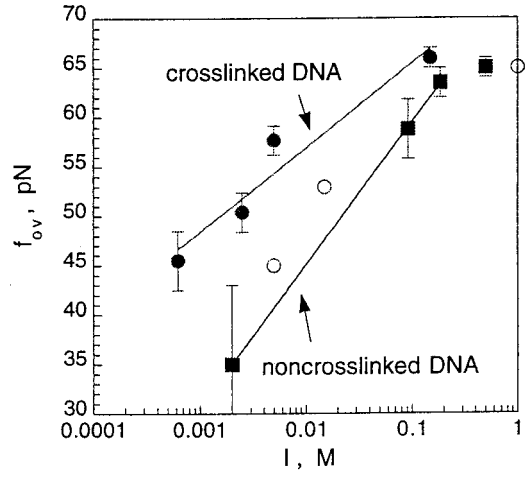

FIGURE 4 DNA overstretching force as a function of solution ionic strength. *Filled circles*: data for crosslinked DNA from (Smith et al., 1996). *Squares*: data from C. G. Baumann, S. B. Smith, V. A. Bloomfield, and C. Bustamante, manuscript in preparation). *Open circles*: data for noncrosslinked DNA from (Clausen-Schaumann et al., 2000). Lines are linear fits of $f_{ov}(\ln(I))$ according to Eq. 13, with $\nu = 0.6$ for crosslinked DNA and $\nu = 1.1$ for noncrosslinked DNA, as discussed in the text.

Another effect, which should make the dependence of f_{ov} on I weaker in crosslinked DNA, thus enhancing its difference from noncrosslinked DNA in low salt, is the increasing stiffness of single-stranded DNA in low salt (Tinland et al., 1997). When the persistence length of ssDNA reaches the distance between crosslinks, the entropic advantage of DNA melting will be strongly reduced and the melting force should increase. This effect is analytically tractable and will be treated elsewhere, but there is not yet enough experimental information for quantitative comparison.

In Fig. 4, the data points at low salt for noncrosslinked DNA have large error bars. This is due to the intrinsic problems with DNA overstretching in low salt, which originally prompted the authors (Smith et al., 1996) to crosslink it. The force versus extension profiles become quite jagged and exhibit strong hysteresis in the relaxation part of the stretch–relax cycle. We believe both of these features are related to the slow kinetics of strand recombination in low salt (Rouzina and Bloomfield, manuscript in preparation). Also, the DNA becomes more fragile in low salt. This can be related to significantly higher cooperativity of DNA melting in lower salt (Kozyavkin et al., 1987). This, in turn, leads to a much larger size of the cooperatively melting fragments (see Eq. 30 of Rouzina and Bloomfield, 2001), which can become equal to the distance between nicks, and lead to frequent DNA breakage at the very beginning of the overstretching transition.

DISCUSSION AND CONCLUSIONS

In this paper, we have explored some of the implications of our general theory of force-induced DNA melting (Rouzina and Bloomfield, 2001) with regard to the effects of solution temperature, pH, and ionic strength. The model predicts that the DNA overstretching force should be a decreasing function of temperature, and, conversely, that the melting temperature should be a decreasing function of applied force. This should not be the case if the transition is into some double-stranded DNA form such as S-DNA. Indeed, any double-stranded B-to-S transition in DNA would involve only restructuring of primarily enthalpic bonds, which should not be temperature sensitive. Although available data for comparison are still limited, the force-induced melting model is consistent with most of the observations. This is particularly true of the effect of pH on the overstretching force (Williams et al., 2001), in which f_{ov} follows the changes in T_m as pH is varied.

The force-induced melting model also quantitatively explains the observation that crosslinking the dsDNA makes little difference to its stretching behavior in high salt, (except for lower cooperativity), but significantly raises f_{ov} in lower salt, compared to noncrosslinked DNA.

The model makes numerous testable predictions. Most generally, changes in the overstretching force should follow changes in melting temperature with DNA composition and varying solution conditions such as different salts, cosolvents, and ligands.

Precise measurement of DNA stretching curves in various solutions offers new possibilities for studying DNA duplex stability. An advantage of force-induced melting over thermal melting is that it is isothermal, thereby avoiding poorly characterized thermal contributions to the transition enthalpy and entropy. The resulting data can be used for independent verification of current ideas about DNA melting thermodynamics, including the issue of the heat capacity increment in DNA melting (Chalikian et al., 1999; Holbrook et al., 1999; Jelesarov et al., 1999; Rouzina and Bloomfield, 1999a,b). Deeper insight can be obtained into basic aspects of the helix-coil transition, such as boundary energies, sequence heterogeneity effects, loop entropy factors, and elastic behavior of single-stranded DNA. Understanding the single-stranded nature of overstretched DNA can affect interpretation of some experimental data on RecA (Hegner et al., 1999) and polymerase (Wuite et al., 2000) proteins binding to DNA.

The force-induced melting model can account for the jagged stretching curves and pronounced relaxation hysteresis observed in many overstretching experiments (Clausen-Schaumann et al., 2000). More systematic study of the effects of varying stretching and relaxation rates should give insight into the kinetics of melting and strand recombination, and should aid understanding of the rate-dependent unbinding of oligomeric DNA (Strunz et al., 1999). Theoretical work on the kinetics of DNA force-induced melting is in preparation.

APPENDIX

Here we show that the second term in Eq. 12 makes an insignificant contribution to the slope $\partial f_{ov}/\partial \ln(I)$. The derivative $\partial\Delta\Phi/\partial \ln(I)$ reflects changes in the work of stretching ds and ssDNA (the Helmholtz free energy as defined in Eq. 1 of Rouzina and Bloomfield, 2001), due to changes in their flexibility with solution ionic strength. According to (Skolnick and Fixman, 1977), the dependence of the persistence length of a polyelectrolyte on ionic strength has the form

$$A(I) = A(I_0) + \delta A \quad \text{where} \quad \delta A(I) = \frac{1}{16\pi l_B^2 I}. \tag{A1}$$

Therefore,

$$\frac{\partial\Delta G}{\partial \ln(I)} = \frac{\partial A}{\partial \ln(I)} \cdot \frac{\partial\Delta G}{\partial A} \approx -\frac{\delta A}{A+\delta A} \cdot \frac{k_B T b}{A+\delta A}, \tag{A2}$$

where we took into account that $\partial A/\partial \ln(I) = -\delta A$ and $\partial\Delta G/\partial A = -(1/A)(\Delta\Phi - \Delta b \cdot f) \approx k_B T b/A^2$. Expression A2 is small in high salt, because $\delta A/(A+\delta A) \ll 1$. It is also small in low salt, because then, $b/(A+\delta A) \ll 1$. Therefore, it is always true that $\partial\Delta\Phi/\partial \ln(I) \ll \partial\Delta G^{el}/\partial \ln(I)$.

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