Force-Induced Melting of the DNA Double Helix 1. Thermodynamic Analysis

Ioulia Rouzina and Victor A. Bloomfield
Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, 1479 Gortner Avenue, St. Paul, Minnesota 55108 USA

ABSTRACT The highly cooperative elongation of a single B-DNA molecule to almost twice its contour length upon application of a stretching force is interpreted as force-induced DNA melting. This interpretation is based on the similarity between experimental and calculated stretching profiles, when the force-dependent free energy of melting is obtained directly from the experimental force versus extension curves of double- and single-stranded DNA. The high cooperativity of the overstretching transition is consistent with a melting interpretation. The ability of nicked DNA to withstand forces greater than that at the transition midpoint is explained as a result of the one-dimensional nature of the melting transition, which leads to alternating zones of melted and unmelted DNA even substantially above the melting midpoint. We discuss the relationship between force-induced melting and the B-to-S transition suggested by other authors. The recently measured effect on T7 DNA polymerase activity of the force applied to a ssDNA template is interpreted in terms of preferential stabilization of dsDNA by weak forces ~7 pN.

INTRODUCTION

Application to DNA of the powerful new technique of single molecule manipulation with optical tweezers has led to the discovery of a striking overstretching transition (Smith et al., 1996). Under moderate forces f and extensions x up to their contour lengths, the double-stranded (ds) and single-stranded (ss) forms of DNA can be characterized as slightly extensible worm-like chains (WLC) and freely jointed chains (FJC), respectively (Marko and Siggia, 1995; Smith et al., 1992, 1996). But at a force of about 65 pN, the dsDNA elongates to about 1.7 times the normal B-DNA contour length (see Fig. 1). The transition is highly cooperative, the width of the $f - x$ plateau being only a few pN. At forces above the overstretching plateau, the dsDNA extension profile converges to that of ssDNA, eventually approaching the ssDNA contour length.

The convergence to the ssDNA $f - x$ curve and contour length led initially to the speculation that dsDNA could be converted to ssDNA (i.e., melted) in the course of the overstretching transition (Marko and Siggia, 1995; Smith et al., 1996). This possibility was discarded because of two arguments: (i) the overstretching transition seems too cooperative for a common melting process; and (ii) dsDNA is able to withstand forces up about 150 pN, much larger than the overstretching force of 65 pN. Since the double-stranded λ-DNA molecule in these experiments was pulled on its two different single strands by attachment of polystyrene latex beads to the 5′ overhangs at each end, it seemed virtually impossible that the two strands would not separate at such high forces if an extensive dissociation of the two strands from each other occurred at the transition force. This argument was later strengthened by measurement via atomic force microscopy (AFM) of the stretching profiles of λ-DNA and of synthetic alternating AT and GC DNAs (Clausen-Schaumann et al., 2000; Rief et al., 1999). It was shown that, while the overstretching plateau seemed to be at equilibrium, the actual unbinding events happened at rate- and sequence-dependent forces in the range 150–300 pN, much higher than the transition force. Therefore, DNA overstretching was attributed (Clausen-Schaumann et al., 2000; Cluzel et al., 1996; Rief et al., 1999; Smith et al., 1996) to a double-stranded form, nearly twice as long as B-DNA, called S-DNA.

This idea inspired several molecular modeling studies of S-DNA (Konrad and Bolonick, 1996; Kosikov et al., 1999; Lebrun and Lavery, 1996). Despite differences in details of molecular structure and energetics, these studies agree that B-DNA can be stretched to about twice its normal length without losing interbase hydrogen bonding, but giving up about every other base stacking interaction. The calculated deformation energies per base pair of the resulting S-DNA are about 10 to 20 kcal/mol or 17 to 34 k_BT where k_B is the Boltzmann constant and T the Kelvin temperature (about 295 K at room temperature). However, these transition energies are about an order of magnitude higher than those estimated from the experimental stretching curves, and the cooperativity of the modeled B-S transition is much less than is seen experimentally. Both of these discrepancies were attributed to the limitations of the finite size, periodic base composition, and restricted coordinate space of the molecular models.

In this paper we return to the interpretation of the overstretching transition in B-DNA as a force-induced melting and show that this interpretation can quantitatively explain most of the observations. In the next section, we show how

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Address reprint requests to Dr. Ioulia Rouzina, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, 1479 Gortner Ave., St. Paul, MN 55108. Tel.: 612-625-2268; Fax: 612-625-5780; E-mail: rouzina@biosci.umn.edu.

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the experimental dsDNA and ssDNA stretching curves can be used to obtain the force-dependent contributions to the free energy of the transition between the helix and coil states of the molecule. Comparing the force-induced destabilization free energy with the free energy change of DNA melting without an applied force, we conclude that melting of B-DNA should occur at 60 to 80 pN of applied force.

In the following section, "Application of helix-coil transition theory," we use the force-dependent transition free energy to calculate the fraction of helix and coil base pairs as a function of the force, using Bragg-Zimm theory (Zimm and Bragg, 1959). This allows calculation of the complete DNA stretching profile, which exactly superimposes on the measured $f - x$ curve with a plausible choice of the cooperativity parameter σ. Then, in "Cooperativity of melting and the overstretch transition," we interpret σ in terms of the standard theory of DNA melting, which involves considerations of boundary free energies, DNA sequence heterogeneity, and loop entropy. We show that the observed width of the overstretching transition agrees reasonably well with expectations from a melting transition. In the section on "Hysteresis and kinetic effects," we discuss the implications of one-dimensional phase transition theory, and of hysteresis and kinetic effects, for interpretation of the overstretching transition. Finally, in the Discussion, we discuss our results and the potential relevance of S-DNA. We note that the B-DNA to S-DNA transition might well occur if DNA is complexed with a protein that rigidly fixes the overstretched conformation, thereby reducing the entropy gain that drives the transition to the coil state.

In the accompanying paper (Rouzina and Bloomfield, 2001), we discuss the application of these ideas to overstretching behavior as a function of solution conditions, including variations in temperature, pH, and ionic strength. All of these factors affect the melting behavior of DNA, and thereby modify its force-extension behavior in a way that can be theoretically predicted and compared with experiment. These results provide additional support for the proposition that the overstretching transition in DNA is a force-induced melting transition.

THERMODYNAMICS OF FORCE-INDUCED B-DNA MELTING

Free energy of a macromolecule subject to an applied force

In this section we show how to use the experimental stretching curves in Fig. 1 for dsDNA and ssDNA to characterize the effect of the applied force f on the relative stability of these two forms. All energies and lengths are calculated per base pair, so $b(f)$ is the average equilibrium projection of a base pair in the direction of the applied force, equal to the end-to-end extension of the molecule divided by its number of base pairs.

Two potentials can be used to describe the state of a macromolecule stretched by an applied force. One is the free energy at a given extension, equal to the work done by the force in stretching the molecule to the fixed length b:

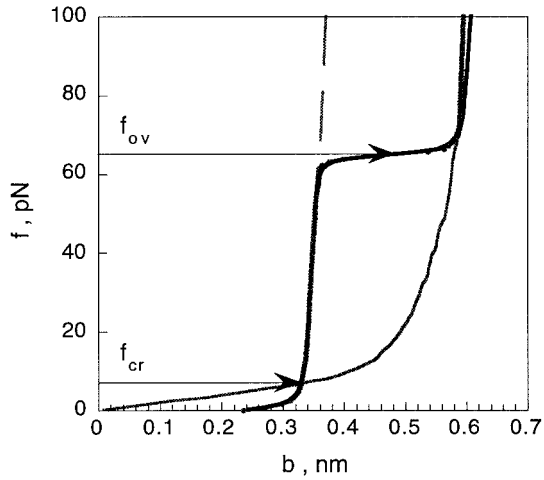

FIGURE 1 Force as a function of extension per base pair for the single-stranded, (*thin solid line*) and double-stranded (*dashed line*) DNA. The latter was obtained as an extension of the experimental dsDNA stretching curve, assuming no overstretching transition, according to slightly extensible WLC theory. The *bold solid line* is the DNA stretching curve, assuming force-induced melting, calculated according to Eq. 3 with $\Delta\Phi(f)$ obtained by numerical integration of the ssDNA and dsDNA stretching curves according to Eq. 4. The parameters are $\Delta G^0 = 2.3\ k_B T_r = 1.36$ kcal/mol and $\sigma = 8 \times 10^{-4}$. The arrows mark two characteristic forces: the crossover force, f_{cr}, at which ssDNA and dsDNA have equal extension, and f_{ov}, at which the overstretching transition occurs. Data from Smith et al. (1996) on λ-DNA, taken at pH 8, 150 mM NaCl, room temperature.

$$F(b) = \int_0^b f(b')db'. \tag{1}$$

This is analogous to the Helmholtz free energy if one substitutes V for b and P for $-f$. $F(b)$ is always positive and equal to the area under the equilibrium $f(b)$ curve (Fig. 2).

The other thermodynamic potential is analogous to the Gibbs free energy and is related to F by

$$\Phi(f) = F(f) - f \cdot b(f)$$

$$= \int_0^{b(f)} f(b')db' - f \cdot b(f) = -\int_0^f b(f')df'. \tag{2}$$

The $-fb(f)$ term is the negative potential energy in the external field f (Fixman and Kovac, 1973). The third equality in Eq. 2 expresses the free energy as an integral over the force rather than the extension. $\Phi(f)$ can be graphically presented as a negative of the area under the $b(f)$ curve, or above the $f(b)$ curve, as in Fig. 2. The equalities 1 and 2 can also be written in differential form as $\delta F = f\delta b$ and $\delta\Phi = -b\delta f$.

Minimization of the appropriate thermodynamic potential $F(b, f)$ or $\Phi(b, f)$ yields the equilibrium stretching profile. For different experimental setups, one or the other of these potentials will be more suitable. A nanomechanical device such as an optical tweezers or AFM usually controls the molecular end-to-end extension and measures the average force. Control of the applied force through a feedback loop by adjusting the extension is also possible (Wuite et al., 2000). But even when the instrument fixes the end-to-end extension, different parts of the molecule can

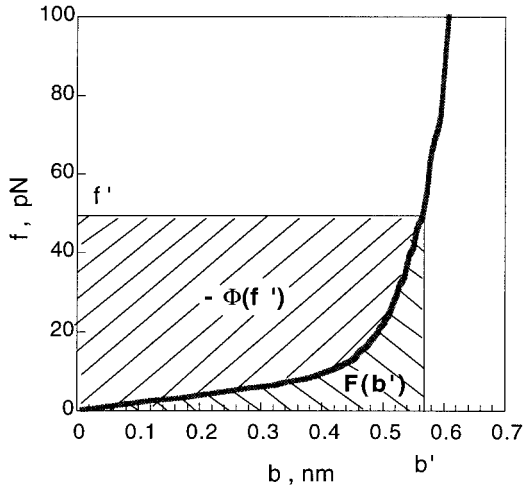

FIGURE 2 Two kinds of thermodynamic free energies characterizing the molecule under tension—the Helmholtz free energy, $F(b)$, and the Gibbs free energy, $\Phi(f)$—calculated as integrals of the equilibrium stretching curve, $f(b)$.

deform independently from each other, so that only the total extension is fixed, while the extension of any particular part of the molecule can fluctuate. Such a situation occurs, for example, when multiple weak bonds in the molecule gradually yield, or when a polymer with conformational flexibility is stretched. Such is true of DNA undergoing the overstretching transition at a defined force. The fixed molecular extension in this case is a weighted average of its extensions in the two possible states $b_1(f)$ and $b_2(f)$:

$$b(f) = \Theta(f)b_1(f) + [1 - \Theta(f)]b_2(f). \quad (3)$$

Here $\Theta(f)$ and $1 - \Theta(f)$ are the fractions of base pairs in the first and second states (e.g., helix and coil, or B and S). Thus, Θ is an internal degree of freedom, which allows the molecule to extend gradually at constant force along its length, while high cooperativity of the transition is ensured by the large boundary energy between the two states.

Formally, minimization of $F(b)$ with respect to internal degrees of freedom of a molecule under the constraint of fixed end-to-end extension $b(f)$ is equivalent to minimization of $\Phi(f)$, if force is used as a Lagrange multiplier to extension, Eq. 2. In other words, $\Phi(f)$ is an appropriate thermodynamic potential of the molecule in the case of constant force. Minimization of $\Phi(f)$ for the freely jointed chain and wormlike chain have been performed analytically, and explicit expressions for $f(b)$ obtained (Birshtein and Ptitsyn, 1966; Fixman and Kovac, 1973; Marko and Siggia, 1995).

Thus, in DNA stretching experiments the force, rather than extension, is the macroscopic variable under experimental control and defined throughout the molecule. Therefore, the phase transition between the two states occurs when their Gibbs free energies become equal at the particular transition force $\Phi_1(f_{ov}) = \Phi_2(f_{ov})$. Such a transition is characterized by abrupt molecular elongation.

In contrast to the above scenario of gradual molecular stretching, there can arise a situation in which all of the soft degrees of freedom in the molecule are pulled out. Further extension can proceed only by abrupt yielding of the structure. Extension of the molecule at the transition point then is a definite, nonfluctuating quantity. The transition itself is determined by equality of the Helmholtz free energies of the two phases at the particular extension b^*, when $F_1(b^*, f_1) = F_2(b^*, f_2)$. The molecular stretching curve in this case displays an abrupt drop in force at b^*. Such jagged force-versus-extension curves are typical of many molecules and often depend on the pulling rate, since most structures seem inextensible only on a certain time scale.

Force-dependent contribution to the transition free energy

According to Eq. 2, the state of the polymer with the larger end-to-end extension will experience a greater reduction in Gibbs free energy and will be preferentially stabilized at any given force. The corresponding contribution to the transition free energy between the two states can be calculated as

$$\Delta\Phi(f) = \Phi_2 - \Phi_1 = -\int_0^f [b_2(f') - b_1(f')]df'. \quad (4)$$

We can obtain analytical estimates of $\Delta\Phi(f)$ from the equation for the extensible worm-like chain (WLC) model (Marko and Siggia, 1995; Smith et al., 1996):

$$\tilde{f} = \frac{1}{4}\left[\frac{1}{(1 + f/K - \tilde{b})^2} - 1\right] + \tilde{b}. \quad (5)$$

Here $\tilde{f} = fA/k_BT$, $\tilde{b} = b(f)/b^{max}$ is the relative extension of the molecule in the direction of the applied force, b^{max} is the length per bp, i.e., the contour length of the molecule divided by the number of bp, A is the persistence length, and K is the elastic modulus allowing for the linear extension of the molecule beyond its contour length. K includes the effects of the molecular elasticity of the nonentropic nature, which can become important at high forces. Eq. 5 is an interpolation formula between the two analytical limiting cases of low $\tilde{f} \ll 1$,

$$\tilde{b} = \tfrac{2}{3}\tilde{f}, \quad (6)$$

and high $\tilde{f} \gg 1$

$$\tilde{b} = 1 - \frac{1}{(4\tilde{f})^{1/2}} + \frac{f}{K}. \quad (7)$$

The best-fit parameters for the B-DNA double helix are $b_{ds}^{max} = 0.34$ nm, $A_{ds} = 50 \pm 5$ nm, and $K_{ds} = 1000 \pm 100$ pN. The change from low to high force behavior should occur at $\tilde{f} \approx 1$, i.e., at $f \approx k_BT/A = 0.08$ pN. The fit to the WLC model for ssDNA is not quite as good, but in high salt, 150 mM NaCl, the parameters $b_{ss}^{maxWLC} = 0.61$ nm, $A_{ss}^{WLC} = 1.05$ nm, and $K_{ss}^{WLC} = 1000 \pm 100$ pN represent the data fairly well.

A slightly better fit for ssDNA can be obtained with the FJC model

$$\tilde{b} = \left[\coth(2\tilde{f}) - \frac{1}{2\tilde{f}}\right]\left[1 + \frac{f}{K}\right], \quad (8)$$

which in the low and high force limits gives

$$\tilde{b} = \tilde{f} \quad (9)$$

and

$$\tilde{b} = 1 - \frac{1}{2\tilde{f}} + \frac{f}{K}, \quad (10)$$

respectively. For the FJC model the best-fit parameters are $b_{ss}^{max} = 0.58$ nm, $A_{ss} = 0.7$ nm, and $K_{ss} = 900 \pm 100$ pN. The value of $A_{ss}^{WLC}/A_{ss}^{FJC} = 1.05/0.7 = 1.5$, as expected from the low force expansions in the two models, Eqs. 6 and 9. The value of K comes from the AFM study of Gaub (Rief et al., 1999), in which forces up to about 800 pN were applied. The

transition between low and high forces comes at about 6 pN. Note from Fig. 1 that dsDNA is already well into the high force regime at this point.

Single-stranded DNA is probably neither purely WLC nor FJC, but rather has some intermediate rotational-isomeric flexibility (Birshtein and Ptitsyn, 1966; Grosberg and Khokhlov, 1994). In addition, its persistence length increases strongly in low salt (Smith et al., 1996; Tinland et al., 1997). In this paper we consider high salt behavior, but in the accompanying one (Rouzina and Bloomfield, 2000) we consider effects of ionic strength variation.

Diffusion measurements on ssDNA in high salt (Tinland et al., 1997) yield $A_{ss}^{FJC} = 0.8$ if b_{ss}^{max} is fixed at 0.43 nm. The latter estimate is in reasonable agreement with A_{ss}^{FJC} determination from ssDNA stretching curves (Rief et al., 1999; Smith et al., 1996). However, this persistence length is significantly lower than the high salt value of 1.5 to 2 nm measured by transient electric birefringence (Mills et al., 1999) for unstacked poly(dT) ssDNA with the rise per base pair $b_{ss}^{max} = 0.5 - 0.7$ nm. Poly(dA) ssDNA stacked at 4°C has even higher $A_{ss}^{FJC} = 5.2$ nm and $b_{ss}^{max} = 0.32$ nm. This strong disagreement in A_{ss}^{FJC} values most likely is due to a significant number of hairpins that form in the long natural ssDNA at low forces. Pulling out a DNA hairpin requires up to 10–15 pN force according to recent studies (Essevaz-Roulet et al., 1997a, 1997b; Rief et al., 1999); see "Unzipping the double helix" section below. This force should strongly depend on DNA sequence and solution conditions. Indeed in a recent study (Maier et al., 2000) it was shown that ssDNA with the higher GC content required a few pN stronger stretching force at $f \leq 10$ pN.

Therefore the FJC is not a very good physical model for natural ssDNA stretching at low forces. The fitted value of A_{ss}^{FJC} should underestimate the actual ssDNA persistence length. Also it seems that the FJC model becomes inapplicable at the very high forces 100 pN $< f <$ 800 pN studied experimentally with AFM by Gaub et al. (Clausen-Schaumann et al., 2000; Rief et al., 1999), when ssDNA extends with significant bond deformation.

Despite the physical inadequacy of these simple models, we can in practice use either Eq. 5 or Eq. 8 to analytically represent the measured ssDNA stretching curve in the range of interest $f \leq 100$ pN. We calculate the Gibbs free energy of a chain from Eq. 2 and the low and high force limiting expressions for $f(b)$ from the WLC Eqs. 6 and 7:

$$\Phi^{WLC}(f) = -\int_0^f b(f')df' = -\frac{k_B T}{3}\frac{b^{max}}{A}\cdot\tilde{f}^2, \quad (11)$$

$$\Phi^{WLC}(f) = -b^{max}f + k_B T\cdot\frac{b^{max}}{A}\cdot\tilde{f}^{1/2} - \frac{1}{2K}b^{max}\cdot f^2, \quad (12)$$

and from the FJC Eqs. 9 and 10:

$$\Phi^{FJC}(f) = -\frac{k_B T}{2}\frac{b^{max}}{A}\cdot\tilde{f}^2, \quad (13)$$

$$\Phi^{FJC}(f) = -b^{max}f + \frac{k_B T}{2}\cdot\frac{b^{max}}{A}\cdot\ln(\tilde{f}) - \frac{1}{2K}b^{max}\cdot f^2. \quad (14)$$

Therefore, the force-dependent difference in Gibbs free energy of the two states in the low force limit is

$$\Delta\Phi = \Phi_{ss} - \Phi_{ds} = -\frac{f^2}{3k_B T}\cdot(b_{ss}^{max}A_{ss} - b_{ds}^{max}A_{ds}) \quad (15)$$

for the WLC model. (The Gibbs free energy difference using FJC parameters is 3/2 this value.) In this regime $\Delta\Phi > 0$, i.e., the double helix is stabilized by a small applied force, because the ssDNA extension is smaller than that of dsDNA. That is, $b_{ss}(f) - b_{ds}(f) = (f/k_B T)(b_{ss}A_{ss} - b_{ds}A_{ds}) <$ 0, due to the much shorter ssDNA persistence length, $A_{ss}/A_{ds} \approx 0.015$, despite its longer contour length, $b_{ss}/b_{ds} \approx 1.7$. The phenomenon of the shortening of DNA and other polymers upon melting with application of a weak force has been observed experimentally (Rupprecht et al., 1994, and early references in Birshtein and Ptitsyn, 1966), and considered theoretically (Birshtein and Ptitsyn, 1966; Buhot and Halperin, 2000).

In contrast, it follows from Eqs. 7 and 10 that at high forces ($f \gg 7$) pN, the DNA duplex is destabilized by the amount $\Delta\Phi \approx -(b_{ss}^{max} - b_{ds}^{max})f +$ const, in accord with our numerical results presented below. Thus the application of a weak stretching force causes a maximum in the relative stability of dsDNA (Fig. 3 *a*).

Simple analytical results are obtained only at low and high forces. In Fig. 3 *a*, we present $\Delta\Phi(f)$ for the entire range of forces, calculated numerically according to Eq. 4 from the experimental stretching curves $f(b)$ for dsDNA and ssDNA in Fig. 1.

Consider first the case in which only one of the two ssDNA strands remains intact and able to exert tension (*solid line* in Fig. 3 *a*). One can see that forces ≤15 pN stabilize dsDNA relative to ssDNA. This stabilization

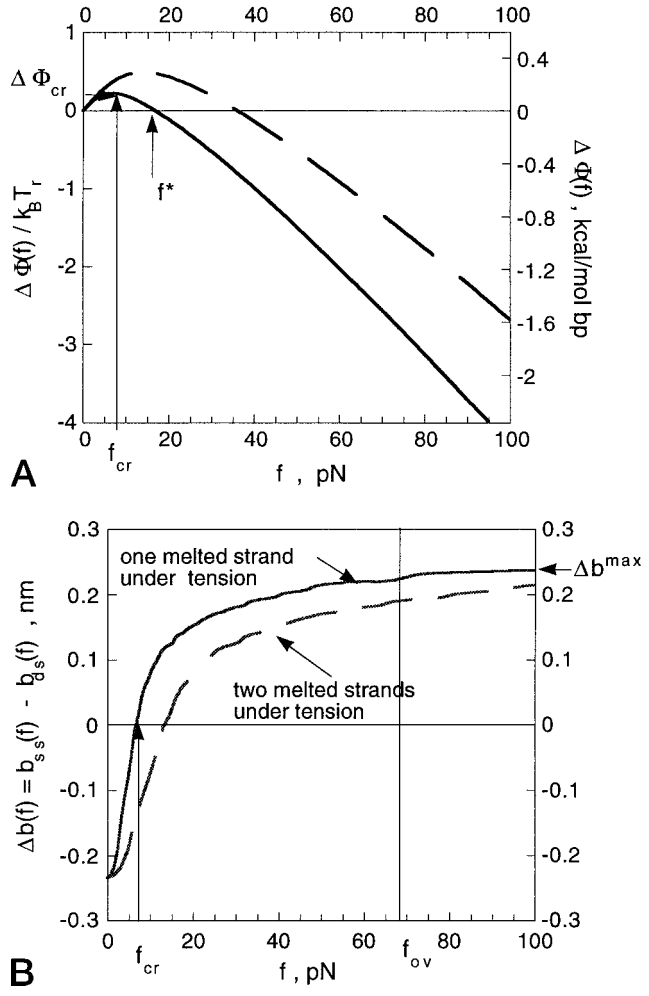

FIGURE 3 (*a*) Force-dependent contribution, $\Delta\Phi(f)$, to the Gibbs free energy of DNA melting, obtained by numerical integration of dsDNA and ssDNA stretching curves according to Eq. 4. *Solid* and *dashed lines* correspond to DNA melting into a state with one or two single strands under tension, respectively. The crossover forces and corresponding maxima in transition free energy are marked by arrows. (*b*) Experimental elongation per base pair $\Delta b(f) = b_{ss}(f) - b_{ds}(f)$ as a function of applied force. Maximum elongation $\Delta b^{max} = \Delta b(f \to \infty)$ corresponding to completely stretched dsDNA and ssDNA is marked by an arrow. Elongation at the transition force $\Delta b^0 = \Delta b(f_{ov}) = 0.22$ nm.

is moderate and reaches its maximum $\Delta\Phi(f_{cr}) \approx 0.23\ k_BT = 0.14$ kcal/mol at $f_{cr} \approx 7$ pN and $T = 295$ K. At this force the derivative $\partial\Delta\Phi/\partial f = -\Delta b(f) = -[b_{ss}(f) - b_{ds}(f)]$ changes its sign, i.e., ssDNA becomes longer than dsDNA, as seen in Fig. 3 *b*. The crossover force $f_{ov}(\Delta b = 0)$ is an important parameter, which lies between the two characteristic forces for ds- and ssDNA, i.e., $k_BT/A_{ds} < f < k_BT/A_{ss}$. As discussed above, A_{ss} has a meaning of an apparent persistence length, which can include the effect of the hairpin formation in ssDNA at low forces. Since flexibility of both forms of DNA depend on solution conditions such as salt, temperature, pH, etc., specific value of f_{ov}, as well as maximum dsDNA stabilization by force, $\Delta\Phi(f_{cr})$, should also vary with these parameters.

At higher forces $\Delta b(f) > 0$ and saturates (see Figure 3 *b*). In the range of forces where the transition happens, 60–80 pN, Δb is almost constant with a value $\Delta b_0 \approx 0.22$ nm. Therefore, $\Delta\Phi$ becomes a linearly decaying function of *f*,

$$\Delta\Phi(f) \approx -\Delta b_0(f - f^*) = 0.8k_BT - \Delta b_0 f. \qquad (16)$$

Eq. 16 is convenient for the estimates of the force-induced destabilization of dsDNA relative to ssDNA. However, in our calculations of the complete DNA overstretching curves below, we will use the exact form of $\Delta\Phi(f)$, given by Eq. 4 with numerically integrated experimental $b_{ds}(f)$ and $b_{ss}(f)$.

Stretching of one or two melted strands

So far we have considered stretching just one single strand in the melted state of DNA, but two single strands are produced upon dsDNA melting. Here we should note that in this paper we consider only stretching of the torsionally unconstrained DNA, in which the two strands can freely rotate around each other. Such a situation is realized when at least one single-stranded end of the DNA is unattached, or DNA has at least one single-stranded nick. In the regular optical tweezer experiments DNA remains torsionally unconstrained even if there are no free ends or nicks in it, because DNA is attached to the polystyrene beads, which are free to rotate within the laser trap.

For the torsionally unconstrained DNA, two qualitatively different situations are possible. First, melting can proceed from the free end of one strand or a nick, so that only one strand is under tension while the other is relaxed. Second, the melted fragment can nucleate to form an interior ssDNA region, so that both melted strands are under tension. In actual stretching experiments with polymeric DNA, both types of melted regions should coexist. Therefore, a significant fraction of melted DNA exists in a state of the first type, which has a force-dependent free energy of single strand stretching and leads to $\Delta\Phi(f)$ given by the solid line in Fig. 3 *a*.

On the other hand, in melted regions of the second type, the force to stretch two parallel single strands should be twice the force needed to extend one single strand to the same extension, i.e., $f_{2ss}(b) = 2f_{ss}(b)$. Therefore, the force-dependent part of free energy for the melted state can be found from the experimental stretching curve of the single DNA stand as follows:

$$\Phi_{2ss}(f) = \int_0^{b_{ss}(f/2)} 2f(b')db' - fb_{ss}(f/2) = -2\int_0^{f/2} b_{ss}(f')df'. \qquad (17)$$

The dashed line in Fig. 3 *a* shows $\Delta\Phi(f) = \Phi_{ds}(f) - \Phi_{2ss}(f)$. This force-dependent contribution to melting free energy applies to the case of DNA with no free ends or nicks, but with the rotationally unconstrained attachment. Obviously the force-induced destabilization of the double helix is much weaker in this case, since it is harder to extend two single strands compared to one. As follows from Fig. 3 *a*, the DNA melting force f_{ov} in these two cases should differ by ~20 pN.

Since the force along the whole molecule is everywhere the same, while the extensions are additive, $\Delta\Phi(f)$ for the whole molecule with both types of melted regions in it will be a weighted average. In other words, the actual force-dependent contribution to the transition free energy will lie between the two limiting cases given by the solid and dashed lines in Fig. 3 *a*. Some information on the actual configuration of the two melted strands in λ-DNA can be obtained from the salt dependence of the overstretching force, which will be discussed in the following paper (Rouzina and Bloomfield, 2001).

The alternative case of torsionally constrained DNA leads to DNA overstretching at much higher force, about 110 pN (Clausen-Schaumann et al., 2000; Leger et al., 1999; Marko, 1998). We believe that the overstretching transition in this case is still a force-induced melting. The ~45 pN increase in the overstretching force in this case has two causes. The first is that both melted DNA strands are under tension along their whole length. The second cause is even more significant: the melted strands of torsionally constrained DNA are at a huge entropic disadvantage compared to the torsionally unconstrained single strands. These issues will be treated in a separate publication.

Force dependence of WLC and FJC entropy

High force has only minor effects on dsDNA structure until it overstretches. But forces typical of the overstretching transition, $f \approx 65$ pN, may significantly reduce the entropy *S* of ssDNA. This is a major factor in understanding the energetics of DNA melting. In the intermediate range of forces 15 pN $\leq f \leq$ 100 pN when all hairpins are pulled out the entropic polymer model of ssDNA elasticity should adequately describe its physical nature. Then the analytical expressions for $\Phi(f)$ obtained above allow calculation of the effect of force on polymer entropy. At high forces for the WLC model one obtains

$$S^{WLC}(f) = -\frac{\partial\Phi^{WLC}}{\partial T} = -k_B(1 - \nu/2)\frac{b^{max}}{A}\tilde{f}^{1/2}. \qquad (18)$$

Here we used Eq. 12 and took into account that only its second term depends on temperature. In addition to the explicit dependence on $T^{1/2}$ this term contains the persistence length *A*, which behaves with *T* as (Grosberg and Khokhlov, 1994):

$$A(T) = \frac{\kappa}{k_BT} = A(T_r)\left(\frac{T_r}{T}\right)^{1-\nu}. \qquad (19)$$

Here $\kappa(T) = \kappa(T_r)\cdot(T/T_r)^{\nu}$ is the weakly *T*-dependent bending elasticity of the polymer in units of energy · length, and T_r is a reference temperature (~room temperature). The power $|\nu|$ is small, <1, so the dominant *T* dependence of *A* is ~1/*T*.

The FJC expression for $\Phi(f)$, Eq. 14 yields an only slightly different result for $S(f)$:

$$S^{FJC}(f) = -\frac{\partial\Phi^{FJC}}{\partial T} = -\frac{k_B}{2}(1 - \nu/2)\frac{b^{max}}{A}\ln(\tilde{f}). \qquad (20)$$

Eqs. 18 and 20 give the force-induced entropy reduction per base pair (or base) of a WLC or FJC polymer. The reduction is A/b^{max}-fold larger per persistence length. Since at $f \approx 65$ pN, $\tilde{f}_{ss} = 11 \gg 1$, $S(f)A/b^{max} \approx -1.5\ k_B$; i.e., the backbone degrees of freedom in the WLC or FJC models for ssDNA are completely pulled out by this force.

For comparison, the conventional thermal melting transition in polymeric DNA at high salt has an entropy increase per base pair of:

$$\Delta S^0 = S^0_{ss} - S^0_{ds} = 25\,\frac{cal}{mol\cdot K} = 12.5\ k_B. \qquad (21)$$

This means that about $\exp(12.5) \approx 2.7 \cdot 10^5$ degrees of freedom are liberated upon melting of the single DNA base pair. This corresponds to about 6 independent rotations per nucleotide, or 12 per basepair, with ~3

preferred rotational-isomeric states for each bond (Cantor and Schimmel, 1980). On this scale, the force-induced entropy reduction of a single strand, $S^f_{ss} = -1.4\ k_B$, is minor, whereas for dsDNA it is essentially negligible: $S^f_{ds} = -0.2\ k_B$. The net effect of the overstretching force on the entropy of the DNA melting transition is therefore a reduction from $\Delta S^0 = 12.5\ k_B$ to $\Delta S = (12.5 - (1.4 - 0.2))k_B = 11.3\ k_B = 22.6$ cal/mol · K. This estimate gives the upper bound of the effect, since it uses the FJC model of ssDNA with the lowest estimated value of A_{ss} and assumes $\nu = 0$ in Eq. 20. This result allows us to consider force-induced DNA melting as essentially the same process as conventional thermal melting, to which the force introduces only a minor perturbation.

Prediction of the melting force

We have shown that high force destabilizes the DNA double helix relative to its single-stranded state. But can it really melt B-DNA? To answer this question we must estimate the force at which the absolute value of the destabilizing free energy $-\Delta\Phi(f)$ becomes equal to the free energy of melting transition without force $\Delta G^0 = G^0_{ss} - G^0_{ds}$:

$$\Delta\Phi(f_{ov}) = -\Delta G^0. \tag{22}$$

Here ΔG^0 should be taken from conventional thermal melting studies. Its value depends on the temperature and all other solution conditions, as well as on DNA composition. In most cases it is not ΔG^0, but rather the melting temperature T_m, which is known experimentally. The dependence of T_m on solution ionic strength I and average base composition $x_{GC} = 1 - x_{AT}$ has been summarized as (Blake and Delcourt, 1998):

$$T_m = 360.31 + 34.47x_{GC} + (20.15 - 6.52x_{GC})\log(I). \tag{23}$$

For a DNA stretching experiment performed at $I = 150$ mM with λ-DNA, $x_{GC} = 0.5$, Eq. 23 predicts $T_m = 360$K $= 87$°C.

The conventional way to estimate ΔG^0 at temperature T from the known T_m is to use the expression

$$\Delta G^0 = \Delta H - T\Delta S = \Delta S(T_m - T) \tag{24}$$

where $T_m = \Delta H/\Delta S$. Using the measured entropy value of $\Delta S = 25$ cal/mol · K at 150 mM salt, we obtain $\Delta G^0 = 1.7$ kcal/mol $= 2.9\ k_B T_r$ at room temperature $T_r = 293$K. From Fig. 3 *a* we find $-\Delta\Phi(f_{ov}) = \Delta G^0 = 2.9\ k_B T_r$ at forces between 80 and 100 pN depending on the state of ssDNA. This is close to, but somewhat higher than, the measured transition force of 65 pN. If we accept that dsDNA melts at $f_{ov} = 65$ pN, the $\Delta\Phi(f)$ function of Fig. 3 *a* can be used to estimate that ΔG^0 lies between $1.1\ k_B T_r$ and $2.3\ k_B T_r$ depending on the state of ssDNA. Again, these numbers are somewhat lower than our estimate according to Eq. 24. We will later discuss the temperature dependence of dsDNA stability in more detail, and show that this is a real effect related to the recently discovered (Chalikian et al., 1999; Holbrook et al., 1999; Rouzina and Bloomfield, 1999a) large positive heat capacity of DNA melting, which leads to a nonlinear dependence of $\Delta G^0(T)$ on T.

This close prediction of the melting force does not by itself prove that the overstretching transition in B-DNA is a melting phenomenon. However, it implies that given enough time for local equilibrium between dsDNA and ssDNA to set in, such forces would necessarily induce DNA melting.

APPLICATION OF HELIX-COIL TRANSITION THEORY

Theory for a single-stranded homopolymer

Using our conclusion that the function $G(f) = \Phi(f) + G^0$ is a proper thermodynamic potential for a molecule under tension, we can introduce the force-dependent free energy of the DNA melting transition:

$$\Delta G(f) = G_{ss}(f) - G_{ds}(f) = \Delta\Phi(f) + \Delta G^0 = \Delta\Phi - \Delta\Phi(f_{ov}), \tag{25}$$

which can be used for a complete description of melting within the conventional Ising model theory (Grosberg and Khokhlov, 1994; Vedenov et al., 1972; Zimm and Bragg, 1959). In its simplest form, strictly applicable only to single-stranded homopolymers, the theory involves only two parameters,

$$s = \exp\left(\frac{\Delta G(f)}{k_B T}\right) \text{ and } \sigma = \exp\left(-\frac{2\Delta G_s}{k_B T}\right). \tag{26}$$

Here s is the equilibrium constant for converting a helical residue to a coil residue at the end of a helical segment, and σ is the cooperativity parameter determined by the extra free energy of the two coil boundaries flanking a helical region, $2\Delta G_S$. (We use this definition of σ, following Grosberg and Khokhlov (1994), rather than the definition by Zimm and Bragg (1959), which involves only one junction.)

The fraction of base pairs which remain in the helical state at force f then is (Grosberg and Khokhlov, 1994):

$$\Theta(f) = \frac{1}{2} + \frac{(s(f) - 1)}{2((s(f) - 1)^2 + 4s(f)\sigma)^{1/2}} \tag{27}$$

where the dependence of s on f is given by Eqs. 25 and 26.

Figure 1 shows the extension per base pair $b(f)$ calculated according to Eq. 3. Here $\Theta(f)$ was obtained with the experimental $\Delta G(f)$, assuming a transition to one single strand under tension. The calculated $b(f)$ for $f \le f_{ov}$ is indistinguishable from the experimental stretching curve if $\sigma = 8 \times 10^{-4}$. This value of σ reflects the small width of the overstretching transition in terms of the force:

$$\delta f = \left(\frac{\partial f}{\partial \Theta}\right)_{s=1} = \left(\frac{\partial s}{\partial \Theta}\right)_{s=1}\left(\frac{\partial f}{\partial s}\right)_{s=1} = 4\sigma^{1/2} \cdot \frac{k_B T}{\Delta b}, \tag{28}$$

which for $\sigma = 8 \times 10^{-4}$ and $\Delta b(f = 65\text{ pN}) = 0.22$ nm is $\delta f = 2$ pN. Although small, our value of σ is still almost ten times larger than the value typical of homopolymer melting, $\sigma \approx 10^{-4}$, which according to Eq. 26 would correspond to a transition width of $\delta f = 0.7$ pN. This is not surprising, since λ-DNA is not a homopolymer.

In the next section we will discuss the two main factors determining the width of the force-dependent melting transition: DNA sequence heterogeneity and the loop factor for the double helix. We will show that the observed δf can be reasonably understood in terms of these conventional DNA melting factors.

Integrity of nicked DNA under stretching

The main argument against interpretation of the overstretching transition as force-induced melting has been that if

strand separation occurs at forces $\approx f_{ov}$, and if each of the single strands has several nicks, as is generally the case, then overstretched DNA should break at forces $\gtrsim f_{ov}$. We believe that there are two answers to this argument. For forces $\gtrsim$100 pN, much higher than the transition force, the endurance of the double-stranded DNA is kinetic (Clausen-Schaumann et al., 2000; Rief et al., 1999). This will be discussed in a forthcoming paper (Rouzina and Bloomfield, in preparation). However, even in thermodynamic equilibrium melted DNA should be able to sustain forces significantly higher than f_{ov}, because of the one-dimensional nature of DNA melting.

In a true phase transition, the two phases tend to completely separate at the transition point. The more stable phase occupies the whole sample, while the less stable phase can only exist as a metastable nucleus. By contrast, in a one-dimensional system the two phases do not separate but instead remain mixed (Grosberg and Khokhlov, 1994; Landau and Lifshitz, 1988), since the energy of the boundary does not depend on the extent of the phase in one dimension. Thus, at any point in the transition there are an equilibration number of boundaries, defining the average sizes of the melted, k_{ss}, and helical, k_{ds}, regions (Buhot and Halperin, 2000; Grosberg and Khokhlov, 1994):

$$k_{ds} = \frac{1 + s + ((s-1)^2 + 4s\sigma)^{1/2}}{1 - s + ((s-1)^2 + 4s\sigma)^{1/2}} \tag{29}$$

$$k_{ss} = \frac{1 + s + ((s-1)^2 + 4s\sigma)^{1/2}}{s - 1 + ((s-1)^2 + 4s\sigma)^{1/2}}. \tag{30}$$

The length $k = k_{ds} + k_{ss}$ containing two boundaries has a minimum $k_{min} = 1 + \sigma^{-1/2}$ at the transition midpoint $s = 1$. For $\sigma = 10^{-4}$, $k_{min} \approx 100$.

In Fig. 4 we plot k_{ss} and k_{ds} as functions of applied force,

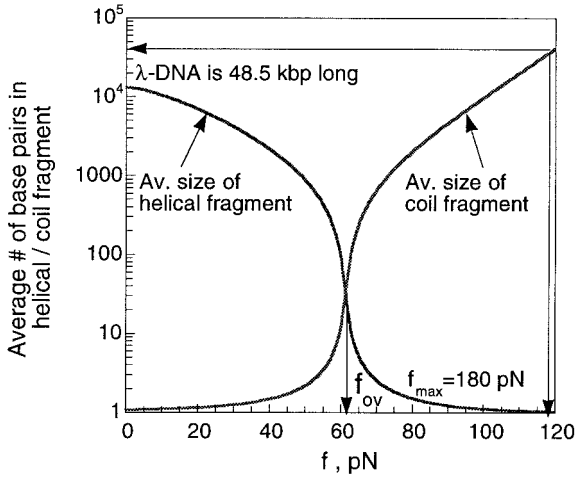

FIGURE 4 Average size of a ss- or dsDNA domain as a function of applied force calculated according to Eqs. 29 and 30 with $\Delta G(f)$ according to Eqs. 25 and 4 and $\sigma = 8 \times 10^{-4}$.

with $s(f)$ obtained as described above from the experimental stretching curves with the fitted value $\sigma = 8 \times 10^{-4}$. The two strands of DNA will not completely separate until k_{ss} becomes equal to the length of DNA under tension, or the distance between two nicks in the same strand. According to Fig. 4, a 10,000-bp-long DNA without nicks should be able to withstand forces up to ~100 pN, whereas a 1000-bp fragment can withstand up to ~80 pN. Both are much higher than $f_{ov} = 65$ pN.

Thus standard helix-coil transition theory predicts strong dependence of the DNA breaking point on DNA length and nicking. This is routinely observed in experiments with λ-DNA. In many cases the two DNA strands either permanently unbind or lose a piece of single strand, leading to permanent alteration of the stretching behavior (Baumann et al., 1997; Smith et al., 1996; Williams et al., 2000a, 2000b), which is different for each DNA molecule. This interpretation will not be qualitatively changed by refinements such as heteropolymer composition and loop statistics (Fixman and Freire, 1977; Grosberg and Khokhlov, 1994).

COOPERATIVITY OF MELTING AND THE OVERSTRETCH TRANSITION

Effects of compositional heterogeneity

To obtain a more realistic fit to the force-induced melting transition in λ-DNA, we must take account of its compositional heterogeneity. The theory of melting of long random heteropolymers (Grosberg and Khokhlov, 1994; Vedenov et al., 1972) gives, for the melting temperature,

$$T_x = x_{GC}T_{GC} + (1 - x_{GC})T_{AT}, \tag{31}$$

and, for the width of the thermal melting transition,

$$\delta T_{hetero} \approx 2\frac{(T_{GC} - T_{AT})^2 \Delta S \cdot x_{GC}(1 - x_{GC})}{\Delta G_S}, \tag{32}$$

which for λ-DNA evaluates to $\delta T_{hetero} = 6$ K with the high-salt homopolymer value of $\sigma = 10^{-5}$ (Kozyavkin et al. (1987) and references cited therein).

This estimated δT_{hetero} value is much smaller than the difference between the melting temperatures of pure AT and GC DNAs, ($T_{GC} - T_{AT} = 40$ K at $I = 0.15$ M according to Eq. 23), because the difference in transition free energy of the two types of base pairs $(T_{GC} - T_{AT})\Delta S \approx 1.7\ k_B T_r$ is smaller than the boundary free energy, $\Delta G_s = -\frac{1}{2} k_B T_r \ln(10^{-5}) = 5.7\ k_B T_r$. However, it is much larger than δT_{homo} for the homopolymer with the same cooperativity parameter:

$$\delta T_{homo} = 4\sigma^{1/2}\frac{k_B T_m}{\Delta S}, \tag{33}$$

which for $\sigma = 10^{-5}$ is just 0.3 K.

Because of the additivity of $\Delta G^0(x_{GC})$ within the force-dependent transition free energy $\Delta G(f)$, and the linearity of

$\Delta G(f)$ with force at the high forces typical of the overstretching transition, this theory can be directly translated into the language of force-induced melting. The melting force as a function of composition for a long random DNA molecule should be

$$f_x = x_{GC} f_{GC} + (1 - x_{GC}) f_{AT}. \quad (34)$$

The variations of duplex stability on the temperature and force scales are related by a Clausius-Clapeyron relationship, $\delta G = \delta T \cdot \Delta S^0 = \delta f \cdot \Delta b(f)$, so

$$\delta f = \delta T \frac{\Delta S^0}{\Delta b} = 0.8 \frac{\text{pN}}{\text{K}} \cdot \delta T. \quad (35)$$

The factor 0.8 comes from $\Delta S^0 = 25$ cal/mol-K and $\Delta b = 0.22$ nm, after conversion from calories to Joules. This yields $f_{GC} - f_{AT} = 0.8(T_{GC} - T_{AT}) = 32$ pN, in very good agreement with the value measured by AFM of $f_{GC} - f_{AT} = 65 - 35 = 30$ pN (Clausen-Schaumann et al., 2000; Rief et al., 1999).

We note and explain an apparent inconsistency here: Gaub and coauthors (Clausen-Schaumann et al., 2000; Rief et al., 1999) measured $f_{ov} \approx 65$ pN for λ-DNA, in agreement with other determinations of f_{ov}. Equation 34 then predicts that $f_{GC} = 80$ pN and $f_{AT} = 50$ pN, rather than the measured values $f_{GC} = 65$ pN and $f_{AT} = 35$ pN (Clausen-Schaumann et al., 2000; Rief et al., 1999). The apparently lower stability of the synthetic DNAs can be attributed to the fact that both polynucleotides are self-complementary, so that the single strands can form hairpin structures. So the DNA duplex may well have melted not into two single strands, but rather into one single strand under tension, and another relaxed strand in the form of a hairpin. Since a hairpin has a much lower energy than a single strand, ΔG^0 should be significantly smaller than for regular melting. The shift-down of the melting force by 15 pN would correspond to the lowering of the double helix stability by about 0.8 $k_B T_r$, which is almost half of the average stability of a base pair without hairpin formation, $\sim 2\ k_B T_r$.

The width of the heteropolymer overstretching transition can be calculated analogously to Eq. 32:

$$\delta f_{hetero} = \frac{(f_{GC} - f_{AT})^2 x_{GC}(1 - x_{GC}) \Delta b}{\Delta G_S}, \quad (36)$$

which for $x_{GC} = 0.5$ evaluates to 4.8 pN. As expected, this value is much larger than the width of the homopolymer transition width $\delta f_{homo} = 0.24$ pN with the same cooperativity parameter $\sigma = 10^{-5}$ calculated according to Eq. 35.

In Fig. 5 we plot two derivative curves from representative dsDNA stretching experiments. The solid line is the derivative of the smoothed stretching curve from Smith et al. (1996) obtained at 150 mM NaCl, 10 mM Tris, and pH 8. The dashed line is the derivative of the original data by Williams et al. (2000b) taken at 250 mM NaCl, 1 mM cacodylate, and pH 6. The change in solution conditions resulted in a shift of the transition midpoint, but the apparent width of both $\partial b/\partial f$ peaks is 4 to 5 pN, in agreement with the simple estimate from Eq. 35 of $\delta f = 0.8\delta T = 0.8 \cdot 6 = 4.8$ pN, or equivalently from Eq. 36.

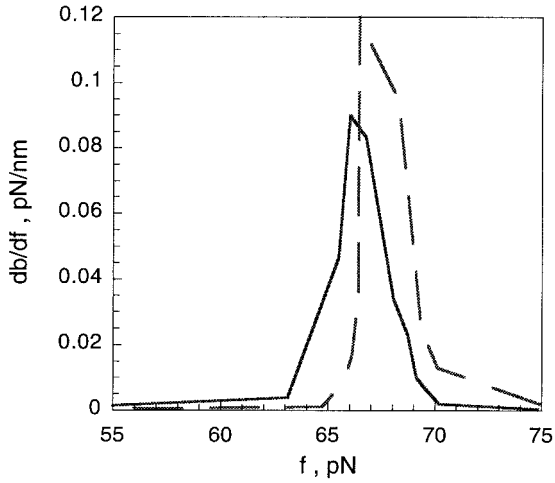

FIGURE 5 Smoothed derivative $\partial b/\partial f$ of typical experimental stretching data $f(b)$ taken under hysteresis-free conditions at 250 mM NaCl, pH 6.1. *Short dashed line*, stretching; *long dashed line*, relaxation. (Data courtesy of M. Williams and J. Wenner.)

The above δf value is larger than $\delta f = (\partial f/\partial \Theta)_{\Theta=1/2} \approx 2$ pN; i.e., the width is not fully determined by the slope of the overstretching plateau at the transition midpoint. This occurs for two physical reasons. The first is that the lengths of both ssDNA and dsDNA depend on applied force, which leads to transition broadening at the beginning and end of the plateau. The second and more important consideration is that the actual width of the peak cannot be described within the homopolymer model. Taking these two factors into account, we may conclude that the width of the overstretching transition in DNA is in general agreement with a force-induced melting model.

Unzipping the double helix

When the DNA double helix is opened like a zipper by pulling on the 3′- and 5′-strand termini at the same end of the molecule, i.e., in the direction perpendicular to the helix axis, the strand separation forces obtained from fitting the equilibrium $f - x$ curve for λ-DNA are $f_{\perp GC} = 15$ pN and $f_{\perp AT} = 10$ pN (Essevaz-Roulet et al., 1997a, 1997b). Experiments on poly(dG) · poly(dC) and poly(dA) · poly(dT) give fairly similar results: $f_{\perp GC} = 20$ pN and $f_{\perp AT} = 9$ pN (Clausen-Schaumann et al., 2000). These perpendicular forces are significantly lower than the parallel overstretching force of 65 pN, but they reflect the same equilibrium free energy change upon DNA melting: $\Delta G^0 \sim f_{\parallel} \cdot \Delta b_{\parallel} = f_{\perp} \cdot \Delta b_{\perp}$. The extension per base pair in the perpendicular geometry is about twice the length per base in a single

strand at the transition force of about 20 pN, i.e., $\Delta b_\perp \approx 2 \cdot 0.5$ nm $= 1$ nm. This is about 4.5 times longer than $\Delta b_\parallel \approx 0.22$ nm. The average perpendicular unbinding force should be lower than the parallel force by the same factor: $f_\perp = f_\parallel/4.5 = 65/4.5 = 14.5$ pN. The difference between AT and GC perpendicular unbinding forces should also be proportionally lower: $\sim$30 pN/4.5 = 7 pN. Both estimates are in reasonable agreement with experiment, supporting the idea that in both stretching experiments the same process of equilibrium DNA strand separation occurs.

Although the DNA melting force applied in parallel to DNA axis is well defined, the unzipping force $f_\perp$ strongly fluctuates through the DNA opening process (Essevaz-Roulet et al., 1997a, 1997b). This is easy to understand, remembering that the overstretching force is averaged over the whole DNA sequence to which it is applied according to Eq. 34, while the local value of $f_\perp$ reflects the composition of the next cooperatively opening end fragment of DNA.

Effects of loop entropy

The value of the cooperativity parameter, $\sigma \sim 10^{-5}$, used for our estimates in the previous subsection is an order of magnitude lower than the experimental homopolymer value $\sigma_{\text{homo}} = 10^{-4}$, and at the upper end of the measured $\sigma = 10^{-8} - 10^{-5}$ for double-stranded DNA melting (Kozyavkin et al., 1987; Lubchenko et al., 1976). The much lower value of the cooperativity parameter for melting of a double- or multi-stranded polymer, compared to σ for a single-stranded polymer, is known to arise from loop entropy effects (Grosberg and Khokhlov, 1994; Zimm, 1960). The low probability that the two ends of a loop, in which each strand contains N monomers (or persistence lengths), will meet each other reduces σ by a factor proportional to $N^{-3/2}$, which can amount to several orders of magnitude for long chains.

There is less reduction of σ by loop formation in stretched DNA, because the stretched single strands in the loop are constrained and have less entropy to lose when forming a loop. We have treated the effect of stretching on σ in detail in a separate paper (Rouzina and Bloomfield, in preparation). We find that for large forces, $(fA_{ss}/k_BT) \gg 1$,

$$\sigma = \sigma_0 N^{-3/2} (fA_{ss}/k_BT)^2, \tag{37}$$

where σ_0 is the value in the absence of both loop formation and stretching. Thus, stretching can partially counteract the sharpening of the transition by loop formation, leading to a breadth more characteristic of the single-stranded helix-coil transition.

This effect of the force-induced transition broadening will not be strong compared to thermal melting for stretching of DNA with free ends or nicks, since a significant fraction of all base pairs melt from the ends. But it will become quite important for broadening of the overstretching transition in torsionally constrained DNA.

HYSTERESIS AND KINETIC EFFECTS

Another argument in favor of force-induced melting comes from the frequent observation of hysteresis during stretching experiments. In all reported experiments the two strands reanneal during the relaxation phase of the stretch-release cycle. If the reannealing reaction is fast enough, the $f(b)$ curve on the way back is the same as on the way out. In this case the process is reversible, with no total energy change or net work done during the cycle. However, it is often observed experimentally that forces measured during release are lower than those during stretching. A plausible explanation is that the reannealing of the two strands is too slow to accommodate the decreasing molecular extension imposed by experiment. Typical rates of shortening are about 1 s per step of 1 to 1000 nm. This is a very long time on the molecular scale, making it unlikely that the observed hysteresis is related to a local structural reorganization, such as would be involved in an S-B transition. However, the renaturation of two single strands can easily take this long. All presently available experimental data show that every solution change which slows down recombination of DNA strands (low salt, high pH, and elevated temperature) also enhances hysteresis in DNA stretching.

In support of the force-induced melting hypothesis is the observation that hysteresis is essentially eliminated by crosslinking the two strands (Smith et al., 1996). Only very weak hysteresis was seen even at very low salt concentrations, $\sim$1 mM. Also, stretching of torsionally constrained DNA (Clausen-Schaumann et al., 2000; Leger et al., 1999; Marko, 1998) does not exhibit hysteresis, as should be the case if it is equivalent to the melting of closed circular DNA.

The connection of hysteresis with melting was mentioned by Bustamante and coworkers (Smith et al., 1996), but their picture was that stretching first produces a double-stranded S-DNA form, and that melting occurs only at higher extensions or longer times. This explanation, however, is inconsistent with the observation that hysteresis was seen in every stretch/release cycle in which DNA was overstretched, independent of the point at which the stretching was reversed. There is no basis in experiment to separate a putative first stage of the double-stranded B-to-S transition from a second stage of melting.

DISCUSSION

Overstretching as force-induced melting

We have shown that essentially all of the published phenomena associated with the overstretching transition can be

explained as force-induced melting. To recapitulate, the main arguments are:

1. When the experimental stretching curves $f(b)$ for dsDNA and ssDNA are used for calculating the free energy of DNA melting as a function of force, one obtains a transition force and stretching profile very similar to that observed experimentally.
2. The cooperativity of the overstretching transition is very high, in agreement with experimental and theoretical melting behavior.
3. Overstretched DNA in which the strands are largely melted is mechanically stable despite the presence of the free ends or single-stranded nicks. This happens due to the one-dimensional nature of DNA melting, which leads to the fact that the average length of the melted fragment is shorter than the average distance between two nicks in the same strand, even at forces significantly above the transition midpoint.
4. The DNA melting force in the perpendicular stretching geometry is lower than the melting force in the parallel stretching geometry by the same factor as the elongations in the two geometries differ.
5. The dependence of the overstretching transition on DNA base composition is consistent with a force-induced melting explanation.
6. The relaxation part of the DNA stretch/release cycle exhibits hysteresis whenever the DNA is stretched beyond its B-DNA contour length, but never before that. The farther into the overstretch transition, the more hysteresis is observed. There is no experimental basis for separating "initial B-S transition without hysteresis" from "subsequent melting." Hysteresis becomes progressively more prominent in lower salt, as would be expected for melting.

In related papers (Rouzina and Bloomfield, 2000; Williams et al., 2000a, 2000b) we show that f_{ov} follows changes in DNA stability as solution temperature, ionic strength, or pH are varied, in accord with a melting explanation of this transition.

Critique of modeling studies

The picture of force-induced DNA melting developed in this study contradicts the generally accepted point of view that the overstretched form of DNA is double-stranded S-DNA. The existence of an S-form was suggested in pioneering works of Bustamante and Lavery and their coworkers (Cluzel et al., 1996; Smith et al., 1996), and several detailed modeling studies of overstretched S-DNA have appeared within the last several years (Bertucat et al., 1999; Cluzel et al., 1996; Konrad and Bolonick, 1996; Kosikov et al., 1999; Lebrun and Lavery, 1996).

The two strands of the DNA double helix are held together by strong hydrogen, van der Waals, and electrostatic forces. Each basepair is bound by two (AT) or three (GC) hydrogen bonds corresponding to about 6 to 10 k_BT of binding enthalpy, and there is about 10 to 20 k_BT per bp of binding enthalpy due to base stacking. It would seem easier to stretch the double helix beyond its B-form contour length by retaining some of these bonds, i.e., by overstretching it into some other double-stranded form. Despite their differences, all of the modeling studies agree that it is possible to extend double-stranded DNA to about twice its B-form contour length, while maintaining most of the hydrogen bonding and giving up only about half of the base stacking interactions (Konrad and Bolonick, 1996; Kosikov et al., 1999; Lebrun and Lavery, 1996). Such deformation has an energy cost of about 10 to 20 k_BT per bp, which is less than half the cost to break all of the bonds.

However, inspection of the experimental $f(b)$ DNA stretching curve in Fig. 1 immediately indicates that the deformation free energies involved are only about 2 k_BT, an order of magnitude smaller than for the putative B-S transition, and similar to the typical energy of DNA melting.

It is relatively easy to melt the DNA double helix because the large loss of enthalpy, $\Delta H \sim 15\ k_BT$, is almost compensated by a similar gain in conformational energy, $T\Delta S \sim 13\ k_BT$, so that the free energy stabilizing the duplex, $\Delta G \sim 2\ k_BT$, is about an order of magnitude smaller than either of its components. This marginal stability of the double helix at physiological conditions is essential for its biological functioning.

In other words, the low stability of the DNA double helix with respect to melting is due to the large entropy of the DNA single strands, $\Delta S^0 = 25$ cal/mol-K for polymeric DNA in high salt. This corresponds to a large number, $N \approx \exp(\Delta S^0/k_B) = \exp^{12.5} \approx 2.7 \times 10^5$, of liberated degrees of freedom per bp upon DNA melting. This makes it difficult to model even a relatively short piece of single-stranded DNA.

The large ΔS^0 of DNA melting is also responsible for the high cooperativity of this transition. In fact, no other known transition between two double-stranded DNA structures, such as B-A or B-Z, is as cooperative as melting. Indeed, the high free energy of the helix-coil boundary, $2\Delta G_s = -\ln(\sigma = 10^{-4}) \approx 9.2\ k_BT \sim \Delta H$, is due to the fact that the base pair at the boundary loses most of its binding enthalpy without gaining enough compensating conformational freedom. This value is much larger than any likely boundary energy between two double-stranded forms, which amounts to only a fraction of the total binding enthalpy per base pair.

On the other hand, if the overstretched DNA is rigidly fixed in space due to its binding to some protein or ligand, as in the case of complexes with RecA or TATA box binding proteins, then the entropic advantage of DNA melting is gone, and the DNA deformation pathways suggested in the modeling studies (Bertucat et al., 1999; Konrad and Bolonick, 1996; Kosikov et al., 1999; Lebrun and Lavery, 1996) should be appropriate.

Dependence of T7 DNA polymerase activity on template tension

Indirect support for our picture of force-induced melting comes from a recent single-molecule study of T7 DNA polymerase activity in which the single-stranded DNA template is under tension (Wuite et al., 2000). The dependence of the polymerization rate $k(f)$ on tension f can be described as

$$k(f) = k_0 \cdot \exp[n\Delta\Phi(f)], \tag{38}$$

where k_0 is the rate without applied force, $n \approx 2$ is a fit to the number of base pairs converted from the ss- to dsDNA form in the elementary step of the polymerase reaction, and $\Delta\Phi(f)$ is calculated according to Eq. 4. (The authors, Wuite et al. (2000), used different notation, but their fitting expression is equivalent to Eq. 38). A pronounced burst of dsDNA polymerization occurs at $\sim f_{cr} = 6$ pN, i.e., when the two forms of DNA have equal extensions and the force-induced stabilization of the dsDNA relative to ssDNA is calculated to be most pronounced (see Fig. 3 *a*). The experimental result embodied in Eq. 38 means that the effect of force on polymerase activity does not contain any specific features of the polymerase itself, except n, but is fully determined by the relative stabilities of the two DNA forms under tension. The same factor seems to control stalling of the polymerase and switching its activity to exonuclease at $\sim$34 pN (Wuite et al., 2000).

Connection with next paper

In the following paper (Rouzina and Bloomfield, 2001), we examine published experimental studies for their consistency with the predictions we can make by assuming that the overstretching transition is force-induced melting. We predict that the overstretching force should be a decreasing function of temperature and, conversely, that the melting temperature should be a decreasing function of the applied force. (However, we predict raising of the melting temperature by weak forces $\leq$7 pN.) This behavior should not be observed if the transition is to some double-stranded form such as S-DNA. We also predict that changes in the overstretching force should follow changes in melting temperature with base composition and with solution factors such as salt concentration and pH. To the extent that such data exist, these predictions are generally confirmed by experiment.

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