Unfolding and unzipping of single-stranded DNA by stretching

Alexei V. Tkachenko*
Michigan Center for Theoretical Physics and Department of Physics, University of Michigan, 500 East University Avenue, Ann Arbor, Michigan 48109, USA

(Received 1 April 2003; revised manuscript received 31 March 2004; published 2 November 2004)

We present a theoretical study of single-stranded DNA under stretching. Within the proposed framework, the effects of base pairing on the mechanical response of the molecule can be studied in combination with an arbitrary underlying model of chain elasticity. In a generic case, we show that the stretching curve of single-stranded DNA exhibits two distinct features: the second-order "unfolding" phase transition, and a sharp crossover, reminiscent of the first-order "unzipping" transition in double-stranded DNA. We apply the theory to the particular cases of wormlike chain and freely jointed chain models, and discuss the universal and model-dependent features of the mechanical response of single-stranded DNA. In particular, we show that variation of the width of the unzipping crossover with interaction strength is very sensitive to the energetics of hairpin loops. This opens another way of testing the elastic properties of ssDNA.

DOI: 10.1103/PhysRevE.70.051901 PACS number(s): 87.14.Gg, 82.37.Rs, 64.90.+b

I. INTRODUCTION

Dramatic progress has been made over the last decade in employing single-molecule micromanipulation techniques for studies of biological materials and processes. Pioneered by the work of Smith *et al.* [1] on stretching of double-stranded DNA (dsDNA), these techniques have later been applied to study proteins, DNA-protein interactions, chromosomes, etc. Chain-stretching experiments were also performed on single-stranded DNA molecules (ssDNA) [2–5].

One could expect the response of ssDNA to stretching to be dramatically different from that of dsDNA, because of the effects of the possible base pairing between complementary segments of the chain. The need for understanding of the resulting mechanical behavior has already attracted considerable attention from theorists [6–10]. In particular, it has been shown [7–9] that in the thermodynamic limit, the ssDNA chain should undergo a second-order phase transition, at a finite critical force. Formally, this phenomenon is very similar to the hypothetical native-molten transition in RNA [11], as well as to the classical (yet unconfirmed) picture of dsDNA denaturation [12].

The predicted critical behavior is qualitatively different from another related phenomenon, the force-induced denaturation (unzipping) of dsDNA [13,14]. The unzipping is a first-order transition, which occurs as a result of competition between the elastic energy of the stretched ssDNA, and the base pairing (hybridization) energy within dsDNA. The very same effects are essential for the above second-order phase transition in stretched ssDNA. As a part of the present work, we will clarify the relationship between the two phenomena.

The theoretical modeling of ssDNA and RNA is traditionally done within a freely jointed chain (FJC) model. Its extensible version was originally used for fitting the early ssDNA stretching data [2]. However, both the microscopic structure of ssDNA and the recent experiments with DNA hairpin constructs [15,16] strongly suggest a picture with a finite bending modulus of its backbone, which feature is reminiscent of the semiflexible wormlike chain (WLC) model [17]. Nevertheless, being a continuous model, the WLC description is unlikely to be valid in the regimes when the discrete nature of chemical bonds becomes relevant (e.g., for a sufficiently high stretching force). To overcome this limitation, a discrete persistent chain (DPC) model has been proposed for ssDNA in recent work by Storm and Nelson [18]. Interestingly, the WLC itself was originally introduced by Kratky and Porod [17] as the continuous limit of a similar discretized model. The authors of [18] have shown that the extensible versions of all three models (FJC, WLC, and DPC) produce a good fit to the experimentally observed stretching curves, as long as the force is not too high ($f \lesssim 200$ pN for the FJC, and $f \lesssim 400$ pN for the WLC and DPC).

The central idea of our paper is to include the effects of base pairing within a theoretical framework compatible with an arbitrary underlying model of ssDNA elasticity. In this way we can separate the two parts of the problem: the search for an adequate elastic description of ssDNA, and the evaluation of the effects of its self-interactions. In fact, one may be able to start with an empirical elastic model extracted from independent experiments, and use our theory to predict the stretching behavior of the chain after "switching on" the base pairing interactions.

II. THEORETICAL FRAMEWORK

We consider a ssDNA chain subjected to an external pulling force f. As an input for our theory, one has to specify two functions characterizing the system without self-interactions: $q_{\mathrm{el}}(f)$, the elastic free energy per unit chain length vs pulling force; and $F_{\mathrm{loop}}(l)$, the free energy of a loop as a function of its contour length. Our goal is to study the effect of interactions between complementary segments of the chain. Similarly to Ref. [7], we assume the interaction strength to be the same for any two chain fragments. It will be characterized by

*Electronic address: alexei@umich.edu

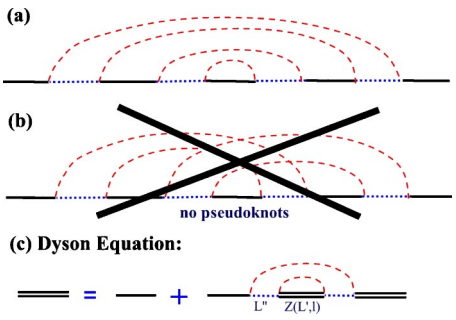

FIG. 1. Schematic representation of partition function calculation. Thin solid lines represent Z_0, while dashed lines indicate base pairing of distant chain segments (dotted).

a single parameter ϵ, defined as the pairing energy per unit chain length. Strictly speaking, this should limit the applicability of our approach to self-complementary periodic sequences of ssDNA (such as ATATAT). However, as discussed in [7,8], this uniform model may be reasonably adequate for random sequences, too. Nevertheless, the effect of randomness remains an important problem for future studies.

Hybridization of distant chain segments results in looping. Therefore, the interactions reduce the effective (free) chain length l exposed to the stretching force. Our goal is to calculate the partition function of the system $Z(L,l)$, parametrized by the total chain length L and free length l. The general form of the interaction-free partition function is $Z_0(L,l)=\exp(\mu_0 L)\delta(L-l)$, because l and L coincide, and different chain segments are statistically independent (we neglect excluded volume effects). Without loss of generality, one can choose $\mu_0=0$. It is useful to perform a double Laplace transform of the partition function $Z(L,l)$. This results in introduction of the parameters μ and q, conjugated to L and l, respectively (the parameter μ conjugated to the total length is often called the fugacity):

$$Z(\mu,q)=\int_0^\infty \int_0^\infty Z(L,l)\exp[-\mu L - ql]dL\, dl. \tag{1}$$

In particular, $Z_0(\mu,q)=1/(\mu+q)$. Since the elastic part of the free energy is $q_{\rm el}(f)l$, the overall free energy may be expressed as

$$F(L,f)=-\log\int_{-i\infty}^{+i\infty} Z[\mu,q_{\rm el}(f)]e^{\mu L}\frac{d\mu}{2\pi i}. \tag{2}$$

Here and below, we take $k_BT=1$.

It is well known that the partition function of uniform RNA or ssDNA may be calculated in a recursive manner, reminiscent of the Hartree approximation [6–11]. This calculation can be represented in a diagrammatic form shown in Fig. 1. The solid lines correspond to the bare partition function Z_0; dotted fragments connected with dashed lines represent pairing (hybridization) of the corresponding chain segments. Within our approach, the energy cost of the pairing is $\epsilon L_{\rm hyb}+F_{\rm loop}(l)$, where $L_{\rm hyb}$ is the length of the hybridized region, and l is the free length of the chain segment being "internalized" due to the looping. In fact, one can extend our approach to a more general form of hybridization energy, $\varepsilon_0+\epsilon L_{\rm hyb}$, with the constant $\varepsilon_0>0$ representing the energy cost of termination of the double-stranded segment. Introduction of ε_0 accounts for the cooperativity of the base pairing interactions.

Traditionally, problems involving DNA or RNA folding or denaturation are studied within a discrete model. Each discrete "monomer" in this approach represents a chain segment which can hybridize independently of its neighbors. Its length is assumed to be equal to the statistical segment, which makes it easy to combine this description with the FJC model. Since we are interested in developing a theory for an arbitrary model of chain elasticity, it is logical to abandon this artificial discretization of hybridized segments. Of course, the base pairing remains fundamentally discrete on the length scale l_0 of a single base. As long as the relevant physics occurs on larger scales, ssDNA can be considered as a continuous chain.

The crucial observation is that one can typically neglect all the diagrams with intersecting dashed lines. Such a situation would correspond to a "pseudoknot," whose probability is low because it requires winding of ssDNA or RNA around itself [Fig. 1(b)]. Thanks to this topological constraint, the self-energy diagram entering the Dyson equation [Fig. 1(c)] may be calculated exactly within the one-loop (Hartree) approximation. Since we have assumed a uniform interaction parameter, the problem has a particularly simple form in the Laplace representation:

$$Z^{-1}(\mu,q)=Z_0^{-1}(\mu,q)-\int\int\int \frac{dL'dL''dl}{l_0^3}Z(L',l) \times\exp[-\mu(2L''+L')-\epsilon L''-F_{\rm loop}(l)-\varepsilon_0]. \tag{3}$$

From here, one can obtain

$$Z^{-1}(\mu,q)=\mu+q-\frac{W(q_\mu)}{2\mu+\epsilon}, \tag{4}$$

were q_μ corresponds to the pole in $Z(\mu,q)$, and

$$W(q)=\frac{e^{-\varepsilon_0}}{l_0^3}\int_0^\infty \exp[-F_{\rm loop}(l)+ql]dl. \tag{5}$$

III. GENERIC BEHAVIOR: "UNZIPPING" VERSUS "UNFOLDING"

In the thermodynamic limit ($L\to\infty$),

$$l=-\frac{\partial}{\partial q}\log\int_{-i\infty}^{+i\infty} Z(\mu,q)e^{\mu L}\frac{d\mu}{2\pi i}\bigg|_{q_{\rm el}(f)}=-L\frac{d\mu_q}{dq}\bigg|_{q_{\rm el}(f)}. \tag{6}$$

Here the function $\mu_q(q)$ again corresponds to the pole in $Z(\mu,q)$, i.e., it is inverse to $q_\mu(\mu)$. Explicitly,

$$\mu_q(q) = -\frac{1}{2}\left[\frac{\epsilon}{2} + q - \sqrt{\left(\frac{\epsilon}{2} - q\right)^2 + 2W(q)}\right]. \quad (7)$$

This yields the following result for the free length as a function of tension:

$$\frac{l}{L} = \frac{1}{2}\left(1 - \frac{q_{\rm el} - \epsilon/2 + W'(q_{\rm el})}{\sqrt{(q_{\rm el} - \epsilon/2)^2 + 2W(q_{\rm el})}}\right). \quad (8)$$

Here $W' \equiv dW/dq$. Note that from the definition of the function W, Eq. (5), one can relate its logarithmic derivative to the average length of the loop:

$$l_{\rm loop}(q) = \frac{W'}{W}. \quad (9)$$

If l is known, one can easily find the relative elongation of the chain, i.e., the ratio of the end-to-end distance R to the total chain length L, which is observable experimentally:

$$\frac{R}{L} = xl = -\frac{1}{2}\frac{\partial q_{\rm el}}{\partial f}\left(1 - \frac{q_{\rm el} - \epsilon/2 + W'(q_{\rm el})}{\sqrt{(q_{\rm el} - \epsilon/2)^2 + 2W(q_{\rm el})}}\right). \quad (10)$$

Here $x = -\partial q_{\rm el}/\partial f$ is the relative elongation of the free portion of the chain.

According to Eq. (8), in the strong stretching limit ($q_{\rm el} \to -\infty$) the chain becomes completely "free:" $l \to L$. Further examination of our expression for the free length reveals its peculiar behavior near the point $q_{\rm el} = \epsilon/2$. In fact, in the limit of vanishing W (i.e., very high energy cost of a loop), $l(q_{\rm el})$ becomes a step function changing from 0 to L at that point. In a realistic situation, it is transformed into a crossover whose width depends on W as

$$\delta_q = \sqrt{2W(\epsilon/2)}. \quad (11)$$

Since the location of that crossover corresponds to the point were the hybridization free energy is exactly equal to the elastic one, we conclude that this behavior is directly related to the first-order *unzipping* transition of dsDNA. The transition is transformed into the crossover due to the finite size of the hybridized segments.

Another important feature of our result is that the free fraction of the chain l/L goes to zero at finite tension $q_{\rm el} = q^*$. According to Eq. (8) the condition for this to happen is

$$q^* - \epsilon/2 = \frac{W(q^*)}{W'(q^*)} - \frac{W'(q^*)}{2} = \frac{1}{l_{\rm loop}(q^*)} - \frac{l_{\rm loop}(q^*)}{l_0^2}W(q^*). \quad (12)$$

This point corresponds to the second-order phase transition that has been reported in the earlier studies of the problem. The crucial observation is that this transition is physically distinct from the unzipping crossover. It may be viewed as a precursor of force-induced denaturation. Indeed, even when the tension in the free segments is still insufficient to overcome the binding energy $-\epsilon$, one can convert a finite fraction of the chain into the free length without breaking any bonds; namely, since there is a finite density of loops in the folded ssDNA, one can simply "move" the unbound bases from the loops to the free segment. This will only result in an entropy loss, but will not reduce the interaction energy.

Indeed, if the base pairing energy is negative, the magnitude of the critical tension is somewhat lower ($|q^*| < -\epsilon/2$) than that at the unzipping point:

$$q^* - \frac{\epsilon}{2} \approx \frac{1}{l_{\rm loop}(q^*)}. \quad (13)$$

The free segments of the chain exposed to the external stretching can be viewed as topologically distinct objects ("vortices"), which separates the system into independently folded domains. The effective interaction potential of two consecutive vortices is determined by the free energy of the folded region between them. At the point q^*, the typical separation between the vortices along the chain coordinate, i.e., the size of the separable domains, diverges. This *folding-unfolding* transition occurs as a result of competition between the elastic tension and folding entropy, which grows with the domain size.

Interestingly, Eq. (12) has physical solutions even for positive interaction energy, i.e., when the base pairing is energetically unfavorable. It follows from that fact that for $l_{\rm loop} \to \infty$, the generic behavior of the looping free energy is $F_{\rm loop}(l) \simeq (3/2)\log l$ (for an ideal Gaussian chain). As a result, $W(q)$ remains finite for $q \to 0$, while W' and $l_{\rm loop}(q^*)$ are diverging as $q^{\nu-2}$. Therefore, according to Eq. (12), there is a finite tension at which the unfolding transition takes place, for $\epsilon > 0$. The asymptotic relationship between ϵ and q^* in this regime is

$$l_{\rm loop}(q^*) \approx \frac{\epsilon l_0^2}{2}W(0). \quad (14)$$

This yields a power law dependence of the critical tension on ϵ: $q^* \sim \epsilon^{-2}$. Note, however, that our conclusion about the unfolding transition at positive values of base pairing energy may well be a result of limitations of the model.

IV. APPLICATION TO WORMLIKE CHAIN MODEL

As an example, we discuss the application of the above approach to the particular case of the WLC model. Its Hamiltonian is given by

$$H = \int_0^L \left[\frac{l_p}{2}\left(\frac{\partial^2 \mathbf{r}}{\partial s^2}\right)^2\right] ds, \quad (15)$$

where l_p is the persistence length, and $\mathbf{r}(s)$ defines the spatial conformation of the chain, subjected to the constraint $|\partial \mathbf{r}/\partial s| = 1$. For this model, Marko and Siggia [17] have proposed the following interpolative relationship between the stretching force f and relative extension $x \equiv R/L$:

$$f(x) = \frac{1}{l_p}\left[\frac{1}{4}\left(\frac{1}{(1-x)^2} - 1\right) + x\right]. \quad (16)$$

From here, the function $q_{\rm el}(f)$ (which is one of the inputs for our theory) can be expressed in parametric form:

$$q_{\rm el}(f) = \int_0^x f(x')dx' - xf(x) = -\frac{x^2}{4l_p}\left(\frac{1}{(1-x)^2} + 2\right). \quad (17)$$

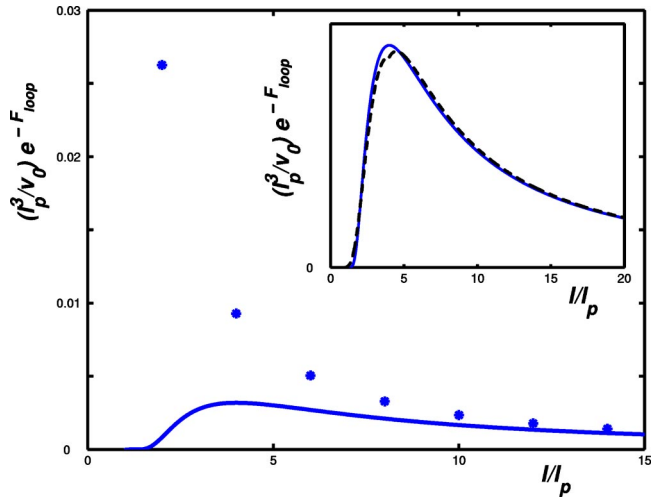

FIG. 2. Looping probability $\exp(-F_{\mathrm{loop}})$ as a function of loop size for different models of ssDNA elasticity. Here the solid line represents the analytic result for the WLC, Eq. (19), and the points correspond to the FJC model. Inset: Comparison of classical interpolative result for looping probability (from Ref. [19], dashed line), with the simplified formula Eq. (19).

One has also to specify the loop free energy. An analytic interpolation for F_{loop} was proposed in Ref. [19], in the context of the ring cyclization problem:

$$e^{-F_{\mathrm{loop}}} \simeq \frac{v_0}{l_p^3} \times \begin{cases} 4\pi^3 \left(\frac{2l_p}{l}\right)^6 \exp\left(-\frac{2\pi^2 l_p}{l} + \frac{l}{4l_p}\right), & l < 4l_p, \\ \left(\frac{3l_p}{\pi l}\right)^{3/2} \left[1 - \frac{11}{4}\frac{l_p}{l} + \frac{l_p^2}{5l^2}\right], & l > 4l_p. \end{cases} \tag{18}$$

Here $v_0 \lesssim 1$ Å^3 is the effective "reaction volume" associated with the localization of the loop ends by the hydrogen bonding. We have found a simpler version of the interpolative expression, which gives a very good fit to the global behavior of the looping probability, $e^{-F_{\mathrm{loop}}}$ (see Fig. 2):

$$e^{-F_{\mathrm{loop}}} \simeq v_0 \left(\frac{3}{\pi l_p (l - l_p)}\right)^{3/2} \exp\left(-\frac{4.5}{l/l_p - 1}\right) \theta(l - l_p). \tag{19}$$

Here θ is the step function.

Within this approximation, the function $W_{\mathrm{WLC}}(q)$ can be found analytically:

$$W(q) \simeq \frac{\alpha}{l_p^2} \exp\left(-3\sqrt{-2ql_p} + ql_p\right). \tag{20}$$

Here

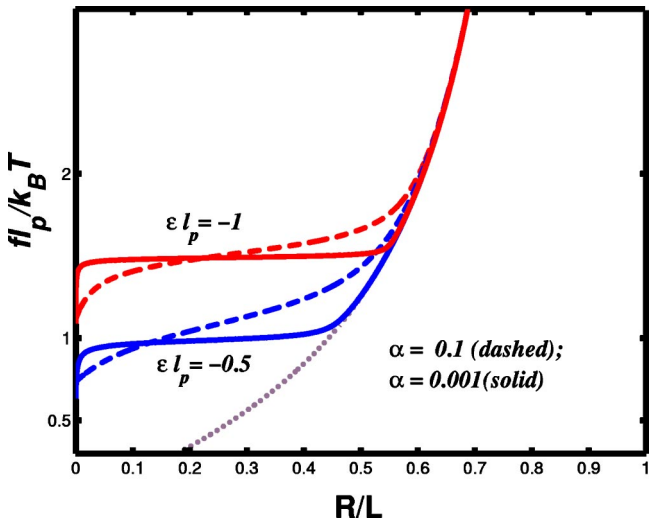

FIG. 3. Theoretical stretching curves for the WLC model with self-interaction. Dotted line corresponds to the interaction-free WLC.

$$\alpha = \frac{\sqrt{6}}{\pi} \frac{v_0}{l_0^3} e^{-\varepsilon_0}. \tag{21}$$

Note that α replaces three parameters of the original model, v_0, l_0, and the energy ε_0. Since $\delta \lesssim l_0$ and $\varepsilon_0 > 0$, this dimensionless parameter is expected to be small. It has the physical meaning of the effective reaction volume of the loop ends, in units of l_0^3.

Now that we have specified $W_{\mathrm{WLC}}(q)$ and $q(f)$, Eqs. (6) and (7) can be used to find $l(f)$, together with force-extension curves $R(f) = l(f)x(f)$. The fact that α is a small parameter allows us to simplify the results. In the regime of negative ϵ, a sharp unzipping crossover is expected. As we have discussed in the previous section, its characteristic width is given by

$$\delta_q = \sqrt{2W(\epsilon/2)} = \frac{\sqrt{2\alpha}}{l_p} \exp\left[\left(-6\sqrt{-\epsilon l_p} + \epsilon l_p\right)/4\right]. \tag{22}$$

In the vicinity of the crossover point $q_{\mathrm{el}} = \epsilon/2$, the change in free length can be well described by a universal function of the rescaled variable $\Delta = (q - \epsilon/2)/\delta_q$:

$$\frac{l}{L} \simeq \frac{1}{2}\left(1 - \frac{\Delta}{\sqrt{\Delta^2 + 1}}\right). \tag{23}$$

For large enough Δ, this universal behavior breaks down due to the proximity of the unfolding phase transition. The corresponding critical tension q^* is given by

$$q^*_{\mathrm{WLC}} \simeq \frac{\epsilon}{2} + \frac{1}{l_{\mathrm{loop}}(\epsilon/2)} \simeq \frac{\epsilon}{2} + \frac{1}{l_p(1 + 3/\sqrt{-\epsilon l_p})}. \tag{24}$$

Figure 3 shows the sharp crossover at $|q(f)| = \epsilon/2$. Consistently with Eq. (22), its sharpness increases with lowering parameter α. This can be associated with growth of the typical length of hybridized regions. The unzipping is clearly separated from the second-order unfolding transition. While

this crossover regime was not present in the early studies of the problem, it has been recently reported in Ref. [9]. In that work, the traditional FJC-based model has been modified to include the effects of cooperativity, analogous to our parameter ε_0. As we have discussed, the sharp crossover should be interpreted as unzipping (force-induced denaturation), while the second-order transition corresponds to topological change (unfolding) which may be viewed as a precursor of the unzipping. This physical picture is consistent with the results of Ref. [9]. On the other hand, our analysis disagrees with the conclusions of Ref. [10], in which a first-order phase transition was predicted for the regime of strong enough hybridization energy.

V. MODEL DEPENDENCE OF THE RESULTS

Here we discuss how the above results may depend on the choice of the elastic description of ssDNA. It should be noted that such important features as the unfolding transition and the unzipping crossover are very robust and nearly independent of the model. Furthermore, it is well known that the stretching curve of ssDNA may be fitted reasonably well by several models, e.g., extensible versions of the FJC, WLC, or DPC. This implies that deduction of $q_{\rm el}(f)$ from existing and future experimental data is unlikely to provide a sensitive test for the possible models. On the other hand, we have seen that the loop free energy $F_{\rm loop}(l)$ and consequently $W(q)$ are significant parameters of the problem. As we shall see, these parameters are very sensitive to the choice of the underlying elastic model.

In the case of the discreet persistent chain model, we do not expect any significant deviation of $F_{\rm loop}(l)$ or $W(q)$ from those obtained for the WLC model, since the typical bending radius significantly exceeds the bond length. The (extensible) FJC model has been the standard framework in which the discussed problem has been studied so far. Nevertheless, here we briefly review the results of our theory for the FJC, in order to identify the major model-dependent features. The freely jointed chain consists of discreet bonds of length $a=2l_p$, whose orientations are mutually independent. The corresponding loop free energy can be written as

$$\exp[-F_{\rm loop}(l)] \simeq 2\left(\frac{3}{2\pi}\right)^{3/2}\frac{v_0}{l_p^2}\sum_{n=1}^{\infty}\frac{\delta(l-2nl_p)}{n^{3/2}}. \qquad (25)$$

The prefactor here ensures that the asymptotic behavior of the free energy at the large-loop limit coincides with the WLC result, given by Eq. (18). Now one can find $W(q)$ for the FJC model:

$$W(q) = \frac{3\alpha}{2\sqrt{\pi}l_p^2}\sum_{n=1}^{\infty}\frac{\exp(2ql_pn)}{n^{3/2}}. \qquad (26)$$

For negative ϵ, the change of the elastic description results in a modest shift of the critical tension q^* at which the unfolding transition occurs:

$$q^*_{\rm FJC} \simeq \frac{\epsilon}{2} + \frac{1}{l_{\rm loop}(\epsilon/2)} \simeq \frac{\epsilon}{2} + \frac{1}{2l_p}. \qquad (27)$$

This should be compared to the WLC result Eq. (24).

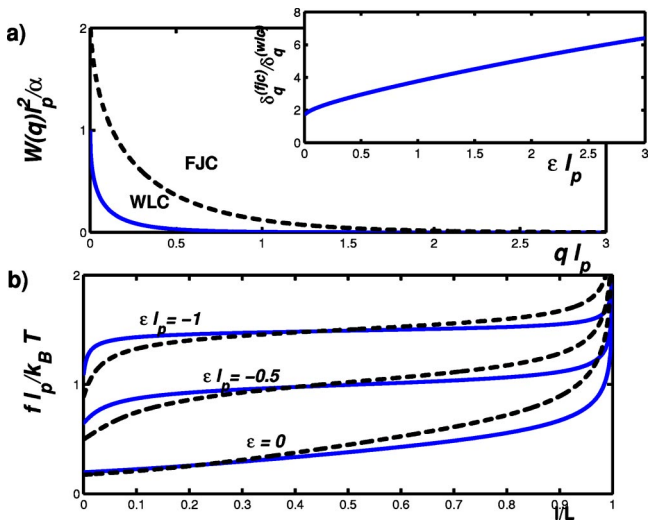

FIG. 4. (a) Comparison of $W(q)$ functions calculated for the WLC and FJC models. Inset: Relative width δ_q of unzipping crossovers, for the FJC and WLC, as a function of base pairing energy. (b) Unzipping behavior of WLC (solid) and FJC (dashed) systems for the base pairing energy changing between $\epsilon=0$ and $\epsilon=-k_BT/l_p$. For both models, we put $\alpha=0.01$.

While the position of the unzipping point $q_{\rm el}=\epsilon/2$ is model independent, the width of the crossover is very sensitive to the behavior of $W(q)$. As one can see in Fig. 4, the shapes of $W(q)$ curves are substantially different for the WLC and FJC models. This difference arrises because of the relative suppression of the short loops in the WLC case. What is especially remarkable is that the width of the unzipping crossover $\delta_q=\sqrt{2W(\epsilon/2)}$ decreases with ϵ in a strongly model-dependent manner. In fact, upon change of ϵl_p from 0 (the dsDNA denaturation point) to $3kT$ (which roughly corresponds to physiological conditions for a - *GCGCGC*- sequence), the ratio of δ_q for the two models changes by a factor of $\simeq$3.5. (Here, *G* and *C* denote guanine and cytosine bases in DNA sequence.) Therefore, an experimental study of the unzipping crossover in variable conditions (e.g., temperature) would open the possibility of testing the plausible models of ssDNA elasticity.

VI. CONCLUSIONS

In this paper, we have discussed the effects of base pairing on the stretching behavior of ssDNA, within a theoretical framework compatible with an arbitrary underlying model of chain elasticity. Our conclusion is that in a generic case, the stretching curves exhibit two related but distinct features: a second-order *unfolding* phase transition and a sharp *unzipping* crossover. The latter is reminiscent of the first-order transition in dsDNA, as well as the mechanical response of nonrandom RNA molecules [6]. On the other hand, we have interpreted the unfolding as a topological transition. At the critical point, the typical size of an independently folded domain diverges (in the thermodynamic limit). This transition is due to the competition of conformational entropy and elastic free energy, and it is expected to occur even in the regime

when base pairing is energetically unfavorable.

In the light of our results, one can see a clear relationship between the three types of force-induced denaturation: (i) unzipping of dsDNA [13,14], (ii) denaturation of RNA with a preferred secondary structure [6], and (iii) stretching of self-complementary or random ssDNA. While in the case of ds-DNA the unzipping occurs as a first-order transition, it becomes a crossover for the two other cases. The width of the crossover is defined by the typical length of a single hybridized region. We expect this width to be sensitive to the sequence of ssDNA or RNA. In fact, the force-induced denaturation of RNA [6] was predicted to show a sequence of unzipping steps. It follows from our discussion that the sharpness of those steps may be even more pronounced than was originally predicted within FJC-based models. In the case of ssDNA, the sequence disorder must result in smearing of the unzipping crossover. Because of its entropic nature, the unfolding transition is expected only in the case of ssDNA (or long RNA, in the molten phase [11]).

At present, the experimental indications of the second-order unfolding transition are not conclusive enough [3,4]. On the other hand, experiments with uniform self-complementary DNA show a clear manifestation of the sharp unzipping crossover [5]. However, their precision is still insufficient to make a quantitative comparison with the theory, and to distinguish between different underlying elastic models. Based on our theory, one may extract this information by performing a systematic experimental study of the unzipping behavior for various values of hybridization energy (e.g., various temperatures). As we have shown, the width of the crossover is very sensitive to the energy cost of the hairpin loop.

ACKNOWLEDGMENTS

The author thanks B. Shraiman, D. Lubensky, J. Marko, and E. Siggia for valuable discussions.

[1] S. B. Smith, L. Finzi, and C. Bustamante, Science **258**, 1122 (1992).
[2] S. B. Smith, Y. Cui, and C. Bustamante, Science **271**, 795 (1996).
[3] B. Maier, D. Bensimon, and V. Croquette, Proc. Natl. Acad. Sci. U.S.A. **97**, 12 002 (2000).
[4] M.-N. Dessinges *et al.*, Phys. Rev. Lett. **89**, 248102 (2002).
[5] M. Rief, H. Clausen-Schaumann, and H. E. Gaub, Nat. Struct. Biol. **6**, 346 (1999).
[6] U. Gerland, R. Bundschuh, and T. Hwa, Biophys. J. **81**, 1324 (2001); **84**, 2831 (2003).
[7] A. Montanari and M. Mézard, Phys. Rev. Lett. **86**, 2178 (2001).
[8] M. Müller, F. Krzakala, and M. Mézard, Eur. Phys. J. E **9**, 67 (2002).
[9] M. Müller, Phys. Rev. E **67**, 021914 (2003).
[10] H. Zhou and Y. Zhang, J. Chem. Phys. **114**, 8694 (2001).
[11] R. Bundschuh and T. Hwa, Phys. Rev. Lett. **83**, 1479 (1999).
[12] D. Poland and H. A. Scheraga, *Theory of Helix-Coil Transitions in Biopolymers* (Academic, New York, 1970).
[13] S. M. Bhattacharjee, J. Phys. A **33**, L423 (2000).
[14] D. K. Lubensky and D. R. Nelson, Phys. Rev. Lett. **85**, 1572 (2000); Phys. Rev. E **65**, 031917 (2002).
[15] N. L. Goddard, G. Bonnet, O. Krichevsky, and A. Libchaber, Phys. Rev. Lett. **85**, 2400 (2000).
[16] S. V. Kuznetsov, Y. Shen, A. S. Benight, and A. Ansari, Biophys. J. **81**, 2864 (2001)
[17] O. Kratky and G. Porod, Recl. Trav. Chim. Pays-Bas **68**, 1106 (1949); J. F. Marko and E. Siggia, Macromolecules **28**, 8759 (1995).
[18] C. Storm and P. C. Nelson, Phys. Rev. E **67**, 051906 (2003).
[19] J. Shimada and H. Yamakawa, Macromolecules **17**, 689 (1984).