Force-induced stretched state: Effects of temperature

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A model of self-avoiding walks with suitable constraint has been developed to study the effect of temperature on a single-stranded DNA (ssDNA) in the constant force ensemble. Our exact calculations for small chains show that the extension (reaction coordinate) may increase or decrease with the temperature depending on the applied force. The simple model developed here, which incorporates semimicroscopic details of base direction, provides an explanation of the force-induced transitions in ssDNA as observed in experiments.

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In recent years, single-molecule force spectroscopy (SMFS) has made it possible to observe force-induced transitions in single molecules [1–5]. These experiments also provide information about, for example, transcription and replication processes involving DNA, mechanical and elastic properties of biopolymers, functional and structural properties of proteins, etc. Moreover, these experiments also present a platform to verify theoretical predictions based on models developed in the framework of statistical mechanics [6–8,10,11]. In many biological processes, there is a large conformational change and the temperature plays a crucial role. Therefore, the efforts of SMFS experiments have now been shifted to study the effect of temperature on these processes keeping the force constant [12–14]. In a recent paper, Danilowicz *et al.* [14] studied the elastic properties of single-stranded DNA (ssDNA) and showed that temperature has a significant impact on the force extension curve. In the low force regime, they found that the extension increases with the temperature. By changing the solvent condition, they could show that the increase in the extension is due to the disruption of hairpins. In fact, using the Poland-Scheraga (PS) model of double-stranded DNA (dsDNA) [15] and the modified freely jointed chain model (mFJC) [16–18] of a polymer, they could nicely represent the force-extension curve in the low force regime. However, for the higher forces, none of these models could explain the outcome of their experiments, where the extension decreases abruptly with the rise of the temperature, and it appears that there is no clear understanding about it. It was suggested that the observed decrease in extension may be because of the sequence-dependent secondary structures [14].

The mechanical properties of biopolymers (e.g., DNA, proteins) are fairly well understood theoretically from applications of the mFJC [16–18] or wormlike chain (WLC) models [19–21]. Indeed one finds that the force-extension curves obtained from these models agree excellently with experiments [1–5,12,14]. As a result, these models have been used as a benchmark to compare the outcome of SMFS experiments. It is important to point out here that the WLC or FJC model ignores the crucial excluded volume effect [22] in its description, and they are therefore not ideal models to probe the entire phase space. The PS model [15] of the DNA does not include the non-native interactions, and so it underestimates the entropy of partial bound states and excludes the possibility of the formation of hairpins. Moreover, the PS model does not incorporate configurational entropy, hence it is not appropriate in the constant force ensemble where the "stretched state" may be induced by a force [23]. Thus these models may only give a limited picture of the unzipping and stretching transitions.

Here, we adopt (from the single molecule of finite length point of view) a more realistic model of ssDNA proposed in Ref. [24]. In this, a self-attracting self-avoiding walk (SASAW) along with orientation of the base pairs has been considered to model the ssDNA. The base pairing takes place only when the nucleotides approach each other directly without the sugar phosphate strand coming in between, as shown in Fig. 1.

This restriction also takes into account that once two bases are bonded, no further hydrogen bonds can be formed with these bases. By applying a force at one end of the chain, the system undergoes a phase transition from the zipped or hairpin state to the coil (extended state). With further rise of the force, the system goes from the extended state to the stretched state, a state solely induced by the applied force [23]. The force-temperature phase diagram of unzipping of dsDNA is known in two dimensions for some simple models [6,8,10]. However, in three dimensions (a ssDNA may form a hairpin loop), the phase diagram is yet to be explored. As

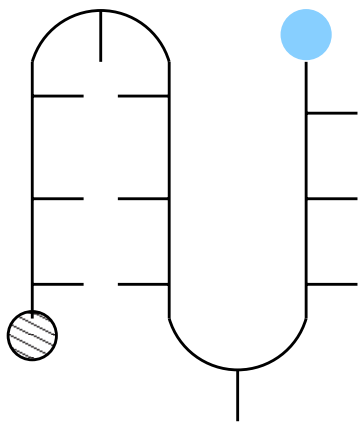

FIG. 1. (Color online) The schematic representation of a ssDNA where bases are on the links of the strand with short stubs representing the direction of the hydrogen bonds. This figure also shows that once two bases are bonded, no further hydrogen bonds can be formed with these bases. The small black circle indicates that one end of the ssDNA is kept fixed while a force F may be applied at the other end.

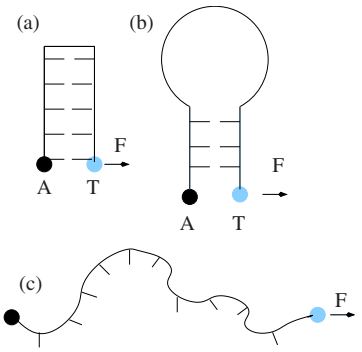

FIG. 2. (Color online) The schematic representation of a ssDNA forming (a) the zipped conformation (dsDNA). In this case, half of the strand is of **A** type nucleotides and the other half consists of complementary nucleotides **T**. (b) A hairpin structure of stems of three bases. (c) shows that by application of a force applied to one end, the system changes from a zipped or hairpin state to an extended state.

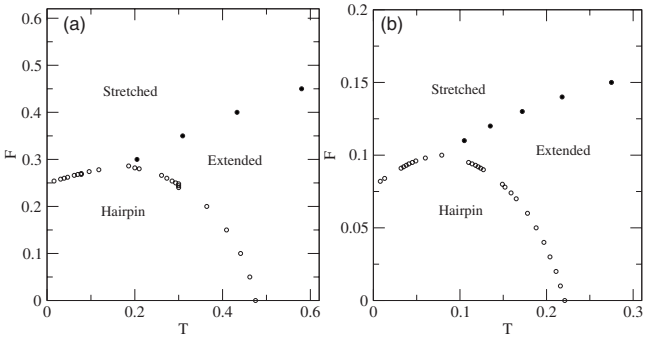

FIG. 3. Force-temperature diagram for (a) zipped case and (b) DNA hairpin. Solid circles show the crossover regime.

of now, experiments using eight and more bases are available [25–27] to describe melting and unzipping of DNA at a coarse-grained level. The purpose of this paper is to provide exact results of a semimicroscopic model of a short ssDNA and then to study the effect of temperature in a constant force ensemble. In order to see whether the abrupt decrease in extension is a sequence-dependent effect [14], we consider two conformational possibilities of a ssDNA. In the first case, we allow monomers (nucleotides **T**) of half of the chain to form base pairs (nucleotides **A**) with the other half of the chain (diblock copolymer). Since in most of the experiments one end of the chain is kept fixed, the ground-state conformation resembles the zipped state of dsDNA as shown in Fig. 2. However, if we allow interaction among (say) the first three (**T**) and the last three monomers (**A**), we have the possibility of the formation of hairpins in a ssDNA at low temperature, as shown in Fig. 2. These two models may be considered as two bounds of ssDNA.

All possible conformations of SASAWs with the base orientations have been enumerated on a cubic lattice. Although the time involved in enumerating these conformations increases as μ^N, where μ is the connectivity constant of the lattice, using parallel processing we were able to enumerate walks of 20 monomers in three dimensions [28]. The thermodynamic properties of ssDNA may be determined from the canonical partition sum

$$Z_N = \sum_{p=0}^{n} \sum_{x=0}^{N} C_N(p,x)(e^{-\epsilon/k_BT})^p (e^{-F/k_BT})^x. \tag{1}$$

Here $C_N(p,x)$ is the total number of configurations corresponding to a walk of N steps with p number of base pairs whose end points are at a distance x. k_B, T, ϵ, and F are the Boltzmann constant, temperature of the system, the base pairing energy, and the applied force, respectively. In the following calculation, we set $\epsilon/k_B=1$ and calculate all the thermodynamic variables in reduced units. Here n is the $N/2$ for the zipped case, while 3 is for the hairpin. Although no true phase transition can occur in the finite-size single-molecule experiments, the "phase transition" observed in such experiments may be considered as real if the length of the chain exceeds the characteristic correlation lengths. We use a sudden change in appropriate average to obtain the different phases in the phase diagram [11,23]. It is pertinent to mention here that the thermodynamic limit may be achieved by using the extrapolation technique developed in [28,29]. For example, the exact phase diagram of the partial-directed self-avoiding walks (PDSAWs) is in excellent agreement with the exact enumeration technique [11,29]. It has also been shown that peak values obtained for PDSAWs from fluctuation in nonbonded nearest-neighbor monomers of finite length chain are also in quantitative agreement (within ± 0.01) with exact values [11]. In view of finite-size experiments, we choose this technique so that the complete state diagram can be probed exactly. The force-temperature diagram for ssDNA for the zipped and the hairpin cases is shown in Fig. 3. It is evident from these plots that unzipping force decreases with the rise of temperature. Moreover, the existence of reentrance at low temperature and the stretched state at a high force observed here are already discussed in another context (unzipping and unfolding) and its physical origin is known [8,9,23]. The upper line shown here (crossover regime) has been obtained in the constant force ensemble; it is absent in the constant temperature ensemble [9].

Here, we focus our studies on the behavior of the force-extension curve at low temperatures as well as in the high force limit, where Monte Carlo and models discussed above fail. The average extension may be obtained from the relation

$$\langle x \rangle = \frac{1}{Z} \sum_{p=0}^{n} \sum_{x=0}^{N} C_N(p,x) x (e^{-\epsilon/k_BT})^p (e^{-F/k_BT})^x. \tag{2}$$

In Fig. 4, we plot the extension versus force for the zipped and the hairpin situation at various temperatures, respectively. It can be seen from these plots that the extension increases with the applied force. This is in agreement with the experiment [14], and qualitative understanding is known in terms of dissociation of base pairs. In a constant temperature ensemble, by varying the force one can go from the zipped state or the hairpin to the extended state. With further rise of the force, one finds the stretched state, i.e., the extension approaches the contour length of the ssDNA as seen in

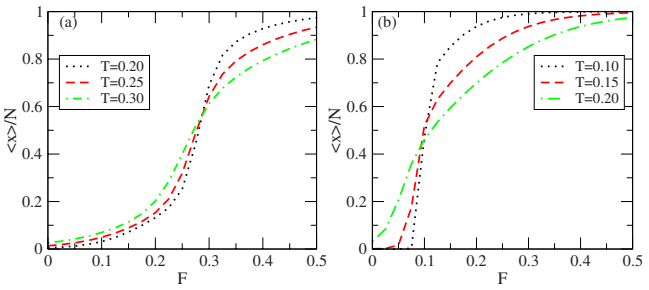

FIG. 4. (Color online) The force-extension curve in the constant temperature ensemble for (a) the zipped state and (b) the DNA hairpin. One can see the extension approaching the contour length when the force is varied.

the case of stretching of polymers [22]. However, for a temperature range, we find that these curves cross at a critical extension L_{cross}. Above this length, the force increases with the temperature [23]. In other words, to keep the extension constant, one has to apply more force because the applied force competes with the entropy of the chain.

It is now known that different ensembles may give different results in single-molecule experiments [5]. In order to see the effect of the temperature in a constant force ensemble, we plot the extension versus temperature curves in Fig. 5. At low force, the extension increases with the temperature and the chain acquires the conformation of a swollen (extended) state, with size $\sim N^{\nu}$ (here ν is the end-to-end distance exponent). In the swollen state (high temperature), its value is given by the Flory approximation $\nu \approx 3/(d+2)$ [22]. At high force and low temperature the system attains the stretched state N^{ν} with $\nu=1$ and it remains stretched up to a certain temperature. As temperature increases, the applied force is not enough to hold the stretched state and the extension falls sharply to the extended state due to the increased contribution of entropy. This is again in agreement with the experiment [14]. Since the present model incorporates the configurational entropy as well as the formation of a hairpin, we are therefore able to show the abrupt decrease in extension with temperature.

If the abrupt fall in extension is the manifestation of entropy, then what is the role of base-pairing energy? To answer this question, in Fig. 6 we also plot the extension versus temperature curve for a situation in which the base-pairing

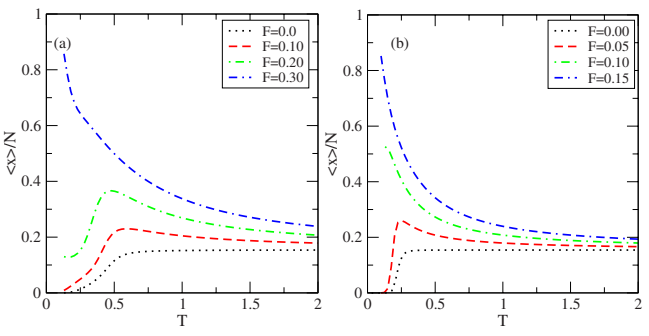

FIG. 5. (Color online) Same as Fig. 4 but in the constant force ensemble. The abrupt decrease in the extension is obvious from these plots.

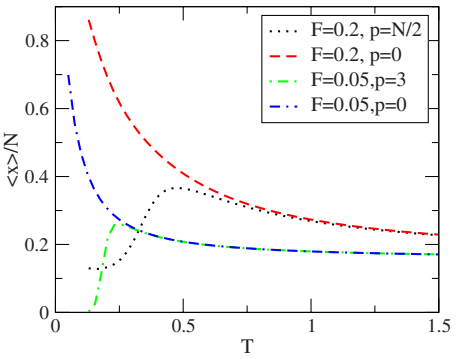

FIG. 6. (Color online) Comparison of extension vs temperature curves with and without base-pairing. For the zipped state, the number of base pairs is $N/2$, while for the hairpin it is 3. $p=0$ corresponds to the noninteracting case.

energy in the partition function is set equal to 0. This is identical to a noninteracting linear polymer chain in a good solvent. It is surprising to see that all these curves have similar behavior at high T, indicating that the chain is in a swollen state. However, the slope of the fall in extension depends on the number of base pairs p. It is pertinent to mention here that at low temperatures, if the formation of base pairing is possible, the system may again go to the zipped state as predicted by reentrance. Since in the experiment the possibility of forming a hairpin is suppressed by the solvent condition, the observed decrease is due to the entropy.

In order to rule out the possibility that the observed effect is due to the small size, we revisited the unfolding of biopolymers, where the data of 55 steps in two dimensions (2D) (sufficiently long) allowed us to settle this issue [23]. In Fig. 7(a), we plot the average extension with temperature for a noninteracting polymer (in this case, the nonbonded nearest neighbor $p=0$) and an interacting polymer ($p \approx N$). This clearly shows that the sequence does not play any role as far as a decrease in extension is concerned. However, for the interacting polymer, the fall is sharper than the noninteracting case. In Fig. 7(b), we plot the $\langle x \rangle / N$ with N for different values of N. The collapse of the curves of various lengths of polymer chain at low temperatures indicates that the chain is in the "stretched state" ($\nu=1$) and the observed decrease is not a phase transition but a crossover effect.

It would be interesting to see the variation of the entropy with temperature, which can be calculated for the unfolding of biopolymers [23] from the following expressions:

$$A = -T \ln Z_N(T), \tag{3}$$

$$S = -\left(\frac{\partial A}{\partial T}\right), \tag{4}$$

where A is the Helmholtz free energy [23,29] and we have set the Boltzmann constant $k_B=1$. In Fig. 8, we show the variation of entropy with temperature for different values of F. It is evident from this plot, at high force ($F=1.2$) and low temperature, that the chain is in the stretched state. Entropy associated with this state is nearly equal to zero. Rise in temperature brings the system to the high entropic state, i.e.,

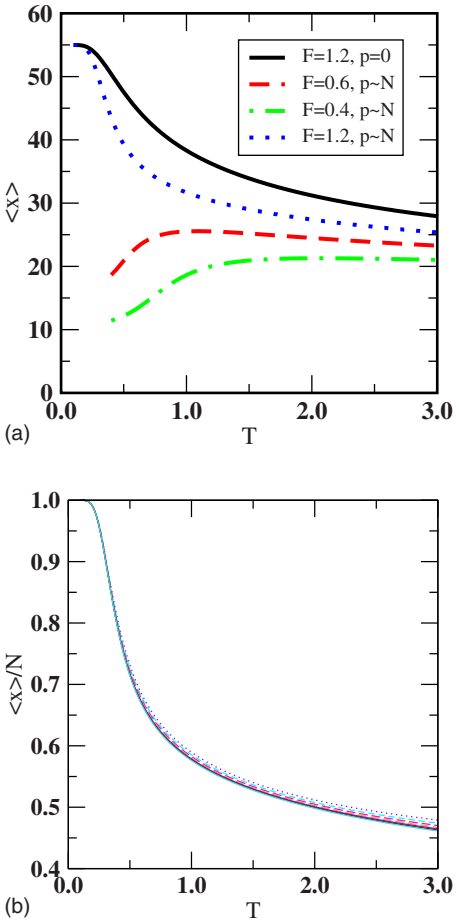

FIG. 7. (Color online) (a) Extension vs temperature curves in 2D for interacting and noninteracting walks of chain length 55 in the constant force ensemble. (b) Extension vs temperature curves for different length ($N=25$, 30, 35, 40, 45, 50, and 55) at $F=1.2$. The collapse of data at low temperature indicates that the chain is in a stretched state.

the extended state. At zero force, the polymer is in the collapsed state and the entropy associated with this is high but much less than the extended state, which is reflected in Fig. 8. It can also be seen that with an increase in temperature, the system again approaches the extended state. In this figure, we have also plotted entropy just above and below the phase boundary [23] at low temperature. A sharp rise in entropy can be seen near the phase boundary around $F=1.0$.

The conversion of the reduced temperature (T) used here and real temperature (T^*) measured in experiments may be obtained through the relation $1/T=\epsilon/(kT^*)$ [11]. It may be noted that the present models are a bit different from the λ-DNA used in experiments. In the experiment, it is not known which bases are making hairpins and their positions. However, in the present case we have used two bounds by putting either the first half and the second half complementary to model the zipped case, or the first and last three complementary bases to model the DNA hairpin loop. Since reaction coordinates are different and the model is coarse-grained, a quantitative comparison is not possible. Moreover, the effect of salt concentration, the pH of the solvent, etc. are experimental parameters that have been generally ignored in

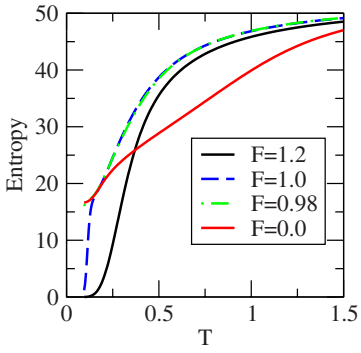

FIG. 8. (Color online) Entropy vs temperature curves in 2D for interacting walks of chain length 55 steps in the constant force ensemble. At high force $F=1.2$ and low temperature, the chain is found to be a stretched state and the entropy of the system is nearly equal to zero. As temperature increases, the system attains a high entropy state. At force equal to zero, one can see that the entropy associated with the globule is higher than the stretched state but much below the extended state. The slight variation in force $F=0.98$ and 1.0 around the phase boundary [23] shows a sharp fall in the entropy from the collapsed state to the extended state. At higher values of T, these two curves ($F=1.0$ and 0.98) overlap.

the description of the coarse-grained model. Even so, the model developed here with minimum parameters shows qualitative agreement with experiments at low as well as at high force regime, and it provides an explanation of the abrupt decrease in the extension.

It would be interesting to see experimentally whether the temperature, where an abrupt decrease in extension occurs, increases with the force. Because the model predicts that at high force the system attains the stretched state and hence is close to the upper boundary, the force should increase with the temperature. Furthermore, it would be useful to repeat these experiments for different chain lengths at higher force in order to see whether the scaling observed in Fig. 7(b) is a genuine phase transition or a crossover.

In conclusion, we have studied a simple but realistic model for ssDNA in 3D that includes the excluded volume effect, non-native base pairing, and the directional nature of the hydrogen bond. The heterogeneity, intrastrand, and interstrand interaction can be incorporated in the description of this model. It is pertinent to mention here that by replacing thymine (T) by uracil (U) and considering intrastrand and interstrand interaction apart from base pairing, the model may be extended to study the unfolding of RNA [30], which is considered as a step toward the understanding of protein folding. We have shown that the formation of a hairpin loop gives rise to the existence of reentrance in ssDNA. In the constant force ensemble, the system attains the swollen state (entropy-dominated state), while in the constant temperature ensemble, it acquires the stretched state (force-induced state). Our results are consistent with the experiment and indicate that the observed decrease in extension with temperature may be observed in other SMFS experiments such as protein unfolding, RNA unfolding, and DNA unzipping.

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