

Stretching of a single-stranded DNA: Evidence for structural transition

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Recent experiments have shown that the force-extension (F-x) curve for single-stranded DNA (ssDNA) consisting only of adenine [poly(dA)] is significantly different from thymine [poly(dT)]. Here, we show that the base stacking interaction is not sufficient to describe the F-x curves as seen in the experiments. A reduction in the reaction coordinate arising from the formation of helix at low forces and an increase in the distance between consecutive phosphates of unstacked bases in the stretched state at high force in the proposed model qualitatively reproduce the experimentally observed features. The multistep plateau in the F-x curve is a signature of structural change in ssDNA.

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The last ten years have witnessed a revolution in single molecule force spectroscopy experiments involving the manipulation of single biomolecules. These experiments allowed detection of inter- and intramolecular forces and their influence on the properties observable at the chain length of biomolecules [1–4]. New challenges have emerged when the semimicroscopic changes in the monomer (nucleotide) found to influence the elastic property of the single-stranded DNA (ssDNA). Attempts have been made to monitor the force-extension (F-x) curves [5,6] of RNA and ssDNA consisting of only one type of nucleotide. It was found that the elastic properties of ssDNA made up with adenine [poly(dA)] are significantly different from thymine [poly(dT)] (or uracil [poly(rU)]). It is known that base stacking is strongest among adenine and weakest among thymine and uracil [7]. As a result, base stacking favors a parallel orientation of consecutive bases in poly(dA), but not in poly(dT) or poly(rU) [8,9]. Notably, theoretical models developed for such studies do not include orientation of bases in their description [10–13] and hence provide a limited picture of the system.

The F-x curves for poly(dT) and poly(rU) show the effect of entropic elasticity, whereas poly(rA) exhibits plateau in the F-x curve [5] which was found to be absent in earlier experiments [1,14,15]. In a recent experiment, Ke *et al.* [6] found the existence of multistep plateau in case of poly(dA). The first plateau occurs at a force 23 ± 1 pN and overstretched the nucleotide by $\sim 74\%$ which has been predicted by the model proposed by Buhot and Halperin [16]. This prediction has also been observed in case of homopolymeric RNA [5]. A plateau obtained in case of ssRNA [poly(rA)] has been explained on the basis of seven parameters [5] in the Zimm-Bragg model [17]. The qualitative understanding of the first plateau has been achieved in terms of the unwinding of helical structure of poly(A) arising due to base stacking. This transition appears to be weakly cooperative, but needs further attention from the statistical-mechanics point of view [5,16,18]. Moreover, the second plateau which occurs at a force 113 ± 1 pN and overstretches ssDNA by an additional 16% [6] was not predicted by the model proposed by Buhot and Halperin [16]. Though Ke *et al.* [6] conjectured that the second plateau is associated with structural transition [19], high level *ab initio* quantum-mechanical calculation did not support it [6,20].

We adopt a more realistic model of DNA where directionality of bases has been included apart from excluded volume and non-native base pairing interaction to study the F-x curve. In order to study the consequence of orientation of bases, we allow bases to orient along phosphate bonds in the model [9]. If two bases are parallel to each other, we say bases are stacked and associate an attractive interaction ϵ between them. The issue of cooperativity can also be studied exactly in this model by associating a cooperativity factor (σ) between stacked and unstacked domains [17]. In this paper, we will show that only base stacking and cooperativity factor are not sufficient to describe the F-x curve, but further semimicroscopic modifications are needed. Our results based on exact enumeration provide unequivocal support that the multistep plateau is the result of structural changes in ssDNA, a field which warrants further studies.

The model discussed above [21] is general and can be defined in any dimension, though for computational limitations, we restrict ourselves in two dimensions. We consider a linear polymer chain consisting of either (A) or (T) which are described by self-avoiding walks on a square lattice. The bases are associated with the link between two monomers as depicted in Fig. 1.

The present model is a coarse-grained one where the microscopic distinction between nucleotide A and C has been ignored. Moreover, Seol *et al.* [5] showed that the normalized F-x curves for poly(rA) and poly(rC) have similar elastic response. In order to study the multistep plateau [6], we

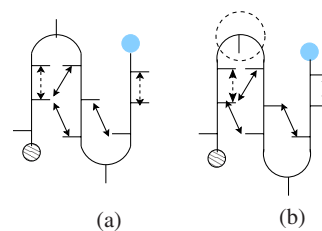


FIG. 1. (Color online) The schematic representation of ssDNA. Two types of stacking interaction may arise, namely, interstrand shown by the solid line and intrastrand shown by dashed line. In (b) we also show the steric repulsion among the adjacent bases. The small black circle indicates that one end of the ssDNA is kept fixed while a force may be applied at the other end.

consider the following cases: (i) In absence of the stacking interaction, bases are free to orient and the ssDNA (or RNA) behaves like a flexible chain. This will correspond to the case of poly(dT) [or poly(rU)]; (ii) due to the stacking interaction, bases can stack at low temperature which may represent the case of poly(dA) [or poly(rA)] [5]. With these constraints, we enumerate all possible conformations of the ssDNA. The partition function (Z_N) of the system can be written as

$$Z_N = \sum_{\text{all walks}} C(N_1, N_2, x) e^{\beta N_1 \epsilon} e^{\beta F x} e^{-\beta N_2 \epsilon_w}, \quad (1)$$

where $\beta = \frac{1}{k_B T}$. Here, ϵ_w , T , F , and k_B are the wall energy, temperature, applied force, and the Boltzmann constant, respectively. $C(N_1, N_2, x)$ is the number of distinct conformations of walk of length N . N_1 and N_2 are the number of intrastacked bases and the number of walls between stacked and unstacked regions of the chain whose ends are at a distance x apart. $\sigma (= e^{-\beta \epsilon_w})$ is the Boltzmann weight for the wall energy which is termed as a cooperative factor. The value of σ in between 0.5 to 1 corresponds to weakly cooperative, while $\sigma \rightarrow 0$ referred as strongly cooperative [17]. Time required to enumerate these conformations increases as $\mu^{N_2 N}$ because of extra degree of freedom associated with bases (two possible orientations) [10,22]. Here μ is the connectivity constant of the lattice. The model also takes care of steric effect among adjacent bases and conformations such as the one shown in Fig. 1(b) have not been incorporated in the partition function. The quantity of experimental interest, i.e., the reaction coordinate [1,16] in this case, is the extension in the chain, which can be obtained from the expression $\langle x \rangle = \frac{1}{Z_N} \sum_{N_1, N_2, x} x C(N_1, N_2, x) e^{\beta N_1 \epsilon} e^{\beta F x} e^{-\beta N_2 \epsilon_w}$.

In the following, we set $k_B = 1$ and plot the F-x curves for different values of ϵ in Fig. 2(a). In absence of stacking interaction ($\epsilon = 0$), we reproduce the entropic response of the chain as seen in case of poly(rU) [or poly(dT)] [5,6]. In Fig. 2(a) one can see that the curve for poly(rA) shifts to the right of poly(rU). Moreover to keep extension, say, at 0.8, one requires less force for poly(rA) compared to poly(rU). In Fig. 2(b), we plot the experimental curve obtained by Seol *et al.* [5]. It is evident that curve for poly(rA) shifts to the left of poly(rU) and to keep extension say 1200 nm, one requires almost the double force than the poly(rU). This indicates that apart from the stacking interaction and cooperativity there must be some other mechanism involved in the elasticity of poly(rA). It was proposed [16] that since base stacking favors formation of helices, the effective end-to-end distance (R') decreases as shown in Fig. 3(b). Since we have the exact information about the number of stacked bases (N_1) participating in the formation of helix, the reduction in length can be calculated exactly in the proposed model. Following the procedure adopted in Ref. [16], we reduce the reaction coordinate x by a certain factor proportional to N_1 , i.e., $x' = x - N_1 \alpha$. The value of proportionality constant $\alpha = b/a \approx 0.63$ has been obtained by setting $a = 5.9 \text{ \AA}$ and $b = 3.7 \text{ \AA}$ [5,16]. Here a and b are the interphosphate distance and rise in length of helix per nucleotide, respectively. The modified F-x curve, with this constraint [23], has been shown in Fig. 2(c). Surprisingly this consideration not only shifts

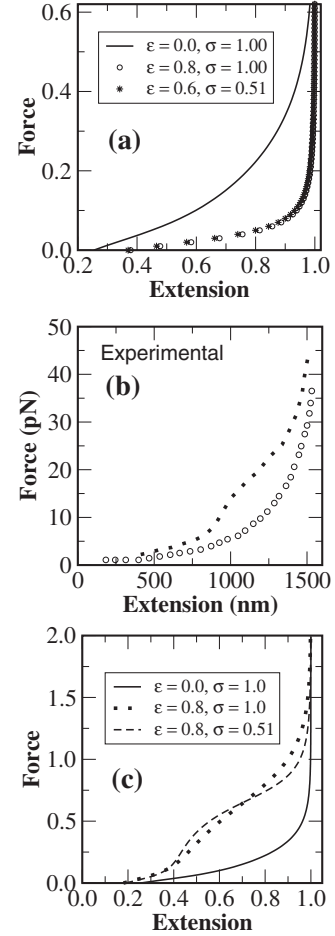


FIG. 2. (a) F-x curve for the model ssDNA for different values of ϵ . $\epsilon = 0$ corresponds to poly(t) [or poly(u)], which shows entropic response [5,6]. $\epsilon \neq 0$ represents the case where stacking play a role in the formation of helix (e.g., adenine); (b) experimental F-x curve [5] for the poly(rA). Here F is in pN. (c) Figure shows the F-x curve obtained from the model which includes formation of helix (dotted line). In (a) and (c), force and extension are in the reduced unit.

the curve to the left as seen in experiment (earlier shifting to the right in absence of helix), but also qualitatively reproduces the F-x curve obtained by Seol *et al.* [5].

In Fig. 2(a), we show that the force-extension curve obtained by using cooperativity parameter $\sigma = 0.51$ and $\epsilon = 0.6$, which overlaps with $\sigma = 1.0$ (no cooperativity) and $\epsilon = 0.8$. Therefore, stacking interaction reproduces the F-x curve for the low cooperativity. In fact, we find that the nature of curve remains almost same up to $\sigma = 0.71$. This is in accordance with earlier studies where it was also found that stacking decreases the cooperativity of melting of homopolymeric DNA [24]

When cooperativity increases, flatness of the curve [Fig. 2(c)] also increases. The reduction in reaction coordinate ($x' = x - N_1 \alpha$) along with σ modifies Eq. (1) which gives rise to such effect. From Fig. 2(c), we also note that the curve with cooperativity ($\sigma = 0.51$) crosses the curve without cooperativity ($\sigma = 1$) at the extension (≈ 0.7). We identified this as a crossover point where chain goes from the extended to the stretched state [22].

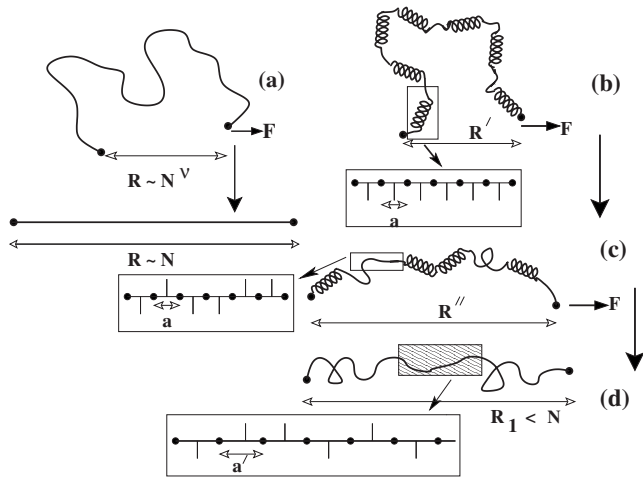


FIG. 3. (a) The schematic representation of poly(t). (b) In case of poly(a), bases can stack and form a helixlike structure. Inset shows the base stacking for specific segment; (c) shows the unwinding of helix where consecutive phosphate distance remains same. (d) Here the chain is in the stretched state where few bases are stacked. Flipping of bases in presence of high force shows the extension in the consecutive phosphate, i.e., $a' > a$.

Existence of the second plateau [Fig. 4(a)] at higher forces as seen in case of poly(dA) [6] requires further refinement in the model. At such a high force (~ 113 pN) chain must be in the stretched state [22] as shown in Fig. 3(d). It was shown [25,26] that there may be increase in distance ($\approx 18\%$) between consecutive phosphates which drive backbone to a new torsional state [19]. However 1–2 kcal/mol energy difference between two states could not reveal which conformation ($c3'$ -endo or $c2'$ -endo) poly(dA) will take under equilibrium condition. In order to see whether the second plateau is a result of structural transition, we calculate the number of unstacked bases exactly in our model which may participate in this transition. We increase the reaction coordinate by a factor proportional to the number of unstacked bases. The proportionality constant for this case has been obtained from Ref. [19]. The modified F-x curve depicted in Fig. 4(b) shows the multistep plateau which is qualitatively similar to the experimental one [6]. This implies that further increase in the extension (second plateau) is associated with structural transition. Moreover, curves for different values of $\sigma > 0.51$ almost overlaps with the curve for $\sigma = 1$ and hence the transition appears to be weakly cooperative.

To get the enhanced understanding of the structural changes, we study the probability distribution $[P(N_1)]$ of poly(dA) from the following expression (for $\sigma = 1$): $P(N_1) = \frac{1}{Z_N} \sum_{N_1, x} C_N(N_1, x) e^{BN_1 \epsilon} e^{BFx}$. In Fig. 4(c), we have shown the probability distribution for stacked bases $P(N_1)$ for different values of force at a fixed temperature $T = 0.3$ for the poly(dA). The maxima of $P(N_1)$ for $F = 0$ occurs at $N_1 \approx 15$. This represents the situation where most of the bases are stacked (helix) and domains are randomly oriented. Slight increase in the force aligns the helix along the force direction and thus the number of stacked bases increases slightly. This can be seen from Fig. 4(c) where maxima of $P(N_1)$ shift toward the right. For low forces, thermal fluctuations are too

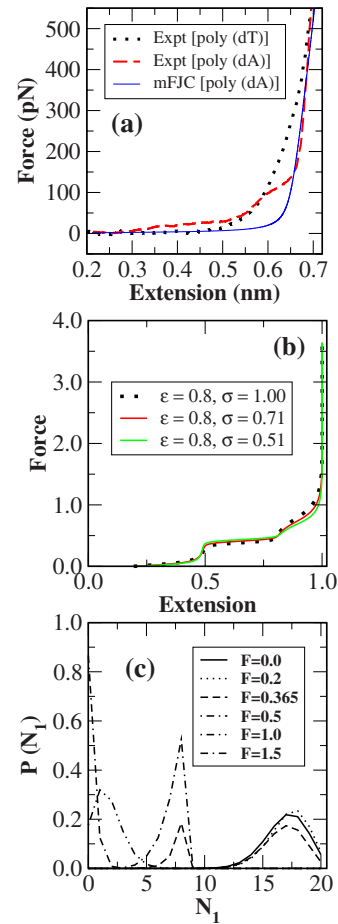


FIG. 4. (Color online) (a) Experimental F-x curve [6] for the poly(dT) [dotted] and poly(dA) [dashed] of ssDNA. Solid line is for poly(dA) using mFJC [1]. (b) shows the F-x curve where the formation of helix as well as increase in consecutive phosphate distance between bases has been taken into consideration (force and extension are in the reduced unit). One can see that inclusion of such considerations qualitatively reproduces the F-x curve obtained by Ke *et al.* [6] and exhibits the multistep plateau. (c) shows the probability distribution curves for poly(dA).

weak to unstack the bases in the strand. We observe that there is an emergence of a new peak around the value $N_1 = 8$ and decrease in the peak heights around $N_1 = 15$ at the intermediate forces ($F = 0.2 - 0.4$). The first plateau took place at $F = 0.365$ as shown in Fig. 4(b). At this force the height of both peaks are found to be equal. Two peaks of equal height in the probability distribution curve show the signature of coexistence of two phases (helix and coil). This is analogous to liquid-gas transition. Here force represents the pressure and extension is analogous to volume [18]. In this region the number of stacked bases decreases which reflects the unwinding of helix. This gives relative extension ($\sim 70\%$) in the backbone. Further rise in force (above $F = 0.5$) shifts the maxima of distribution curve toward left. This reflects that the number of stacked bases in the stretched state decreases gradually and gives additional increase in extension which is about 16% as shown in Fig. 4(b).

In order to resolve the issue associated with structural transition in poly(dA), it is essential to calculate the change

in energy associated with this transition. Ke *et al.* [6] obtained the total energy (3.6 ± 0.2 kcal/mol per base) from the area under F-x curve for adenine (Fig. 3(b) of Ref [6]) and interpreted it as base stacking energy. In fact total area under the curve [poly(dA)] should be attributed to entropic, enthalpic, structural, and elastic contributions. However, in case of F-x curve of thymine [poly(dT)], the contributions come from entropic and elastic parts only. Therefore, at low force (neglecting the elastic contribution) one can get the rough estimate of enthalpic contribution (0.6 kcal/mol per base) associated with coil-helix transition corresponding to first plateau by subtracting area under II from I below extension 0.58. This value is in agreement with the known values available in the literature [7]. However, at high force, elastic contribution for thymine is different than the adenine and hence one cannot get the energy required for structural transition from the same curves. This may be visualized that area under thymine is more than the adenine. By using the same persistence length for adenine [15], one can get F-x curve from modified freely jointed chain (mFJC) which contains only entropic and elastic contributions which are shown in Fig. 4. The energy (1.2 ± 0.2 kcal/mol per base) associated with structural transition above the extension 0.58 has been obtained by subtracting the mFJC curve from the experimental F-x curve. This value is in excellent agreement with quantum calculation [20].

We have studied effect of cooperativity in the framework of exact enumeration. Our analysis exactly showed that

stacking reduces the cooperativity [Fig. 2(a)] and F-x curve for σ up to 0.26 can be reproduced by stacking alone. For high cooperativity ($\sigma < 0.26$), stacking is not sufficient enough to compensate cooperativity factor. However, formation of helix reduced the reaction coordinate which gives an additional factor (αN_1) in the partition function. We find that this term has significant impact on cooperativity which makes plateau broader in case of adenine. Response of cooperativity on the applied forces below and above the cross-over point is also evident from Fig. 2(c).

The exact results on short chain of a semimicroscopic model presented here provide unequivocal support for the structural transition. This has been substantiated by the correct analysis of experimental curve along with the mFJC curve that gives the required energy associated with structural transition which is in good agreement with quantum calculation. This also resolves that c3'-endo pucker conformation is most likely in the equilibrium condition and at high force poly(dA) goes from c3'-endo to c2'-endo conformation.

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