Statistical Mechanics of DNA Unzipping under Periodic Force: Scaling Behavior of Hysteresis Loops

Sanjay Kumar and Garima Mishra
Department of Physics, Banaras Hindu University, Varanasi 221 005, India
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A simple model of DNA based on two interacting polymers has been used to study the unzipping of a double stranded DNA subjected to a periodic force. We propose a dynamical transition where, without changing the physiological condition, it is possible to bring DNA from the zipped or unzipped state to a new dynamic (hysteretic) state by varying the frequency of the applied force. Our studies reveal that the area of the hysteresis loop grows with the same exponents as of the isotropic spin systems. These exponents are amenable to verification in the force spectroscopic experiments.

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The mechanism involved in the separation of a double stranded (ds) DNA into two single stranded (ss) DNA is a prerequisite for understanding processes like replication and transcription. *In vitro*, the opening of DNA is achieved either by increasing the temperature (85–90 °C) termed as thermal melting or by changing the pH value of the solvent, called DNA denaturation [1]. However, such a drastic change in the physiological condition is not possible in living systems. The mechanism of the opening of dsDNA *in vivo* is quite complex and is initiated by helicases, DNA and RNA polymerase, etc., which exert a force of the order of piconewtons and as a result DNA unwinds. It is now possible to unzip the two strands of a DNA using techniques like optical tweezers, magnetic tweezers, etc. [2,3]. The theoretical understanding of unzipping is mostly based on equilibrium conditions [4–7].

However, living systems are open systems and never at equilibrium. Understanding the separation of DNA in equilibrium is one approach, but another route is to perform the analysis in a situation which closely resembles the living systems, i.e., in nonequilibrium conditions. Moreover, helicases are adenosine triphosphate (ATP) driven molecular motors. The periodic hydrolysis of ATP to adenosine diphosphate (ADP) can generate a continuous push and pull kind of motion. As a direct consequence of these chemomechanical cycles, biological machines act like repetitive force generators, and it is believed that forces with periodic signatures are experienced by biomolecules in many physiological contexts. For instance, it has been postulated that DNA-B, a ringlike hexameric helicase, pushes through the DNA like a wedge and produces unidirectional motion and strand separation [8]. The active rolling model and the inchworm model are two mechanisms which suggest that plasmid copy reduced (PcrA) goes through a cycle of pulling the ds part of the DNA and then moving on the ss part during ATP hydrolysis [9]. Viral RNA helicase NPH-II hops cyclically from the ds to the ss part of DNA and back during the ATP hydrolysis cycle [10]. There are several studies [11–14], which suggest that the force acting on DNA (at the junction of the Y fork, i.e., ssDNA and dsDNA) is periodic in nature rather than constant. Surprisingly, in most of the studies, the applied force or loading rate is kept constant [15], and hence the results provide a limited picture of the unzipping. The application of a periodic force would introduce new aspects, which are not possible in the steady force case.

In DNA unzipping, the equilibrium response of the reaction coordinate (extension y) to the constant force is well understood [4–7,15–17]. However, when a dsDNA is driven by an oscillatory force, y will also oscillate and lag behind the force due to the relaxation delay. This relaxation delay induces hysteresis in the force-extension ($f - y$) curve, which has been recently observed in simulations and experiments [18–20]. The nature of hysteresis and its dependence on the amplitude F and frequency ν of the applied force is well studied in the context of spin systems [21–24]. It is found that the area under the hysteresis loop $A_{\rm loop}$ scales as $F^{\alpha}\nu^{\beta}$. The values of α and β differ from system to system [24]. However, for DNA unzipping, the nonequilibrium response of y to the oscillatory force remains elusive.

In this Letter, we show that under a certain physiological condition, a dsDNA remains in the steady and stable (zipped or open) state for an extended period of time. Furthermore, without any change in temperature T or pH of the solvent, by varying ν alone, a dsDNA may be brought from the time averaged open or zipped state to a new dynamic (hysteretic) state, oscillating between the zipped and unzipped states, which is dynamical in origin and vanishes in the quasistatic limit [24]. We evaluate the scaling exponents α and β associated with $A_{\rm loop}$, which are amenable to verification in the force spectroscopic experiments. We also show that using the work theorem [25], it is possible to extract the equilibrium $f - y$ curve from the nonequilibrium pathways.

We consider DNA as a string of beads (see Fig. 1) with restrictive (native) base pairing among complementary nucleotides. The model captures some of the essential

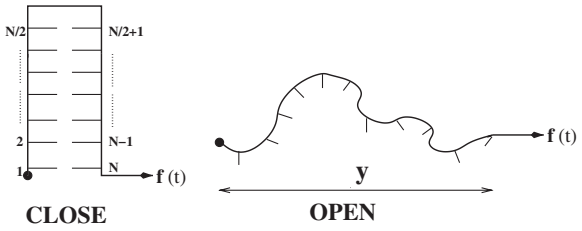

FIG. 1. DNA in zipped and unzipped state. One end is fixed and the other end is subjected to a periodic force.

properties of DNA and the equilibrium force-temperature diagram is in good agreement with the two state model in the entire range of f and T [18]. In order to study the dynamical stability of DNA under the periodic force $f(t)$, we add an energy $-f(t)y(t)$ to the total energy of the system and perform a Langevin dynamics (LD) simulation to monitor the separation y of the terminal base pairs (see the Supplemental Material [26] and Ref. [27]). The random force Γ (see the Supplemental Material [26] and Ref. [27]), has also been superimposed on the periodic force to take account of stochastic fluctuations of the system. Here, one may fix ν and vary F or vice versa. The value of f increases to its maximum value F in m_s steps at interval $\Delta f(= 0.01)$ and then it is taken to 0 in the same way [27]. Since, we are interested in the nonequilibrium regime, we allow only n LD time steps ($\ll$ equilibrium time) in each increment of Δf. We keep a sum of the time spent $\tau(= 2nm_s)$ in each force cycle constant to keep $\nu(= 1/\tau)$ constant. In the following, we keep $T = 0.1$ and $F > 0.32$ [28,29].

In Fig. 2, we plot the value of $\langle y(f)\rangle$ (averaged over $C = 1000$ cycles) with f for different values of ν. It is interesting to note that $\langle y(f)\rangle$ for different initial conformations remains almost the same, showing that the system is in the steady state [32]. All plots show hysteresis. The area of the loop is the measure of the energy dissipated over a cycle and is defined as a dynamic order parameter [24] $A_{\text{loop}} = \oint y df$, which depends upon F and ν. If $\langle y(f)\rangle$ is less than 5, we consider the system to be in the zipped state, whereas if $\langle y(f)\rangle > 5$, it is in the unzipped state [27]. At high ν, it is evident that for small F, dsDNA remains in the zipped state [see Fig. 2(a)], whereas at high F, it is in the unzipped state [see Fig. 2(b)], irrespective of initial conformations. Moreover, the path of $y(f)$ for the force 0 to F is different from that of the path for F to 0, which constitutes a hysteresis loop. A decrease in ν leads to a bigger path of the hysteresis loop (see the Supplemental Material [26]). Depending on the amplitude, the system starts from the zipped conformation as shown in Fig. 2(a) (or open conformations shown in Fig. 2(b)) and then gradually approaches the open state (or the zipped state) and back to the initial state.

One may note that even though f decreases from F to 0 [see Fig. 2(a)], $y(f)$ increases and there is some lag, after which it decreases. Recall that the relaxation time is much higher compared to the time spent at each interval of Δf. Therefore, an increase in $y(f)$ with decreasing f indicates that the system gets more time to relax. As a result $y(f)$ approaches a path which is close to equilibrium. Once the system gets enough time, the lag disappears. A similar lag is expected when the system starts from the open state at high ν. However, in this case as ν decreases, $y(f)$ decreases with increasing f [see Fig. 2(b)]. In both cases, whether dsDNA starts from the zipped or open state, as $\nu \to 0$ the system approaches the equilibrium $f - y$ curve and A_{loop} vanishes (see the Supplemental Material [26]). Moreover, at high ν, A_{loop} also vanishes [see Figs. 2(a) and 2(b)], but the system goes away from the equilibrium. The other dynamic order parameter $Q = 1/\tau \oint y(t)dt$, studied in the context of magnetic systems [24], has recently been applied to obtain the $F - \nu$ diagram of a DNA hairpin [27]. In Fig. 3, we plot Q with cycles for different ν and F. The distribution shows that the path remains in the zipped or open or dynamic (hysteretic) state, depending on F and ν. In contrast to the DNA hairpin, which shows the coexistence of different states, a dsDNA shows a continuous

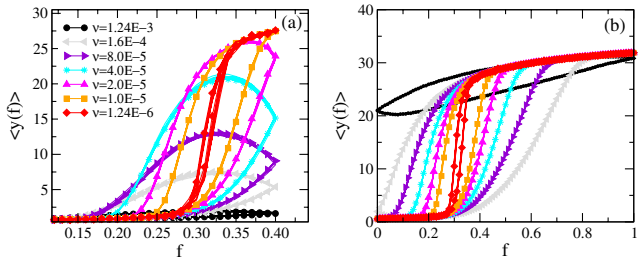

FIG. 2 (color online). $\langle y\rangle$ of DNA as a function of the cyclic force of amplitudes (a) 0.4 and (b) 1.0 at different ν. (a) At high ν, DNA remains in the zipped state with a small hysteresis loop. As ν decreases, the system extends from the zipped state to the open state with a bigger loop. For $\nu \to 0$, the hysteresis loop vanishes and the system approaches the equilibrium path. (b) DNA remains in the open state at high ν and approaches the equilibrium path from below as $\nu \to 0$.

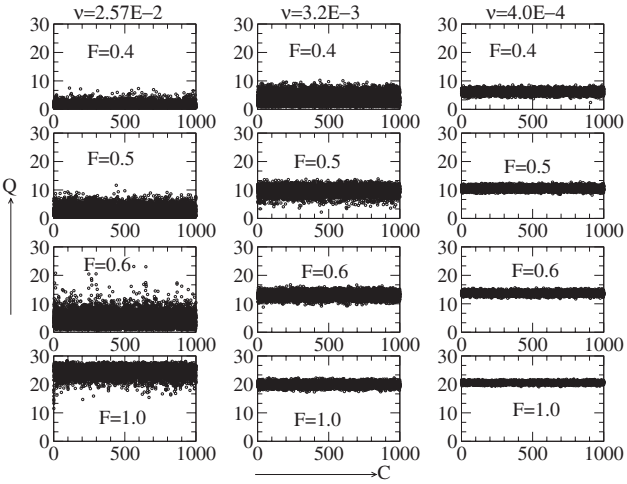

FIG. 3. The time sequence of Q for different ν and F.

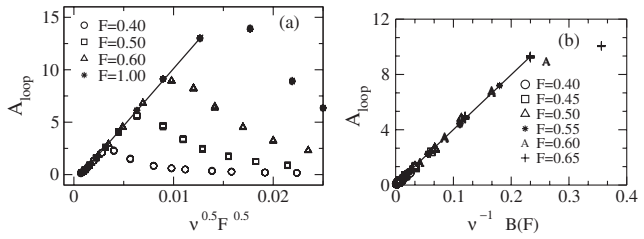

FIG. 4. The scaling of the loop area of hysteresis (A_{loop}) with respect to $\nu^{0.5}F^{0.5}$ in the low frequency limit (a) and with respect to $\nu^{-1}B(F)$ in the high frequency limit. Here, $B(F) \sim (F - f_c)^{2\pm0.1}$ (b).

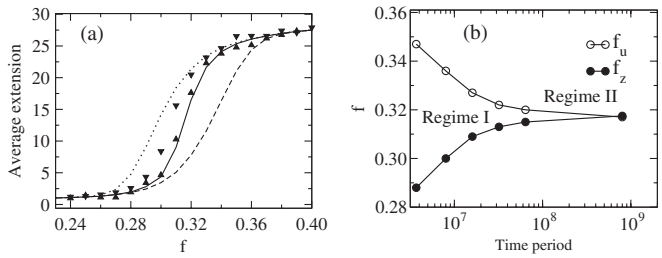

FIG. 5. (a) The variation of the average extension as a function of force f. Dotted and dashed lines correspond to simple average of backward and forward paths, respectively. The weighted averages of backward and forward paths are shown by up and down triangles, respectively. The solid line represents the equilibrium $f - y$ curve. (b) Simulated values of unzipping force f_u and rezipping force f_z as a function of time. I and II correspond to the nonequilibrium and equilibrium regime.

transition from the zipped state to the new dynamic state as frequency decreases.

We now focus on the scaling of A_{loop}. In Fig. 4(a), we have plotted A_{loop} as a function of $(F\nu)^{0.5}$. For low ν, all plots for different F collapsed on a straight line. This gives the value of $\alpha = 0.5 = \beta$. At high ν, depending on the amplitude, the system remains either in the zipped state (low F) or in the open state (high F). In contrast to the spin system, where the average applied field is zero over a cycle, here the average applied force is finite over a cycle because the two states are asymmetric. In fact, at low F, we find that A_{loop} scales as $\nu^{-1}(F - f_c)^{2.0\pm0.1}$, where f_c is the equilibrium critical force at that temperature. The proposed scaling is consistent with the mean field values for a time dependent hysteretic response to a periodic force in the case of the isotropic spin [23] and is found to be independent of length [33].

In single-molecule experiments, measurements are taken at nonequilibrium conditions. It is possible to infer the equilibrium properties of the system from these data. For this, measurements have been taken in the quasistatic limit [3] so that the techniques involved in thermodynamics can be employed. There is considerable work to extract equilibrium properties from the nonequilibrium data, e.g., the Jarzynski equality which relates the free energy differences between two equilibrium states through nonequilibrium processes [34], a dominant reaction pathway algorithm which computes the most probable reaction pathways between two equilibrium states [35], etc.

Here, we use the work theorem to derive the equilibrium path between the two states [25]. Instead of repeating the force cycle C times, we now randomly choose C initial conformations, which belong to equilibrium conformations at that T and $f(= 0)$. We follow a similar protocol as described above to reach the final state ($f = F$) from the initial state ($f = 0$). No attempt is made to achieve equilibrium during this process. The total work performed on the system going from the zipped to open state (forward path) is $w_{m_s} = -\Delta f \sum_{i=1}^{m_s} y_i$. When the applied force decreases (backward path) from F to 0, we start with C initial conformations, which belong to equilibrium conformations at that T and $f(= F)$. The work done by the system from the open to zipped state can be written as $w_1 = \Delta f \sum_{i=m_s}^{1} y_i$. The equilibrium distance y_k for the force f_k for the forward path can be obtained by assigning the weight $\exp(-\beta w_k)$ to all forward C paths [25] at that instant k, which can be written as

$$y_k = \frac{\sum_i^C y_k \exp(-\beta w_k)}{\sum_i^C \exp(-\beta w_k)}. \quad (1)$$

Similarly, y_k for the reverse path can also be obtained.

Figure 5(a) shows the simple average of extension over many ($C = 1000$) forward paths as well as backward paths ($n = 10^4$ LD time steps) and the existence of hysteresis. For equilibrium, we have used 2×10^9 time steps out of which the first 5×10^8 steps have not been taken in the averaging. The results are averaged over many trajectories, which are almost the same within the standard deviation. The weighted averages of $y(f)$ for the forward and the backward paths obtained from Eq. (1) have also been depicted in this plot. One can see from these plots that the weighted average even for $n = 10^4$ LD steps is quite close to the equilibrium (solid line) path (1.5×10^9 LD steps). We further note that the weighted average of the backward path almost overlaps with the equilibrium path. Since, two strands of DNA are in the open state, therefore, the system can access more configurational space. This gives the higher probability of choosing rare conformations, which have dominant contributions in Eq. (1). The underlying assumption behind the work theorem relies on the fact that the initial state of the system should be in the thermal equilibrium. Whereas for the scaling, the system need not be in equilibrium, but in the steady state. Moreover, scaling involves frequency, whereas the equilibrium path obtained from the work theorem is independent of frequency.

Chattopadhyay and Marenduzzo [36] studied the dynamics of a polymer chain whose ends are anchored. An oscillatory force was applied at the intermediate bead. They also observed hysteresis for the flexible polymer chain. However, they showed a crossover from a periodic limit cycle (hysteresis) to an aperiodic dynamics as the polymer gets stiffer. Since the unzipping experiments

[3,19] usually are performed on a long chain (few kilo base pairs) much greater than the persistence length of DNA, their model studies [36] also imply the existence of hysteresis under a periodic force [37].

Are the dynamic transition and the scaling proposed here observable in single molecule experiments? To answer this, in Fig. 5(b) we show how the system approaches equilibrium (regime II) from nonequilibrium (regime I). This is in accordance with experiment followed by simulation [19]. For a two state model, the time needed to cross the energy barrier $\Delta E(10\text{–}20k_BT)$ depending upon the length and sequence of DNA lies between 4 s and 15 min [38]. The equilibrium response of DNA unzipping (regime II), which has been studied in experiments, belongs to this time scale [3,39] as we obtained in our simulation but in the microsecond range. There is a mismatch in the time scale because of the coarse grained description of the model. One of the possible ways to check the feasibility of the experiment from our simulation is to compare the ratio of time needed for the equilibrium (shown in Fig. 2 by the diamonds) and the nonequilibrium regime (say the filled circle in Fig. 2). From our simulation, this ratio turns out to be $\sim$1000. If the experimental equilibrium time is 900 s [3] then the lower limit of time is $900/1000 \sim 1$ s. Hence, by manipulating the amplitude and the frequency in the intermediate time scale (1 s–15 min), it is possible to perform experiments where the dynamical transition may take place.

In conclusion, we have studied DNA unzipping under a periodic force. We showed the existence of a dynamic transition, where by varying ν, a dsDNA can go from the zipped or unzipped state to a new hysteretic state. We find A_{loop} scales with the same exponents as of spin systems. The scaling exponents are found to be quite robust and independent of length [33] and friction coefficient (see the Supplemental Material [26]). Using the work theorem, we extracted the equilibrium properties of the system from the nonequilibrium data. At this stage, additional investigations are needed to establish a connection between the dynamical transition in the spin systems and a polymer under a periodic force. Since, the role of hysteresis in biological processes remains unexplored territory, our work calls for further experiments on periodically driven DNA to explore such hitherto unknown dynamical phase transitions and related scaling.

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$\sim$308 g/mol and $\sigma = 5.17$ Å, we get $t \approx 3.0\ t^*$ ps. However, this conversion does not work in the entire range of f and T [27].

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