A Motor that Makes Its Own Track: Helicase Unwinding of DNA

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We study the unwinding of DNA by helicase proteins as a representative system in which a motor protein interacts with a mobile obstacle. In our discrete model, the interaction between the helicase and the DNA fork is characterized by an interaction potential. For the case of a hard-wall potential, the helicase opens the DNA by rectifying thermal fluctuations which spontaneously open base pairs. A potential with nonzero range describes the destabilization of the double strand by the enzymatic action of the helicase. We derive solutions for the opening speed as a function of the potential shape and relate our results to experiments on helicase motion.

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Many active processes in living cells require motion and the generation of forces. Motor proteins which move along cytoskeletal filaments have been extensively studied as a prototype system for force generation on the molecular scale [1]. The energy released from breaking a covalent bond (hydrolysis of adenosine-triphosphate, or ATP) drives this motion, while the direction of motion is defined by polar asymmetry of the filaments.

A number of motor proteins operate on DNA molecules. Examples include polymerases, which duplicate and transcribe the base sequence [2], and helicases, which separate a double strand into two single strands [3,4]. Because single-stranded (ss) DNA is polar, the helicases we consider here can use the direction defined by the sugar phosphate backbone to advance along ssDNA. For example, a 3'–5' helicase moves towards the 5' end of a ssDNA strand. While the helicase advances, it may encounter a ss-ds junction where two complementary single strands meet to form a double strand. The junction is an obstacle to this type of helicase, which cannot move along the double strand. The helicase transduces the energy of ATP hydrolysis to move the ss-ds junction and thus unwind the double strand [5]. During this process, the helicase moves forward on the additional "track" it has created by advancing the ss-ds junction (Fig. 1).

In this Letter, we discuss the principles governing a motor which advances against a mobile obstacle. Helicase unwinding of DNA is an important example of this more general process. The position of the motor (the helicase) is labeled by the integer n (indexing the base to which the helicase is bound). Similarly, we denote by m the position of the obstacle (the ss-ds junction). Since the helicase cannot move on the dsDNA, we expect $n \leq m$ (if the helicase moves toward increasing n). The interaction between helicase and the junction can be characterized by an interaction potential $U(m - n)$ which depends only on the separation of the helicase and the junction along the DNA. The simplest such potential is a hard wall with $U = 0$ for $m > n$ and $U = \infty$ for $m \leq n$, to ensure that the helicase does not move on the dsDNA. For a hard-wall potential, the helicase which is near the junction ($n = m - 1$) can advance only if the DNA opens due to a fluctuation which increases m. In this case, motion generation is limited by the requirement that fluctuations of a particular size occur. This situation has been termed the "thermal ratchet" in earlier work. In the context of polymerization forces against an obstacle, Peskin *et al.* argued that the rate of polymerization is limited by the time required for the obstacle to diffuse one monomer size [6]. This scenario corresponds to a hard-wall interaction potential between the growing tip and the obstacle.

In general, the interaction between a motor and an obstacle has nonzero range. For example, the presence of the helicase near the junction influences both the kinetics of DNA opening and the helicase motion. The enzymatic action of the helicase may directly destabilize the dsDNA by changing the DNA conformation upon binding. In this scenario, the helicase acts as a catalyst which facilitates dsDNA opening when bound near the junction. We study the interplay between such enzymatic activity and motion generation, and derive analytical expressions for the opening speed for different potentials. We discuss the maximal speed that can be reached by

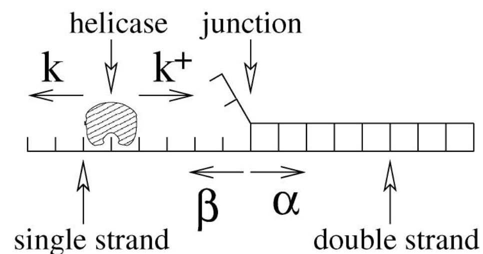

FIG. 1. Schematic of a helicase moving along DNA. Motion is described by hopping rates k^+ and k^- (helicase) and α and β (junction).

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catalytic assistance of opening and compare our results to experimental observations of helicase motion on ssDNA and unwinding of dsDNA.

Our description is based on the rates of forward and backward hopping, k^+ and k^-, for the helicase moving on ssDNA far from the ss-ds junction. (We average over sequence-induced variations so that all rates are sequence independent.) The hopping rates for forward and backward motion of the junction in the absence of the helicase are denoted α and β. The ratio of opening and closing rates of the DNA is given by $\alpha/\beta = e^{-E/k_BT}$ where E is the free-energy difference per base between dsDNA and two complementary ssDNA strands; this energy difference is positive when dsDNA is thermodynamically stable. For simplicity, we write a similar expression for the ratio of forward and backward hopping rates of the helicase, $k^+/k^- = e^{\Delta\mu/k_BT}$. Here, $\Delta\mu$ denotes the chemical free energy of the ATP hydrolysis which drives helicase motion. This expression applies when each hydrolysis event is strongly coupled to a forward step on the DNA, as suggested by translocation experiments on PcrA helicase on ssDNA [7,8]. The tight-coupling assumption may not apply to all helicases.

The interaction between the helicase and the ss-ds junction is described by the interaction energy $U(m-n)$. The rates are modified according to

$$\frac{\beta_j}{\alpha_{j-1}} = \frac{\beta}{\alpha} e^{-[U(j-1)-U(j)]/k_BT}, \tag{1}$$

$$\frac{k_j^+}{k_{j-1}^-} = \frac{k^+}{k^-} e^{-[U(j-1)-U(j)]/k_BT}, \tag{2}$$

where α_j, β_j, k_j^+, and k_j^- are the position-dependent rates when the helicase and the junction are separated by $j = m - n$ bases.

For the simple case of a hard-wall potential [Fig. 2(a)], we see that $\beta_1 = 0$ if $U(0)$ is infinite. In this case, the helicase prevents DNA closing when $j = 1$ (or $m = n + 1$). Of course, infinite $U(0)$ also implies $k_1^+ = 0$: the helicase must wait until a thermal fluctuation opens the DNA before advancing.

A more realistic interaction potential has nonzero range and corresponds to enzymatically assisted opening.

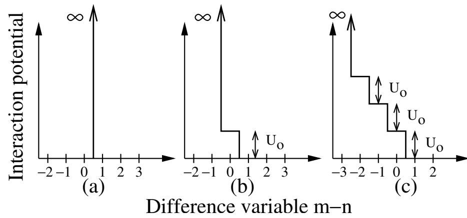

FIG. 2. Interaction energies between the helicase and ss-ds junction as a function of their distance $m - n$ in number of bases. (a) Hard-wall potential. (b) Potential with a single step. (c) Potential with three steps.

For simplicity, we use in our discrete description potential shapes as shown in Figs. 2(b) and 2(c), where the potential energy increases in N steps, each of energy U_0, before a hard wall is reached. This allows the helicase to take N steps beyond the point $m = n$. The increase in energy due to this potential facilitates opening of the dsDNA: $U(j-1) > U(j)$ implies $\beta_j/\alpha_{j-1} < \beta/\alpha$. In addition, the interaction energy slows helicase forward motion. We show below that this trade-off leads to an optimal choice of interaction potential for fast unwinding. Note that we require (i) $U \to 0$ for $j \to \infty$ and (ii) $U \to \infty$ for $j \to -\infty$. The former condition implies that no interaction occurs for large separations, and the latter ensures that the helicase always retains some "footing" on the ssDNA. The helicase thus becomes localized in the vicinity of the junction.

Having defined the local rates, we can write the equation for the probability $P(j, l, t)$ that the helicase and junction are at separation $j = m - n$ and midpoint position $l = m + n$. The probability distribution satisfies

$$\begin{aligned}\frac{\partial P(j,l)}{\partial t} = &-(\alpha_j + \beta_j + k_j^+ + k_j^-)P(j,l) \\ &+ \alpha_{j-1}P(j-1,l-1) + \beta_{j+1}P(j+1,l+1) \\ &+ k_{j+1}^+P(j+1,l-1) + k_{j-1}^-P(j-1,l+1).\end{aligned} \tag{3}$$

Since the rates depend only on j, we can sum over l to obtain the distribution $\mathcal{P}_j = \sum_l P(j,l)$. This difference-variable probability relaxes to its stationary distribution within a characteristic relaxation time which depends on the rates α, β, k^+, and k^-. Since the potential diverges as $j \to -\infty$, the stationary probability distribution satisfies the recursion relation

$$\mathcal{P}_{j+1} = \frac{k_j^- + \alpha_j}{k_{j+1}^+ + \beta_{j+1}} \mathcal{P}_j. \tag{4}$$

The distribution $\mathcal{P}_j$ can be used to calculate the mean velocity (bp/sec) of DNA opening

$$v = \frac{1}{2}\sum_j (k_j^+ + \alpha_j - k_j^- - \beta_j)\mathcal{P}_j. \tag{5}$$

Note that v is the steady-state current in the midpoint variable l. This expression for v has a simple physical interpretation—the quantity in parentheses is the unwinding rate at separation j, which is multiplied by the probability $\mathcal{P}_j$ of finding the complex at separation j. The effective diffusion coefficient which characterizes velocity fluctuations is

$$D = \frac{1}{4}\sum_j (k_j^+ + \alpha_j + k_j^- + \beta_j)\mathcal{P}_j. \tag{6}$$

A hard-wall potential ($N = 0$) with the wall at $j = 0$ has $k_1^+ = \beta_1 = 0$. Then the probability distribution for

$j > 0$ is $\mathcal{P}_j = Ac^j$, where $c = (\alpha + k^-)/(\beta + k^+)$ and A is a normalization constant. The average velocity has the simple form

$$v_{HW} = \frac{\alpha k^+ - \beta k^-}{\beta + k^+}. \quad (7)$$

This expression corresponds to the force-velocity relation for polymerization derived in Ref. [6]. The velocity is positive whenever $k^+/k^- > \beta/\alpha$, that is, the free-energy change E which drives DNA closing must be smaller than the free-energy change $\Delta\mu$ of ATP hydrolysis.

We now consider the case $N = 1$ for which the potential has one step of height $U_o = [U_0 - U(1)]/k_B T$ at $j = 0$ and a hard wall at $j = -1$ [Fig. 2(b)]. The junction and helicase can overlap if the energetic cost U_0 is paid. Equations (1) and (2) determine only the ratios of the rates. The individual rates depend on the energy barrier that separates the states with energy $U(1)$ and $U(0)$. The effect of this energy barrier associated with the transition can be represented by a dimensionless coefficient f with $0 < f < 1$ [9].

$$k_1^+ = k^+ e^{-fU_0}, \quad (8)$$

$$k_0^- = k^- e^{-(f-1)U_0}, \quad (9)$$

$$\beta_1 = \beta e^{-fU_0}, \quad (10)$$

$$\alpha_0 = \alpha e^{-(f-1)U_0}. \quad (11)$$

Since the hopping rates vary exponentially with U_0, we expect a strong dependence of the velocity on the interaction potential. The velocity increase relative to the hard-wall case is

$$\frac{v_1}{v_{HW}} = \frac{c + (1-c)e^{-fU_0}}{c + (1-c)e^{-U_0}}. \quad (12)$$

with $c = (\alpha + k^-)/(\beta + k^+)$. The ratio v_1/v_{HW} varies with f as shown in Fig. 3(a). Small values of f cause a large change in the velocity. Initially, the unwinding rate increases with step height U_0 because the presence of the step facilitates DNA opening. As the step height increases further, the unwinding rate begins to decrease, because the step reduces the speed of helicase motion.

For given values of f and c, the maximum unwinding rate occurs at the step height $U_0 = U_*$ which satisfies

$$fe^{U_*} - e^{fU_*} = \frac{(1-c)(1-f)}{c}. \quad (13)$$

The catalytic activity of the helicase cannot increase the unwinding rate beyond a certain limit. Using Eq. (13) and assuming $0 < f < 1$, the unwinding rate has an upper bound $v_1 \leq c^{-1} v_{HW} = (\alpha k^+ - \beta k^-)/(\alpha + k^-)$.

We can generalize to an interaction potential with N identical steps, each of height U_0 [Fig. 2(c)]. The increase of the unwinding rate relative to the hard-wall velocity is given by

$$\frac{v_N}{v_{HW}} = \frac{c^N + (1-c)e^{-(f-1)U_0}\sum_{j=1}^N c^{N-j}e^{-jU_0}}{c^N + (1-c)\sum_{j=1}^N c^{N-j}e^{-jU_0}}, \quad (14)$$

which reduces to Eq. (12) for $N = 1$. The upper bound remains $v_N \leq c^{-1} v_{HW}$. The velocity for different N is shown in Fig. 3(b) as a function of the step height U_0. For increasing N, the opening rate is more sensitive to U_0 and the maximum occurs at higher speeds.

For large N, the maximum unwinding rate occurs for $U_* \approx -\ln c \approx -\ln \alpha/\beta$. This optimal step height approaches the free-energy change of opening of one base. Thus, fastest opening occurs when the interaction energy and the base-pairing energy of the DNA match.

In general, the step height of the potential could also be negative. Then the potential hinders unwinding because it accelerates closing of the DNA. In this case, the curves for potentials with a varying number of identical steps are similar. If we define $W = -U_0 > 0$, then for all N Eq. (14) is well approximated by the expression

$$\frac{v_N}{v_{HW}} \approx e^{-(1-f)W}. \quad (15)$$

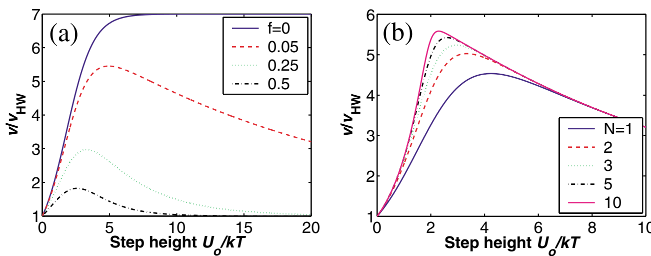

FIG. 3 (color online). (a) Step potential unwinding rate as a function of step height (relative to the hard-wall rate) for $N = 1$. The different curves correspond to different values of f, as discussed in the text. (b) Unwinding rate as a function of step hight for staircase potentials with N steps for $f = 0.05$. In both figures, $c = 1/7$.

We can now discuss the magnitude of parameter values and rates assuming that the helicase advances a single base for each ATP hydrolyzed. The sequence-averaged value of $\beta/\alpha \approx 7$ for DNA [3], corresponding to a free energy per base pair of $2k_B T$. Thus, by Eq. (7), opening requires $k^+ > 7k^-$, as observed in experiments on PcrA helicase [7,8]. These measurements found the single-strand translocation rate of PcrA to be $k^+ \simeq 80$ bases/sec. On the other hand, DNA hairpin experiments [10] have found that α and β are several orders of magnitude faster. An estimate using the observed cooperative opening of a 5-bp hairpin [11] suggests $\alpha \approx 10^7$/sec, so $c \approx \alpha/\beta \approx 1/7$. We can use this value to find an upper bound for the rate of hard-wall unwinding, because the maximum of Eq. (7) occurs if $k^- = 0$. With

$\beta \gg k^+$, $v_{HW} \leq \frac{\alpha}{\beta} k^+$. Note that the rate of hard-wall opening is significantly slower than the single-strand translocation rate k^+. However, an interaction potential with optimal choice of $U_0 \simeq U_*$ increases the opening rate to values that approach the translocation rate along single strands (Fig. 3). The value N of the number of steps the helicase takes beyond the point $m = n$ in our description can be motivated by crystal structures which reveal that a helicase can interact with 5–10 bases in the ds region of the ss-ds junction [12–18].

We have discussed the increase of the unwinding rate due to an interaction potential with nonzero range relative to a hard-wall potential. This increase is independent of the values of k^+ and k^-. Experimentally, the rates k^+ and k^- can be changed (e.g., by changing the ATP concentration). Our description provides expressions for the strand opening rate as a function of hopping rates which can be compared to experiments. Similarly, the effect of changes of the free energy of unwinding, $c \approx \alpha/\beta$, given by Eqs. (7), (12), and (14), can be compared to experiments.

Our simplified description neglects several effects which may be important in experiments. Here, we have averaged over DNA sequence; thus our description should describe the average behavior while not capturing fluctuations correctly. While sequence effects are believed to be weak for helicases [3], recent work by Lubensky and Nelson [19] shows that interesting effects can arise if a random DNA sequence is opened by pulling its ends. Furthermore, we have ignored the finite processivity of a helicase, which detaches from the DNA; and we have neglected the effects of DNA elasticity on unwinding. Finally, we have assumed an oversimplified description of force generation and have neglected details of the biochemistry of the helicase hydrolysis cycle. A more detailed description of force generation and ATP hydrolysis is left for future work. Such a model could capture situations where forward stepping is not strongly coupled to ATP hydrolysis.

The situation described here represents a class of problems where a motor molecule interacts with a mobile obstacle. We analyze the physical principles governing such a situation and describe the interplay of two distinct effects within a common framework: the effects of local enzymatic action together with mechanical action and nonequilibrium fluctuations. This situation is relevant for other systems. Examples include two cytoskeketal motor proteins which walk on microtubules with different speeds and the interaction of a motor protein with a polymerizing filament end [20]. Finally, our description represents a generalized version of the polymerization ratchet [6] and could also be relevant for the properties of polymerization forces.

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