Opening of DNA double strands by helicases.
Active versus passive opening

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Abstract

Helicase opening of double-stranded nucleic acids may be “active” (the helicase directly destabilizes the dsNA to promote opening) or “passive” (the helicase binds ssNA available due to a thermal fluctuation which opens part of the dsNA). We describe helicase opening of dsNA, based on helicases which bind single NA strands and move towards the double-stranded region, using a discrete “hopping” model. The interaction between the helicase and the junction where the double strand opens is characterized by an interaction potential. The form of the potential determines whether the opening is active or passive. We calculate the rate of passive opening for the helicase PcrA, and show that the rate increases when the opening is active. Finally, we examine how to choose the interaction potential to optimize the rate of strand separation. One important result is our finding that active opening can increase the unwinding rate by 7 fold compared to passive opening.

1 Introduction

A helicase is a molecular-motor protein which opens double-stranded nucleic acid (NA) molecules. The strand separation is fueled by nucleoside triphosphate (NTP) hydrolysis, typically of ATP. Since the discovery of the first helicase in 1976 [1], more than 60 distinct helicase proteins have been found [2], including 12 apparent helicases solely in the bacterium \textit{E. coli}. Helicases play a role in nearly every cellular process which involves NA, including replication, transcription, translation, repair, and RNA processing [2]. All helicases share the ability to move along NA strands, and to couple that motion to strand separation. For this reason, helicases are often referred to as DNA translocases [3]. Certain helicases, because of their role in repair of DNA damage, are crucial to maintain a stable (undamaged) genome. For example, helicase mutations play a role in the human diseases Xeroderma Pigmentosum, Bloom syndrome and Werner syndrome, all of which are associated with a predisposition to cancer [4].

Given the ubiquity of helicases in cellular processes which access nucleic acids and the large number of different types of helicase, it is not surprising that a variety of different helicase structures exist. We note that the many different examples of helicases are not all similar, and their unwinding mechanisms are not necessarily the same. The earliest known helicases were ring-shaped hexamers[2]; other helicases have been shown to function as dimers. In the
last 5 years, the helicase superfamilies I and II (SF1 and SF2, based on sequence comparison) have been discovered; this work led to a revision of the view that helicase function requires a multimeric protein. For a review of the SF1 and SF2 helicases see [3].

This paper was inspired by experiments on the SF1 and SF2 helicases. The monomeric proteins are known to bind to single-stranded (ss) NA and translocate directionally along it. Upon reaching the ss-double strand (ds) junction, the helicase continues and moves the junction forward, creating additional ssNA “track” as it goes. In this paper, we describe the physical principles of this process and show how to optimize performance of the enzyme. The coupling between the helicase translocation and NA opening is loosely classified in the literature [2] as “passive” or “active.” Passive unwinding means that the helicase unwinds indirectly, by binding ssNA available due to a fluctuation which opens part of the dsNA. We also call this “hard” unwinding for reasons discussed below, and to avoid confusion with other meanings of passive/active. In active unwinding (which we refer to as “soft” unwinding) the helicase directly destabilizes the dsNA, presumably by binding to the dsNA and changing the free energy of the ds state.

Experiments on the SF1 protein PcrA support a soft mechanism. Velankar et al. [5] solved crystal structures of the enzyme bound to a forked (part ss and part ds) DNA substrate. In one of these structures, the protein binds the non-hydrolyzable ATP analog ADPNP and represents the helicase prior to ATP hydrolysis. A second structure contains a sulfur ion and represents the conformation after ATP hydrolysis. These structures showed that the helicase binds ssDNA and tracks along the single strand as it moves. However, in one crystal, a portion of the PcrA also binds to the duplex DNA ahead of the ss-ds junction, causing a distortion of the helical structure. The authors suggested that this distortion promotes melting of the helix, thereby facilitating strand separation. The crystal structures and footprinting studies [6] showed that the contact between the protein and the double helix occurs 4-5 base pairs (bp) from the junction and again 12-13 bp from the junction. Sultanas et al. mutated the residues of PcrA which interact with the duplex portion of the DNA [6]. The mutant proteins unwound dsDNA 10-30 fold more slowly than the wild-type protein, depending on which residue is mutated. These experiments provide evidence that PcrA unwinds softly and suggest that hard unwinding may be slower and less effective than soft.

In this paper, we use a simple model to explore the theoretical consequences of soft and hard opening. We suppose that the helicase and the NA ss-ds junction interact. Physically, the interaction corresponds to an interaction potential which describes how the presence of the helicase modifies the free-energy change of the NA opening. In our formulation, passive opening corresponds to a hard-wall potential: the helicase cannot advance beyond the ss-ds junction, but the presence of the helicase does not affect the energetics of dsNA opening. Softer potentials correspond to active opening, because the helicase changes the local energetics at the junction.

We study how NA strand separation depends on the interaction. In particular, we show that passive opening is always slower than a simple form of active opening. We calculate the rate of hard opening for the helicase PcrA, and discuss how the rate increases for soft opening. Finally, we examine how to choose the interaction potential to optimize the rate of strand separation. One important result is our finding that soft opening can increase the unwinding rate by 7 fold compared to hard opening. This result is consistent with the mutation studies of PcrA discussed above.

Although extensive biochemical and structural studies of helicases have been performed, relatively little has been done to characterize the function of helicases theoretically. An important review of helicase mechanism by von Hippel and Delagoutte [7] synthesizes the thermodynamic and kinetic properties of helicases into a general framework and emphasizes the importance of considering helicase-NA binding, translocation, and strand-separation independently. Doering et al. [8] developed a “flashing field” model specific to hexameric ring helicases. Recently Bhattacharjee and Seno [9] considered the coexistence of ss and dsDNA in the presence of a
Chen et al. [10] described a helicase as a biased random walk, and considered how the density of histones affects the random walk. However, we know of no theoretical work which addresses how the interaction between helicase and NA affects the unwinding in general, or the difference between active and passive unwinding in particular. Helicases offer a particularly appealing choice for a simplified physical description because they are an important class of enzyme which interacts with NA, yet they have many relatively simple features. For example, many helicases have unwinding rates which are independent of the NA sequence unwind[2, 11]. Thus, the information content of the NA does not play an essential role in helicase operation—a large simplification compared to RNA polymerases, for example[12, 13]. Monomeric helicases are a relatively simple system that may serve as a building block for future theoretical studies of helicases. However, the principles discussed here are quite general and could also apply to multimeric helicases.

### 1.1 Helicase properties

Extensive experiments in the last thirty years have characterized several important properties of helicases[2]. A helicase may bind a ss tail of NA adjoining a duplex, a forked NA containing both a ds region and two ss arms, or the blunt end of dsNA. Most helicases bind and translocate with polarity: they move—either on a single strand, or on one of the two strands of a duplex—in a preferred direction. A 3′-5′ helicase moves toward the 5′ end of the NA strand to which it is bound. The monomeric SF1 helicases we consider here bind to a ssNA tail. Thus after binding, the helicase moves along the ss tail to the ss-ds junction, then continue moving to unwind.

Once a helicase has bound to a strand and begins moving, it unwinds at a measurable average rate, ranging from a few (for PcrA) to thousands (for RecBCD) of bp/sec. The average number of base pairs unwound before the helicase falls off the NA is known as the processivity; measured values for different types of helicases vary from 40 to 30,000 bases unwound[2]. Both the rate and the processivity of a helicase are strongly affected by the presence of accessory proteins as well as solution conditions. For example, *E. coli* single-strand binding protein (SSB) decreases the average free energy cost of unwinding a segment of dsDNA, and has also been observed to increase the processivity of helicases. High values of processivity, such as the 30,000 bp measured for RecBCD, are usually observed in the presence of SSB[2]. Hexameric ring helicases also tend to have higher processivities than dimeric or monomeric helicases. In the absence of any accessory proteins, the monomeric helicase PcrA translocates on ssDNA at a rate of about 80 bases/s [14] and has a low processivity of about 50 bp unwound[15].

A helicase is able to unwind dsNA because it uses the energy released by nucleotide hydrolysis (usually by ATP) for its motion. The average number of bp unwound per ATP molecule hydrolyzed is called the efficiency of unwinding (this quantity is often called the “efficiency” of the helicase in the literature; we prefer to call it the efficiency of unwinding to distinguish it from the efficiency of energy transduction, which is related but not exactly the same). Again, the presence of SSB changes the free energy balance and increases helicase efficiency of unwinding. Under *E. coli* conditions at room temperature, ATP hydrolysis releases about 23 kT, and the average free-energy change from melting one base pair is approximately 1.7–2.5 kT [2]. The theoretical limit on efficiency of unwinding is thus 9–12 bp unwound per ATP hydrolysis. Experimentally measured values are typically smaller, usually 0.33–1 bp unwound/ATP hydrolyzed[2]. For PcrA, the measured efficiency of unwinding is approximately 1 bp unwound/ATP [11].

### 2 Hopping Model

We formulate a discrete model for helicase motion and strand separation (Fig. 1). We consider two degrees of freedom: the helicase position *n* along the NA strand and the position *m* of the
NA ss-ds junction. Since we are considering only single-base hops, both \( n \) and \( m \) are integers. (In our units the length of a ss base is 1.) The helicase hops one base forwards towards the dsNA with rate \( k^+ \), and backwards with rate \( k^- \). Since the helicase hydrolyzes ATP, it is out of equilibrium\(^1\) and has \( k^+ > k^- \). Similarly, the NA opens by one base\(^2\) with rate \( \alpha \) and closes with rate \( \beta \). Because the NA tends to close (in the absence of melting agents [7]) we have \( \beta > \alpha \). Thus the motion of the helicase and the junction tend to drive their positions closer together.

Our simple picture neglects several effects. We ignore binding and unbinding of the helicase, and thus consider the motion of the helicase along the NA strand only. The NA strand we treat as a rigid, one-dimensional track (twist relaxation is fast compared to the unwinding rate). We neglect effects of the NA sequence; each NA base is identical. We ignore the different biochemical states of the helicase and describe it only by forward and backward rates.

For a helicase to unwind a NA duplex, it must interact with (and move) the ss-ds junction. The form of this interaction determines whether the unwinding is soft or hard. We describe this interaction by an potential \( U(m - n) \) between the helicase and the NA ss-ds junction which depends only on the difference between \( m \) and \( n \). For large separations \( m - n \gg 1 \) we assume that the junction and the helicase have no effect on each other, so the coupling potential tends to zero. However, when \( m - n \) is small the coupling potential \( U \) changes both the helicase motion and the junction motion: \( U > 0 \) inhibits the forward motion of the helicase and increases the relative opening rate of the junction.

We use detailed balance (the law of mass action) to write how this coupling potential changes the rates. If the NA closes (so \( m \rightarrow m - 1 \), the change in interaction energy is \( U(m - n) - U(m - n - 1) \). The opening and closing of the NA happens without an external energy source, so detailed balance applies. The ratio of the opening and closing rates is

\[
\frac{\beta_m}{\alpha_{m-1}} = \frac{\beta}{\alpha} e^{-\frac{U(m-n-1)-U(m-n)}{kT}},
\]

where \( \alpha \) and \( \beta \) are the sequence-averaged rates when the helicase and ss-ds junction are far apart. (See Section 4 for a discussion of the values of the different rates.)

We now apply the same argument to the helicase motion. The change in energy \( U(m - n - 1) - U(m - n) \) represents the effective force acting on the helicase. We describe the force

\(^1\)The rates \( k^+ \), and \( k^- \) can be derived from a more complicated model which explicitly treats the nonequilibrium nature of the enzyme. A forthcoming article will address this issue.

\(^2\)We consider only the breathing modes of the NA in which it opens or closes by one base pair. Modes in which larger number of bases open are present, but they are much less probable than the single-base opening or closing.
dependence of helicase motion by writing the ratio of forward and backward rates as

$$\frac{k_{n+1}^+}{k_{n+1}^-} = \frac{k^+}{k^-} e^{-\frac{U(m-n-1)-U(m-n)}{kT}}$$

Eq. (2) is not valid in general, because the helicase is driven by ATP hydrolysis and detailed balance cannot be used. However, it holds for certain cases in a model for motion generation by ATP hydrolysis and is very useful for a discussion of the principles.

The essence of passive opening is to suppose that the helicase provides a steric inhibition of NA closing when it is near the junction. If the junction is at position $m = n+1$, then the helicase covers the ss base adjacent to the junction and prevents base-pairing with the complementary NA strand. Thus hard opening requires $\beta_m$ to equal 0 when $m = n+1$. By equation (1), we see that $\beta_m = 0$ if $U(0)$ is infinite. This is the hard-wall potential sketched in Fig. 2a.

The effect of this potential on the helicase motion makes intuitive sense, as seen in Eq. (2). If $U(0)$ is infinite then $k_n^+ = 0$ when $m = n+1$. In other words, the helicase is unable to advance into the double-stranded NA region and must wait until a thermal fluctuation opens the NA before advancing. This example shows how we describe hard opening: when the helicase and dsNA cannot overlap, the interaction energy $U$ is infinite for $n \geq m$.

The interaction potential $U(m-n)$ can be “softer” than the hard wall form. Such a potential (as in Fig. 2b) leads to active opening, because the interaction with the helicase increases the opening rate of the dsNA. Recall Eq. (1) for the position dependence of the rates. Whenever the NA closing increases the interaction energy ($U(m-n-1) > U(m-n)$), the ratio of NA closing to opening rate decreases, relative to $\beta/\alpha$. Thus the interaction with the helicase can increase the rate of opening or decrease the rate of closing (or both). The crystal structure of PcrA discussed above, which shows that the enzyme can bind to the dsDNA and distort it suggests active opening. In addition, point mutations of the region of PcrA which binds the duplex DNA decrease the unwinding rate [6]. In our picture, the mutations could alter the potential so it approaches a hard-wall form (which corresponds to hard opening).

For a real helicase such as PcrA we do not know the exact form of the potential. We explore different forms of $U(m-n)$ subject to the requirements (i) $U \to 0$ for $m \gg n$ (when the helicase and the junction are far apart, there is no interaction) and (ii) $U \to \infty$ for $n \gg m$. The second requirement means that the helicase cannot bind solely to dsNA and always retains

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3 A forthcoming article will address this issue.

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Figure 2: Schematic interaction potentials between the helicase and ss-ds junction (a) Left, hard wall potential corresponding to passive opening. (b) Middle, a three-step potential, which corresponds to active opening. (c) Right, a three-step potential.
some “footing” on the ssNA. An increase in the energy for $n > m$ is necessary to confine the helicase near the ss-ds junction and prevent it from wandering off into the ds region.

In the analysis of this model, we address several questions. How does changing the potential affect the unwinding rate? We showed above that an increasing interaction potential has two effects: it leads to relatively faster NA opening and relatively slower helicase forward motion. We wish to understand how this trade-off can be resolved. In particular, is there a choice of interaction potential which leads to the fastest possible rate of opening? We address these questions below for certain simplified forms of the potential $U$.

2.1 Solving for the opening rates

We wish to determine the rate of dsNA unwinding for the hopping model, given the rates $k^+, k^−, \alpha, \beta$, and the potential $U(m−n)$. We consider the probability $P(n,m,t)$ that the helicase is at base $n$ and the ss-ds junction at base $m$ at time $t$. We give the details of this calculation in App. A and outline the main features here. Our approach is similar to the polymerization ratchet of Peskin, Odell, and Oster, who examined how a polymerizing filament is able to produce a force [16]. In their work, the two fluctuating degrees of freedom are the tip of a filament and a wall, which are analogous to our helicase and ss-ds junction. However, they considered a hard-wall interaction; thus our work may be considered a generalization of their approach. The probability distribution satisfies the Eq. (17), which describes the changes to $P(n,m,t)$ due to opening/closing of the NA (which changes $m$) and helicase hopping (which changes $n$).

We rewrite this equation using difference and midpoint variables $j = m − n$ and $l = m + n$ (Eq. (18)). This allows an important simplification since the coefficients in Eq. (18) are independent of $l$. By summing Eq. (18) over $l$, we obtain Eq. (19) for the difference-variable distribution $P_j = \sum_l P(j,l)$. This equation describes the relaxation of the difference-variable distribution to a stationary one, which corresponds to a constant current in the difference variable $j$. The potential diverges as $j \to -\infty$, which implies that the helicase cannot pass the junction. Therefore, the $j$-current must vanish, which leads to a simple recursion relation for $P_j$ that depends on the interaction potential.

The strand separation velocity can be calculated assuming that the distribution $P_j$ is stationary. The distribution $P_j$ reaches its steady state after a relaxation time related to the rates $\alpha, \beta, k^+, k^-$. Summing Eq. (18) over $j$ leads to a discrete drift-diffusion equation for $\Pi_l = \sum_j P(j,l)$ (Eq. (24)). The corresponding current in $l$ does not vanish and determines the mean strand separation velocity

$$v = \frac{1}{2} \sum_j (k_j^+ + \alpha_j - k_j^- - \beta_j) P_j.$$  

The expression for $v$ has a simple physical interpretation. The quantity in parentheses is the unwinding rate at separation $j$—either a forward hop of the helicase ($k_j^+$) or NA opening ($\alpha_j$) move the helicase/junction complex forward. Similarly, a backward hop of the helicase ($k_j^-$) or NA closing ($\beta_j$) move the complex backwards. We then take the unwinding velocity at separation $j$ and multiply it by the probability $P_j$ of finding the complex at separation $j$. Repeating this addition for all possible $j$, we arrive at the total unwinding rate. Thus we see that, under our assumptions, solving Eq. (21) for the stationary probability distribution $P_j$ is sufficient to calculate the mean helicase opening velocity. The diffusive part of the equation for $\Pi_l$ allows us to determine the effective diffusion coefficient which characterizes velocity fluctuations

$$D = \frac{1}{4} \sum_j (k_j^+ + \alpha_j + k_j^- + \beta_j) P_j.$$  

6
3 Hard versus soft opening

Consider the hard wall model for passive opening, with the wall at \( j = 0 \). As described above, this means \( k^+, k^-, \alpha \) and \( \beta \) are constant in the region \( j > 0 \) except at \( j = 1 \), where \( k_1^+ = \beta_1 = 0 \). Since the rates are constant, we have

\[
P_j = A \left( \frac{\alpha + k^-}{\beta + k^+} \right)^j = A c^j,
\]

(5)

where we have defined \( c = \frac{\alpha + k^-}{\beta + k^+} \) and the constant \( A \) is determined from normalization: \( A = (\beta + k^- - \alpha - k^-)/(\alpha + k^+) \). Evaluating Eq.(3) for the helicase opening velocity, we find

\[
v_{HW} = \frac{\alpha k^+ - \beta k^-}{\beta + k^+}
\]

(6)

The opening velocity is positive whenever \( k^+ / k^- > \beta / \alpha \), that is, the free energy change which drives DNA closing must be smaller than the effective free energy change of the helicase. This requirement intuitively makes sense: the helicase must have a sufficiently high forward rate to overcome the energetically favorable DNA closing. The sequence-averaged value of \( \beta / \alpha \) for DNA is 7 (corresponding to an average free energy change per base closed of 2 kT). Thus, even by this simple calculation we see that for passive opening to be possible we must have \( k^+ > 7k^- \).

The backward rate \( k^- \) must be considerably smaller than the forward rate, as observed in experiments[11, 14].

3.1 Soft opening: the staircase

Next we consider a simple example of soft opening shown in Fig. 2b. Instead of a hard-wall potential, we insert a step of height \( U_o \) at \( j = 0 \) and a hard wall at \( j = -1 \). Thus the DNA junction and the helicase can overlap by one base if the energetic cost is paid. The ratios of the forward and backward rates at the step must satisfy the relations

\[
\frac{k^-}{k^+} = \frac{k^- e^{fU_o}}{k^+ e^{fU_o}},
\]

(7)

\[
\frac{\alpha_0}{\beta_1} = \frac{\alpha e^{-fU_o}}{\beta e^{-fU_o}},
\]

(8)

\[
k_0^+ = \beta_0 = 0.
\]

(9)

These relations affect only the ratios of the rates. We also have to specify how the rates themselves change. This is determined by the position of the barrier which must be crossed for the helicase to move or for the NA to open/close. We describe this by a weighting factor \( 0 < f < 1 \). (Note that \( f \) is the same for the helicase motion and the NA breathing since both phenomena involve the same barrier.) Smaller values of \( f \) imply that the barrier influences mainly backward rates, while for large \( f \) the barrier affects forward rates. We have

\[
k_1^+ = k^+ e^{-fU_o},
\]

(10)

\[
k_0^- = k^- e^{-(f-1)U_o},
\]

(11)

\[
\beta_1 = \beta e^{-fU_o},
\]

(12)

\[
\alpha_0 = \alpha e^{-(f-1)U_o}.
\]

(13)

With these values, we can calculate the opening velocity \( v_1 \) for one step. For comparison, we present the opening velocity relative to \( v_{HW} \) of Eq. (6):

\[
\frac{v_1}{v_{HW}} = \frac{c + (1 - c)e^{-fU_o}}{c + (1 - c)e^{-U_o}}.
\]

(14)
Figure 3: Left, Ratio of the opening velocity with one-step potential to the hard-wall opening velocity versus height of the step. The different curves show different values of the weighting parameter $f$. The maximum opening speed increases as $f \to 0$, which corresponds to leaving the forward helicase and DNA closing rates unchanged, while increasing the helicase backward rate and the DNA opening rate. Right, the velocity for $f = 0.1$ versus height of the step, shown for staircases with different number of steps.

where $c = \frac{\alpha + k^-}{k^+ + \beta}$. Since $0 < f < 1$, the opening velocity $v_1$ for a single step is always larger than $v_{HW}$. However, the size of $v/v_{HW}$ depends strongly on the choice of $f$ (Fig. 3a). Smaller values of $f$ increase the opening velocity. The maximal opening rate occurs when $f = 0$. From Eq. (14), we see that for $f = 0$ and large $U_o$, $v_1 \to v_{HW}/c \approx 7v_{HW}$. For small values of $f$ the helicase forward rate $k^+$ and the DNA closing rate $\beta$ do not change much due to the step, whereas the helicase backward rate $k^-$ and the DNA opening rate $\alpha$ increase significantly. The main advantage of active opening is that it increases the opening rate of the DNA. It is more advantageous to increase the opening rate than to decrease the closing rate, because with an increased opening rate the strand-separation timescale is faster.

The unwinding rate also depends on the step height $U_o$. For small $U_o$, the velocity is little changed from the hard-wall velocity. As the step height increases, so does the opening velocity, as a result of the increase in DNA opening rate caused by the presence of the step. For a height of 1 kT and $f = 0.05$, the opening velocity is approximately twice the hard-wall velocity. We draw particular attention to the barrier height of 2 kT. This means that the helicase at $j = 0$ causes the base-pair nearest the junction to be indifferent to opening. A 2 kT step height with $f = 0.05$ increases the opening velocity by approximately a factor of 3 relative to the hard-wall velocity. For a further comparison of our calculation to experimental data, please see the Discussion below.

### 3.2 Multi-step staircase

Evidence from the crystal structures of PcrA bound to a forked DNA substrate discussed above suggests that we consider more than one “step” in the potential. The structures indicate that the helicase and dsDNA interact 4–5 bp from the junction, and again 12–13 bp from the junction. Thus we should consider an interaction potential with a variable number of steps. For a multi-step staircase (with $n$ identical steps, each of height $U_o$, see Fig. 2c) the unwinding velocity is
\[
\frac{v_n}{v_{HW}} = \frac{c^n + (1 - c)e^{-(f-1)U_o} \sum_{j=1}^{n} c^{n-j}e^{-jU_o}}{c^n + (1 - c) \sum_{j=1}^{n} c^{n-j}e^{-jU_o}}.
\]

This expression can be simplified by performing the summation, but as written one can see
the similarity between this expression and Eq. (14) for the one-step unwinding velocity. In
particular, note that for \( n = 1 \) the expression here reduces to Eq. (14). Fig. 3b shows how the
velocity changes with step number (for \( f = 0.1 \)). Adding additional steps allows the opening
velocity to increase faster, and the maximum opening speed increases with the number of steps.
We note that as the number of steps becomes large, the step size \( U_o \) corresponding to the
maximum opening velocity approaches \( U_{max} \approx -\ln c \). In other words, in the limit of a large
number of steps, the optimal step height approaches the free energy change of melting one base
of NA: the fastest opening occurs when the interaction between helicase and NA causes the
melting of one NA base pair to be \textit{energetically neutral}.

For a step height of 2 kT, adding one step increase the opening velocity by a factor of 3.5,
whereas for 5 steps of height 2 kT the opening velocity is 5 times the hard-wall velocity. This
is a significant increase over the hard-wall velocity. The PcrA mutation studies [6] found that
mutating the residues which interact with the dsDNA decreased the unwinding rate by a factor
of 10-30. While our model shows a decrease in the opening velocity (when opening changes from
soft to hard) of at most a factor of \( 1/c \approx 7 \), we find it striking that such a simple model can
demonstrate such a large change in the velocity. We emphasize that because our result looks at
the ratio of active to passive unwinding rates, it is \textit{independent} of particular helicase properties
such as \( k^+ \) and \( k^- \). Our result depends only on the sequence-averaged ratio of DNA opening
and closing rates \( c \) as well as the number of steps \( n \) and the step size \( U_o \).

Indeed, while hard opening is significantly slower than the PcrA single-strand translocation
rate, the helicase that chooses (or evolves) an appropriate interaction potential can open NA
nearly as fast as the rate it moves on ssNA.

4 Discussion

In this paper, we have described the opening of NA by a helicase in terms of two hopping degrees
of freedom: the position of the helicase, which hops along ssNA driven by ATP hydrolysis, and
the position of the ss-ds NA junction, which fluctuates passively. This simplified model allows us
to focus on how the motion is affected by the interaction between these two degrees of freedom.
We demonstrate that active opening in general leads to a faster overall unwinding rate that can
approach the velocity of motion of helicase along the ssNA.

We show that the interaction between helicase and dsNA determines the speed of unwinding.
If rapid unwinding is advantageous, helicases will evolve towards interaction potentials which
optimize this speed. In our calculations we can make quantitative predictions of how different
potentials change the unwinding rate.

Because the helicase moves primarily forward, the backward rate \( k^- \) is small compared to \( k^+ \).
Dillingham, Wigley, and Webb used two different techniques[11, 14] to measure the rate of PcrA
motion on ssDNA. In our notation, this is \( k^+ - k^- = 80 \text{ bases/sec} \). They found that their data
were well-fit by a model with no backward steps \( (k^- = 0) \), although our analysis of their results
found a comparably good fit with values of \( k^- \) up to 10% of \( k^+ \). The free-energy change \( \Delta F \) of
opening one base determines the ratio \( \alpha/\beta = e^{-\Delta F/kT} \). This ratio is approximately \( \Delta F \approx 2kT \)
for a base-pair near a junction[17]. This value is consistent with bulk melting-curve results [2]
and is the origin of our number \( \alpha/\beta = e^{-2} \approx 1/7 \). For the actual values of \( \alpha \) and \( \beta \), we deduce
a number using the experimental results of Bonnet, Krichevsky, and Libchaber [18] who found
that the opening rate of a 5-bp hairpin loop at 300 K and at 0.1 M NaCl was \( k \approx 3000/\text{sec} \).
Using a simple kinetic model\textsuperscript{4} we estimate from this value of $k$ that $\alpha \approx 1.4 \times 10^6$/sec. We use this value of $\alpha$ in our calculations below. This estimate can be compared with theoretical estimates of the base-pair opening rate $\alpha$, which range from $10^5$/sec to $10^6$/sec \cite{19, 20}.

Here we compare the results of our model to experimental data, using the experimental values of $k^+, k^-$, $\alpha$, and $\beta$ discussed above. The maximum possible hard-wall opening velocity occurs in the case where $k^- = 0$. Then

$$v_{HW} = \frac{\alpha}{\beta} \left( \frac{k^+}{1 + k^+/\beta} \right) \approx 11 \text{ bases/sec.}$$  \hspace{1cm} (16)

Note that this is an upper bound on the velocity: any non-zero value of $k^-$ decreases the hard-wall velocity. Unfortunately, to our knowledge the unwinding rate has not been directly measured for SF1 helicases such as PcrA. Existing unwinding assays \cite{6} use gel electrophoresis to determine the fraction of dsDNA molecules completely unwound at a given time. Therefore we cannot make a direct comparison between our calculation and measured unwinding velocities.

Adding a single-step-staircase potential allows the velocity to increase by up to a factor of 7. We choose a modest step height of $2kT$, which gives a maximum increase over the hard-wall velocity of a factor of 3 (for $f = 0.1$). Thus the DNA opening velocity could be up to 33 bases/sec for a single-step-staircase potential. For a multi-step staircase, 5 steps of height $2kT$ increase the opening rate by a factor of 5. Thus the helicase unwinding rate can increase to 55 bases/sec from the hard rate of 11 bases/sec. This velocity is nearly as large as the single-strand translocation speed itself. Thus we demonstrate that soft opening—despite the extremely simplified form of the interaction potential we have chosen—can significantly increase the strand-separation rate.

We emphasize that changes to the helicase hopping rates $k^+$ and $k^-$, for example due to changes in the ATP concentration, lead to changes in the unwinding rate which our model predicts quantitatively. Similarly, the free energy change of melting one NA base pair can be changed by altering solution conditions or by applying a force to the NA molecule. Such a change directly alters the parameter $c \approx \alpha/\beta$ in our model, and we quantitatively predict the resulting change in the unwinding rate. Here, we have neglected the effects of the base sequence on NA opening which are believed to be weak for helicases\cite{2}. However, work by Lubensky and Nelson \cite{21} suggest that interesting effects can arise if a random DNA sequence is opened.

The simplified structure of this model means it may be useful in other situations where two degrees of freedom interact. For example, two motor proteins which walk on a microtubule may affect each other’s motion. As mentioned above, our work may be viewed as a generalization of the work of Peskin et al. \cite{16} to include an arbitrary type of interaction potential. This generalization may also be relevant to the problem originally addressed in Ref. \cite{16}: the production of a force by a polymerizing filament. Introducing a potential (which describes the interaction between the growing filament tip and the obstacle against which the polymer pushes) affects the polymerization speed and the force-velocity relation of the filament.

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\textsuperscript{4}We assume that each of the 5 bases has an opening rate $\alpha$ and a closing rate $\beta$ and the ratio $\alpha/\beta = c$. With $P_n$ denoting the relative probability of having $n$ base pairs open, the opening rate is given by $k = J/\sum P_n = \alpha c^3/(1 + 2c + 3c^2 + 4c^3 + c^4(1 + c^2 + c^3 + c^4))$, where $J = \alpha P_0 - \beta P_1 = \alpha P_4$ is the current. With this expression we estimate $\alpha$, given $k$ and $c$. 

10
A Calculation of the Unwinding Velocity

Here we determine the rate of dsNA opening for the hopping model, given the rates \( k^+, k^-, \alpha, \beta \), and the coupling potential \( U(m - n) \). Consider the probability \( P(n, m, t) \) that the helicase is at position \( n \) and the ss-ds junction at position \( m \) at time \( t \). The probability satisfies

\[
\frac{\partial P(n, m)}{\partial t} = -(k^+_{m-n} + k^-_{n-m} + \alpha_{m-n} + \beta_{m-n})P(n, m) + \alpha_{m-n-1}P(n, m-1) + \beta_{m-n+1}P(n, m+1) + k^+_{m-n+1}P(n-1, m) + k^-_{m-n-1}P(n+1, m). \tag{17}
\]

Note that the subscript \( m - n \) means the hop starts at \( m - n \) and moves either right or left. After rewriting using the difference and midpoint variables \( j = m - n \) and \( l = m + n \), we have

\[
\frac{\partial P(j, l)}{\partial t} = -(\alpha_j + \beta_j + k^+_j + k^-_j)P(j, l) + \alpha_{j-1}P(j-1, l-1) + \beta_{j+1}P(j+1, l+1) + k^+_{j+1}P(j+1, l-1) + k^-_{j-1}P(j-1, l+1). \tag{18}
\]

Since the coefficients in Eq. (18) do not depend on \( l \), we can obtain an equation for the difference variable distribution \( P_j = \sum_j P(j, l) \) by summing over \( l \)

\[
\frac{\partial P_j}{\partial t} = -(k^+_j + k^-_j + \alpha_j + \beta_j)P_j + \alpha_{j-1}P_{j-1} + \beta_{j+1}P_{j+1} + k^+_{j+1}P_{j+1} + k^-_{j-1}P_{j-1}. \tag{19}
\]

This is the equation for a position-dependent hopping model with forward rate \( k_j^- + \alpha_j \) and backward rate \( k_j^+ + \beta_j \). The stationary probability distribution for \( j \) satisfies the recursion relation \( I(j) = I(j-1) \), where we have defined the current

\[
I(j) = (k^+_{j+1} + \beta_{j+1})P_{j+1} - (k^-_j + \alpha_j)P_j. \tag{20}
\]

The stationary probability distribution corresponds to a constant current solution. In the case of a potential \( U(j) \to \infty \) as \( j \to -\infty \), the current must be zero (no helicases can escape to \( j \to -\infty \)). This gives the zero-current relation

\[
P_{j+1} = \frac{k^-_j + \alpha_j}{k^+_{j+1} + \beta_{j+1}}P_j. \tag{21}
\]

In the zero-current case, and if the rates are are constant (independent of \( j \)), \( P_j \) has a power law form with a constant determined by normalization. For the general case where the rates \( k_j^+, k_j^-, \alpha_j, \beta_j \) vary with \( j \), we can solve Eq. 21 iteratively, using the conditions (i) \( P \to 0 \) as \( j \to \infty \), and (ii) \( \sum_j P_j = 1 \).

The difference variable distribution relaxes to a steady state in a finite time while the midpoint variable \( l \) undergoes a drift with diffusion. We can find a simple equation for the midpoint motion if we focus on times longer than the relaxation time of \( P_j \). (Since the DNA opening/closing times are of order of microseconds, we expect the relaxation time to be at most hundreds of microseconds. This time is short compared to the overall rate of unwinding). In this limit, the probability \( P(j, l) \) becomes of the form \( P(j, l) = P_j \Pi_l \). If we define

\[
p = \sum_j (\alpha_j + k^+_j)P_j, \tag{22}
\]

\[
q = \sum_j (\beta_j + k^-_j)P_j. \tag{23}
\]
then the equation for $\Pi_l$ reduces to a hopping model with effective forward and backward rates $p$ and $q$:

$$\frac{\partial \Pi_l}{\partial t} = -(p + q)\Pi_l + p \Pi_{l-1} + q \Pi_{l+1}.$$  \hspace{1cm} (24)

The rates $p$ and $q$ are independent of $l$, because of our assumption that all rates depend only on the difference variable $j$. As above, defining the current $\Upsilon(l) = p\Pi_l - q\Pi_l$ the equation for the stationary distribution $\Pi$ becomes $\Upsilon(l) = \Upsilon(l - 1)$. For the midpoint motion, there is no confining potential which localizes the probability distribution. Thus we expect a solution with non-zero current. The solution is $\Pi_0 = \frac{1}{N}$ and $\Upsilon = (p - q)\Pi_0$, where $N$ is the total number of NA bases. The mean opening velocity of a single helicase is

$$v = \frac{1}{2}(p - q) = \frac{1}{2} \sum_j (k_j^+ + \alpha_j - k_j^- - \beta_j)P_j.$$  \hspace{1cm} (25)

As discussed in the text, $v$ is the unwinding rate at separation $j$ multiplied by the probability $P_j$ of finding the complex at separation $j$. Repeating this addition for all possible $j$, we arrive at the total unwinding rate. The factor of $1/2$ appears because we use $l = m + n$ for convenience, while the true midpoint location is $l/2$. Thus we see that, under our assumptions, solving Eq. 21 for the stationary probability distribution $P_j$ immediately gives the exact mean helicase opening velocity.

We can also determine the effective diffusion coefficient which characterizes velocity fluctuations

$$D = \frac{1}{4} \sum_j (k_j^+ + \alpha_j + k_j^- + \beta_j)P_j.$$  \hspace{1cm} (26)

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