Kinetic Measurement of the Step Size of DNA Unwinding by Escherichia coli UvrD Helicase
Janid A. Ali, et al.
Science 275, 377 (1997);
DOI: 10.1126/science.275.5298.377

The following resources related to this article are available online at www.sciencemag.org (this information is current as of May 1, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/275/5298/377

This article has been cited by 48 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/275/5298/377#otherarticles

This article appears in the following subject collections:
Biochemistry
http://www.sciencemag.org/cgi/collection/biochem

Information about obtaining reprints of this article or about obtaining permission to reproduce this article in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl
The kinetic mechanism by which the DNA repair helicase UvrD of *Escherichia coli* unwinds duplex DNA was examined with the use of a series of oligodeoxynucleotides with duplex regions ranging from 10 to 40 base pairs. Single-turnover unwinding experiments showed distinct lag phases that increased with duplex length because partially unwound DNA intermediates are highly populated during unwinding. Analysis of these kinetics indicates that UvrD unwinds duplex DNA in discrete steps, with an average “step size” of 4 to 5 base pairs (approximately one-half turn of the DNA helix). This suggests an unwinding mechanism in which alternating subunits of the dimeric helicase interact directly with duplex DNA.

**DNA helicases are motor proteins that function to unwind duplex (ds) DNA during DNA replication, recombination, and repair and are also components of eukaryotic transcription complexes (1, 2). These enzymes use the chemical energy obtained from nucleoside triphosphate binding or hydrolysis (or both) to perform the mechanical work of unwinding dsDNA, which also requires translocation of the helicase along DNA for processive unwinding.**

The *Escherichia coli* UvrD helicase [helicase II (3)] plays essential roles in both methyl-directed mismatch repair (4) and nucleotide excision repair of DNA (5), and in humans, defects in these processes are linked to increased susceptibility to cancer (6). UvrD protein [720 amino acids; molecular mass of 81,989 (7)] forms dimers in the absence of DNA (8), and the dimeric form of the enzyme is functional in DNA unwinding (9). In fact, the functional forms of most DNA helicases are oligomeric (mainly dimers or hexamers), most likely because multiple DNA binding sites are needed for helicase function (1, 10, 11). UvrD displays a 3'-to-5' polarity in DNA unwinding (12), in that a 3' single-stranded (ss) DNA flanking the duplex facilitates initiation of unwinding in vitro (9); however, UvrD can also initiate unwinding at a nick (13). UvrD shares about

**REFERENCES AND NOTES**

2. S. F. Dermott and C. D. Murray, Nature **301**, 201 (1983). A MMR occurs when the orbital period of an asteroid is a simple multiple of Jupiter’s orbital period. In addition to 1:2 and 2:3, interior MMRs are also associated with other gaps in the asteroid belt.
14. Integration of a test asteroid was stopped, and the asteroid was presumed to have been removed from the population, if it came within 1 Hill radius \( r_H \) of a planet. Once an object is less than 1 \( r_H \) from a planet, the dominant perturber is the planet, not the sun. The Hill radii of Jupiter and Saturn are 0.36 and 0.44 AU, respectively.
17. Because of the geometric locations of the 4:7, 3:5, and 5:8 interior MMRs, Jupiter has to migrate inward, by about 0.2 AU, to remove asteroids in the 3.5- to 3.9-AU region. However, the migration time scale and the migration scheme (linear, exponential, or other) are less constrained on the basis of the population of asteroids in this region. The long-term dynamics of the asteroids at 2:3 and 1:2 interior MMRs after the planet migration ceased may provide more insight into these questions.
18. We thank H. Zook for enthusiastic discussions. This research was performed while J.-C.L.H. held a National Research Council–NASA Johnson Space Center Associateship and R.M. was a staff scientist at the Lunar and Planetary Institute (LPI), which is operated by the Universities Space Research Association under contract NASW-4574 with NASA. Support was also provided by NASA’s Origins of Solar Systems Research Program under grant 4474. This paper is LPI contribution 905.
19. 4 September 1996; accepted 15 November 1996.
This lag phase is not due to reannealing of the fully unwound DNA because the half-life for reannealing is >2.5 hours at 1 nM DNA (21). It also does not reflect a slow protein-DNA binding step because the UvrD-DNA complexes were preformed before the addition of ATP and the rate of the lag phase is independent of UvrD concentration (22). Although DNA unwinding requires an oligomeric form of the UvrD protein (most likely a dimer (9)), the lag phase does not result from a linkage to protein assembly, because the excess ΔT(pT)15 adduced with the ATP prevents binding of free UvrD to the DNA substrate. Time courses determined at lower concentrations of ATP (as low as 5 μM), where the unwinding rate becomes limited by ATP binding, also showed distinct lag phases (9). Therefore, the lag phase does not result from a fortuitous situation in which DNA unwinding is preceded by unrelated processes that have comparable rates because the lag phase would be eliminated by lowering the ATP concentration into a range such that unwinding is limited by the rate of ATP binding. Rather, this lag phase suggests that DNA unwinding occurs by a multistep process with highly populated intermediate states along the pathway to fully unwound DNA. The rate constants for the formation and decay of these intermediates must also be comparable in magnitude, otherwise a lag phase would not be observed (23).

One likely explanation for the lag phase is that partially unwound DNA molecules are formed as intermediates during unwinding. Even though the “all or none” assay used in these experiments cannot directly detect such partially unwound intermediate states, their presence would be detectable as a lag phase, if they are sufficiently populated. If this hypothesis is correct, then the magnitude of the lag phase should increase with the DNA duplex length. We therefore examined the unwinding kinetics of a series of DNA substrates varying in duplex length, L, with L = 10, 18, 24, and 40 bp (substrates I through IV, respectively, in Table 1). A lag phase was observed with each DNA substrate, and the lag phase increased with duplex length (Fig. 2A).

We analyzed the time courses in Fig. 2A to determine the number of “steps” required by UvrD to unwind each duplex and thus the number of base pairs unwound in each step, that is, the unwinding step size, m. The simplest mechanism that yields a lag phase for unwinding is the sequential n-step mechanism outlined in Scheme 1, where preformed productive UvrD-DNA complexes, (U-DNA)n, form the partially unwound DNA intermediates, I1(L−m), I2(L−2m), I3(L−3m), and so on along the pathway to fully unwound ssDNA, where the subscripts refer to the number of base pairs remaining in the duplex.

Table 1. DNA unwinding substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Structure</th>
<th>Sequence of top strand of duplex</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3'-(dT)40 18 bp DNA (substrate I)</td>
<td>5'-GCCTCGGTGCGAAGGTAC3'</td>
</tr>
<tr>
<td>II</td>
<td>3'-(dT)40 18 bp DNA (substrate II)</td>
<td>5'-GCCTCGGTGCGAAGGTAC3'</td>
</tr>
<tr>
<td>III</td>
<td>3'-(dT)40 18 bp DNA (substrate III)</td>
<td>5'-GCCTCGGTGCGAAGGTAC3'</td>
</tr>
<tr>
<td>IV</td>
<td>3'-(dT)40 18 bp DNA (substrate IV)</td>
<td>5'-GCCTCGGTGCGAAGGTAC3'</td>
</tr>
</tbody>
</table>

Fig. 1. Single-turnover kinetic time course for UvrD-catalyzed unwinding of a 3'-(dT)40 18 bp duplex shows a distinct lag phase. Experiments were performed with 80 nM UvrD (monomer concentration) and 1 nM DNA substrate I (Table 1) at 25°C in buffer U (19). Reactions were initiated by the addition of 1.5 mM ATP and 5 μM ΔT(pT)15 and quenched by Na3EDTA (20). (A) Image of a 20% nondenaturing polyacrylamide gel electrophoresis of the 32P-end-labeled DNA obtained from the rapid quench experiments. The time after addition of ATP is indicated for each lane. (B) Unwinding time course plotted as the fraction of DNA molecules unwound. The lines are simulations based on the nonlinear least-squares fits (27) to Eq. 1 with n = 2 (kobs = 7.8 ± 1.2 s−1, xL = 0.70 ± 0.79), n = 3 (kobs = 12.9 ± 1 s−1, xL = 0.67 ± 0.08), or n = 4 (kobs = 18.4 ± 1 s−1, xL = 0.64 ± 0.07).
In our analysis we have assumed that the net unwinding rate constants, \( k_{\text{up}} \), are equivalent for each step because the lag will be largest when all the rate constants are equal (23, 24). Although each \( k_{\text{up}} \) may depend on base composition or sequence (or both), this assumption seems justified (24). Scheme 1 also assumes that UvrD can dissociate with rate constant \( k_{\text{d}} \) at each step in the unwinding reaction. In that these experiments were performed at saturating ATP concentrations, we have not explicitly included any steps associated with ATP binding, hydrolysis, or release of adenosine diphosphate (ADP) and inorganic phosphate. Hence, \( k_{\text{up}} \) and \( k_{\text{d}} \) are macroscopic rate constants with contributions from nucleotide binding and release.

Scheme 1 also incorporates the fact that some fraction of the DNA is bound to UvrD in nonproductive complexes, (U-DNA)_{\text{NP}}, which must slowly isomerize with rate constant \( k_{\text{iso}} \) to form productive complexes before the DNA can be unwound (25).

On the basis of Scheme 1, the expression for the fraction of DNA molecules unwound as a function of time, \( F(t) \), is given in Eq. 1, where \( k_{\text{obs}} = k_{\text{up}} + k_{\text{d}} \), \( n \) is the number of steps in the unwinding reaction, \( x \) is the fraction of DNA molecules bound to UvrD in complexes that are productive for DNA unwinding, \( A_1 \) is the total amplitude of the unwinding reaction, and \( k_{\text{up}} \) is a macroscopic rate constant for conversion of nonproductive to productive UvrD-DNA complexes (26).

\[
F(t) = A_1 \left[ 1 - \sum_{r=1}^{n} \frac{(k_{\text{obs}} t)^{r-1}}{(r-1)!} e^{-k_{\text{obs}} t} \right] + (1 - x)(1 - e^{-k_{\text{up}} t}) \tag{1}
\]

Figure 1B shows the nonlinear least squares fits of Eq. 1 to the unwinding time course for the 18-bp duplex (substrate II) obtained by constraining \( n \) to values of 2, 3, or 4 steps (27). It is clear that \( n = 4 \) provides a better fit than either \( n = 2 \) or 3. However, because of the existence of the second slow unwinding phase, analysis of a single time course only provides a minimum estimate of the number of steps and thus a maximum estimate of the step size (\( L/n \)) (28). Therefore, from analysis of this single experiment, we can only conclude that there are at least three intermediates in the pathway to fully unwind an 18-bp duplex and thus the step size \( m \leq 4.5 \) bp.

Simultaneous fitting to Eq. 1 of all four time courses in Fig. 2A for the DNA substrates varying in duplex length, \( L \), does provide additional constraints to obtain upper limits on \( n \) (28). A consistent set of \( n \) values (28, 29) was chosen for the four duplexes, and we performed a nonlinear least squares global fit using all four time courses to obtain the best estimates of \( k_{\text{up}} \), \( k_{\text{obs}} \), \( x \), and \( A_1 \) (Fig. 2A). The smooth curves in Fig. 2A were simulated by using Eq. 1 and the best fit parameters for each substrate (see legend to Fig. 2). As expected, the number of steps, \( n \), increases with duplex length, \( L \), with the best fit (integer) values of \( n \) determined to be 2, 4, 6, and 10 for \( L = 10, 18, 24, \) and 40-bp duplexes, respectively. For all four DNA substrates, the unwinding step size, \( m \), determined from the ratio \( L/n \) varied from 4.0 to 5.0 bp, with an average value of 4.4 bp. Therefore, UvrD unwinds about one-half turn of a B-form DNA duplex in each step. The fact that all time courses in Fig. 2A are well described by the same value of \( k_{\text{obs}} = 18.6 \pm 1.3 \) s\(^{-1}\) for the observed per step unwinding rate, independent of duplex length, provides further support for the proposed mechanism.

Figure 2A also shows that the fraction of DNA molecules unwind decreases with increasing duplex length. These experiments were performed in the presence of excess dT(pT)\(_{15}\) that prevents reinitiation of unwinding by dissociated UvrD; therefore this observation indicates that the fraction of UvrD dissociating during unwinding increases with duplex length. Thus, DNA unwinding by UvrD is not highly processive under these conditions. The processivity of unwinding per step, defined as \( P = k_{\text{up}} (k_{\text{d}} + k_{\text{up}}) \) or equivalently as \( P = (N - 1)/N \), where \( N \) is the average number of steps taken before dissociation (1), is the probability of unwinding the next \( m \) base pairs. A low processivity results in some fraction of the DNA molecules not being unwound because of dissociation of the helix before the DNA is fully unwound. The amplitudes of the lag phase \( (A_1) \) as a function of \( L \) are plotted in Fig. 2B. On the basis of the mechanism in Scheme 1, \( A_1 \) is equal to \( xP^{L/m} \), where \( x \) is the initial fraction.
dissociation. In that containing takes an average of productively bound duplex (U)
unwound ssDNA occurs with a lag phase.

1. T. M. Lohman and K. P. Bjornson, Annu. Rev. Bio-


18. Kinetics experiments were performed (25°C, buffer U) with a three-syringe, quenched-flow apparatus (KinTek RGF-3, University Park, PA). UvR at twice the final concentration was premixed with DNA substrate (2 nM) in buffer U and MgCl₂, 2.5 mM MgCl₂, 5 mM 2-mercaptoethanol, 10% (v/v) glycerol, and 0.1 mg/ml bovine serum albumin (BSA). Buffer A is 25 mM Tris- HCl, pH 7.5, 6 mM NaCl, 2.5 mM MgCl₂, 5 mM 2-mercaptoethanol, 10% (v/v) glycerol, and this may contribute to our non-

19. Buffer U is 25 mM Tris- HCl, pH 7.5, 6 mM NaCl, 2.5 mM MgCl₂, 5 mM 2-mercaptoethanol, 10% (v/v) glycerol, and this may contribute to our non-

20. Kinetics experiments were performed (25°C, buffer U) with a three-syringe, quenched-flow apparatus (KinTek RGF-3, University Park, PA). UvR at twice the final concentration was premixed with DNA sub-


22. Single-turnover unwinding rates (measured with DNA substrates I and II) were independent of the UvR concentration (3 nM to 150 nM (monomer)); hence, unwinding initiates from prebound complexes. The apparent equilibrium dissociation constant for UvR binding to the ssDNA substrate is 1 to 2 nM; hence, at 80 nM UvR, all the DNA is bound to UvR.


24. This assumption is reasonable because each DNA sub-
strate has a similar base composition (high G+C con-
tent), and the time courses with different length duplexes can be globally fitted to Eq. 1 with the same kh and step size. Also, experiments with 18-bp substrates (II and V in Table 1) differing in A+T content both fit to mechanisms with n = 4 steps with similar kN Global simulation of simulated time courses also showed that the step size determination for the unwinding rate constants of alternate steps differed by twofold, although kN was affected.

25. Even in the presence of a high concentration of d(T²pT)₉, a second slower unwinding phase was observed with a small amplitude remains, which represents unwinding by UvR bound in a nonproductive form that must first isomerize with rate constant kEₐ prior to the replication of the helicase, but rather proceeded forward to complete unwinding, then a lag phase would not be observed for the 10-bp substrate.

26. The first term in Eq. 1 is the fraction of DNA molecules unwound by the productive (U-DNA) complex (23), where x = [U-DNA]₁/[U-DNA]₀ ([U-DNA]₀ is the amplitude of the lag phase). The second term in Eq. 1, with amplitude (1 - Aₐ), reflects slower unwinding by nonproduc-
tive complexes (25).

27. No linear least squares analyses were performed with Scientist (MicroMath Scientific Software, Salt Lake City, UT) and plotted with KaleidaGraph (Syn-
ergy Software, Reading, PA). Uncertainties are re-
ported as 95% confidence limits.

28. Because of the second slow phase (25), analysis of a single time course provides only a minimum estimate of the number of steps, n. Equally good fits are ob-
tained for greater values of n, which increases the am-
plitude and increasing the rate of the lag phase, re-
spectively, because these changes can be compen-
sated by increases in both the amplitude and the value of kN for the slow phase. Simulta-
aneous solution of all four time courses in Fig. 2A (floating each value of Aₐ) and globally fitting for the same values of kₐₕ₊ and kₐₕ₋ provides additional constraints on the upper limit of n for each duplex, assuming the step size, m = L/n, is independent of L. Global fits with m = 2 or 3 gave poorer fits.

29. Analysis of data in Fig. 2B assumed a constant av-
average step size of m = L/n = 4.4, and that the am-
plitude, Aₐ, a, are a smooth function of duplex length; however, Aₐ may decrease in a step-wise manner with L, and this may contribute to our non-
integer (4.4) estimate of the step size.


32. B. A. Barshop, R. F. Wrenn, C. Frieden, Anal. Bio-
chem. 130, 134 (1983).

33. We thank I. Wong, K. Bjornson, and K. Moore for critical discussions; M. Amarutanga for preliminary experiments; W. van Zanten and T. Ho for DNA syn-
thesis; and P. Burgers, R. Gregorian, and J. Hsieh for comments on the manuscript. Supported in part by NIH grant GM45948.

1 July 1996; accepted 25 November 1996