Displacement of a DNA binding protein by Dda helicase

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ABSTRACT

Bacteriophage T4 Dda helicase has recently been shown to be active as a monomer for unwinding of short duplex oligonucleotides and for displacing streptavidin from 3'-biotinylated oligonucleotides. However, its activity for streptavidin displacement and DNA unwinding has been shown to increase as the number of Dda molecules bound to the substrate molecule increases. A substrate was designed to address the ability of Dda to displace DNA binding proteins. A DNA binding site for the Escherichia coli trp repressor was introduced into an oligonucleotide substrate for Dda helicase containing single-stranded overhang. Here we show that a Dda monomer is insufficient to displace the E.coli trp repressor from dsDNA under single turnover conditions, although the substrate is unwound and the repressor displaced when the single-stranded overhang is long enough to accommodate two Dda molecules. The quantity of product formed increases when the substrate is able to accommodate more than two Dda molecules. These results indicate that multiple Dda molecules act to displace DNA binding proteins in a manner that correlates with the DNA unwinding activity and streptavidin displacement activity. We suggest a cooperative inchworm model to describe the activities of Dda helicase.

INTRODUCTION

Helicases are ubiquitous molecular motors which utilize the energy of nucleotide triphosphate (NTP) hydrolysis to separate the strands of duplex nucleic acids or move along DNA (1–4). This strand separation ability makes them essential for virtually all processes in DNA and RNA metabolism, including replication, recombination, transcription and DNA repair (5). Helicases have been classified into five families based on sequence homology (6) and a number of helicase motifs have been identified (7). It has become clear that the roles of enzymes containing helicase motifs extend beyond simply separating dsDNA into ssDNA. For example, removing proteins from nucleic acids is now believed to be an important role for both DNA helicases (8,9) and RNA helicases (10,11).

Helicases and other enzymes that move along DNA or RNA are likely to encounter proteins that are bound to the nucleic acid. The collision between these proteins can lead to stalling of the motor, displacement of the motor or displacement of the DNA binding protein. The probable outcome of such collisions may depend on the relative affinity of the motor protein compared with the DNA binding protein, as well as the force imparted by the molecular motor. The biological relevance of such collisions is becoming increasingly apparent. For example, RNA polymerase can encounter protein roadblocks that prevent transcription of essential genes. In Escherichia coli, stalled RNA polymerase can be ‘pushed’ by the translocase activity of a helicase-like protein called Mfd (12). Mfd can push RNAP to correctly position the polymerase active site after stalling. Alternatively, Mfd can completely displace RNAP from the DNA. In other cases, the translocase activity of a polymerase can accelerate the rate of a helicase. Another clear example of a collision between a DNA-dependent ATPase and a DNA binding protein occurs with chromatin remodeling proteins such as those in the SWI2/SNF2 class (13). Chromatin remodelers disrupt the interaction between DNA and histones but do not necessarily unwind dsDNA. A number of models have been proposed for how remodeling can occur including nucleosome sliding, nucleosome dissociation or histone replacement with a variant histone. Chromatin remodelers share regions of homology to DNA helicases (14).

The mechanism for helicases has been under intense study by a number of laboratories over the past decade. One aspect of the mechanism that has provided much discussion is in regards to the optimal functional form of helicases. For those enzymes of superfamily 1 (SF1) and SF2, different
models have been proposed. Evidence for functional, dimeric forms of helicases has been reported for the E.coli Rep helicase (15,16) and E.coli UvrD helicase (17). The proposed inchworm mechanism for helicase activity (18,19) places no requirements on the oligomeric structure of the active helicase species. A model for the inchworm mechanism for PcrA helicase has been provided based on biochemical and X-ray crystallographic evidence (19,20). The SF2 helicase, NS3 helicase domain, from the hepatitis C virus has been proposed to function as a monomer (21), although functional cooperativity between monomers has been suggested for optimal activity (22). The full-length NS3 helicase has been proposed to function as a dimer (23), although optimal activity may require a larger species (24).

Dda helicase has been shown to be capable of functioning as a monomer for unwinding of short duplex oligonucleotides (25,26) and for displacing streptavidin from 3'-biontylated oligonucleotides (27). Dda also does not appear to form stable oligomers even in the presence of ATP, Mg2+ and ssDNA (25). Dda’s activity for displacement of streptavidin from 3'-biontylated oligonucleotides (27) and for unwinding of short duplex oligonucleotides has been shown to increase as the number of Dda molecules bound to the DNA substrate increases (28). This led to the proposal of a model for Dda whereby monomeric forms of the enzyme can function in an inchworm-like fashion, yet multiple molecules can function together to enhance the overall activity of the enzyme (27).

Although unwinding of duplex DNA is a physiologically relevant reaction, displacement of streptavidin from biontylated oligonucleotides is a process which Dda does not perform in vivo. However, the displacement of streptavidin may mimic Dda’s ability to displace DNA binding proteins in the path of the replication complex (29–31). We sought to determine whether the cooperativity observed for streptavidin displacement (27) was also exhibited for displacement of a protein bound to duplex DNA. The E.coli trp repressor was chosen because it binds to the trpEDCBA operator sequence with an equilibrium dissociation constant of 3.2 \( \times 10^{-10} \) (32) in the presence of L-Trp. This value is intermediate in the range of dissociation constants for DNA binding proteins present in E.coli (Table 1).

### MATERIALS AND METHODS

**Reagents**

Poly(dT) was purchased from Amersham Pharmacia Biotech. BSA, L-Trp and Sephadex G-25 were from Sigma. ATP, Hepes, KOAc, BME and EDTA were obtained from Fisher. [\( \gamma^{32} \)P]ATP was purchased from PerkinElmer Life Sciences and T4 polynucleotide kinase was obtained from New England Biolabs. Fluorescein-labeled oligonucleotides were purchased from Operon Technologies. Unlabeled oligonucleotides were purchased from Integrated DNA Technologies. Dda (25) and E.coli trp repressor (33) were overexpressed and purified as described.

**Oligonucleotides**

All the substrates used contain a 30 bp duplex (Figure 1B). The first 18 nt are the sequence of the E.coli trpEDCBA operator (a palindromic sequence) and the remaining 12 nt are a random, nonpalindromic sequence. The 5'-single-stranded overhang consists of a varying number of thymidines. DNA duplexes and oligonucleotides were purified by PAGE as described previously (25). Concentrations were determined using the absorbance at 260 nm in 0.2 M KOH and calculated extinction coefficients (34). The loading strand (top strand in Figure 1B) was radiolabeled (25).

### Displacement of and unwinding in the presence of trp repressor

Reactions in which the E.coli trp repressor was displaced from its binding site were performed at 25°C using a Kintek rapid chemical quench-flow instrument maintained. An illustration of the reaction appears in Figure 1A. All concentrations listed are after mixing unless otherwise noted. Dda (at the concentrations indicated in the figure legends) was incubated with the indicated concentration of [\( ^{32} \)P]-labeled DNA substrate (Figure 1B) in reaction buffer (25 mM Hepes pH 7.5, 10 mM KOAc, 0.1 mM EDTA, 2 mM BME, 0.1 mg/ml BSA and 0.5 mM L-Trp). The reaction was initiated by the addition of ATP (5 mM), Mg(OAc)₂ (10 mM), 75 μM (in nucleotides) poly(dT) and a 25-fold excess of a re-anneging trap. The reaction was quenched at various times with 200 mM EDTA (concentration before mixing). A control sample to test for the efficiency of the annealing trap was heated to 95°C for 10 min and cooled on ice. Samples were mixed with 0.1% xylene cyanol, 0.1% bromophenol blue and 10% glycerol and loaded immediately on a 20% native polyacrylamide gel containing 10 mM TrisCl pH 7.5 and 0.1 mM L-Trp while running at 250 V in a buffer containing 10 mM TrisCl pH 7.5 and 0.1 mM L-Trp with a circulating water bath maintaining the temperature at 4°C. The fraction of trp repressor bound dsDNA, free dsDNA and ssDNA in each sample were determined using a Molecular Dynamics 445-SI PhosphorImager and ImageQuant software.

### Table 1. Equilibrium dissociation constants of selected cellular and phage DNA binding proteins in E.coli

| DNA binding protein constant (M) | Binding site |
|---------------------------------|-------------|
| lac repressor\(^{a}\)           | lac operator|
| P22 mnt repressor               | mnt operator|
| \( \lambda \) cl repressor       | Oₙ₁        |
|                                | O₂         |
| \( \lambda \) trp repressor     | trpEDCBA operator|
| RNA polymerase                  | E.coli and phage promoters |
| \( \lambda \) Cro                | \( \lambda \), Oₙ operator/\( \lambda \), O₁ operator |
| Factor H₁                       | \( \lambda \) or \( \phi \) DNA |
| P22 Arc repressor\(^{b}\)       | mnt operator|

\(^{a}\)One of the tightest binding E.coli proteins (51).

\(^{b}\)Riggs et al. (52).

\(^{c}\)Yousifian et al. (53).

\(^{d}\)Havley et al. (54).

\(^{e}\)Liu and Matthews(32).

\(^{f}\)McClure (51).

\(^{g}\)Takeda et al. (55).

\(^{h}\)Spasovsky and Buc (56).

\(^{i}\)One of the weakest repressors (51).
Unwinding reactions were performed at 25°C using a Kintek rapid chemical quench-flow instrument as described previously (26) in reaction buffer. Dda (750 nM) and 100 nM DNA (final concentrations) were pre-incubated and the reactions were initiated by addition of ATP and Mg(OAc)₂, poly(dT) (75 μM, in nucleotides) and DNA trap (5 μM). Data were fit to a stepping equation and a minimum of three steps were required to fit the data (Equation 2).

\[
y = A \left\{ 1 - \left( 1 + k_{obs}t + \frac{(k_{obs}t)^2}{2} + \frac{(k_{obs}t)^3}{6} \right) e^{-k_{obs}t} \right\}
\]

Protein–DNA binding. The equilibrium dissociation constant for Dda binding to the substrate with an 8 nt ssDNA tail was calculated after measuring the polarization of 0.5 nM fluorescein-labeled DNA pre-incubated with Dda in reaction buffer using a Beacon fluorescence polarization spectrophotometer (PanVera) as described previously (25) in the presence and absence of 25 nM trp repressor. The substrate used was identical to the displacement substrate with the 8 nt single-stranded region except that the 5' end of the single-stranded region was labeled with fluorescein.

RESULTS

Displacement of the trp repressor is dependent on ATP and Dda

A substrate was designed to allow examination of helicase-catalyzed displacement of a DNA-binding protein along with DNA unwinding in the same assay. The standard helicase assay (Figure 1) must allow the ssDNA products to be separated from dsDNA by native polyacrylamide gel electrophoresis. This is done by adding a strand of DNA that can hybridize to one of the ssDNA products produced by the helicase activity. This additional strand is referred to as the re-annealing trap. Another feature of helicase experiments described here is that single turnover conditions with respect to the DNA substrate were maintained. Helicases require ATP hydrolysis to unwind dsDNA, therefore the enzyme can be incubated with the DNA substrate prior to initiating the reaction. Single turnover conditions can be obtained by introducing a large amount of ssDNA such as poly dT at the same time as the ATP. The poly dT serves as a protein trap that binds to any enzyme that is free in solution or dissociates from the substrate. Thus, in addition to the DNA substrate, the reaction contains a re-annealing trap and a protein trap.

The trp repressor was chosen based on the ease of the protein purification and the well-characterized affinity for duplex dsDNA (Table 1) (36). The addition of a DNA-binding protein contributes additional complications to the assay. The trpEDCBA operator is a palindromic sequence which could be problematic for trapping of the ssDNA reaction products in the helicase assay. For this reason, the substrate was designed with 12 nt of non-palindromic sequence adjacent to the trpEDCBA operator sequence to aid in substrate preparation and increase trapping efficiency (Figure 1B). To test the effectiveness of the DNA trap, an unwinding reaction was performed with 750 nM Dda and 100 nM substrate containing a 12 nt single-strand overhang in the absence of a protein trap. Under these conditions, all of the substrate would be

\[
F(T) = \text{TACTATGTTAATCGTACGGCTGATGTGCCATGATCATTGGCATGTCGGCCG?CAACAGCG}
\]
expected to be converted to product, allowing the trapping efficiency to be determined. No substrate was present after 1 s (Figure 2A), indicating that the displaced strand can be effectively trapped using a re-annealing trap complimentary to the 12 nt of non-palindromic sequence in the displaced strand (Figure 1A).

Next we sought to determine whether displacement of trp repressor from the substrate was dependent on ATP and Dda. In the absence of ATP, no displacement was observed after 20 s, but the substrate was converted to product in the presence of 5 mM ATP (Figure 2B). Product was also not formed in the absence of Dda, but substrate was completely converted to product in the presence of 750 nM Dda (Figure 2C). This indicates that the trp repressor is displaced by Dda as it unwinds the DNA substrate.

Helicase-catalyzed displacement of the E.coli trp repressor and DNA unwinding

Dda sequesters 6 nt when bound to ssDNA (27). Based on this, a substrate with an 8 nt single-strand overhang should easily be able to accommodate one Dda molecule. Since the trp repressor binding site is at the ssDNA–dsDNA junction, it is unlikely that much fraying occurs, so binding of more than one Dda molecule would be unlikely. Dda helicase exhibits higher processivity when more than one molecule of the enzyme binds to the substrate (28). We wished to compare the protein displacement activity of monomeric Dda with that of multiple molecules of Dda, so substrates with 12 and 24 ssDNA overhangs were designed that should be able to bind two and four Dda molecules when saturated, respectively.

Displacement of trp repressor from and unwinding in the presence of trp repressor of substrates with 8, 12 and 24 nt single-stands were compared. Dda was incubated with substrate, and the reactions were initiated by addition of ATP, Mg$^{2+}$, and protein and re-annealing traps. The products were separated by PAGE in the presence of L-Trp (Figure 3A). The trp repressor bound, dsDNA substrate could be separated from the unbound dsDNA substrate, allowing for protein displacement to be measured in the absence of DNA unwinding. However, the unbound dsDNA appeared only transiently in the reaction, and almost all of dsDNA substrate from which trp repressor was displaced and was quickly melted by the helicase. Reaction progress curves (Figure 3B) show that the unwinding reaction is completed rapidly after the protein is displaced. The same quantities of product are formed in both reactions, which illustrates that Dda is capable of displacing a protein and continuing with the unwinding reaction after displacement since the trp repressor must be displaced before the final 12 bp can be unwound. No unwinding or displacement is seen when the substrate contains only 8 nt of single-strand, suggesting that a single Dda molecule is not capable of displacing the trp repressor while unwinding DNA under single cycle conditions. A monomer is capable of unwinding naked DNA (25,26) but for unwinding of this substrate to occur, the bound protein must first be displaced. The displacement of trp repressor and unwinding of the DNA require multiple steps, as indicated by the initial lag in product formation (Figure 3C). The lag phases are identical, again suggesting that protein displacement limits the overall reaction rate.

In order to ensure that the substrate was saturated with respect to Dda, the reaction with the three substrates was conducted at two concentrations of Dda with each substrate (Figure 3D). No increase in the rate or amplitude was observed when Dda was raised from 750 nM to 1 µM (Figure 3D). Therefore, 750 nM Dda is sufficient to saturate the rate on 100 nM of all the three substrates.

Unwinding of unbound dsDNA

To determine whether protein displacement limits the reaction rate and quantity of product formed, unwinding reactions were performed in the absence of trp repressor on
each substrate. Reactions were performed as described for the trp repressor displacement experiments except the trp repressor was omitted. Samples were analyzed by PAGE (Figure 4A). Reaction progress curves (Figure 4B) show that the quantity of product formed increases as the length of the ssDNA overhang increases. Interestingly, product is observed with the substrate containing 8 nt ssDNA overhang (filled circles in Figure 4B), whereas in the presence of trp repressor, no product was observed (filled circles in Figure 3B). Unwinding of the free 30 bp duplex also exhibits a lag phase (Figure 4C), although the lag phase is shorter than that for unwinding in the presence of the trp repressor (Figure 3C), indicating that fewer steps are required for unwinding of free DNA.

**Comparison of quantities of product formed and rates in the presence or absence of trp repressor**

The quantity of product formed (Figure 5A) and the rate at which it is formed (Figure 5B) are compared in Figure 5. The major difference between DNA unwinding and trp repressor displacement occurs on the substrate containing the 8 nt ssDNA overhang. Little product for DNA unwinding or trp repressor displacement was observed with this substrate in the presence of the repressor. However, the substrate was rapidly unwound in the absence of trp repressor. The quantity of product formed for protein displacement and DNA unwinding are similar in the presence of trp repressor with the substrates containing the 12 nt ssDNA overhang, but the rate for DNA unwinding is somewhat faster in the absence of trp repressor. The magnitude of these differences decreases as the length of the single-stranded portion of the substrate increases from 12 to 24 nt, suggesting that the more Dda molecules bound, the more efficient the displacement of the protein bound in its path. The rate and quantity of product formation in the presence and absence of trp repressor are nearly identical when the substrate can accommodate up to four Dda molecules. The fact that unwinding occurs at nearly identical rates to trp repressor displacement in the presence of trp repressor, compared with somewhat faster rates for unwinding in the absence of trp repressor, indicates that the unwinding reaction is more rapid than protein displacement.

Dda does bind to the substrate with an 8 nt single-strand when trp repressor is bound

No displacement or unwinding in the presence of trp repressor was observed from the substrate with an 8 nt single-strand. Since the trp repressor binding site is at the ssDNA–dsDNA junction, it is possible that the trp repressor protein overhangs the single-stranded region of the substrate somewhat and prevents Dda from binding. The equilibrium
dissociation constants for Dda from a substrate containing an 8 nt single-strand in the presence and absence of bound trp repressor were found to be nearly identical (Figure 6), suggesting that Dda is bound to the substrate with the 8 nt single-strand even when the trp repressor is bound. However, a single Dda molecule is not able to displace the bound protein.

**DISCUSSION**

DNA and RNA helicases are motor proteins that manipulate nucleic acids in virtually all aspects of nucleic acid metabolism. The primary role for DNA helicases is believed to be in melting of dsDNA, which is likely to be the role in most cases. The role for helicases in RNA metabolism is not as clear, although for both RNA and DNA, collisions between helicases and proteins bound to nucleic acids are highly probable to occur. These collisions may lead to dissociation of the translocating helicase, or the bound protein might be displaced by the helicase. Numerous examples of collisions between DNA or RNA translocases have been reported, but the mechanism for protein displacement has not been described. For example, DNA replication termination in *E.coli* has been shown to occur at defined sequences due to specific interaction between the DnaB helicase and the replication terminator protein Tus (37). Other protein–nucleic acid complexes do not impede DnaB helicase. Kaplan and O’Donnell showed that DnaB can displace dsDNA-binding

**Figure 4.** DNA unwinding kinetics in the absence of bound trp repressor. (A) Products of unwinding reactions were separated by electrophoresis on 20% polyacrylamide gels (shown is unwinding of 100 nM substrate containing a 24 nt ssDNA overhang by 750 nM Dda). (B) Product formation was plotted using Kaleidagraph software for unwinding of 100 nM substrate by 750 nM Dda. Unwinding rates obtained from fits to a three-step mechanism (Equation 2) were 55.0 ± 0.4, 44.1 ± 1.0 and 30.0 ± 1.3 s⁻¹ for unwinding of substrates containing 8 (filled circles), 12 (filled squares) and 24 (filled diamonds) nt of single-strand, respectively. (C) Data in panel B are shown to 0.25 s to illustrate the initial lag phase in product formation.

**Figure 5.** Comparison of the quantities of product formed and the rates at which it was formed. The fraction of substrate converted to product (A) and the rate at which this occurs (B) for DNA unwinding in the absence of trp repressor (black), in the presence of trp repressor (dark gray). For comparison, the quantity and rate of displacement of trp repressor are shown (light gray). The error bars represent the SD of three experiments.

**Figure 6.** Determination of equilibrium dissociation constants in the presence and absence of bound trp repressor. The $K_D$ values for Dda binding to the substrate containing an 8 nt single-strand were 0.7 ± 0.2 and 0.9 ± 0.3 nM in the presence (filled circles) and absence (filled squares) of *E.coli* trp repressor bound to the duplex.
proteins during translocation on dsDNA (38). This activity of DnaB was proposed to facilitate branch migration in DNA recombination or to strip proteins away from sites of DNA repair. In *Saccharomyces cerevisiae*, termination of replication forks is controlled in a sequence-specific fashion by the interaction of the Fob1p replication terminator protein (39). Stalled replication forks can be released by the Rrm3p helicase (termed a ‘sweepase’). The action of Rrm3p is modulated by other proteins such as Top1p-Csm3p complex (40). Hence, a complex series of protein collisions controls the number of replication forks that can proceed through normal termination sites in this organism.

Removal of proteins from RNA was shown in vitro in the case of NPH-II helicase illustrating that RNA helicas can participate in structural reorganization of ribonucleoprotein complexes (11). The ability of RNA helicas to remove proteins from dsRNA in the absence of RNA unwinding has also been demonstrated (10). Evidence has been presented for a functionally significant role for two putative yeast helicases, Sub2 and Prp28, in disruption of protein–RNA complexes during spliceosomal assembly (41,42).

We have designed a substrate for evaluation of the biochemical mechanism of displacement of protein–DNA complexes by Dda helicase. The substrate was designed so that the monomeric form of Dda could be compared with multimeric forms of the enzyme by increasing the length of the ssDNA loading strand. Dda has been found to function as a monomer in unwinding short oligonucleotide substrates, but the processivity of the enzyme is enhanced by increasing the number of molecules bound to a DNA substrate (28). Dda also displaces streptavidin from biotin labeled oligonucleotides, and the activity exhibits positive cooperativity, again illustrating the idea that more molecules of helicase appear to function together. The data for streptavidin displacement activity support a model whereby two or more Dda molecules interact to enhance the activity of the enzyme. This might occur in a manner where a trailing molecule of helicase pushes a leading molecule along the nucleic acid strand, or prevents the lead molecule from slipping during translocation. The data for DNA unwinding by Dda can be interpreted in terms of ‘functional’ cooperativity, which does not rely on protein–protein interactions per se. The latter term has been applied to the NS3 helicase domain to explain how multiple molecules can work on the same substrate to enhance the rate and quantity of ssDNA formed from dsDNA substrates (22). Functional cooperativity can occur when the trailing molecule of helicase continues unwinding dsDNA after the lead molecule dissociates, thereby leading to increased product formation. Also, the trailing molecule can prevent re-annealing of the ssDNA products that might occur after the lead molecule unwinds a given number of base pairs. It is likely that some of the biochemical activities of Dda require interactions between helicase monomers and other activities do not require protein–protein interactions.

A single Dda molecule was not capable of displacing the *E.coli trp* repressor from the trpEDCBA operator sequence or unwinding DNA with a trp repressor molecule bound under single turnover conditions (Figure 3), although, more unwinding was observed in the absence of bound protein (Figure 4). The longest ssDNA overhang used here (24 nt) can bind up to four Dda molecules. When this substrate was examined, almost all of the trp repressor was displaced and nearly all of the substrate unwound (Figures 3 and 4). In fact, the quantity of product formed and rate of product formation for protein displacement, unwinding in the presence of bound *trp* repressor, and unwinding of naked DNA were nearly identical when four Dda molecules were bound to the substrate. This suggests that although a Dda monomer is sufficient to unwind the 30 bp of DNA, it cannot produce the force necessary to unwind DNA and displace a protein bound to the duplex at the same time under single turnover conditions. Two Dda molecules can accomplish this task, although the presence of the protein slows the unwinding process. When more than two molecules of Dda are bound to the substrate, progression of the unwinding reaction is unaffected by the presence of the bound protein, indicating that multiple Dda molecules have increased activity in protein displacement.

The unwinding reaction was limited by protein displacement, as illustrated by the faster DNA unwinding in the absence of *trp* repressor. The longer lag phase in the presence of *trp* repressor indicates that Dda needed additional catalytic cycles to displace the *trp* repressor (compare Figure 3C with Figure 4C). This observation is consistent with the idea that Dda may slip when encountering a block or challenge in its path, therefore requiring additional steps to complete the process. This also illustrates the need for multiple Dda molecules to remove *trp* repressor, because monomeric Dda is not highly processive, therefore it is not able to undergo as many catalytic cycles after encountering the protein block. The fact that protein displacement and ssDNA unwinding were correlated so tightly for the substrates with longer ssDNA overhangs indicates that the displacement reaction was probably a direct consequence of the DNA unwinding. This suggests that the DNA melting activity of Dda is responsible for protein displacement.

Others have shown that Dda can displace proteins from DNA including the *E.coli lac* repressor (30) and the *E.coli* Ter protein (43). However, not all protein–DNA complexes are displaced by Dda. Dda was unable to dislodge a GAL4–DNA complex, even under conditions which should favor binding of more than one molecule of Dda to the substrate (44). Therefore, some specific protein–DNA complexes are able to sequester Dda in a manner that appears to trap Dda and reduce the ATP hydrolysis activity of the enzyme. It is possible that the GAL4–DNA complex adopts a unique structure that perturbs Dda’s interaction with DNA.

A model for Dda helicase activity is emerging that illustrates a role for multiple helicase molecules per substrate, whether DNA unwinding is measured or protein displacement. The work here illustrates that the idea of cooperative function of Dda put forth for streptavidin displacement applies for displacement of DNA-binding proteins as well; i.e. increasing the number of Dda molecules leads to enhanced activity. This model suggests that multiple helicase molecules can cooperate to unwind DNA and displace proteins more effectively than a single molecule. The activity continues to increase as more helicase molecules bind to the same substrate. We suggest the term ‘cooperative
Figure 7. Model depicting displacement of the trp repressor by Dda helicase. (A) Monomeric Dda is shown bound to a substrate to which trp repressor is bound (i). Upon hydrolyzing ATP, the helicase moves forward while adopting an open conformation (ii). Upon binding ATP, the domains change conformations to adopt the closed conformation (iii). The presence of the trp repressor prevents the helicase from moving forward after ATP hydrolysis, therefore the enzyme slips backward or dissociates from the substrate. (B) When two or more helicase molecules are bound to the substrate, the trailing molecule can act as a brake to prevent slipping by the lead molecule (i). The lead molecule is able to translocate forward, thereby unwinding the DNA and simultaneously displacing the trp repressor (ii).

inchworm’ to describe Dda, which illustrates the ability of individual monomers to function, but the increased activity as more than one molecule binds to a substrate. Some challenges presented to the helicase, such as protein displacement or unwinding of long stretches of dsDNA, require the action of multiple helicase molecules, whereas short duplexes can readily be melted by the monomeric form of this enzyme.

A cartoon depicting a possible mechanism by which two helicases can function together to displace the trp repressor is shown in Figure 7. Monomeric Dda is shown in Figure 7A. Of the helicases for which structures are available, Dda is most similar to RecD (45). RecD contains two domains that include the known helicase motifs found in super family 1 helicases, and like Dda, translocates in a 5'-to-3' direction. Based on the reported structure of RecD, and by making analogous comparison with the inchworm model proposed for PerA helicase (19), the two domains in RecD are thought to adopt open and closed conformations as a function of binding ATP. Dda is also thought to contain two major ‘helicase domains’ based on limited proteolysis experiments (L. Blair and K. D. Raney, unpublished observations). The two domains of Dda are depicted as a leading domain and a trailing domain in Figure 7. Further, these two domains can adopt an open conformation in the absence of ATP binding and closed conformation in the presence of ATP binding. Movement of the helicase along nucleic acid is proposed to be driven by cycling between these conformations as a function of ATP binding and hydrolysis. When monomeric Dda encounters the trp repressor, movement of the helicase is impeded by the presence of the repressor, which serves as a protein block. Monomeric Dda can continue to hydrolyze ATP upon encountering a protein block, without immediately displacing the protein block (46). Therefore, it is likely that Dda is able to ‘slip’ backwards, which is indicated by the small reverse arrow in Figure 7A. Slipping by Dda occurs as a result of the trailing domain losing its grip on the nucleic acid, thereby re-establishing the open conformation without forward movement. When two molecules of Dda are bound to the same substrate, trp repressor can be displaced as a result of the second Dda molecule acting as a brake or an anchor for the first molecule, thereby preventing the backwards slipping of the first molecule (Figure 7B). This model for Dda is similar to a model recently reported for the interaction between T7 polymerase and the T7 helicase. DNA synthesis by the T7 polymerase provides the driving force that speeds up the DNA unwinding rate for T7 helicase by ~10-fold (47). The enhanced rate of the helicase has been interpreted in terms of a ‘push’ by the polymerase or as a ‘brake’ by the polymerase to prevent the helicase from sliding backwards.

The fact that multiple molecules of Dda are required for protein displacement may illustrate an additional level of regulation available for determining the outcome of collisions between translocases and DNA binding proteins. The number of helicase molecules that can be loaded onto a particular DNA sequence is likely to be dependent on whether DNA repair, replication or recombination or transcription is being catalyzed. Dda helicase has been implicated in playing some role in disrupting biological significant protein–nucleic acid interactions. The first step in the homologous pairing reaction catalyzed by the T4 uvsX recombinase is inhibited by Dda helicase, presumably by disruption of the uvsX protein–DNA filament (48). However, Dda is known to bind to uvsX and stimulate branch migration activity (49). The protein displacement activity of Dda is believed to allow branch migration through a DNA–protein complex containing an RNA polymerase promoter complex (50). The number of Dda molecules required for these processes in vivo remains to be determined. In addition to binding to uvsX, Dda is also known to bind to the T4 single-stranded binding protein, gp32. Each of these proteins is known to form filaments along nucleic acid. If these proteins play a role in loading Dda onto ssDNA, then it is possible that multiple Dda molecules are loaded onto specific DNA sites in vivo as a result of the interaction of Dda with multiprotein filaments such as uvsX and gp32.

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REFERENCES

1. Delagoutte,E. and von Hippel,P.H. (2002) Helicase mechanisms and the coupling of helicases within macromolecular machines. Part I: structures and properties of isolated helicases. Q. Rev. Biophys., 35, 431–478.

2. Lohnam,T.M. and Bjornson,K.P. (1996) Mechanisms of Helicase-Catalyzed DNA Unwinding. Annu. Rev. Biochem., 65, 169–214.

3. Patel,S.S. and Picha,K.M. (2000) Structure and function of hexameric helicases. Annu. Rev. Biochem., 69, 651–697.

4. Soultanas,P. and Wigley,D.B. (2001) Unwinding the ‘Gordian knot’ of helicase action. Trends Biochem. Sci., 26, 47–54.

5. Delagoutte,E. and von Hippel,P.H. (2003) Helicase mechanisms and the coupling of helicases within macromolecular machines. Part II: integration of helicases into cellular processes. Q. Rev. Biophys., 36, 1–69.

6. Gorbalenya,A. and Koonin,E.V. (1993) Helicases: Amino Acid Sequence Comparisons and Structure-function Relationships. Curr. Opin. Struct. Biol., 3, 419–429.

7. Caruthers,J.M. and McKay,D.B. (2002) Helicase structure and mechanism. Curr. Opin. Struct. Biol., 12, 123–133.

8. Flores,M.J., Sanchez,N. and Michel,B. (2005) A fork-clearing role for UvrD. Mol. Microbiol., 57, 1664–1675.

9. Macris,M.A. and Sung,P. (2005) Multifaceted role of the Saccharomyces cerevisiae Srs2 helicase in homologous recombination regulation. Biochem. Soc. Trans., 33, 1447–1450.

10. Fairman,M.E., Maroney,P.A., Wang,W., Bowers,H.A., Gollnick,P., Jankowsky,E., Gross,C.H., Shuman,S. and Pyle,A.M. (2001) Active E. coli UvrD is the active form of the helicase Escherichia coli. J. Mol. Biol., 269, 33063–33068.

11. Nilsen,T.W. and Jankowsky,E. (2004) Protein displacement by DEAH/D RNA helicases’ without duplex unwinding. Science, 304, 730–734.

12. Jankowsky,E., Gross,C.H., Shuman,S. and Pyle,A.M. (2001) Protein displacement by an assembly of hexacell helicase molecules aligned along single-stranded DNA. Nat. Struct. Mol. Biol., 11, 531–538.

13. Mohrmann,L. and Verrijzer,C.P. (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Q. Rev. Biophys., 38, 69–100.

14. Lusser,A. and Kadonaga,J.T. (2003) Chromatin remodeling by the dda DNA helicase is required to pass a bound RNA polymerase molecule. Cell, 134, 115–123.

15. Velankar,S.S., Soultanas,P., Dillingham,M.S., Subramanya,H.S. and Tackett,A.J. (2002) Initiation and re-initiation of DNA unwinding by the Escherichia coli Rep oligomers are required to initiate DNA unwinding in vitro. J. Mol. Biol., 310, 327–350.

16. Byrd,A.K. and Raney,K.D. (2005) Increasing the length of the single-stranded overhang enhances unwinding of duplex DNA by bacteriophage T4 dda helicase. Biochemistry, 44, 12990–12997.

17. Kaplans,D.L. and O’Donnell,M. (2002) DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands. Proc. Natl Acad. Sci. USA, 99, 2675–2680.

18. Mohanty,B.K. and Bastia,D. (2004) Binding of the replication terminator protein Fob1p to the Ter sites of yeast causes polar fork arrest. J. Biol. Chem., 279, 1932–1941.

19. Mohanty,B.K., Bairwa,N.K. and Bastia,D. (2006) The Toflp-Csm3p protein complex counteracts the Rrm3p helicase to control replication termination of Saccharomyces cerevisiae. Proc. Natl Acad. Sci. USA, 103, 907–912.

20. Chen,J.Y., Stal, L., J. P. S. T. S. J. P., Jackups,R.R., Latus J.L. and Chang,T.H. (2001) Specific alterations of U1-C protein or U1 small nuclear RNA can eliminate the requirement of Prp28p, an essential DEAD box splicing factor. Mol. Cell, 7, 227–232.

21. Kistler,A.L. and Guthrie,C. (2001) Deletion of MUD2, the yeast DEAD box splicing factor. Genes Dev., 15, 42–49.

22. Levin,M.K., Wang,Y.H. and Patel,S.S. (2004) The functional interaction of the hepatitis C virus helicase molecules is responsible for unwinding processivity. J. Biol. Chem., 279, 26005–26012.

23. Serebrov,V. and Pyle,A.M. (2004) Periodic cycles of RNA unwinding and pausing by hepatitis C virus NS3 helicase. Nature, 430, 476–480.

24. Tackett,A.J., Chen,Y., Cameron,C.E. and Raney,K.D. (2005) Multiple full-length NS3 molecules are required for optimal unwinding of oligonucleotide DNA in vitro. J. Biol. Chem., 280, 10797–10806.

25. Morris,P.D., Tackett,A.J., Babb,K., Nanduri,B., Chick,C., Scott,J. and Raney,K.D. (2001) Evidence for a functional monomeric form of the bacteriophage T4 Dda helicase. Dda does not form stable oligomeric structures. J. Biol. Chem., 276, 19691–19698.

26. Nanduri,B., Byrd,A.K., Esfiri,L.R., Tackett,A.J. and Raney,K.D. (2002) Pre-steady-state DNA unwinding by bacteriophage T4 Dda helicase reveals a monomeric molecular motor. Proc. Natl Acad. Sci. USA, 99, 14722–14727.

27. Byrd,A.K. and Raney,K.D. (2004) Protein displacement by an assembly of helicase molecules aligned along single-stranded DNA. Q. Rev. Biophys., 38, 69–100.

28. Fairman,M.E., Maroney,P.A., Wang,W., Bowers,H.A., Gollnick,P., Jankowsky,E., Gross,C.H., Shuman,S. and Pyle,A.M. (2001) Active E. coli UvrD is the active form of the helicase Escherichia coli. J. Mol. Biol., 269, 33063–33068.

29. Liu,Y.C. and Matthews,K.S. (1993) Dependence of trp repressor-operator affinity, stoichiometry, and apparent cooperativity on DNA sequence and size. J. Biol. Chem., 268, 23239–23249.

30. Byrd,A.K. and Raney,K.D. (2005) Increasing the length of the single-stranded overhang enhances unwinding of duplex DNA by bacteriophage T4 dda helicase. Biochemistry, 44, 12990–12997.

31. Francis,P., Jankowsky,E., Gross,C.H., Shuman,S. and Pyle,A.M. (2001) Active E. coli UvrD is the active form of the helicase Escherichia coli. J. Biol. Chem., 276, 19691–19698.

32. Mohanty,B.K. and Bastia,D. (2004) Binding of the replication terminator protein Fob1p to the Ter sites of yeast causes polar fork arrest. J. Biol. Chem., 279, 1932–1941.

33. Mohanty,B.K., Bairwa,N.K. and Bastia,D. (2006) The Toflp-Csm3p protein complex counteracts the Rrm3p helicase to control replication termination of Saccharomyces cerevisiae. Proc. Natl Acad. Sci. USA, 103, 907–912.

34. Bellingham,M.A., Lin,C. and Caron,P.R. (1998) Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. Structure, 6, 89–100.
44. Maine, I.P. and Kodadek, T. (1994) Inhibition of the DNA unwinding and ATP hydrolysis activities of the bacteriophage T4 DDA helicase by a sequence specific DNA-protein complex. *Biochem. Biophys. Res. Commun.*, **198**, 1070–1077.

45. Singleton, M.R., Dillingham, M.S., Gaudier, M., Kowalczykowski, S.C. and Wigley, D.B. (2004) Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. *Nature*, **432**, 187–193.

46. Raney, K.D. and Benkovic, S.J. (1995) Bacteriophage T4 Dda Helicase Translocates in a Unidirectional Fashion on Single-stranded DNA. *J. Biol. Chem.*, **270**, 22236–22242.

47. Stano, N.M., Jeong, Y.J., Donmez, I., Tummalapalli, P., Levin, M.K. and Patel, S.S. (2005) DNA synthesis provides the driving force to accelerate DNA unwinding by a helicase. *Nature*, **435**, 370–373.

48. Kodadek, T. (1991) Inhibition of protein-mediated homologous pairing by a DNA helicase. *J. Biol. Chem.*, **266**, 9712–9718.

49. Hacker, K.J. and Alberts, B.M. (1992) Overexpression, Purification, Sequence Analysis, and Characterization of the T4 Bacteriophage Dda DNA Helicase. *J. Biol. Chem.*, **267**, 20674–20681.

50. Salinas, F. and Kodadek, T. (1994) Strand exchange through a DNA–protein complex requires a DNA helicase. *Biochem. Biophys. Res. Commun.*, **205**, 1004–1009.

51. McClure, W.R. (1985) Mechanism and control of transcription initiation in prokaryotes. *Annu. Rev. Biochem.*, **54**, 171–204.

52. Riggs, A.D., Suzuki, H. and Bourgeois, S. (1970) Lac repressor–operator interaction. I. Equilibrium studies. *J Mol. Biol.*, **48**, 67–83.

53. Youderian, P., Vershon, A., Bouvier, S., Sauer, R.T. and Susskind, M.M. (1983) Changing the DNA-binding specificity of a repressor. *Cell*, **35**, 777–783.

54. Hawley, D.K., Johnson, A.D. and McClure, W.R. (1985) Functional and physical characterization of transcription initiation complexes in the bacteriophage lambda OR region. *J. Biol. Chem.*, **260**, 8618–8626.

55. Takeda, Y., Folkmanis, A. and Echols, H. (1977) Cro regulatory protein specified by bacteriophage lambda. Structure, DNA-binding, and repression of RNA synthesis. *J. Biol. Chem.*, **252**, 6177–6183.

56. Spassky, A. and Buc, H.C. (1977) Physico-chemical properties of a DNA binding protein: Escherichia coli factor H1. *Eur. J Biochem.*, **81**, 79–90.