Helicase-catalysed translocation and strand separation

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Abstract

Helicases are molecular-motor enzymes that manipulate DNA or RNA during replication, repair, recombination, transcription, translation and processing of nucleic acids. The mechanisms for helicase activity have been studied intensely over the past decade. Recent advances in our understanding of the helicase mode of action have led to a general convergence of models that describe this diverse class of enzymes. One mechanism has been proposed that appears to have withstood the test of time, namely the inchworm mechanism. As the name implies, this mechanism involves a process whereby a helicase maintains at least two sites of contact with the nucleic acid. These binding sites can move relative to one another in a sequential fashion, resulting in net movement of the enzyme along the nucleic acid. The inchworm mechanism appears to be applicable to oligomeric states beyond the simple monomeric molecular motor. Although there are certainly many pertinent questions that remain unanswered, striking similarities in both form and function of seemingly disparate enzymes are becoming evident.

Introduction

Life as we know it is dependent upon a multitude of cellular processes that serve to utilize and perpetuate the information stored in the genetic code, while simultaneously preventing the loss of genomic integrity [1–3]. NA (nucleic acid) metabolism often requires enzymes that are able to catalyse the thermodynamically unfavourable separation of ds (double-stranded) helices, to form ss (single-stranded) intermediates transiently, for the purpose of cellular events such as replication, recombination, repair, transcription, translation and splicing. Helicases fulfil such a requirement, in that they possess the ability to couple energy associated with NTP hydrolysis with translocation along the NA lattice and manipulation of NA structure [4]. Some helicases can function as monomeric species, providing the simplest model system for translocation and strand separation [5,6]. A general helicase model requires binding of the NTP·Mg2+ complex and the NA substrate to the enzyme (Figure 1). A series of discrete NTP hydrolysis events is coupled with translocation along the NA lattice and separation of the complementary strands. How closely these two events (translocation and strand separation) are related is still a topic of reasonable debate.

Helicases are thought to function in vivo as integral components of various macromolecular complexes [7]. As such, it has become apparent that every form of life codes for at least one and usually several distinct helicases. If the function of a helicase is absent or defective within the context of a higher-order biological system, then pathological states can arise. Known disease states involving defective helicases are characterized by traits such as an increased susceptibility to cancer, premature aging, and/or severe mental retardation [8]. Mutations that give rise to the human disease state xeroderma pigmentosum are thought to result from a defect in CSB (Cockayne syndrome group B) protein. While dsDNA unwinding activity has not been observed for CSB yet, the protein does possess seven conserved helicase motifs and may be involved in the nucleotide excision-repair pathway and possibly rescue of stalled transcription machinery [9]. Several studies have revealed the importance of helicase action within the context of recombination repair and rescue of stalled replication forks [10,11]. The RecQ helicase from Escherichia coli has been implicated in the events of homologous recombination as mediated by the RecF pathway [12]. Importantly, there are five human homologues of the E. coli RecQ helicase, and mutations in two of these enzymes, the WRN and BLM helicases, result in manifestation of Werner’s and Bloom’s syndromes respectively [8]. Thus defining the mechanism(s) of action for this class of enzymes is an active topic of great interest.

Mechanism of NTP binding and hydrolysis

Helicases have been classified into four large super-families (SF1–SF4) based on sequence identity, with SF4 being subdivided into two smaller families (DnaB-like and Rho) [13,14]. SF1 and SF2 helicases possess seven characteristic helicase motifs. SF3 possesses three conserved motifs, while SF4 has five conserved helicase motifs. All helicases possess a putative NTP-binding domain, comprising the Walker A and B motifs. These two motifs contain a conserved lysine and ‘DEAD’ (or DExH) box moieties that are required...
for NTP binding and metal ion co-ordination by Walker A and B respectively. Base-catalysed ester hydrolysis is facilitated through co-ordination of the metal ion and a water molecule by electronegative side chains in the DEAD box motif. Specifically, a conserved glutamic acid appears to serve as the catalytic base that activates the water molecule for in-line nucleophilic attack on the γ-phosphate of the bound NTP [15,16]. Stimulation of the helicase NTPase activity often requires the presence of a DNA substrate cofactor. It should be noted that this rule does not apply to the NTPase activity of every helicase, as is the case with the *E. coli* helicase, DnaB [17].

The initial crystal structure of the PcrA helicase revealed four regions that are now identified as the canonical 1A, 1B, 2A, and 2B domains associated with SF1 helicases [15]. The helicase NTP-binding domain, located within domain 1A, is similar to that of the RecA recombination protein, which catalyses strand exchange and re-annealing reactions. In contrast to RecA, helicases also possess domain 2A, which has a ‘core’ that is almost identical in topology to domain 1A. Subsequent PcrA structures determined in complexes with a ssDNA/dsDNA junction and in the presence or absence of p[NH]ppA (adenosine 5′-[β,γ-imido]triphosphate)·Mg2+ suggest that the conformational change that accompanies NTP binding results in the closure of a cleft between domains 1A and 2A [16]. Closing of this cleft is mediated solely through interactions between the bound metal ion with domain 1A and the γ-phosphate with domain 2A. The positions of domains 1B and 2B are also altered as a function of NTP binding. The resulting motion that occurs as a function of ATP binding, hydrolysis and release has been likened to an inchworm crawling along a tree branch and has significant mechanistic applications towards understanding translocation and strand-separation (Figure 1).

The crystal structure of the *E. coli* RepSF1 helicase revealed two monomers bound to ssDNA [18]. ADP (without metal ion) was bound in the active site of one of the Rep monomers, resulting in a dramatic swivelling of domain 2B (by 130° to form the ‘closed’ form of Rep). Later experiments completely deleted the 2B domain from the Rep sequence, resulting in an increase in both unwinding rate and amplitude [19]. The Rep 2B-deletion-mutant studies suggest that only the motor domains may in fact be necessary for unwinding. By analogy, the bound NTP therefore serves as a ‘bridge’ between domains 1A and 2A. This tether holds the conformational change until this molecular bridge is ‘burned’.

Some helicases function as hexamers. The hexameric structure encircles the DNA to form either a ‘ring’ or a ‘pinwheel’ shape [20]. It is thought that this strongly inhibits premature dissociation of the helicase from the substrate, a property that is necessary for timely completion of events such as replication. There are currently three variations on the model for translocation and strand separation by hexameric helicases (Figure 2): wedge, torsional, and helix-destabilizing [21]. In each of these models, a single strand of NA passes through the centre of the hexameric ring. The wedge and torsional models differ in the requirement for interactions with the excluded strand. In the wedge model, the helicase does not interact tightly with the excluded strand, and in the torsional model, the helicase uses the excluded strand as a fulcrum that supplies the capability for helicase action. The helix-destabilizing model proposes that the outer surfaces of the helicase interact with and actively destabilize the helix. Neither the wedge nor the torsional model requires any specific contacts with dsDNA.

The process of NTP binding and hydrolysis for hexameric helicases is quite complex and a number of models have been proposed to explain the order and co-ordination of each nucleotide-binding event [21]. The bacteriophage T4 helicase, gp41, undergoes the transition from a dimeric form to the active hexameric form of the enzyme in the presence of ATP [22]. The hexameric helicase DnaB has six NTP-binding sites and nucleotide binding exhibits biphasic kinetics [23]. There is evidence suggesting that not all six NTP-binding sites are occupied at once [24]. Studies with DnaB suggest that three sites bind NTP with high affinity and three sites bind NTP with decreased affinity, illustrating the increased complexity of helicases [23].

**Modes of translocation and strand separation**

Some helicases can separate tens of thousands of base pairs in a single binding event [25,26], while others are only able to unwind a few base pairs at a time [5,27]. Helicases perform...
Figure 2 | Hexameric helicase unwinding models

(A) In the wedge model, hexameric helicase action involves specific interactions with the one strand of NA that passes through the central channel of the hexamer, often referred to as the included strand. The excluded strand is displaced through non-specific forces, while no specific interactions with dsNA are proposed for the wedge model. (B) In the torsional model, the hexamer interacts with both the included and excluded strands. The excluded strand serves as a fulcrum that promotes rotation of the hexamer as it translocates down the NA lattice. (C) The helix-destabilizing model is the only hexameric model that proposes specific interactions between the helicase and dsNA. In this model, the surface of the helicase in direct contact with dsNA serves to separate the complementary strands.

this action through a combination of translocation and strand separation. The rates of translocation versus strand separation have been compared for several helicases. Recent studies with the E. coli helicase UvrD reveal translocation rates of approx. 190 nt·s⁻¹, compared with UvrD unwinding rates of approx. 70 bp·s⁻¹ [28]. Helicase–polymerase coupling between gp41 and T4 DNA polymerase holoenzyme (gp43, gp45, and gp44/62) was shown to result in strand separation/DNA synthesis rates of approx. 250 nt·s⁻¹ [29]. In the absence of the polymerase holoenzyme, gp41 and gp43 perform strand displacement and DNA synthesis at a rate of approx. 90 nt·s⁻¹, highlighting the influence that a macromolecular complex may have upon helicase function.

With regard to strand separation, two general models have been proposed. In the first model, helicases utilize the energy of NTP hydrolysis to drive translocation alone, and strand separation occurs as the enzyme sequentially traps single base pairs as they separate momentarily because of thermal fluctuations. This type of model would consider the helicase a passive motor that performs no direct action upon the NA, other than a directed walk along the lattice. A second general model for helicase catalysis proposes that the enzyme actively drives the thermodynamically unfavourable strand-separation. Proteins and enzymes that bind NA interact with a substrate that is often very large, usually much larger than the protein itself, creating the possibility that helicases could utilize binding energy to unwind dsNA. In the absence of NTP hydrolysis, helicases could then in theory separate dsNA through stoichiometric binding. However, helicases translocate, and they do so at the expense of NTP hydrolysis. A series of crystal structures with PcrA led the authors to propose a mechanism in which strand separation was the direct result of conformational changes that actively promoted helix destabilization [15,16]. These conformational changes are also thought to drive translocation. Thermodynamic calculations suggest that a single NTP hydrolysis event could theoretically separate 6–8 bp [30]. However, estimates for helicase efficiency (i.e. number of bp separated per ATP hydrolysed) have generally been near 1 bp per ATP [4]. Why then does nature incorporate such an inefficient enzyme into universal cellular processes?

Translocate, separate and displace

Some helicases possess other enzymatic properties such as protease and/or nuclease activity, as determined by their respective biological duties. An additional function of helicase action, disparate from unwinding, lies in the ability of certain helicases to displace proteins bound to NA from the path of the macromolecular complex in which they are engaged [31–33]. Displacement is thought to result from the force generated by translocation of the enzyme along the ssNA lattice, similar to other translocating motors [34]. Work with the E. coli translocase FtsK showed that the bidirectional translocation of the enzyme can generate a force of up to 60 pN [35]. This level of linearly applied force is similar to the torque generated by the F₁-ATPase flagellar motor [36]. Such similarities conform with a theory that maximal-force output scales with the mass of the motor over several orders of magnitude, from the molecular to the macroscopic [37].

By analogy, a helicase may be thought of as a molecular ‘snow-plough’ that utilizes non-specific forces to displace unbound NA from the path of the translocating helicase (Figure 2) [38,39]. The ‘snow-plough’ model could actively promote strand separation through a non-specific steric exclusion that results from translocation along one of the strands of NA. This is consistent with studies in our own laboratory, indicating that Dda interacts primarily with one strand of NA [40,41]. Other studies with vaccinia virus NPH-II indicated that this SF2 helicase was only sensitive to modifications in the backbone of the strand to which the enzyme was bound initially [42]. This type of one-strand interaction may not be applicable to every helicase, as revealed from studies with DnaB and NS3 [41,43].

The apparent lack of efficient conversion of energy into work may therefore result from the biological requirements placed upon helicases, namely that they must translocate, separate, and displace. The end result is an enzyme that can perform multiple tasks in a single reaction. Alterations
in either the oligomeric state or motif characteristics might enhance or diminish the ability of a specific helicase to perform one or more of the aforementioned tasks. With regard to the apparent lack of helicase efficiency, it is important to note that the majority of helicase studies have been performed with the enzyme in isolation. Incorporating any helicase into the appropriate macromolecular context is very likely to alter the efficiency of the motor. However, dissecting out this efficiency has proven to be difficult.

**Dda: transitioning from monomer to multimer**

The mechanism of unwinding for Dda has been characterized biochemically in great detail [6,40,41,44–46]. The Dda helicase can function as a monomeric molecular motor and does not readily form higher-order oligomeric species in solution [6,44]. Dda translocates with a 5′ to 3′ directional bias and can remove several protein blocks from its path [46,47]. Increasing the nt length of ssDNA incubated with excess Dda results in higher rates and higher amplitudes of streptavidin displacement that drives streptavidin displacement [32]. The enhancement does not result from co-operative binding of Dda to the oligonucleotide. The simple co-operative inchworm model describing the resulting displacement from a biotinylated oligonucleotide [32] results in higher rates and higher amplitudes of streptavidin displacement [46,47]. Increasing the nt length of ssDNA incubated with excess Dda and can remove several protein blocks from its path [46,47].

**Conclusions**

The convergence of helicase models towards different variations of the inchworm mechanism provides insight into both the subtle differences and similarities observed for this class of enzymes. Certain elements, such as the motor domains 1A and 2A, are requisite not only for NTP hydrolysis but also for subsequent conformational changes that drive translocation and strand separation. Electrostatic interactions between helicases and DNA have been shown to be important for the action of both SF1 and SF2 helicases. The recently solved RecBCD structure piques the imagination by revealing a defunct helicase that is joined at the hip with two functional motors, while simultaneously answering questions that define the polarity of translocation [48]. Directional bias appears to involve a reversal of translocation direction rather than a reversal of the binding of the enzyme to the DNA. A precise mechanism describing strand separation is still rather elusive, though a ‘snow-plough’ or ‘wire-stripper’ model may prove more than adequate. Finally, studies with Dda may begin to illuminate a transition from a functional monomer to a functional multimeric species, illustrating the versatility of helicase catalysis.

**References**


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