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Dynamics of monomeric and hexameric helicases

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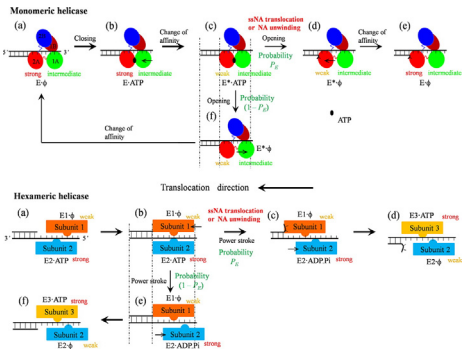
HIGHLIGHTS

- Models of NA unwinding by monomeric and hexameric helicases are presented.
- Both monomeric and hexameric helicases use active mechanism to unwind NA duplex.
- Experimental data on NA unwinding dynamics are quantitatively explained.
- Experimental data on ssNA translocation dynamics are quantitatively explained.

GRAPHICAL ABSTRACT



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ABSTRACT

Helicases are a ubiquitous class of enzymes that use the energy of ATP hydrolysis to unwind nucleic acid (NA) duplex. According to the structures, helicases can be classified as the non-ring-shaped (or monomeric) and ring-shaped (or hexameric). To understand the NA unwinding mechanism, here we study theoretically the unwinding dynamics of both the monomeric and hexameric helicases based on our proposed model. Various available single-molecule experimental data on unwinding speed of both the monomeric and hexameric helicases versus the external force applied to the ends of the NA duplex to unzip the duplex or versus the stability of the NA duplex are consistently and quantitatively explained. We provide quantitative explanations of the experimental data showing that while the unwinding speeds of some monomeric helicases are insensitively dependent on the external force they are sensitively dependent on the stability of the NA duplex. The experimental data showing that wild-type Rep translocates along ssDNA with a lower speed than RepΔ2*B* (removal of the 2B subdomain of Rep) and that RepΔ2*B* monomer can unwind DNA whereas the wild-type monomer is unable to unwind DNA are also quantitatively explained. Our studies indicate that although the monomeric and hexameric helicases show very different features on the dependence of NA unwinding speed upon the external force, they use much similar active mechanisms to unwind NA duplex.

1. Introduction

Helicases are a ubiquitous class of enzymes that use the energy of NTP hydrolysis, typically of ATP, to unwind nucleic acid (NA) duplex, providing single-stranded NA (ssNA) template required in many biological events such as replication, repair and recombination [1–6]. During NA unwinding, helicases translocate processively along ssNA in one specific direction. From a physical point view, helicase functions as a molecular motor, utilizing the chemical energy from NTP hydrolysis to drive the mechanical motion of the motor along ssNA and unwind the downstream NA duplex. Thus, understandings of the chemomechanical coupling mechanism and NA unwinding dynamics are the most fundamental issues for the helicase.

According to the structures, helicases can be classified as the non-ring-shaped (or monomeric) [7–11] and ring-shaped (or hexameric)

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[12–14]. For the former group, in vitro biochemical assays showed that some helicases such as *Escherichia coli* RecQ [15,16], hepatitis C virus (HCV) NS3 [17–19] and bacteriophage T4 Dda [20–22] function as monomers to unwind NA duplex, while others such as *E. coli* Rep [23,24], *E. coli* UvrD [25,26] and *Bacillus stearothermophilus* PcrA [27] show very weak NA unwinding activities in monomeric forms and only in dimeric or oligomeric forms can they show efficient unwinding activity. To understand the NA unwinding mechanism and dynamics, the unwinding speed of helicase monomers such as RecQ, HCV NS3 and Dda which can unwind NA duplex as monomers versus the external force applied to the ends of NA duplex to unzip the duplex and/or versus the stability of the NA duplex was elaborately studied by using single-molecule optical or magnetic trappings [16,18,19,22].

Bacteriophage T7 gp4 [28–31] and T4 gp41 [16,32,33] helicases are two model enzymes for understanding the hexameric helicases. In the presence of dTTP, T7 gp4 assembles usually into a hexamer that has a central channel [28,29,34]. After the binding of ssDNA into the central channel [35], the hexamer translocates processively along the ssDNA in the 5′ to 3′ direction by dTTP hydrolysis [36,37] and thus unwinds the downstream DNA duplex. Using single-molecule optical or magnetic trappings, the DNA unwinding speed versus the external force applied to the ends of DNA duplex and/or versus the stability of the DNA duplex was also systematically studied, showing that both the external force and the DNA stability affect sensitively the unwinding speed [16,30,31,33].

Using kinetic models which were modified from that originally proposed by Betterton and Julicher [38], these single-molecule data for the hexameric helicases were also fitted well. From the fittings it was concluded that T7 gp4 uses an active mechanism whereas T4 gp41 uses a passive mechanism to unwind DNA [16,30,33]. Considering that the two enzymes have the very similar structures, it is puzzling that the two have sharply different mechanisms. In addition, the single-molecule experimental data on the dependence of the unwinding speed of the monomeric helicases upon the external force applied to the ends of NA duplex have not been explained quantitatively. Since both the external force and the GC content in the NA duplex affect sensitively the stability of the NA duplex, it is puzzling that the while NA unwinding speeds of some monomeric helicases such as RecQ and HCV NS3 are insensitively dependent on the external force, they are sensitively dependent on the stability of the NA duplex [16,18,19]. For example, while the RNA unwinding speed of HCV NS3 is kept nearly unchanged in the large range of the external force (5–17 pN), the unwinding speed for the RNA of 100% GC content is reduced by about 3-fold relative to that for the RNA of 100% AU content [18,19]. Moreover, it is still uncertainty if the helicases use active or passive mechanism to unwind NA duplex. It is unclear why the monomeric and hexameric helicases show very different features on the dependence of their NA unwinding speeds upon the external force [16,18,19,22,30,33].

To address the above-mentioned unclear issues, here we study the dynamics of both monomeric and hexameric helicases using our proposed models [39]. We provide a consistent and quantitative explanation of a lot of different, independent single-molecule experimental data. The studies thus have important implications for understanding the NA mechanism of the helicases.

2. Models

2.1. Model of NA unwinding and/or ssNA translocation by the monomeric helicase

Some monomeric helicases such as PcrA, Rep and UvrD consist of two domains [7,40,41], with each domain comprising two subdomains (1A, 1B and 2A, 2B) (Fig. 1, upper panels). Others such as HCV NS3 helicase domain and RecQ catalytic core have no the subdomain that is equivalent to subdomain 2B of the four-subdomain helicases [42,43].

The model for the monomeric helicase is built up based on the following two hypotheses or pieces of experimental or structural evidence. (i) It is hypothesized that subdomain 2A has a high affinity for ssDNA in nucleotide-free state whereas has a low affinity in ATP state, while subdomain 1A has an intermediate affinity which is independent (or weakly dependent) on the nucleotide state. This hypothesis is supported by the following pieces of experimental evidence. Biochemical data showed that the nucleotide-free helicase has a high affinity for ssDNA, while the ATP-helicase complex has a low affinity [11,44,45]. For example, for HCV NS3, the equilibrium dissociation constant K_d is about 0.4 nM (equivalent to the binding energy of about $21.6k_BT$) in nucleotide-free state, while K_d is reduced by about 100-fold in ATP state, equivalent to the reduction of the binding energy by about $4.6k_BT$ [45]. These biochemical data can be understood as follows. In nucleotide-free state the total free energy of the strong interaction of subdomain 2A and intermediate interaction of subdomain 1A with ssDNA is about $21.6k_BT$, while in ATP state the total free energy of the weak interaction of subdomain 2A and intermediate interaction of subdomain 1A with the ssDNA is reduced by about $4.6k_BT$ to a value of about $17k_BT$. The reduction of the total free energy of $4.6k_BT$ is due to the change in the binding affinity of subdomain 2A. (ii) ATP binding in the cleft between subdomains 1A and 2A results in closure of the cleft, while the release of ATP-hydrolysis products—ADP and Pi—leads to opening of the cleft. This is consistent with the available structural data [7,41,46].

The model is schematically shown in Fig. 1 (upper panels) [39]. We begin with the helicase in nucleotide-free state, with subdomain 2A binding strongly and subdomain 1A binding intermediately to the ssDNA (Fig. 1a). Since subdomain 2A binds strongly to the ssDNA, the closing of the cleft induced by ATP binding causes subdomain 1A to move close to subdomain 2A along the ssDNA by overcoming the intermediate binding energy of subdomain 1A with the ssDNA (Fig. 1b). The closing of the cleft in turn induces the change in the interaction between subdomain 2A and the ssDNA from the strong to weak, with unactivated E·ATP complex (Fig. 1b) transiting to activated E*·ATP complex (Fig. 1c). After ATP hydrolysis, the release of products ADP and Pi leads to opening of the cleft. Since now subdomain 2A has the weak interaction with the ssDNA whereas subdomain 1A has the intermediate interaction, the opening of the cleft would cause most probably subdomain 2A to move away from subdomain 1A along the ssDNA if no downstream DNA duplex is present to impede the movement of subdomain 2A. If the downstream DNA duplex is present, the movement of subdomain 2A would result in DNA unwinding (Fig. 1d), which is called effective unwinding. However, the DNA unwinding would impede the downstream movement of subdomain 2A. Thus, it is also possible that the opening of the cleft would cause the upstream movement of subdomain 1A away from subdomain 2A by overcoming the intermediate binding energy of subdomain 1A with the ssDNA (Fig. 1f), which is called futile unwinding. In other words, the opening of the cleft would cause either the effective unwinding (with a probability P_E) or the futile unwinding (with a probability $1 - P_E$). The opening of the cleft in turn induces the change in the interaction between subdomain 2A and the ssDNA from the weak to strong, with activated E*·nucleotide-free state (Fig. 1d) transiting to unactivated E·nucleotide-free state of Fig. 1e that is the same as that of Fig. 1a except that the helicase has moved downstream by one step or with activated E*·nucleotide-free state (Fig. 1f) transiting to unactivated E·nucleotide-free state of Fig. 1a. Since available structural studies for PcrA, UvrD and HCV NS3 showed that the opening of the cleft between subdomains 2A and 1A increases the distance between the two subdomains by about 1 nucleotide (nt) [7,41,46], we take the step size to be 1 nt for the monomeric helicase.

As biochemical data showed, the transition from the strong to weak interaction of subdomain 2A with the ssDNA (from Fig. 1b to c) accompanies the transition of subdomain 2B from the weak to strong interaction with the downstream DNA duplex [11,44]. We argue here