Helicase activity and substrate specificity of RecQ5β*

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RecQ5β is an essential DNA helicase in humans, playing important roles in DNA replication, repair, recombination and transcription. The unwinding activity and substrate specificity of RecQ5β is still elusive. Here, we used stopped-flow kinetic method to measure the unwinding and dissociation kinetics of RecQ5β with several kinds of DNA substrates, and found that RecQ5β could well unwind ss/dsDNA, forked DNA and Holiday junction, but was compromised in unwinding blunt DNA and G-quadruplex. Rec5β has the preferred unwinding specificity for certain DNA substrates containing the junction point, which may improve the binding affinity and unwinding activity of RecQ5β. Moreover, from a comparison with the truncated RecQ5β^{1-467}, we discovered that the C-terminal domain might strongly influence the unwinding activity and binding affinity of RecQ5β. These results may shed light on the physiological functions and working mechanisms of RecQ5β helicase.

Keywords: helicase, RecQ5β, DNA, substrate specificity, unwinding kinetics

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1. Introduction

Helicases are a ubiquitous class of motor proteins that move directionally along the nucleic acid phosphodiester backbone, separating duplex nucleic acid strands (DNA, RNA, or RNA-DNA hybrid) by using energy derived from the hydrolysis of NTP or dNTP.[1,2] RecQ family is a member of superfamily 2 with 3′–5′ DNA unwinding activity,[3] which is named after *Escherichia coli* RecQ helicase.[4] The RecQ family helicases are highly evolutionary conserved from bacteria to human, and play important roles in the maintenance of genome integrity and stability.[5–8] There are five identified members of the RecQ family in human cells, namely RecQ1, RecQ2 (known as BLM), RecQ3 (WRN), RecQ4 (RTS), and RecQ5.[9–12] Importantly, the deficiencies of BLM, WRN, and RTS could lead to serious human hereditary diseases: Bloom syndrome, Werner syndrome and Rothmund–Thomson syndrome, respectively.[13] These diseases are all attributed to autosomal recessive disorders characterized by genomic instability, premature aging and cancer predisposition.[14]

Although RecQ5 deficiency has not been found to be directly involved in any hereditary disease in humans until now, RecQ5 is believed to provide important backup functions in the absence of other DNA helicases.[10,15] Furthermore, RecQ5 knockout is prone to cause cancer in mice, in which the cells show chromosomal abnormalities and elevated levels of sister chromatid exchanges.[16] Although the biological role of RecQ5 is only partially understood, RecQ5 is established to be involved in various DNA metabolic pathways including DNA replication, repair, recombination, and transcription by interacting with multiple functional proteins, such as Rad51, PCNA, and topoisomerase.[17–19]

There are three different isoforms of human RecQ5 helicase, namely RecQ5α (410 aa), RecQ5β (991 aa), and RecQ5γ (435 aa), resulted from the alternative splicing of the RecQ5 transcript.[20] RecQ5β, which is the largest isomer, has DNA unwinding, strand-annealing and strand-exchange activities,[21–23] and contains a conserved helicase core domain, a zinc-binding motif and a long C-terminal domain. The C-terminal domain exists in RecQ5β exclusively, and shares no homology with the other RecQ family helicases. The C-terminal domain is believed to contribute greatly to the annealing and strand-exchange activities of RecQ5β.[24] However, the influence of the C-terminal domain on RecQ5β unwinding activity is still not clear. In previous studies, RecQ5β was

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characterized to partially unwind forked and Holiday junction DNA, and was unable to unwind D-loop and bubble DNA.[23] Besides that, BLM and WRN are identified to efficiently unwind G-quadruplex, bubble, D-loop, Holiday junction, and forked DNA.[25] DmRecQ5, which is a highly homologous helicase with RecQ5β in *Drosophila melanogaster*, has a substrate specificity to unwind junction structures.[26] Despite these studies on the substrate specificity of members of the RecQ family, relatively less is known about human RecQ5 helicases. Moreover, although previous studies spent much effort on the biochemical characteristics and cellular functions of RecQ5β, the substrate specificity and unwinding kinetics of RecQ5β are still elusive, requiring more concern and attention.

Stopped-flow kinetic method is often used to study the kinetics of fast reactions at the millisecond level, which is carried out by rapid mixing of multiple solutions. By measuring the change of fluorescence signals, one could accomplish the continuous and real-time observations of fast biochemical reaction processes. To examine the DNA unwinding kinetics, we used the fluorescence resonance energy transfer (FRET) technique. FRET is widely used in life science to detect nanoscale distance changes between biological macromolecules *in vivo* and *in vitro*. In this assay, the double-stranded DNA (dsDNA) substrate was typically labeled with fluorescent molecule fluorescein (Flu, donor) at the 3′ end and with hexachlorofluorescein (Hex, acceptor) at the opposing 5′ end. The overlap between the fluorescence emission spectrum of Flu and the excitation spectrum of Hex results in an energy transfer from Flu to Hex. If the dsDNA is unwound by helicases, Flu and Hex are no longer in close proximity, so the energy transfer is disrupted, resulting in an enhancement of fluorescence emission from the Flu.[27]

In addition, we also used the fluorescence polarization method to examine the helicase dissociation kinetics. In this assay, DNA substrates were only labeled with Flu at the 3′ end. The fluorescence anisotropy signal is proportional to the volume of fluorescent molecules. If a helicase is binding to the DNA substrate, the volume of fluorescent molecule, which currently is a complex of Flu, DNA substrate and the bound helicase, is large, and the fluorescence anisotropy is high. If the helicase has dissociated from the DNA substrate, the volume would decrease, inducing the decrease of fluorescence anisotropy. So we could monitor the dissociation kinetics by measuring the change of fluorescence anisotropy. By combining the two kinds of fluorescence detections with the stopped-flow method, we have previously studied the DNA unwinding activities and annealing activities of some helicases, such as *E. coli* RecQ, BLM, RecQ5, etc.[28–32]

In this work, we used the rapid stopped-flow method based on FRET to study the DNA unwinding kinetics catalyzed by full-length RecQ5β (RecQ5β^{fl}) and the N-terminal fragment RecQ5β^{1-467}. By comparisons of the unwinding kinetics with five kinds of DNA substrates, we discovered that RecQ5β helicase might prefer to unwind specific DNA structures, and the C-terminal domain might have important effects on the unwinding efficiency and DNA binding affinity of RecQ5β. Our work may be helpful for further studies on RecQ5β.

2. Materials and methods

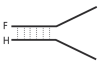
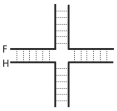
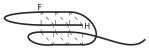
2.1. Reagents and buffers

All chemicals were of reagent grade and all solutions were prepared in high quality deionized water from a Milli-Q Ultrapure Water Purification System (Millipore, France) with resistivity $>$ 18.2 MΩ·cm. ATP was purchased from Sigma (St. Louis, USA), and was dissolved as a concentrated stock at pH 7.0. The ATP concentration was determined by using an extinction coefficient at 259 nm of 1.54×10^4 $\mathrm{cm^{-1}\cdot M^{-1}}$. All the unwinding and dissociation assays were performed in reaction buffer containing 25-mM Tris-HCl (pH 7.5, 37 °C), 10-mM NaCl, 2-mM $MgCl_2$, 0.1-mM dithiothreitol (DTT), and $ZnCl_2$ at given concentrations. The RecQ5β^{fl} and RecQ5β^{1-467} we used in this assays both have zinc-binding motifs. Zinc-binding motifs have important influence on the binding and unwinding activities of the RecQ5β helicase.[24] When Zn^{2+} was at the same concentration as the helicase, the unwinding activities of RecQ5β were better (data not shown). So in the unwinding and dissociation assays, we added $ZnCl_2$ to the unwinding buffer at the same concentration as the helicase.

2.2. RecQ5β helicases and DNA substrates

RecQ5β^{1-467} is a truncated fragment of human RecQ5β that lacks the C-terminal domain. The RecQ5β^{fl} and RecQ5β^{1-467} were prepared as previously described.[24] The concentrations of the purified proteins were determined by Bradford assay and stored at −80 °C.[33] DNA substrates used in the unwinding assays were labeled by Flu and Hex. RecQ5β has 3′–5′ unwinding activity, and a single-stranded 3′ tail with appropriate length will enhance the unwinding efficiency of RecQ5β.[31] So we used 20-nt 3′ tail for some of the DNA substrates. In total, five DNA substrates were used to study the substrate specificity of RecQ5β helicase (Table 1). In dissociation experiments, DNA substrates were only labeled by Flu.

Table 1. The sequences and labels of DNA molecules used in this study.

Substrate	DNA sequence (5′–3′)	Structure
ss/dsDNA	ATCTGCATTGGTGATCACGG-F[a]	
	H[b]-CCGTGATCACCAATGCAGATTGACGAACCTTTGCCCACGT	
Forked DNA	GCAGTAGGCCTAGCATGACAATCTGCATTGGTGATCACGG-F[a]	
	H[b]-CCGTGATCACCAATGCAGATTGACGAACCTTTGCCCACGT	
Blunt DNA	ATCTGCATTGGTGATCACGG-F[a]	
	H[b]-CCGTGATCACCAATGCAGAT	
Holiday junction	GCAGTAGGCCTAGCATGACAATCTGCATTGGTGATCACGG-F[a]	
	H[b]-CCGTGATCACCAATGCAGATTGACGAACCTTTGCCCACGT	
	ACGTGGGCAAAGGTTCGTCAATGGACTGACAGCTGCATGG	
	CCATGCAGCTGTCAGTCCATTGTCATGCTAGGCCTACTGC	
G-quadruplex	H[b]-AGGGTTTTTTTT(F[a])GGGTTAGGGTTAGGG-dT$_{20}$	
Protein trap	dT$_{56}$	

[a]F, Fluorescein; [b]H, hexachlorofluorescein.

2.3. Stopped-flow measurements

All the kinetic assays were carried out using a Bio-Logic SFM-400 stopped-flow instrument. The unwinding kinetic assays were measured in a two-syringe mode. The DNA substrates (2 nM) and RecQ5β were pre-incubated in reaction buffer in one syringe, and ATP (2 mM) was stored in another syringe. The reaction was initiated by rapidly mixing the two syringes (within 6 ms). When DNA substrates are unwound by the RecQ5β, the distance between Flu and Hex would increase, reducing the efficiency of FRET between the two fluorescence molecules. Then the donor fluorescence intensity would increase. To monitor the unwinding process, Flu was excited at 492 nm (2-nm bandwidth) and its emission was monitored at 525 nm using a high pass filter with 20-nm bandwidth (D525/20, Chroma Technology Co., USA). All concentrations indicated in this study are final values after the mixing. The unwinding kinetic curves represented averages of over 10 individual traces, which were analyzed using Bio-Kine (version 4.26, Bio-Logic) with a single- or double-exponential function for one or two unwinding phases, as shown below:

$$F(t) = A(1 - e^{-kt}), \tag{1}$$

$$F(t) = A_1(1 - e^{-k_1 t}) + A_2(1 - e^{-k_2 t}), \tag{2}$$

where F is the fluorescence intensity, A is the unwinding amplitude, k is the unwinding rate constant.

Multiple-turnover kinetic assays were used in the experiments. With no protein trap in the unwinding reactions, the helicase molecules could repetitively bind and work after dissociating from the DNA substrates.

In the dissociation kinetic assays, when RecQ5β (100 nM) dissociate from DNA substrates (10 nM), the fluorescence polarization anisotropy of Flu would reduce, so we could measure it to monitor the dissociation kinetics of RecQ5β. In these assays, we use dT$_{56}$ (2 μM) as the protein trap to capture the dissociated helicases. The DNA substrates and RecQ5β were pre-incubated in reaction buffer in one syringe, and dT$_{56}$ was stored in another syringe. All the reactions are performed in 37 °C.

3. Results

3.1. Unwinding of ss/dsDNA

First, we studied the unwinding kinetics of RecQ5β^{fl} and RecQ5β^{1-467} with a 20-nt 3′-tailed 20-bp ss/dsDNA. We monitored the unwinding processes of RecQ5β^{fl} in different concentrations (5 nM–250 nM). The fluorescent intensities of Flu were measured as shown in Fig. 1(a). The unwinding kinetic curves for RecQ5β^{fl} are well fitted by double-exponential functions, suggesting that two phases (fast and slow phases) are involved in the unwinding process. The unwinding amplitude and rate obtained through the fittings are given in Figs. 1(b) and 1(c). In the case of RecQ5β^{1-467}, the unwinding kinetics are also in two phases (Figs. 1(d)–1(f)). From the unwinding amplitudes in Figs. 1(b) and 1(e), we noticed that the saturated value for RecQ5β^{1-467} ($A_{\max} = 0.35$) is lower than that for RecQ5β^{fl} ($A_{\max} = 0.40$), and there is a jump at low helicase concentrations for RecQ5β^{1-467} (10 nM–50 nM).

As reported previously, the multiple unwinding phases may suggest the presence of different binding sites on the ss/dsDNA substrate for the helicases.[30] The binding of RecQ5β at a preferred site could lead to a tight interaction between the RecQ5β and DNA substrate, which may promote the unwinding process and thus cause the occurrence of fast unwinding phase. On the contrary, the loose binding in other sites may lead to a weak interaction, which may lead to slow unwinding process. In addition, DmRecQ5, as well as BLM, which are both highly homologous with RecQ5β, are inclined to bind to the junction sites on DNA substrates.[26,30] So we could suppose that RecQ5β would also prefer to bind to the junction sites on ss/dsDNA. The RecQ5β molecules bound to the junctions may contribute to the fast phase, while that bound to the other sites may contribute to the slow phase.

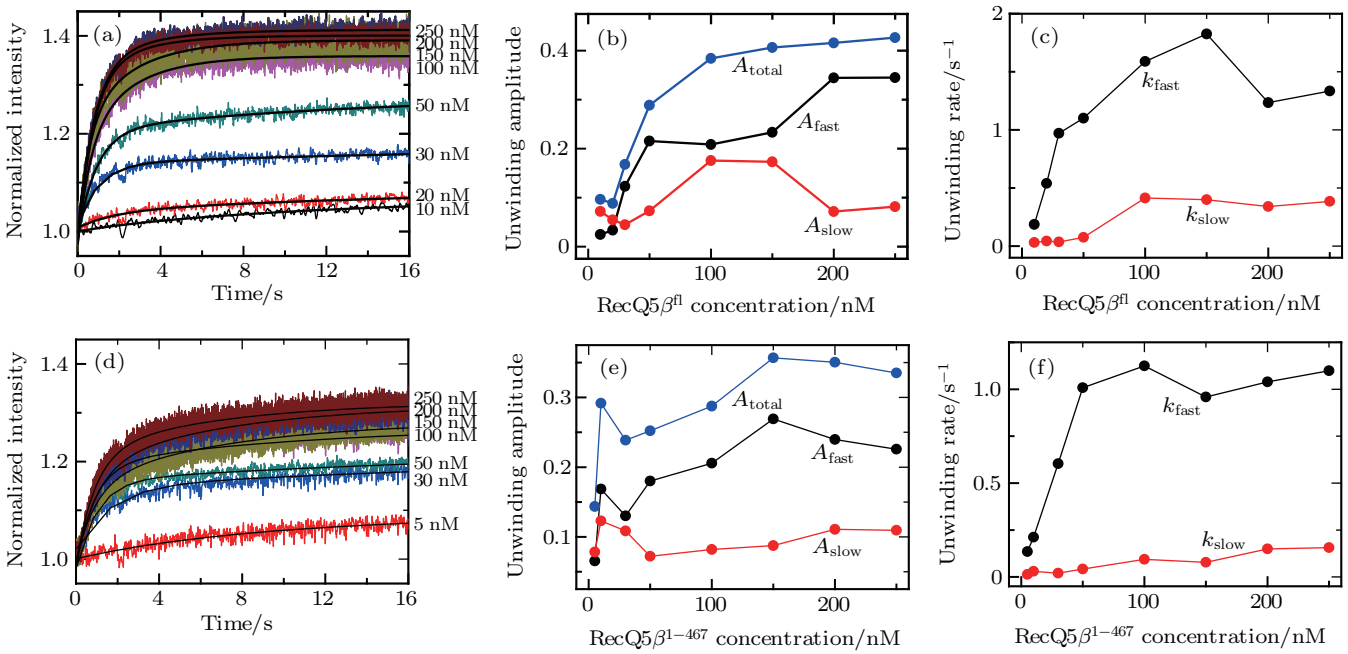


Fig. 1. (color online) ss/dsDNA unwinding kinetic curves and kinetic parameters (unwinding amplitude and rate). (a)–(c) RecQ5β^{fl}, (d)–(f) RecQ5β^{1-467}.

3.2. Unwinding of forked DNA

Next, we used a 20-nt tailed 20-bp forked DNA to study the unwinding kinetics of RecQ5β. The assays were carried out under the same conditions as before. The unwinding kinetic curves for RecQ5β^{fl} and RecQ5β^{1-467} are both well fitted by double-exponential functions, indicating that two unwinding phases are involved in these processes (Fig. 2).

For the two helicases, the unwinding amplitudes were similar at high helicase concentrations ($>$ 50 nM). However, the unwinding amplitudes for RecQ5β^{1-467} at low helicase concentrations (10, 20 nM) are much higher than that at 50-nM helicase concentration, forming a jump change at 10 nM–50 nM (Fig. 2(e)). This phenomenon is also observed for RecQ5β^{1-467} in the ss/dsDNA case (Fig. 1(e)) as well. It is probably due to the interactions between the ssDNA tail and RecQ5β^{1-467} helicase, as it is not observed in the unwinding amplitudes for blunt DNA and Holiday junction DNA (shown below). The underlying mechanism might be quite complex, which need further studies in the future.

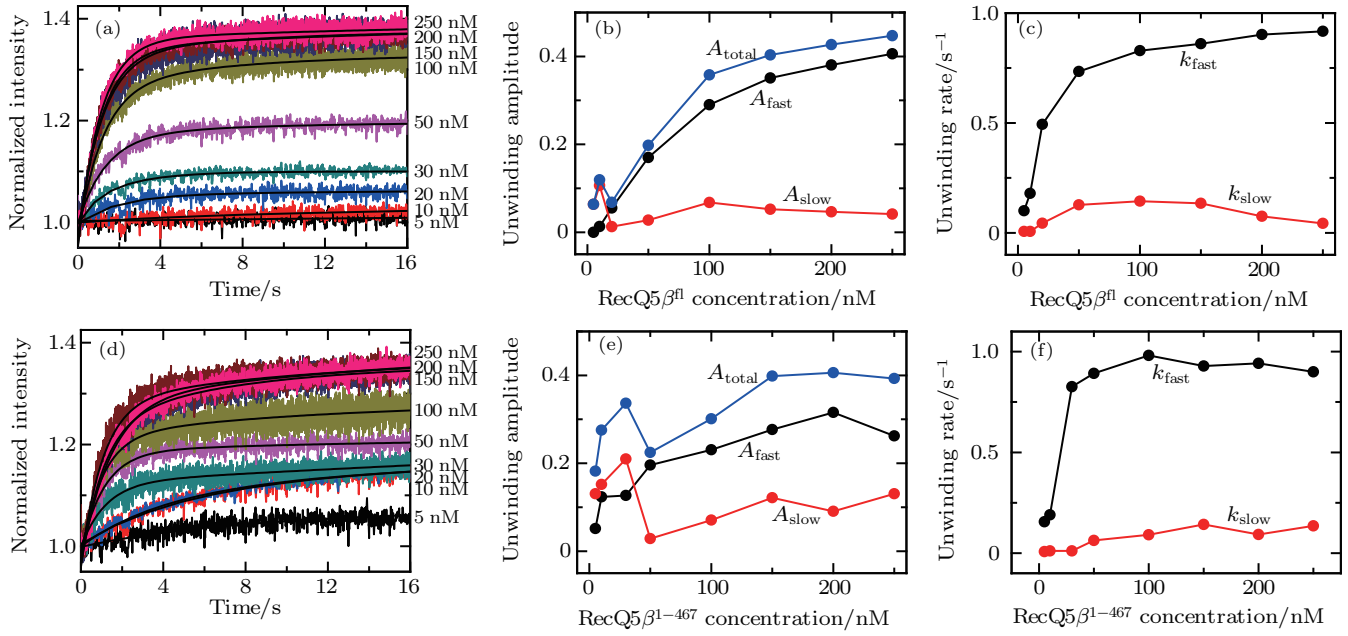


Fig. 2. (color online) Forked-DNA unwinding kinetic curves and kinetic parameters. (a)–(c) RecQ5β^{fl}, (d)–(f) RecQ5β^{1-467}.

3.3. Unwinding of blunt DNA

We next tried to study the blunt DNA unwinding kinetics by RecQ5β (Fig. 3). In this case, single-exponential functions could fit the kinetic curves best, indicating that the blunt DNA unwinding has only one phase. As the highest value for the unwinding rate (~ 0.08 s^{-1}) here is much lower than that for the fast unwinding rate ($k_{\rm fast} \approx 1.0$ s^{-1}) in the cases of ss/dsDNA and forked DNA, the single phase is a slow phase. As there is no fast unwinding phase for blunt DNA with no junction, and there are fast unwinding phases for ss/ds- and forked DNA both with ss/dsDNA junctions, we are more certain that RecQ5β helicases bound to the ss/dsDNA junctions in the ss/ds- and forked DNA contribute to the fast unwinding phase. Note that at low RecQ5$\beta^{\rm fl}$ concentrations (5, 10, 20 nM), the unwinding amplitudes are in decline and the unwinding rate is quite low (Figs. 3(b) and 3(c)). This phenomenon probably is due to the DNA strand-annealing activity of RecQ5$\beta^{\rm fl}$. According to previous reports, the strand-annealing activity is high with 10 nM–20 nM RecQ5$\beta^{\rm fl}$.[21] As RecQ5$\beta^{\rm fl}$ has both strand-annealing and unwinding activities, the competitive effect appears. The decline in the unwinding amplitude could only be detected in the case of blunt DNA, because the unwinding rates for blunt DNA (0.042 s^{-1}, 20 nM) are much lower compared with those for ss/dsDNA ($k_{\rm fast} = 0.54$ s^{-1}, 20 nM) and forked DNA ($k_{\rm fast} = 0.49$ s^{-1}, 20 nM). As RecQ5β^{1-467} is hard to mediate strand-annealing activity,[23] there is no decline of the unwinding amplitude for it (Fig. 3(e)).

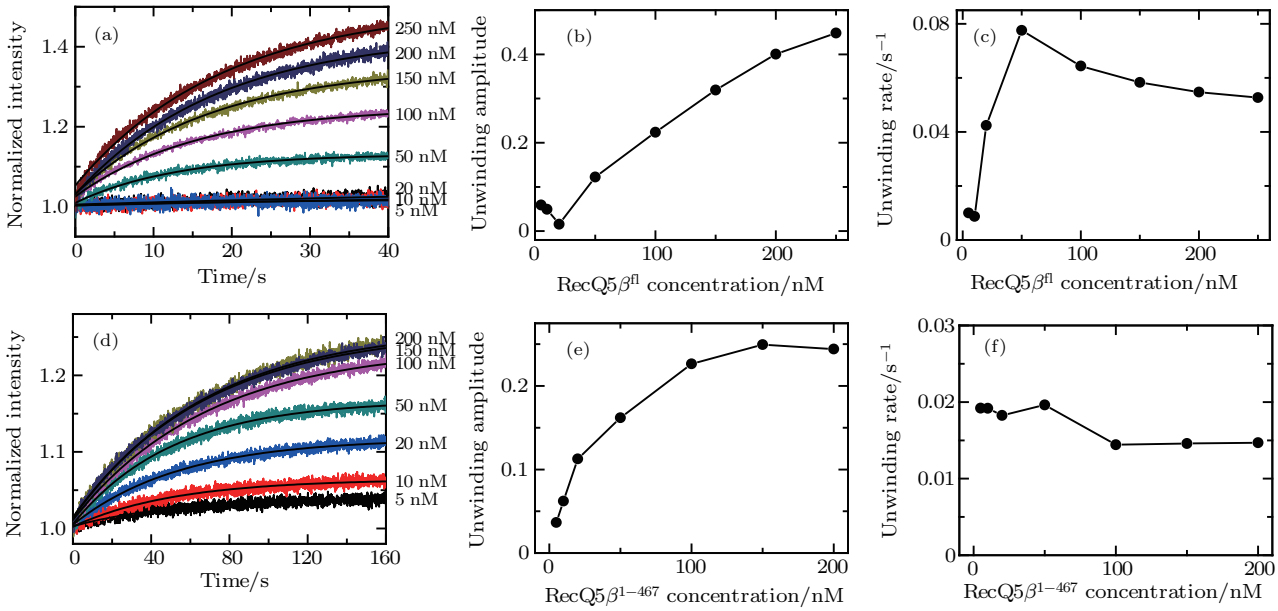


Fig. 3. (color online) Blunt-DNA unwinding kinetic curves and kinetic parameters. (a)–(c) RecQ5$\beta^{\rm fl}$, (d)–(f) RecQ5β^{1-467}.

Comparing the unwinding kinetics for the two helicases with blunt DNA, we could find that the unwinding amplitude for RecQ5β^{1-467} ($A_{\rm max} = 0.25$) is much lower than that for RecQ5$\beta^{\rm fl}$ ($A_{\rm max} = 0.45$), and the unwinding rate for RecQ5β^{1-467} ($k_{\rm max} = 0.015$ s^{-1}) is much lower than that for RecQ5$\beta^{\rm fl}$ ($k_{\rm max} = 0.078$ s^{-1}). These results indicate that the C-terminal domain may play an important role in the unwinding of blunt DNA. This is supported by previous studies. For example, BLM and WRN, both of which lack the C-terminal domain, were identified as being unable to unwind blunt DNA.[25] DmRecQ5 helicase with a deficiency of the C-terminal domain was also found to be incapable of unwinding 80-bp blunt DNA.[34]

3.4. Unwinding of Holiday junction DNA

Holiday junction DNA is a special structure appearing in DNA homologous recombination. We used a Holiday junction DNA with four 20-bp arms as the DNA substrate in the unwinding assay. The unwinding kinetic curves are best fitted by double-exponential functions, indicating fast and slow unwinding phases coexist in this process. As shown in Fig. 4, the unwinding amplitudes and rates for Holiday junctions are all lower than those for forked DNA and ss/dsDNA. Besides, at low helicase concentrations (5, 10, 20 nM), Holiday junctions are hard to unwind (Figs. 4(a) and 4(d)). These phenomena might be due to the complex structures of the Holiday junction. This structure, which contains four 20-bp arms and a junction, may need more helicases to unwind from multiple sites simultaneously. Otherwise, the ssDNA released from unwinding would re-anneal to form duplexes and the unwinding process need to restart, thus leading to low unwinding efficiencies. It is consistent with a previous model that the FRET efficiency would not change unless the two arms con-

taining the fluorescence labels of the Holiday junction DNA are unwound.[35] Holiday junction DNA could also be unwound by BLM, WRN, and DmRecQ5 of the RecQ family, but the unwinding efficiencies are all low, this indicates that it is difficult for RecQ family helicases to unwind the Holiday junction.[25,26]

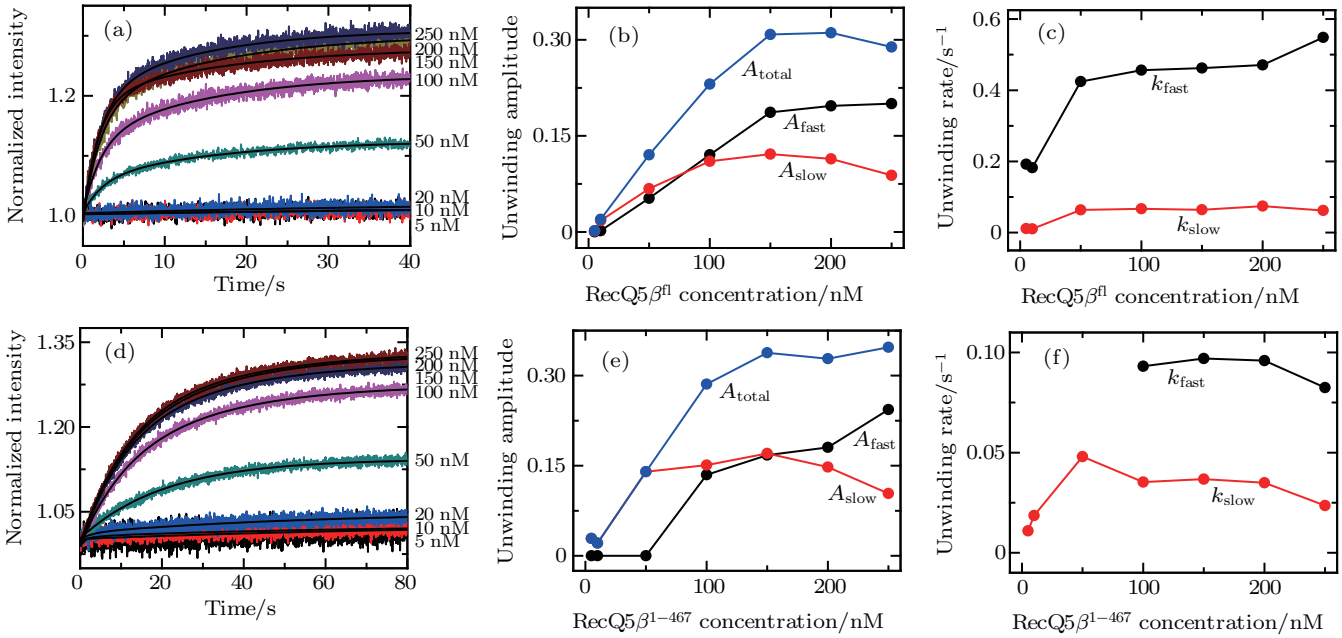


Fig. 4. (color online) Holiday-junction-DNA unwinding kinetic curves and kinetic parameters. (a)–(c) RecQ5β^{fl}, (d)–(f) RecQ5β^{1-467}.

Although the total unwinding amplitudes for the two helicases are similar (Figs. 4(b) and 4(e)), we noticed that the unwinding rate for RecQ5β^{fl} is much higher than that for RecQ5β^{1-467} (Figs. 4(c) and 4(f)), indicating that the C-terminal domain could enhance the RecQ5β unwinding efficiency for Holiday junctions. Moreover, the unwinding rates for Holiday junction are higher than those for blunt DNA, which might be caused by the junction site in the center of the structure, to which RecQ5β might prefer to bind. This speculation is also mentioned in the studies of DmRecQ5 helicase for unwinding three-way junction and Holiday junction structures.[26]

3.5. Unwinding of G-quadruplex DNA

G-quadruplex DNA is the specific DNA structures in human telomeres, having the functions of protecting the chromosome ends from deterioration and the cells from aging.[36] BLM and WRN can efficiently unwind the G-quadruplex DNA.[25,26] For this substrate, we labeled the fluorescence molecule Hex on the 5′-end and Flu on the end of the first loop of the G-quadruplex, so as to increase the distance between the donor and acceptor. We also extend the length of the first loop to 8 nt to increase the change of FRET efficiency as much as possible.

The unwinding assays were similar to the above experiments, except that there was 10-mM KCl in the unwinding buffer to increase the stability of the G-quadruplex structure.[37] As shown in Fig. 5, the unwinding kinetic courses for RecQ5β^{fl} are fitted well with single-exponential functions, indicating there is only one phase, while the kinetic curves for RecQ5β^{1-467} are fitted better with double-exponential functions, indicating two phases exist (Fig. 5(d)). We noticed that the unwinding activities of both RecQ5β^{fl} and RecQ5β^{1-467} are much lower for this substrate than for the other four DNA substrates mentioned above. This may be due to the fact that the ssDNA released from unwinding tends to refold spontaneously and rapidly to G-quadruplex.[38] In fact, a recent smFRET study demonstrated that the G-quadruplex unfolding activity of RecQ5β is an order of magnitude weaker than BLM and WRN.[39]

Interestingly, compared with RecQ5β^{fl}, RecQ5β^{1-467} shows better abilities in unwinding G-quadruplexes. Both the unwinding amplitude and rate for RecQ5β^{1-467} (Figs. 5(e) and 5(f)) are greater than those for RecQ5β^{fl} (Figs. 5(b) and 5(c)). The phenomenon is entirely different from that for the other four DNA substrates, in which cases RecQ5β^{fl} are better than RecQ5β^{1-467}. It implies that the C-terminal domain probably inhibits the unwinding of G-quadruplex by the RecQ helicases.

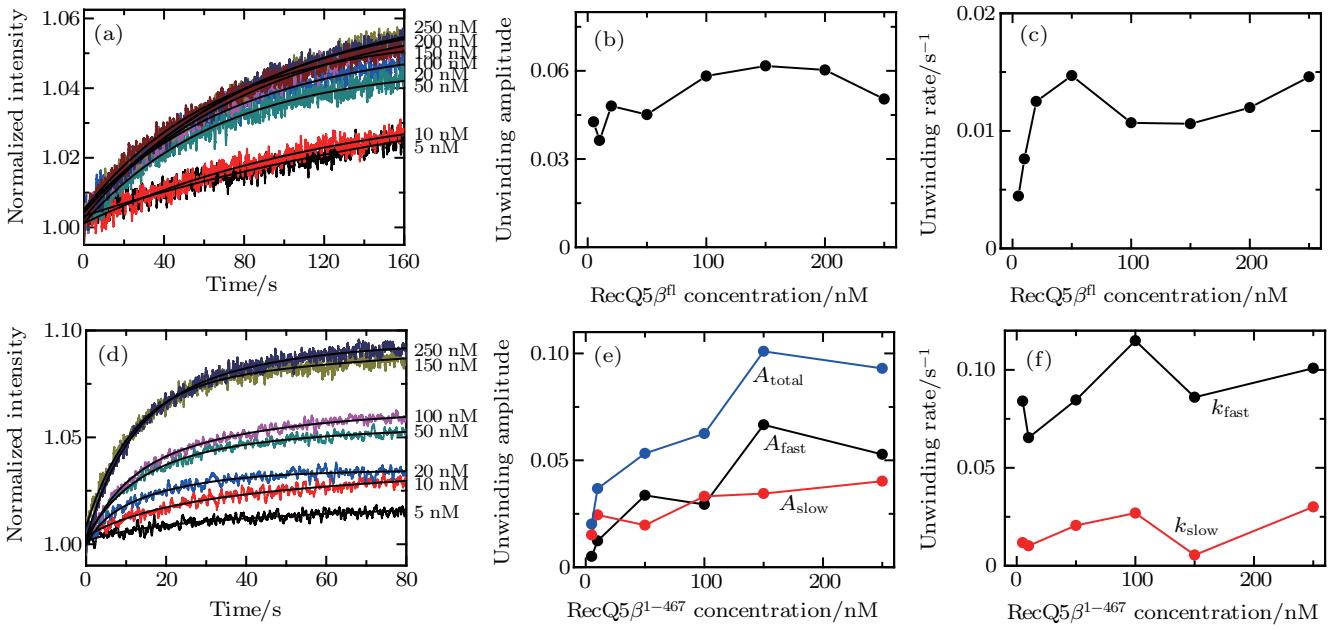


Fig. 5. (color online) G-quadruplex DNA unwinding kinetic curves and kinetic parameters. (a)–(c) RecQ5$\beta^{\rm fl}$, (d)–(f) RecQ5β^{1-467}.

3.6. Kinetics of dissociation from DNA substrates

The unwinding efficiency of a helicase is related to its binding affinity for the DNA substrate.[30] To determine the affinity of RecQ5β for different DNA substrates, we used fluorescence polarization anisotropy assay to measure the kinetics of dissociation of bound RecQ5β from different DNA substrates. As shown in Figs. 6(a) and 6(b), the dissociation processes for RecQ5$\beta^{\rm fl}$ and RecQ5β^{1-467} are fitted well with single- or double-exponential functions, respectively, indicating that there exist one or two phases (fast and slow phases). Because the dissociation rate in the one-phase case is relatively low, that phase should be a slow phase. As the unwinding process is mainly influenced by the tight binding affinity, which corresponds to the slow dissociation phase, here we mainly analyze and compare the slow dissociation rates (Fig. 6(c)). We noticed that for all the DNA substrates, the dissociation rate of RecQ5$\beta^{\rm fl}$ is much higher than that of RecQ5β^{1-467}, indicating that the C-terminal domain might reduce the binding affinity of RecQ5β. It is just because RecQ5$\beta^{\rm fl}$ is easy to fall off from DNA substrates, we need to use the multiple-turnover assay, which allows the helicase to work repetitively, in order to enhance the unwinding efficiencies.

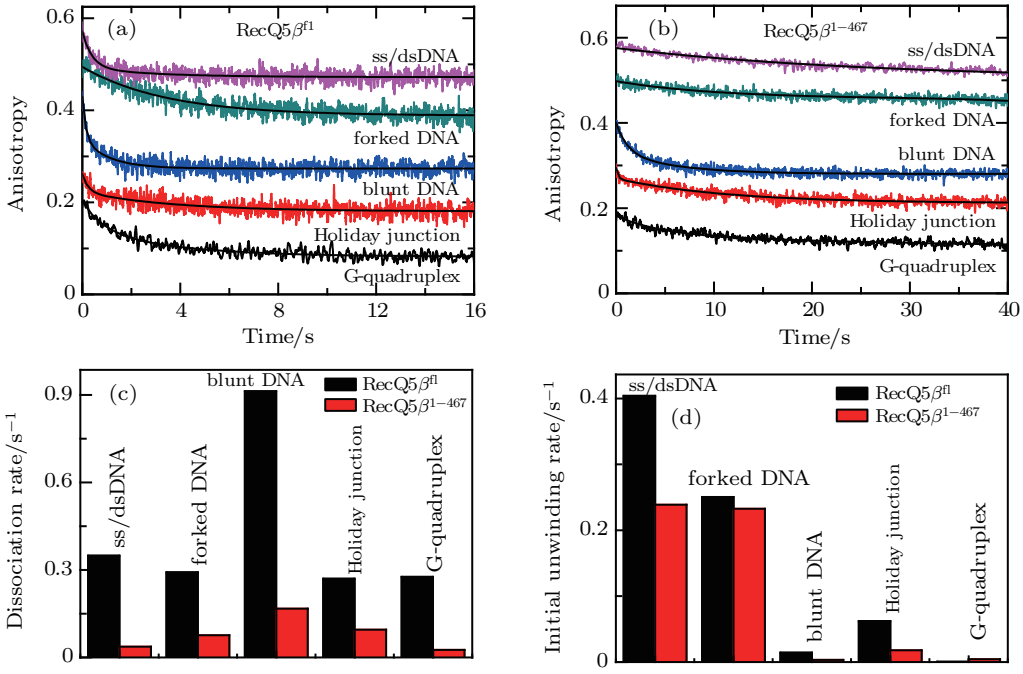


Fig. 6. (color online) Kinetics of helicase dissociation from different DNA substrates. (a) Dissociation kinetic curves for RecQ5$\beta^{\rm fl}$. (b) Dissociation kinetic curves for RecQ5β^{1-467}. (c) Slow dissociation rates obtained with 100-nM helicase. (d) Initial unwinding rates with 100-nM helicase.

The initial unwinding rates (at 100-nM helicase concentration) calculated from the kinetic parameters in the above unwinding kinetic experiments are presented in Fig. 6(d). The initial unwinding rate corresponds to the slope of an unwinding kinetic curve in the initial stage. By comparing Figs. 6(c) and 6(d), we may notice some interesting points. (i) The binding affinity of RecQ5β^{fl} is much weaker than that of RecQ5β^{1-467} for all the DNA substrates, however, the initial unwinding rates of RecQ5β^{fl} are higher than that of RecQ5β^{1-467} for all substrates except for G-quadruplex. This phenomenon is opposite to our usual idea that a weak affinity results in a lower initial unwinding rate, and it could only be explained by a high unwinding activity of RecQ5β^{fl} contributed by the C-terminal domain. (ii) For both RecQ5β^{fl} and RecQ5β^{1-467}, the dissociation rate for blunt DNA is the highest, but the initial unwinding rate for blunt DNA is the lowest (except for G-quadruplex). It suggests that the binding affinity of RecQ5β for blunt DNA is quite weak, resulting in the slow unwinding rate for blunt DNA. Among the five DNA substrates, only blunt DNA have no junction site, and the related dissociation rate is the highest, further indicating that tight binding occurs with the junction site. (iii) For G-quadruplex, the binding affinity is similar to that for ss/dsDNA, thus there exists probably a junction site on G-quadruplex for RecQ5β. But the initial unwinding rate is much lower, indicating the low unwinding capacity of RecQ5β for G-quadruplex.

4. Conclusions

In this work, we studied the unwinding and dissociation kinetics of RecQ5β^{fl} and RecQ5β^{1-467} with different DNA substrates: ss/dsDNA, forked DNA, blunt DNA, Holiday junction, and G-quadruplex. Like other RecQ family members, RecQ5β helicases are able to unwind specific DNA structures. From the unwinding amplitudes and rates, we found that RecQ5β prefers unwinding ss/dsDNA, forked DNA, and Holiday junction, but is compromised in unwinding blunt DNA and G-quadruplex.

Although BLM and WRN are both highly homologous with RecQ5β in human, we should note that they are different in substrate specificities. For example, BLM and WRN cannot unwind blunt DNA,[13] while RecQ5β can slowly unwind this substrate; BLM and WRN are efficient in unwinding G-quadruplex structure,[39] while RecQ5β is not. In the case of ss/dsDNA, forked DNA and Holiday junction DNA, the unwinding efficiencies of BLM, WRN, and RecQ5β have the same magnitude.

The substrate specificity of RecQ5β may be attributed to the preferred binding sites at the junctions, because the unwinding rates for ss/dsDNA and forked DNA are much higher than that for blunt DNA, which lacks a junction. Moreover, Holiday junction also has a junction site in the center of the structure, and the unwinding rate for it is also much higher than that for blunt DNA. In fact, the dissociation assays also support this suggestion: the dissociation rate for blunt DNA is the highest and thus the binding affinity of RecQ5β for it is the weakest. For the G-quadruplex with a unique structure, RecQβ is intrinsically difficult to unwind.

The C-terminal domain, which exists particularly in RecQ5β and has no homology in other RecQ family helicases, plays a great role in the unwinding activities of RecQ5β. From the comparison between RecQ5β^{fl} and RecQ5β^{1-467}, we found that the C-terminal domain could enhance the unwinding efficiencies with ss/dsDNA, forked DNA, blunt DNA, and Holiday junction DNA in our assays. And from the dissociation experiments, we also found that the C-terminal domain may weaken the RecQ5β binding affinity for DNA substrates. The two aspects of C-terminal of RecQ5β may possibly be relevant to the biological functions of RecQ5β. It might be beneficial for RecQ5β to easily dissociate from DNA substrates after unwinding certain specific sites, as the main function of RecQ5β is the regulation of cellular metabolism, which does not require long continuous unwinding activity. So the C-terminal domain is essential for RecQ5β to catalyze specific DNA unwinding efficiently. However, for unwinding G-quadruplex, the C-terminal has the negative effect. The unwinding ability of RecQ5β^{fl} for G-quadruplex is weaker than that of RecQ5β^{1-467}. This is quite different from the other substrates, which might need further studies for detailed mechanism.Since human RecQ family helicases have essential functions in the maintenance of genome integrity and stability,[7] RecQ5β attracts great attention on its structures and functions. Although previous studies made certain progresses on the understanding of the unwinding activity, strand-annealing and strand-exchange activities of RecQ5β,[21–23] the underlying mechanisms are still elusive. Our studies mainly focus on the helicase activity and substrate specificity of RecQ5β using stopped-flow method to monitor the real-time kinetics, making further steps on characterizing the DNA unwinding properties of human RecQ5β. The contrast of RecQ5β^{fl} and RecQ5β^{1-467} suggests the possible functions of the C-terminal domain that is particularly conserved in RecQ5β in the RecQ family, which is critically important for further exploring the physiological functions and working mechanisms of RecQ5β *in vivo*.

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