African Journal of Biotechnology Vol. 11(26), pp. 6795-6801, 29 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2983
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Characterization of DNA unwinding properties of three N-terminal fragments of RecQ5β helicase

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Accepted 23 January, 2012

RecQ5β is one member of the human RecQ family helicases that belong to superfamily 2 (SF2) and are critical for the maintenance of genomic stability. Here, the DNA unwinding kinetics of three N-terminal fragments of RecQ5β helicase, RecQ5β$^{1-467}$, RecQ5β$^{1-567}$ and RecQ5β$^{1-662}$, were studied with stopped-flow method based on fluorescence resonance energy transfer (FRET). Under single-turnover kinetic conditions, we found that both the unwinding amplitude and rate increased with the increase of the 3'-tail length of the DNA substrate for each fragment. The maximum amplitudes were 73.5, 57.6 and 35.5% for RecQ5β$^{1-467}$, RecQ5β$^{1-567}$ and RecQ5β$^{1-662}$, respectively. Obviously, the unwinding amplitude decreased with the increase of the fragment length. For each RecQ5β fragment, when the 3'-tail length of the DNA substrates was short, essentially only one slow unwinding process occurred. When the 3'-tail length was increased, the unwinding amplitude of the fast unwinding process increased obviously; that is, the RecQ5β-catalyzed DNA unwinding depended on the 3'-tail length of the DNA substrate. It indicates that RecQ5β molecules are cooperative in DNA unwinding. This is an interesting new feature for a SF2 helicase.

Key words: RecQ5β helicase, stopped-flow technique, fluorescence resonance energy transfer (FRET), DNA unwinding kinetics.

INTRODUCTION

Helicases are a ubiquitous class of motor proteins that catalyze the unwinding of nucleic acid duplexes (DNA-DNA, DNA-RNA and RNA-RNA) in an ATP-dependent manner (Matson and Kaiser-Rogers, 1990; West, 1996; Waksman et al., 2000). They play essential roles in a variety of biological processes including DNA replication, transcription, DNA repair and recombination (Matson and Kaiser-Rogers, 1990; Matson et al., 1994; Lohman and Bjornson 1996; Bachrati and Hickson, 2003; Sharma et al., 2006; Vindigni et al., 2010; Warmerdam and Kanaar, 2010). RecQ family helicases are named after *Escherichia coli* RecQ which was a 3′ to 5′ DNA helicase (Umezu et al., 1990). The are highly conserved in evolution from bacteria to humans and belong to helicase superfamily 2 (SF2) (Abdel-Monem et al., 1976; Matson and Kaiser-Rogers, 1990; Umezu et al., 1990; Cheok et al., 2005; Machwe et al., 2005). They have been shown to be important for the maintenance of genome integrity through their participation in DNA metabolic processes (Bachrati and Hickson, 2003; Hickson 2003; Sharma et al., 2006; Singleton et al., 2007; Lohman et al., 2008; Warmerdam and Kanaar, 2010; Masai, 2011). In human, five RecQ-related helicases have been identified to date, including RecQ1, BLM, WRN, RTS (RecQ4) and RecQ5. Notably, loss of function of BLM, WRN, and RTS would cause genetic disorders, namely Bloom's syndrome, Werner's syndrome and Rothmund–Thomson syndrome, respectively, that are characterized by cancer predisposition and premature aging (German et al., 1977; Karow et al., 2000; Kyng et al., 2005; Sharma et al., 2007; Speina et al., 2010).

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Table 1. The structures and sequences of oligonucleotides used in this study.

Substrate	DNA sequence (5' - 3')	Structure
16-bp duplex with a 3'-ssDNA tail	CTC TGC TCG ACG GAT T – F[a] H – AAT CCG TCG AGC AGA G (dT_n)[b]	
Forked duplex	ATA TCT CCG AAT GGC AAA GAT GTC CTA G– F H –CTA GGA CAT CTT TGC CCA CGT TGA CCC G CGG GTC AAC GTG GGC AAA GTC TAT GCT TAG CGC CAG AAT TCG GCA GCG TC–F	
DNA bubble	H–GAC GCT GCC GAA TTC TGG CTT GCT AGG ACA TCT TTG CCC ACG TTG ACC CG	
DNA trap	CTC TGC TCG ACG GAT T	
Protein trap	dT_{56}	

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

RecQ5 is a small one of the five human RecQ helicases, containing only a core part of RecQ helicase. And it exists in three isoforms, RecQ5α (corresponding to the original RecQ5 with 410 amino acids), RecQ5β (991 amino acids) and RecQ5γ (435 amino acids), which result from alternative splicing of the *RECQ5* transcript (Shimamoto et al, 2000; Garcia et al., 2004; Speina et al., 2010). So far, RecQ5 has not been observed to relate directly to any diseases; but it is believed to functionally act as a backup for the others (Wang et al., 2003; Garcia et al., 2004; Sharma et al., 2006; Speina et al., 2010). RecQ5β contains a conserved helicase core, a RQC (RecQ C-terminal) region and a longer C-terminal region with no homology to the other RecQ helicases (Shimamoto et al., 2000; Ren et al., 2008). It has been predicted that a mutation in RecQ5β may cause a disease that shows the phenotypes shared by BS, WS and RTS (Shimamoto et al., 2000).

Here, we used the rapid stopped-flow method, which is based on fluorescence resonance energy transfer assay (FRET) and allows the continuous and real time observations of the DNA unwinding process, to analyze the DNA unwinding kinetics catalyzed by the three N-terminal fragments of RecQ5β, obtaining some interesting results.

MATERIALS AND METHODS

Reagents and enzymes

All chemicals were of reagent grade and all solutions were prepared in high quality de-ionized water from a Milli-Q water purification system (Millipore Corporation, France) with a resistivity of up to 18.2 MΩ/cm. The unwinding reaction is performed in 25 mM Tris-HCl, pH 7.5 (25°C), 10 mM NaCl, 1.5 mM $MgCl_2$ and 0.1 mM dithiothreitol (DTT), unless noted elsewhere. ATP purchased from Sigma (St. Louis, USA) was dissolved as a concentrated stock and adjusted to a pH value of 7.0 with NaOH. The ATP concentration was determined by using an extinction coefficient at 259 nm of 1.54×10^4 cm^{-1} M^{-1}.

The three N-terminal fragments of RecQ5β helicase, $RecQ5\beta^{1-467}$, $RecQ5\beta^{1-567}$ and $RecQ5\beta^{1-662}$, were prepared as previously described (Ren et al., 2008). The concentrations of the purified proteins were determined using Bradford assay (Xu et al., 2003; Ren et al., 2008) and stored at -80°C until use.

Oligonucleotide substrates

The oligonucleotides used in this research were labeled with fluorescein (F) in the 3'-terminal and hexachlorofluorescein (H) in the 5'-terminal, respectively. Their sequences and structures are shown in Table 1. The protein trap used for single-turnover kinetic experiments is 56 nt poly (dT), dT_{56}, without fluorescent label. The DNA trap is a 16-nt single-stranded oligonucleotide having the same sequence as the short single strand of the ss/dsDNA substrate. They were purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, China). The synthetic oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis and stored at -20°C.

The single-stranded DNA (ssDNA) was dissolved in DNA annealing buffer: 10 mM Tris-HCl, pH = 8.0, 50 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA). Then a 50 μM working stock of double-stranded DNA (dsDNA) was prepared by mixing the same amounts of complementary single-stranded oligonucleotides, followed by heating to 90°C. After equilibrating for 3 min, annealing was allowed by slowly cooling to room temperature. Duplexes were stored at –20°C until use.

Monitoring DNA unwinding with FRET

Previously, we have employed a FRET assay to observe in real time the DNA unwinding by helicases (Zhang et al., 2005; Zhang et al., 2006; Yang et al., 2008). One ssDNA strand was labeled with fluorescein (donor) at the 3' end, whereas a complementary ssDNA strand was labeled with hexachlorofluorescein (acceptor) at the 5' end. As there is a large spectrum overlap between the fluorescein emission and hexachlorofluorescein excitation spectra (Zhang et al., 2005), after annealing and formation of dsDNA, the fluorescein and hexachlorofluorescein are in close proximity and FRET will occur between the two fluores cent molecules. As a result, the fluorescence emission of fluorescein (at 525 nm) becomes reduced and that of hexachlorofluorescein (at 556 nm) enhanced. On the

contrary, when dsDNA is unwound to ssDNA by the helicase, fluorescence emission of fluorescein (at 525 nm) becomes enhanced and that of hexachlorofluorescein (at 556 nm) reduced. By monitoring the fluorescence signal changes at 525 nm, the helicase-catalyzed DNA unwinding kinetics can be observed in real-time.

Stopped-flow kinetic measurements

The stopped-flow kinetic experiments were carried out using a Bio-Logic SFM-400 mixer with a 1.5 mm × 1.5 mm cell (Bio-Logic FC-15) and a Bio-Logic MOS450/AF-CD optical system equipped with a 150 W mercury-xenon lamp. Fluorescein was excited at 492 nm (2 nm in slit width) and the fluorescence emission was monitored using a 525 nm high pass filter with 20-nm bandwidth (D525/20; Chroma Technology Co.). Unwinding kinetics were measured in a two-syringe mode, where RecQ5β and dsDNA substrates were pre-incubated in a large syringe (syringe #1, 10 ml volume) for 5 min, while ATP and protein trap (and DNA trap in some cases) were in a small syringe (syringe #4, 1.9 ml volume). The mixing ratio of syringe #1 versus syringe #4 was set as 4:1. All indicated concentrations were after mixing. The unwinding reaction was initiated by rapid mixing of the two syringes. To convert the output fluorescence signal in volts to the fraction unwound, a calibration experiment was performed in a two-syringe mode, where RecQ5β and hexachlorofluorescein-labeled single-stranded oligonucleotides were in syringe #1, and ATP, fluorescein-labeled single-stranded oligonucleotides, dT_{56}, (and DNA Trap) were in syringe #4. The fluorescent signal of the mixed solution from the two syringes corresponds to 100% unwinding.

Kinetics data analysis

All stopped-flow kinetic traces were averages of several individual traces. The kinetics data of DNA unwinding were fitted to equation 1,

$$A(t) = A_1(1-e^{-k_1 t}) + A_2(1-e^{-k_2 t}) \quad (1)$$

In the equation 1, $A(t)$ is the unwinding amplitude at time t, A_1 and A_2 correspond to the unwinding amplitudes of the fast and slow phases, respectively. k_1 and k_2 correspond to the rate constants of the two phases.

RESULTS

The conditions for the single-turnover kinetic analysis

Here, two fragments of RecQ5β, RecQ5β$^{1-467}$ and RecQ5β$^{1-662}$, were used to define the single-turnover unwinding kinetic conditions. The factors to consider include the substrate structure, reaction temperature, concentrations of the protein trap (dT_{56}), ATP, helicase and DNA trap. It was found that under the same conditions, RecQ5β preferred to unwind the 16-bp ss/dsDNA with a 3'- tail of 30-nt (Figure 1a). Then we performed experiments with this 30-nt 16-bp ss/dsDNA to determine the other optimal reaction conditions. Our data indicate that, as *in vivo*, RecQ5β functioned efficiently at 37°C *in vitro*. Although the unwinding amplitude was much higher at 25°C than at 37°C, a slow process with lower unwinding rate occurred (Figure 1b). The two concentrations of dT_{56} (2 and 5 μM) gave similar results (Figure 1c), thus we used 2 μM dT_{56} in our studies. When the concentration of ATP was 1.5 mM, RecQ5β could unwind the substrate with a higher efficiency (Figure 1d). The optimal concentration for RecQ5β was 100 nM (Figure 1e). When it was further increased (120 nM), a slow process appeared. As to the larger fragment RecQ5β$^{1-662}$, to inhibit its annealing activity (Garcia et al., 2004), a DNA trap was been applied. The presence of 2 μM DNA trap enhanced the unwinding efficiency significantly (Figure 1f). Therefore, the determined single-turnover unwinding conditions are: 16-bp ss/dsDNA as the substrate, temperature at 37°C, 2 μM dT_{56}, 1.5 mM ATP, 100 nM helicase, 2 μM DNA trap.

DNA unwinding catalyzed by the three fragments of RecQ5β

Under this optimized conditions, the single-turnover unwinding kinetics for the three fragments RecQ5β$^{1-467}$, RecQ5β$^{1-567}$and RecQ5β$^{1-662}$, were investigated. 2 nM 16-bp duplex DNA substrates with 3' tails of 10, 15, 20, 30, 40 and 50 nt were used. In addition, for the largest fragment (RecQ5β$^{1-662}$), 2 μM DNA trap was used. For each fragment, the unwinding amplitude increased with the increase of the 3'-tail length of the DNA substrate. The maximum efficiencies for RecQ5β$^{1-467}$, RecQ5β$^{1-567}$ and RecQ5β$^{1-662}$, were 73.5, 57.6 and 35.5%, respectively. It indicated that the unwinding efficiency of RecQ5β depends on the fragment length: the longer the fragment is, the lower the unwinding efficiency is. This is an unexpected phenomenon because it is generally believed that a helicase with a full length should function the best. The interesting results for RecQ5β will be discussed later.

Analyses of unwinding kinetics for the three fragments of RecQ5β

By exponential fitting of the unwinding data curves to equation 1, the unwinding amplitude and unwinding rate were obtained. As shown in Figures 2 and 3, almost all the obtained unwinding kinetic traces contained two phases, a fast one and a slow one. Some characters can be noticed: (a) for each of the three RecQ5β fragments, when the 3'-tail length of the DNA substrates was short, one slow unwinding process appeared. With increasing 3'-tail length, the unwinding amplitude of the fast unwinding process increased obviously. For RecQ5β$^{1-567}$, when the 3'-tail was 10 or 15 nt, only one slow unwinding process was present. (b) For each of the three RecQ5β fragments, the unwinding amplitude of the fast and the slow unwinding phase increased with the increase of 3'-tail length, and the fast phase increased more obviously. (c) For each of the three RecQ5β fragments, the

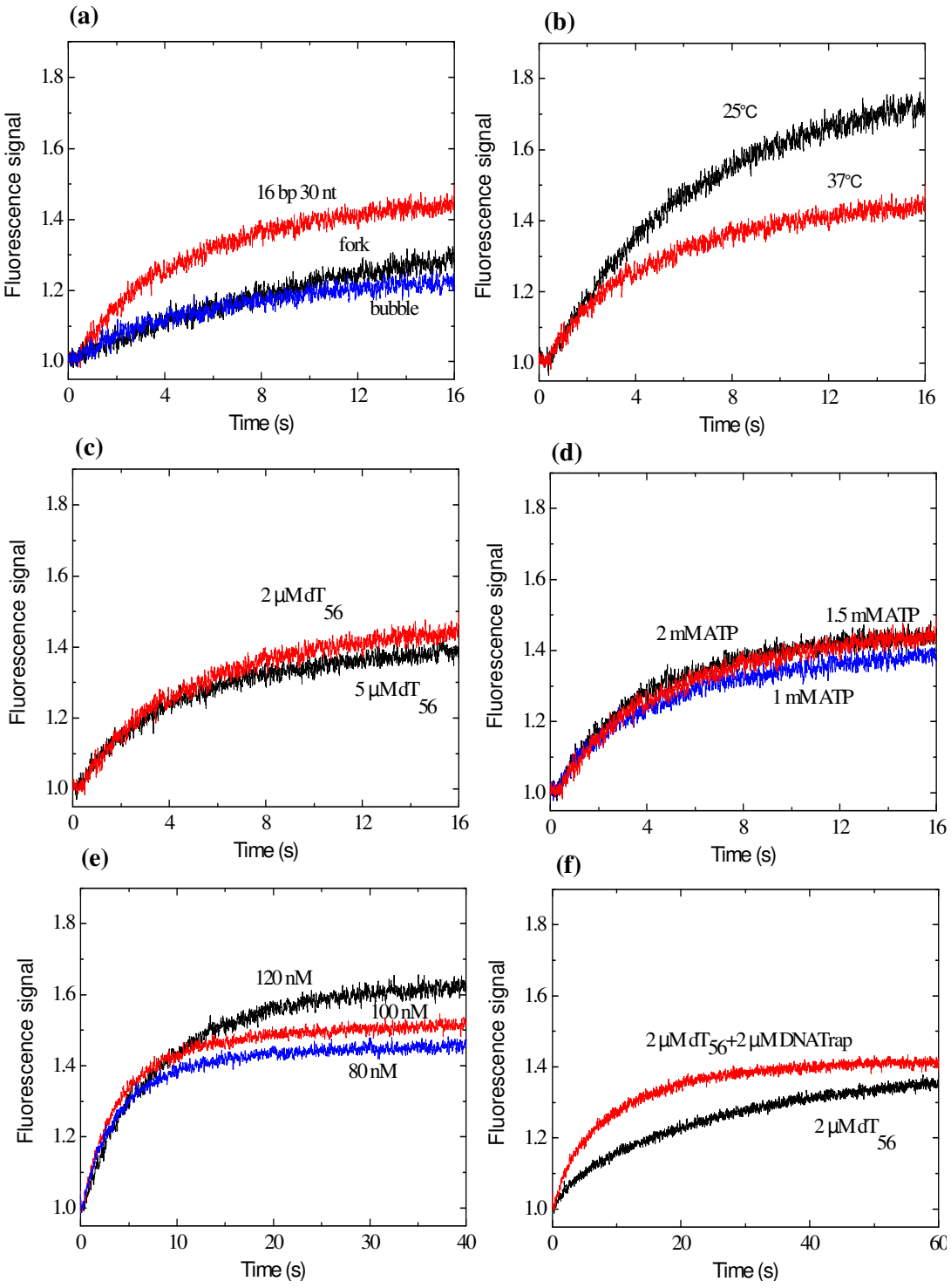

Figure 1. Determination of the conditions for the single-turnover unwinding kinetic assay. RecQ5β$^{1-467}$ was used to determine: (a) the optimum substrate; (b) temperature; (c) the concentration of dT_{56}; (d) ATP; (e) enzyme. For RecQ5β$^{1-662}$, 2 μM DNA trap was needed for a better performance (f).

unwinding rate of the fast and the slow unwinding phase also increased with the increase of 3'-tail length. (d) For a given DNA substrate, the unwinding amplitude of the slow unwinding phase decreased with the increase of the fragment length, whereas that of the fast phase did not vary much.

Clearly, with the increase of the N-terminal RecQ5β fragment length, the unwinding efficiency decreased.

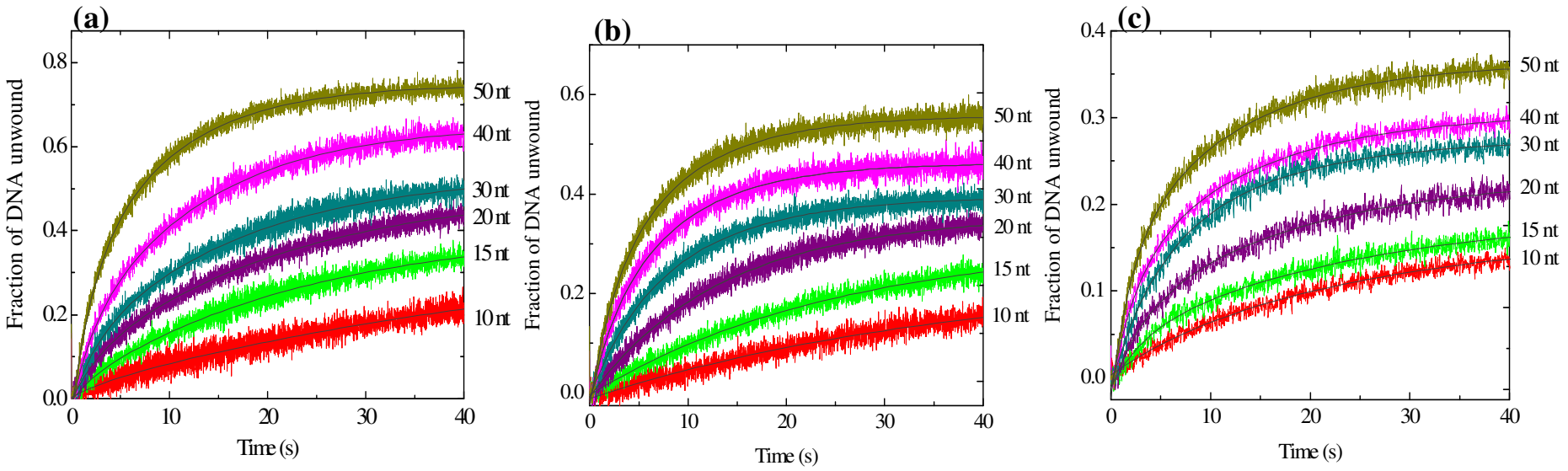

Figure 2. Kinetic time-courses of single-turnover unwinding of 16-bp duplex DNA substrates with different 3'-ssDNA tail lengths. (a) RecQ5β$^{1-467}$; (b) RecQ5β$^{1-567}$; (c) RecQ5β1^{-662}. 2 nM ss/ds DNA substrate was first pre-incubated with excess helicase (100 nM) in the reaction buffer at 37℃ for 5 min. The unwinding reaction was initiated by adding 1.5 mM ATP and 2 μM protein trap (dT_{56}) which prevents, after initiation, any free or dissociated helicase molecules from rebinding to the duplex DNA substrates. 2 μM DNA trap was used in the case of RecQ5β$^{1-662}$. The solid lines were best fits of the data to equation 1 with fitting parameters given in Figure 3.

Among the three fragments, RecQ5β$^{1-467}$ displayed the highest unwinding efficiency (73.5%). In contrast, the largest fragment displayed a much lower unwinding efficiency (35.5%). Obviously, the fragment of 467 amino acids in the N-terminal of RecQ5β was enough to mediate the unwinding activity. Thus we may infer that in the enzyme RecQ5β, the core of the unwinding activity is located within the 467 amino acids in the N-terminal, and the other amino acids in the C-terminal do not contribute positively to the helicase activity, but rather, have an inhibiting effect.

It should be noted that, in the unwinding process of DNA by a helicase, the first step for the helicase is to bind to the single strand in the 3' tail of the DNA substrates (Bachrati and Hickson, 2003; Cheok et al., 2005; Machwe et al., 2005). When the 3' tail is short, such as 10 nt, only one single enzyme molecule can bind efficiently to the substrate. For substrates with longer 3' tails, more enzyme molecules may bind (Yang et al., 2008). Therefore, it was inferred that RecQ5β molecules are cooperative in the DNA unwinding process, leading to an enhanced unwinding efficiency of substrates with long tails.

DISCUSSION

Stopped-flow kinetic method is an effective one with a high time resolution of millisecond. Together with FRET assay, it can be used to characterize the helicase-catalyzed unwinding of dsDNA continuously and in real time. It could provide much valuable kinetic information for elucidating the unwinding mechanism of helicases. In this study, we found that, under single-turnover kinetics conditions, the unwinding features of RecQ5β fragments were absolutely different from that of *E. coli* RecQ (Zhang et al., 2006), although both of them could function as monomers. For RecQ5β, when the 3'-tail length of DNA substrate was short, only one slow unwinding process was observed, while for substrate with longer tails, another obvious fast process appeared. In contrast, for *E. coli* RecQ, when the 3' tails of the DNA substrates are short (5 and 10 nt), only one rapid and efficient unwinding phase course is observed, while with long-tailed DNA substrates (≥15 nt), an additional slow process appears

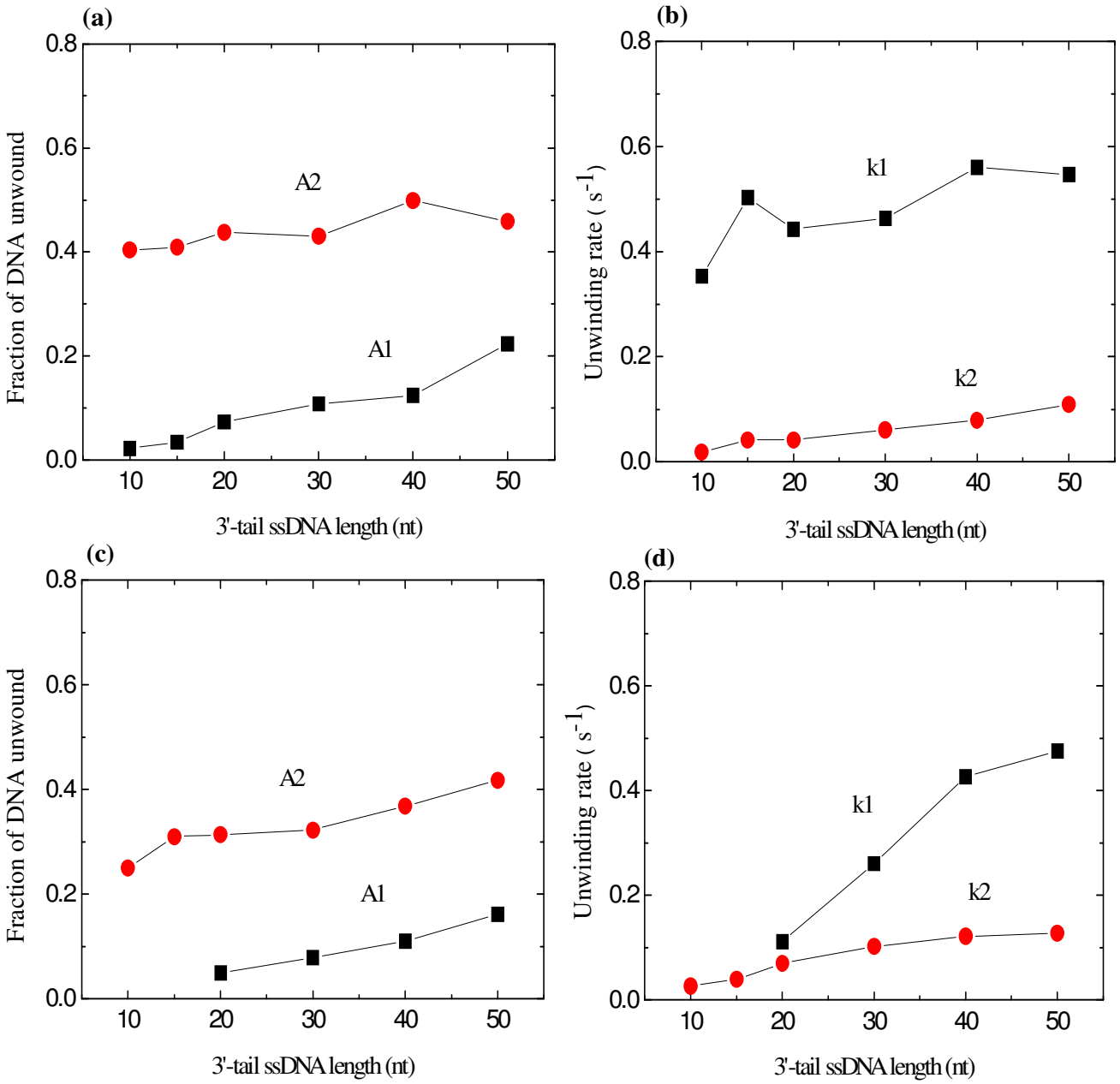

Figure 3. The kinetic parameters of single-turnover DNA unwinding catalyzed by RecQ5β$^{1-467}$, RecQ5β$^{1-567}$and RecQ5β$^{1-662}$. The data were obtained by fitting the unwinding time courses in Figure 2 using equation 1. A_1 (A_2) and k_1 (k_2) are, respectively, the unwinding amplitude and rate of the fast (slow) unwinding phase. (a) and (b) RecQ5β$^{1-467}$; (c) and (d) RecQ5β$^{1-567}$; (e) and (f) RecQ5β$^{1-662}$.

(Zhang et al., 2006). Among the three fragments of RecQ5β in this research, the N-terminal fragment of 467 amino acids exhibited a highest unwinding efficiency of 73.5%, while *E. coli* RecQ has a highest unwinding efficiency of ~95% (Zhang et al., 2006). Different from the other human RecQ helicases such as BLM and WRN that can form oligomers in solution, RecQ5β exists as monomers *in vivo* (Garcia et al., 2004; Machwe et al., 2005). The results in our present study indicate that RecQ5β may function cooperatively *in vitro*. Note that *E. coli* UvrD and *Bacillus stearothermophilus* PcrA also function as oligomers *in vitro*, but they all belong to helicase superfamily 1 (SF1) (Ali et al., 1999; Maluf et al., 2003; Fischer et al., 2004; Niedziela-Majka et al., 2007; Yang et al., 2008).

As mentioned earlier, the full length of RecQ5β is 991aa in human, and previous studies indicated that its unwinding activity is located in the N-terminus (Garcia et al., 2004); but the precise domain partition is not yet well defined. In this study, we have observed that among the

three fragments, the largest one, RecQ5β$^{1-662}$, has the lowest unwinding efficiency, while the smallest one, RecQ5β$^{1-467}$, has the highest efficiency. Thus, it could be inferred that the catalytic core is located within the 467aa N-terminus. The more accurate number of amino acids needed to sustain the structure and the helicase activity deserves further research.

Though much great effort has been made to characterize the biochemical properties of RecQ helicases, so far the biological functions of RecQ family helicase *in vivo* are not yet completely understood. In this study we observed that the efficiency of RecQ5β-catalyzed DNA unwinding depended on the 3'-tail length of the DNA substrates and with its increase an obvious fast unwinding process appeared. This indicates that RecQ5β may be cooperative in DNA unwinding. In addition, the N-terminal 467aa of RecQ5β could function well to unwind DNA and the remaining amino acid sequence could inhibit the unwinding activity of the helicase. Thus it could be inferred that RecQ5β has a certain regulatory mechanism to control its helicase activity. This study provides new insight into the functions of RecQ5β *in vivo*, the relation of RecQ5β and genetic disorders, and the mechanism RecQ family helicase-mediated maintenance of genome integrity.

REFERENCES

Abdel-Monem M, Durwald H, Hoffmann-Berling H (1976). Enzymic unwinding of DNA. 2. Chain separation by an ATP-dependent DNA unwinding enzyme. Eur. J. Biochem. 65: 441-449.

Ali JA, Maluf NK, Lohman TM (1999). An oligomeric form of *E. coli* UvrD is required for optimal helicase activity. J. Mol. Biol. 293(4): 815-834.

Bachrati CZ, Hickson ID (2003). RecQ helicases: suppressors of tumorigenesis and premature aging. Biochem. J. 374(Pt 3): 577-606.

Cheok CF, Wu L, Garcia PL, Janscak P, Hickson ID (2005). The Bloom's syndrome helicase promotes the annealing of complementary single-stranded DNA. Nucleic Acids Res. 33(12): 3932-3941.

Fischer CJ, Maluf NK, Lohman TM (2004). Mechanism of ATP-dependent translocation of *E.coli* UvrD monomers along single-stranded DNA. J. Mol. Biol. 344(5): 1287-1309.

Garcia PL, Liu Y, Jiricny J, West SC, Janscak P (2004). Human RECQ5β, a protein with DNA helicase and strand-annealing activities in a single polypeptide. EMBO J. 23(14): 2882-2891.

German J, Schonberg S, Louie E, Chaganti RS (1977). Bloom's syndrome. IV. Sister-chromatid exchanges in lymphocytes. Am. J. Hum. Genet. 29(3): 248-255.

Hickson ID (2003). RecQ helicases: caretakers of the genome. Nat. Rev. Cancer, 3(3): 169-78.

Karow JK, Constantinou A, Li JL, West SC, Hickson ID (2000). The Bloom's syndrome gene product promotes branch migration of Holliday junctions. Proc. Natl. Acad. Sci. USA. 97(12): 6504-6508.

Kyng KJ, May A, Stevnsner T, Becker KG, Kølvrå S, Bohr VA (2005). Gene expression responses to DNA damage are altered in human aging and in Werner Syndrome. Oncogene, 24(32): 5026-5042.

Lohman TM, Bjornson KP (1996). Mechanisms of Helicase-Catalyzed DNA Unwinding. Annu. Rev. Biochem. 65: 169-214.

Lohman TM, Tomko EJ, Wu CG (2008). Non-hexameric DNA helicases and translocases: mechanisms and regulation. Nat. Rev. Mol. Cell Biol. 9(5): 391-401.

Masai H (2011). RecQL4: a helicase linking formation and maintenance of a replication fork. J. Biochem. 149(6): 629-631.

Machwe A, Xiao L, Groden J, Matson SW, Orren DK (2005). RecQ family members combine strand pairing and unwinding activities to catalyze strand exchange. J. Biol. Chem. 280(24): 23397-23407.

Maluf NK, Fischer CJ, Lohman TM (2003). A Dimer of *Escherichia coli* UvrD is the active form of the helicase *in vitro*. J. Mol. Biol. 325(5): 913-935.

Matson SW, Bean DW, George JW (1994). DNA helicases: Enzymes with essential roles in all aspects of DNA metabolism. Bioessays, 16: 13-22.

Matson SW, Kaiser-Rogers KA (1990). DNA helicases. Annu. Rev. Biochem. 59: p. 289-329.

Niedziela-Majka A, Chesnik MA, Tomko EJ, Lohman TM (2007). Bacillus stearothermophilus PcrA monomer is a single-stranded DNA translocase but not a processive helicase *in vitro*. J. Biol. Chem. 282(37): 27076-27085.

Ren H, Dou S X, Zhang X D, Wang PY, Kanagaraj R, Liu JL, Janscak P, Hu JS, Xi XG (2008). The zinc-binding motif of human RECQ5β suppresses the intrinsic strand-annealing activity of its DExH helicase domain and is essential for the helicase activity of the enzyme. Biochem. J. 412(3): 425-433.

Sharma S, Doherty KM, Brosh RMJr (2006). Mechanisms of RecQ helicases in pathways of DNA metabolism and maintenance of genomic stability. Biochem. J. 398(3): 319-337.

Singleton MR, Dillingham MS, Wigley DB (2007). Structure and mechanism of helicases and nucleic acid translocases. Annu. Rev. Biochem. 76: 23-50.

Sharma S, Stumpo DJ, Balajee AS, Bock CB, Lansdorp PM, Brosh Jr. RM, Blackshear PJ (2007). RECQL, a member of the RecQ family of DNA helicases, suppresses chromosomal instability. Mol. Cell Biol. 27(5): 1784-1794.

Speina E, Dawut L, Hedayati M, Wang Z, May A, Schwendener S, Janscak P, Croteau DL, Bohr VA (2010). Human RECQL5β stimulates flap endonuclease 1. Nucleic Acids Res. 38(9): 2904-2916.

Shimamoto A, Nishikawa K, Kitao S, Furuichi Y (2000). Human RecQ5β, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3α and 3β. Nucleic Acids Res. 28(7): 1647-1655

Umezu K, Nakayama K, Nakayama H (1990). *Escherichia coli* RecQ protein is a DNA Helicase. Proc. Natl. Acad. Sci. USA. 87(3): 5363-5367.

Vindigni A, Marino F, Gileadi O (2010). Probing the structural basis of RecQ helicase function. Biophys. Chem. 149: 67-77.

Waksman G, Lanka E, Carazo JM (2000). Helicases as nucleic acid unwinding machines. Nat. Struct. Biol. 7(1): 20-22.

Wang W, Seki M, Narita Y, Nakagawa T, Yoshimura A, Otsuki M, Kawabe Y, Tada S, Yagi H, Ishii Y, Enomoto T (2003). Functional relation among RecQ family helicases RecQL1, RecQL5, and BLM in cell growth and sister chromatid exchange formation. Mol. Cell Biol. 23(10): 3527-3535.

Warmerdam DO, Kanaar R (2010). Dealing with DNA damage: relationships between checkpoint and repair pathways. Rev. Mutat. Res. 704(1-3): 2-11.

West SC (1996). DNA helicases: new breeds of translocating motors and molecular pumps. Cell. 86(2): 177-180.

Xu HQ, Deprez E, Zhang AH, Tauc P, Ladjimi MM, Brochon JC, Auclair C, Xi XG (2003). The *Escherichia coli* RecQ helicase functions as a monomer. J. Biol. Chem. 278(37): 34925-34933.

Xu HQ, Zhang AH, Auclair C, Xi XG (2003). Simultaneously monitoring DNA binding and helicase-catalyzed DNA unwinding by fluorescence polarization. Nucleic Acids Res. 31(14): e70.

Yang Y, Dou SX, Ren H, Wang PY, Zhang XD, Qian M, Pan BY, Xi XG (2008). Evidence for a functional dimeric form of the PcrA helicase in DNA unwinding. Nucleic Acids Res. 36(6):1976-1989.

Zhang XD, Dou SX, Xie P, Wang PY, Xi XG (2005). RecQ helicase-catalyzed DNA unwinding detected by fluorescence resonance energy transfer. Acta Biochim. Biophys. Sin. (shanghai). 37(9): 593-600.

Zhang XD, Dou SX, Xie P, Hu JS, Wang PY, Xi XG (2006). *E. coli* RecQ is a rapid, efficient and monomeric helicase. J. Biol. Chem. 281(18): 12655-12663.