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Short communication

Characterization of recombinant malarial RecQ DNA helicase

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ABSTRACT

RecQ DNA gene of multi-drug resistant *Plasmodium falciparum* K1 (PfRecQ1) was cloned, and the recombinant C-terminal-decahistidine-tagged PfRecQ1 was expressed in *Escherichia coli*. The purified enzyme could efficiently unwind partial duplex DNA substrate in a 3′ to 5′ direction. The malarial RecQ1 could not unwind substrates with both 5′ and 3′ overhangs, those with a 5′ overhang, or blunt-ended DNA duplexes. Unwinding of DNA helicase activity was driven by the hydrolysis of ATP. The drug inhibitory effects of six compounds indicated that only doxorubicin and daunorubicin could inhibit the unwinding activity.

RecQ DNA helicases are a family of highly conserved DNA helicases that play a key role in protecting and stabilizing the integrity of the genome [1]. They have been recognized as the guardian of genomic integrity and shown to be a hallmark of both cancer and aging [2]. The RecQ helicases have gained significant interest in the past decade due to the association of three human RecQ helicases in rare genetic disorders, i.e. Bloom syndrome (BLM helicase), Werner syndrome (WRN helicase), and Rothmund-Thomson syndrome (RECQ4) helicases [1]. The roles of other two human RecQ helicases in humans, i.e. RECQ1 and RECQ5, are not yet known, although it is likely that more links between defects in DNA helicases and human diseases will be identified. So far, only RecQ DNA helicases from human, *E. coli*, yeast, and a few others bacteria and fungi have been investigated [3]. No RecQ homolog has been reported in any protozoa or parasite.

Helicases have been previously suggested to be one of the feasible drug targets for the control of malaria, one of the most devastating diseases worldwide [4]. However, since there are a number of helicases in *Plasmodium falciparum* and other living organisms, understanding the detailed mechanism of the unwinding reaction of different helicases is important to evaluate whether the enzyme is one of the potential candidates for drug targets.

The helicase gene of 2211 bp from the genomic DNA of *P. falciparum* K1 [5] was cloned using a PCR-based method, using genomic DNA as a template (GenBank accession number KM213514). The primers used for cloning were designed based on the DNA sequence of the putative helicase gene of *P. falciparum* 3D7, Gene ID PF3D7_0918600 (previous ID PFI0910w) from the PlasmoDB database.

Amino acid sequence analysis revealed that the RecQ1 helicase from *P. falciparum* strain K1 and 3D7 are almost identical, with 99.73% sequence identity. *P. falciparum* RecQ1 (designated PfRecQ1) shares low sequence identity with human BLM, RECQ1, and WRN at 24.47%, 23.11%, and 19.87%, respectively. PfRecQ1 from *P. falciparum* strain K1 contains two inserts that are missing in strain 3D7. Only human BLM contains a long N-terminal portion containing three acidic domains (see Supplementary material I).

The schematic diagram of the four aligned RecQ helicases is shown in the upper panel of Fig. 1, while the predicted 3D structure of the parasite PfRecQ1 in this study is illustrated in the bottom panel. The organization of the conserved domains is similar. There are two conserved domains in all RecQ helicases, namely helicase ATP-binding domain, helicase C-terminal or RQC domain. The DEAH box on human BLM was replaced by the DEVH box in human RECQ1 and plasmodium RecQ. Human RecQ BLM contains an additional 595 and 51 amino acids at the N-terminus and C-terminus,

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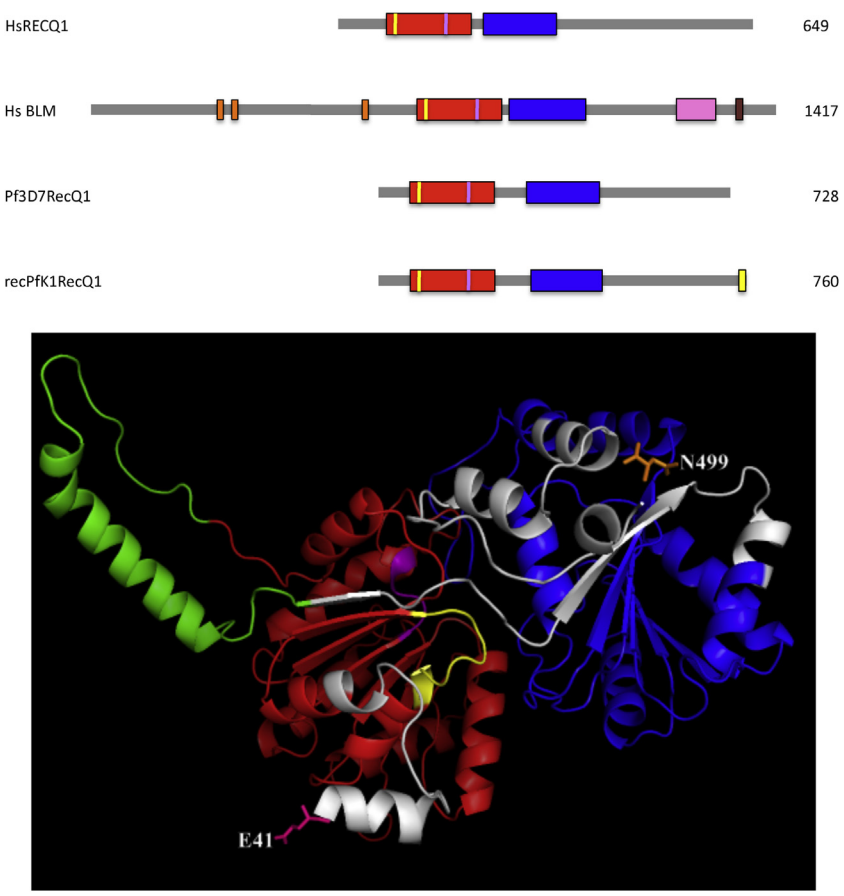

Fig. 1. Structural diagram of different RecQ helicases and 3D structure of PfRecQ1. *Upper panel* illustrates the schematic diagram of human and *Plasmodium* RecQ helicases referred to in this study. Yellow and purple amino acids indicated ATP binding domain and DExH box, respectively. Red box indicates the main helicase domain; blue box indicates the helicase C-terminal domain or RQC domain. Only human BLM contains HRDC and the nuclear localizing signal, which are indicated by the pink and brown boxes, respectively. Yellow box at the C-terminal of recombinant PfRecQ1 represents 10xHis, followed by FLAG tags. The number of amino acids in each protein is indicated on the right. The diagram is drawn to scale. The position of each bar is based on the CLUSTALW alignment in Fig. 2. *Bottom panel* illustrates the 3D structure of PfRecQ1 generated by SWISS-MODEL homology modeling software, using human RECQ1, PDB code: 2wwyA as a template. Only partial structure with high confidence (based on Phyre2) is shown, i.e., E41 to N499 are shown. Different colors indicate different conserved domains, which are indicated in the schematic model below. The green helix indicates an extra-loop that is only found in *Plasmodium* RecQ.

respectively. In addition, the putative nuclear localization signal and the HDRC domain were only found in human BLM. The 3D structure of PfRecQ1 was predicted using Swiss-model server and visualized by Pymol software, using human RECQ1 as a template [6]. The green helix protruding out of the compact structure of the compact helicase is a non-conserved additional insert that was only found in *P. falciparum* RecQ1.

The expression and purification of PfRecQ1 from *P. falciparum* strain K1 in this study was based on our previous successful method for the expression of various bacterial enzymes [7]. The recombinant PfRecQ1 could be purified by one-step Ni^{2+} affinity chromatography to apparent homogeneity (see supplementary material II). Biochemical characterization indicated that the helicase reaction required the presence of ATP as a cofactor, although dATP supported the unwinding at 89% of the efficiency of ATP. Other dNTPs, i.e. dCTP, dGTP, and dTTP, could support partial unwinding at 30%, 21%, and 13%, respectively. $MgCl_2$ is required for maximum helicase activity. $CuCl_2$, $NiCl_2$, $CoCl_2$, $ZnCl_2$ and $FeSO_4$ could partially provide the unwinding activity at approx. 70–80% of activity in the absence of $MgCl_2$. In contrast, $CaCl_2$, $CuSO_4$ and $MnSO_4$ could slightly support the unwinding activity in the range of 44–61%. DNA helicase activity was significantly decreased in the presence of 8 mM EDTA, 200 mM KCl or 200 mM NaCl.

The helicase activity of recombinant PfRecQ1 on various substrates is shown in Fig. 2. The enzyme could unwind DNA substrates with 17- and 34-base pair duplex regions, as indicated in Fig. 2 A and B. The enzyme could not unwind 17-mer partial duplex DNA substrates with both 5′ and 3′ protruding ends (Fig. 2E) or 5′ protruding ends (Fig. 2C), and 41-mer duplex blunt end substrate (Fig. 2F). However, the enzyme could unwind 17-mer partial duplex DNA substrate with 3′ protruding end (Fig. 2D). These results indicated that the *Plasmodium* RecQ1 catalyzes DNA unwinding unidirectionally in the 3′ to 5′ direction along the bound strand, and could not unwind blunt-ended duplex DNA substrate.

The effects of various drugs on the activity of recombinant PfRecQ1 DNA helicase in a standard duplex DNA dissociation reaction were reported in Table 1. All drugs were pre-incubated with duplex M13-17-mer DNA substrate at the concentration of 50 μM. Only anthracycline antibiotics, i.e. doxorubicin and daunorubicin, could inhibit the DNA unwinding activity of the enzyme, at IC_{50}

Table 1
Effect of inhibitors on PfRecQ1 DNA helicase activity.

Inhibitors	IC_{50} (μM)
Aphidicolin	>50
Daunorubicin hydrochloride	30
Doxorubicin hydrochloride	23
Genistein	>50
Mitoxantrone hydrochloride	>50
Netropsin dihydrochloride	>50

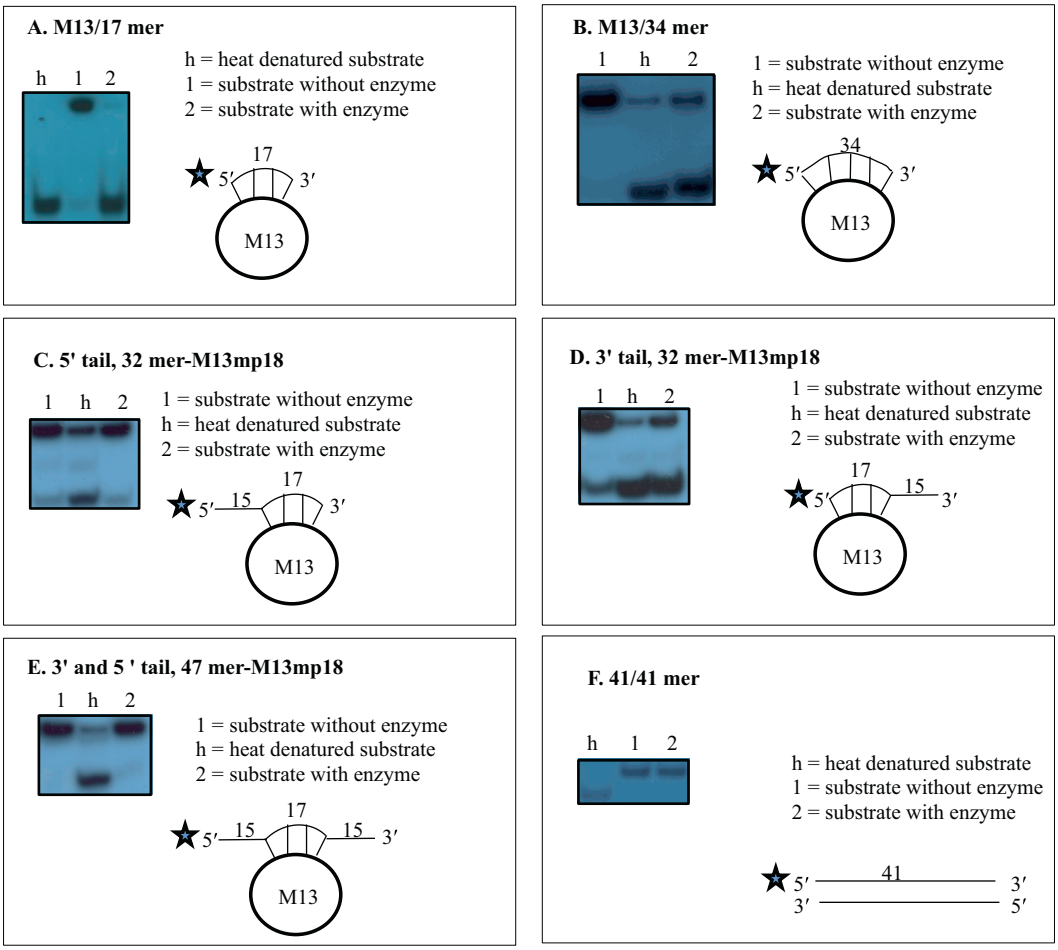

Fig. 2. Substrate specificity of PfRecQ1 helicase activity. Each panel shows the structure of the substrate used and the autoradiogram of the results from various conditions described. Top bands indicate DNA duplex substrates; bottom bands indicate ssDNA products. Asterisk denotes the end of DNA labeled with ^{32}P. Purified recombinant PfRecQ1 was analyzed as previously described [7,11]. The recombinant enzyme was fused with 10xHis tag, followed by FLAG tag. The enzyme was harvested after induction with 0.5 mM IPTG when the culture reach OD_{600} of 0.6, and further cultivated for 20 h at room temperature. The enzyme was further purified by one-step Ni^{2+} affinity chromatography to apparent homogeneity. The apparent molecular weight (MW) of the recombinant enzyme was approximately 87 kDa. The yield of the purified helicase was approx. 0.5 mg/L culture. Detail description of the expression construct and expression condition can be found in supplementary material.

values of 23 and 30 μM, respectively. Other drugs including mitoxantrone, a type II topoisomerase inhibitor, netropsin, an antibiotic that can bind to the minor groove of AT-rich sequence of ds DNA, aphidicolin, an inhibitor of DNA polymerase A and D, and genistein, an isoflavone that showed several biological activities including antihelmintic and inhibitor of topoisomerase II, could not inhibit the activity of PfRecQ1.

So far BLM and WRN RecQ helicases from human have been relatively well-studied [1] and only a few RecQ helicases from *E. coli*, yeast and some others bacteria and fungi have been investigated [3]. Recent research on the genome-wide identification of *P. falciparum* helicases indicated that there are two putative homologs of human RecQ helicases, i.e. BLM homolog and WRN homolog [8]. The PFI0910w or the PfRecQ1 from this study were indicated as homologs of human BLM [8]. However, our results demonstrated that the PfRecQ1 is indeed likely to be the homolog of human RECQ1 because the organization of the conserved domains and the size of the enzyme are much more similar than those between human BLM and PfRecQ1. Moreover, the results from homology modeling indicated that the three-dimensional structure of human RECQ1 is best fitted with the 3D structure of PfRecQ1. Human BLM and RECQ1 have been shown to display distinct DNA substrate specificity and different functions [9]; therefore, it can be proposed that human BLM helicase evolved from RECQ1 helicase, through the addition of N-terminal and HRDC domains, resulting in the diverse biological roles within each eukaryotic cell. Moreover, it can be suggested that the N-terminal part found in BLM may be required for the maintenance of more complex organisms, which is not necessary in parasites.

The unwinding characteristics of PfRecQ1 are similar to those of human BLM and WRN [10]. The ability of PfRecQ1 helicase to unwind a 3′-tailed duplex confirms the previous observation that a single-stranded 3′-tail is not a structural requirement for the unwinding of standard B-form DNA by these helicases [10]. Further analysis on the substrate preference of this enzyme, such as substrates with internal nicks, a blunt-ended duplex containing a centrally located single-stranded 'bubble', as well as a synthetic X-structure (a model for the Holliday junction recombination intermediate), should be conducted to try to understand the specific functions and interactions of these enzymes in the cells. In addition, since human RECQ1 has been shown to have a unique role in restoring active replication forks that have regressed by DNA topoisomerase I inhibition [2], it would be interesting to investigate whether the *Plasmodium* RecQ1 could possess a similar activity.

Previously PfDH A helicase has been identified from a crude extract of *P. falciparum* K1 [11]. The apparent molecular weight of the recombinant PfRecQ1 was approximately 87 kDa while that of PfDH A was 90 kDa. The polarity of both DNA helicases was 3′ to 5′ direction and both of them could not unwind blunt-ended duplex DNA. The unwinding activity of both PfRecQ1 and PfDH A depended

on hydrolysis of ATP or dATP, and could be inhibited by 200 mM KCl, 200 mM NaCl, and 8 mM EDTA.

The anthracyclines were used as antitumor antibiotics. These drugs will intercalate into double stranded DNA and generate DNA structural change. Various DNA-intercalating drugs have been used for the effect on prokaryotic and eukaryotic DNA helicase [12]. Drug inhibition assays indicated that only anthracycline antibiotics could inhibit recombinant PfRecQ1 helicase from *P. falciparum* strain K1 at higher IC_{50} values when compare to those of *P. falciparum* from the parasite culture [11], *P. cynomolgi* DEAD-box DNA helicase 45 (PcDDH45) [13], and *P. falciparum* helicase 45 (PfH45) [14]. This anti-cancer drug is an intercalating agent, which can also inhibit topoisomerase II. However, other inhibitors of topoisomerase II, i.e. mitoxantrone and genistein, did not inhibit the plasmodium RecQ1, indicating its distinctive mode of function. Mitoxantrone, an intercalator that places amino and hydroxyl groups into the major groove, and netropsin, a minor groove binder, have been shown to inhibit human WRN and BLM helicases activity at a concentration of $\geq$10 μM [15]. However, mitoxantrone and netropsin did not inhibit PfRecQ1 activity at a concentration of 50 μM. The different in drug sensitivity from human RecQ helicase indicated its potential use as a novel target for anti-malarial drug.

Even if PfRecQ1 is similar to human RECQ1 and BLM, 3D structure analysis indicated an extra loop is only present in malarial helicase. This could be the basis for difference in drug sensitivity. In addition, it also suggested that it is possible to use this enzyme as a potential anti-malarial drug target, provided that the drug is targeted to this extra loop.

The knowledge obtained from this study could provide more insight into the function of the RecQ family of DNA helicase in various eukaryotic cells and suggested that this enzyme could be a potential anti-malarial drug target.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molbiopara.2014.07.013.

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