Title: Studies on 'DEAD'-box helicase in *Plasmodium falciparum*

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Abstract

Malaria parasite *P. falciparum* causes most problematic disease in humans as a result of its prevalence, virulence and drug resistance. *P. falciparum* has developed resistance to nearly all available anti-malarial drugs. The search for novel drug targets for malaria caused by *P. falciparum* is one of the most important tasks to pursue. Helicases are enzymes, which contain nucleic acid–dependent ATPase activity and are capable of unwinding DNA or RNA duplex substrates. As a consequence, in conjunction with other components of the macromolecular machines helicases play roles in almost every process in cells that involves nucleic acids, including DNA replication and repair, transcription, translation, ribosome synthesis, RNA maturation and splicing, and nuclear export processes. Bioinformatics analysis of *P. falciparum* genome suggested that it contains 22 putative DEAD-box (Asp-Glu-Ala-Asp, or DEAD in one-letter code) helicases. Twelve out of these 22 helicases are homologues of eIF4A having roles in different aspects of nucleic acid metabolism. On the basis of the highest degree of homology to eukaryotic translation initiation factor-4A (eIF4A) PF14_0655 DEAD-box helicase was selected for cloning and detailed characterization.

The PF14_0655 helicase encodes a protein with a predicted molecular mass of approximately 45.3 kDa [hence named as *Plasmodium falciparum* helicase 45 kDa in size (PfH45)]. Southern and northern blot analysis suggested that PfH45 exists as a single

copy gene and the transcript size is ~1.2 kb. The purified PfH45 protein showed ATP binding, ssDNA or RNA- and Mg^{2+}-dependent ATPase activity, DNA and RNA unwinding in an ATP and Mg^{2+} dependent manner and bipolar helicase activity. Characterization of the truncated derivatives of PfH45 suggests that its ATPase activity mainly resides in the N-terminal domain and the RNA-binding activity predominantly resides in the C-terminal domain. This study also suggests that neither PfH45-N nor PfH45-C alone was able to show any unwinding activity. The unwinding activity of PfH45 showed preference for replication fork like structure of the substrate. Immunodepletion study suggested that the anti-PfH45 antibody (IgG) and anti-His antibody abolishes the DNA unwinding and ATPase activities of PfH45 in vitro. Immunofluorescence assay using purified antibody to PfH45 followed by confocal microscopy showed that PfH45 protein was localized mostly in cytosol. Metabolic labeling of total proteins followed by immunoprecipitation data suggests that although the PfH45 expression was observed throughout the intra-erythrocytic stages of development of the parasite but maximal synthesis was observed ~24–32 hrs post synchronization. Transcription of PfH45 gene measured by semi-quantitative RT-PCR also confirmed that PfH45 is transcribed throughout the intraerythrocytic stages. PfH45 specific dsRNA inhibited the parasite growth due to specific down-regulation in the synthesis of PfH45 protein. The purified anti-PfH45 antibody (IgG) inhibited in vitro translation. Furthermore, the addition of the purified PfH45 protein in standard in vitro translation reaction resulted in an enhancement of the translation suggesting its role in this process. The ssDNA-dependent ATPase and DNA unwinding activity of PfH45 was effectively inhibited by actinomycin, DAPI, daunorubicin, ethidium bromide, netropsin and nogalamycin. The effect of these inhibitors in parasite culture suggested that *P. falciparum* blood forms do not die by apoptosis. The characterization of such an important bipolar and dual helicase with multiple intrinsic activities and roles in protein synthesis may help in better understanding of DNA and RNA metabolism in the malaria parasite *P. falciparum.*