ABSTRACT

*Plasmodium falciparum*, the etiological agent of malaria, is the most lethal of all the protozoan parasites. The lifecycle of the parasite involves the mosquito and mammalian hosts and it faces a wide range of temperatures therein. An important way in which the parasite combats stress involves the use of heat shock proteins (HSPs). *P. falciparum* codes for about 6 homologues of Hsp70, which are known to be important. The present work mainly involves PfHsp70-1 (PF08_0054) that has been annotated as an Hsp70 homolog in the database. At the time when the work was initiated, the protein had drawn wide attention for its novel role in the transport of over 500 nuclear encoded proteins to the apicoplast, an essential relict plastid of the parasite. Nuclear encoded proteins destined to the apicoplast possess a leader sequence at the N-terminus, also called transit peptide, which has specific Hsp70 binding features. Hence Hsp70 and its interacting proteins destined to the apicoplast represent exciting therapeutic targets for the development of novel anti-malarials. In the first instance, we were interested in characterizing the protein biophysically and biochemically and had to clone and purify the protein in large quantities. PfHsp70-1 is a multi-domain protein and we were interested in the functions of the individual domains in protein function and generated domain deleted versions of the protein and studied their respective activities also. We also wanted to probe the determinants of the interactions of the protein with specifically designed peptides endowed with features similar to that of transit peptides. The reported results are important both from the standpoint of dissecting the functions of the HSP and also against the longer-term goal of exploiting its potential as a novel therapeutic target.

The first chapter is an introduction to the various heat shock proteins present in the parasite and the functions they play in other organisms. A background about the characteristics of nuclear encoded and apicoplast targeted proteins is also provided to place the reported results against the backdrop of the work carried out by other groups.

The second chapter details the materials and methods used to carry out the reported work; this includes the protocols used to clone, purify and characterize the proteins. The biochemical and biophysical experiments performed to characterize the full-length protein and the various truncated mutants are also detailed. Crystallization protocols that were employed, as also the methods used to study the protein-peptide interactions, are also reported.
The purification of the full-length protein, the domain truncated versions and the characterization of the ATPase and chaperone activities are reported in the third chapter. As with many parasite proteins, the cloning and purification of PfHsp70-1 was a challenge. It was essential to get large quantities of the purified protein and its domains for the subsequent characterizations including crystallization trials. The first direct demonstration of functional interactions between Pfj1 (an Hsp40/DnaJ parasite homolog) and PfHsp70-1 is also reported. The results show that the former enhances the chaperone activity of PfHsp70-1 in the reported in vitro assays.

The contributions of the individual domains to the overall protein function and stability have been elaborated in chapter four. The N-terminal nucleotide-binding domain (NBD) is highly unstable. The substrate binding domain (SBD) and also the SBD along with the extended C-terminal tail (P-Ctr) are more stable. Addition of SBD, especially P-Ctr provides stability to the otherwise unstable NBD. The results suggest that the C-terminal domain functions as the stabilization domain in PfHsp70-1.

Protein trafficking of nuclear encoded proteins to apicoplast requires the presence of transit peptides that contain Hsp70 binding sites. In the fifth chapter, we report the design and synthesis of peptides endowed with transit peptide like characteristics. The in vitro interactions of these peptides with PfHsp70-1 have been studied using spectroscopic techniques. The relative affinities of various peptides have been determined. The results give information about the positional importance of select residues in the designed peptides. Amongst other things, they are also expected to be useful in the design of novel therapeutics that acts by disrupting interactions between PfHsp70-1 and nuclear encoded proteins destined to the apicoplast.

The author also worked on the evaluation of novel inhibitors of the P. falciparum Enoyl acyl carrier protein reductase (PfENR). The protein has drawn a flurry of recent attention as a novel therapeutic target. Development of new inhibitors might result in therapeutics that can overcome current resistance issues. The protein is also destined for the apicoplast and should interact with the PfHsp70-1 as revealed by the features of the transit peptide at the N-terminus. In the sixth chapter, we report the characterization of the inhibitory activity of several anti PfENR compounds by our group that were designed and synthesized in a collaborative manner. The in vitro mechanism of action of the best
compound (with a new scaffold) is explained in detail. The compound has an enzyme inhibition comparable to that of Triclosan, a known standard inhibitor of the enzyme.

During the course of the work, the author was also involved in a project involving the structure solution and analysis of the Nucleoside diphosphate kinase from *B. anthracis*. The crystal structure and analysis reveals that the enzyme, which has been implicated in sporulation, is well adapted to perform under stress conditions. These results have been included as an Appendix. Co-ordinates and structure factors of the *B. anthracis* Nucleoside diphosphate kinase have been submitted to the Protein Data Bank (http://www.rcsb.org) with accession numbers 2VU5 and R2VU5SF respectively.

The details pertaining to the chemicals, strains, media and buffer composition have been described in the Annexure.

**A part of the results of this thesis have already resulted in the following publications:**

1. **Gauri Misra** & Ravishankar Ramachandran (2009)
   Hsp70-1 from *Plasmodium falciparum*: Protein stability, domain analysis and chaperone activity.

   Purification, crystallization and preliminary structural analysis of Nucleoside diphosphate kinase from *Bacillus anthracis*.

   Crystal structure of the *Bacillus anthracis* nucleoside diphosphate kinase and its characterization reveals an enzyme adapted to perform under stress conditions
   *PROTEINS* doi: 10.1002/PROT.22364