Summary and Conclusions
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Malaria is a major parasitic disease caused by the protozoan parasites of the genus *Plasmodium* of which, *P. falciparum* causes most of the malaria-related fatalities. *Plasmodium* is an obligate intracellular parasite and completes its life cycle in two hosts. Sexual and asexual phases of *Plasmodium* life cycle take place in female *Anopheles* mosquito and human hosts respectively. Asexual erythrocytic cycle of malaria parasite is responsible for the clinical symptoms of malaria. Although drugs are available against malaria but drug resistance of the parasites and toxicity associated with the available antimalarials necessitate the need for novel drugs against the disease. Malarial proteases are attractive drug targets due to their roles in many parasite-specific processes involved in parasite growth and maturation. During erythrocytic cycle, malarial proteases are involved in hemoglobin digestion, merozoite released from infected RBCs (egress) and entry of released merozoites into fresh RBCs (invasion).

In this study, we have characterized two *P. falciparum* proteases, subtilisin-like protease 3 (PfSUB3) and serine repeat antigen 5 (SERA5) and demonstrated their functional significance at asexual erythrocytic stages. The major findings of this study are:

1. Mature protease domain spans from T516 to E769 at the C-terminal of PfSUB3.
2. *E. coli*-expressed PfSUB3 mature protein (PfSUB3m) showed serine protease activity in *in vitro* assays. PfSUB3m possessed autolytic activity which was inhibited by serine protease inhibitor, PMSF. PfSUB3m showed dose-dependent proteolytic activity on general protease substrates, azocasein and azoalbumin. Purified PfSUB3m underwent autocatalytic cleavage at N-terminal to produce the truncated form, PfSUB3mt. PfSUB3mt showed time-dependent autolytic activity which was inhibited by PMSF.
3. By quantitative real-time PCR, *pfsub3* transcript abundance was found in the ratio of 1.0000:0.7240:0.0934 at schizont, trophozoite and ring stages, confirming the predominant expression at schizont stage.
4. By immunofluorescence assay, PfSUB3 was localized in schizont stage parasites.
5. By yeast two-hybrid screening with cDNA library of mature trophozoite and schizont stage parasite, *P. falciparum* profilin (PfPRF) was found to the interacting protein of PfSUB3 mature protein.

6. PfSUB3 mature protein was found to hydrolyze recombinant PfPRF in *in vitro* assays.

7. SERA5 prodomain was inhibitory to parasite growth up to nanomolecular concentrations (1 µM to 1 nM) and delayed the rupture of schizonts in a dose-dependent manner.

8. A 7-residue peptide, DNSDNMF derived from the C-terminal of SERA5 prodomain (D\(^{560}\) to F\(^{566}\) in SERA5 primary sequence) was inhibitory to enzyme activity of SERA5 proenzyme domain in micromolar concentrations.

9. Peptide, DNSDNMF interacted with SERA5 catalytic domain and induced conformational changes in it as demonstrated by CD spectroscopy and spectrofluorimetry.

10. Peptide, DNSDNMF was also inhibitory to parasite maturation and delayed the rupture of schizonts but at a much higher concentration as compared to SERA5 prodomain (100 µM).

Our studies have shown that PfSUB3 possesses serine protease activity and is expressed in the late stages of erythrocytic schizogony. PfSUB3 undergoes autocatalytic processing to form the mature proteolytically active form. In blood stages of the parasite, *P. falciparum* profilin was found to interact with PfSUB3 mature protein and recombinant profilin was shown to be hydrolyzed by PfSUB3 mature protein. Profilin is a cytoskeleton protein and in higher eukaryotes which promotes the polymerization of actin filaments but it also acts as a ligand for Toll-like receptors in *T. gondii* and *P. falciparum*. We suggest the proteolytic activity of PfSUB3 on profilin as a parasite defense mechanism to evade the innate immune response of the host. This protective response may facilitate the survival of the released merozoites and hence implicated in egress and/or invasion processes. Time and stage of PfSUB3 expression is also suggestive of its role in these processes.
SERA5 prodomain and its derived peptide inhibited the growth of the parasite in a dose-dependent manner and delayed the rupture of schizonts. Prodomain, when incubated with the parasite culture in ring stages, caused severe morphological defects and compromised the maturation of the parasite. Upon incubation with the late trophozoite stage culture, it delayed the rupture of schizonts without any morphological defects. Since, SERA5 has been implicated in schizont rupture; these findings suggest the functional significance of the SERA5 prodomain for the enzyme domain. Prodomains carry out a variety of functions for their cognate enzyme domains like inhibition of enzyme activity, chaperone function, trafficking of the enzyme to its final destination in the cell and substrate recognition.

We speculate that SERA5 prodomain may be involved in substrate recognition by the protease and hence, addition of exogenous prodomain may sequester the physiological substrate from the enzyme domain and hence inhibiting the physiological effect of the enzyme. Since the peptide, DNSDNMF was inhibitory to the enzyme activity of SERA5 proenzyme domain, we suggest that it interacts with the active site of the enzyme or hinders with the substrate entry into the active site of the enzyme. The inhibitory effect of SERA5 prodomain and the peptide derived from it, suggests that SERA5 is a potential drug target against malaria and possesses some functionally important elements within its prodomain.

The work in this thesis has demonstrated the functional importance of PfSUB3 and SERA5 for the parasite. Our results have shown that PfSUB3 and SERA5 are two important asexual erythrocytic stages proteases of *P. falciparum*. Both of these proteases have the potential of drug targets against malaria.