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Malaria is a major infectious disease in tropical and subtropical regions of the world and a cause of suffering and economic losses for the developing countries (Snow et al., 2005). According to WHO report of 2008, there were an estimated 247 million cases of malaria among 3.3 billion people, resulting in nearly a million deaths, mostly of children under 5 years of age (World Malaria Report, 2008). Malaria is a vector-borne infectious disease caused by the protozoan parasites of the genus Plasmodium. Human malaria is caused by four species of Plasmodium namely P. falciparum, P. vivax, P. malariae and P. ovale. Malaria parasites are transmitted to humans through the bites of female Anopheles mosquitoes which inoculate parasite forms called sporozoites into the bloodstream that infect liver where the parasite develops into liver stage merozoites. Upon release from the liver cells, merozoites infect red blood cells and start the erythrocytic cycle that is responsible for the clinical symptoms of malaria. Of the four species of Plasmodium, P. falciparum is the causative agent of severe malaria including cerebral and placental malaria. Cerebral malaria is often fatal due to the blockade of blood capillaries of the brain by adherence of parasitized red blood cells on endothelial cells. Symptoms of malaria can be absent to very mild or severe or even cause death. Although antimalarial drugs are available, drug resistance and toxicity associated with many antimalarials necessitate the development of new therapeutics against malaria (Rosenthal, 1998).

Parasite-derived proteases are a group of molecules that play vital roles in many parasite-specific processes essential for parasite multiplication and development. The human malaria parasite, P. falciparum has a complex life cycle with many obligate intracellular stages in human and mosquito. Clinical manifestations of malaria result from the cyclical asexual replication of the parasite in the erythrocytes of human host. The erythrocytic cycle begins when free merozoites infect red blood cells and develop from small ring-stage parasites to larger, more metabolically active trophozoites and then to multinucleated schizonts. The erythrocytic cycle completes with the rupture of schizonts and release of invasive merozoites. Proteases belonging to various mechanistic classes
play crucial roles during different stages of erythrocytic stages of *P. falciparum* and are considered as potential drug targets due to the feasibility of designing inhibitors against them. Serine and cysteine protease activities have been implicated in merozoite release from schizonts (Arastu-Kapur *et al.*, 2007) and serine protease activity has been shown to be essential for invasion of fresh erythrocytes by merozoites (Blackman and Holder 1992; Harris *et al.*, 2005).

Subtilisin-like proteases or subtilases are a class of serine proteases widely distributed in organisms through evolution. Three subtilisin-like proteases have been annotated in the *Plasmodium* genome database “PlasmoDB”. Of these, *P. falciparum* subtilisin-like protease 1 (PfSUB1) is essential in blood stages. It is expressed at schizont stages in exonemes which are apical organelles with morphological characteristics of dense granules (Yeoh *et al.*, 2007). PfSUB1 as one of the key proteases involved merozoite release from infected RBCs. It carries out the proteolytic processing of members of serine repeat antigen (SERA) family; of which serine repeat antigen 5 (SERA5) is a protease involved in merozoite release from erythrocytes (Arastu-Kapur *et al.*, 2007). In addition, PfSUB1 primes the merozoites for invasion by carrying out the primary processing of all the members of merozoite surface protein 1 (MSP1) complex (MSP1, MSP6 and MSP7) in the parasitophorous vacuole (Koussis *et al.*, 2008). Thus, since PfSUB1 is involved in two critical steps of parasite maturation, it qualifies as a potential drug target against malaria. The second subtilase, subtilisin-like protease 2 (PfSUB2) is also essential in blood stages. It is expressed at schizont stages and has been shown to cause the shedding of MSP1 and apical membrane antigen 1 (AMA1), two adhesins involved in merozoite invasion of red blood cells. PfSUB2 traverses across the merozoite surface in an actin-dependent manner and sheds these adhesins as the merozoite takes entry into the host erythrocytes (Harris *et al.*, 2005).

Subtilisin-like protease 3 (PfSUB3) is the third *P. falciparum* subtilase annotated in PlasmoDB. It is encoded by a 2310-bp, single exon open reading frame which translates to an 88048 Da-protein. Microarray studies have suggested that *pfsub3* is transcribed at asexual blood stages with significant levels of expression in gametocytes and liver stages (Le Roch *et al.*, 2003). In this study, we have characterized PfSUB3 with
respect to its proteolytic activity, expression in different asexual erythrocytic stages and its interacting partners.

*P. falciparum* serine repeat antigens (SERAs) are a group "cysteine-like proteases" secreted into the parasitophorous vacuoles of mature erythrocytic stage parasites (Delplace et al., 1987; Bzik et al., 1988; Miller et al., 2002). Nine *sera* genes are found in *P. falciparum*, eight located on chromosome 2 (*sera1* to *sera8*) and one on chromosome 9 (*sera9*) (Gardner et al., 2002). All the SERA proteins possess a central papain-like protease domain flanked by two cysteine-rich domains. Several SERA proteins possess an atypical serine residue in place of the active site cysteine. In *P. falciparum*, SERAs 1-5 and SERA9 possess active site serine, whereas the rest three possess more classical active site cysteine. Among all the *P. falciparum* SERAs, SERA5 is expressed at much higher levels than any other family member and is indispensable for parasite survival (Crabb et al., 2004; McCoubrie et al., 2007). SERA5 has been implicated in merozoite egress from infected RBCs (Arastu-Kapur et al., 2008; Fairlie et al., 2008)

The central protease domain of SERA5 consists of pro and enzyme domains and possesses chymotrypsin-like proteolytic activity but its biological relevance is uncertain (Hodder et al., 2003). SERA5 proenzyme domain consists of 187-amino acid residue long prodomain and 251-amino acid residue long catalytic domain. The prodomain remains associated with the catalytic domain after proteolytic processing of SERA5 at the time of schizont rupture and hence, it is supposed to play some important role in regulating the enzyme activity. Prodomains are associated with numerous regulatory functions like prevention of premature activation of enzyme (Nirasawa et al., 1999), proper folding of their cognate enzyme domain (Yabuta et al., 2001), trafficking of the enzyme to its destination (Harper et al., 2006) and substrate recognition (Roy et al., 1998). Attachment of SERA5 prodomain with the enzyme domain raises the question of its biological relevance. In this study, we have assessed the functional importance of SERA5 prodomain for the enzyme and its physiological significance.