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# Synthetic peptides derived from the C-terminal 6 kDa region of *Plasmodium falciparum* SERA5 inhibit the enzyme activity and malaria parasite development

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ABSTRACT

*Background: Plasmodium falciparum* serine repeat antigen 5 (PfSERA5) is an abundant blood stage protein that plays an essential role in merozoite egress and invasion. The native protein undergoes extensive proteolytic cleavage that appears to be tightly regulated. PfSERA5 N-terminal fragment is being developed as vaccine candidate antigen. Although PfSERA5 belongs to papain-like cysteine protease family, its catalytic domain has a serine in place of cysteine at the active site.

*Methods:* In the present study, we synthesized a number of peptides from the N- and C-terminal regions of PfSERA5 active domain and evaluated their inhibitory potential.

*Results:* The final proteolytic step of PfSERA5 involves removal of a C-terminal ~6 kDa fragment that results in the generation of a catalytically active ~50 kDa enzyme. In the present study, we demonstrate that two of the peptides derived from the C-terminal ~6 kDa region inhibit the parasite growth and also cause a delay in the parasite development. These peptides reduced the enzyme activity of the recombinant protein and co-localized with the PfSERA5 protein within the parasite, thereby indicating the specific inhibition of PfSERA5 activity. Molecular docking studies revealed that the inhibitory peptides interact with the active site of the protein. Interestingly, the peptides did not have an effect on the processing of PfSERA5.

*Conclusions:* Our observations indicate the temporal regulation of the final proteolytic cleavage step that occurs just prior to egress.

*General significance:* These results reinforce the role of PfSERA5 for the intra-erythrocytic development of malaria parasite and show the role of carboxy terminal ~6 kDa fragments in the regulation of PfSERA5 activity. The results also suggest that final cleavage step of PfSERA5 can be targeted for the development of new anti-malarials.



## 1. Introduction

Recent advances in the malaria control efforts have been promising and have helped in preventing around 1 million deaths, in the past decade, according to the recent data [1]. World malaria report estimated 216 million malaria cases and approximately 660,000 malaria deaths in year 2010, suggesting that malaria is still a serious disease prevalent in the tropics [1]. The emergence of drug resistant parasites from time to time poses a constant challenge to combat the disease. Continuous efforts are thus required to understand the biology of the parasite at the molecular level, which may help in identifying novel vaccine candidates or new drug targets.

Egress of merozoites from the blood stage schizont is an important event in the asexual stages of the parasite life cycle. Merozoites thus released from the infected RBCs invade fresh erythrocytes resulting in propagation of the disease. In *Plasmodium falciparum*, a number of proteases have been suggested to play a key role in the process of egress by degradation of parasite and RBC membranes [2]. One of the major families of proteases known to be involved in this process, in *P. falciparum*, is a large multigene family of serine repeat antigens (SERAs). The *falciparum* SERA family consists of nine members, out of which *sera*1–8 genes are arranged on chromosome 2 in a tandem array and *sera*9 gene is arranged on chromosome 9 [3]. All the members of the family have a papain protease like central domain and are transcribed at trophozoite as well as schizont stages [4–7]. Two members of this family, PfSERA5 and 6, are

*Abbreviations:* SERA, serine repeat antigen; SUB1, subtilisin1; hpi, hours post invasion; kDa, kilodalton

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refractory to deletion and appear to be essential for parasite growth and viability [7–9].

PfSERA5 is one of the most abundant proteins in the schizont stage of the parasite [6,10] and a well studied member of this family. It is one of the promising asexual blood stage vaccine candidates and therapeutic drug target [11]. PfSERA5 could induce antibodies that either protected against blood stage infection in vivo [12] or inhibited parasite replication in vitro [13]. Recombinant proteins made from the N-terminal 47-kDa domains of PfSERA5 have been shown to be immunogenic and elicited antibodies that inhibited erythrocyte invasion and parasite replication in vitro as well as in vivo in rodent and primate models [14–17]. In addition, serum antibody titer against PfSERA5 in Ugandan adult sera from a malaria endemic area correlated with the protection against malaria symptoms and sera from these patients were effective in in vitro parasite growth inhibition [4,18,19]. Recently, BK-SE36 malaria vaccine was shown to confer malaria symptomatic protection over 70% for one year in Ugandan children [19]. Also, inhibitors of PfSUB1, an enzyme involved in processing of PfSERA5 have been shown to stall the process of merozoite egress and parasite maturation [20,21]. Together, all these studies emphasize the role of PfSERA5 in parasite growth, maturation and its potential as a drug target.

Phylogenetic and evolutionary studies of SERA genes from eight *Plasmodium* species have revealed that SERA genes can be categorized into four groups [22,23]. SERA proteins from groups I to III possess cysteine residue in their catalytic site, while members of group IV posses serine residue at that position [22]. In *P. falciparum* SERA1–5 and 9 have serine and SERA 6–8 have cysteine at the key catalytic position. PfSERA5 is approximately a 120 kDa protein, which undergoes proteolytic processing. It is cleaved into a 47 kDa N terminal domain, a central 56 kDa and 18 kDa C terminal fragments. 47 kDa and 18 kDa domains remain linked via a disulfide bond [24]. In certain alleles of SERA5, the 47 kDa domain is further processed into two 25 kDa fragments [25], both of which remain linked to the 18 kDa fragment. The 56 kDa domain is further cleaved to a 50 kDa catalytically active domain having a chymotrypsin like activity [26] and a 6 kDa fragment [24,27,28] which are shed into the culture supernatant after schizont rupture. This cleavage is sensitive to leupeptin and E-64 inhibition. It was recently speculated that this additional processing of P56 to P50 may serve as a proteolytic inactivation step [2].

The proteolytic activity of PfSERA5 has been an issue of continuous debate among different groups because of replacement of cysteine-to-serine within the catalytic triad of the protein. A single report has demonstrated the chymotrypsin-like activity of *Escherichia coli* expressed recombinant SERA5 central domain [26]. This final processing event, cleavage of 56 kDa domain to 50 kDa, is an enigma and significance of its cleavage just prior to egress is an important mechanism that needs to be studied. In the present study, we provide evidence for the enzymatic activity of PfSERA5 ~ 50 kDa protein fragment (PfSERA5$P_{50}$) and its inhibition by two peptides derived from the 6 kDa fragment. These peptides significantly inhibited the growth and development of *P. falciparum* parasites in vitro as well as co-localized with the protein in the parasitophorous vacuole. These results thus have implications in understanding the tight temporal regulation of PfSERA5 proteolytic activity and the importance of the final cleavage step just before release of the merozoites.

## 2. Results

### 2.1. Design and synthesis of peptides derived from the PfSERA5 sequence

We have previously shown that peptides derived from pro-region of falcipain-2 can block the activity of the parasite cysteine protease [29]. To identify similar sequence(s) for the SERA5 protein, we selected and synthesized nine different peptides across its entire length (Fig. 1). The peptides were designed based primarily on three criteria. First, the peptide sequences were selected either from the unique serine rich region of PfSERA5 (SE5 P4, P5 P6) or from the unique region of serine rich stretch from the full length protein (SE5 P9). Secondly, the peptides were designed from the regions flanking the various processing sites of the protein (SE5 P5, P7, P8) (Additional File 1) [2]. Finally, peptides SE5 P1, P2 and P3 were synthesized to span the entire 6 kDa region from the 56 kDa segment of PfSERA5 that gets cleaved to generate an active 50 kDa protein. Sequences of each peptide used in the present study are shown in Table 1.

### 2.2. Effect of PfSERA5 derived peptides on P. falciparum development and growth

We next evaluated the biological activity of PfSERA5 derived peptides by assessing their effects on parasite growth, development and invasion. Parasite cultures were incubated with various peptides at a concentration of 50 μM and parasite smears were examined by microscopy and flow cytometry ~20 hpi to assess new ring formation. As shown in Fig. 2a, peptides SE5 P1 and SE5 P2 (derived from the C-terminal 6 kDa region of the P56 domain of PfSERA5) inhibited the parasite growth and invasion (~60–70%) considerably in the first cycle. In comparison, peptide SE5 P3, also from the C-terminal 6 kDa region, did not affect the parasite invasion. Peptides from other domains especially the N-terminal domain (SE5 P5 and SE5 P7) as well as from the extreme C-terminal region (SE5 P9), reduced the parasitemia only by ~30–45%. They were half as effective as peptides SE5 P1 and SE5 P2. Peptide SE5 P8 did not show any effect on the parasite culture. Since the PfSERA5 protein has been known to express at late stage of the intra-erythrocytic cycle [5], we next assessed the effect of peptides SE5 P1 & SE5 P2 on parasite growth in culture by incubating these peptides with late trophozoite/early schizont stages. The addition of either of these peptides to doubly synchronized culture of *P. falciparum* 3D7 parasites (30–34 h) resulted in the accumulation of late stage parasites

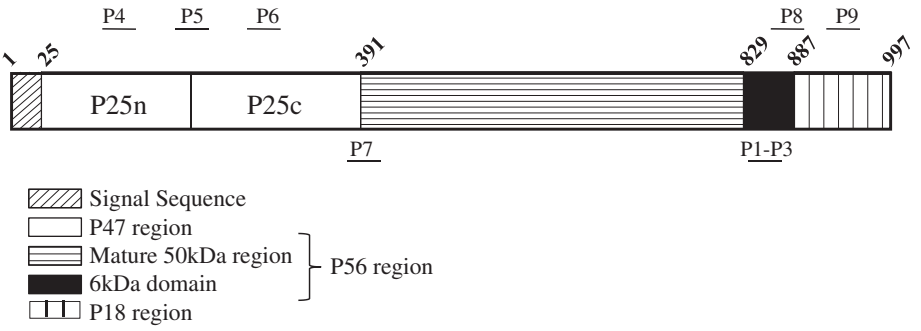


**Fig. 1.** Schematic representation of full length PfSERA5 protein. The numbers above indicate the amino acid positions defining the various domains of the protein. The peptides used in the study are marked across the length of the protein in the region from which they are derived.