Abstract

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Project Title: Enzymatic and structural study of the PEXEL protease, Plasmepsin V From *Plasmodium falciparum*

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Abstract:

Plasmodium falciparum is the causative agent of the most severe form of human malaria, which results in approximately one million deaths annually. The parasite exports several hundreds of effector proteins that remodel the host erythrocyte and enable parasites to acquire nutrients and evade immune response. The majority of exported proteins contain the *Plasmodium* export element (PEXEL) in their N-terminus, which is proteolyically cleaved in the parasite ER by plasmepsin V (PMV). Since the activity of PMV is essential for parasite viability, PMV is a novel target for antimalarial drugs. In this study, two versions of *Pf*PMV were constructed: *Pf*PMVp37, premature form containing residues E37 to N521 and *Pf*PMVm84, mature form, covering residues D84 to N521. The optimal condition for overexpressing the proteins were at 16°C over night with 0.2 mM IPTG in *E. coli* BL21(DE3). Two-step purifications of the recombinant Trx-*Pf*PMV were required. Soluble proteins were first purified over Ni^{2+} affinity and incubating the Ni^{2+}-Trx-*Pf*PMV purified fractions with ATP for the purpose of eliminating the *E. coli* HSP60 resulted in more purified protein. Even though only around 150 μg of purified proteins were obtained, the specific activity of Trx-*Pf*PMVp37Trx-*Pf*PMVm84 for HRPII and *Pf*EMP2peptides was determined. Both recombinant enzymes could hydrolyze the peptide substrates with more or less the same rate. However, both enzymes showed the substrate preference on *Pf*EMP2 peptide over HRPII. This indicated that there was a preference of amino acid residues at P_2 and P_1' position. In addition, inhibition study revealed that pepstatin hardly inhibited the activity of Trx-*Pf*PMVm84, whereas Nelfinavir, an HIV-1 protease inhibitor, phenyl-methylsulphonyl fluoride, a serine protease inhibitor, inhibited the activity of Trx*Pf*PMVm84 by 30% and 50%, respectively. Taken together, this research provided new insights on enzyme activity and substrate specificity of the recombinant *Pf*PMV, which may lead to the design of the peptidomimetic inhibitor.

Keywords: Malaria, PEXEL motif,Plasmepsin