Structural Analysis and Strategic Truncation of Plasmodium BEM-46 Like Protein (PBLP), a Malarial Hydrolase

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Abstract

Malaria is a leading cause of death across developing nations; the World Health Organization documented 610,000 fatalities in 2024 alone. Bud emergence 46 (BEM46) proteins are transmembrane α/β hydrolases, and despite being highly conserved across all eukaryotic organisms, their cellular functions remain elusive. In the malaria parasite *Plasmodium yoelii*, the membrane-associated Plasmodium BEM-46 like protein (PBLP) is expressed throughout all life stages and modulates the replication of invasive forms. Characterizing PBLP'S structure and function could aid in development of anti-malarial therapy and shed light on the enzymatic function of other BEM46 proteins. A preexisting expression protocol for a truncated form of PBLP, lacking the first 49 amino acids, required troubleshooting. Protein from the supernatant could not be measurably or consistently retrieved as evidenced in SDS-PAGE and enzymatic assay data. We hypothesized that the 49 amino acid truncation destroyed much of the substrate-binding pocket and was severe enough to interfere with protein folding. To resolve this, we designed a truncation construct using Alphafold3 and PyMOL which removed only the first 16 residues, including much of the transmembrane domain, while retaining near-native binding pocket formation. Using site-directed mutagenesis, we generated plasmids to express this less-truncated construct and will run our established protein purification protocol for the new mutant. Future enzymatic assays using this construct should provide more specific insight into PBLP's binding specificity and substrate preference as well as shed light on its catalytic role.