Expression, Purification, and Hydrolase Characterization of PBLP, a Protein from the Malaria Parasite *Plasmodium yoelii*

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Abstract:

Plasmodium parasites are transmitted from mosquitoes to mammalian hosts upon a blood meal and are the pathogens causative of malaria, a significant public health burden. The *Plasmodium* BEM46-like protein (PBLP) contributes to the maturation and formation of invasive-stage parasites, which advance the development of malaria. Characterizing the enzyme activity of PBLP is an ongoing research goal, driven by the potential for new antimalarial drug designs. PBLP is currently known as a membrane-associated member of the α/β-hydrolase superfamily, but information regarding its substrate and specific biological function remains poorly understood. Here, we present a study aimed at determining the catalytic function and substrate molecule(s) of PBLP, as well as the steps taken to optimize its yield from transformed *E. coli* cells. We used homology searches and computational structural predictions to gain insight into potential mechanistic features, including PBLP's conserved Ser-His-Asp catalytic triad and sequence matches to lipase and esterase families. We hypothesize that PBLP functions as a lipid esterase, and we have experimentally confirmed hydrolase activity. Additionally, we found that adding dithiothreitol (DTT) as a reducing agent to isolation, nickel column chromatography, and dialysis buffers facilitates a greater recovery of soluble PBLP. This project is a foundation for future studies working to confirm PBLP's biological significance in the *Plasmodium* parasite life cycle.