Optimizing the Isolation of Plasmodium BEM46-Like Protein, an Enzyme from Malarial Parasite *Plasmodium yoelii*

Jennifer Nguyen, Dia Sharma, and Hannah Baughman

TBIOMD 495

Background

- Malaria is an infectious disease caused by *Plasmodium* parasites, with no current cure
- Plasmodium BEM46-Like Protein (PBLP) is constitutively expressed & conserved throughout the parasitic life cycle in all Plasmodium species (Groat-Carmona et al. 2015)
- The sequence of PBLP matches the α/β-hydrolase superfamily, which has a wide range of functions (Groat-Carmona et al. 2015)
- PBLP has an important role in modulating the maturation of invasive stages in the parasite (Groat-Carmona et al. 2015)

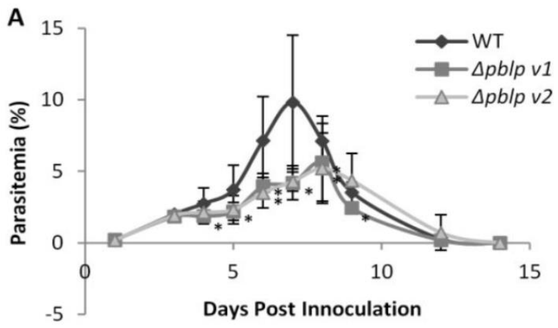
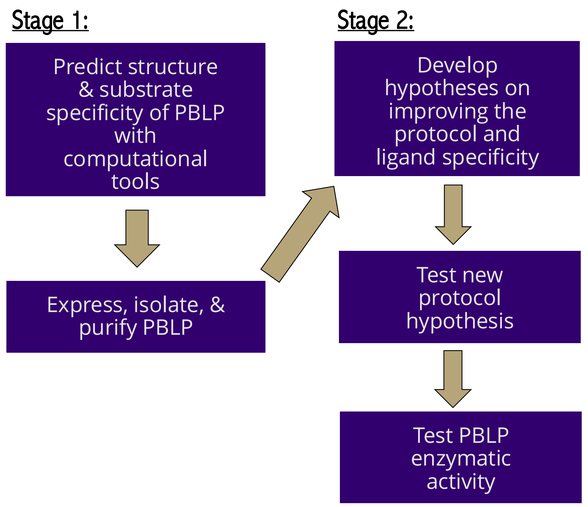


Figure 1. *Relationship of parasitemia percentage of wild type pblp versus knockout strains.* Mice were monitored over 15 days post infection with each strain of *Plasmodium yoelii*. When pblp was knocked out, there was a significant decrease in infectivity, indicating pblp has a role in the invasive stages of the parasite. This figure was adapted from "A Plasmodium α/β-hydrolase modulates the development of invasive stages" (Groat-Carmona et al. 2015).

Research Objectives



- Hypothesis 1: Combining enzymatic lysis by using lysozyme with sonication will result in a more efficient cell lysis and greater protein yield (Kielkopf et al. 2021).
- Hypothesis 2: PBLP will have greater binding affinity with larger ester-containing ligands, since substrate specificity depends on size among other factors.

Experimental Methods

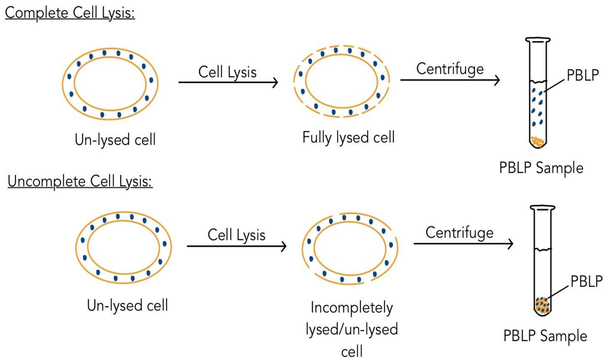


Figure 2. *Comparison of un-lysed E. coli cell versus lysed E. coli cell.* In Stage 1, we attempted to isolate PBLP from the E. coli cell. It was discovered through SDS-PAGE that the protein had been lost in the pellet. This may have been a result of incomplete cell lysis or the cell remained un-lysed. Due to this, a new procedure for isolation of PBLP from E. coli was developed in Stage 2.

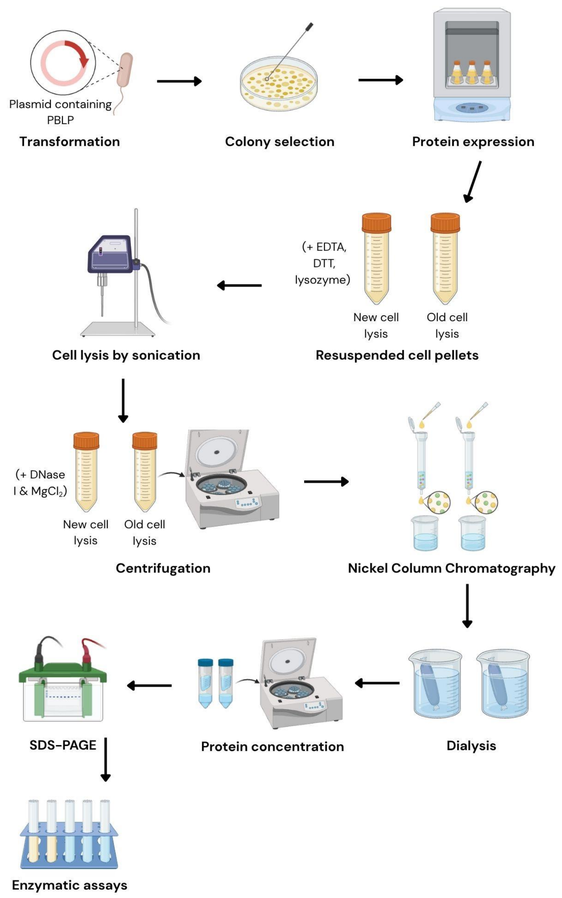


Figure 3. *Schematic of experimental methods workflow used throughout the experiment.* The cell lysis step was changed from Stage 1, which only had sonication. E. coli cells with truncated wild type PBLP had additions of EDTA, DTT, lysozyme, DNase I, and MgCl2 to aid in lysis for the new cell lysis sample. The original Stage 1 procedure was replicated in another sample for comparison. Schematic was created using BioRender illustration software.

Experimental Results

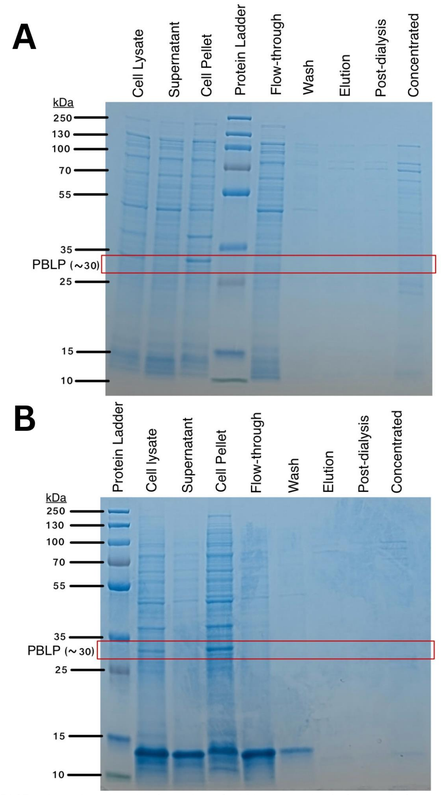


Figure 4. *SDS-PAGE polyacrylamide gels of new lysis & old lysis samples before and after purification.* (A) Old lysis procedure using sonication. PBLP is lost in the cell pellet and is not seen in the concentrated protein sample. (B) New lysis procedure with addition of lysozyme. PBLP is also lost in the cell pellet.

Computational Results

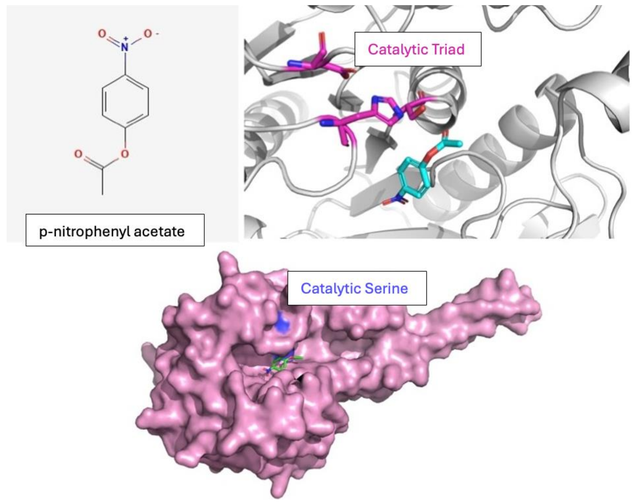


Figure 6. *PBLP wild type full length molecule with p-nitrophenyl acetate as the ligand substrate.* P-nitrophenyl acetate is a promising substrate for PBLP since it fits in the active site of the protein and is in proximity with the catalytic triad, where it can interact with the catalytic serine. Images were created using PyMOL-generated visualization along with AlphaFold3 structure predication of PBLP. The ligand was docked using SwissDock protein docking.

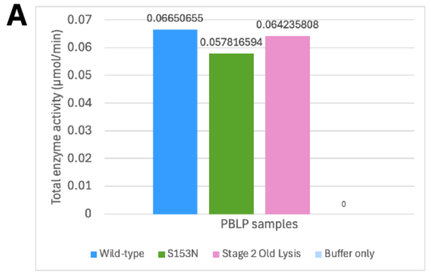


Figure 5. *Enzymatic assay activity results for PBLP with p-nitrophenyl acetate and p-nitrophenyl butyrate.* (A) PBLP activity on p-nitrophenyl acetate. (B) PBLP activity on p-nitrophenyl butyrate. In both enzyme assays, the enzymatic activity was observed to be similar among the wild type and mutant PBLP samples, suggesting issues with the viability of the construct used.

Discussion

Did combining sonication with enzymatic lysis by lysozyme improve cell lysis?

- PBLP remains lost in the cell pellet even with enzymatic assistance in cell lysis, suggesting that there may be issues with the sonication step.
- Low yield of protein expressed from the construct also may be a factor in the procedure failing.

What we conclude about PBLP specificity?

- Due to the failed purification of PBLP, the enzymatic results are inconclusive, and we cannot determine the substrate specificity of PBLP.
- Since the mutant and wild type samples had similar activity, the viability of the construct is called into question.

Future Directions

- Use new constructs for PBLP expression in E. coli to improve protein yield
- Identify new experimental techniques to aid in isolation of PBLP from the periplasmic space of E. coli
- Further purify PBLP to remove excess proteins remaining in the sample
- Run additional enzymatic assays with ester-containing ligands such as p-nitrophenyl benzoate

Acknowledgements

I would like to extend an earnest thank you to my mentor, Dr. Baughman, for her support and guidance throughout this experiment. I would also like to thank Dr. Groat-Carmona for her prior research on PBLP, which provided the extensive background material for my research. Furthermore, I am grateful to my lab partner Dia for collaborating with me on the experiment.

References

- Groat-Carmona AM, Kain H, Brownell J, Douglass AN, Aly ASI, Kappe SHI. 2015. A Plasmodium α/β hydrolase modulates the development of invasive stages. Cellular Microbiology 17(12):1848-1867. doi:10.1111/cmi.12477
- Kielkopf CL, Bauer W, Urbatsch IL. 2021. Preparation of cell extracts for purification of soluble proteins expressed in *E. coli*. Cold Spring Harb Protoc. 2021(2):[1 p.]. doi:10.1101/pdb.prot102718