Solubilization and Refolding of Plasmodium BEM-46-Like Protein (PBLP) from Inclusion Bodies

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TBIOMD 495

BACKGROUND

- PBLP is found in *Plasmodium yoelii* parasites that cause malaria (Groat-Carmona et al. 2015).
- Malaria treatment and cures are limited.
- Knockout of PBLP reduces Plasmodium infectivity (Groat-Carmona et al. 2015). PBLP can be a potential target for drug development.
- PBLP contains an α/β hydrolase domain and is predicted to possess lipid hydrolase activity.

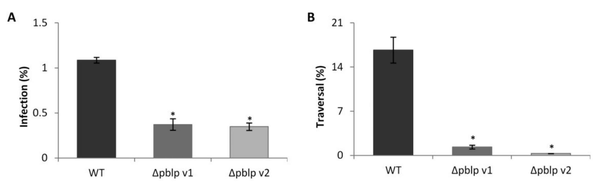

Fig. 1. Knockouts of PBLP showed a significant reduction in Plasmodium parasites' **(A)** infectivity and **(B)** traversal, suggesting PBLP as a promising drug target (Groat-Carmona et al. 2015).

COMPUTATIONAL RESULTS

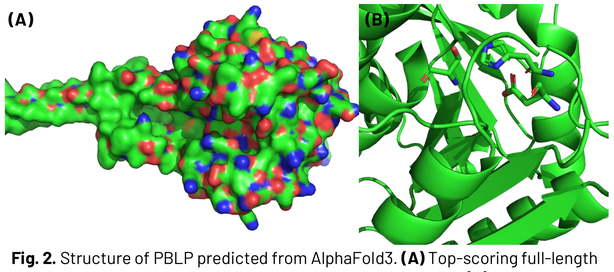

Fig. 2. Structure of PBLP predicted from AlphaFold3. **(A)** Top-scoring full-length PBLP structure surface, displaying the substrate binding site. **(B)** Active site of the full-length PBLP, showing the catalytic triad (Serine 153, Histidine 258, and Aspartate 229).

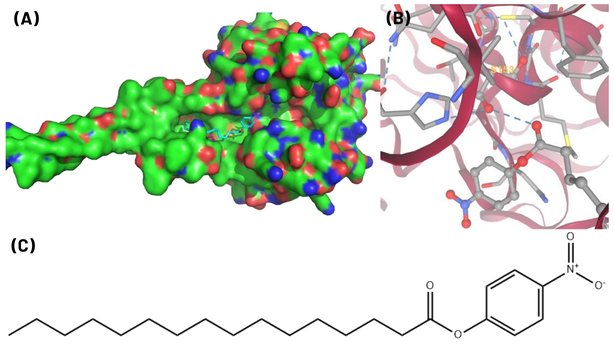

Fig. 3. Promising SwissDock prediction of the molecular interaction between the full-length PBLP and P-nitrophenyl Palmitate. **(A)** P-nitrophenyl Palmitate is perfectly in the binding pocket of the full-length PBLP. **(B)** The catalytic serine (S153) is close to and interacts with the Oxygen atom of the electrophile of P-nitrophenyl Palmitate. **(C)** Chemical structure of P-nitrophenyl Palmitate.

EXPERIMENTAL METHODS

STAGE 1: Initial PBLP expression, isolation, and purification → Unsuccessful (low yield of PBLP)

STAGE 2: Developed and tested a new protocol to solubilize PBLP from inclusion bodies and refold it.

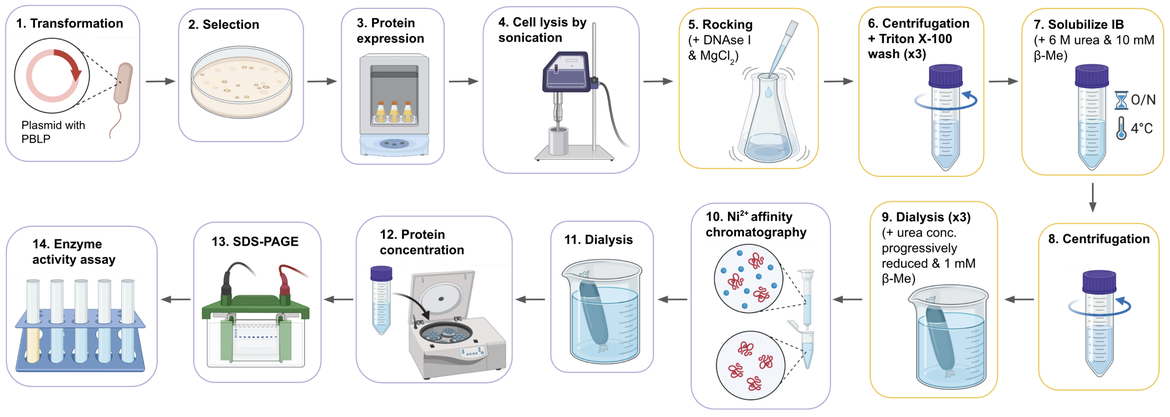

Fig. 4. Schematic diagram of Stage 2 workflow. The purple outlines indicate the same steps performed as in Stage 1. The gold outlines indicate the steps adapted from Torres-Paris et al. (2023) for unfolding and refolding PBLP to release it from inclusion bodies in Stage 2.

EXPERIMENTAL RESULTS

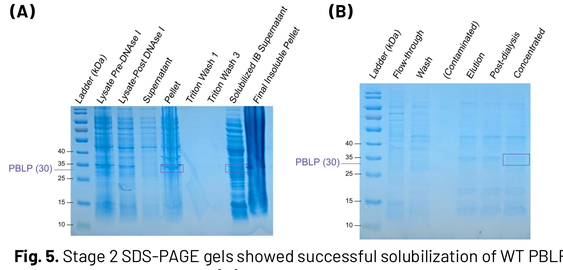

Fig. 5. Stage 2 SDS-PAGE gels showed successful solubilization of WT PBLP ΔTM from inclusion bodies. **(A)** PBLP was lost in the pellet, but only a small amount was successfully re-solubilized after a series of centrifugations and washes using urea. **(B)** A faint 30 kDa band indicates that a small amount of PBLP was resolubilized; additional bands present in the concentrated lane suggest partial purification.

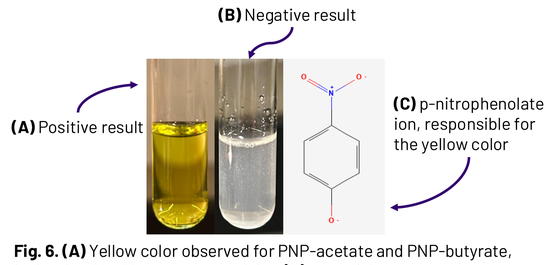

Fig. 6. (A) Yellow color observed for PNP-acetate and PNP-butyrate, indicating PBLP's hydrolase activity. **(B)** No activity was observed towards PNP-palmitate. **(C)** Chemical structure of the p-nitrophenolate ion, responsible for the yellow color seen in the hydrolase activity of PNP-acetate and PNP-butyrate.

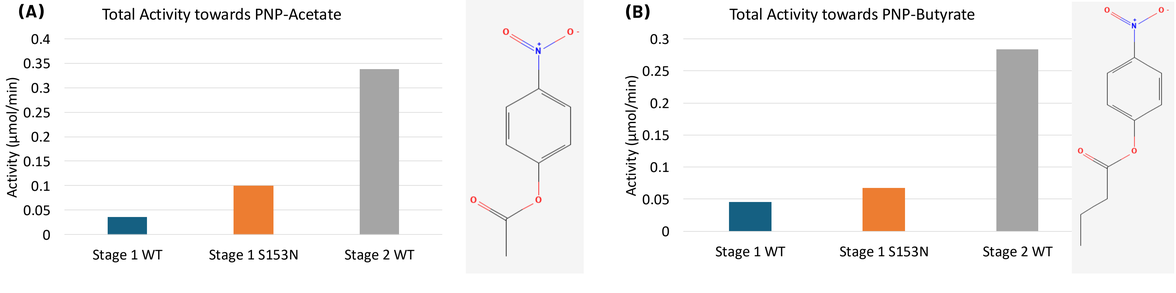

Fig. 7. (A) Left: Total activity towards PNP-acetate in Stage (ST) 1 WT, ST 1 S153N, and ST 2 WT. ST 1 S153N had higher enzyme activity than ST 1 WT. ST 2 WT had higher activity than ST 1 WT. Right: Chemical structure of PNP-acetate. **(B)** Left: Total activity towards PNP-butyrate in ST 1 WT, ST 1 S153N, and ST 2 WT. ST 1 S153N had higher enzyme activity than ST 1 WT. ST 2 WT had higher activity than ST 1 WT. Right: Chemical structure of PNP-butyrate.

DISCUSSION

- PBLP was successfully solubilized and recovered from the pellet.
- SDS-PAGE analysis supports that PBLP was localized in inclusion bodies.
- A relatively low recovery of PBLP from inclusion bodies implies incomplete solubilization.
- Purified WT PBLP from the pellet showed much higher activity towards PNP-acetate and PNP-butyrate than purified WT and S153N PBLP from the supernatant, supporting that PBLP possesses hydrolase activity.
- No activity towards PNP-palmitate suggests that PBLP might prefer shorter fatty acid chains.

FUTURE DIRECTIONS

- Redesign the PBLP expression plasmid to improve protein yield.
- Develop a more efficient protein extraction protocol.
- Additional short-to-medium-length p-nitrophenyl substrates can be tested via enzyme activity assays:
  - PNP-valerate (C5 fatty acid ester)
  - PNP-caproate (C6 fatty acid ester)

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