Towards the Expression of the Pheromone-Binding Protein from the Cockroach *Leucophaea maderae*

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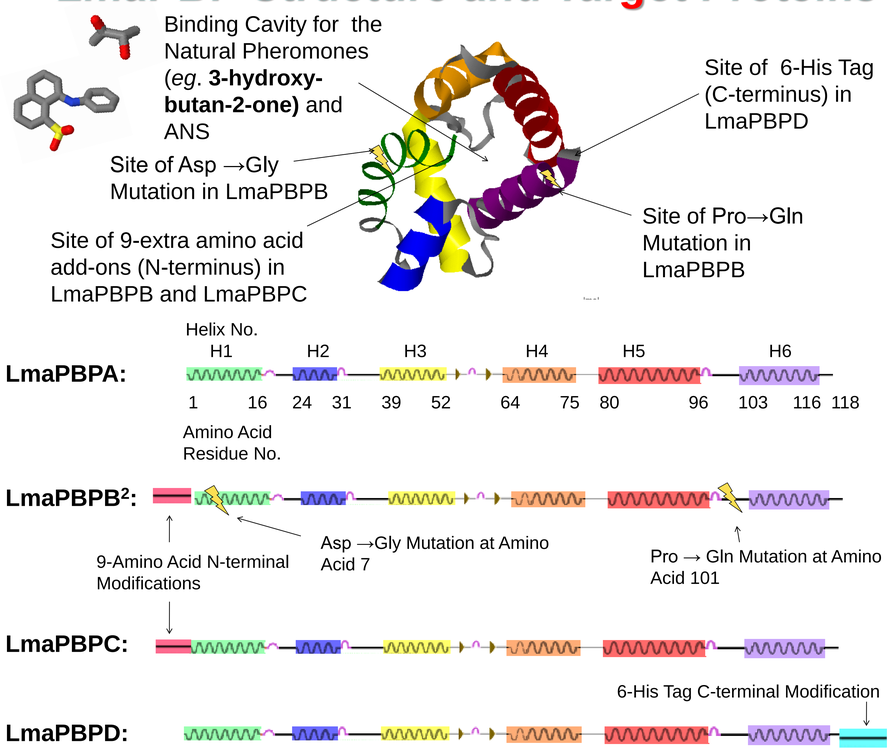
Abstract

The pheromone-binding protein from the cockroach *Leucophaea maderae* (LmaPBP) is believed to play a critical role in odor reception, although the details of its biochemical mechanism are unclear. To study this protein *in vitro*, we sought to overexpress LmaPBP in *E. coli.* To this end, four unique expression vectors carrying the LmaPBP gene were constructed, and evaluation of the ability of these vectors to allow for the overexpression of LmaPBP in *E. coli* were initiated. All four plasmid constructs encode a full-length LmaPBP, however, they differ in engineering at the N- and C-termini. One expression vector (pET22b+-LmaPBPA) encoding LmaPBP with minimal engineering at the N- and C-termini failed to express under the conditions examined. A construct that bears a nine-amino acid leader at the N-terminus was successfully expressed (pET22b+-LmaPBPB), however, mutations in the LmaPBP sequence resulted in expression of mutant protein products. To obtain the wildtype protein sequence, two additional expression vectors were prepared. The first contained N-terminal modifications identical to pET22b+-LmaPBPB (pET22b+-LmaPBPC) and the second with a C-terminal 6-His tag (pET22b+-LmaPBPD). Expression studies of these final constructs are ongoing.

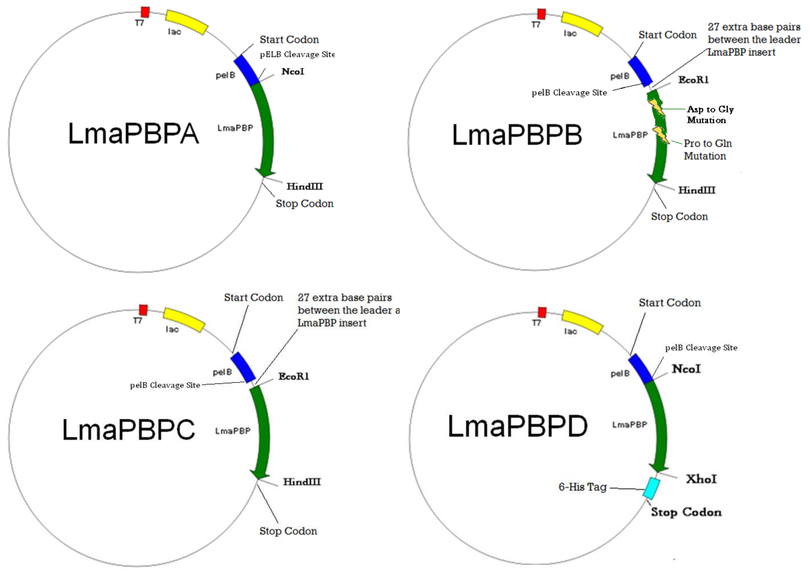
Background

Insects rely on odors to find mates, identify enemies, and communicate. Odor-binding proteins are believed to play a critical role in odor reception.[1] The pheromone-binding protein from the cockroach *Leucophaea maderae* (*Lma*PBP) represents a subclass of odor-binding proteins that specifically binds pheromones. The biochemical role of LmaPBP is uncertain; however, it may play a role in the specificity of pheromone detection, transfer of odors from the air to the pheromone receptor on the dendrite, and signal attenuation at high odor concentrations. The goal of this study is to overexpress, isolate, and purify LmaPBP in order to characterize its binding properties with natural and unnatural ligands.

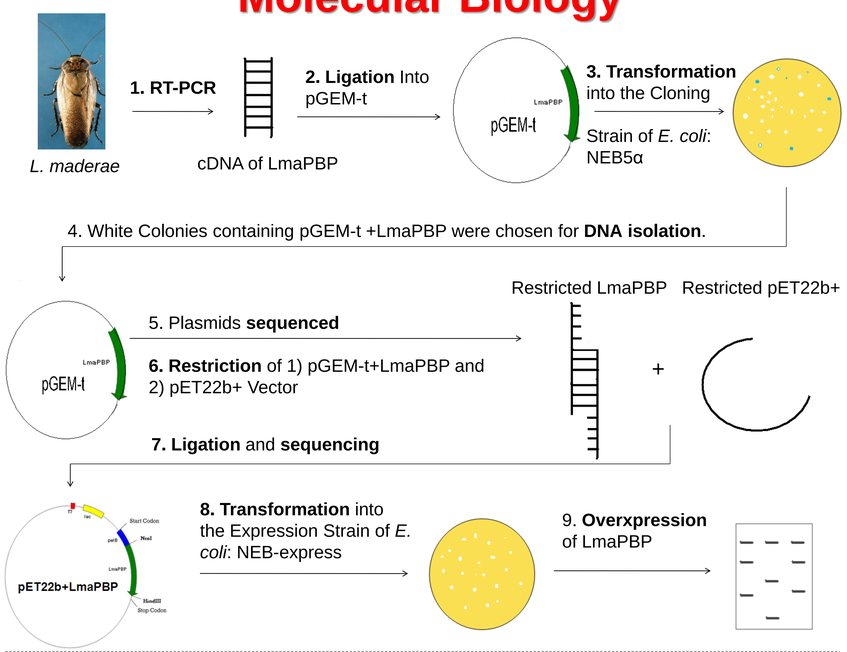
LmaPBP Structure and Target Proteins



Vector Design



Molecular Biology



Results:

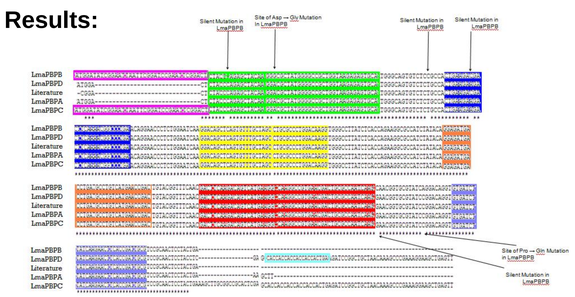


Fig. 1. Sequence Alignment of LmaPBPB, LmaPBPD, Literature, LmaPBPA, and LmaPBPC. Colors indicate LmaPBP helices and/or N- and C-terminal modifications.

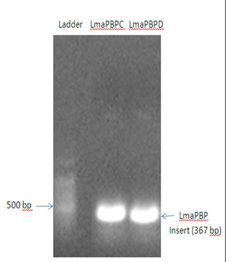
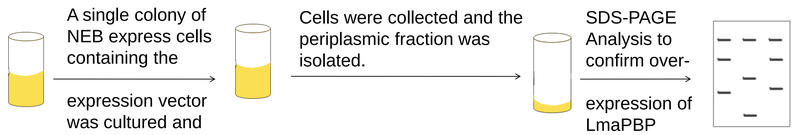


Fig. 2. Agarose gel of PCR-amplified inserts of LmaPBPC and LmaPBPD. Size of insert is approximately 367 bp.

Expression



Results:

❖The mutated LmaPBPB was overexpressed in the periplasm (Fig. 3).

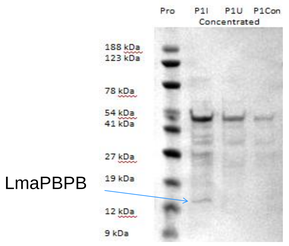


Fig. 3. SDS-PAGE gel of the first periplasmic fractions (I: induced, U: uninduced, Con: control, Pro: Prosieve ladder) of the mutated *Lma*PBP. Overexpression of the mutated protein is suggested by the band at ~13 kDa.

❖The wild-type LmaPBPA may have been overexpressed in the periplasm (Fig. 4), but this result was not reproducible. Purification of the second periplasmic fraction via anion exchange was unsuccessful (Fig. 5).

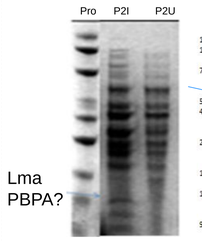


Fig. 4. SDS-PAGE gel of the second periplasmic fractions of wild-type protein. Overexpression may be suggested by the band at 13kDa.

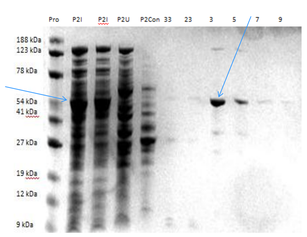


Fig. 5. SDS-PAGE gel of the second periplasmic fractions and anion exchange chromatography of the fractions. It seems that the only protein overexpressed had a mass of about 54 kDa.

Ongoing and Future Research

While the mutated *Lma*PBPB was overexpressed, the wild-type protein LmaPBPA was not. Therefore, modified plasmids (LmaPBPC and LmaPBPD) were constructed, and the ability of these vectors to express the protein are ongoing.

❖Once LmaPBP is obtained, we will perform steady state binding assays and kinetic studies with ANS, dansyl amide, and natural ligands to better understand the role of PBPs in olfactory signaling.[3]

❖We will obtain and compare the binding constants of LmaPBP for natural and unnatural ligands. We will also be able to determine if the protein acts under thermodynamic and/or kinetic control.

Acknowledgements

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References

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2. Thurber, A. E., and Hofferberth, J. (2007, Honors thesis) Investigating odorant signal attenuation in the cockroach *Leucophaea maderae*.

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