2014-08

2014 REU Poster: Expression and Characterization of WT BMUL4434 from Burkholderia multivorans

Expression and Characterization of WT BMUL4434 from *Burkholderia Multivorans*

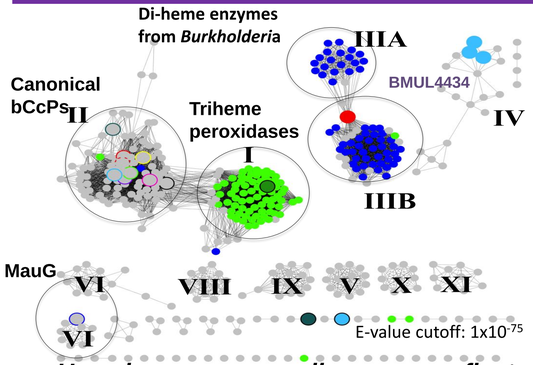
Santina Cierra Johnson, Kimberly Rizzolo, and Sean Elliott

Department of Chemistry, Boston University, Boston, MA

Abstract

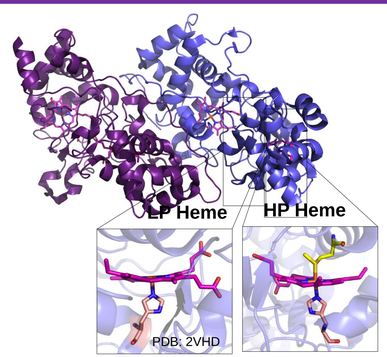
Bacterial cytochrome *c* peroxidases (bCcPs) are di-heme enzymes that protect the cell from peroxide by reducing it to water. Alternatively, MauG is a di-heme enzyme that is similar in structure to bCcPs, yet is a poor peroxidase. Instead, MauG catalyzes the formation of tryptophan tryptophyl quinone (TTQ) the catalytic cofactor required for MADH. Previous work in the Elliott lab used bioinformatics to investigate how sequence divergence within peroxidase superfamily reflects functionality. The bioinformatics analysis led to the discovery of unreported di-heme enzymes found in all strains of *Burkholderia.* BMUL4434 from *Burkholderia multivorans* was gene synthesized and codon optimized for expression in *E. coli.* We expressed and purified WT BMUL4434 and obtained initial optical characterization to understand where it falls in the peroxidase superfamily.

BMUL 4434 from *Burkholderia Multivorans*



How does sequence divergence reflect functionality? (1)

Bacterial Cytochrome c Peroxidase

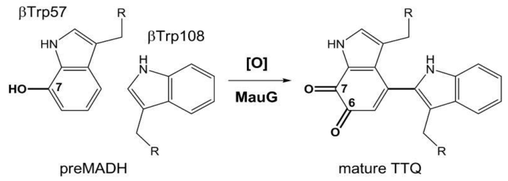


$$2Cc_{(reduced)} + H_2O_2 + 2H^+ \longrightarrow 2Cc_{(oxidized)} + 2H_2O$$

Bacterial CcPs contain one low potential heme that is the site of peroxide binding and a high potential heme known as the electron transfer heme (2).

MauG from *Paracoccus denitificans*

MauG is a di-heme enzyme that catalyzes formation of TTQ (catalytic cofactor required for MADH. MauG reacts with peroxide to form a *bis*-Fe(IV) intermediate where both hemes are oxidized to Fe(IV).MauG can bind O_2, NO, and CO at the active site heme, a property unusual for c-type hemes (3, 4).



Di-heme center of MauG

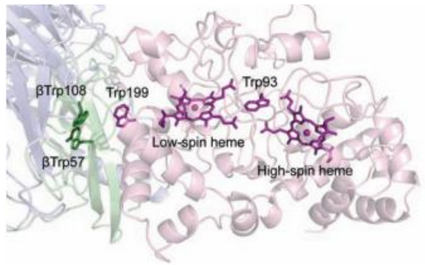


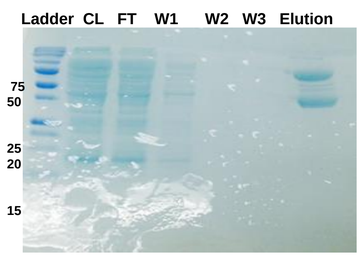
Figure adapted from Wilmot *et al*.

Purification of WT BMUL

WT BMUL was purified in a two-step purification protocol. The first step was to purify a maltose-binding protein fusion version of BMUL using Amylose resin:

Affinity Chromatography

- Lyse cells to get protein into solution
- MBP:BMUL binds to the amylose resin
- Fusion protein is eluted with a maltose buffer which binds to MBP:BMUL to remove from resin.
- TEV protease is added to cleave MBP from BMUL

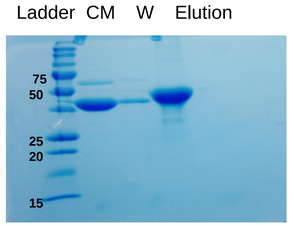


CL – clarified lysate
FT – Flow through
W1 – wash 1
W2 – Wash 2
W3- Wash 3
Elution – Eluted MBP-BMUL protein

Purification of WT BMUL 4434

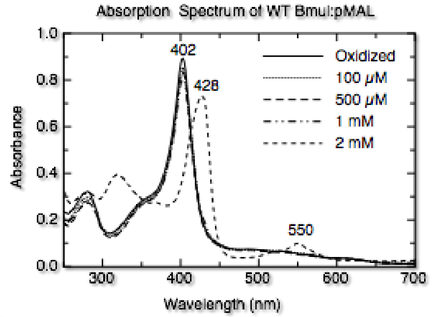
Ion-exchange Chromatography

- Cleaved protein is loaded on High S column
- Positively charge BMUL binds to the negatively charged resin
- The column is equilibrated with a low salt concentrated buffer
- BMUL is eluted off resin with a high salt concentrated buffer



FT – cleavage mixture (MBP-BMUL ~90kDa and free MBP ~42 kDa)
W – wash
Elution – Eluted cleaved BMUL (~50 kDa)

Optical Characterization

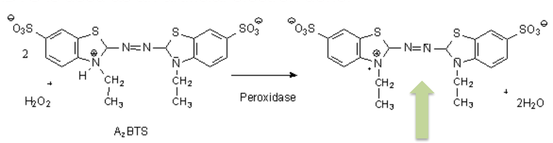


BMUL Spectra:

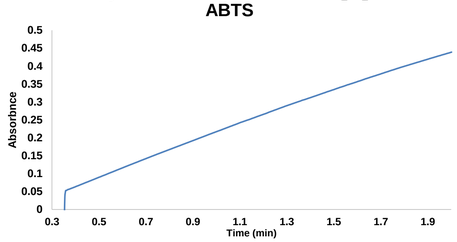
- Soret shifts 26 nm
- Alpha/beta bands show single broad feature

Activity of WT BMUL

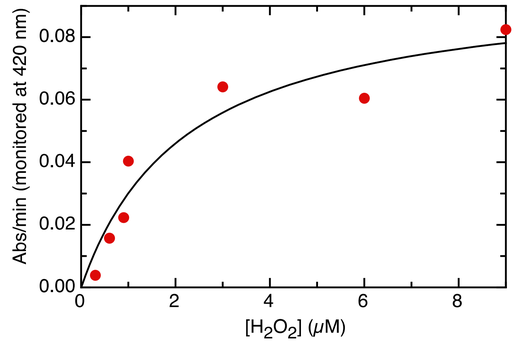
ABTS is used as an artificial electron donor



Activity of BMUL in presence of H_2O_2 and ABTS



BMUL 4434 Activity



Michaelis-Menten parameters:

$k_{cat}/K_M = 3.8x10^4\ M^{-1}\ s^{-1}$

Activity data indicates BMUL can turnover peroxide, suggesting in may have a role in peroxide removal.

Conclusion and Future Experiments

BMUL was purified in an oxidized state. Reduction of the heme centers generates broad feature at 550 nm. Activity with ABTS shows BMUL is a fairly good peroxidase.

How to further characterize BMUL as bCcP-like or MauG-like?

If similar to MauG: Ligand studies with BMUL binding to CO and NO. See if bis-Fe(IV) intermediate is formed when BMUL reacts with peroxide. **If similar to bCcPs:** Determine reduction potentials of the HP heme and LP heme

Acknowledgements

This work was made possible by funding from the National Science Foundation. Thanks is also extended to Boston University and the Elliott Research Group.

References

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(3) Bradley, *et al J. Biol. Chem* (2004) 279, 13297
(4) Wilmot *et.al. Science* (2010) 327(5971)