E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester 1824 · Biotechnology and Biological Sciences Research Council

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University of Huddersfield Inspiring global professionals

Oxford Biotrans

Switching the iron in cytochromes P450 to expand their catalytic repertoire

"Given the high-risk nature of iron-centre modification and the difficulty in achieve successful protein folding, Oxford Biotrans would have been unable to pursue this research without the partnership this funding enabled."

PROJECT AIMS: Cytochrome P450 enzymes are increasingly used in biomanufacturing to add oxygen atoms to diverse molecules. One P450, BM3 (CYP102A1), is particularly attractive due to its high efficiency. The crux of BM3's reactivity is its heme cofactor that contains an iron centre. Here we propose to make novel versions of BM3 with different metal centres, starting with cobalt, to expand upon the enzyme's capabilities and to generate new molecules of commercial interest.

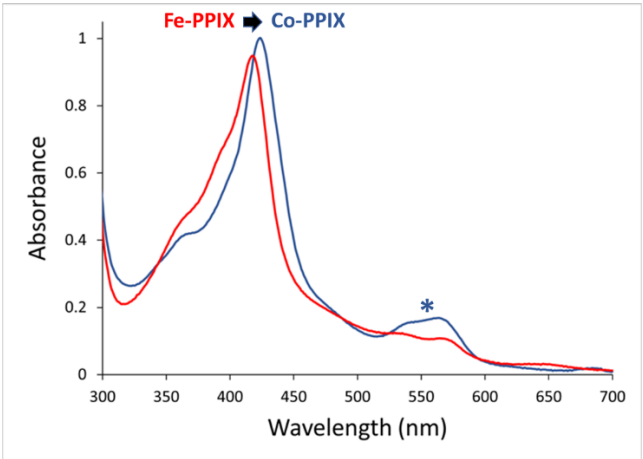
OUTCOMES & NEXT STEPS:

- A placement student is expanding these studies, characterising the enzymatic products of the Co-PPIX proteins with diverse substrates and develop the generation of new metal containing BM3 variants.
- We are exploring collaborative opportunities and hope to recruit a PhD student to further investigate enzyme engineering combined with metal-ion substitution.
- We have a ICP bid in preparation to the BBSRC.
- This work will be disseminated with a poster presentation at the tetrapyrrole discussion group meeting in April 2023.
- An outreach activity was carried out with year 6 pupils in Luke Lane Junior School, Huddersfield

RESULTS:
Overall, we generated variants of P450 BM3 with the iron centre of the heme cofactor replaced with cobalt. Overexpression of BM3 in iron-free minimal media containing cobalt under controlled growth conditions enabled us to produce a variant containing cobalt protoporphyrin IX (PPIX). The protein was purified, and ICP-MS confirmed we had made Co-PPIX BM3. In conjunction with ICP-MS, pyridine hemochromagen analysis of the PPIX content and protein-content assays assay confirmed near-complete Co-PPIX incorporation at stoichiometric levels.
UV-visible spectroscopic analysis showed that the typical porphyrin spectral signal for wild-type BM3 (Fe-PPIX) peaked at 418nm, while the signal was shifted for Co-PPIX, with a peak at 422 nm and a dominant visible feature at 547 nm (see figure). This oxidised form could be reduced with sodium dithionite to a species with a peak at 407 nm with a reduction in the extinction coefficient. These spectral differences confirm a novelty in the PPIX cofactor.

We then expaned our study to include the BM3 A82F/F87V double mutant (DM), which has an open active site and accepts many more substrates than wild-type BM3, which predominantly hydroxylates fatty acid. The Co-PPIX DM had better overexpression yields than wild-type Co-PPIX.Initial enzyme assays with the Co-PPIX DM variant demonstrated consumption of NADPH in the presence of a fatty acid substrate, indicating that the enzymes were active. Turnover studies looking for oxygenated products by GC-MS are ongoing.

Change in technology readiness level: 1 to 2



A cobalt-containing protoporphyrin IX variant of P450 BM3 was produced in this study. The red line shows the UV-visible spectrum of the iron-containing form, and the blue lines shows the cobalt-containing form. The visible region is indicated by *