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Manipulation of redox partner protein expression levels in cytochrome P450 systems

AUTHORS

Kinga NYTKO / UCL & HYPHA DISCOVERY, GOWER STREET, LONDON
John WARD / UCL, GOWER STREET, LONDON
Jack JEFFRIES / UCL, GOWER STREET, LONDON
Vincent POON / HYPHA DISCOVERY, 154B BROOK DR, ABINGDON

PURPOSE OF THE ABSTRACT

Cytochrome P450 monooxygenases (P450s) are one of the most versatile biological catalysts involved in the oxidative transformation of endogenous and exogenous substrates. Mammalian P450s play a major role in the metabolism of xenobiotic compounds and bacterial P450 systems can mimic mammalian biotransformations and convert the drugs to the same metabolites. Microbial P450s are also objects of interest in biotechnology due to their extreme biodiversity and numbers.

Hypha Discovery's PolyCYPs® metabolite screening kit mainly contains recombinant microbial Class I P450 enzymes mined from talented filamentous bacteria called actinomycetes. PolyCYPs® enzymes can be used for the generation of P450s-derived metabolites of drugs, agrochemicals and other small molecules. The Class I microbial P450 systems contain three enzymes: a P450 and two redox partners – ferredoxin and ferredoxin reductase. The redox partners are essential for electron transfer from NAD(P)H to P450's heme cofactor. However, the selection of suitable redox partners (native or surrogate) and their amount are known to be a bottleneck of Class I P450 activity and their usage in biotechnology.

This poster describes efforts to enhance the activity of the 3-component system through modification of the expression level of the three enzymes. In this study, surrogate redox partners from Pseudomonas putida and PolyCYP-6 were used; these redox partners are co-expressed together with P450 within a single polycistronic operon under a T7 promoter. Six plasmids with different modifications within the operon, such as altering gene orders and changing RBS sequences, had a high impact on the production level of proteins and biotransformation activity. Systems with higher amounts of redox partners showed improved biotransformation activity against selected drug compounds. However, the molar ratio between ferredoxin and ferredoxin reductase also plays an important role in biotransformation activity.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

P450 | redox partners | oxidation | polycistronic operon

BIBLIOGRAPHY