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make history.

Bespoke biocatalysts for selective hydroxylation reactions

The development of new biocatalysts to produce high-value fine chemicals

Benefits

New routes for highly selective chemical oxidations using mild conditions to generate desirable high-value fine chemicals or to activate recalcitrant chemicals for easier removal from the environment. Selected examples include:

- The selective oxidation of steroids to generate chemically inaccessible hydroxylated compounds as intermediated for the synthesis of medicinally active compounds (Figure 1).
- The selective oxidation of natural products to generate sought after high-value flavours, fragrances and biologically active compounds.
- The facile oxidation of drug candidates to mimic the generation of drug metabolites that would be formed in humans/animals enabling their isolation. This is essential for the assessment of their toxicity and pharmaceutical properties during the approval process.
- The oxidation of recalcitrant chemicals such as polyaromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) and other toxic chemicals (drug molecules, alkane and steroids) to facilitate their removal from the environment.

Background

We have developed of a new set of proteins known as 'biocatalysts' with altered function that catalyze hydrogen peroxide-driven and selective oxidation reactions. These are challenging reactions which are often not possible using chemical processes as they require the input of a high amount of energy. The biocatalysts we have developed can function under mild conditions. They also overcome several significant barriers associated with using these proteins by removing the requirement for expensive nicotinamide cofactors and additional electron transfer partners. Several of the new biocatalysts developed in this project are obtained from microorganisms which are found in extreme environments (Figure 2). This enables them to function at high temperatures or to be stored without cryoprotection which is crucial to their optimisation for larger scale production. This enables a simple, cheap, clean, and robust method by which to undertake this challenging reaction to generate bespoke chemicals.

The technology can be applied to a wide range of proteins from the same protein superfamily (cytochrome P450 enzymes) with different functions. This will enable the synthesis of diverse range new chemicals such as flavour and fragrance compounds, drug metabolites and complex natural products with medicinal properties.

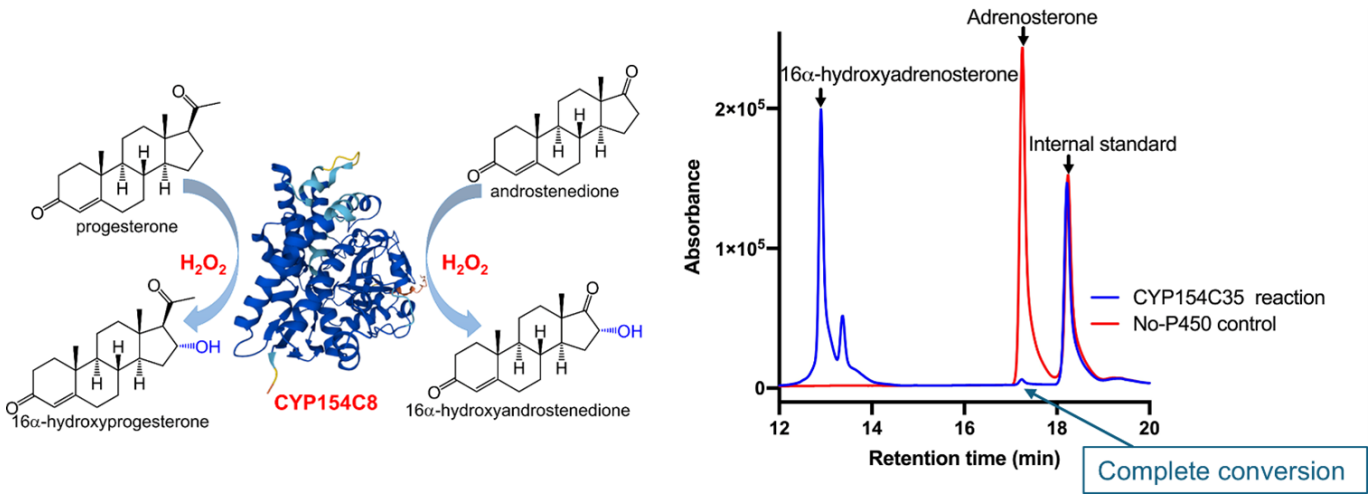

Figure 1. The stereoselective oxidation of steroids (progesterone, androstendione and andrenosterone) by CYP154 enzymes from different bacteria to generate valuable hydroxylated steroid metabolites.

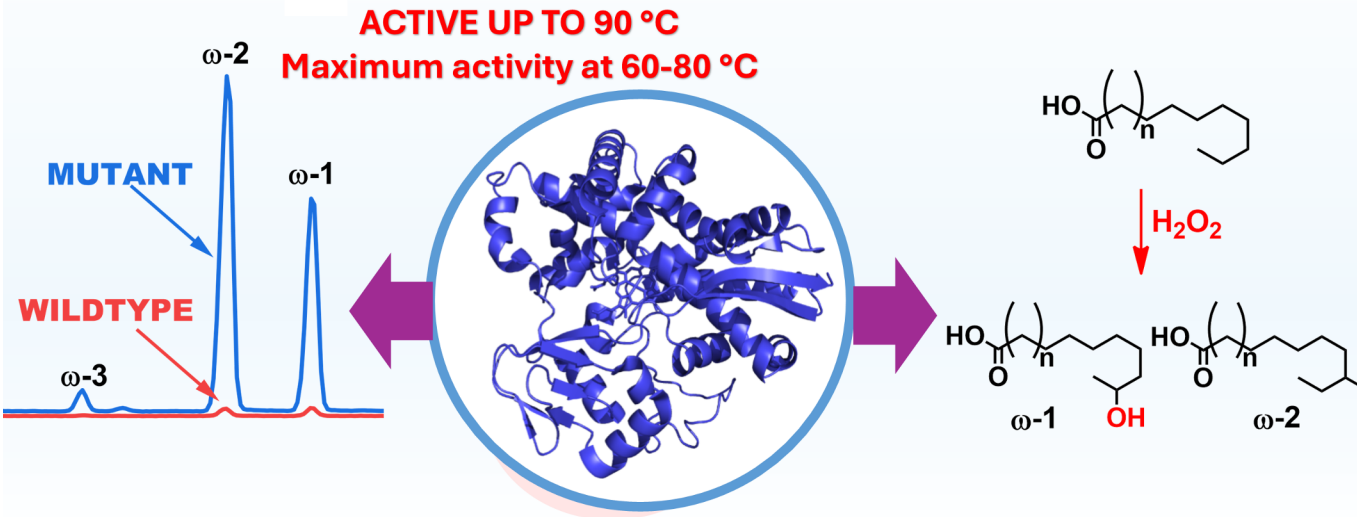

Figure 2. The identification and engineering of a thermophilic peroxygenase enzyme from a hot spring for lipid oxidation at elevated temperatures.

Technology

We have already developed biocatalysts for the selective oxidation of steroids, fatty acids (lipids), aromatic compounds, norisoprenoid and terpenoid natural products to generate chemicals of interest.

We have developed methods to use these biocatalysts with hydrogen peroxide using clarified cell lysates or whole-cells without the requirement for protein purification to facilitate larger scale applications and the development of high-throughput methods for biocatalyst optimisation.

The next steps are to extend the technology to other proteins to develop new biocatalysts with specific industrial applications. These include the generation of flavour and fragrance compounds, natural product based medicinal compounds, drug metabolites and the oxidation of recalcitrant and toxic compounds.

Stage

We have demonstrated that the biocatalysts work with purified protein. We have also developed new methods to expand the biocatalytic function into in bacterial whole cells (*E. coli*) and unpurified cell lysates simplifying biocatalyst production. We have optimised this for several biocatalyst/substrate combinations and are in the process of scaling up the biocatalyst production and reactions. We are seeking to extend the method to new biocatalyst/substrate combinations to target products molecules of industrial/commercial interest.

Applications

- Synthesis of hydroxylated steroids, diterpenoids and vitamins with biological function/importance.
- Hydroxylation of terpenes, sesquiterpenes and norisoprenoids for the generation of flavour and fragrance compounds.
- Synthesis of drug metabolites for toxicity testing/trials.
- The oxidation of lignin derived aromatics and polyaromatic hydrocarbons for bioremediation/valorization.
- The ability to design bespoke biocatalysts for selective hydroxylation reactions on a wide range of molecules on request.

Opportunity

We are seeking development partners and investment in our efforts to scale-up the process (biocatalyst production and activity) and to identify new compounds and biocatalysts to target.

IP status

Provisional patent PCT/AU2024/050934 | Mutant Cytochrome P450 enzymes with enhanced peroxygenase activity and/or altered product selectivity.

Relevant Publications

Das, T., Hayball, E. F., Harlington, A. C., & Bell, S. G. (2024). A Thermostable Heme Protein Fold Adapted for Stereoselective C-H Bond Hydroxylation Using Peroxygenase Activity. ChemBioChem, 26(2), e202400737-1-e202400737-9.

Podgorski, M. N., Akter, J., Churchman, L. R., Bruning, J. B., De Voss, J. J., & Bell, S. G. (2024). Engineering Peroxygenase Activity into Cytochrome P450 Monooxygenases through Modification of the Oxygen Binding Region. ACS Catalysis, 14(10), 7426-7443.

Gee, A. R., Stone, I., Stockdale, T. P., Pukala, T. L., De Voss, J. J., & Bell, S. G. (2023). Efficient biocatalytic C-H bond oxidation: an engineered heme-thiolate peroxygenase from a thermostable cytochrome P450. Chemical Communications, 59(90), 13486-13489.

Inventors

Associate Professor Stephen Graham Bell and collaborators at the University of Adelaide.
e: stephen.bell@adelaide.edu.au

Commercial Contact

Aisha Sirop, Commercial Manager
Innovation and Commercialisation Services
The University of Adelaide SA 5005 Australia
e: aisha.sirop@adelaide.edu.au
p: +61 8 8313 1336
w: www.adelaide.edu.au/ics