Novel and more robust fungal peroxidases as industrial biocatalysts

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OBJECTIVES

1. Search for peroxidases with novel properties of interest (such as self-sufficient monooxygenation and oxidation of chemically-inaccessible compounds) by screening microbial cultures and the exponentially-increasing genomic resources.

2. Structural-functional characterization of selected peroxidases to understand the bases of their catalytic properties, and to engineer them by a rational design based on the above information using site-directed mutagenesis and other techniques.

3. To perform directed evolution, and related tools, as a non-rational alternative with the aim of solving some of the drawbacks that cannot be rationally addressed because a direct structure-function relationship cannot be established.

4. To optimize strategies for the production of peroxidases adapted to the needs of gene screening and rational or non-rational design, as well as the use of industrial hosts for large-scale production of the selected enzyme candidates.

5. Detailed chemical analysis of reaction products from the new peroxidases to evaluate their industrial interest in biodegradation and oxyfunctionalization reactions using both aromatic and lipophilic substrates.
Enzymes catalyzing redox reactions (oxidoreductases) represent an environmentally friendly alternative to harsh chemicals in industrial processes that include oxidative transformations for production of chemicals and other value-added products with large markets in developed and emerging economies.
Fungi and other microorganisms provide the wider and more easily exploitable source for oxidative enzymes.

However, the penetration of microbial oxidoreductases in the chemical markets is still low despite the recent discovery of very promising enzymes.

The use of these enzymes as biocatalysts requires tuning their catalytic and operational properties (a type of manipulation that is possible nowadays using protein engineering tools).

In addition to microbial screening, the huge amount of genomic resources available nowadays, and to be generated during the course of the project, will be exploited in the search for new fungal peroxidase/peroxygenases.
Some of the main issues presently limiting the industrial application of peroxidases will be addressed, such as:

- Their suicide \textit{inactivation} by H2O2
- Low functional \textit{expression}
- Limited \textit{oxygen transfer} potential

Moreover, the \textit{catalytic properties} of the most interesting enzymes will be \textit{modulated} to adapt them to the industrial processes.

A combination of \textit{rational} and \textit{non-rational} design will be used, based on directed mutagenesis, and random mutagenesis (together with high-throughput screening), respectively.

The reaction mechanisms and industrial interest of the selected peroxidases/peroxygenases will be studied using \textit{modern analytical techniques}, including two-dimensional NMR, that are able to provide information on the modifications produced on both simple and complex substrates.

In this way novel and robust peroxidases/peroxygenases will be obtained with high potential both in \textit{bulk chemistry}, e.g. for hydrocarbon \textit{oxyfunctionalization} and \textit{oxidation} of recalcitrant compounds, and \textit{fine chemistry}, e.g. substituting costly hydroxylation reactions in the pharmaceutical sector.
Peroxidases as biocatalysts

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More information: www.peroxicats.org