BIOTRANS
LA ROCHELLE 2023

N°663 / OC / PC
TOPIC(s) : Enzyme discovery and engineering

Oxidation of indole derivatives by engineered fungal peroxygenases

AUTHORS
Alejandro BELTRAN-NOGAL / INSTITUTE OF CATALYSIS, CSIC, MARIE CURIE 2, MADRID
Ivan MATELJAK / EVOENZYME, FARADAY 7, MADRID
Patricia GOMEZ DE SANTOS / EVOENZYME, FARADAY 7, MADRID
David GONZALEZ-PEREZ / INSTITUTE OF CATALYSIS, CSIC, MARIE CURIE 2, MADRID
Corresponding author : Miguel ALCALDE / malcalde@icp.csic.es

PURPOSE OF THE ABSTRACT
Indigo blue is one of the oldest dyes used throughout Human History since early times (as far as 6,000 years ago) [1, 2]. A precursor of indigo is indole, an aromatic heterocycle constituted by a benzene ring bonded to an imidazole moiety, that is widely present in biomolecules. Indole can be oxidized to indoxyl (3-oxindole) and then undergo spontaneous oxidation towards the formation of indigo (Figure 1) [3]. Traditionally, this reaction has been achieved by fermentation of indican-producing plants – a protected O-glycosylated version of indoxyl – and later by fossil-based processes relying on aniline and hazardous chemicals [1].

The biotechnological synthesis of indigo is an alternative to the chemical processes that depart from unrenewable resources. These processes constitute microbial fermentations and enzymatic reactions [1]. Among the enzymes that produce indigo are naphthalene dioxygenases, multicompetent phenol hydroxylases, P450s, and flavin-dependent monooxygenases. However, most of these depend on expensive cofactors and/or electron transport chains that can hamper their industrial implementation. On the contrary, fungal unspecific peroxygenases (UPOs) are stable biocatalysts that simply require hydrogen peroxide to bring about selective oxygen-insertion reactions in unactivated C-H bonds, including indole and indole-derivative compounds [3, 4].

Here we present a directed evolution campaign focused on obtaining UPO variants with high activity to produce indigo and halogenated indigo-derivatives. The UPO from Daldinia sp. EC12 (DspUPO) was subjected to iterative rounds of mutagenesis and selection in order to increase its secretion levels in the heterologous host Saccharomyces cerevisiae. Upon increasing DspUPO functional expression, a study of the amino acids lining the active site channel was carried out. Five residues were selected to be targets of combinatorial saturation mutagenesis and codon degeneracy with NDTs codons in independent libraries that were evaluated by their ability to produce indigo, 5,5'-dicloroindigo and 6,6'-dibromoindigo (also known as Tyrian purple or Royal purple). The best mutant hits were isolated and biochemically characterized aiming to assess the potential of these enzyme candidates for the synthesis of different indigo derivatives.

FIGURES

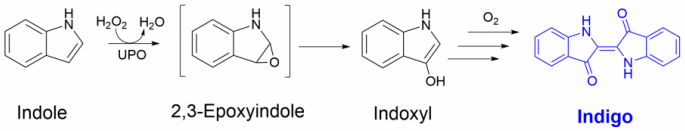

FIGURE 1

Oxidation of indole.

Proposed reaction of the oxidation of indole to indigo by UPOs [3].

FIGURE 2

KEYWORDS

directed evolution | UPO | indigo | combinatorial saturation mutagenesis

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