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Engineering of a bacterial cytochrome P450 monooxygenase for the synthesis of (-)-podophyllotoxin

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PURPOSE OF THE ABSTRACT

The lignan (-)-podophyllotoxin is a natural plant precursor for the synthesis of the chemotherapeutics teniposide and etoposide, but its limited supply is dependent on the endangered plant Podophyllum hexandrum [1]. Therefore, the biosynthetic pathway towards (-)- podophyllotoxin was reconstituted in Escherichia coli [1]. An exception still is the unidentified enzyme catalyzing the last step of this cascade, the hydroxylation of ( ) deoxypodophyllotoxin to (-)-podophyllotoxin [1]. To overcome this boundary a cytochrome P450 monooxygenase (P450) was identified to catalyze the hydroxylation of (-)-deoxypodophyllotoxin to (-)-podophyllotoxin [2]. Still, the chemoselectivity is quite low and the reaction yields several products besides (-)-podophyllotoxin [2].

P450s are well described for their ability to introduce oxygen into non-activated C-H bonds of a vast range of chemically diverse compounds in the presence of molecular oxygen [3]. Their ability to catalyze regio- and stereoselective oxidations of complex molecules under mild conditions outcompetes many chemical catalysts [4] and makes them the right candidate to selectively hydroxylate complex compounds like (-)-deoxypodophyllotoxin. To achieve this reaction the two required electrons for the activation of molecular oxygen are delivered by NAD(P)H via redox partner proteins [4]. To extend activity or chemoselectivity of P450s, many enzymes were characterized over the last decades and optimized for specific applications using rational design and directed evolution methods [4].

In this work, we aimed to improve activity and regio- and stereoselectivity of a bacterial P450 towards the production of (-)-podophyllotoxin by means of rational protein design and saturation mutagenesis. Therefore, 12 residues in the active site identified from literature and docking studies were chosen for site-directed mutagenesis. The resulting mutants where analyzed in an implemented medium-throughput screening based on the whole cell conversion in E. coli in 96 deepwell plates and a LC/MS high-throughput screening. An identified mutant may allow to extend the enzyme cascade for the selective production of (-)-podophyllotoxin in E. coli.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

P450 | protein engineering | (-)-podophyllotoxin

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