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Anti-Markovnikov oxidation of unactivated, aliphatic alkenes by directed enzyme evolution

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PURPOSE OF THE ABSTRACT

The direct aerobic oxidation of alkenes to carbonyls is an important, yet, in part very challenging transformation in synthesis.[1] Our research group has recently started to engineer cytochrome P450 enzymes for direct alkene to carbonyl oxidations. This includes enzymes that perform anti Markovnikov alkene oxidation of styrenes to generate the corresponding aldehydes,[2] as well as regioselective oxidation of internal aryl alkenes to the corresponding ketones.[3] The engineered enzymes fully exploit a catalytic cycle that has largely eluded small molecule catalysis. This is achieved by conformational control over a key radical intermediate to prevent the dynamically favored alkene epoxidation.[4,5] Here we expand this chemistry towards unactivated aliphatic alkenes through directed enzyme evolution.[6] The initially low activity and selectivity for the aliphatic 1-alkene was improved over 14 rounds of directed evolution, introducing 21 beneficial mutations. The final catalyst was characterized by exploring the substrate scope and its applicability in synthesis as well as determining kinetic parameters and solving the crystal structure.

FIGURES

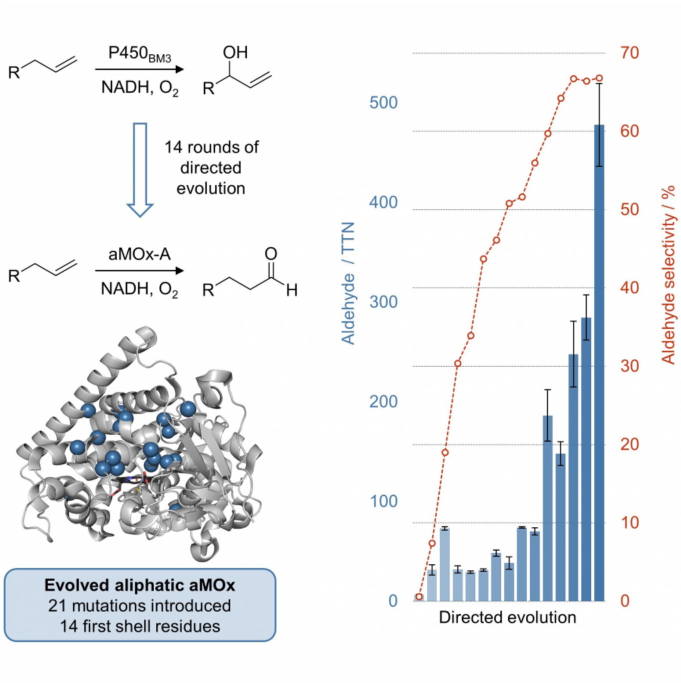

FIGURE 1

Directed evolution of an anti-Markovnikov oxidase for unactivated, aliphatic alkenes.

Starting from P450BM3 which converts aliphatic 1-alkenes into the corresponding allylic alcohol an aldehyde selective anti-Markovnikov oxygenase (aMOx-A) was obtained in 14 rounds of directed evolution.

FIGURE 2

KEYWORDS

Enzyme engineering | Biocatalysis | Directed evolution | Cytochrome P450

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