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Catalytically self-sufficient CYP116B5: domain switch for improved peroxygenase activity

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Abstract

Self-sufficient cytochromes P450 of the sub-family CYP116B have gained great attention in biotechnology due to their ability to catalyze challenging reactions towards a wide range of organic compounds without the need of a separate reductase partner. However, these P450s are often unstable in solution and their activity is limited to short reaction time. As the isolated heme domain of CYP116B5 has been shown to work as a peroxygenase with H2O2 without the need for expensive NAD(P)H, in this work protein engineering was used to generate a chimeric enzyme (CYP116B5-SOX), in which the native reductase domain is replaced by a monomeric sarcosine oxidase (MSOX) that is able to produce H2O2 with a controlled and continuous release in time. The full-length form enzyme (CYP116B5-fl) is expressed and characterized for the first time, allowing a detailed comparison to both the isolated heme domain (CYP116B5-hd) and CYP116B5-SOX. The catalytic activity of the three forms of the enzyme was studied using p-nitrophenol as substrate, and adding NADPH (CYP116B5-fl), H2O2 (CYP116B5-hd) and sarcosine (CYP116B5-SOX) as direct or indirect source of electrons. CYP116B5-SOX outperforms CYP116B5-fl by 10 folds and CYP116B5-hd by 3 folds, in terms of p-nitrocatechol produced per mg of enzyme per minute. CYP116B5-SOX represents an optimal model to exploit CYP116B5 and the same protein engineering approach could be used for P450s of the same class.

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