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Stabilization and application of a Baeyer-Villiger monooxygenase as protein scaffold in photochemical reactions

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PURPOSE OF THE ABSTRACT
Baeyer-Villiger-Monooxygenases (BVMOs), belong to the large family of Flavoprotein-Monooxygenases (FPMOs) and can catalyze the enzymatic Baeyer-Villiger oxidation reaction under mild conditions, leading to a sustainable chemical process, often accompanied with high enantio- and regioselectivity. However, there are many limitations to the application of BVMOs on an industrial scale, due to their low thermo-, pH-, solvent- and oxidative stability.

Herein, we propose that flavin-dependent Type I BVMOs can act as protein-based photosensitizers for singlet oxygen generation, which can be utilized in synthetically useful photochemical reactions. The biochemical characterization of several wild-type enzymes available in house, highlighted the instability of these enzymes under conditions employed in photochemical reactions. Thus, Rational Design Mutagenesis was performed on the residues susceptible to oxidation, in order to enhance the stability of the proteins.

We managed to construct a thermostable variant of the 2-oxo-Δ3-4,5,5-trimethylcyclopentenylacetyl-CoA monooxygenase from Pseudomonas putida (OTEMO), with the single mutation C444S. Applying this variant in photochemical reactions, we were able to provide the proof-of-principle of our hypothesis, which was the generation of singlet oxygen in the active site of the BVMO. The photochemical reactions with furan substrates consisted the benchmark reactions, that helped us to understand the principles governing these reactions and expand the substrate portfolio on nonfuran substrates.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS
BVMO | photocatalysis | stabilization | singlet oxygen

BIBLIOGRAPHY