ENGINEERING A ROBUST CYCLOHEXANONE MONOOXYGENASE FOR THE PRODUCTION OF METHYL PROPANOATE

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Cyclohexanone monooxygenase (EC 1.14.13.22) from *Acinetobacter calcoaceticus* (AcCHMO) catalyzes the Baeyer-Villiger oxidation of 2-butanone, producing both ethyl acetate and methyl propanoate (Fig. 1A).[1] Methyl propanoate is of industrial interest as a precursor of acrylic plastic. We have replaced various residues near the substrate and $NADP^+$ binding sites in AcCHMO using saturation mutagenesis with the aim of increasing both the activity on 2-butanone and the methyl propanoate/total product ratio. Whole cell biotransformations were prepared for the resulting libraries and the analyses were carried out by headspace GC analysis. A higher conversion yield (92%) and k_{cat} value (0.5 s^{-1}) than wild type AcCHMO (52% and 0.3 s^{-1}, respectively) were observed for T56S AcCHMO. I491A AcCHMO exhibited a significant improvement over the wild type enzyme in the desired regioselectivity using 2-butanone as a substrate (40% *vs.* 26% methyl propanoate, respectively). The T56S/I491A double mutant combined the beneficial effects of both mutations (Fig. 1B).[2]
Recently, we reported on the discovery, characterization, and crystal structure determination of a CHMO from *Thermocrispum municipale* (TmCHMO).[3] A Ser residue was found in TmCHMO at the equivalent position to that of AcCHMO T56. The TmCHMO I493, equivalent to AcCHMO I491, was replaced with an Ala by site-directed mutagenesis. The resulting mutant exhibited a similar activity and regioselectivity to those observed for T56S/I491A AcCHMO using the substrate 2-butanone. This study shows that even for a relatively small aliphatic substrate, regioselectivity can be tuned by structure-inspired enzyme engineering in two different CHMOs. Beneficial mutations previously carried out for AcCHMO, or other CHMOs, may be repeated in TmCHMO achieving similar effects. This is very attractive for biocatalysis since TmCHMO is significantly more thermostable and solvent tolerant than all CHMOs described so far.

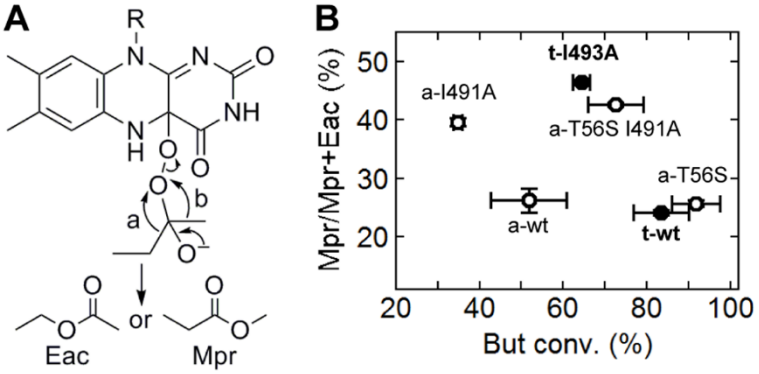

Figure 1 – Conversion of 2-butanone (But) into ethyl acetate (Eac) and methyl propanoate (Mpr) catalyzed by wild type AcCHMO (a-wt) and TmCHMO (t-wt) and their mutants

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