Baeyer-Villiger Monooxygenases : A Promising Route to Chiral Enol- and Ene-lactones

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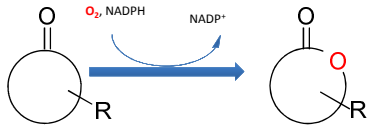
I. Baeyer-Villiger Monooxygenases (BVMOs)

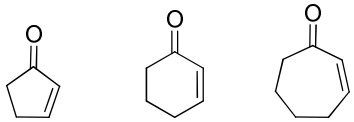
- More than sixty BVMOs described in literature [1]



- Active on a large range of substrates : ketones, but also sulfides, amines....
- High regioselectivity and high enantioselectivity

II. Screening of putative BVMOs

- High throughput cloning (HTC) in *E. coli* BL21
- High throughput screening (HTS) of 60 new BVMOs

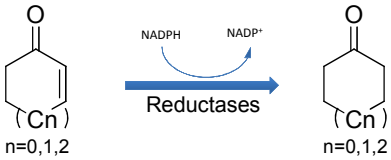


2-cyclopentenone 2-cyclohexenone 2-cycloheptenone

- UV assays based on NADPH consumption (340nm) by extracts of recombinant cells
- 2 relevant hits only against 3 cyclic enones
- Difficult reading due to high background activity

III. An unwanted activity : reduction

- Background activity due to reductase activity on enone from the enzymes of the host microorganism[2]



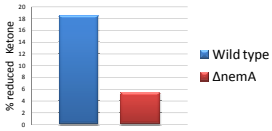
- Competition between BVMO and reductase(s) for NADPH
- Substrate hydrogenation reduced unsaturated lactone yields

➡ **Strategy to limit the side reaction ?**

Solution: find and remove the reductase(s)

- Searching for enone reductase in the literature
  ➡ the old yellow enzyme family (OYE)
- BLAST using OYE against the *E. coli* genome
  ➡ N-ethylmaleimide reductase (NEMA)
- Deletion of *nemA* gene (Keio knock-out mutants) :

Biotransformation of cyclohexenone with wild-type and *ΔnemA* strains



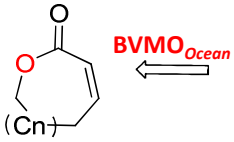
➡ Strong decrease of the reduction

- Introduction of the mutation in *E. coli* BL21 strain by phage transduction

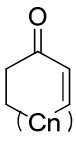
➡ knock-out *E. coli* BL21 strain : unsaturated lactone yield from 39 to 80 %

IV. New BVase activity on enones[3]

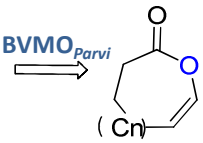
Abnormal regio-selectivity



- From *Oceanicola batsensis*
- Regiochemistry opposite to that of chemical BV oxidation : Ene-lactone formation



Normal regio-selectivity

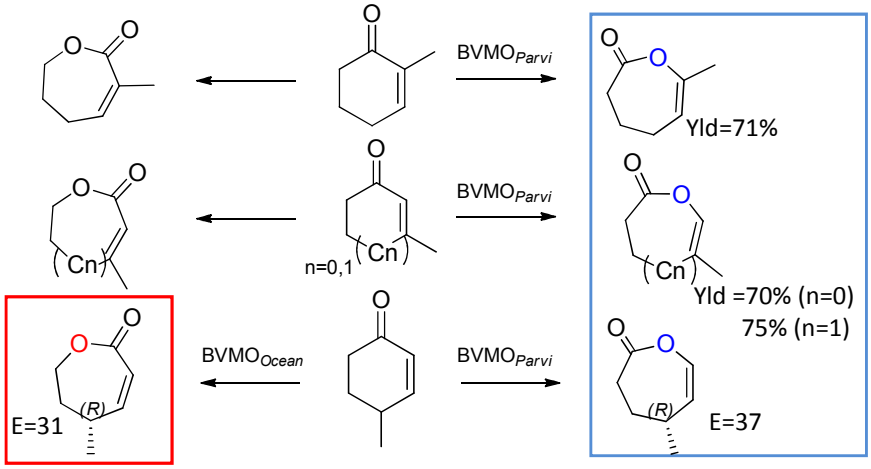


- From *Parvibaculum lavantivorans*
- Regiochemistry similar to that of chemical BV oxidation : Enol-lactone formation

V. Broadening the scope of activities

Activities against substituted cyloalkenones

- $BVMO_{Parvi}$: activity on 2, 3 and 4-methylated cycloalkenones, enol-lactone formation
- $BVMO_{Ocean}$ and $BVMO_{Parvi}$ still regiodivergent enzymes on 4-methyl-cyclohexenone
- High enantioselectivity (E>30) : promising route to new enantiopure synthons
- $CPMO_{Coma}$[5] showed the same actvity as $BVMO_{Parvi}$ on these substrates : consistent with high sequence identity (53%)



[1] a) V. Alphand, R. Wohlgemuth *Curr. Org. Chem.* **2010**. 14, 1928. b) H. Leisch, K. Morley, and P.C.K. Lau *Chem. Rev.* **2011**. 111, 4165. c) K. Balke, M. Kadow, H. Mallin, S. Saß, U. T. Bornscheuer *Org. Biomol. Chem.* **2012**, 10, 6249.
[2] the reductase activity prevented BVase activity detection (see M.D. Mihovilovic, R. Snajdrova, and B. Grötzl. *J. Mol. Catal. B: Enz.* **2006**, 39, 135–140)
[3] the only unambiguous mention of a BVMO activity on enone was the oxidation of 5-hexyl-2-cyclopentenone by pure $CPMO_{Coma}$ (see M. T. Bes, R. Villa, S. M. Roberts, P. W. H. Wan and A. Willetts *J. Mol. Catal. B: Enz.* **1996**, 1, 127)
[4] S.S. Canan Koch, A.R. Chamberlin *Synth. Commun.* **1989**, 19, 829.
[5] $CPMO_{Coma}$ was overexpressed in the deleted strain *E. coli* BL21Δ*nem*A.