Abstract for oral lecture at the the NCCC Netherlands Catalysis Meeting, www.n3c.nl, March 2018, Netherlands

Redox-neutral enzymatic cascade for the synthesis of ursodeoxycholic acid (UDCA)

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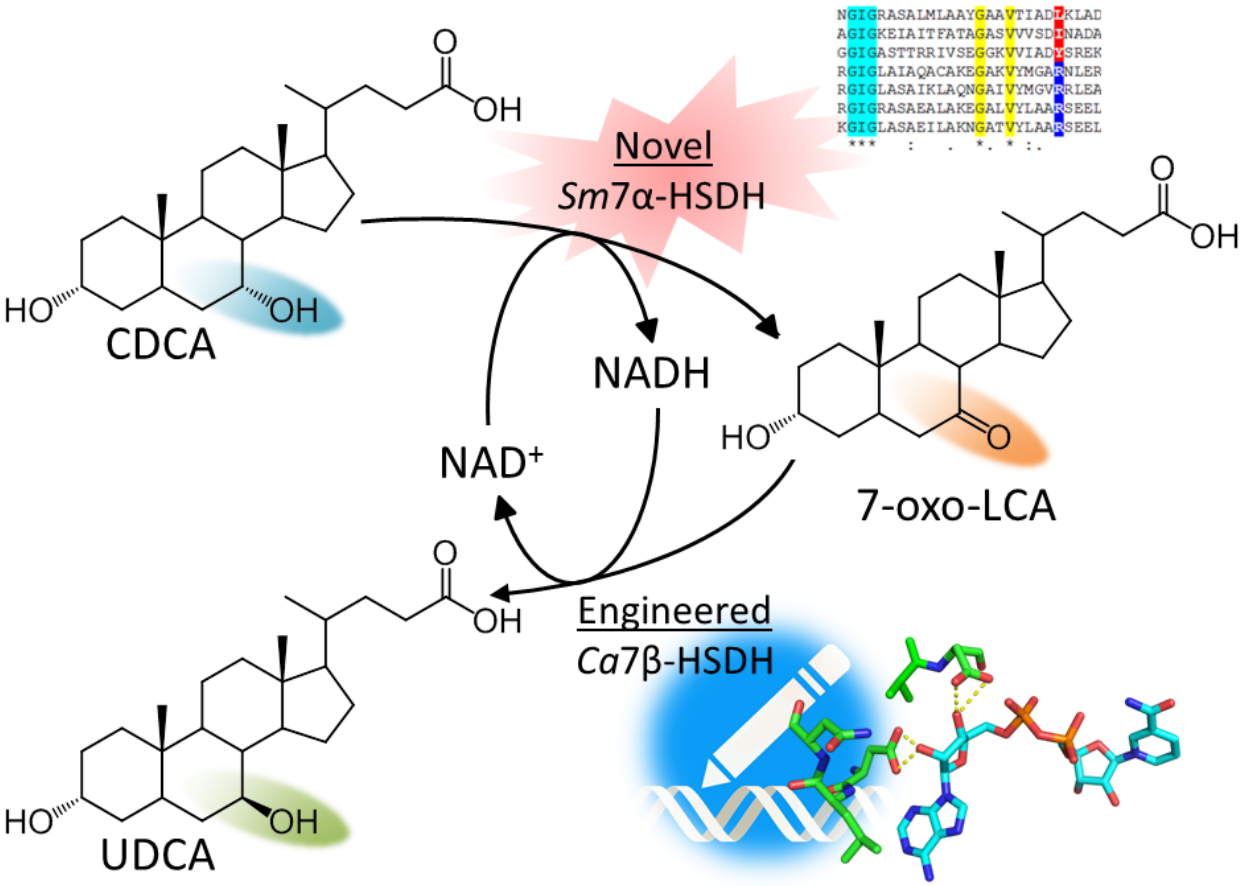
In the European project ONE-FLOW (www.one-flow.org) the objective is to develop sustainable catalytic cascade processes in flow-reactors for pharmaceuticals. Here ursodeoxycholic acid (UDCA) is the subject of study. As bile acid it solubilizes cholesterol gallstones and improves liver function in cholestatic diseases. UDCA can be obtained from cholic acid (CA), which is the most abundant and least expensive bile acid available. This transformation requires several protection and de-protection steps and the use of toxic and dangerous reagents, resulting in an overall yield of about 30%. For that reason, studies have been done on the development of microbial transformations or chemo-enzymatic procedures for the synthesis of UDCA employing CA or chenodeoxycholic acid (CDCA) [1, 2].

In this paper we report the development of an enzymatic cascade for the production of UDCA from CDCA, using both known and unknown enzymatic activities. This epimerization reaction can be carried out with two specific hydroxysteroid (alcohol) dehydrogenases (HSDH): the 7α-OH group is firstly oxidized to the ketone by 7α-HSDH and subsequently re-reduced with opposite stereochemistry (7β-OH) by 7β-HSDH. All procedures up till now require a set of a respectively NAD^+ and $NADP^+$ dependent enzyme. Here we report the first example of a fully NAD^+ mediated cascade, thus circumventing the need for cofactor regeneration [3].

A novel NAD^+ dependent 7α-HSDH from *Stenotrophomonas maltophilia* was recombinantly expressed, purified and biochemically characterized. This enzyme shows good properties in terms of activity (430 $U/mg_{protein}$ on CDCA as substrate) and stability to co-solvents. A second enzyme, the $NADP^+$ dependent 7β-hydroxysteroid dehydrogenase from *Clostridium absonum* was engineered in order to change the cofactor specificity of this enzyme, obtaining a redox-neutral cascade reaction. In addition, the first natural NAD^+ dependent 7β-HSDH from *Lactobacillus spicheri* was identified, expressed and characterized.



In preliminary bio-transformation trials high activity was observed for the UDCA production in a simple batch modus, leading to 65% yield (in 40 min). Near complete conversion (98%) with 100% selectivity was observed when applying a sequential cascade approach. Further studies are underway to develop a flow process for this industrially relevant biotransformation.

1. Eggert, T., D. Bakonyi, and W. Hummel, *Enzymatic routes for the synthesis of ursodeoxycholic acid.* Journal of biotechnology, 2014. **191**: p. 11-21.
2. Tonin, F., L.G. Otten, and I.W. Arends, *Mini-review: bottlenecks in the synthesis of ursodeoxycholic acid (UDCA).* In preparation, 2018.
3. Hollmann, F., I.W. Arends, and K. Buehler, *Biocatalytic redox reactions for organic synthesis: nonconventional regeneration methods.* ChemCatChem, 2010. **2**(7): p. 762-782.