COFACTOR SWITCH: DEVELOPMENT OF A NAD\(^+\)-DEPENDENT CASCADE FOR THE PRODUCTION OF URSODEOXYCHOLIC ACID (UDCA)

Fabio Tonin, Department of Biotechnology, Delft University of Technology
F.Tonin@tudelft.nl
Linda G. Otten, Department of Biotechnology, Delft University of Technology
Ulf Hanefeld, Department of Biotechnology, Delft University of Technology
Isabel W.C.E. Arends, Department of Biotechnology, Delft University of Technology

Key Words: Cofactor switching, hydroxysteroid, Ursodeoxycholic acid, redox-neutral, dehydrogenase

The employment of alcohol dehydrogenases in cascade reactions is often limited by the different cofactor specificity of the enzymes involved: the employment of additional cofactor regeneration systems and the excess amount of sacrificial substrates frequently increase the environmental impact and the costs of biocatalytic processes. Additionally, a NADP\(^+\)-dependent process is generally less desirable, inasmuch this cofactor is more expensive, unstable and less naturally available than NAD\(^+\), leading to an increase of the process costs. Nowadays, protein engineering offers the possibility to switch the cofactor dependency of enzymes introducing few targeted mutation\(^1\).

We applied this methodologies for the development of the first fully NAD\(^+\) mediated cascade for the production of Ursodeoxycholic acid (UDCA), a widely used pharmaceutical ingredient for the clinical treatment of cholesterol gallstones and liver diseases\(^2\). The enzymatic epimerization of CDCA into UDCA, can be carried out with two specific hydroxysteroid 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 to now require a set of a respectively NAD\(^+\) and NADP\(^+\) dependent enzymes. Nevertheless, gene sequences of NAD\(^+\)-dependent 7β-HSDH were not reported. In order to obtain a full-NAD\(^+\)-dependent process, the NADP\(^+\)-dependent 7β-HSDH from Clostridium absonum was engineered. Employing a semi-rational mutagenesis approach, we obtained a variant with shifted cofactor preference. Importantly, this study allows to identify the residues responsible of the cofactor recognition in other 7β-HSDH homologues and, thus, to the identification of the gene coding for a wild-type NADH-dependent homologue from Lactobacillus spicheri (Ls7β-HSDH). These novel NAD\(^+\)-dependent 7β-HSDH enzymes in combination with 7α-HSDH from Stenotrophomonas maltophilia permitted the redox-neutral biotransformations of CA and CDCA in the presence of catalytic amounts of NAD\(^+\), resulting in high yields (>90 %) of UCA and UDCA\(^3\).

Further studies are underway to develop a flow process for this industrially relevant biotransformation.