ENZYME ENGINEERING OF A MEMBRANE-BOUND MONOOXYGENASE AS KEY STEP OF AN ARTIFICIAL METABOLIC PATHWAY TOWARDS TULIPALIN A

Robert Kourist, Austrian Centre of Industrial Biotechnology
kourist@tugraz.at
Andrea Nigl, Austrian Centre of Industrial Biotechnology
Kamela Myrtollari, Henkel AG & Co. KGaA, Adhesive Technologies
Andreas Taden, Henkel AG & Co. KGaA, Adhesive Technologies

Key Words: biobased polymers; alpha-methylene lactone; alkane monooxygenase, rational design.

The synthesis of renewable and sustainable polymeric materials as replacement of petroleum-based raw materials has been receiving increasing attention. The alpha-methylene lactone Tulipalin A has two polymerizable functional moieties and is a potential substitute of (meth)acrylates in vinyl-addition polymerization and (co)monomer for lactone ring-opening polymerization. While Tulipalin A can be isolated from the flowers of tulips and alstroemerias, its biosynthesis remains unknown (Figure 1).

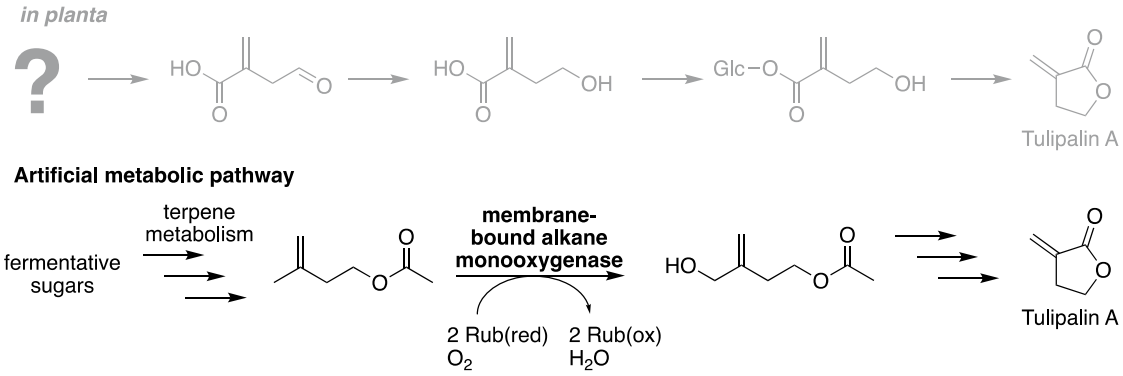

Figure 1: Proposed pathway for the synthesis of Tulipalin A

We propose a synthesis from isoprenyl acetate, which itself can be produced via the microbial hemiterpenoid metabolism. Selective hydroxylation of isoprenyl acetate in C4-position and subsequent oxidation of the intermediate hydroxy group gives rise to 4-acetoxy-2-methylene butyric acid, whose hydrolysis and cyclization then leads to Tulipalin A. We identified bacterial alkane monooxygenases that catalyze the hydroxylation. Undesired epoxidation of the exo-methylene group was not observed.

In order to increase the activity of the membrane-bound dioxygenase, we used de novo structure prediction to generate a structural model. Site-directed mutagenesis of the active-site cavity inspired by molecular docking allowed a substantial increase of the activity of the monooxygenase. We envision that the engineered enzyme variants will find application in a future whole-cell process, unlocking the supply of Tulipalin A as future bio-based monomer.

.