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Enzyme engineering of alkane monooxygenase for improved activity in a key reaction step of the synthesis of the alpha-methylene lactone Tulipalin A

AUTHORS
Robert KOURIST / GRAZ UNIVERSITY OF TECHNOLOGY, PETERSGASSE 12, GRAZ

PURPOSE OF THE ABSTRACT

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.

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 without, albeit with lower activity than the terminal hydroxylation of linear alkanes. 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.

FIGURES

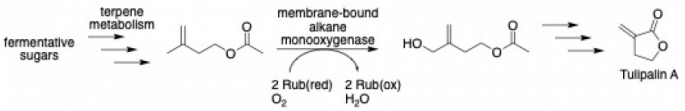

FIGURE 1

FIGURE 2

Figure 1

Proposed pathway for the synthesis of Tulipalin A

KEYWORDS

bio-based polymer | membrane-bound enzyme | alkane monooxygenase | rational protein desigh

BIBLIOGRAPHY