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Synthesis of Deoxysugars from Aliphatic Ketoacids and Aldoses Catalysed by Thermostable Transketolase Variants from Geobacillus stearothermophilus

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PURPOSE OF THE ABSTRACT

Transketolase (TK), catalyzes the synthesis of a wide range of ketoses by stereoselective C-C bond formation in the presence of thiamine diphosphate (ThDP) and Mg2+ as cofactors.7 Indeed, wild type TK transfers a ketol unit from 3-hydroxypyruvate (HPA), which is the most commonly used synthetic donor substrate, to an aldehyde as acceptor substrate and the product of the reaction is a (3S)-ketose or an analog depending on the acceptor substrate. The reaction is rendered irreversible by the release of carbon dioxide. Recent studies showed that aliphatic alpha-ketoacids can be recognized as donor substrates by specially designed TK variants.[1] Particularly, the thermostable TK from Geobacillus stearothermophilus (TKgst)[2] has been engineered to accept pyruvate, 2-oxobutyrate as donors in place of HPA (scheme 1).[3]
We present a strategy for the enzymatic synthesis of 1-deoxy and 1,2 deoxyketoses from the aliphatic alpha-ketoacids, pyruvate and 2-oxobutyrate, as donors, and natural aldoses of variable chain length as acceptors, catalyzed by TKgst variants.[4] Analytical studies have been firstly carried out with the appropriate substrates allowing to select the best combinations of mutations. The preparative scale synthesis allowed to obtain 1-deoxy and 1,2 deoxy ketoses with good to excellent isolated yields (61%-86%).
To optimize the strategy, and as a proof of principle, the alpha-ketoacids pyruvate and 2-oxobutyrate were generated in situ from the corresponding D-aminoacids D-alanine and D-homoalanine respectively, using a thermostable D-aminoacid oxidase DAAO4536 that was selected from a screening of 55 putative DAAOs provided by Prozomix Limited. Hence, a one-pot one step procedure was performed at 50 °C by coupling DAAO4536 and the best TKgst variant H102L/L118I/H474S in the presence of D-alanine or D-homoalanine as alpha-ketoacids precursors and D-erythrose as acceptor substrate. The corresponding 1-deoxy and 1,2-dideoxydeoxyketoses were isolated with good yields (64% and 72% respectively, out of two steps).[4]

FIGURES

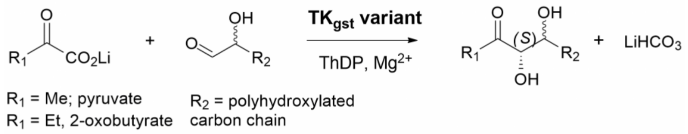

FIGURE 1

Transketolase reaction

scheme 1

FIGURE 2

KEYWORDS

Biocatalysis | transketolase | stereoselectivity | D-amino acid oxidase

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