ISGC 2022

N°906 / OC
TOPIC(s) : Homogenous, heterogenous and biocatalysis / Alternative technologies

Cleavage and synthesis of aliphatic ?-Hydroxy Ketones by Evolved Transketolase from Geobacillus stearothermophilus

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PURPOSE OF THE ABSTRACT

The synthesis of aliphatic acyloins (alpha-hydroxy ketones) is of particular interest because these compounds are largely used as fine chemicals or building blocks for the production of larger molecules, particularly for construction of heterocycles required in pharmaceuticals and also offer unique properties as flavors or nonionic surfactants. On another side, the cleavage of aliphatic alpha-hydroxy ketones yields free aldehydes highly valuable as aromas, intermediates for the preparation of polyesters and polyamides. A wide range of multi-enzyme or chemical ways have been developed for the cleavage or the synthesis of aliphatic alpha-hydroxy ketones. We proposed here an attractive alternative catalyzed in one step by a thiamine pyrophosphate-dependent enzyme, transketolase (TK).

In cells, TK is involved in the pentose phosphate pathway where it reversibly catalyzes the transfer a ketol group from the phosphorylated ketoses (C5?C7) to the phosphorylated aldoses (C3?C5). To improve TK activity toward aliphatic substrates, our present study was performed from the thermostable TK from Geobacillus stearothermophilus (TKgst) discovered recently by our group. refs 1-6 TKgst variant libraries obtained by structure-guided studies of the active site were constructed and screened on one hand toward donor and acceptor substrates to catalyze the synthesis of aliphatic alpha-hydroxy ketones (C5-C10) by C-C bond formation (Figure 1) ref 7 and on the other hand toward aliphatic alpha-hydroxy ketones with varying carbon backbone lengths (C5-C10) to release the corresponding aldehydes (Figure2).ref 8 Specific and efficient TKgst variant were identified with enhanced activities for the synthesis or cleavage of aliphatic alpha-hydroketones.

The single variant L382F was able to catalyze efficiently the transfer of the ketol group from hydroxypyruvate on aliphatic aldehydes (C3-C8) to give the corresponding 1,3-dihydroxy ketones with good yields and excellent enantioselectivity while the new variant H102L/H474S/F435I transferred the acyl goup of 2-oxobutyrate and 2-oxovalerate to aliphatic aldehydes, giving the corresponding mono alpha-hydroxylated ketones.ref 7

The single variant F435I gave the best activity toward the cleavage of (3S)-1,3-dihydroxyhexan-2-one leading to

the corresponding product with 92% yield after only 2 h reaction time. Three triple variants H102L/H474 (S, G or A) / F118I were found to cleave (±)-4-hydroxyhexan-3-one giving the corresponding products with 90, 82 and 79 % yield respectively after 24 h, whereas wild-type TK was almost ineffective.ref 8

This biocatalytic strategy offers a promising one-step alternative to other enzymatic or chemical ways for the highy valuable synthesis or cleavage of aliphatic alpha-hydroxy ketones.

FIGURES

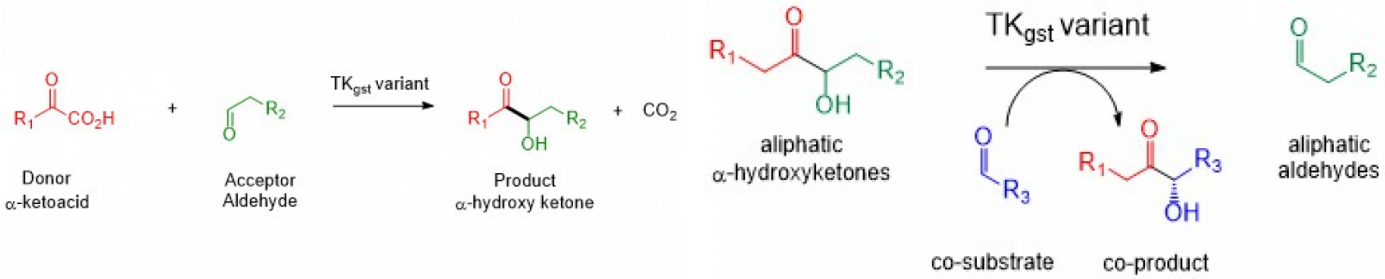

FIGURE 1

Figure 1

Synthesis of alpha-hydroxyketones with TKgst variant in the presence of aliphatic donor and acceptor substrates.

FIGURE 2

Figure 2

Cleavage of alpha-hydroxy ketones into corresponding aldehydes with TKgst variant.

KEYWORDS

transketolase | biocatalysis | alpha-hydroxyketones | rational mutagenesis

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