PURPOSE OF THE ABSTRACT

In polyol and carbohydrate chemistry, the inherent problems of regioselectivity, chemoselectivity and stereoselectivity is a challenge for traditional methods of organic synthesis. By contrast, regio-and stereoselectivity of enzymes avoid the use of protective groups and enable reaction pathways with fewer steps in mild reaction conditions. The enzyme transketolase (TK), a thiamine diphosphate (ThDP)-dependent enzyme, yields D-threo ketoses having a (3S,4R)-configuration by C-C bond formation. TK is highly specific for ketol donor substrates, stereospecific for forming the new asymmetric center in (S) configuration and enantioselective towards 2-hydroxyaldehyde acceptor substrates with an (R) configuration. Interestingly, commercial lithium hydroxypyruvate (Li-HPA) can be used as a donor substrate, rendering the ketol transfer irreversible owing to its decarboxylation catalyzed by TK.

We recently cloned and overexpressed the first thermostable TK from the thermophilic bacterium Geobacillus stearothermophilus (TKgst).[1] Thermostable enzymes offer many advantages, such as robustness against unusual conditions (T°C, pressure)[2], improved solubility of organic substrates at elevated temperatures and higher reaction rate [3], increased tolerance toward unconventional media and greater resistance to protein destabilizing factors introduced by mutagenesis.[4,5] TKgst, like other TK sources, preferentially accepts (2R)-hydroxylated aldehydes. Remarkably, at high temperature, TKgst was also able to accept (2S)-hydroxylated aldehydes yielding (3S,4S) ketoses stereospecifically.[4] None of the mesophilic TKs have been reported to catalyze the conversion of (2S)-configured hydroxylaldehydes (Figure 1).

For synthetic purposes, the main problem of TKgst-catalyzed reactions at high temperature is the limited stability of the synthetic donor substrate Li-HPA.[6] We will report on the identification and characterization of a novel thermostable serine-glyoxylic acid L-?-transaminase (TA) from the thermophilic bacterium Thermosinus carboxydvorans DSM 14886 (TAtca) and its use for the in situ biocatalyzed synthesis of HPA at high temperature from natural L-serine and pyruvate. TAtca-catalyzed reaction is shifted towards HPA by coupling to the
irreversible TKgst-catalyzed reaction in an efficient one-pot two-step simultaneous cascade at elevated temperature (Figure 2). This procedure is applied to the synthesis of highly valuable and naturally rare L-erythro (3S,4S)-ketoses, L-ribulose, 5-deoxy-L-ribulose, D-tagatose and L-psicose obtained with excellent stereoselectivity and good yields. Such a configuration is currently inaccessible with mesophilic TKs. TKgst activities towards the (2S)-?-hydroxylated aldehydes, which are generally poor TKgst substrates, were greatly enhanced by performing the reactions at high temperature, leading to excellent conversion rates within a reasonable time (24 h to 96 h). Overall, this cascade synthesis prevents the thermal decomposition of the labile HPA and offers an efficient, environmentally friendly procedure.
FIGURES

FIGURE 1
Irreversible TK reaction?-
lithium -hydroxypyruvate (Li-HPA) ; (2R)
?-hydroxyaldehydes at room temperature (RT) using
TKsce, TKeco, and TKgst ; (2S) ?-hydroxyaldehydes
at 60 °C using TKgst.

FIGURE 2
Enzymatic cascade synthesis of L-erythro (3S,4S)
ketoses catalyzed by TAtca and TKgst at 60 °C
1 R= -CH2OH (L-ribulose )
2 R= -CH3 (5-deoxy-L-ribulose )
3 R= -CHOH(R)-CH2OH (D-tagatose)
4 R= -CHOH(S)-CH2OH (L-psicose)

KEYWORDS
thermostability | enzymatic cascade reactions | chiral polyols | carboligation

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