EXPLORING DONOR SUBSTRATE PROMISCUITY OF A THERMOSTABLE TRANSKETOLASE BY DIRECTED EVOLUTION

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Enzymes catalyzing asymmetric carboligation reactions typically show very high specificity for their nucleophilic substrate. Transketolase (TK, EC 2.2.1.1) catalyses a reversible transfer of a hydroxylated C_2 fragment among phosphorylated ketoses and aldoses. [1] Native TK converts a large variety of (2R)-hydroxyaldehydes as the electrophilic acceptor substrates, but apart from its natural phosphoketose donors TK accepts only hydroxypyruvate (hydroxylated donor) (Figure 1). In contrast, 1-deoxy-D-xylulose-5-phosphate synthase (DXS, EC 2.2.1.7) catalyzes the specific decarboxylative transfer of the acetyl moiety from pyruvate (non-hydroxylated donor) to glyceraldehyde-3-phosphate to yield 1-deoxy-D-xylulose 5-phosphate (DXP), which constitutes the first step into the non-mevalonate biosynthesis of terpenoids (Figure 1).[2] Reactions of native TK and DXS are mutually exclusive in vivo.

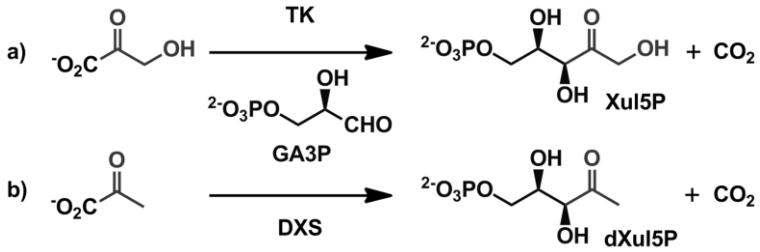

Figure 1. Comparison of carboligation reactions catalyzed by TK and DXS: a) directed carboligation of TK by using hydroxypyruvate in vitro; b) native carboligation of DXS by using pyruvate in vivo.

We have performed a structure-guided saturated mutagenesis of transketolase from *Geobacillus stearothermophilus* (TK_{gst}) to create thermostable variants that are able to utilize pyruvate and related 2-oxoacids as nucleophilic components. From this study, we discovered several new variants that were far superior in imitating the native reaction catalyzed by DXS. Even sterically more demanding oxoacids such as oxobutyrate and branched-chain 3-methyl-2-oxobutyrate could be converted with good rates, and products were obtained with high enantio- and diastereoselectivity.[3] The best variant was able to complement for the *dxs* gene in the engineered auxotrophic *E. coli* strain,[4] by resurrecting the biosynthesis of DXP.

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