EXPLORING DIASTEREOSELECTIVITY MECHANISM OF L-THREONINE ALDOLASE

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L-Threonine aldolase (LTA), a PLP-dependent enzyme, is an attractive tool in organic chemistry for catalyzing the asymmetric formation of β-hydroxy-α-amino acids with two chiral centers from aldehyde and glycine. β-Hydroxyl-α-amino acids are widely used in the fields of medicine, food and agriculture as a kind of chiral building blocks. The wild enzyme has a strict selectivity for C_α of β-hydroxy-α-amino acids but a moderate selectivity for C_β, limiting its wide application in stereospecific carbon-carbon bond synthesis. In this work, diastereoselectivity mechanism of LTA was explored using molecular dynamics simulations. And then, the diastereoselectivity of *Cp*LTA from *Cellulosilyticum sp* was engineered based on the insights. Guided by the molecular dynamics simulations, “path hypothesis” and “Prelog rule” were proposed to elucidate diastereoselectivity mechanism of LTA. We assumed that the active pocket of LTA has two substrate access paths named *syn* path and *anti* path (Fig 1A). L-*syn* configuration products were formed by the substrate aldehyde entering the active center from *syn* path, as the electron of C_α anion of PLP-Gly (quinonoid intermediate form) transferred to carbonyl carbon atom of aldehyde from *si*-face. On the contrary, L-*anti* configuration products were formed by the substrate aldehyde entering the active center from *anti* path, as the electron of C_α anion transferred to carbonyl carbon atom of aldehyde from *re*-face (Fig 1B). Furthermore, with *Cp*LTA as an object, a mutability landscape was first constructed by performing saturation mutagenesis at substrate access tunnel amino acids. CAST/ISM strategy was then performed to tune diastereoselectivity. As a result, diastereoselectivity of mutant H305L/Y8H/V143R was improved from 37.2%$_{syn}$ to 99.4%$_{syn}$. Besides, diastereoselectivity of mutant H305Y/Y8I/W307E was inverted to 97.2%$_{anti}$ (Fig 1C). The study would be useful to expand LTA applications and guide the engineering of other C-C bond formation enzymes. The work has been published in *Angew. Chem. Int. Ed.* (doi.org/10.1002/anie.202213855)

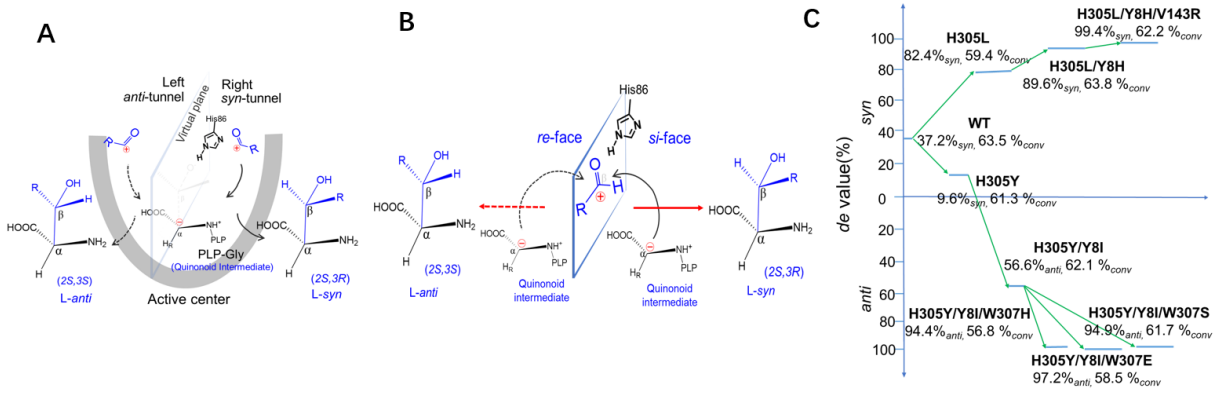

Figure 1. Diastereoselectivity mechanism and Directed evolution of LTA. (A): path hypothesis for illustrating the diastereoselectivity of LTA; Dark arrows indicate the entry direction of substrate. Define virtual plane consists of atoms including C_α, H_R of PLP-Gly (quinonoid intermediate) and N_ε, H of catalytic base His86. (B): Prelog rule for illustrating the diastereoselectivity of LTA. electronic transfer to the re- or si-face of substrate aldehyde, respectively, with the formation of corresponding configuration of β-hydroxya-amino acid. (C): The evolutionary process of CpLTA in diastereoselectivity. syn: mutant with L-syn-MTPS preference. anti: mutant with L-anti-MTPS preference. conv: conversion rate.

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