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Exploring diastereoselectivity mechanism of L-threonine aldolase

AUTHORS

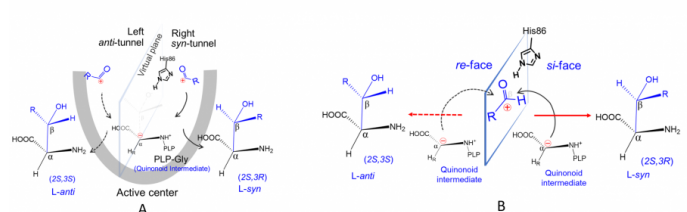
Wenlong ZHENG / ZHEJIANG UNIVERSITY, 733 JIANSHE SAN ROAD, XIAOSHAN DISTRICT, HANG ZHOU

Jianping WU / ZHEJIANG UNIVERSITY, YUHANGTANG ROAD 866, HANG ZHOU, CHINA, HANGZHOU

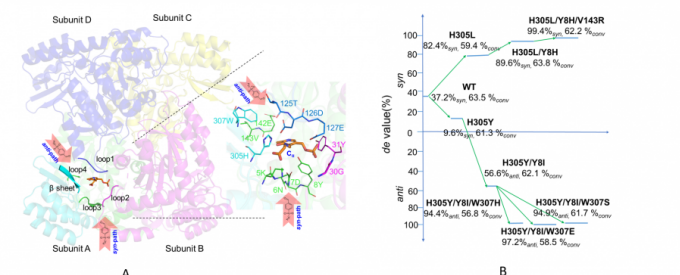
PURPOSE OF THE ABSTRACT

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 -Hydroxy- 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 CpLTA 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. 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. Furthermore, with CpLTA 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. 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 online in Angew. Chem. Int. Ed. (doi.org/10.1002/anie.202213855)

FIGURES



(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_α, 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-hydroxyα-amino acid.



(A) Models of CpLTA with the docked ligands PLP-Gly.(B): The evolutionary process of CpLTA in diastereoselectivity. *syn*: mutant with Lsyn-MTPS preference. *anti*: mutant with L-anti-MTPS preference. *conv*: conversion rate.

FIGURE 1

diastereoselectivity mechanism of LTA

FIGURE 2

Directed evolution

(A) Models of CpLTA with the docked ligands PLP-Gly.(B): The evolutionary process of CpLTA in diastereoselectivity. *syn*: mutant with Lsyn-MTPS preference. *anti*: mutant with L-anti-MTPS preference. *conv*: conversion rate.

KEYWORDS

L-threonine aldolase | directed evolution | diastereoselectivity | protein engineering

BIBLIOGRAPHY

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