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Rational Design of Transketolase with Non-Natural Amino Acids to Accelerate a Novel Reaction for Pharmaceutical Synthesis

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PURPOSE OF THE ABSTRACT

Transketolase (TK) catalyses the reversible transfer of a C2-ketol unit from a donor substrate to an aldehyde acceptor, but its narrow substrate scope limits its potential for industrial applications. It was initially engineered by our research group to accept both the unnatural donor substrate pyruvate and acceptor substrate 3-formylbenzoic acid (3-FBA) to produce an analogue of phenylacetylcarbinol (PAC) [1], a crucial pharmaceutical intermediate. To accelerate this reaction, we first established a "push and pull" rational design strategy that incorporated non-natural amino acids to reshape the enzyme's binding pocket and guide substrates into its active sites. In-silico approaches, including the Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) method, were employed to evaluate the binding affinities of the transketolase-cofactor complex and substrates. Subsequently, a smart library of transketolase mutants was designed and screened, resulting in the identification of a lead mutant exhibiting a 25.6% improvement in substrate conversion in comparison to the best-performing historical variant [2]. Additionally, this mutant displayed a significant increase in stability, reflected by a 6.7°C enhancement in its melting temperature [2]. Furthermore, our investigations revealed a strong correlation between the root-mean-square deviation (RMSD), obtained through molecular dynamic simulations, and the thermal stability of transketolase within diverse scaffolds, providing important insights for designing thermally stable proteins. Overall, the significant improvements identified demonstrate the potential of this strategy in computer-aided protein design. These findings are not only valuable for the development of transketolase-based biocatalysts but also hold great promise for the engineering of other proteins with enhanced properties for a wide range of industrial and biotechnological applications.

[1] Yu, H., Hernández López, R. I., Steadman, D., Méndez-Sánchez, D., Higson, Cázares-Körner, A., Sheppard, T. D., Ward, J. M., Hailes, H. C., & Dalby, P. A. (2020). Engineering transketolase to accept both unnatural donor and acceptor substrates and produce α-hydroxyketones. The FEBS journal, 287(9), 1758-1776.
[2] Li, Y., Dalby, P. A. Rational Design of Transketolase with Non-Natural Amino Acids to Accelerate a Novel Reaction for Pharmaceutical Synthesis. unpublished results

FIGURES

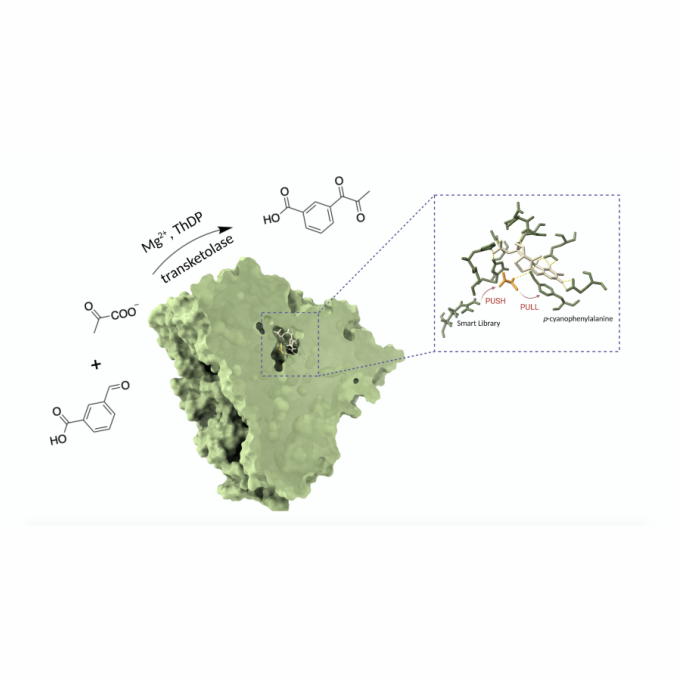

FIGURE 1

The push and pull mechanism of transketolase catalysing a Novel Reaction for Pharmaceutical

The push and pull rational design strategy mechanism of transketolase catalysing a Novel Reaction for Pharmaceutical

FIGURE 2

KEYWORDS

Rational design | non-natural amino acid | smart library | industrial biocatalysis

BIBLIOGRAPHY