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Rational Design of Carbonyl Reductase CgKR1 Based on Molecular Simulation Methods

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PURPOSE OF THE ABSTRACT
Chiral alcohols are important chiral blocks for the synthesis of chiral drugs, fine chemicals, etc. and have high application value. Carbonyl asymmetric reduction is an important method for the biocatalytic synthesis of chiral alcohols, which has the advantages of mild reaction conditions, environmental friendliness and better stereoselectivity. In our previous work, the carbonyl reductase CgKR1-F92C/F94W from Candida glabrata (as the starting parent M0) was discovered with high catalytic activity and stereoselectivity. However the unsatisfying thermal stability has become the bottleneck for its practical application.
Here in this work, we focused on computer-based rational design of the enzyme to address the problem of its low thermal stability. Since the interactions between residues in the protein structure play an important role in maintaining the thermal stability of proteins. The RINVES (Residue Interaction Network-based Virtual Design for Enzyme Stability) strategy based on residue interaction networks was constructed. Firstly, three-dimensional structure of proteins was converted into a two-dimensional network with residues as nodes and inter-residue interaction forces as edges. A virtual mutation library with site-saturated mutations was then established, and the changes in the topology and other network parameters were calculated and investigated to select mutations that were beneficial to the stability improvement. Using this virtual screening strategy, the best mutation CgKR1-M3 with significantly improved thermal stability was obtained, representing a 62.3-fold increase in t1/2 at 50 °C and a 13 °C increase in T15 50 compared to the parent, and maintained a high viability. The structural mechanism of the enhanced thermal stability of the mutant was further investigated based on molecular dynamics simulation analysis.

Subsequently, the optimal mutant CgKR1-M3 were coupled with glucose dehydrogenase to achieve a coenzyme cycle, resulting in the asymmetric reduction of 100 g L-1 of N-Boc-3-piperidone. 95% conversion of the mutant CgKR1-M3 was achieved in about 4 h, whereas the parent M0 was only converted to 56% in the end due to rapid inactivation. Validation of the reaction showed that the resulting dominant mutant strain was able to efficiently synthesize (S)-N-Boc-3-hydroxypiperidine, a key chiral intermediate of the anticancer drug ibrutinib, with promising applications.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

ketoreductase | residue interaction networks | rational design | thermal stability

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