Multi-Enzyme Cascade Catalysis for the Synthesis of Ursodeoxycholic Acid from Chenodeoxycholic Acid Postprint

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Abstract

Bear bile powder has been used as a health food and medicine for nearly a millennium. Ursodeoxycholic acid (UDCA) is the main active ingredient of bear bile powder (with an annual demand of approximately 45 tons/year), and is widely used for cholestatic liver diseases such as fatty liver disease, drug-induced hepatitis, and viral hepatitis. 7 -hydroxysteroid dehydrogenase (7 -HSDH) is the key bottleneck enzyme in the dual-enzyme coupling method (Fig. 1) that catalyzes the conversion of chenodeoxycholic acid (CDCA) to synthesize ursodeoxycholic acid, and its activity, stability, and coenzyme affinity significantly affect the technical and economic feasibility of the enzymatic biotransformation route. We obtained a novel enzyme, 7 -HSDHRt, through gene mining approaches. However, nearly all reported 7 -hydroxysteroid dehydrogenases suffer from common defects such as low activity, poor stability, and incompatible working pH with 7 -HSDH, resulting in very limited space-time yield of the target product UDCA [1]. Therefore, employing enzyme engineering technology to substantially improve the catalytic performance of relevant enzymes is key to determining whether biotechnology can save black bears. In this report, we propose a multiobjective directed evolution strategy (MODE) for engineering the catalytic activity, thermal stability, and optimal pH of 7 -hydroxysteroid dehydrogenase [2]. Through methods such as error-prone PCR, DNA shuffling, and site-directed mutagenesis, the final mutant V3-1 achieved a 5.5-fold higher specific activity than the wild-type enzyme, while its half-life was extended by 3-fold. Additionally, the optimal pH of the mutant shifted toward weakly alkaline conditions (pH 6.5 $\rightarrow$ pH 7.5), thus approaching closer to the pH of the dehydrogenation reaction catalyzed by the upstream enzyme 7 -HSDH (pH 8.0). When using the aforementioned co-evolved mutant enzyme V3-1 for cascade conversion reactions, the space-time yield of UDCA reached 942 g L^{-1} d^{-1}, significantly higher than that of the native enzyme at 141 g L^{-1} d^{-1}. Thus, protein engineering technology for enzyme catalysts will exert powerful impact in enhancing the catalytic synthesis

potential of enzymes, promoting the application of biocatalytic processes in the green chemical and pharmaceutical industries, as well as in serving ecological civilization construction, advancing environmental protection, and maintaining ecological balance.

Full Text

Preamble

Multi-enzyme Cascade Catalysis for Synthesis of Ursodeoxycholic Acid from Chenodeoxycholic Acid

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Bear bile powder has been used as both a health supplement and medicinal agent for nearly a millennium. Ursodeoxycholic acid (UDCA), the principal active component of bear bile powder (with an annual demand of approximately 45 tons), is widely employed in treating cholestatic liver diseases including fatty liver disease, drug-induced hepatitis, and viral hepatitis. 7 -hydroxysteroid dehydrogenase (7 -HSDH) represents the key bottleneck enzyme in the two-enzyme coupling system (Figure 1 [Figure 1: see original paper]) that catalyzes the conversion of chenodeoxycholic acid (CDCA) to UDCA. Its activity, stability, and cofactor affinity significantly influence the technical and economic viability of the enzymatic biotransformation route. We obtained a novel enzyme, 7 -HSDHRt, through gene mining. However, nearly all previously reported 7 -hydroxysteroid dehydrogenases suffer from common limitations including low activity, poor stability, and incompatible working pH with 7 -HSDH, resulting in very limited space-time yields of the target product UDCA [1]. Therefore, employing enzyme engineering technology to substantially improve the catalytic performance of relevant enzymes is crucial for determining whether biotechnology can save black bears.

Figure 1 [Figure 1: see original paper]. Multi-enzyme cascade catalyzing the chiral inversion of chenodeoxycholic acid (CDCA) to synthesize ursodeoxycholic acid (UDCA). The system comprises *E. coli* 7 -HSDH with NAD /NADH recycling via lactate/pyruvate, and 7 -HSDHRt with NADPH/NADP recycling via gluconate/glucose, with 7-oxo-LCA as the intermediate.

In this report, we propose a multiobjective directed evolution (MODE) strategy to engineer the catalytic activity, thermal stability, and optimal pH of 7 -hydroxysteroid dehydrogenase [2]. Through error-prone PCR, DNA shuffling, and site-directed mutagenesis, the final mutant V3-1 achieved a specific activity 5.5-fold higher than the wild-type enzyme, while its half-life was extended threefold. Additionally, the optimal pH of the mutant shifted toward weak alkalinity (pH 6.5 → pH 7.5), making it more compatible with the pH requirement

(pH 8.0) of the preceding 7 -HSDH-catalyzed dehydrogenation reaction. When using the co-evolved mutant enzyme V3-1 for cascade conversion reactions, the space-time yield of UDCA reached 942 g L^{1} d^{1}, significantly higher than the 141 g L^{1} d^{1} achieved with the native enzyme. This demonstrates that protein engineering of enzyme catalysts will play a powerful role in enhancing enzymatic synthesis potential, promoting the application of biocatalytic processes in green chemical and pharmaceutical manufacturing, and serving ecological civilization construction while advancing environmental protection and ecological balance.

[1] Zheng, M.M.; Wang, R.F.; Li, C.X.; Xu, J.H. Process Biochem. 2015, 50, 598–604.
[2] Zheng, M.M.; Chen, K.C.; Wang R.F.; Li, H.; Li, C.X.; Xu, J.H. J. Agric. Food Chem. 2017, 65, 1178–1185.

Note: Figure translations are in progress. See original paper for figures.