ChinaRxiv

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201709.00085

Postprint: Highly Stereoselective Asymmetric Reduction of Long-Chain Aliphatic -, -Keto Acids/Keto Esters Catalyzed by a Novel Enzyme

Authors: Bai Yunpeng, Zhang Chao, Li Chunxiu, Xu Jianhe, Bai Yunpeng

Date: 2017-09-20T00:00:00+00:00

Abstract

Optically pure aliphatic hydroxy acids and their derivatives are important chiral building blocks that can be used to prepare various natural products or bioactive molecules. For example, chiral lactones obtained through intramolecular cyclization of long-chain -, -hydroxy acids are important fragrance molecules with significant application value in the food and fine chemical industries. Studies have shown that asymmetric reduction of keto acids or keto esters is the most efficient and atom-economical route for synthesizing chiral hydroxy acids/hydroxy esters. However, to date, the substrate scope reported in the vast majority of studies on enzymatic or chemical catalysts has been limited to -, -aromatic keto esters or short-chain keto esters. Apart from whole-cell catalysis using Saccharomyces cerevisiae, Pichia pastoris, and other microorganisms, highly stereoselective enzymatic asymmetric reduction of long-chain aliphatic -, -keto acids/keto esters remains a major challenge. Therefore, the discovery and engineering of novel enzymes with high activity and high selectivity toward long-chain aliphatic -, -keto acids/keto esters hold significant scientific and application value.

In this thesis, a novel catalyst, Pseudomonas panipatensis, capable of asymmetrically reducing 4-oxodecanoic acid, was first isolated from soil using conventional methods. The wild-type strain was then subjected to whole-genome sequencing, and the target enzyme PpCR (Pseudomonas panipatensis carbonyl reductase) was successfully cloned from this strain using a gene hunting approach. This carbonyl reductase is NADPH-dependent. Subsequently, using PpCR as a probe, a carbonyl reductase with superior catalytic performance, SmCR (Serratia marcescens carbonyl reductase), was identified through database mining. The catalytic properties of SmCR were investigated, revealing an optimal reaction pH of 6.0 and an optimal reaction temperature of 40°C. SmCR was relatively stable at 30°C, with a half-life of 90 h. The half-lives at 40°C and 50°C were 50

h and 15 h, respectively. For 4-oxodecanoic acid, SmCR exhibited a Km value of 1.26 mM and a kcat of 0.128 min [1].

Preparative reactions for chiral lactones were carried out in 100 mL of 100 mM sodium phosphate buffer at pH 6.0, with a 4-oxodecanoic acid concentration of 2 mM, affording the product (R)- -decalactone with an ee value of 99% and an isolated yield of 72%. Using 5-oxodecanoic acid as the substrate under the same conditions, (R)- -decalactone was obtained with an ee value of 95% and an isolated yield of 70%. The results of this thesis demonstrate that SmCR is the first reductase capable of asymmetrically reducing both 4-oxodecanoic acid and 5-oxodecanoic acid, and holds potential application value in the production of (R)- -decalactone and (R)- -decalactone.

Full Text

Preamble

Research on Highly Stereoselective Asymmetric Reduction of Long-Chain Aliphatic -, -Keto Acids/Keto Esters Catalyzed by Novel Enzymes

Yunpeng Bai*, Chao Zhang, Chunxiu Li, Jianhe Xu

Optically pure aliphatic hydroxy acids and their derivatives serve as important chiral building blocks for the synthesis of various natural products and bioactive molecules. For instance, chiral lactones derived from intramolecular cyclization of long-chain -, -hydroxy acids represent valuable fragrance compounds with significant applications in the food and fine chemical industries. Asymmetric reduction of keto acids or keto esters has been established as the most efficient and atom-economical pathway for synthesizing chiral hydroxy acids and their esters. However, the vast majority of reported enzymatic and chemical catalysts to date have been limited to -, -aromatic keto esters or short-chain keto esters. With the exception of whole-cell catalysis using *Saccharomyces cerevisiae*, *Pichia pastoris*, and related organisms, highly stereoselective enzymatic asymmetric reduction of long-chain aliphatic -, -keto acids and keto esters remains a formidable challenge. Consequently, the discovery and engineering of novel enzymes exhibiting high activity and selectivity toward these substrates hold considerable scientific and practical significance.

This study commenced with conventional soil screening to isolate a novel biocatalyst, *Pseudomonas panipatensis*, capable of asymmetrically reducing 4-oxodecanoic acid. Whole-genome sequencing of this wild-type strain enabled the successful cloning of the target enzyme PpCR (*Pseudomonas panipatensis* carbonyl reductase) via a gene hunting approach. PpCR was determined to be NADPH-dependent. Subsequently, employing PpCR as a molecular probe, database mining identified a carbonyl reductase with superior catalytic performance, designated SmCR (*Serratia marcescens* carbonyl reductase). Biochemical characterization of SmCR revealed an optimal reaction pH of

6.0 and optimal temperature of 40°C. The enzyme demonstrated reasonable stability at 30°C with a half-life of 90 h, while half-lives at 40°C and 50°C were 50 h and 15 h, respectively. For 4-oxodecanoic acid, SmCR exhibited a Km of 1.26 mM and a kcat of 0.128 min ^{1}.

Preparative-scale reactions for chiral lactone synthesis were conducted in 100 mL of 100 mM sodium phosphate buffer (pH 6.0) containing 2 mM 4-oxodecanoic acid, producing (R)- -decalactone with 99% ee and an isolated yield of 72%. Under identical conditions, 5-oxodecanoic acid served as substrate to afford (R)- -decalactone with 95% ee and an isolated yield of 70%. These findings establish SmCR as the first reductase capable of asymmetrically reducing both 4-oxodecanoic acid and 5-oxodecanoic acid with high stereoselectivity, demonstrating its potential utility in the production of (R)- -decalactone and (R)- -decalactone.

Author Information

Yunpeng Bai* (born May 1982) holds a Ph.D. and is currently an Associate Professor at the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology.
Correspondence address: Room 911, Building 18, 130 Meilong Road, Shanghai 200237, China.
Email: ybai@ecust.edu.cn

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

Source: ChinaXiv —Machine translation. Verify with original.