Assessing the stereoselectivity of carbonyl reductases toward the reduction of OPBE and docking analysis

Rong Chen[1,2]
Jian Deng[1]
Jinping Lin[1*]
Xiaopu Yin[2]
Tian Xie[2]
Shengli Yang[1]
Dongzhi Wei[1*]

[1] *State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai, People's Republic of China*

[2] *Center for Biomedicine and Health, Division of Basical Medicine, Hangzhou Normal University, Hangzhou, People's Republic of China*

Abstract

The asymmetric reduction of prochiral carbonyl compounds by NAD(P)H-dependent carbonyl reductases represents a powerful method for the production of optically active alcohols. The stereoselectivity of a series of carbonyl reductases were evaluated toward the reduction of ethyl 2-oxo-4-phenylbutyrate (OPBE). A majority of reductases produced the ethyl (*R*)-2-hydroxy-4-phenylbutyrate ((*R*)-HPBE) with low to excellent enantiomeric excess (e.e.), whereas about 30% reductases catalyzed OPBE to form (*S*)-HPBE. Among them, the carbonyl reductase from *Saccharomyces cerevisiae* (SeCR) and short-chain dehydrogenase from *Gluconobacter oxydans* (GoKR) exhibited 100% e.e., yielding the corresponding (*R*) and (*S*)-HPBE, respectively. However, the SeCR showed relative higher activity (29.0 U/mg) and affinity (K_m of 0.22 mM) than those of GoKR. Docking analysis found that the interaction of OPBE with enzyme-NADPH complex determined the NADPH-provided hydrogen transfer and the configuration of reductive product. These results indicated that the three-dimensional (3D) structure of enzymes controlled the stereoselectivity of the reductive product based on the geometry of the substrate and cofactor. Volume 00, Number 0, Pages 1–6, 2015

Keywords: *asymmetric reduction, carbonyl reductase, docking analysis, Prelog's rule, chiral alcohol*

Abbreviations: *ACE, angiotensin in converting enzyme; ADH, alcohol dehydrogenase; AKRs, aldo-keto reductases; CpKR, polyketone reductase from Candida parapsilosis; 3D, three-dimensional; e.e., enantiomeric excess; GoKR, short-chain dehydrogenase from Gluconobacter oxydans; HPBE, ethyl-2-hydroxy-4-phenylbutyrate; HPLC, high-performance liquid chromatography; LB, Luria-Bertani; NMN, nicotinamide mononucleotide; OPBE, ethyl 2-oxo-4-phenylbutyrate; PDB, Protein Data Bank; SDR, short-chain alcohol dehydrogenase/reductase; SeCR, carbonyl reductase from Saccharomyces cerevisiae.*

**Address for correspondence: Jinping Lin, PhD, State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, People's Republic of China. Tel.: +86 21 64253287; Fax: +86 21 64250068; e-mail: jplin@ecust.edu.cn; or Dongzhi Wei, PhD, State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, People's Republic of China. Tel.: +86 21 64252078; Fax: +86 21 64250068; e-mail: dzhwei@ecust.edu.cn.*

Received 2 February 2015; accepted 14 May 2015

DOI: 10.1002/bab.1397

Published online in Wiley Online Library (wileyonlinelibrary.com)

1. Introduction

Biocatalysts have drawn considerable attention not only because of the advantage of their unparalleled stereoselectivity and regioselectivity, but also because of the economic viability and environmental friendliness. Among these biocatalysts, carbonyl reductases or dehydrogenases, which catalyze asymmetric reduction of ketones to give optical active secondary alcohols, play an increasingly important role in the synthesis of enantiomeric pure pharmaceuticals, agrochemicals, and natural products [1–3]. Several approaches to exploit suitable biocatalysts have been explored via screening and identifying from diverse sources, such as metagenomics, commercial enzymes, culture microorganism, or a clone bank [4]. The use of isolated enzymes is advantageous because the undesirable enantiomer formation mediated by multiple enzymes can be minimized. However, the application of isolated carbonyl reductases in synthesizing chiral alcohols is still hampered by their limited availability [5]. Moreover, the most prochiral ketones are not native substrates for enzymes; the discovery of promising and new enzymes may pose a huge challenge [6]. In this regard, we have been interested in searching for reported and putative carbonyl reductases and then expressing

these enzymes in an *in vitro* system. The part of library include the enzymes that can catalyze reductive reaction regardless of their substrate spectrum, such as reductase from *Candida macedoniensis*, (*R*)-specific alcohol dehydrogenase (ADH) from *Lactobacillus kefir*, ADH from *Leifsonia* sp., and so on [7–9].

Homology-driven genome mining is an effective strategy to search for novel biocatalysts from the genomes of organisms [10]. Yeast cells have been the popular biocatalyst for asymmetric reduction of ketone, and their genomes include multiple genes that encode enzymes with divergent chemo-, stereo-, and enantio-selectivity [11]. Therefore, gene isolation and expression at higher level in host is an effective method. For example, Nie et al. [12] identified four anti-Prelog stereospecific carbonyl reductase genes from *Candida parapsilosis* through genome mining. Yeasts, such as *Saccharomyces cerevisiae*, *Lodderomyces elongisporus*, and so on, together with another bacterium, *Gluconobacter oxydans*, are commonly applied in many industries for their abilities of stereoselective oxidoreduction [13, 14]. Their genomes reveal the presence of many sequences encoded putative oxidoreductases based on sequence analysis. Therefore, these candidates are selected through homology-driven genomic mining, cloned and overexpressed in an *in vitro* system, consisting of another part of the library.

Herein, we present the reduction results of the prochiral precursor ethyl 2-oxo-4-phenylbutyrate (OPBE) through the carbonyl reductases in the library. The reductive product, ethyl (*R*)-2-hydroxy-4-phenylbutyrate ((*R*)-HPBE or (*S*)-HPBE) is a common building block for the synthesis of a variety of angiotensin in converting enzyme (ACE) inhibitors, such as cilazapril, enalapril, benazepril, ramipril, and quinapril [15, 16]. Further, the homologies of (*R*)-HPBE-producing reductase and (*S*)-HPBE-producing dehydrogenase were modeled in complex with NADPH. OPBE was subjected to docking analysis for understanding the catalytic mechanism of stereoselective reduction.

2. Materials and Methods

2.1. Chemicals and cultivation of cells in library

OPBE and HPBE were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Other reagents and solvents were from local commercial suppliers, and were of analytical grade or biochemical reagents. NADPH was purchased from Sigma–Aldrich.

The reported genes and candidate sequences were synthesized, ligated into pET vector, and then transformed to *Escherichia coli* BL21 (DE3). The engineered *E. coli* BL21 (DE3) cells in the library were routinely cultured in Luria-Bertani medium at 37 °C. Ampicillin and kanamycin were added to the medium at 100 and 50 μg mL^{-1} when necessary. When cell density at OD_{600} reached at 0.6, 0.25 mM of isopropyl β-D-thiogalactopyranoside was added; and the culture was shaken overnight at 25 °C for protein production. The induced cells were harvested by centrifugation at 7,104*g* for 10 Min at 4 °C, rinsed twice with phosphate buffer (20 mM, pH 7.4), and then used for stereoselective reduction of OPBE as biocatalyst. Two enzymes, carbonyl reductase from *S. cerevisiae* (SeCR) and short-chain dehydrogenase from *G. oxydans* (GoKR), were purified by Ni-NTA agarose according to the operation manual (Qiagen, Hilden, Germany).

2.2. Stereoselective reduction of OPBE

The reaction mixture (10 mL) contained 50 mM phosphate buffer (pH 8.0), NADPH (10 mM), the whole cells (0.3 g/L), and OPBE dissolved in ethanol (1.0 g/L, 10% (v/v)). The reaction mixtures were shaken at 30 °C and further extracted using an equivalent volume of ethyl acetate for three times. The organic layer was dried, diluted in 2-propanol, and then subjected to chiral high-performance liquid chromatography (HPLC) analysis to determine enantiomeric excess (e.e.). The absolute configurations were assigned by chiral HPLC analysis using authentic (*R*)- and (*S*)-HPBE as standard samples under the same conditions.

Chiral HPLC analyses were performed on an Agilent 1100 series HPLC system with a UV detector. Chiral HPBE was analyzed on a chiralcel OD-H column at 210 nm by using hexane/2-propanol (98/2, v/v) as eluent at a flow rate of mL Min^{-1} and a temperature of 30 °C. The e.e. was calculated according to the peak areas of *R* and *S* enantiomers according to the HPLC. The conversion of HPBE was determined based on their peak areas by gas chromatography (Agilent Technologies Co., Los Angeles, CA, USA) on a pEG-20M column.

2.3. Activity and kinetic parameters analysis

The activities of purified SeCR and GoKR were measured on the basis of OPBE reduction in the coupled conversion of NADPH to $NADP^+$. The change in NADPH absorbance was monitored at 340 nm using an ultraviolet-visible spectrophotometer with a temperature-controlled cuvette holder (Shimadzu Co., Kyoto, Japan). Reaction mixtures (1.0 mL) consisted of 5 mM OPBE, 50 mM PBS (pH 7.0), 0.1 mM NAD(P)H, and enzyme. One unit (U) was defined as the amount of enzyme catalyzing the oxidation of 1.0 μmol NADPH per minute at 30 °C. Protein concentrations were measured through bicinchoninic acid assay using bovine serum albumin as a standard. Enzyme kinetic parameters were assayed in PBS (pH 7.0) at 30 °C by adding OPBE ranging from 0.1 to 10 mM. The apparent values of Michaelis constant (K_m), maximum velocity (V_{max}), and k_{cat} were calculated by fitting the data into the Michaelis–Menten equation using the SigmaPlot program (Systat Software Inc., San Jose, CA, USA).

2.4. Protein modeling and docking analysis

The amino acid sequences of SeCR were used to search for homologous sequences in the Protein Data Bank (PDB) with the BLASTp program. The three-dimensional homology model of SeCR was carried out based on the X-ray structure of CpKR in complex with NADPH using Discovery Studio 3.0 (Accelrys, San Diego, CA, USA). Polyketone reductase from *C. parapsilosis* IFO 0708 (CpKR, PDB ID: 4H8N), was a NADPH-dependent reductase, which belongs to the aldo-keto reductase (AKR)

TABLE 1 ***Conversion and stereoselectivity of carbonyl reductases toward the reduction of OPBE***

Entry	*Protein ID.*	*Source*	*Conversion (%)*	*e.e. (%)*	*Type*
001	NP_010159.1	*Saccharomyces cerevisiae*	>99	>99	*R*
002	NP_014490	*Saccharomyces cerevisiae*	>99	44.4	*R*
003	NP_012630	*Saccharomyces cerevisiae*	58.2	42.0	*R*
004	NP_010656	*Saccharomyces cerevisiae*	88.1	23.2	*R*
005	AFI38947	*Lodderomyces elongisporus*	66.7	31.1	*R*
006	XP_001526453	*Lodderomyces elongisporus*	>99	25.5	*R*
007	AFI38949	*Lodderomyces elongisporus*	>99	5.06	*S*
008	XP_001523546	*Lodderomyces elongisporus*	>99	15.9	*S*
009	XP_715609	*Lodderomyces elongisporus*	>99	1.49	*R*
010	BAE46987	*Candida macedoniensis*	>99	76.3	*R*
011	BAP71734	*Candida macedoniensis*	69.5	43.0	*R*
012	XP_456023	*Candida albicans*	>99	10.2	*R*
013	XP_454964	*Kluyveromyces lactis*	45.2	18.2	*R*
014	AFI38946	*Yarrowia lipolytica*	35.8	32.8	*R*
015	WP_011158613	*Rhodopseudomonas palustris*	60.9	46.9	*R*
016	BAD99642	*Leifsonia* sp.	95.9	47.0	*S*
017	AAP94029	*Lactobacillus kefiri*	>99	91.6	*S*
018	WP_004658895	*Pseudomonas syringae*	50.9	45.6	*R*
019	WP_011241052	*Zymomonas mobilis*	55.1	37.7	*R*
020	WP_000168720	*Zymomonas mobilis*	34.8	11.4	*R*
021	WP_010866880	*Aeropyrum pernix*	58.7	0.17	*R*
022	YP_001469824	*Thermotoga lettingae*	79.3	67.1	*R*
023	YP_001471191	*Thermotoga lettingae*	>99	96.1	*S*
024	YP_192428	*Gluconobacter oxydans*	62.3	25.7	*R*
025	YP_190959	*Gluconobacter oxydans*	93.1	>99	*S*
026	YP_191077	*Gluconobacter oxydans*	49.1	91.4	*S*
027	YP_192012	*Gluconobacter oxydans*	31.5	61.6	*R*
028	YP_191995	*Gluconobacter oxydans*	17.8	75.0	*S*
029	YP_191867	*Gluconobacter oxydans*	15.2	76.3	*R*
030	YP_190729	*Gluconobacter oxydans*	10.9	40.7	*S*

superfamily [17]. The crystal structure data of GoKR was obtained and submitted in PDB (PDB ID: 3WTB) (unpublished work). The NADPH docked into SeCR or GoKR was obtained via setting parameters of "copy ligand from templates" by Discovery Studio 3.0 software. The generated model was geometrically refined using the Princeton TIGRESS workspace (http://atlas.princeton.edu/refinement/). The final model was evaluated using Procheck program [18], and the lowest energy conformational model was chosen for docking studies. The docking of OPBE into enzyme–NADPH complexes was performed with Autodock program following previously described procedures [19].

3. Results and Discussion

3.1. Screening of carbonyl reductases for stereoselective reduction of OPBE

The library of carbonyl reductases included thirty members belonging to the short-chain ADH/reductase (SDR), AKR, and medium-chain dehydrogenase families. Except from seven enzymes from *G. oxydans*, the enantioselectivity toward OPBE reduction catalyzed by those enzymes was not investigated. As shown in Table 1, nine reductases (about 30%) can asymmetrically reduce OPBE to (*S*)-HPBE, whereas majority of reductases gave (*R*)-HPBE with low to excellent enantioselectivity. Ten reductases gave approximately 100% conversion with fluctuant e.e. In all the tested enzymes, SDR from *G. oxydans* (Genbank accession numbers: NC_006677) produced (*S*)-HPBE with 100% e.e. and 93.1% conversion. The diketoreductases from *Acinetobacter baylyi* ATCC 33305 synthesized (*S*)-HPBE with 99.5% e.e [20], whereas hydroxyl acid dehydrogenase from Enterobacter sp. BK2K produced (*S*)-2-hydroxy phenylbutanoic acid ((S)-HPBA) with >94% e.e. [21]. Both (*S*)-HPBE and (*S*)-HPBA are important chiral building blocks in synthesizing of ACE inhibitors [15]. The SeCR (Genbank accession numbers: NM_001180183) exhibited 99% e.e. and 100% conversion toward OPBE, yielding (*R*)-HPBE. Two yeasts, namely, *Candida boidinii* CIOC2 and *Candida krusei* SW202 (whole cells), were proven to effectively reduce of OPBE, leading to the (*R*)-enantiomer with >99% e.e. However, the enzymes at work were uncovered [22, 23]

3.2. Activity and kinetic parameters analysis

Further, the activities of the purified SeCR and GoKR were investigated. As shown in Table 2, the SeCR showed relatively higher activity of 29.0 U/mg compared with GoKR. Further, the kinetic constants toward OPBE reduction were determined by nonlinear regression using a sigmaplot program. The K_m value of SeCR was 0.22 mM, which was similar to that of AKRs from *C. krusei* SW 2026 (0.1 mM) and *C. glabrata* (0.319 mM), but much lower than that of GoKR (2.97 mM) [24]. GoKR showed a good catalytic efficiency with having a k_{cat}/K_m value of 17.19 s^{-1} mM^{-1} (Table 2). In terms of coenzyme specificity, both SeCR and GoKR exclusively utilized NADPH as a cofactor.

TABLE 2 *Activity and kinetic constants of SeCR and GoKR toward OPBE*

Parameter	SeCR	GoKR
Activity (U/mg)	29.0	7.2
K_m (mM)	0.22	2.97
k_{cat} (Sec^{-1})	2.45	51.04
k_{cat}/K_m (Sec^{-1} mM^{-1})	11.14	17.19

3.3. Protein modeling and docking studies

To gain insight into the understanding of how enzyme-substrate-cofactor interaction affects the stereoselectivity, homology modeling of the two HPBE-producing enzymes and docking analysis were performed. SeCR belonged to the aldo-keto superfamily [25]. In PDB database, the CpKR shared 41.4% of amino acid sequence identities and 56.4% of similarities with SeCR and served as the model template. The modeled 3D structure, conserved catalytic residues, and binding pockets of SeCR were similar to the homology template. OPBE was respectively docked into the binding pocket of the modeled structure of SeCR–NADPH and GoKR–NADPH complexes, which were obtained as previously described.

As shown in Figs. 1A and 2A, NADPH was located in the active site groove and OPBE was bonded to the catalytic residues of enzymes. The nicotinamide mononucleotide (NMN) ring was positioned at the bottom of the OPBE binding cavity. In Fig. 1B, the spatial arrangement of Tyr64, His122, and the nicotinamide ring was proposed to create an oxyanion hole and define the specificity and stereoselectivity. Likewise, His122 residue played a role in orienting the substrate carbonyl [26]. Tyr64 and His122 as proton donors formed hydrogen bonds with carbonyl oxygen of OPBE, resulting that the oxygen atom was protonated. Then Lys95 promoted hydrogen atom transfer [27]. The lowest-energy conformation of OPBE into SeCR–NADPH complex showed that the hydride transfer from NMN ring of NADPH occurred at *re* face of OPBE, producing the corresponding (*R*)-HPBE.

According to the proposed catalytic mechanism of the enzymes belonging to the SDR family, three catalytic residues (Tyr, Ser, and Lys) are essential for the catalytic reaction [28, 29]. In lowest energy conformation of GoKR–NADPH–OPBE, Tyr152 and Ser138 formed hydrogen bonds with the carbonyl oxygen of OPBE, resulting that the oxygen atom was protonated. Then, Lys154 promoted the hydrogen atom of NMN ring to attack the carbonyl carbon of OPBE, following the hydrogen transferred from the *si* face of OPBE to carbonyl oxygen, forming the (*S*)-HPBE (Fig. 2B).

The stereospecificity of enzyme-catalyzed ketone reduction depends on the steric situation of carbonyl substrates and coenzyme NADPH [30, 31]. Hydride from NADPH can attack the carbonyl oxygen atom of the substrate in four possible

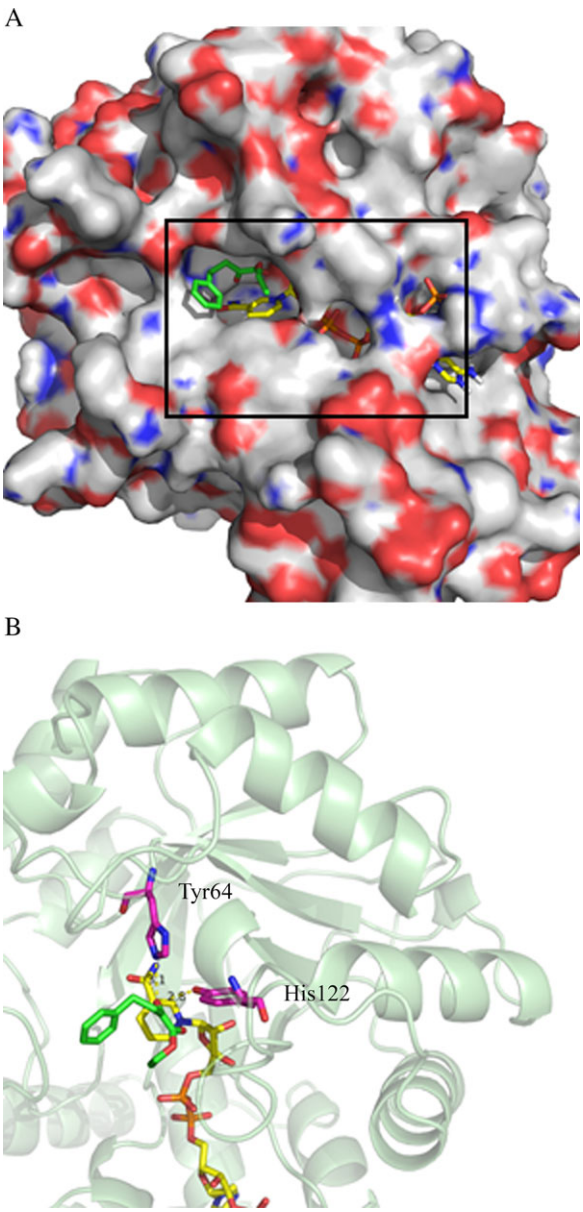

FIG. 1 *(A) Structural observation of SeCR–NADPH–OPBE model. (B) Structural insights into the SeCR–NADPH–OPBE model and key residues involved in substrate catalysis. The bound NADPH is highlighted in stick and colored in yellow. The catalytic tetrad residues are highlighted in stick and colored in magenta. Substrate OPBE is colored in green.*

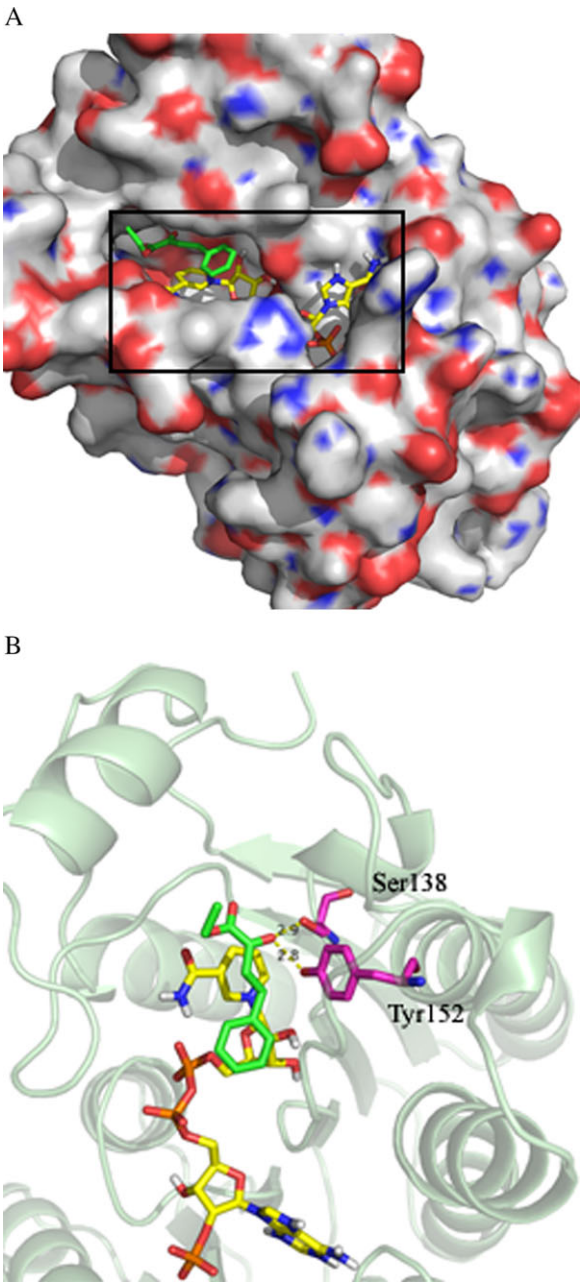

FIG. 2 *(A) Structural observations of GoKR–NADPH–OPBE model. (B) Structural insights into the SeCR–NADPH–OPBE model and key residues involved in substrate catalysis. The bound NADPH is highlighted in stick and colored in yellow. The catalytic tetrad residues are highlighted in stick and colored in magenta. Substrate OPBE is colored in green.*

pathways. When the hydride transfers from NADPH to oxygen atom at the *re* face of the pro-chiral ketone, producing the corresponding (*S*)-alcohol, the enzyme-catalyzed reduction follows the Prelog's rule [32]. Most carbonyl reductases (about 70%) in the library are Prelog stereoselective enzymes. The SeCR-catalyzed reduction follows Prelog's rule. Few carbonyl reductases have anti-Prelog stereopreference, and the GoKR shows anti-Prelog's stereoselectivity. This type of enzyme is rare in nature and in demand in the pharmaceutical industry [33]. In a word, the configuration of chiral alcohol produced by enzymatic asymmetric reduction can be predicted according to the structure of the substrate.

4. Conclusion

The library includes a set of reported and uncharacterized carbonyl reductases. The prochiral ketoester (OPBE) was used to evaluate the stereoselectivity of those enzymes. A majority of the reductases produced the (*R*)-HPBE, whereas about 30% of the reductases catalyzed OPBE to (*S*)-HPBE with low to excellent e.e. Among them, SeCR and GoKR exhibited 100% e.e., yielding the corresponding (*R*) and (*S*)-HPBE, respectively. According to Cahn-Ingold-Prelog priority rules for naming stereoisomers HPBE, the SeCR yielded (R)-HPBE, which means that SeCR is a Prelog stereoselective reductase. On the contrary, the GoKR yielded (S)-HPBE. Thus, GoKR is an anti-Prelog one. Homology modeling and docking studies showed that the interaction of OPBE with enzyme–NADPH complex determined the NADPH-provided hydrogen transfer and the configuration of reductive product. These results may provide a valuable insight on the effect of enzyme–substrate–cofactor interaction in the stereoselectivity of reduction.

5. Acknowledgements

This work was financially supported by the National Key Basic Research Development Program of China ("973" Program, No. 2012CB721003), the Natural Science Foundation of China (No. 21276084 and 21002029), the National Major Science and Technology Projects of China (No. 2012ZX09304009), and the Technology Research and Development Program for Institute of Hangzhou (20130432B05 and 20142013A63).

6. References

[1] Hölsch, K., and Weuster-Botz, D. (2010) Biotechnol. Appl. Biochem. 56, 131–140.
[2] Huisman, G. W., Liang, J., and Krebber, A. (2010) Curr. Opin. Chem. Biol. 14, 122–129.
[3] Xu, F. (2005) Ind. Biotechnol. 1, 38–50.
[4] Nakamura, K., Yamanaka, R., Matsuda, T., and Harada, T. (2003) Tetrahedron: Asymmetry 14, 2659–2681.
[5] Zhu, D., Yang, Y., Buynak J. D., and Hua, L. (2006) Org. Biomol. Chem. 4, 2690–2695.
[6] Wahler, D., and Reymond, J. L. (2001) Curr. Opin. Chem. Biol. 5, 152–158.
[7] Kataoka, M., Hoshino-Hasegawa, A., Thiwthong, R., Higuchi, N., Ishige, T., and Shimizu, S. (2006) Enzyme Microb. Technol. 38, 944–951.
[8] Weckbecker, A., and Hummel, W. (2006) Biocatal. Biotransfor. 24, 380–389.
[9] Inoue, K., Makino,Y., and Itoh, N. (2005) Appl. Environ. Microbiol. 71, 3633–3641.
[10] Ferrer, M., Martínez-Abarca, F., and Golyshin, P. N. (2005) Curr. Opin. Biotechnol. 16:588–593.
[11] Kaluzna, I. A., Matsuda, T., Sewell, A. K., and Stewart, J. D. (2004) J. Am. Chem. Soc. 126(40), 12827–12832.
[12] Nie, Y., Xiao, R., Xu, Y., and Montelione, G. T. (2011) Org. Biomol. Chem. 9, 4070–4078.
[13] Chen, R., Liu, X., Lin, J. P., and Wei, D. Z. (2014) Biosci. Biotechnol. Biochem. 78, 1350–1356.
[14] Pscheidt, B., and Glieder, A. (2008) Microbiol. Cell Factories 7, 25.
[15] Oda, S., Inada, Y., Kobayashi, A., and Ohta, H. (1998) Biosci. Biotechnol. Biochem. 62, 1762–1767.
[16] Gröger, H. (2001) Adv. Synth. Catal. 343(6–7), 547–558.
[17] Qin, H. M., Yamamura, A., Miyakawa, T., Kataoka, M., Nagai, T., and Kitamura, N. (2014) Appl. Microbiol. Biotechnol. 98, 243–249.
[18] Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. App. Crystallogr. 26, 283–291.
[19] Abagyan, R., Totrov, M., and Kuznetsov, D. (1994) J. Comput. Chem. 15(5), 488–506.
[20] Wu, X., Wang, Y., Ju, J., Chen, C., Liu, N., and Chen, Y. (2009) Tetrahedron: Asymmetry 20, 2504–2509.
[21] Cha, M., Kim, E. J., Yun, H., Cho, B. K., and Kim, B. G. (2007) Biotechnol. Prog. 23, 606–612.
[22] Chen, Y., Lin, H., Xu, X., Xia, S., and Wang, L. (2008) Adv. Synth. Catal. 350, 426–430.
[23] Zhang, W., Ni, Y., Sun, Z., Zheng, P., Lin, W., Zhu, P., and Ju, N. (2009) Process Biochem. 44, 1270–1275.
[24] Li, N., Ni, Y., and Sun Z. H. (2010) J. Mol. Catal B Enzym. 66, 190–197.
[25] Hyndman, D., Bauman, D. R., Heredia, V. V., and Penning, T. M. (2003) Chem. Biol. Interact. 143–144, 621–631.
[26] Jez, J. M., Bennett, M. J., Schlegel, B. P., Lewis, M., and Penning, T. M. (1997) Biochem. J. 326, 625–636.
[27] Ellis. (2002) FEMS Microbiol. Lett. 216, 123–131.
[28] Filling, C., Berndt, K. D., Benach, J., Knapp, S., Prozorovski, T., Nordling, E., Ladenstein, R., Jörnvall, H., and Oppermann, U. (2002) J. Biol. Chem. 277, 25677–25684.
[29] Höffken, H. W., Duong, M., Friedrich, T., Breuer, M., Hauer, B., Reinhardt, R., Rabus, R., and Heider, J. (2006) Biochemistry 45, 82–93.
[30] Prelog, V. (1964) Pure Appl. Chem. 9, 119–130.
[31] Liang, P., Qin, B., Mu, M., Zhang, X., Jia, X., You, S. (2013) Biotechnol. Lett. 35, 1469–1473.
[32] Musa, M. M., and Phillips, R. S. (2011) Catal. Sci. Technol. 1, 1311–1323.
[33] Tasnádi, G., and Hall, M. (2013). In Synthetic Methods for Biologically Active Molecules (Brenna, E, ed.). pp. 329–374, Wiley, Weinheim , Germany.