Appl Biochem Biotechnol (2010) 160:868–878
DOI 10.1007/s12010-009-8528-9

Improved Production of (*R*)-1-phenyl-1,2-ethanediol by a Codon-optimized *R*-specific Carbonyl Reductase from *Candida parapsilosis* in *Escherichia coli*

Rongzhen Zhang · Yan Xu · Yawei Geng · Shanshan Wang · Ying Sun · Rong Xiao

Received: 27 October 2008 / Accepted: 14 January 2009 /
Published online: 10 February 2009

Abstract An *R*-specific carbonyl reductase from *Candida parapsilosis* (CprCR) catalyzes the transformation of (*R*)-1-phenyl-1,2-ethanediol from 2-hydroxyacetophenone. The gene *rcr* coding CprCR contains a few codons rarely used by *Escherichia coli*. In order to improve chiral alcohol production, three codon variants Δ24, aRCR, and mRCR of CprCR were designed through truncation of 4–27 bp disorder sequence at the 5′-terminus or/and adaption of nine rare codons. The effects of codon optimization on enzyme activity, protein production, and biotransformation were studied. Among these three types, the disorder sequence-truncated and rare codon-adapted variant mRCR presents the highest enzyme activity. When compared with CprCR, mRCR showed an increase of 35.6% in the total activity of cell-free extracts. The specific activity of mRCR presented similar increase in the cell-free extract with purified protein, which suggested that the codon optimization caused positive effect on protein productivity of variant enzyme. When microbial cells concentration was 30% (*w*/*v*), the molar conversion yield and enantiomeric excess of the mRCR variant reached 86.4% and 93.6%, which were increased 36.5% and 15.8% than those of wild-type at a high substrate concentration of 5 g/L. The work will supply a new method for improving chiral alcohol preparation with codon engineered microorganisms.

Keywords (*R*)-1-phenyl-1,2-ethanediol · 2-ethanediol · Carbonyl reductase · Codon optimization · *Candida parapsilosis*

Abbreviations

ADHs	alcohol dehydrogenases
CprCR	*R*-specific *c*arbonyl *r*eductase from *Candida parapsilosis*
e.e.	enantiomeric excess
MDRs	medium-chain dehydrogenases/reductases

R. Zhang · Y. Xu (✉) · Y. Geng · S. Wang · Y. Sun
Key Laboratory of Industrial Biotechnology of Ministry of Education and School of Biotechnology, Jiangnan University, Wuxi 214122, People's Republic of China
e-mail: biosean@yahoo.com.cn

R. Xiao
Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854, USA

NAD(H)	nicotinamide adenine dinucleotide
PED	1-phenyl-1, 2-ethanediol
rcr	*R*-specific *c*arbonyl *r*eductase gene
SCR	*S*-specific *c*arbonyl *r*eductase
WT	wild-type

Introduction

The optically active (*R*)-1-phenyl-1, 2-ethanediol (PED) is a versatile chiral building block for the synthesis of pharmaceuticals, agrochemicals, pheromones, liquid crystals, etc. [1–4] although the chiral alcohol can be produced by fermentation with natural selected organisms including bacteria [5], yeasts [6], plants [7], tissues from several mammalian species [8], and recombinant organisms. The medium-chain carbonyl reductase from *Candida parapsilosis* (CprCR) has a high potential of biotechnological application in catalyzing the reversible reduction of 2-hydroxyacetophenone to (*R*)-PED with high yield and purity [9]. Given the importance of CprCR in organic synthesis, CprCR was purified from the primary organism and the gene *rcr* has been cloned from *C. parapsilosis*. A review on the properties of this enzyme has become recently available [10, 11]. Recently gene recombination technique offers a new choice to increase CprCR expression for improving (*R*)-PED productivity. The production of (*R*)-PED using recombinant *Escherichia coli* has been studied by several groups [11–14]. It was found that the CprCR expression level and (*R*)-PED productivity were still low. and the yield and optical purity of (*R*)-PED reached only about 61% and 81% using genetic engineered *E. coli*, respectively [11, 15].

It has been shown that each kind of microorganisms has their own favorable codons [16]. Synonymous replacement of rare codons with those used at higher frequency can improve target protein yields in host cells [17, 18] or increase the specific enzyme activity [19]. It is clear that a few codons are underrepresented. In particular, Arg codons (AGA, CGA), Ile codon (AUA), Leu codon (CUA), Gly codon (GGA), and Pro codon (CCC) are rarely used by *E. coli*. The excessive rare codon usage in the target protein has been implicated as a cause for low expression level [16, 20]. The effect seems to be most severe when multiple rare codons occur near the amino start and terminus [16, 21]. Several laboratories have shown that the yield of protein whose coding genes contain rare codons can be improved when the cognate transfer RNA is increased within the host [20, 22]. For example, in the human codon-optimized variant, codon engineering had a positive impact on the expression level and production cell lines [18]. Liu reported that optimization of rare codons use results in enhanced recombinant quinate 5-dehydrogenase expression [17, 23]. Disordered protein sequences may also affect on the protein folding, and their function in some cases mechanically uncouples structured domains, making their dynamics less constrained [24].

Based on a complete compilation of codon usage in the GenBank database (http://www.kazusa.or.jp/codon/) [25], there are six rare codons (AGA/CGA) in the *rcr* gene (1,008 bp, 336 codons) from *C. parapsilosis* (about 2% frequencies) and three successive AGAs near the amino terminus. This work reported the improved (*R*)-PED production by a codon optimized CprCR whose coding gene *rcr* was engineered by truncating its disorder sequence of 4–27 bp at 5′-terminus and adaption of codon usage bias in *E. coli*. It will provide a new method to establish an effective expression system for improving chiral alcohol productivity by codon optimization.

Materials and Methods

Materials and Microorganisms

C. parapsilosis CCTCC M203011 was obtained from the American Type Culture Collection (ATCC, USA).The organisms were cultivated as described previously [10, 26]. The enzyme and cofactors were purchased from the Sigma–Aldrich Chemical Co. Inc. The protein marker was purchased from SIBAS Biotechnology Co., Shanghai Institute of Biochemistry (Lot No. 0301). The substrate β-hydroxyacetophenone was prepared using the method described by Liese et al. [27]. The recombinant plasmids and bacterial strains containing different antibiotic resistances used in this study are listed in Table 1.

Construction of the Expression Vectors

The disorder sequence in secondary structure was predicted with the program GlobPlot (http://www.expasy.org/). In order to improve the chiral alcohol production, three codon optimized variants (Δ24, aRCR, and mRCR) were designed. For the variant *Δ24*, its disorder sequence of 4–27 bp at the 5′-terminus was truncated; for *aRCR*, its nine out of 11 rare codons were adapted according to the codon bias in *E. coli* [28]; for *mRCR*, its disorder sequence of 4–27 bp at the 5′-terminus was truncated and nine out of 11 rare codons were adapted.

The genes were generated using the oligonucleotide primers (with restriction sites underlined) in Table 1 using the overlap polymerase chain reaction (PCR) method [29]. The primers RCR-F and RCR-R were used for *rcr*, RCR-F1 and RCR-R for *Δ24*, RCR-F, F2, F3, F4 and RCR-R1, R2, R3, R4 for *aRCR*, RCR-F1, F2, F3, F4 and RCR-R1, R2, R3, R4 for *mRCR*. The pETRCR plasmid [15] was used as a template to synthesize the genes *rcr*, *Δ24*, *aRCR*, and *mRCR*. The *Sma*I-*Hin*dIII sites of the purified *rcr* gene (GenBank ID: DQ295067) and its modified sequences were inserted into the corresponding sites of pQE30a, respectively. The recombinant plasmids pQERCR, pQEΔ24, pQEaRCR, and pQEmRCR were confirmed by colony PCR and digestion with restriction enzymes *Sma*l I/*Hin*dIII. All the recombinant plasmids were then transformted into the competent *E. coli* BL21 (AI™) cells.

Expression of CprCR and its Variants

The positive transformants were grown at 37 °C in 500-ml baffled shake flasks containing 100 ml LB liquid medium. The protein expression was induced with addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 50 mmol/L $ZnCl_2$ [30–32] when the OD_{600} value of culture reached 0.6–0.8. After continuous cultivation for 8 h at 37 °C, the cells were harvested by centrifugation at 10,000×*g* for 15 min for further protein sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, purification of recombinant enzyme, and enzyme activity assay.

Purification of Recombinant Proteins

The recombinant proteins were expressed as His_6-tagged proteins in *E. coli* strain BL21 (AI™) and purified first by affinity chromatography on Ni^{2+}-Sepharose column (His-Trap Kit, Pharmacia). The pooled fractions as further loaded on a Resource Q column (1 × 1 cm) equilibrated with the buffer (20 mM Tris-HCl, pH 8.5) with an ÄKTA Protein Purifier

Table 1 Plasmids, bacterial strains, and primers used in this study.

Plasmids or strains or primers	Discription	Sources
Plasmids		
pETRCR	6.5 kb, pET21c containing *rcr*, Amp^r	This laboratory
pQE30a	3.4 kb, Amp^r	Novagen
pQERCR	4.5 kb, pQE30a containing *rcr*, Amp^r	This work
pQEΔ24	4.5 kb, pQE30a containing disorder sequence-truncated *rcr*, Amp^r	This work
pQEaRCR	4.5 kb, pQE30a containing rare codon-optimized *rcr*, Amp^r	This work
pQEmRCR	4.5 kb, pQE30a containing disorder sequence-truncated and rare codon-optimized *rcr*, Amp^r	This work
Strains		
E. coli DH5α	F-mcrA Δ(mrr-hsdRMS-mcrBC) j80 lacZΔM15Δ lacΔ 74 recA1 deoR araD139Δ(ara-leu)7697 galU galK rpsL (StrR) endA1 nupG	This laboratory
E. coli BL21-AI™	F-ompT gal dcm lon $hsdS_B(r_B^- m_B^-)$ araB::T7RNAP-tetA	Novagen
E. coli AI/ pQE30	*E. coli* with plasmid pQE30a	This work
E. coli AI/pQERCR	*E. coli* with recombinant plasmid pQERCR	This work
E. coli AI/pQEΔ24	*E. coli* with recombinant plasmid pQEΔ24	This work
E. coli AI/pQEaRCR	*E. coli* with recombinant plasmid pQEaRCR	This work
E. coli AI/ pQEmRCR	*E. coli* with recombinant plasmid pQEmRCR	This work
Primers		
RCR-F	5′-ATCGATCGCCCGGGATGTCAATTCCATCAAGC CAG-3′ (*Sma*I)	This work
RCR-R	5′-TGATAAGCTTCTATGGATTAAAAACA ACTC-3′ (*Hind*III)	This work
RCR-F1	5′-ATCGATCGCCCGGGATGTTCGTATTCAATA AGCAATCAGGACTTAAGTTGCGTAAT-3′ (*Sma*I)	This work
RCR-R1	5′-CAATCCAACAGCATCAACTTTCAACAACAA TTGACCCGCTTTAGGCTT-3′	This work
RCR-F2	5′-GCTGTTGGATTGTGTCATTCTGAT-3′	This work
RCR-R2	5′-ATTAAACGATAAATTAGGAGCACCGAGTCCCACA GGCAT-3′	This work
RCR-F3	5′-TTTAATTTGGGAGATTTGGCATTGCGTGAAATTCGT ATCTTG-3′	This work
RCR-R3	5′-ACTTCTCACAACGGGTTTAACTTTACC-3′	This work
RCR-F4	5′-AAACCCGTTGTGAGAAGTGCCAAATTG-3′	This work
RCR-R4	5′-TGATAAGCTTCTATGGATTAAAAACAACTCTAC CTTCATAAGCATTGTTTCTCAA-3′ (*Hind*III)	This work

system (Pharmacia, Uppsala, Sweden). It was followed by a Superdex 200 (HiLoad 26/60, preparation grade) chromatography in a buffer containing 20 mM Tris-HCl (pH 8.5) and 150 mM NaCl. Molecular mass of CprCR was calculated by comparing its elution volume with that of appropriate standard proteins. All mutant enzymes were purified to homogeneity as judged by Coomassie Brilliant Blue staining of SDS-PAGE gels. The extinction coefficient of 1.9 AU/M was used for all CprCR variants.

Analytical Ultracentrifugation

CprCR at a concentration of 0.5−1.0 mg/ml was used for the analytical ultracentrifugation (AUC) analysis with a buffer of 20 mM Tris-HCl (pH 8.5) and 150 mM NaCl. Sedimentation experiments were performed using an XL-A analytical ultracentrifuge (Beckman Coulter) equipped with a four-cell An-60 Ti rotor at 20 °C. Sedimentation velocity analysis was performed at 65,000 rpm. Data were analyzed with the SEDFIT program [33].

Enzyme Assay

The enzyme assay mixture in 220 mL comprised 0.2 M potassium phosphate buffer (pH 6.0), 1 mM NADH, 0.1 mM 2-hydroxyacetophenone, 2 mM $ZnSO_4$, and an appropriate amount of purified enzymes. The decrease in the concentration of NADH was recorded spectrophotometrically at 340 nm. One unit of enzyme activity is defined as the amount of enzyme catalyzing the reduction/oxidation of 1 mM of NAD(H)/min under the assay conditions. The protein concentration was determined using Bradford reagents (Bio-Rad) with bovine serum albumin as a standard [34]. The reported values represent the average of at least three independent measurements.

Biotransformation

For asymmetric reaction with the recombinant *E. coli* cells, the reaction mixture in 1 mL comprised 0.1 M potassium phosphate buffer (pH 6.5), 1 mg 2-hydroxyacetophenone, 2 mM $ZnSO_4$ and 0.1 g washed cells of the *E. coli* transformant. The product (*R*)-PED was extracted with ethyl acetate and the organic layer was used for analysis. The optical purity and yield of (*R*)-PED was determined by HPLC on a Chiralcel OB-H column (Daicel Chemical Ind. Ltd., Japan), as described previously [26].

Results

Based on codon usage bias in *E. coli* (http://www.kazusa.or.jp/codon/) [25] and disorder sequence in secondary structure predicted with the program GlobPlot (http://www.expasy.org/), three variants Δ24, aRCR, and mRCR of CprCR were designed to improve chiral alcohol production. The mutations were designed in their coding genes *Δ24*, *aRCR* and *mRCR* as follows: in *Δ24*, the 4–27 bp disorder sequence at the 5′-terminus was truncated; in a*RCR*, nine out of 11 rare codons were replaced with the synonymous ones used at the highest frequency which was determined by codon usage tabulated from the international DNA sequence databases [17, 35], in details, six AGA/CGAs and three CCCs in *rcr* were substituted by CGTs and CCTs, respectively (Fig. 1); in *mRCR*, the 4–27 bp disorder sequence at the 5′-terminus was truncated and six AGA/CGAs and three CCCs were substituted by CGTs and CCTs. The mutated genes were generated using the primers in Table 1 and recombinant plasmid pETRCR as template by SOE-PCR method [29]. Expression plasmids construction of CprCR and its variants were performed as described in the "Materials and Methods," as shown in Fig. 2. The four recombinant expression plasmids pQERCR, pQEΔ24, pQEaRCR, and pQEmRCR obtained were transformed into the competent *E. coli* BL21 (AI™) cells. Then, the positive clones *E. coli* AI/pQERCR, *E. coli* AI/pQEΔ24, *E. coli* AI/pQEaRCR, and *E. coli* AI/pQEmRCR were achieved after

Fig. 1 Codon usage analysis in the *rcr* gene sequence. Coding sequences are shown for each of the three codon variants that were expressed. Codons *marked in box* are disorder sequence (4–27 bp) was truncated, rare codons *underlined* was adapted based on the codon bias in the *E. coli* database, in details, AGA/CGAs and CCCs were substituted to CGTs and CCTs, respectively

atg tca att cca tca agc cag tac gga ttc gta ttc aat
aag caa tca gga ctt aag ttg AGA aat gat ttg cct
gtc cac aag CCC …… CCC gtg gga ctc ggt gct
cct aat tta tcg ttt aat ttg gga gat ttg gca ttg AGA
gaa att CGA atc ttg ggt agt ttt tgg gga act act
aat gat ttg gat gat gtt ttg aaa ttg gtt agt gaa ggt
aaa gtt aaa CCC gtt gtg AGA agt gcc aaa ttg
aag gaa ttg cca gag tat att gaa aaa ttg AGA aac
aat gct tat gaa ggt AGA gtt gtt ttt aat cca tag

verified by sequencing. All these genes are following lac operon for expression and under control of T5, a powerful phage promoter.

The cultivation and inducing conditions of the recombinant strains were carried out as described by Nie et al. [11], except that 50 mmol/L $ZnCl_2$ was added to the culture for inducing protein expression together with 1 mmol/L IPTG when the OD_{600} value reached

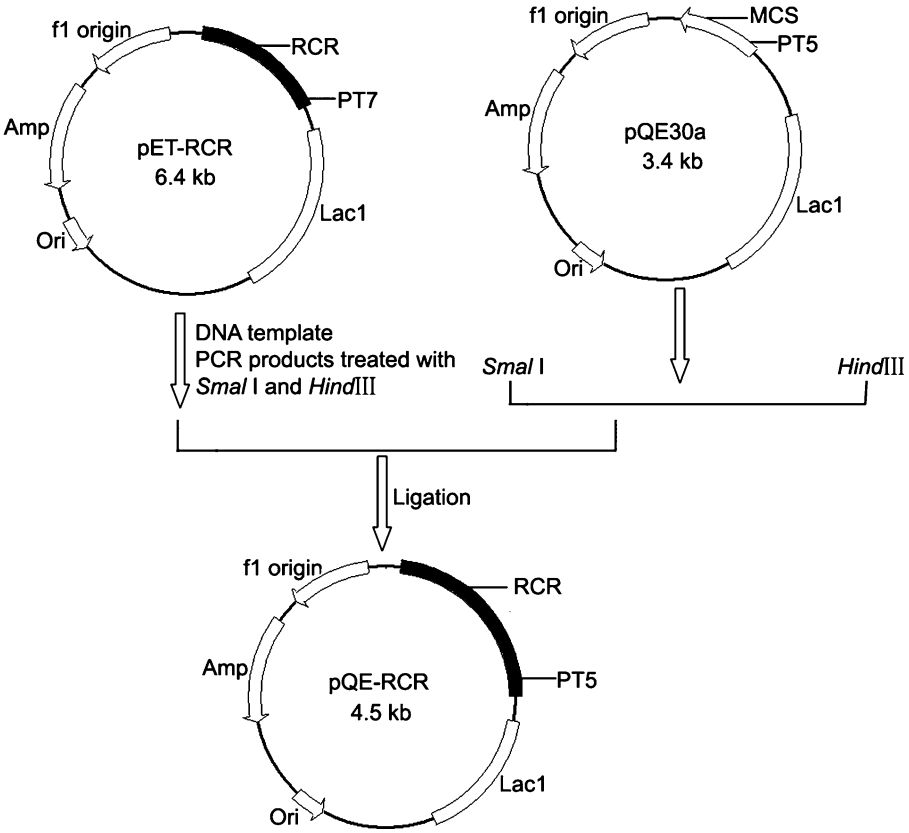

Fig. 2 The sketch map of the recombinant plasmid pQEmRCR construction. The recombinant vector pQEmRCR harbored the optimized gene *mrcr* (987 bp) in which the 4–27 bp of disorder sequence was truncated and rare codons were adapted based on codon preference in *E. coli*. The *Sma*I site in the 5′-end and *Hin*d III site in the 3′-end were created to facilitate cloning. The construction of pQERCR, pQEΔ24, and pQEaRCR was the same as pQEmRCR except that the inserted fragments were different

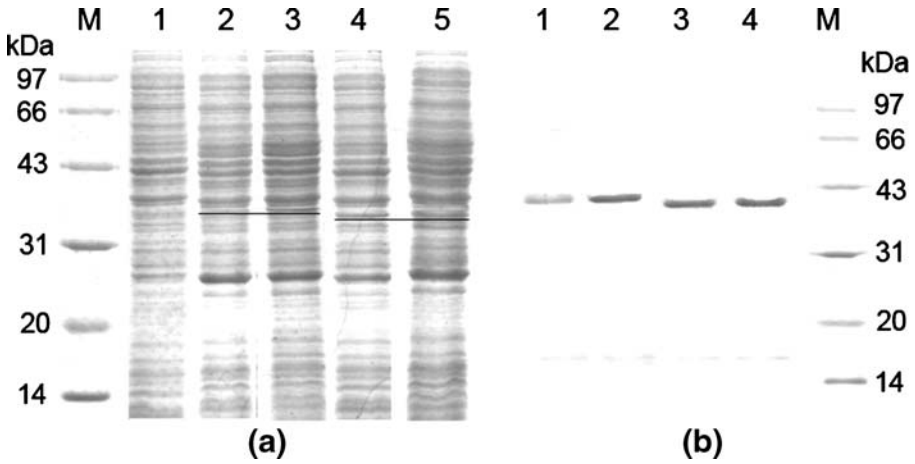

Fig. 3 **a** SDS-PAGE of crude extracts of *E. coli* cells transformed with pQERCR, pQEΔ24, pQEaRCR, and pQEmRCR plasmids. Crude extract of *E. coli* BL21 (AI™) before IPTG induction; *2* cell-free extract of *E. coli* AI/pQERCR; *3 E. coli* AI/pQEaRCR; *4 E. coli* AI/pQEΔ24; *5 E. coli* AI/pQEmRCR; *M* low molecular weight protein markers (rabbit phosphorylase B: 97.4 kDa; calf albumin: 66.2 kDa; rabbit actin: 43 kDa; carbonic anhydrase: 31 kDa; trypsin inhibitor: 20 kDa; egg white lysozyme: 14.4 kDa); **b** SDS-PAGE of purified enzymes. *Lanes*: *1* RCR; *2* aRCR; *3* Δ24; *4* mRCR; *M* low molecular weight protein markers. The target proteins were marked with *lines*. Proteins were visualized with Coomassie Brilliant Blue R-250 and destained in a 50% (*v*/*v*) methanol/10% (*v*/*v*) acetic acid solution

0.6~0.8. The protein expression was checked by SDS–PAGE analysis. The results (Fig. 3a) showed that the protein CprCR and its codon variants were all expressed in *E. coli* AI strains, and they had the molecular weights of about 36 or 37 kDa (including the additional N-terminal His_6-tag) which were consistent with their theoretical masses. The codon variants Δ24, aRCR, and mRCR were expressed at the higher levels than the wild-type (WT) in *E. coli*. And among the four types, the variant mRCR was expressed at the highest level obviously (Fig. 3a).

Because the recombinant proteins were extended with N-terminal His_6-tag, they were purified to homogeneity with a three-step procedure including the first Ni-affinity chromatography as described in "Materials and Methods." During purification, samples were analyzed for enzymatic activity by measuring the decrease in NADH fluorescence with 2-hydroxyacetophenon as substrate. After purification, one single peak of the active dehydrogenase was found on Superdex™ 200 (10/300 GL) chromatography. SDS-PAGE analysis showed that the purified CprCR and variant proteins were around 36 and 37 kDa (Fig. 3b), in agreement with their calculated molecular weights. The molecular weight distribution of all the recombinant proteins showed a single dimer peak (~72 or ~74 kDa) by AUC analysis (Fig. 4). The results suggested that the recombinant proteins were purified to homogeneity and all mutations caused no any changes in protein oligomerizations.

Using the cell-free extracts and purified proteins of wild-type and variants the specific 2-hydroxyacetophenone-reducing activity was detected. The reductive activity identification indicated all codon variants had been functionally expressed in *E. coli*. The variants Δ24, aRCR, and mRCR showed the higher enzyme activity (302.44, 346.52, 376.98 U) in the cell-free extracts, which were increase to 108.8%, 124.7%, and 135.6% of WT (Table 2). Their specific activities of cell-free extract presented increase of 5.8%, 15.4%, and 26.9%, which were similar to those of the purified enzymes (5.6%, 14.5%, and 24.3%), as shown in Table 2. The data suggested the enzyme activities of the variants were improved as well

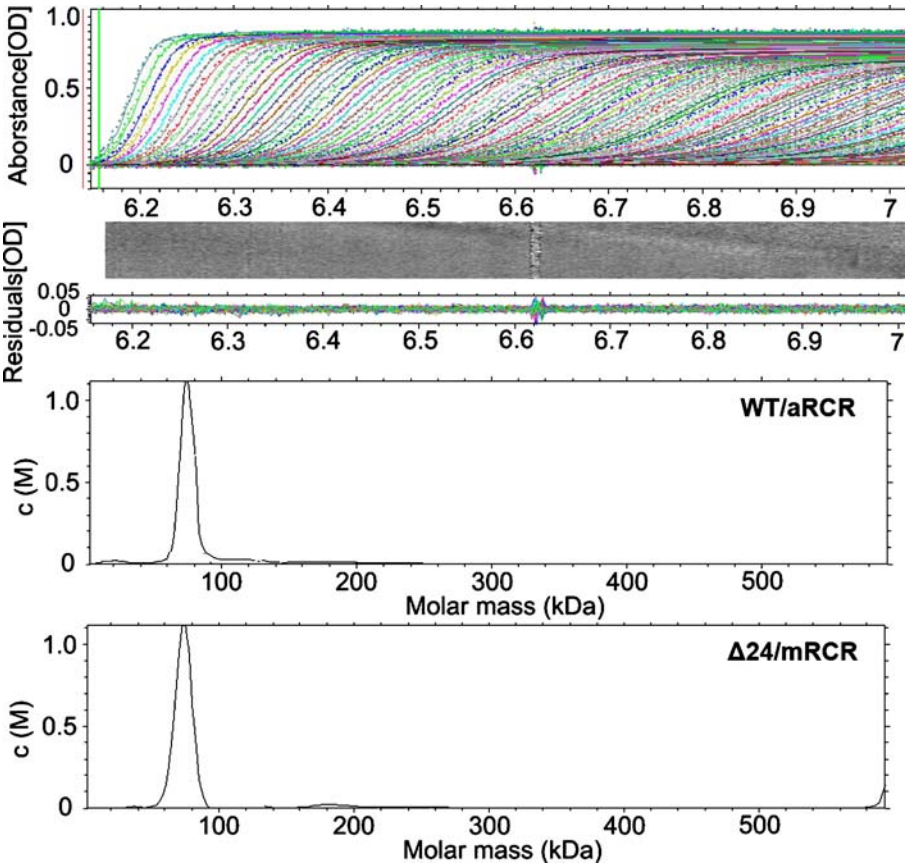

Fig. 4 Analytical ultracentrifugation analysis of purified WT CprCR and its variant enzymes. The *top three panels* suggest the sedimentation velocity data and the systematic noise decomposition. The *next panel* is the transformation to molar mass distributions from the sedimentation velocity profile: 74 kDa for WT and aRCR and 72 kDa for Δ24 and mRCR in a buffer containing 20 mM Tris (pH 8.5) and 150 mM NaCl

as their protein productions by codon optimization. And the variant mRCR showed the higher total activity and specific activity than others.

The biotransformation identification was performed using the whole cells as catalysts. When microbial cells concentration was 30% (*w*/*v*), the (*R*)-PED preparation from

Table 2 The enzyme assay of CprCR and its variant enzymes.

Samples	Cell-free extracts		Purified protein	
	Total activity (U)	Protein (mg)	Specific activity (U/mg)	Specific activity (U/mg)
pQE30a	0	0	0	0
pQERCR	277.95	534.5	0.52	14.92
pQEΔ24	302.44	549.9	0.55	15.75
pQEaRCR	346.52	587.3	0.59	17.08
pQEmRCR	376.98	571.2	0.66	18.54

2-hydroxyacetophenone was examined. The results showed that three variants Δ24, aRCR, and mRCR presented the higher molar conversion yield and enantiomeric excess than WT (Table 3). When 2-hydroxyacetophenone concentration was 5 g/L, they showed the high molar conversion yields of 68.3%, 78.5%, and 86.4% with increase of 8.1%, 24.0%, and 36.5% than WT, respectively. The higher enantiomeric excess (e.e.) of 85.3 ~ 93.6% for (*R*)-PED was also obtained by the variants than WT CprCR (80.8%), as shown in Table 3. Among the four types, the variant mRCR possessed the highest stereoselectivity of bioconversion. The chiral alcohol production was improved by the codon-optimized variants.

Discussion

It was reported that codon usage difference between the target protein source and expression host affects on the protein translation rate, which is frequently the case for low level of the recombinant protein expression [22, 28, 36, 37]. Beier et al. and Chen et al. reported that the high-level expression and specific enzyme activity of alcohol dehydrogenases were achieved by synonymous substitution of codon bias [19, 23, 38]. The other elements such as the splice signals, secondary structure, GC content, codon contexting, and regulatory sequence can also regulate translation rate and protein expression levels. Secondary structure may have physical implications on gene transcript that can impede the progression on the translation machinery. For example, disordered protein sequences make their dynamics less constrained [24]. So codon engineering could change these components, regulate the translation frequency, and improve the protein production [23, 39] and enzyme activity [19].

Previously, CprCR expressions in *E. coli* were attempted by Nie et al. [11]. Yet, the challenges were still presented to produce recombinant CprCR at an acceptable level. So, the high CprCR production with full activity has been a major problem and needs to be solved urgently. By analysis of total codon usages in the *rcr* gene, there are 11 rare codons and three of them are consecutive AGA's near the 3′-terminus, and the latter may more seriously interfere the protein translation rate. The crystal structure of (*S*)-specific carbonyl reductase (also known as *S*-specific alcohol dehydrogenase) from *C. parapsilosis* was determined (PDB ID: 3CTM) by our labs [40]. It was found that the truncation of N-terminal 31-residue would not affect the enzyme activity [40]. This work reported positive effects of protein expression and enzyme activity by truncation of disorder sequence or codon usage adaptation.

The variant aRCR presented the higher specific enzyme activity than the wild-type although they had the same amino acid sequence, which may be due to the more quick and correct protein-folding in the variant aRCR than WT. The variant mRCR was designed that 4–27 bp disorder sequence at the 5′-terminus was truncated and six AGAs /CGAs and three

Table 3 Comparison on stereospecific reduction of 2-hydroxyacetophenone between CprCR and its variants.

Samples	Molar yield (%)	Enantiomeric excess (%)
pQE30a	0	0
pQERCR	63.3	80.8
pQEΔ24	68.3	85.3
pQEaRCR	78.5	89.2
pQEmRCR	86.4	93.6

CCCs were adapted to CGTs and CCTs. The variant mRCR presented an increase of 35.6% in total activity of cell-free extract as compared to WT and showed similar increase in the specific activity between their cell-free extracts and purified proteins. This suggested codon optimization played an important role in a high protein production as reported by other groups [16, 17, 19, 23]. Furthermore, the codon variants showed higher bioconversion of (*R*)-PED than WT with β-hydroxyacetophenone as substrate. When microbial cells concentration was 30% (*w*/*v*), the molar conversion yield and e.e. of the mRCR variant reached 86.4% and 93.6%, which were increased 36.5% and 15.8% than those of WT at a high substrate concentration of 5 g/L. The high bioconversion ability may stem of the initial reaction rate for its high protein production and enzyme activity of the mRCR variant. The enhanced protein expression and enzyme activity by codon optimization may not only facilitate further studies of CprCR in the near future but can also allow possible production of pure (*R*)-PED in industry.

Acknowledgements This project was supported in part from the National Science Foundation of China (No. 20776060), the National Key Basic Research and Development Program of China (973 Program Nos. 2003CB716008 and 2009CB724706) and Hi-Tech Research and Development Program of China (863 Program No. 2007AA02Z226). This work was also carried out as part of the Project for the Ministry of Education, People's Republic of China under the Program for Changjiang Scholars, Innovative Research Team in University (PCSIRT, IRT0532), and the Program of Introducing Talents of Discipline to Universities 111-2-06.

References

1. Schmid, A., Dordick, J. S., Hauer, B., Kiener, A., Wubbolts, M. G., & Witholt, B. (2001). *Nature*, *409*, 258–268. doi:10.1038/35051736.
2. Kroutil, W., Mang, H., Edegger, K., & Faber, K. (2004). *Current Opinion in Chemical Biology*, *8*, 120–126. doi:10.1016/j.cbpa.2004.02.005.
3. Panke, S., Held, M., & Wubbolts, M. G. (2004). *Current Opinion in Biotechnology*, *15*, 272–279. doi:10.1016/j.copbio.2004.06.011.
4. Schoemaker, H. E., Mink, D., & Wubbolts, M. G. (2003). *Science*, *299*, 1694–1697. doi:10.1126/science.1079237.
5. Itoh, N., Mizuguchi, N., & Mabuchi, M. (1999). *Journal of Molecular Catalysis. B, Enzymatic*, *6*, 41–50. doi:10.1016/S1381-1177(98)00118-0.
6. Cappaert, L., & Larroche, C. (2004). *Biocatalysis and Biotransformation*, *22*, 291–296. doi:10.1080/10242420400011992.
7. Melis, L. E. D., Whiteman, P. H., & Stevenson, T. W. (1999). *Plant Science*, *143*, 173–182. doi:10.1016/S0168-9452(99)00044-8.
8. Wsól, V., Skálová, L., Szotáková, B., Trejtnar, F., & Kvasnicková, E. (1999). *Chirality*, *11*, 505–509. doi:10.1002/(SICI)1520-636X(1999)11:5/6<505::AID-CHIR25>3.0.CO;2-5.
9. Cao, L., Lee, J., Chen, W., & Wood, T. K. (2006). *Biotechnology and Bioengineering*, *94*, 522–529. doi:10.1002/bit.20860.
10. Yang, M., Xu, Y., Mu, X. Q., & Xiao, R. (2006). *Chinese Chemical Industry and Engineering Progress*, *25*, 1082–1088.
11. Nie, Y., Xu, Y., Wang, H. Y., Xu, N., Xiao, R., & Sun, Z. H. (2008). *Biocatalysis and Biotransformation*, *26*, 210–219. doi:10.1080/10242420701661537.
12. Ernst, M., Kaup, B., Muller, M., Bringer-Meyer, S., & Sahm, H. (2005). *Applied Microbiology and Biotechnology*, *66*, 629–634. doi:10.1007/s00253-004-1765-5.
13. Yamamoto, H., Kawada, N., Matsuyama, A., & Kobayashi, Y. (1999). *Bioscience, Biotechnology, and Biochemistry*, *63*, 1051–1055. doi:10.1271/bbb.63.1051.
14. Yamamoto, H., Matsuyama, A., & Kobayashi, Y. (2002). *Bioscience, Biotechnology, and Biochemistry*, *66*, 481–483. doi:10.1271/bbb.66.481.
15. Nie, Y., Xu, Y., Mu, X. Q., Wang, H. Y., Yang, M., & Xiao, R. (2007). *Applied and Environmental Microbiology*, *73*, 3759–3764. doi:10.1128/AEM.02185-06.
16. Zhang, S. P., Zubay, G., & Goldman, E. (1991). *Gene*, *105*, 61–72. doi:10.1016/0378-1119(91)90514-C.

17. Sharp, P. M., & Li, W. H. (1987). *Nucleic Acids Research*, *15*, 1281–1295. doi:10.1093/nar/15.3.1281.
18. Carton, J. M., Sauerwald, T., Hawley-Nelson, P., Morse, B., Peffer, N., Beck, H., et al. (2007). *Protein Expression and Purification*, *55*, 279–286. doi:10.1016/j.pep.2007.05.017.
19. Chen, H. Z., Wang, H. X., Yang, H. N., Wu, W. S., & q Ni, Y. (2005). *Chinese Journal of Biochemistry and Molecular Biology*, *21*, 171–175.
20. Ikemura, T. (1981). *Journal of Molecular Biology*, *146*, 1–21. doi:10.1016/0022-2836(81)90363-6.
21. Kane, J. F. (1995). *Current Opinion in Biotechnology*, *6*, 494–500. doi:10.1016/0958-1669(95)80082-4.
22. Sorensen, M. A., Kurkland, C. G., & Pedersen, S. (1989). *Journal of Molecular Biology*, *207*, 365–377. doi:10.1016/0022-2836(89)90260-X.
23. Liu, L. B., Liu, Y., He, H. Q., Li, Y. H., & Xu, Q. S. (2006). *Sheng Wu Gong Cheng Xue Bao*, *22*, 198–203.
24. Charles, R., Dunker, A. K., & Sharkhnovich, E. (1999). *Pacific Symposium on Biocomputing*, *4*, 517–519.
25. Nakamura, S., Oda, M., Kataoka, S., Ueda, S., Uchiyama, S., Yoshida, T., et al. (2006). *The Journal of Biological Chemistry*, *281*, 31876–31884. doi:10.1074/jbc.M604226200.
26. Nie, Y., Xu, Y., & Mu, X. Q. (2004). *Organic Process Research & Development*, *8*, 246–251. doi:10.1021/op0341519.
27. Liese, A., Karutz, M., amphuis, J., Wandreyand, C., & Kragl, U. (1996). *Biotechnology and Bioengineering*, *51*, 544–550. doi:10.1002/(SICI)1097-0290(19960905)51:5<544::AID-BIT6>3.0.CO;2-C.
28. Ikemura, T., Nakamura, Y., & Gojobori, T. (2000). *Nucleic Acids Research*, *28*, 292. doi:10.1093/nar/28.1.292.
29. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., & Pease, L. R. (1989). *Gene*, *77*, 51–59. doi:10.1016/0378-1119(89)90358-2.
30. Nordling, E., Jornvall, H., & Persson, B. (2002). *European Journal of Biochemistry*, *269*, 4267–4276. doi:10.1046/j.1432-1033.2002.03114.x.
31. Bertini, I., Lanini, G., Luchinat, C., Haas, C., Maret, W., & Zeppezauer, M. (1987). *European Biophysics Journal*, *14*, 431–439. doi:10.1007/BF00254867.
32. Pauly, T. A., Ekstrom, J. L., Beebe, D. A., Chrunyk, B., Cunningham, D., Griffor, M., et al. (2003). *Structure* (*London*, *England*), *11*, 1071–1085. doi:10.1016/S0969-2126(03)00167-9.
33. Pekar, A., & Sukumar, M. (2007). *Analytical Biochemistry*, *367*, 225–237. doi:10.1016/j.ab.2007.04.035.
34. Bradford, M. M. (1976). *Analytical Biochemistry*, *72*, 248–254. doi:10.1016/0003-2697(76)90527-3.
35. Nakamura, Y. G. T., & Ikemura, T. (2000). *Nucleic Acids Research*, *28*, 292. doi:10.1093/nar/28.1.292.
36. Dos Reis, M., Savva, R., & Wernisch, L. (2004). *Nucleic Acids Research*, *32*, 5036–5044. doi:10.1093/nar/gkh834.
37. Oxender, D. L., Zurawski, G., & Yanofsky, C. (1979). *Proceedings of the National Academy of Sciences of the United States of America*, *76*, 5524–5528. doi:10.1073/pnas.76.11.5524.
38. Beier, D. R., & Young, E. T. (1982). *Nature*, *300*, 724–728. doi:10.1038/300724a0.
39. Trinh, R., Gurbaxani, B., Morrison, S. L., & Seyfzadeh, M. (2004). *Molecular Immunology*, *40*, 717–722. doi:10.1016/j.molimm.2003.08.006.
40. Zhang, R. Z., Zhu, G. Y., Zhang, W. C., Cao, S., Ou, X. J., Li, X. M., et al. (2008). *Protein Science*, *17*, 1412–1423.