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Asymmetric reduction of ketones using recombinant *E. coli* cells that produce a versatile carbonyl reductase with high enantioselectivity and broad substrate specificity

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Abstract—The gene encoding a versatile biocatalyst that shows high enantioselectivity for a variety of ketones, SCR (*Saccharomyces cerevisiae* carbonyl reductase), has been identified, cloned, and expressed in *Escherichia coli*. Two types of expression systems with high NADPH-regenerating capacities have been constructed. One is the tandem system, where the genes encoding SCR and GDH (glucose dehydrogenase) are located in the same plasmid, and the other is the two-plasmid system, where each of the SCR and GDH genes is located in separate plasmids that can coexist in one *E. coli* cell. Asymmetric reduction of ketones with the recombinant *E. coli* cells gave synthetically useful 20 alcohols, 11 of which were enantiomerically pure. The productivity of one of these products was as high as 41 g/L.

1. Introduction

Asymmetric synthesis with biocatalysts has attracted much attention from the viewpoint of green chemistry.[1–2] Among them, versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously have found broad utility because this characteristic feature is useful for asymmetric synthesis of various compounds needed in a laboratory as well as a quite important compound.[3] The discovery and development of a new versatile biocatalyst with high efficiency is therefore an important subject, contributing to the significant enlargement of the library of synthetically useful biocatalysts.

Carbonyl reductases from various microorganisms have been used to prepare optically active alcohols from carbonyl compounds.[2] A *Saccharomyces cerevisiae* (bakers' yeast) carbonyl reductase (SCR) previously reported by us showed catalytic activity for various ketones, such as α-chloro ketones, α-acetoxy ketones, α-keto esters, β-keto esters, γ-keto esters, and β-diketones, and 13 out of 20 alcohols obtained had enantiomeric purities of >98% ee.[4a,b] Despite the potential of this enzyme as a versatile biocatalyst, its utility was restricted by its low expression level in *S. cerevisiae* and the laborious purification procedure. These drawbacks can be overcome by constructing an SCR gene expression system. The synthetic power of recombinant carbonyl reductases from various origins has recently been demonstrated.[5–13] In particular, a number of recombinant whole-cell reductions with high efficiency have been reported.[6–8] Recently, we have identified and cloned the gene coding for SCR, and have investigated the capabilities of the recombinant SCR by conducting both enzymatic and whole-cell reductions of various ketones.[4c] Here we report asymmetric reduction of various ketones with recombinant *Escherichia coli* expressing the gene encoding SCR, demonstrating the utility and power of the versatile biocatalyst.

2. Results

2.1. Identification, cloning, and expression of the gene

The amino-acid sequence analysis of purified SCR suggested strongly that the gene encoding SCR is *Gre2* (*YOL151w*). Stewart and co-workers have reported a library of *S. cerevisiae* reductases, which includes Gre2.[5] In this study, the SCR gene was PCR-cloned from the genomic DNA of an *S. cerevisiae* strain that we have used previously, and an expression plasmid, pESCR, was constructed (Fig. 1). As shown in Table 1, enzymatic activity (90 U per 1 g of wet cells) of SCR heterologously expressed in *E. coli* BL21(DE3) harboring pESCR was 800-fold higher than that (0.11 U per 1 g of wet cells) of SCR in *S. cerevisiae*.

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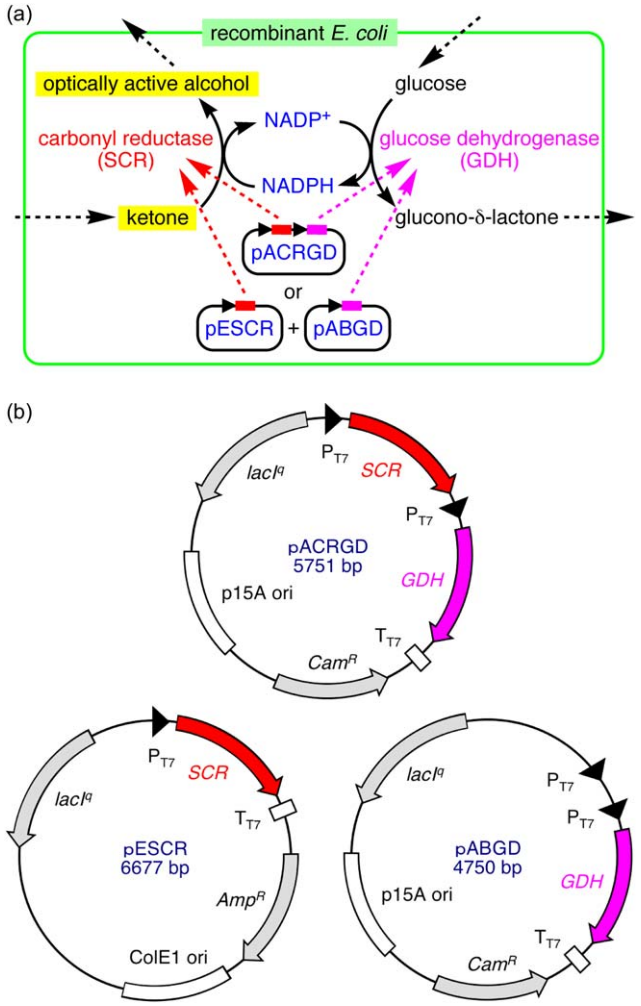

Figure 1. (A) Recombinant *E. coli* cell coexpressing SCR and GDH genes to produce optically active alcohols. In this paper, *E. coli* harboring pACRGD is called the tandem system, while that harboring pESCR and pABGD is called the two-plasmid system. (B) Structures of expression plasmids used in this study. *SCR*: SCR gene, *GDH*: GDH gene, Amp^R: ampicillin-resistance gene, Cam^R: chloramphenicol-resistance gene, P_{T7}: T7 promoter, and T_{T7}: T7 terminator.

Cofactor regeneration is essential for high productivity of alcohols. Kataoka and co-workers have successfully employed a glucose dehydrogenase (GDH) from *Bacillus megaterium* to regenerate NADPH in vivo.[6] We constructed an expression plasmid having the SCR and GDH genes (tandem system), pACRGD, as shown in Figure 1. pACYCDuet-1 (Novagen) was selected as an expression vector because it has two multiple cloning sites, each preceded by a T7 promoter, which is suitable for coexpression of two genes. The GDH gene was subcloned into pACYCDuet-1 to give pABGD, into which the SCR gene was subcloned to give pACRGD. Using *E. coli* BL21(DE3) harboring pACRGD, a high level of production of SCR and GDH was confirmed by SDS-PAGE (Fig. 2), and the SCR and GDH activities were 60 and 15,000 U per 1 g of wet cells, respectively (Table 1).

Table 1. Enzymatic activity per 1 g of wet cells

Host	Plasmid	SCR activity (U)	GDH activity (U)
E. coli BL21(DE3)	pESCR	90	—
E. coli BL21(DE3)	pACRGD	60	15,000
E. coli BL21(DE3)	pESCR+pABGD	100	5800
Bakers' yeast	—	0.11	—

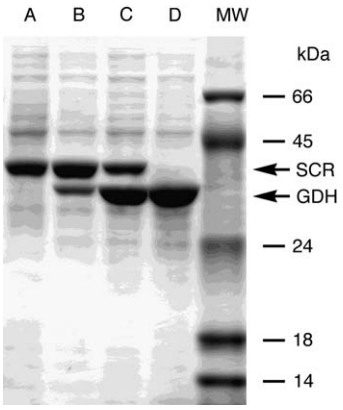

Figure 2. SDS-PAGE of the cell lysates of *E. coli* BL21(DE3) transformed with (A) pESCR, (B) pESCR+pABGD, (C) pACRGD, and (D) pABGD after cultivation and induction.

2.2. Asymmetric reduction of ketones with the tandem system

The whole-cell reductions of various ketones were conducted with the recombinant *E. coli* (tandem system). The experimental procedure is described in Section 5.7, and the results are summarized in Figure 3. Although 3 mmol of ketone was typically used, the amount of a very reactive one, ethyl pyruvate (**7a**), was increased to 6 mmol, and that of some ketones with modest reactivity was decreased to 1 mmol. Although the reaction did proceed without addition of $NADP^+$, the addition of a catalytic amount (0.4 mol %) of $NADP^+$ increased the conversion (not optimized). Figure 3 clearly shows that the inherent characteristic features of SCR are retained in the recombinant SCR. The absolute configurations of all the 16 alcohols **1b**–**16b** obtained with the recombinant *E. coli* were the same as those obtained previously with SCR purified from *S. cerevisiae*. The enantiomeric purities in the former are as high as those in the latter in most cases. Both aliphatic and aromatic ketones were successfully reduced. Not only enantioselectivity but also regioselectivity for 2,4-hexanedione (**16a**) was complete, with the less hindered carbonyl group being reduced exclusively, as observed previously.[4a,b]

As a control, *E. coli* BL21(DE3) harboring pACYCDuet-1 was used, and very low conversions (0% conversion in many cases) were confirmed by ^1H NMR in all cases. This suggests that although *E. coli* produces its own enzymes, its ketone-reducing and/or cofactor-regenerating capacity for 1–6 mmol of ketone is very low. Furthermore, *E. coli* BL21(DE3) strains harboring either pESCR or pABGD, which lack either GDH or SCR gene, were also examined for α-acetoxyacetophenone (**5a**) under the same reaction conditions, which resulted in very low conversions (4% and 0%). All these results indicate that SCR is responsible for the reduction of all the ketones and that both SCR and GDH are essential for the high-turnover catalysis.

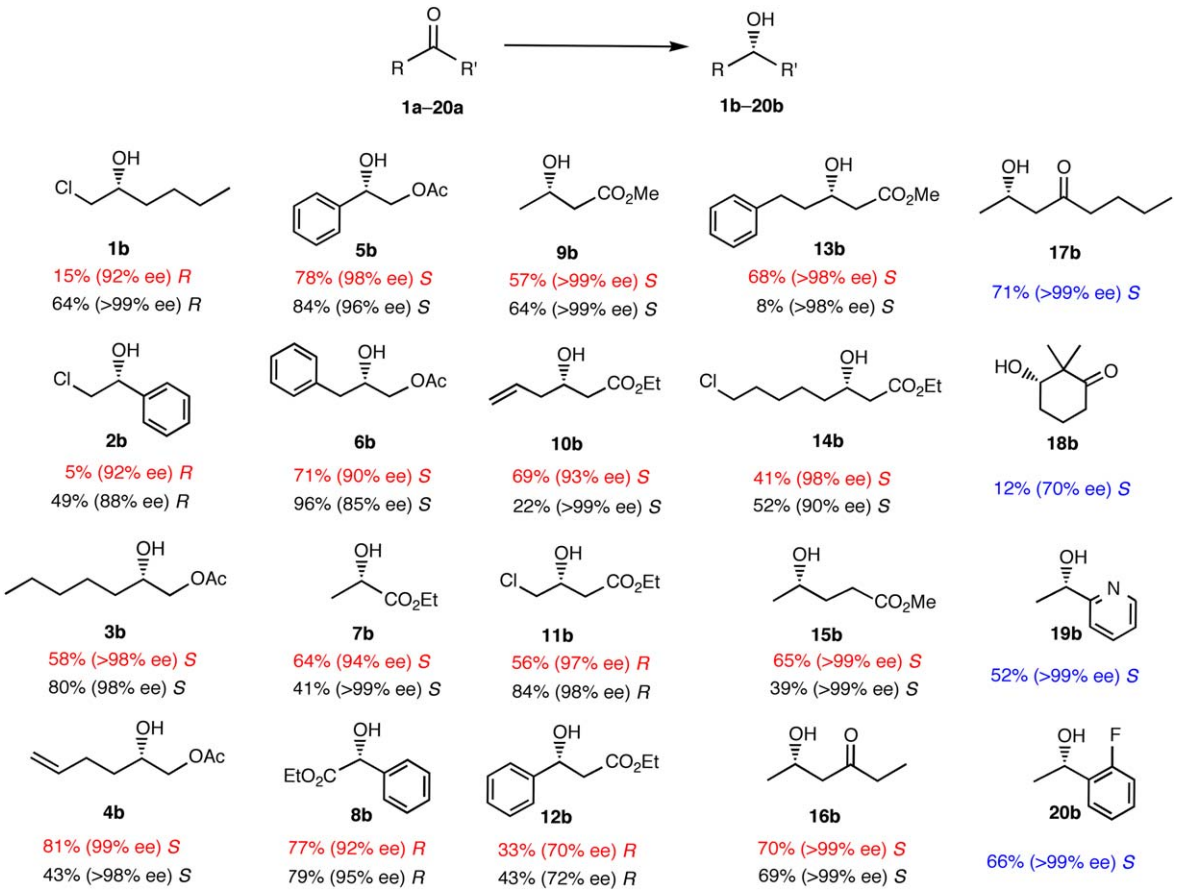

Figure 3. Asymmetric reduction of ketones with SCR. The data are shown in the following order: isolated yield, enantiomeric purity, and absolute configuration. The results obtained with recombinant *E. coli* (tandem system) and that (two-plasmid system) are shown in red and blue, respectively. The results obtained with SCR purified from bakers' yeast are taken from the lit.[4b] and are shown in black. Conditions for the tandem system: substrate (3.0 mmol for **1a**–**6a**, **8a**–**9a**, **11a**, and **15a**–**16a**, 6.0 mmol for **7a**, 1.0 mmol for **10a** and **12a**–**14a**), wet cells of *E. coli* BL21(DE3) harboring pACRGD (2 g), glucose (6.0 mmol), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 50 mL), 30 °C. Conditions for the two-plasmid system: substrate (4.0 mmol for **17a**, 1.0 mmol for **18a**–**20a**), wet cells of *E. coli* BL21(DE3) harboring pESCR and pABGD (2 g), glucose (2 equiv), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL), 30 °C. The pH of the reaction mixture was kept constant by adding 2 N NaOH. The experimental procedure is described in Section 5.7.

Mori and co-workers have demonstrated that (*S*)-3-hydroxy-2,2-dimethylcyclohexanone ((*S*)-**18b**) can be used in the total synthesis of more than 10 natural products.[14] Based on the stereochemical trend shown in Figure 3, we expected that (*S*)-**18b** might be obtained by the asymmetric reduction of 2,2-dimethylcyclohexane-1,3-dione (**18a**) using SCR. As a result of the reduction with the recombinant *E. coli* (tandem system), unfortunately, we detected only a trace amount of product by ^{1}H NMR.

2.3. Asymmetric reduction of ketones with the two-plasmid system

In view of the very low conversion of **18a**, we tried to improve the reducing power by increasing the expression level of SCR. We had two plasmids that can coexist in one *E. coli* cell, pESCR and pABGD (Fig. 1). Because the copy number of the former is higher than that of the latter, *E. coli* cells harboring the two plasmids (two-plasmid system) may produce SCR more efficiently than that harboring pACRGD (tandem system). As expected, enzymatic activity (Table 1) and SDS-PAGE (Fig. 2) clearly indicate that SCR is produced more efficiently in the two-plasmid system than in the tandem system. Because the whole-cell reduction was found to be faster in higher concentrations, the amount of buffer solution was decreased from 50 mL to 10 mL (data not shown). When the time courses of the reduction of a substrate with moderate reactivity, 2,4-octanedione (**17a**), were compared (Fig. 4), the two-plasmid system showed the best performance, giving (*S*)-2-hydroxy-4-octanone ((*S*)-**17b**) with complete enantioselectivity and regioselectivity in 71% isolated yield, which corresponds to the relatively high productivity of 41 g/L. In contrast, *E. coli* transformants harboring either pESCR or pABGD resulted in very low conversions (Fig. 4), which indicates again that both SCR and GDH are essential for the high-turnover catalysis.

Using the two-plasmid system, we conducted the reduction of cyclic β-diketone **18a** again, and found that the reaction proceeded slowly. The product (*S*)-**18b** was isolated in 12% yield with 70% ee (Fig. 3). Despite the long reaction time (24 h), the substrate **18a** remained in the reaction mixture (18% conversion). Using the two-plasmid system, we further examined other substrates **19a** and **20a**, which contain nitrogen and fluorine atoms, respectively, and obtained optically pure alcohols (*S*)-**19b** and (*S*)-**20b** successfully (Fig. 3).

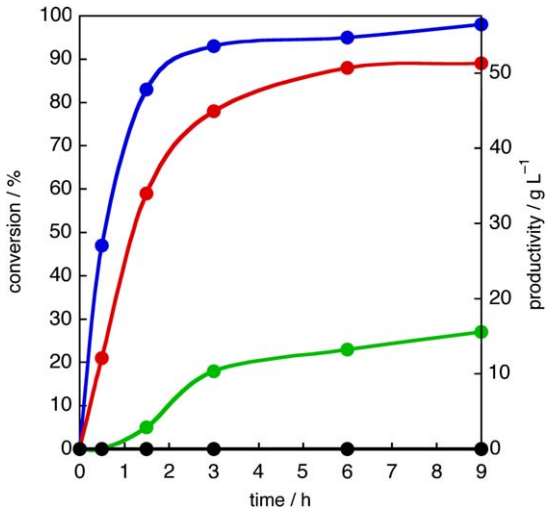

Figure 4. Time courses for reductions of **17a** with *E. coli* BL21(DE3) transformants harboring pACRGD (red), both pESCR and pABGD (blue), pESCR (green), or pABGD (black). Conditions: **17a** (4.0 mmol), wet cells of *E. coli* BL21(DE3) transformant (2 g), glucose (8.0 mmol), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL), 30 °C. The pH of the reaction mixture was kept constant by adding 2 N NaOH. The reactions were monitored by ^{1}H NMR.

3. Discussion

Versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously are very useful for asymmetric syntheses of various chiral compounds. In this study, the gene encoding a versatile biocatalyst, SCR, has been identified, cloned, and expressed in *E. coli*. Two types of systems coproducing SCR and GDH (tandem and two-plasmid systems) have been constructed (Fig. 1), and these recombinant *E. coli* cells afforded 20 optically active alcohols, 11 of which had enantiomeric purities of >98% ee (Fig. 3). Some of the obtained alcohols have been used in the total synthesis of natural products and biologically active compounds: e.g., (*R*)-**11b** for carnitine,[15] (*R*)-**12b** for fluoxetine,[16] (*S*)-**13b** for dihydrokawain,[17] (*S*)-**14b** for xestospongin A,[18] and (*S*)-**15b** for pyrenophorin.[19] (*S*)-**18b** is an extremely useful intermediate for the total synthesis of various natural products such as polygodial, dihydroactinidiolide, *O*-methyl pisiferic acid, juvenile hormone III, and (−)-K-76.[14] SCR, which can produce these practical alcohols, is a synthetically useful, versatile biocatalyst.

Coenzyme regeneration is essential for an efficient catalytic reduction of ketones.[6] NADPH is oxidized to $NADP^+$ by the SCR-catalyzed reduction of ketone, while $NADP^+$ is reduced back to NADPH by the GDH-catalyzed oxidation of glucose (Fig. 1). This catalytic cycle is realized only when both the two enzymes are produced in vivo. Indeed, only the tandem and two-plasmid systems, coexpressing the two genes, gave the product in good yields (Fig. 4). In the latter case, the relatively high productivity (41 g/L based on the isolated yield) of (*S*)-**17b** was achieved; the productivity based on the conversion was 56 g/L. In contrast, *E. coli* strain harboring pESCR showed much lower conversions, which suggests that $NADP^+$ formed by the SCR-catalyzed reaction was slowly reduced to NADPH by small amount of enzymes originating from *E. coli* itself. *E. coli* strain harboring pABGD exhibited 0% conversion because of the absence of SCR.

The two-plasmid system showed a higher reducing power than the tandem system did. Figure 4 shows that the initial rate of the former is about 2-fold faster than that of the latter. This is a result of the modulation of the production level of SCR and GDH. Because glucose is the natural substrate for GDH, GDH activity in the tandem system is sufficiently high (Table 1). In contrast, because the ketones used in this study are unnatural substrates for SCR, SCR activity is low in the tandem system. Obviously, a lower amount of GDH and a higher amount of SCR would be ideal. In the two-plasmid system, the copy number of pESCR in the cell is higher than that of pABGD. Indeed, SDS-PAGE (Fig. 2) indicated that SCR was produced more efficiently in the two-plasmid system than in the tandem system, and in the former case, improved SCR activity (100 U per 1 g of wet cells) and reduced GDH activity (5800 U per 1 g of wet cells) were observed (Table 1). This modulation of the production level of SCR and GDH led to the higher reducing power of the two-plasmid system.

Some products were isolated in low yields with incomplete enantiomeric purities (Fig. 3). For example, the useful synthetic intermediate (*S*)-**18b** was isolated in 12% yield with 70% ee despite the use of the two-plasmid system. The structural alteration of SCR itself may be effective for further improvement. For example, SCR can be used as a parent enzyme for directed evolution or rational design approaches.

4. Conclusion

The gene encoding a versatile biocatalyst that shows high enantioselectivity for a variety of ketones, SCR, has been identified, cloned, and expressed in *E. coli*. Two types of expression systems with high NADPH-regenerating capacities have been constructed. One is the tandem system, where the genes encoding SCR and GDH are located in the same plasmid, and the other is the two-plasmid system, where each of the SCR and GDH genes is located in separate plasmids that can coexist in one *E. coli* cell. These recombinant *E. coli* cells coproducing SCR and GDH are easy-to-use, synthetically useful biocatalysts to give a variety of optically active alcohols, and 11 out of 20 alcohols obtained had enantiomeric purities of >98% ee. When the two coexpression systems were compared in terms of the conversion of 2,4-octanedione, the two-plasmid system showed better performance, giving (*S*)-2-hydroxy-4-octanone with complete enantioselectivity and regioselectivity in 71% isolated yield, which corresponds to the productivity of 41 g/L.

5. Experimental

The pressed bakers' yeast was purchased from Oriental Yeast, and SCR from bakers' yeast was purified to homogeneity as described previously.[4a] Two internal amino-acid sequences of SCR were determined at APRO Life Science Institute, and the homology search was performed by BLAST analysis. All the DNA manipulations and bacterial transformations were carried out according to the standard

protocols or manufacturers' instructions, unless otherwise stated.[20] *E. coli* JM109 (Toyobo) and pGEM-T (Promega) were routinely used as a host and a cloning vector, respectively, and *E. coli* BL21(DE3) (Stratagene) was used as a host for pET-11a (Stratagene), pACYCDuet-1 (Novagen), and their derivatives. *TaKaRa Ex Taq* DNA Polymerase was purchased from Takara Bio. Restriction enzymes were purchased from Toyobo, Stratagene, or Roche, and T4 DNA ligase was purchased from Toyobo or New England Biolabs. Agarose for electrophoresis was purchased from Nacalai Tesque. QIAprep Spin Miniprep Kit, QIAquick PCR Purification Kit, QIAquick Nucleotide Removal Kit, and QIAquick Gel Extraction Kit (Qiagen) were routinely used for DNA isolation and purification. Synthetic oligonucleotides were obtained from Sigma Genosys Japan. DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) at Advanced Science Research Center of Okayama University, and then analyzed with MacVector 7.0 (Oxford Molecular). All the genes cloned into the plasmids were fully sequenced in both directions. NADPH and $NADP^+$ were purchased from Oriental Yeast. The amount of proteins was determined by the method of Bradford using BSA as the standard.[21] TLC was performed on Merck silica gel 60 F_{254}, and silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). ^{1}H NMR spectra were measured in $CDCl_3$ at 600, 500, 300, or 200 MHz.

5.1. Cloning of the SCR gene and construction of an expression plasmid, pESCR

Two internal peptides of the purified SCR were sequenced: AAWEFLEENR and DLLIPAVDGVK, both of which hit Gre2 (YOL151w) of *S. cerevisiae* by BLAST analysis. The SCR gene (*GRE2*, *YOL151w*) was amplified by PCR from the genomic DNA of bakers' yeast purchased from Oriental Yeast. The primers used for PCR are as follows: SC-CR-3F (5′-CGATTTTCAAACAAACAGATAGCAG-3′) and SC-CR-4R (5′-AAAATGCGCAGAGATGTACT AGATG-3′), which correspond to 5′- or 3′-noncoding regions encompassing the gene. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, genomic DNA (400 ng), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 1 min. The amplified DNA fragment was directly cloned into pGEM-T to yield pGSCR from transformed *E. coli* JM109 cells. The SCR gene was subcloned into an expression vector, pET-11a. The primers used for PCR are as follows: SC-CR-5F (5′-ATCACACGCCCTTA *CATATG*TCAG-3′) and SC-CR-6R (5′-CG*GGATCC*TTAA AGTTTATATTCTGCCCTC-3′), where the restriction sites for *Nde*I and *Bam*HI are italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGSCR (100 pg), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 1 min. The amplified DNA fragment was digested with *Nde*I and *Bam*HI, and then ligated into pET-11a that had been treated with the same restriction enzymes. *E. coli* BL21(DE3) harboring pESCR was obtained by transformation of the competent cells.

5.2. Construction of a coexpression plasmid having SCR and GDH genes, pACRGD

Genomic DNA of *B. megaterium* NBRC (formerly IFO) 15308 was isolated according to the lit.[22] The glucose dehydrogenase (GDH) gene was amplified by PCR with the primers BM-GD-1F (5′-ATGTATACAGATTTAAAAGAT AAAGTAGTT-3′) and BM-GD-2R (5′-TTAGCCTCTTC CTGCTTGG-3′).[23] The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, genomic DNA (80 ng), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 50 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was directly cloned into pGEM-T to yield pGBGD from transformed *E. coli* JM109 cells. The GDH gene was subcloned into an expression vector, pACYCDuet-1. The primers used for PCR are as follows: BM-GD-10F (5′-CCATGGCCGCGGGCATA-3′) and BM-GD-11R (5′-CGC*GGTACC*GATTTTAGCCTCTTC-3′), where the restriction site for *Kpn*I is italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGBGD (3.7 ng), 2.5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 48 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was digested with *Nde*I and *Kpn*I, and then ligated into pACYCDuet-1 that had been treated with the same restriction enzymes. *E. coli* BL21(DE3) harboring pABGD was obtained by transformation of the competent cells. The SCR gene was then subcloned into pABGD. The primers used for PCR are as follows: SC-CR-10F (5′-CACGCCC TTAA*TCATGA*GTGTTTTTGTTTC-3′) and SC-CR-6R (5′-CG*GGATCC*TTAAAGTTTATATTCTGCCCTC-3′), where the restriction sites for *Rca*I and *Bam*HI are italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGSCR (370 ng), 2.5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was digested with *Rca*I and *Bam*HI, and then ligated into pABGD that had been treated with *Nco*I and *Bam*HI. *E. coli* BL21(DE3) harboring pACRGD was obtained by transformation of the competent cells.

5.3. Preparation of *E. coli* harboring pESCR and pABGD

E. coli BL21(DE3) harboring pABGD was transformed with pESCR and grown on an LB plate containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). The transformation was confirmed by agarose gel electrophoresis and enzymatic activity.

5.4. Sole expression of SCR gene

After *E. coli* BL21(DE3) cells harboring pESCR were grown in LB medium (3 mL) containing ampicillin (100 μg/mL) at

37 °C for 16 h with shaking at 230 rpm, 2 mL of the culture was transferred to the same medium (200 mL) in a 1-L Erlenmeyer flask. The culture was shaken at 200 rpm at 37 °C, and IPTG (0.1 mM) was added when OD_{600} reached 0.6–0.8. The cells were further incubated at 30 °C for 16 h with shaking at 200 rpm, harvested by centrifugation (9000 rpm, 4 °C, 5 min), and washed with 0.1 M phosphate buffer (pH 7.0, 50 mL). The cell pellet was stored at −20 °C until it was used.

5.5. Coexpression of SCR and GDH genes

E. coli BL21(DE3) cells harboring pACRGD (tandem system) were grown in LB medium (3 mL) containing chloramphenicol (34 μg/mL) at 37 °C for 15 h with shaking at 230 rpm, and those harboring pESCR and pABGD (two-plasmid system) were grown in LB medium (3 mL) containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL) in the same way. The culture (3 mL) was transferred to the same medium (300 mL) in a 1-L Erlenmeyer flask, and shaken at 200 rpm at 37 °C. IPTG (0.1 mM) was added when OD_{600} reached 0.6–0.8. The cells were further incubated at 28 °C for 18 h with shaking at 200 rpm, harvested by centrifugation (7000 rpm, 4 °C, 10 min), and washed with 0.1 M phosphate buffer (pH 7.0, 50 mL). The cell pellet was stored at −20 °C until it was used for asymmetric reduction.

5.6. Enzyme activity

SCR activity was determined as described previously,[4a] after cell disruption by sonication followed by centrifugation. SCR activity of 1.0 U is defined as the amount of enzyme that oxidizes 1.0 μmol of NADPH per minute at 25 °C.[4a] GDH activity was determined as follows. *E. coli* cells having GDH activity were lysed by sonication for 1 min in an ice bath (×10). Centrifugation (20,000 rpm, 4 °C, 30 min) gave a cell-free extract (CFE) as the supernatant. After appropriately diluted, CFE (300 μL) was added to a solution (2.5 mL) of glucose (100 mM) and $NADP^+$ (2.0 mM) in 60 mM Tris buffer (pH 8.0) in a UV-cuvette thermostated at 25 °C. After the solution was quickly shaken, the reaction rate was measured by following the increase in the absorbance of NADPH at 340 nm as a function of time. In this paper, 1.0 U of GDH activity is defined as the amount of enzyme that reduces 1.0 μmol of $NADP^+$ per minute at 25 °C under the above reaction conditions.

5.7. General procedure for whole-cell asymmetric reduction

In the case of the tandem system, to a mixture of glucose (1.08 g, 6.0 mmol), $NADP^+$ (10 mg, 12 μmol), and *E. coli* BL21(DE3) cells harboring pACRGD (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 50 mL) was added the substrate (typically, 3.0 mmol). In the case of the two-plasmid system, to a mixture of glucose (0.36 g, 2.0 mmol), $NADP^+$ (10 mg, 12 μmol), and *E. coli* BL21(DE3) cells harboring pESCR and pABGD (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 10 mL) was added the substrate (typically, 1.0 mmol). The mixture was stirred with a magnetic stirrer in a water bath at 30 °C. The progress of the reaction was monitored by TLC and/or ^{1}H NMR. In the two-plasmid system, the pH of the reaction mixture was kept constant by adding 2 N NaOH. After an appropriate reaction time, the cells were precipitated by centrifugation (10,000 rpm, 4 °C, 10 min), and solid NaCl was added. The products were extracted with EtOAc or Et_2O several times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product was purified by column chromatography and/or distillation. Although the control reaction with *E. coli* BL21(DE3) harboring pACYCDuet-1 was performed in a similar way, the isolation of the product was not attempted because of a very low conversion as determined by ^{1}H NMR.

5.8. Identification of products

Alcohols **1b**–**16b** were characterized as described previously.[4a,b] **17b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.90 (t, J=7.8 Hz, 3H), 1.18 (d, J=6.0 Hz, 3H), 1.31 (sext, J=7.8 Hz, 2H), 1.56 (quint, J=7.8 Hz, 2H), 2.42 (t, J=7.8 Hz, 2H), 2.50 (dd, J=9.0, 17.4 Hz, 1H), 2.60 (dd, J=3.0, 17.4 Hz, 1H), 3.14 (br s, 1H), 4.20–4.23 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 13.8, 22.26, 22.27, 25.7, 43.3, 50.3, 63.8, 212.6; $[\alpha]_D^{27}$ +64.3 (*c* 1.84, $CHCl_3$), >99% ee (*S*), lit.[24] $[\alpha]_D$ +50 (*c* 1.8) for (*S*)-**17b** with >99% ee. **18b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.13 (s, 3H), 1.18 (s, 3H), 1.61 (br d, J=4.2 Hz, 1H), 1.64–1.70 (m, 1H), 1.81–1.86 (m, 1H), 2.00–2.07 (m, 2H), 2.37–2.45 (m, 2H), 3.70–3.73 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 19.5, 20.5, 22.7, 28.8, 37.1, 51.1, 77.7, 214.5; $[\alpha]_D^{27}$ +15.0 (*c* 0.80, $CHCl_3$), 70% ee (*S*), lit.[14] $[\alpha]_D^{21}$ +23.0 (*c* 2.0, $CHCl_3$) for (*S*)-**18b** with 96.0–98.8% ee. **19b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.50 (d, J=6.6 Hz, 3H), 4.30 (br s, 1H), 4.89 (q, J=6.6 Hz, 1H), 7.18–7.21 (m, 1H), 7.27–7.28 (m, 1H), 7.67–7.70 (m, 1H), 8.53 (d, J=4.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.2, 68.8, 119.8, 122.2, 136.8, 148.1, 163.0; $[\alpha]_D^{25}$ −27.4 (*c* 1.03, $CHCl_3$), >99% ee (*S*), lit.[25] $[\alpha]_D$ −24.5 (*c* 0.50, $CHCl_3$) for (*S*)-**19b** with 98% ee. **20b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.52 (d, J=6.6 Hz, 3H), 1.95 (br s, 1H), 5.20 (q, J=6.6 Hz, 1H), 7.00–7.03 (m, 1H), 7.14–7.16 (m, 1H), 7.22–7.26 (m, 1H), 7.47–7.50 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.0, 64.6, 115.3 (d, J=21.9 Hz), 124.3 (d, J=3.5 Hz), 126.6 (d, J=4.7 Hz), 128.8 (d, J=8.0 Hz), 132.6 (d, J=13.3 Hz), 159.7 (d, J=245.7 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −121.1 (m); $[\alpha]_D^{25}$ −47.8 (*c* 1.46, $CHCl_3$), >99% ee (*S*), lit.[26] $[\alpha]_D^{21}$ +47.2 (*c* 1.35, $CHCl_3$) for (*R*)-**20b** with 97% ee.

5.9. Determination of enantiomeric purities

The enantiomeric purities of the obtained alcohols **2b**, **3b**, **5b**–**9b**, **12b**, **13b**, **15b**, and **16b** were determined as reported previously.[4a,b] The enantiomeric purities of **1b** (acetate form), **17b** (acetate form), **18b** (acetate form), **19b**, and **20b** were determined by capillary GC with a CP-cyclodextrin-β-2,3,6-M-19 column (Chrompack, ϕ 0.25 mm×25 m). GC for **1b** (acetate form): Inj. 250 °C, Col. 80 °C, Det. 220 °C, (*R*) 32.7 min, (*S*) 37.8 min. GC for **17b** (acetate form): Inj. 250 °C, Col. 110 °C, Det. 220 °C, (*S*) 30.2 min, (*R*) 31.6 min. GC for **18b** (acetate form): Inj. 250 °C, Col. 110 °C, Det. 220 °C, (*S*) 45.0 min, (*R*) 47.5 min. GC for **19b**: Inj. 300 °C, Col. 70 °C, Det. 250 °C, (*R*) 82.6 min, (*S*) 85.4 min. GC for **20b**: Inj. 300 °C, Col. 99 °C, Det. 250 °C, (*R*) 24.2 min, (*S*) 27.4 min. Alcohol **4b** was

converted to the corresponding tosylate, which was analyzed by HPLC with a chiral column (Daicel Chemical Industries): Chiralpak AD-H, hexane/*i*-PrOH=9/1, 0.5 mL/min, 254 nm, (*S*) 21.2 min, (*R*) 22.1 min. The enantiomeric purities of **10b**, **11b**, and **14b** were determined by 600 MHz ^{1}H NMR after conversion to the corresponding MTPA esters.

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