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Production of chiral compound using recombinant *Escherichia coli* cells co-expressing reductase and glucose dehydrogenase in an ionic liquid/water two phase system

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ABSTRACT

(*S*)-3-Chloro-1-phenyl-1-propanol ((*S*)-CPPO) is a useful chiral building block for the synthesis of anti-depressant drugs. The yeast reductase, YOL151W, evidences enantioselective reduction activity, converting 3-chloro-1-phenyl-1-propanone (3-CPP) into (*S*)-CPPO. *Escherichia coli* whole cells co-expressing YOL151W and *Bacillus subtilis* glucose dehydrogenase were employed for the synthesis of CPPO following permeabilization treatment. A reaction system employing these recombinant *E. coli* whole cells could convert 30 mM 3-CPP enantioselectively into (*S*)-CPPO. In an effort to enhance substrate solubility and to prevent substrate/product inhibition during the enzyme reaction process, a variety of ionic liquids were tested and [Bmim][NTf_2] was ultimately selected for the ionic liquid/water two phase system. Tween 40 was added to accomplish the efficient mixing of the two phases. Using these recombinant *E. coli* whole cells and the [Bmim][NTf_2]/water two phase system, 100 mM (*S*)-CPPO was generated with an enantiomeric excess of >99%.

1. Introduction

Enantioselective enzymatic reductions are important reactions in the asymmetric syntheses of pure chiral alcohols, which are frequently employed as pharmaceutical intermediates [1–3]. Some enantioselective biotransformations of ketone substrates have been conducted using *Saccharomyces cerevisiae* (Baker's yeast) reductase enzymes. Enzymatic reduction reactions require stoichiometric quantities of the NAD(P)H cofactor. However, owing to the relatively high cost of NAD(P)H, the reductase reaction should be conducted in combination with cofactor regeneration systems [4–8]. Cofactor regeneration can be achieved via an 'enzyme-coupled' [9,10] or 'substrate-coupled' reaction [11]. Between them, the 'enzyme-coupled' reaction utilized the second enzyme for cofactor regeneration, and is thus used more frequently. Furthermore, *Escherichia coli* whole cells co-expressing a variety of reductases and dehydrogenases have been previously constructed, and are frequently employed in the production of a variety of chiral compounds [12,13]. In these cases, *E. coli* cells must be rendered permeable to the substrates and cofactors by controlled treatment with EDTA and toluene, while maintaining the necessary enzymes within the cells [14–16].

Enantiopure (*R*)- or (*S*)-3-chloro-1-phenypropanol are useful building blocks of fluoxetine [1], tomoxetine [1], and nisoxetine [17], which are prescription anti-drugs employed in the treatment of greater depressive disorders [2,3]. Fluoxetine is a member of a class of medications referred to as selective serotonin reuptake inhibitors (SSRI) [18,19]. In the previous literature, yeast reductase YOL151W was shown to be able to convert 3-chloro-1-phenyl-1-propanone (3-CPP) exclusively into (*S*)-3-chloro-1-phenyl-1-propanol ((*S*)-CPPO) [20]. In the experiment, commercial *Thermoplasma acidophilum* glucose dehydrogenase (GDH) was employed for cofactor regeneration. One problem with this system was that the low solubility of 3-CPP in the aqueous phase maintained the yield of (*S*)-CPPO at a level below 30 mM.

Ionic liquids are a class of solvents which are nonvolatile and nonflammable, and dissolve a broad range of chemical compounds, including a variety of hydrophobic compounds [21–24]. Additionally, ionic liquids have been identified as biocompatible solvents that support a highly efficient whole cell biocatalytic process in a biphasic ionic liquid/water system [25–27].

In this study, permeabilized *E. coli* cells expressing yeast reductase YOL151W and *Bacillus subtilis* GDH were employed to carry out the enantioselective reduction of 3-CPP into (*S*)-CPPO. Additionally, a variety of ionic liquid/water two phase systems were applied to the *E. coli* whole cell reaction system to increase the solubility of 3-CPP. We determined that the ionic liquid, [Bmim][NTf_2], could be utilized in this coupling reaction and dramatically increase the

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concentration of substrate and the yield of the target compound, (*S*)-CPPO.

2. Experimental

2.1. Chemicals

3-Chloro-1-phenyl-1-propanol (3-CPP), (*S*)-3-chloro-1-phenyl-1-propanol ((*S*)-CPPO), NADPH, and $NADP^+$ were purchased from Sigma–Aldrich Co. (St. Louis, MO, U.S.A.). 1-*n*-Butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]), 1-*n*-butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide ([Bmim][NTf_2]), 1-methyl-3-methylimidazolium methylsulfate ([Mmim][$MeSO_4$], 1-ethyl-3-methylimidazolium tetrafluoroborate ([Emim][BF_4]) were purchased from C-TRI (Suwon, Korea). All other chemicals were of analytical grade.

2.2. Reductase and glucose dehydrogenase activity assay

Reductase activity was evaluated at 30 °C by measuring the decrease in absorbance at 340 nm for 10 min with a spectrophotometer. The reaction mixture (1 mL) consisted of 1 mM 3-CPP (100 mM stock in DMSO), 0.2 mM NADPH, 100 mM Tris–HCl buffer (pH 7.5), and 5 μL of permeabilized cells. One unit of enzyme was defined as the quantity of enzyme required to catalyze the oxidation of 1 μmol NADPH in 1 min at 30 °C.

The oxidation reaction mixture (1 mL) of glucose dehydrogenase consisted of 10 mM glucose, 0.2 mM $NADP^+$, 100 mM Tris–HCl buffer (pH 7.5), and 1 μL of permeabilized cells. The reaction rate was monitored with a spectrophotometer on the basis of the increase in absorbance at 340 nm for 10 min at 30 °C. One unit of enzyme was defined as the quantity required to reduce 1 μmol of $NADP^+$ in 1 min at 30 °C.

2.3. Preparation of permeabilized cells and enzymatic coupling reaction

Recombinant *E. coli* BL21 (DE3) cells harboring one plasmid (pETR151, pACGDH, pACR151-GDH) or two plasmids (pETR151 plus pACGDH) were cultured at 18 °C in 600 mL of LB medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter) containing 100 μg/mL of ampicillin (for pETR151) or 170 μg/mL of chloramphenicol (for pACGDH or pACR151-GDH). When the $OD_{600\,nm}$ reached 0.5, isopropyl thio-β-D-galactoside was added to a final concentration of 1 mM and cultured for an additional 24 h. The cultured cells were then harvested via centrifugation (6000 × *g*, 10 min) and resuspended in 12 mL of a 100 mM Tris–HCl buffer (pH 7.5). Toluene (0.1%, v/v) was added, and the mixture was shaken for 10 min at 300 rpm at 30 °C. Permeabilized cells were harvested via centrifugation (6000 × *g* for 10 min) at 4 °C.

For the coupling reaction using the whole-cell systems, 30 mM 3-CPP, 1 mM NADPH, 45 mM glucose, and 10 units (calculated on the basis of reductase activity) of the permeabilized cells harboring one plasmid (pETR151 or pACR151-GDH) or two plasmids (pERT151 plus pACGDH) were mixed in a total volume of 10 mL of Tris–HCl buffer (100 mM, pH 7.5), and the mixture was incubated at 30 °C. The pH of the reaction mixture was monitored with a pH meter and maintained at 7.0–7.5 via the addition of 1 M NaOH.

Four hundred microliter aliquots of the reaction mixture were sampled, mixed with 1.2 mL of ethyl acetate, and centrifuged for 10 min at 12,000 × *g*. The upper phase (1 mL) was obtained, combined with $MgSO_4$, filtered, and then dried via evaporating the organic solvent using Vacuum Centrifugal Evaporator (CVE-2000, EYELA, Tokyo, Japan). The sample was resuspended in ethyl acetate and analyzed with an HPLC system equipped with a CHIRALPAK IB column (Daicel Chemical Industries, Ltd., Tokyo, Japan). *n*-Hexane and 2-propanol (95:5, v/v) were used as the mobile phase at a flow rate of 0.8 mL/min. Relative quantities of 3-CPP and (*R*)- and (*S*)-CPPO were calculated based on the peak area, which was appropriately calibrated with standards of known concentration. Enantiomeric excess (e.e.) values were calculated from the alcohol products.

2.4. Selection of suitable ionic liquids and detergents

Biotransformation reactions using the *E. coli* cells harboring pETR151 plus pACGDH were conducted in ionic liquid/water systems. The ionic liquids employed in this research included [Bmim][PF_6], [Bmim][NTf_2], [Mmim][$MeSO_4$], and [Emim][BF_4]. The two-phase system was composed of 800 μL of Tris–HCl buffer (100 mM, pH 7.5) and 200 μL of each ionic liquid. To the reaction system, 30 mM 3-CPP, 1 mM NADPH, 45 mM glucose, 0.5% Tween 40 and permeabilized *E. coli* cells (corresponding to 3 units reductase) were added, and the reaction mixtures were incubated at 30 °C with shaking at 300 rpm. After 6 h, the quantity of (*S*)-CPPO generated was measured via HPLC.

To select a suitable detergent, the bioconversion reaction was conducted using various detergents, including Triton X-100, Tween 20, Tween 40, and Tween 80. The two phase system was composed of 800 μL of Tris–HCl buffer (100 mM, pH 7.5) and 200 μL of [Bmim][NTf_2]. To this reaction system, 50 mM 3-CPP, 1 mM NADPH, 75 mM glucose, 0.5% of various detergents and permeabilized *E. coli* cells (3 units reductase) were added, and the reaction mixtures were incubated at 30 °C with shaking at 300 rpm. After 12 h, the quantity of (*S*)-CPPO produced was measured via HPLC.

To determine an appropriate concentration of Tween 40, the bioconversion reaction was conducted with 0–5% of Tween 40. To the same two-phase system, 100 mM 3-CPP, 1 mM NADPH, 150 mM glucose, 0–5% of Tween 40 and permeabilized *E. coli* cells (3 units reductase) were added and the reaction mixtures were incubated at 30 °C with shaking at 300 rpm. After 24 h, the quantity of (*S*)-CPPO produced was measured by HPLC.

Each sample was centrifuged for 10 min at 12,000 × *g* and a portion (50 μL) of the lower phase was mixed with 150 μL of ethyl acetate, then filtered and dried via evaporating the organic solvent using Vacuum Centrifugal Evaporator. The sample was analyzed using an HPLC system as previously described.

2.5. (S)-CPPO production by whole cell-[Bmim][NTf_2] system

The final bioconversion reaction conditions were as follows: 100 mM 3-CPP, 1 mM NADPH, 150 mM glucose, 4% Tween 40, and permeabilized *E. coli* cells containing pETR151 plus pACGDH (30 units reductase activity) were added to the two-phase reaction system containing 8 mL of Tris–HCl buffer (100 mM, pH 7.5) and 2 mL of [Bmim][NTf_2]. The reaction mixture was incubated for 14 h at 30 °C and the pH was monitored and maintained within 7.0–7.5 via the addition of 1 M NaOH. The quantities of 3-CPP and (*S*)-CPPO in the reaction mixture were analyzed by HPLC as previously described.

The partition coefficient of the 3-CPP and (*S*)-CPPO between [Bmim][NTf_2] and water phases was measured as described in Supplementary data 1. Based on these results, the concentration of 3-CPP and (*S*)-CPPO produced during the bioconversion time course was calculated.

3. Results and discussion

3.1. Enzyme activity assay of E. coli whole cells

In a previous study, the yeast reductase YOL151W was generated in *E. coli* BL21 (DE3) cells and the isolated reductase was employed for the production of (*S*)-CPPO [20]. In that

Table 1
Enzyme activity of recombinant *E. coli* whole cells.

Plasmid	Reductase activity (U/mL)	GDH activity (U/mL)
pETR151	10.89 ± 0.73	0.25 ± 0.06
pACGDH	0.50 ± 0.004	61.02 ± 0.73
pACR151-GDH	1.38 ± 0.02	113.96 ± 0.85
pETR151 + pACGDH	2.86 ± 0.21	45.78 ± 0.33

experiment, a reductase YOL151W-glucose dehydrogenase (commercial enzyme from *T. acidophilum*) coupling reaction system was employed for the regeneration of the NADPH cofactor. Recently, recombinant *E. coli* cells co-expressing both reductase YOL151W and *B. subtilis* glucose dehydrogenase were developed [28]; the YOL151W gene was inserted downstream of the T7 promoter in the pET22 vector (pETR151), whereas the *B. subtilis* GDH gene was inserted downstream of the T7 promoter in the pACYCDuet vector (pACGDH). Additionally, both YOL151W and *B. subtilis* GDH genes were sequentially inserted downstream of each T7 promoter in pACYCDuet-1 vector (pACR151-GDH) [28].

In this study, we attempted to apply the above *E. coli* whole cell system for the production of (*S*)-CPPO (Fig. 1). Each cell free extracts of *E. coli* cells carrying one vector or two vectors were analyzed (Supplementary data 2). YOL151W was generated either with His-tag (pETR151) or without His-tag (pACR151-GDH). The molecular masses (39 kDa and 41 kDa, respectively) corresponded well with the molecular masses (38,168 and 39,688, respectively) calculated on the basis of the protein sequences. The molecular mass (~33 kDa) of *B. subtilis* GDH observed on SDS–PAGESDS–PAGE was, on the other hand, slightly higher than the calculated one (28,175).

The relative quantities of the expressed soluble enzymes differed somewhat among them (Supplementary data 2), although their gene expressions were regulated strictly by the T7 promoters. In the one-vector system (pACR151-GDH), the quantity of expressed GDH was higher than that of YOL151W. In the two-vector system (pETR151 plus pACGDH), on the contrary, YOL151W was produced at much higher levels than GDH. This latter result might have been attributable to the fact that the copy number (15–25 per cell) of the pET22 vector (pBR322 replication origin) was higher than that (10–12 per cell) of the pACYCDuet-1 vector (P15A replication origin).

The intact recombinant *E. coli* whole cells evidenced little reductase and/or GDH activity (not shown data). The recombinant *E. coli* cells were subsequently permeabilized using 0.1% (v/v) toluene and the whole cells' reductase and GDH activities were assayed (Table 1). The *E. coli* BL21 (DE3) cells harboring pETR151 or pACGDH revealed that the wild-type *E. coli* cells evidenced very low GDH activity (0.25 U/mL) and reductase activity toward 3-CPP (0.50 U/mL). *E. coli* cells with the one-vector system (pACR151-GDH) and the two-vector system (pETR151 plus pACGDH) exhibited both reductase and GDH activities, as anticipated from the SDS–PAGESDS–PAGE results. In both cases, the GDH activities were much higher than reductase activities, which suggested that reductase activity would function as a limiting factor in the coupling reaction. Accordingly, between the two systems, the two-vector system appeared to be superior to the one-vector system, as the former evidenced higher reductase activity (2.86 U/mL) than the latter (1.38 U/mL).

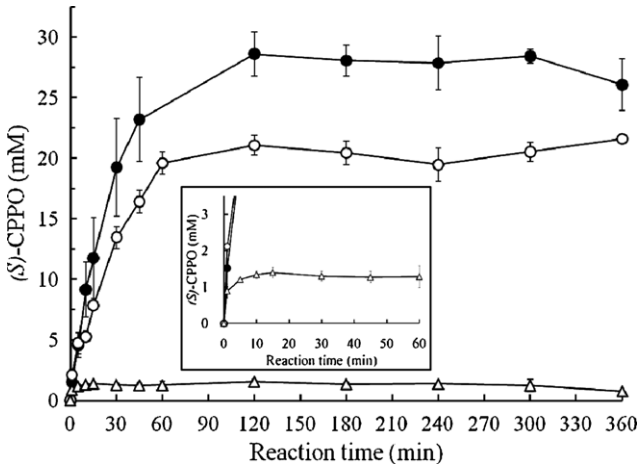

Fig. 2. Production of (*S*)-CPPO. Bioconversion reactions were conducted using permeabilized *E. coli* whole cells and the levels of (*S*)-CPPO were measured over the time course. In the initial reaction mixture (10 mL), 30 mM 3-CPP, 45 mM glucose, 1 mM NADPH, and 10 units (calculated on the basis of reductase activity) of each permeabilized cells were added and incubated at 30 °C. The inlet box showed the amount of (*S*)-CPPO produced using *E. coli* cells harboring pETR151. (○) the amount of (*S*)-CPPO produced via the one plasmid (pACR151-GDH) system, (●) the amount of (*S*)-CPPO produced via the two plasmid (pETR151 plus pACGDH) system, (△) the amount of (*S*)-CPPO produced via the pETR151 plasmid system.

3.2. Coupling reaction with E. coli whole cells

In a previous study, the recombinant reductase YOL151W and commercial *T. acidophilum* glucose dehydrogenase were shown to produce 30 mM (*S*)-CPPO via the 3-step feeding of 10 mM 3-CPPO substrate [20]. In this study, the above-described permeabilized *E. coli* cells harboring one vector (pETR151 or pACR151-GDH) or two vectors (pETR151 plus pACGDH) were employed for the target conversion reaction (Fig. 2). In this case, 30 mM 3-CPP was added to the reaction mixture at the initial stage. *E. coli* cells harboring pETR151 produced only approximately 1 mM of (*S*)-CPPO, which was similar to the quantity (1 mM) of exogenously-added NADPH. This result shows that, in this case, no coupling reaction was conducted. *E. coli* cells harboring pACR151-GDH generated approximately 20 mM of (*S*)-CPPO within 60 min in a pH-controlled reaction. This result implied that 3-CPP, glucose, and NADPH entered into the permeabilized cells and that the coupling reactions of YOL151W and GDH occurred continuously in the cells. When *E. coli* cells harbor-

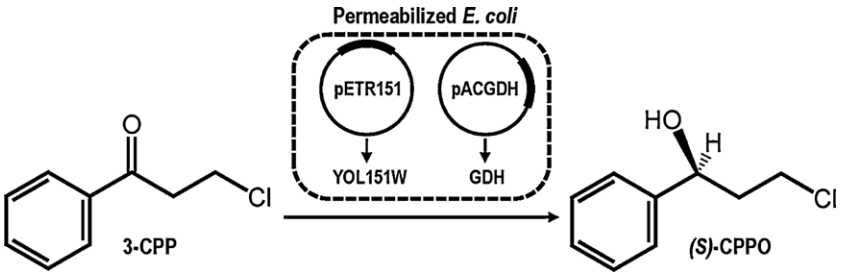

Fig. 1. Schematic reduction of 3-CPP into (*S*)-CPPO. In the *E. coli* cell, YOL151W and GDH were expressed from the pETR151 and pACGDH vectors, respectively. In the permeabilized *E. coli* cell, YOL151W reductase catalyzed the enantioselective reduction of 3-CPP into (*S*)-CPPO. NADPH used in the reduction reaction was supplied by the oxidation reaction of GDH.

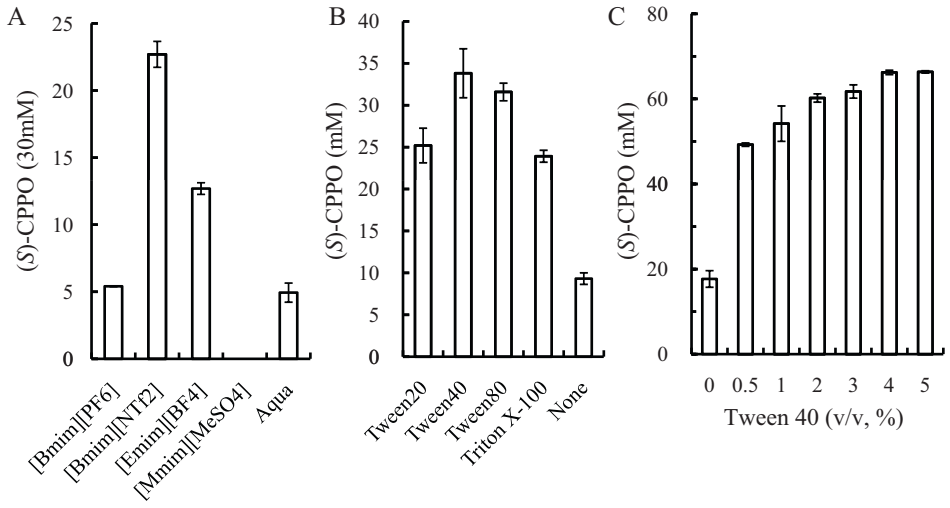

Fig. 3. Effects of various ionic liquids and detergents. (A) Effects of various ionic liquids on the whole cell biphasic reduction were studied. The reaction mixtures contained 30 mM 3-CPP, 45 mM glucose, 1 mM NADPH, 0.5% Tween 40, and 3 reductase units of *E. coli* cells (pETR151 plus pACGDH). After 6 h of reaction, the amount of (*S*)-CPPO was measured. (B) The effect of various detergents was studied. The reaction mixtures contained 50 mM 3-CPP, 75 mM glucose, 1 mM NADPH, 20% [Bmim][NTf_2], 0.5% detergents, and 3 reductase units of *E. coli* cells (pETR151 plus pACGDH). After 12 h of reaction, the amount of (*S*)-CPPO was measured. (C) The effects of various concentrations of Tween 40 were assessed. The reaction mixtures contained 100 mM 3-CPP, 150 mM glucose, 1 mM NADPH, 20% [Bmim][NTf_2], 0-5% Tween 40, and 3 reductas units of *E. coli* cells (pETR151 plus pACGDH). After 24 h of reaction, the amount of (*S*)-CPPO was measured.

ing two vectors (pETR151 plus pACGDH) were used, the produced (*S*)-CPPO reached a concentration of 27 mM within 120 min. This result demonstrated that the two-vector system showed higher initial conversion rate than the one-vector system did, as we had anticipated from the enzyme activities (Table 1).

3.3. Selection of ionic liquids suitable for whole cell reaction

Although the permeabilized *E. coli* cells harboring pETR151 plus pACGDH could successfully produce (*S*)-CPPO via the coupling reaction, the concentration of (*S*)-CPPO produced was limited to less than 30 mM. The low yield seemed to be caused by inactivation of the enzyme in the presence of high substrate concentration [20]. In an effort to increase substrate solubility, ionic liquid solvents were employed in this reaction mixture. In the previous literature, [Bmim][PF_6] and [Bmim][NTf_2] were used in the enantioselective reduction of ketones and octanone by Baker's yeast and alcohol dehydrogenases [22–26,29]. In this study, four different ionic liquids were tested for their suitability in this system. Among them, [Bmim][PF_6] and [Bmim][NTf_2] were hydrophobic ionic liquids and [Emim][BF_4] was less hydrophobic, and [Mmim][$MeSO_4$] was the hydrophilic solvent. As described in Section 2, *E. coli* cells harboring two vectors (pETR151 plus pACGDH) and 30 mM 3-CPP were added to the coupling reaction mixtures containing 20% (v/v) ionic liquids. After 6 h of reaction, the quantity of (*S*)-CPPO was measured. Two ionic liquids ([Bmim][NTf_2] and [Emim][BF_4]) systems yielded high levels of (*S*)-CPPO in comparison with the aqueous system (Fig. 3A). The [Bmim][PF_6] system generated an amount of (*S*)-CPPO similar to that of the aqueous system, whereas no (*S*)-CPPO was produced in the [Mmim][$MeSO_4$] system. Therefore, the [Bmim][NTf_2] system was selected as an appropriate solvent for the conversion reaction of 3-CPP into (*S*)-CPPO. The reason for the relatively low observed yield (5 mM) of (*S*)-CPPO in this aqueous system in comparison with that shown in Fig. 2 (27 mM) was that the pHs of these reaction systems were not adjusted in this screening process.

3.4. Selection of detergents suitable for whole cell-ionic liquid reaction

To increase the ionic liquid dispersion in aqueous phase, a variety of detergents were tested, as follows. *E. coli* cells harboring two vectors (pETR151 plus pACGDH) and 50 mM 3-CPP were added to the reaction mixtures containing 20% (v/v) [Bmim][NTf_2]. Four different detergents were then added to each of the reaction mixtures. After 12 h of reaction, the quantity of (*S*)-CPPO was measured. All four detergents yielded higher levels of (*S*)-CPPO in comparison with the system not containing detergent (Fig. 3B). Among them, Tween 40 yielded the highest levels of 34 mM product.

To optimize the concentration of Tween 40, various concentrations of Tween 40 were tested as follows. *E. coli* cells harboring pETR151 plus pACGDH and 100 mM 3-CPP were added to the reaction mixtures containing 20% (v/v) [Bmim][NTf_2]. Subsequently, 0–5% (v/v) of Tween 40 was added to each reaction mixture. After 24 h of reaction, the quantity of (*S*)-CPPO was measured (Fig. 3C). Tween 40 exerted a positive effect on the production of (*S*)-CPPO. When 4–5% Tween 40 was used, approximately 67 mM (*S*)-CPPO was produced. Finally, 4% Tween 40 was selected for the whole cell-ionic liquid system.

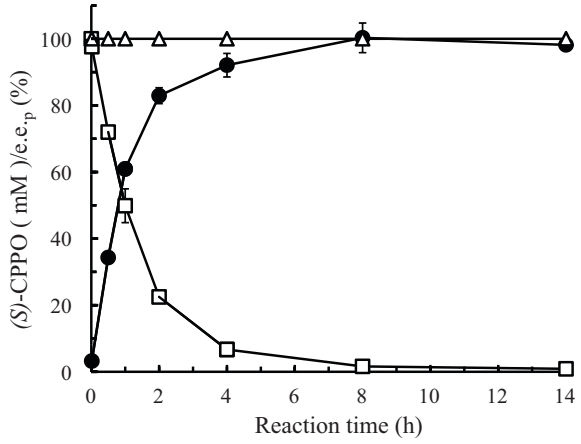

Fig. 4. Production of (*S*)-CPPO by the biphasic system. The bioconversion reaction was conducted using 30 reductase units of permeabilized *E. coli* cells harboring pETR151 plus pACGDH in a [Bmim][NTf_2]/water system containing 100 mM 3-CPP, 150 mM glucose, 1 mM NADPH, 20% [Bmim][NTf_2], 4% Tween 40. The reaction pH was adjusted within pH 7.0–7.5 by 1 M NaOH. The levels of (*S*)-CPPO and the enantiomeric excess (e.e) values were measured over the time course. (△) $e.e._p$ values, (□) 3-CPP, (●) (*S*)-CPPO.

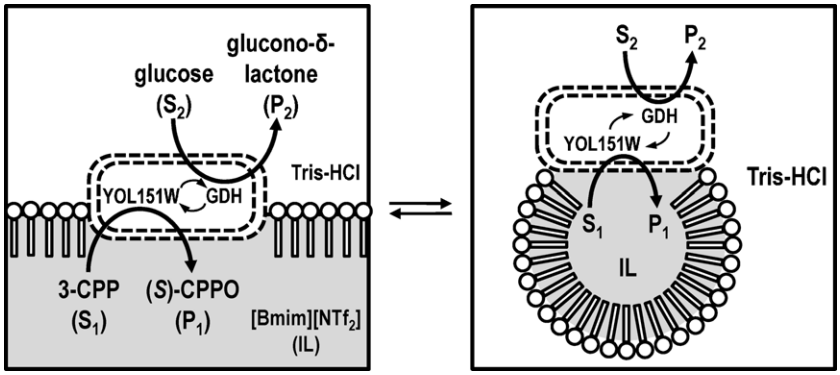

Fig. 5. Model of biphasic reaction system. Ionic liquid ([Bmim][NTf_2]) and water tend to make two separate phases. Detergents (Tween 40) also tend to locate on the interface and sometimes they form micelles/emulsions. Non-polar chemical (3-CPP) dissolves in the non-polar IL phase and glucose in water phase. When permeabilized *E. coli* cell locates on the interface, the reduction of 3-CPP and the oxidation of glucose are conducted by YOL151W and GDH in the cell, respectively. As NADPH is recycled between the two reactions, the reduction and oxidation reactions could be continued during the reaction time.

3.5. (S)-CPPO production using whole cell-ionic liquid system

We selected the appropriate ionic liquid and detergent for the *E. coli* whole cell reaction. The final reaction components were as follows; permeabilized *E. coli* cells harboring pETR151 plus pACGDH, 100 mM 3-CPP, 1 mM NADPH, 150 mM glucose, 20% (v/v) [Bmim][NTf_2], 4% Tween 40, 100 mM Tris–HCl buffer (pH 7.5). The whole cell reaction conducted using the above reaction mixture was conducted on a 10 mL scale using *E. coli* cells corresponding to 30 U of reductase activity. The reaction continued for 14 h with pH adjusted within a value range of 7.0–7.5. In this system, 100 mM 3-CPP was converted to (*S*)-CPPO with an >99% enantiomeric excess within 8 h (Fig. 4). As described previously, as the coupling reaction continued, gluconic acid accumulated and the solution pH was reduced as a consequence. Thus, NaOH was added to adjust the reaction pH to a range within 7.0–7.5.

Fig. 5 provides a schematic drawing of our reaction system. In this [Bmim][NTf_2]-aqueous biphasic system, 3-CPP and (*S*)-CPPO were soluble in the ionic liquid phase, whereas glucose and gluconic acid were soluble in the aqueous phase (Fig. 5). When the permeabilized *E. coli* cell located in the interface, the substrate, 3-CPP, entered the cells and was converted into (*S*)-CPPO. The cofactor required for this reduction reaction was supplied continuously from the GDH in the same *E. coli* cells. This whole cell-ionic liquid biphasic system can be applied for the production of a variety of useful water-insoluble chiral compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.02.013.

References

[1] G. Fronza, C. Fuganti, P. Grasselli, A. Mele, J. Org. Chem. 56 (1991) 6019–6023.
[2] E.B. Kurbanoglu, K. Zibeyaz, N.I. Kurbanoglu, M. Ozdal, M. Taskin, O.F. Algur, Tetrahedron: Asymmetry 21 (2010) 461–464.
[3] W.Y. Lou, L. Chen, B.B. Zhang, T.J. Smith, M.H. Zong, BMC Biotechnol. 9 (2009) 90.
[4] H. Groeger, C. Rollmann, F. Chamouleau, I. Sebastien, O. May, W. Wienand, K. Drauz, Adv. Synth. Catal. 349 (2007) 709–712.
[5] T.W. Johannes, R.D. Woodyer, H. Zhao, Biotechnol. Bioeng. 96 (2007) 18–26.
[6] J. Zhang, B. Witholt, Z. Li, Chem. Commun. (2006) 398–400.
[7] K. Schroer, U. Mackfeld, I.A. Tan, C. Wandrey, F. Heuser, S. Bringer-Meyer, A. Weckbecker, W. Hummel, T. Daussmann, R. Pfaller, A. Liese, S. Lütz, J. Biotechnol. 132 (2007) 438–444.
[8] A. Weckbecker, H. Gröger, W. Hummel, Adv. Biochem. Eng. Biotechnol. 120 (2010) 195–242.
[9] T. Ema, H. Yagasaki, N. Okita, M. Takeda, T. Sakai, Tetrahedron 62 (2006) 6143–6149.
[10] M. Ernst, B. Kaup, M. Müller, S. Bringer-Meyer, H. Sahm, Appl. Microbiol. Biotechnol. 66 (2005) 629–634.
[11] W. Stampfer, K. Edegger, B. Kosjek, K. Faber, W. Kroutil, Adv. Synth. Catal. 346 (2004) 57–62.
[12] J. Jung, H.J. Park, K.N. Uhm, D. Kim, Kim.F H.K., Biochim. Biophys. Acta 1804 (2010) 1841–1849.
[13] M.J. de Smet, J. Kingma, B. Witholt, Biochim. Biophys. Acta 506 (1978) 64–80.
[14] M. Cánovas, T. Torroglosa, J.L. Iborra, Enzyme. Microb. Technol. 37 (2005) 300–308.
[15] Y. Ni, R.R. Chen, Biotechnol. Bioeng. 87 (2004) 804–811.
[16] W. Zhang, K. O'Connor, D.I.C. Wang, Z. Li, Appl. Environ. Microbiol. 75 (2009) 687–694.
[17] M.P. Schneider, U. Goergen, Tetrahedron: Asymmetry 3 (1992) 525–528.
[18] M. Andrews, A. Brown, J.Y. Chiva, D. Fradet, D. Gordon, M. Lansdell, M. MacKenny, Bioorg. Med. Chem. Lett. 19 (2009) 2329–2332.
[19] M. Vaswani, F.K. Linda, S. Ramesh, Prog. Neruopsychopharmacol. Biol. Psychiatry 27 (2003) 85–102.
[20] Y.H. Choi, H.J. Choi, D. Kim, K.N. Uhm, H.K. Kim, Appl. Microbiol. Biotechnol. 87 (2010) 185–193.
[21] Z. Yang, Y.J. Yue, W.C. Huang, X.M. Zhuang, Z.T. Chen, M. Xing, J. Biochem. 145 (2009) 355–364.
[22] W.Y. Lou, W. Wang, R.F. Li, M.H. Zong, J. Biotechnol. 143 (2009) 190–197.
[23] C. Roosen, P. Müller, L. Greiner, Appl. Microbiol. Biotechnol. 81 (2008) 607–614.
[24] F. van Rantwijk, R.A. Sheldon, Chem. Rev. 107 (2007) 2757–2785.
[25] H. Pfruender, R. Jones, D. Weuster-Botz, J. Biotechnol. 124 (2006) 182–190.
[26] S. Bräutigam, D. Dennewald, M. Schürmann, J. Lutje-Spelberg, W.R. Pitner, D. Weuster-Botz, Enzyme Microb. Technol. 45 (2009) 310–316.
[27] H. Pfruender, M. Amidjojo, U. Kragl, D. Weuster-Botz, Angew. Chem. Int. Ed. 43 (2004) 4529–4531.
[28] H.J. Park, J. Jung, H.J. Choi, K.N. Uhm, H.K. Kim, J. Microbiol. Biotechnol. 20 (2010) 1300–1306.
[29] J.Y. He, L.M. Zhou, P. Wang, L. Zu, Process. Biochem. 44 (2009) 316–321.