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An effective method to use ionic liquids as reaction media for asymmetric reduction by *Geotrichum candidum*

Tomoko Matsuda,[a,*] Yusuke Yamagishi,[a] Shinichi Koguchi,[a]
Noritaka Iwai[b] and Tomoya Kitazume[a]

[a]*Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan*
[b]*Frontier Collaborative Research Center, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan*

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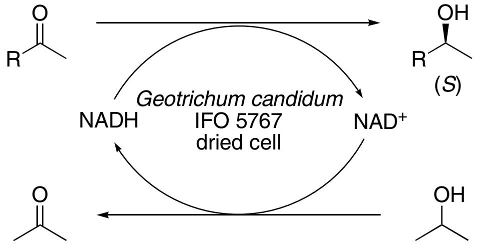
Abstract—An effective method was developed to use an enzyme in ionic liquids; the asymmetric reduction of ketones by *Geotrichum candidum* in ionic liquids proceeded smoothly with excellent enantioselectivity when the cell was immobilized on water-absorbing polymer containing water, while the reaction without the polymer did not proceed.

Ionic liquids have been considered as environmentally friendly solvents for organic synthesis due to their non-volatility and recyclable feature, so the reaction using chemical catalysts in ionic liquids has been fully investigated and has gained great success.[1] The reaction using biocatalysts in ionic liquids has also been developed since biocatalysts are renewable natural catalysts with high enantio-, regio- and chemo-selectivities.[2] However, the kinds of biocatalysts used in ionic liquids have been very limited, and most of those reported so far are hydrolytic enzymes such as lipases. There are only a few reports for alcohol dehydrogenase catalyzed reaction using ionic liquids[3] in spite of the fact that asymmetric reduction of ketones and oxidation of alcohols are very important reactions for organic synthesis. Therefore, a method to use alcohol dehydrogenase in ionic liquids is necessary to be developed. Here we report an effective method to use alcohol dehydrogenase in ionic liquids. The asymmetric reduction of ketones by *Geotrichum candidum* in ionic liquids proceeded smoothly with excellent enantioselectivity when the cell was immobilized on water-absorbing polymer containing water, while the reaction without the polymer did not proceed.

An alcohol dehydrogenase preparation from *G. candidum* IFO 5767 (dried cell)[4] was used for the reduction because the microorganism has suitable enzymes for transformation in nonaqueous solvent; for example, the alcohol dehydrogenase can catalyze the reduction in supercritical carbon dioxide[5] as well as in organic solvents[6] using water-absorbing polymer. It is also known that the enzyme is NADH dependent, and NAD^+ can be recycled to NADH by using 2-propanol as shown in Scheme 1.[4]

Reduction of *o*-fluoroacetophenone was examined first as a model reaction to test the viability of the reaction in ionic liquids. The results are shown in Table 1. First, dried cell, NAD^+ and 2-propanol were added to an ionic liquid, $[bmim]PF_6$ or $[emim]BF_4$, followed by the



Scheme 1. Asymmetric reduction of ketone and recycling of coenzyme catalyzed by *Geotrichum candidum* dried cell containing alcohol dehydrogenases.

Keywords: Asymmetric reduction; Alcohol dehydrogenase; Ionic liquid; *Geotrichum candidum*; Water-absorbing polymer.

[*] Corresponding author. Tel./fax: +81 45 924 5757; e-mail: tmatsuda@bio.titech.ac.jp

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Table 1. Effect of reaction conditions on the reduction of *o*-fluoroacetophenone

Entry	Ionic liquid	H_2O	Water-absorbing polymer	Yield[a] (%)	ee[a] (%)
1	[bmim]PF_6	−	−	0	—
2	[emim]BF_4	−	−	0	—
3	[bmim]PF_6	+	−	84	>99
4	[emim]BF_4	+	−	<1	—
5	[bmim]PF_6	+	+	92	>99
6	[emim]BF_4	+	+	96	>99

[bmim]: 1-butyl-3-methylimidazolium, [emim]: 1-ethyl-3-methylimidazolium. Reaction conditions: 16 h at 30 °C.

[a] Determined by GC analyses. The absolute configuration was determined to be *S* for all samples examined by comparing the GC retention times with those of the authentic samples prepared in the literature methods.[4]

substrate, and the mixture was stirred for 16 h at 30 °C. Unfortunately, the reaction did not proceed at all (entries 1 and 2). To improve the activity, water was added to the reaction mixture, then the yield increased drastically to give (*S*)-1-(*o*-fluorophenyl)ethanol when [bmim]PF_6 was used (entry 3), while the reaction in [emim]BF_4 did not proceed (entry 4). The difference in yield between the two reactions can be attributed to the difference in the water miscibility; water is not miscible with [bmim]PF_6 so that water and [bmim]PF_6 form two separate layers (a two-layer reaction), while [emim]BF_4 is completely miscible with water. The results imply that the water layer around the cell is necessary for the reaction to proceed. Therefore, to create the water layer in [emim]BF_4, water-absorbing polymer was used, so that polymer layer holding water can be formed strongly. To realize the above idea, water-absorbing polymer was added to the mixture of water and cell to immobilize the cell around the polymer physically. The resulting powder holding water and enzyme was added to the ionic liquids and used for the reaction. The yield for the reaction using both ionic liquids (entries 5 and 6) was improved. For the case of water immiscible [bmim]PF_6, the yield improves because of the increase in the surface area between the ionic liquid and water layers (entry 5).[7] For the case of [emim]BF_4, the stabilizing effect of the enzyme by keeping water around it using the polymer improves the reaction yield. The water-absorbing polymer, sodium polyacrylate, may also play the pH-stabilizing role. After all, the use of water-absorbing polymer holding water improved the yield for the reduction in ionic liquids for both water miscible and immiscible from 0% to up to 96%. Importantly, the enantioselectivity was always very high as far as the reaction proceeded. The absolute configuration of the product was same as that for the reaction in water and supercritical solvent.

Figure 1 shows time course of the reduction of *o*-fluoroacetophenone in [emim]BF_4 by immobilized *G. candidum* cell on water-absorbing polymer. The yield increased with the reaction time, which proved that the alcohol dehydrogenase surely catalyzed the reduction in the ionic liquid, eliminating major contribution of the progress of the reaction during the work up.

Other ionic liquids were also tested for the reaction, and the results are shown in Table 2. The yield was strongly affected by the anion; PF_6^- and BF_4^- gave the highest yield (entries 1, 2, 5 and 11), $(CF_3SO_2)_2N^-$ gave moderate yield (entries 3 and 6), and when there was a sulfur functionality on the anion, the reaction hardly proceeded (entries 4, 7, 9 and 10). On the other hand, the kind of imidazolium cation did not affect the reaction outcome. The hydrophobic property of ionic liquids did not relate to the yield, either, when the cell was immobilized on water-absorbing polymer, although it strongly affected the yield of the reaction without using water-absorbing polymer (Table 1, entries 3 and 4). Enantioselectivity of the reaction was independent on the kind of ionic liquids, and whenever the reaction proceed, it was excellent (ee >99%).

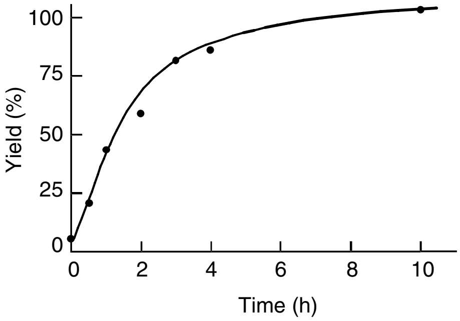


Figure 1. Time course of the reduction of *o*-fluoroacetophenone in [emim]BF_4 by immobilized *G. candidum* cell on water-absorbing polymer. Reaction conditions: 30 °C; 2′-fluoroacetophenone: 0.041 mmol, ionic liquid: 2 mL, immobilized cell: 1.0 g (immobilized cell was prepared from dried cell: 30 mg, NAD^+: 5 mg, H_2O: 1.0 mL, 2-propnol: 0.10 mL and water-absorbing polymer: 150 mg). Yield and ee were determined by GC analyses. Ee of the products was >99% for all samples examined. The absolute configuration was determined to be *S* for all samples examined by comparing the GC retention times with those of the authentic samples prepared in the literature methods.[4]

The substrate specificity was investigated. As listed in Table 3, the reduction in an ionic liquid, [emim]BF_4, by immobilized *G. candidum* cell on water-absorbing polymer proceeded for various ketones. Acetophenone derivatives, benzyl acetone, 2-hexanone, β-keto ester and fluorinated epoxy ketone were used as substrates, and it was found that all of them were reduced with moderate to excellent yield, and the enantioselectivity was excellent for all of the substrates tested (ee >99%). This wide substrate specificity together with the excellent enantioselectivity with the green solvent, ionic liquid, will be a very valuable synthetic tool.

In conclusion, an effective method using water-absorbing polymer to immobilize the cell was developed for

Table 2. Effect of ionic liquids on the reduction of *o*-fluoroacetophenone by immobilized *G. candidum* cell on water-absorbing polymer

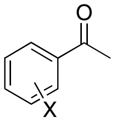
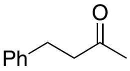
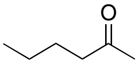
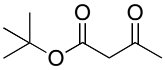
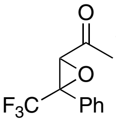
Entry	Ionic liquid	Hydrophobic property[a]	Yield[b] (%)	ee[b] (%)
1	[bmim]PF_6	○	92	>99
2	[bmim]BF_4	▲	82	>99
3	[bmim]$(CF_3SO_2)_2N$	○	49	>99
4	[bmim]CF_3SO_3	▲	1	—
5	[emim]BF_4	▲	96	>99
6	[emim]$(CF_3SO_2)_2N$	○	61	>99
7	[emim]CF_3SO_3	▲	0	—
8	[emim]NO_3	▲	0	—
9	[emim]CH_3OSO_3	▲	0	—
10	[emim]$C_2H_6OSO_3$	▲	<1	—
11	[omim]PF_6	△	88	>99

[bmim]: 1-butyl-3-methylimidazolium, [emim]: 1-ethyl-3-methylimidazolium, [omim]: 1-octyl-3-methylimidazolium. Reaction conditions are described in Figure 1. Reaction time: 16 h.

[a] ○: Hydrophobic, △: half hydrophobic, ▲: hydrophilic. The hydrophobicity was determined by the methods in the literature.[2d]

[b] Determined by GC analyses. The absolute configuration was determined to be *S* for all samples examined by comparing the GC retention times with those of the authentic samples prepared in the literature methods.[4]

Table 3. Substrate specificity of the reduction system in [emim]BF_4 by immobilized *G. candidum* cell on water-absorbing polymer

Substrate	Yield[b] (%)	ee[b] (%)	Config.[e]
 X = *o*-F	96	>99	*S*
o-Me	50	>99	*S*
p-F	27	>99	*S*
H	81	>99	*S*
	49	>99	*S*
	78	>99	*S*
	87	>99	*S*
 [a]	23, 23[c,d]	>99, >99[d]	nd

Reaction conditions are described in Figure 1. Reaction time: 16 h.

[a] Racemic mixture.

[b] Determined by GC analyses.

[c] Determined by ^{19}F NMR.

[d] Product is a mixture of two diastereomers.

[e] Determined by comparison of the GC retention times with those for the authentic samples prepared in the literature methods.[4]

the asymmetric reduction using alcohol dehydrogenases in ionic liquids. The asymmetric reduction of ketones by *G. candidum* in ionic liquids proceeded smoothly with excellent enantioselectivity when the cell was immobilized on water-absorbing polymer, while the reaction without the polymer did not proceed. Due to the wide substrate specificity and excellent enantioselectivity, this reduction system promoting green chemistry will be a powerful tool for the asymmetric synthesis.

Experimental procedure: *G. candidum* IFO 5767 was cultivated, and the dried cell was prepared as described previously.[4] The dried cell (30 mg) and NAD^+ (5 mg) were suspended in H_2O (1.0 mL) and 2-propanol (0.10 mL). The water-absorbing polymer (BL-100, 150 mg) was added and stirred quickly. The resulting immobilized cell (1.0 g) was added to an ionic liquid (2.0 mL), and then followed by addition of the substrate (0.041 mmol). The reaction mixture was stirred for 16 h at 30 °C, extracted with ether (5 mL × 4), and analyzed by GC with a chiral column (Chirasil-DEX CB or CP-Cyclodextrin-B-2,3,6-M-19) and/or ^{19}F NMR using undecane (0.028 mmol) or α,α,α-trifluorotoluene (0.041 mmol) as an internal standard.

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7. For the reaction in hexane, the surface area between the water layer and hexane was increased by immobilization with water-absorbing polymer as in Ref. 6.