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Ionic Liquid-Coated Enzyme for Biocatalysis in Organic Solvent

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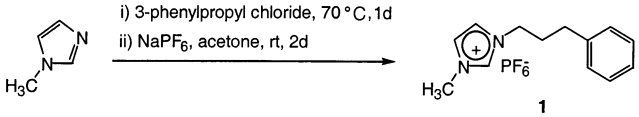
Abstract: Ionic liquid-coated enzyme (ILCE) is described as a useful catalyst for biocatalysis in organic solvent. An ionic liquid, [PPMIM]-[PF_6] (**1**, [PPMIM] = 1-(3′-phenylpropyl)-3-methylimidazolium), which is solid at room temperature and becomes liquid above 53 °C, was synthesized in two steps from *N*-methylimidazole. The coating of enzyme was done by simply mixing commercially available enzyme with **1** in the liquid phase above 53 °C and then allowing the mixture to cool. A representative ILCE, prepared with a lipase from *Pseudomonas cepacia*, showed markedly enhanced enantioselectivity without losing any significant activity.

Nonaqueous biocatalysis provide a useful component of methodology in organic synthesis.[1] For example, lipase catalysis in organic solvents is of great use for the synthesis of optically active compounds such as chiral alcohols, acids, and their esters.[2] However, biocatalysis in nonaqueous media often suffers from reduced activity, selectivity, or stability of enzyme.[3] To overcome these limitations, many approaches have focused on the development of more efficient enzymes by enzyme modification, molecular imprinting, additive addition, or substrate matching.[4] Some recent examples include cross-linked enzyme crystals[4a,b] and aggregates,[4c] ligand[4d−g] or inorganic salt[4h] co-lyophilized enzymes, and enzyme-coated microcrystals.[4i] Although these modified enzymes exhibit better activity, selectivity, or stability, the procedures for their preparations in most cases are rather complicated. We herein wish to report for the first time an ionic liquid-coated enzyme (ILCE) that is readily prepared and exhibits markedly enhanced enantioselectivity and reliable stability.

SCHEME 1. Synthesis of Ionic Liquid



Recently, a few groups including us[5] have reported that ionic liquids[6,7] have great potential as alternative reaction media for biocatalysis and biotransformation. It was observed that their use enhanced the selectivity of the enzyme.[5c,d] It was also demonstrated that they are useful as media for the enzymatic reaction of polar substrates, which are difficult to dissolve in conventional organic solvents.[5f] One of the interesting properties of ionic liquids, we think, is their insolubility in water or organic solvents, which led us to envisage that they might be suitable as the coating materials for immobilizing biocatalysts. Particularly, we thought that room-temperature solid-phase ionic liquids, which become liquid at elevated temperature, would be of great use for such a purpose. To test our idea, a novel ionic liquid [PPMIM]-[PF_6] (**1**, [PPMIM] = 1-(3′-phenylpropyl)-3-methylimidazolium) was synthesized in good yields via two steps from *N*-methylimidazole (Scheme 1). It was observed that the ionic liquid was solid at room temperature and became liquid over 53 °C.

As a representative enzyme for the preparation of ILCE, *Pseudomonas cepacia* lipase (PCL)[8] was chosen since it had been frequently used for biotransformations in organic solvents. In a typical procedure for the preparation of ILCE, solid **1** was converted to its liquid phase by heating above 53 °C. To this liquid were added enzyme powders (0.1 mass equiv) and the resulting mixture was stirred with a glass rod to get a uniform heterogeneous solution. The solution was then allowed to cool to room temperature until the enzyme−ionic liquid mixture solidified. The solid phase was broken down to a small size of particles with a spatula. The small ILCE particles were then used without any further treatment in the next experiments for testing their activity and selectivity.

The enantioselectivity of ILCE was examined with the transesterification reactions of secondary alcohols **2a**−**e**

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(8) This enzyme is available from some commercial suppliers such as Fluka, Roche, and Amano. We used the one provided by Amano.

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SCHEME 2. Lipase-Catalyzed Transesterification

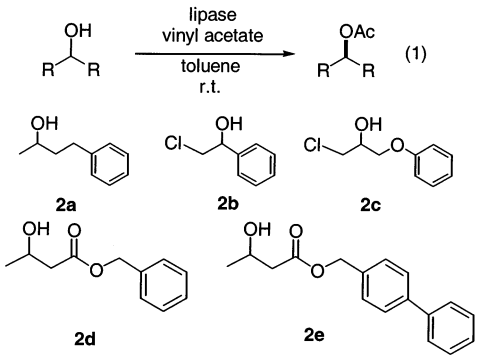

TABLE 1. The Enantioselectivities in the Lipase-Catalyzed Transesterifications of 2a−e in Toluene[a,b]

entry	substrate	lipase	ee_s	ee_p	*E*
1	**2a**	native	0.545	0.987	265
2		ILCE	0.295	0.995	532
3	**2b**	native	0.340	0.986	198
4		ILCE	0.220	0.986	176
5	**2c**	native	0.563	0.988	293
6		ILCE	0.370	0.995	574
7	**2d**	native	0.459	0.971	107
8		ILCE	0.300	0.983	156
9	**2e**	native	0.575	0.978	161
10		ILCE	0.275	0.989	237

[a] Experimental procedure: The ILCE-catalyzed reaction of **2a** is described as a representative procedure. **2a** (15 mg, 0.1 mmol), vinyl acetate (28 μL, 0.3 mmol), and ILCE (165 mg) were mixed with toluene (0.5 mL), and the resulting heterogeneous mixture was stirred at 25 °C for 1 day. The reaction mixture was then filtered to remove enzymes, concentrated, and finally subjected to silica gel chromatography to provide the unreacted substrate (*S*)-**2a** (11 mg, 0.075 mmol, 75%, 29.5% ee) and the acetylated product (*R*)-**3a**, (4 mg, 0.021 mmol, 21%, 99.5% ee). [b] The optical purities were determined by HPLC using a chiral column. Analytical conditions: Chiralcel OD, hexane/2-propanol 90/10 (**2c**), 95/5 (**2a**, **2d**), and 97/3 (**2e**), flow rate = 1.0 mL/min (**2a**, **2c**-**e**), UV 250 (**2c**), 217 nm (**2a**, **2d**, **3e**); Whelk-O1, hexane/2-propanol 99/1 (**2b**, **3a**, **3c**, **3b**), 95/5 (**3e**), and 94/6 (**3d**), flow rate = 1.0 mL/min (**2b**, **3a**-**e**), UV 250 (**3c**), 217 nm (**2b**, **3a**,**b**,**d**,**e**).

in the presence of vinyl acetate in toluene at 25 °C (Scheme 2). For comparison, the same reactions were carried out with native enzyme. In typical experiments,[9] an enzymatic reaction was performed with a solution containing substrate (0.1 mmol), lipase (native, 15 mg; ILCE, 165 mg), and vinyl acetate (3 equiv) in toluene (0.5 mL) at 25 °C for 1 day. The enzymes were then removed by filtration and the resulting solution was concentrated. The organic residue was subjected to silica gel chromatography to obtain unreacted substrate and acetylated product. Their optical purities (ee_s and ee_p) were then determined by HPLC with use of a chiral column. The *E* values were calculated by using the following equation, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$, where $c = ee_s/(ee_s + ee_p)$.[10] The results are given in Table 1.

The ILCE-catalyzed transesterifications of **2a**−**e** except one case proceeded with better enantioselectivity than those catalyzed by native PCL. In the reactions of **2a** and **2c**, the enantioselectivity enhancement by the use of ILCE was about 2-fold (see entries 1−2 and 4−5, respectively). In the reactions of **2d**−**e**, the enantioselectivity enhancements by the use of ILCE were about 1.5-fold. In the reaction of **2b**, however, the enantioselectivities in both cases were similar (compare entries 3 and 4). Overall, these results indicate that the ionic liquid-coated enzyme in most cases is more selective than its native counterpart.

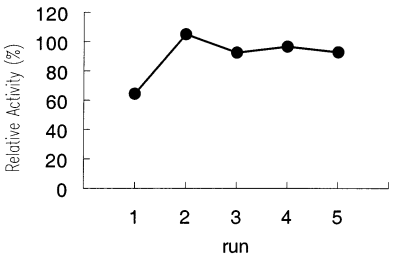

FIGURE 1. The relative activity of ILCE. One control reaction with native enzyme and five ILCE-catalyzed reactions were performed in the presence of **2a** and vinyl acetate in toluene at 25 °C for 1day. In the ILCE-catalyzed reactions, the first run was carried out with fresh ILCE and the second to fifth runs were done with recycled ILCE. The conversion percent in each run was compared with that in the control reaction to obtain the relative activity.

The catalytic efficiency and stability of ILCE were examined with conversion percent in the transesterification reaction of **2a**, which was carried out in the presence of vinyl acetate in toluene at 25 °C for 1 day. The ILCE-catalyzed reactions were repeated five times with the recycling of enzyme. In first run, the fresh ILCE displayed 65% of the activity expected from native enzyme used. However, the catalytic activity of ILCE increased up to the level of native enzyme in the second run and then was slightly reduced in the following runs. After the fifth run, ILCE retained 93% of the activity of its native counterpart (Figure 1). The reduced activities of fresh ILCE in the first run seem to be due to some diffusion difficulty, which should be relieved with a decrease in its particle size as the reaction proceeds. These observations indicate that lipase loses practically no significant activity during the coating process and its coated form has satisfactory stability.

In conclusion, this work has demonstrated that lipase shows enhanced enantioselectivity without losing any significant activity when it is coated with an ionic liquid **1**. The ionic liquid is readily available and the coating procedure is simple and straightforward. Furthermore, the coated lipase is easy to reuse and retains its full activities even after several runs. Accordingly, it is believed that ILCE should find use as a new type of immobilized biocatalyst for biotransformations in organic solvents. Further studies to broaden the scope of ILCE toward other enzymes and organic solvents are in progress at this laboratory.

Experimental Section

Preparation of Compound 1. 3-Phenylpropyl chloride (19 g, 0.125 mol) was dissolved in 1-methylimidazole (10 mL, 0.125 mol) and then reflux for 24 h at 70 °C. To the reaction mixture was added acetone (100 mL) and $NaPF_6$ (21 g, 0.125 mol) at 0 °C and then the resulting solution was stirred for 48 h at room

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temperature. After filtering out precipitate, the organic layer was dried and concentrated in vacuo to afford the desired product (**1**, 45 g, 0.119 mol, 95%): mp 52−53 °C; ^{1}H NMR (CD_3CN, 300 MHz, ppm) 2.14 (m, 2H, CH_2), 2.65 (*t*, 2H, $J = 7.4$, CH_2), 3.80 (s, 1H, NCH_3), 4.14 (t, 2H, $J = 7.2$, CH_2), 7.19−7.35 (m, 7H), 8.34 (s, 1H, CH). Anal. Calcd for $C_{13}H_{17}F_6N_2P$: C, 45.09; H, 4.95; N, 8.09. Found: C, 45.16; H, 4.94; N, 8.08.

Preparation of ILCE. Solid **1** (1 g) was heated above 53 °C in a flask to get a liquid phase, followed by the addition of lipase (0.1 g). The resulting heterogeneous solution was stirred with a glass rod for 1 min and then cooled to room temperature to yield ILCE (1.1 g) in a solidified solution.

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