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Biocatalysis in Ionic Liquids: Markedly Enhanced Enantioselectivity of Lipase

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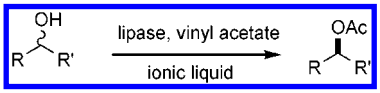
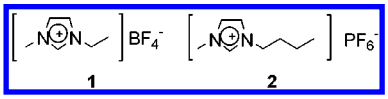
ABSTRACT



Lipase-catalyzed transesterifications in ionic liquids proceeded with markedly enhanced enantioselectivity. It was observed that lipases were up to 25 times more enantioselective in ionic liquids than in conventional organic solvents.

Room-temperature ionic liquids are attracting growing interest as alternative reaction media for chemical transformations.[1,2] One of several advantages of ionic liquids is that they are environmentally benign since they have no detectable vapor pressure. Accordingly, they are emerging as novel replacements for volatile organic solvents in industrial organic synthesis. They are particularly promising as solvents for catalysis. Their use can enhance activity, selectivity, and stability of catalysts. Most of the studies in this area have so far focused on transformations using transition-metal catalysts.[3] Very recently, two groups have reported the use of ionic liquids as solvents in enzymatic reactions.[4] Erbeldinger et al. used ionic liquid in the synthesis of (*Z*)-aspartame using thermolysin.[4a] Lau et al. reported that lipase-catalyzed alcoholysis, ammonolysis, and perhydrolysis in ionic liquids and reaction rates were generally comparable with, or better than, those observed in organic media.[4b] As a part of our "green chemistry" research program, we became interested in ionic liquids as alternative solvents for biotransformations using cell-free enzymes. We herein wish to report the preliminary results from our studies that the use of ionic liquids enhanced significantly the enantioselectivity of lipase in transesterifications.[5] It was observed that lipases were up to 25 times more enantioselective in ionic liquids than in conventional organic solvents.



Two ionic liquids, [EMIM]-[BF_4] (**1**, [EMIM]$^+$ = 1-ethyl-3-methylimidazolium)[6] and [BMIM]-[PF_6] (**2**, [BMIM]$^+$ =

(1) Reviews: (a) Seddon, K. R. *J. Chem. Technol. Biotechnol.* **1977**, *68*, 351. (b) Welton, T. *Chem. Rev.* **1999**, *99*, 2071. (c) Wasserscheid, P.; Wilhelm, K. *Angew. Chem., Int. Ed.* **2000**, *39*, 3772.

(2) (a) Ford, W. T.; Hauri, R. J.; Hart, D. J. *J. Org. Chem.* **1973**, *38*, 3916. (b) Bolkan, S. A.; Yoke, J. T. *Inorg. Chem.* **1986**, *25*, 3587. (c) Wilkes, J. S.; Zaworotko, M. J. *J. Chem. Soc., Chem. Commun.* **1992**, 965. (d) Fuller, J.; Carlin, R. T.; Osteryoung, R. A. *J. Electrochem. Soc.* **1997**, *144*, 3881. (e) Larsen, A. S.; Holbrey, J. D.; Tham, F. S.; Reed, C. A. *J. Am. Chem. Soc.* **2000**, *122*, 7264.

(3) (a) Chauvin, Y.; Mussmann, L.; Olivier, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2698. (b) Dullis, J. E L.; Suarez, P. A. Z.; Einloft, S.; de Souza, R. F.; Dupont, J.; Fisher, J.; DeCian, A. *Organometallics* **1998**, *17*, 815. (c) Carmichael, A. J.; Earle, M. J.; Holbrey, J. D.; McCormac, P. B.; Seddon, K. R. *Org. Lett.* **1999**, *1*, 997. (d) Dyson, P. J.; Ellis, D. J.; Parker, D. G.; Welton, T. *Chem. Commun.* **1999**, 25. (e) Chen, W.; Xu, L.; Chatterton, C.; Xiao, J. *Chem. Commun.* **1999**, 1247. (f) Xu, L,; Chen. W.; Xiao, J. *Organometallics* **2000**, *19*, 1123. (g) Brasse, C. C.; Englert, U.; Salzer, A. *Organometallics* **2000**, *19*, 3818. (h) Song, C. E.; Roh, E. J. *Chem. Commun.* **2000**, 837. (i) Owens, G. S.; Abu-Omar, M. M. *Chem. Commun.* **2000**, 1165. (j) Carmichael, A. J.; Haddleton, D. M.; Bon, S. A. F.; Seddon, K. R. *Chem. Commun.* **2000**, 1237. (k) Mathews, C. J.; Smith, P. J.; Welton, T. *Chem. Commun.* **2000**, 1249. (l) Song, C. E.; Shim, W. H.; Roh, E. J.; Choi, J. H. *Chem. Commun.* **2000**, 1695. (m) Song, C. E.; Oh, C. R.; Roh, E. J.; Choo, D. J. *Chem. Commun.* **2000**, 1743.

(4) (a) Erbeldinger, M.; Mesiano, A. J.; Russel, A. *Biotechnol. Prog.* **2000**, *16*, 1131. (b) Lau, R. M.; Van Rantwijk, F.; Seddon, K. R.; Sheldon, R. A. *Org. Lett.* **2000**, *2*, 4189.

(5) During the preparation of this manuscript, a report describing the improved enantioselectivity of lipase in ionic liquids has appeared (Schoefer, S. H.; Kaftzik, N.; Wasserscheid, P.; Kragl, U. *Chem. Commun.* **2001**, 425).

(6) For the preparation of **1**, see: (a) Wilkes, J. S.; Levisky, J. A.; Wilson, R. A.; Hussey, C. L. *Inorg. Chem.* **1982**, *21*, 1263. (b) Fuller, J.; Carlin, R. T.; De long, H. C.; Haworth, D. *J. Chem. Soc., Chem. Commun.* **1994**, 299.

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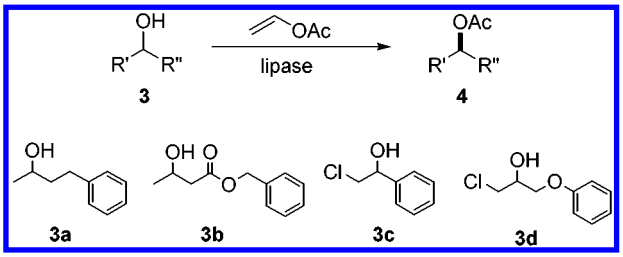
1-butyl-3-methylimidazolium),[7] were tested as media for lipase-catalyzed transesterification (Scheme 1) and compared

Scheme 1. Lipase-Catalyzed Transesterification



with two organic solvents, THF and toluene, in terms of enzyme enantioselectivity. The evaluations of enzyme enantioselectivity in these media were carried out with alcohols **3a**−**d** as the substrates in the presence of vinyl acetate at room temperature. Two lipases, *Candida antarctica* lipase B (CALB, immobilized)[8] and *Pseudomonas cepacia* lipase (PCL, native),[9] were chosen as the enzymes. The enantioselectivity of CALB was evaluated for the reactions of **3a** and **3b**, and the enantioselectivity of PCL was evaluated for the reactions of **3c** and **3d**.

In typical experiments, an enzymatic reaction was performed with a solution containing substrate (0.15 mmol), lipase (20 mg), and vinyl acetate (1.5−3 equiv) in solvent (1 mL) at room temperature. After the reaction reached 10−50% completion, the enzymes were removed by filtration and the resulting solution was concentrated. In the case of ionic liquid solution, the solution mixture was first extracted with ethyl ether and the ethereal phase was concentrated. The organic residues were subjected to silica gel chromatography to obtain unreacted substrate and acetylated product. Their optical purities were then determined by HPLC using a chiral column, which allowed us to measure the enantiomeric excess (ee) up to >99.5%. The E values were calculated using the equation, $E = \ln[1 - c(1 + \mathrm{ee_p})]/\ln[1 - c(1 - \mathrm{ee_p})]$, where $c = \mathrm{ee_s}/(\mathrm{ee_s} + \mathrm{ee_p})$.[10] The results are given in Table 1.

The transesterification of **3a** catalyzed by CALB proceeded with better enantioselectivity in ionic liquids than in organic solvents (Table 1, entries 1−4). The best enantioselectivity (E = >967) was observed for the reaction in **2**, which was 5−7 times higher than for those in organic solvents. The high enantioselectivity (E = 648) was observed for the reaction in **1**, which was 3−4 times higher compared to those in organic solvents. The CALB-catalyzed transesterification

Table 1. The Enantioselectivities for the Lipase-Catalyzed Transesterification in Organic Solvents and Ionic Liquids[a]

entry	substrate	lipase	medium	$\mathrm{ee_s}$	$\mathrm{ee_p}$	E
1	**3a**	CALB	THF	0.916	0.955	141
2			toluene	0.976	0.958	207
3			**1**	0.915	0.990	648
4			**2**	0.789	>0.995	>967
5	**3b**	CALB	THF	0.803	0.827	26
6			toluene	0.975	0.954	187
7			**1**	0.941	0.989	651
8			**2**	0.677	0.941	155
9	**3c**	PCL	THF	0.182	0.958	56
10			toluene	0.420	0.981	158
11			**1**	0.397	0.984	183
12			**2**	0.123	>0.995	>450
13	**3d**	PCL	THF	0.420	0.980	150
14			toluene	0.418	0.965	85
15			**1**	0.499	0.981	172
16			**2**	0.854	>0.995	>1000

[a] On the basis of analyses by HPLC using a chiral column. Analytical conditions: Chiralcel OD, hexane/2-propanol = 95/5 (**3a**), 98/2 (**3b**), 90/10 (**3d**, **4d**), flow rate = 1.0 (**3a**,**b**), 0.5 mL/min (**3d**, **4d**), UV 217 (**3a**,**b**), 250 nm (**3d**, **4d**); Whelk-O1, hexane/2-propanol = 99/1 (**3c**, **4a**−**c**), flow rate = 0.5 (**4a**,**b**), 1.0 mL/min (**3c**, **4c**, UV 217 (**3c**, **4a**−**c**).

of **3b** showed a different pattern in enantioselectivity (entries 5−8). The highest enantioselectivity (E = 651) was observed in **1**, but the enantioselectivity in **2** was moderate (E = 155) and comparable to that in toluene. The lowest enantioselectivity was observed in THF. Accordingly, the enantioselectivity enhancements were 25-fold between THF and **1**, 3.5-fold between toluene and **1**, and 6-fold between THF and **2**.

The PCL-catalyzed transesterifications of **3c** and **3d** proceeded with high enantioselectivity (E = >450 and >1000) in ionic liquid **2** (entries 12 and 16). The enantioselectivities in ionic liquid **1** were modest (E = 183 and 172) but comparable to the best (E = 158 and 150) in organic solvents (entries 10, 11, 13, and 15). Accordingly, the use of **2** enhanced the enantioselectivity by 3−8 times for the reaction of **3c** and by 7−12 times for the case of **3d**.

The enantioselectivities of lipases, in general, were higher in hydrophobic **2** than in hydrophilic **1** except for the reaction of **3b**. In several cases, the lipase enantioselectivity in ionic liquid reached a synthetically desirable level (E = >400, entries 4, 7, 12, and 16). As an illustrative example, the reaction of **3d** corresponding to the entry 16 was performed twice on a 0.6 mmol scale.[11] In the first run, the reaction

(7) For the preparation of **2**, see: (a) Suarez, P. A. Z.; Dullius, J. E. L.; Einloft, S.; De Souza, R. F.; Dupont, J. *Polyhedron* **1996**, *15*, 1271. (b) Bonhôte, P.; Dias, A.-P.; Papageorgiou, N.; Kalyanasundaram, K.; Grätzel, M. *Inorg. Chem.* **1996**, *35*, 1168.

(8) We used the enzyme from Roche, whose trade name is Chirazyme L-2, c-f, C3, lyo. Its price is 406 DM for 150 kU.

(9) This enzyme is available from some commercial suppliers such as Fluka, Roche, and Amano. We used the one provided by Amano, whose price is not available. The price of the Roche enzyme (trade name, Chirazyme L-1, Lyo) is 406 DM for 5.0 MU.

(10) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

(11) **Experimental detail**: **3d** (109 mg, 0.6 mmol), vinyl acetate (1.8 mmol), and PCL (218 mg) were mixed with ionic liquid **2** (3 mL), and the resulting heterogeneous mixture was stirred at 25 °C. When the reaction reached slightly over 50% completion (48 h), the reaction mixture was extracted with ethyl ether (5 times with a 20 mL portion). The ethereal phase was concentrated and subjected to silica gel chromatography to provide the unreacted substrates ((*R*)-**3d**, 47.3 mg, 0.26 mmol, 43%, >99.5% ee) and the acetylated products ((*S*)-**4d**, 63 mg, 0.28 mmol, 47%, 97.7% ee). The second reaction was performed under the same conditions, except that the reaction was carried to about 46% completion (36 h), to afford the unreacted substrates (46.5 mg, 0.256 mmol, 43%, 85.8% ee) and the acetylated product (56.9 mg, 0.255 mmol, 42%, >99.5% ee). The optical purities were determined by HPLC using a chiral column. See the footnote in Table 1 for the HPLC conditions.

was carried to slightly over 50% completion to obtain the unreacted substrates of >99.5% ee in 43% yield. In the second run, the reaction was carried to 46% completion to get the acetylated products of >99.5% ee in 42% yield.

It was observed that in most cases the activities of lipases in ionic liquids were as good as or slightly lower than in organic solvents.[12] One exceptional case was the reaction of **3c** in **2**, which proceeded at much lower rate. An advantage of ionic liquids as solvents is that they can be readily reused together with catalysts. For the CALB-catalyzed reaction of **3a** in **2**, we were able to reuse ionic liquid and enzyme twice without loss in enantioselectivity and reactivity after the first run.

All of these results clearly prove that the lipase-catalyzed transesterifications took place effectively in ionic liquids **1** and **2**, in some cases with impressively high enantioselectivity. Now the question is why lipases show such high enantioselectivity in ionic liquids. At this time, no satisfactory answers are available since the enzyme enantioselectivity could be affected by several factors, including the nature of medium, the diffusion rates of substrate and product, the water content, and the conformational rigidity of enzymes. Accordingly, we should wait until further systematic studies disclose the effects of these factors with some detail in the near future.

In summary, this work has demonstrated that ionic liquids can serve as the solvents for lipase-catalyzed transesterifications with an advantage of enhancing the enantioselectivity, suggesting that they have great potential as alternative media for biocatalysis and biotransformations. Several ionic liquids including those used in this work are commercially available.[13] The lipase reactions in ionic liquids can be scaled up without a major difficulty.[14] We believe that the use of ionic liquids now opens up a new field in nonaqueous enzymology as the use of organic solvents did in the early 1980s.[15]

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(12) The activities of lipases decreased roughly in the following orders: toluene > THF≈ **1** ≈ **2** for the reaction of **3a**, toluene > THF ≈ **1** > **2** for the reaction of **3b,** toluene > THF ≈ **1** >> **2** for the reaction of **3c**, and toluene ≈ THF ≈ **2** > **1** for the reaction of **3d**.

(13) From Solvent Innovation GMBH, Cologne. The prices of **1** and **2** are, respectively, 529.30 and 647.50 DM for 100 mL. In this work, we used chemically prepared ionic liquids. See ref 7.

(14) When the reaction corresponding to entry 4 in Table 1 was scaled up by 10-fold (**3a**, 225 mg, 1.5 mmol), the same level of enantioselectivity was realized.

(15) Reviews: (a) Klivanov, A. M. *Chemtech* **1986**, *16*, 354. (b) Klibanov, A. M. *Acc. Chem. Res.* **1989**, *23*, 114. (c) *Enzymatic Reactions in Organic Media*; Koskinen, A. M. P., Klibanov, A. M., Eds.; Blackie Academic & Professional: Glasgow, 1996.