Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 26 (2003) 41–45
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

1-Butyl-2,3-dimethylimidazolium tetrafluoroborate: the most desirable ionic liquid solvent for recycling use of enzyme in lipase-catalyzed transesterification using vinyl acetate as acyl donor

Toshiyuki Itoh*, Yoshihito Nishimura, Nozomi Ouchi, Shuichi Hayase

Department of Materials Science, Faculty of Engineering, Tottori University, 4-101 Koyama Minami, Tottori 680-8552, Japan

Received 31 May 2003; received in revised form 2 July 2003; accepted 2 July 2003

Abstract

1-Butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim]BF_4) was found to be an excellent solvent to realize a lipase-recycling system using vinyl acetate as acyl donor. No accumulation of an acetaldehyde oligomer was observed in this solvent system and it was possible to use the lipase repeatedly 10 times while still maintaining perfect enantioselectivity and high reactivity.

Keywords: [bdmim]BF_4; Enantioselectivity; Lipase; Recycling; Transesterification

1. Introduction

Ionic liquids have very good properties as a reaction medium in chemical reactions: they are non-volatile, non-flammable, have low toxicity and good solubility for many organic and inorganic materials [1–3]. The use of ionic liquids to replace organic solvents in biocatalytic processes has recently gained much attention [4–21]. We focused our attention on the recycling use of enzyme in the ionic liquid solvent system [5–8]; it was indeed possible to use the enzyme repeatedly in 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6) as solvent, though the reaction rate gradually dropped with repetition of the reaction process when vinyl acetate was used as acyl donor [5]. This drop in reactivity was caused by the inhibitory action of acetaldehyde oligomer which was accumulated in the solvent system [5,7]. We solved this problem by developing a system in which lipase-catalyzed transesterification was carried out using methyl esters as acyl donors under reduced pressure in the [bmim]PF_6 solvent system [6]; this made it possible to use the lipase repeatedly because there was no drop in the reaction rate despite five repetitions of the process in the [bmim]PF_6 solvent system [6,7]. However, it remained a problem that the system could not be applied to volatile substrates. Since it is well known that physical properties of imidazolium salts are drastically clanged by modification of their structure [1], we attempted to evaluate the imidazolium salts again to see if they were suitable for recycling use of the enzyme using vinyl acetate as acyl donor. We report here that no accumulation of acetaldehyde oligomer occurred when the lipase-catalyzed reaction

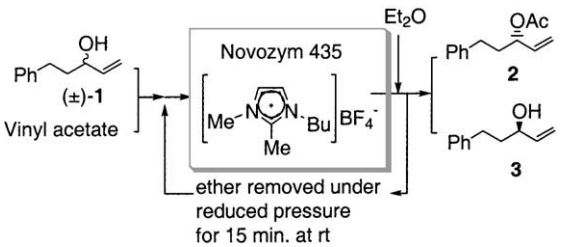
* Corresponding author. Tel.: +81-857-31-5259; fax: +81-857-31-5259.
E-mail address: titoh@chem.tottori-u.ac.jp (T. Itoh).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1381-1177(03)00147-4

was conducted in 1-butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim]BF_4) as solvent in the presence of vinyl acetate as acyl donor.

2. Results and discussion

We hypothesized that oligomerization of acetaldehyde may be caused by the proton derived from 2-position of the 1-butyl-3-methylimidazolium salts, because it was suggested that the acidity of the 2-position of imidazolium cation is very high [1,3]. Hence, we focused on using an imidazolium salt which lacked hydrogen at the 2-position as a reaction medium of lipase-catalyzed transesterification. The enantioselective transesterification of 5-phenyl-1-penten-3-ol ((±)-**1**) [22] was carried out in [bdmim]BF_4 or [bdmim]PF_6 solvent and the results are shown in Table 1. To a mixture of Novozym 435 (50 wt.% based on the substrate) in the ionic liquid (0.2 M) were added (±)-**1** and vinyl acetate (1.5 eq.) The resulting mixture was stirred at 35 °C. The reaction course was monitored by GC analysis and the product (*S*)-**2** [22] and unreacted alcohol (*R*)-**1** [22] were extracted with diethyl ether and purified by silica-gel thin layer chromatography (TLC). To the remaining ionic liquid phase, which was placed under reduced pressure for 15 min to remove the ether, a mixture of the substrate and vinyl acetate was again added and the mixture was stirred at 35 °C (Eq. (1)).



(1)

As we expected, no accumulation of an acetaldehyde oligomer was observed in this solvent system by ^{1}H NMR analysis: ^{1}H NMR analysis of recovered [bdmim]BF_4 showed no signal at δ: 5.1–5.2, while intensity of this signal increased for [bmim]BF_4 recovered when vinyl acetate was used as acyl donor.

Table 1
Recycled use of lipase in an ionic liquid solvent system

Entry	Solvent	Recycle run	Time (h)	e.e. (%) of **2** (yield, %)[a]	Conversion (%)/$c \times 100$	Relative rate[b]	*E*-value[c]
1	[bdmim]BF_4	1	2	>99 (29)	33	16	>200
2	[bdmim]BF_4	2	3	>99 (33)	34	11	>200
3	[bdmim]BF_4	3	3	>99 (29)	34	11	>200
4	[bdmim]BF_4	4	3	>99 (30)	31	10	>200
5	[bdmim]BF_4	5	3	>99 (29)	30	10	>200
6	[bdmim]BF_4	6	3	>99 (26)	30	10	>200
7	[bdmim]BF_4	7	3	>99 (30)	34	11	>200
8	[bdmim]BF_4	8	3	>99 (28)	36	12	>200
9	[bdmim]BF_4	9	3	>99 (27)	36	12	>200
10	[bdmim]BF_4	10	3	>99 (32)	36	12	>200
11	[bdmim]PF_6	1	24	No reaction	0	0	–
12	*i*-Pr_2O	1	3	>99 (47)	50	17	>200
13	[bmim]BF_4	1	3.5	>99 (44)	48	14	>200
14	[bmim]BF_4	3	4	>99 (35)	36	9	>200
15	[bmim]BF_4	5	48	>99 (27)	38	0.8	>200
16	[bmim]PF_6	1	3	>99 (47)	47	16	>200
17	[bmim]PF_6	3	6	>99 (38)	47	8	>200
18	[bmim]PF_6	5	91	>99 (28)	41	0.5	>200

[a] Isolated yield.

[b] Relative rate = conversion (%)/reaction time (h).

[c] $E = \ln[(1-c)(1+\text{e.e. of } \mathbf{2})]/\ln[(1-c)(1-\text{e.e. of } \mathbf{2})]$, where c means conversion which was calculated by the following formula: $c = \text{e.e. of } \mathbf{3}/(\text{e.e. of } \mathbf{2} + \text{e.e. of } \mathbf{3})$ [23].

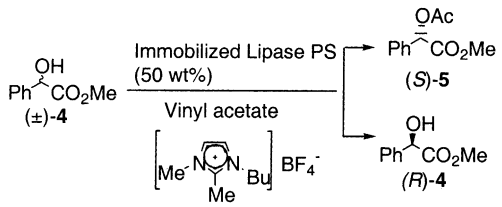
Table 2
Acetylation of methyl mandelate in a [bdmim] salt solvent system

Entry	Supporting material	Solvent	Time (h)	e.e. (%) of **5** (yield, %)[a]	Relative rate[b]	*E*-value [23]
1	200M	[bdmim]BF_4	24	66 (8)	0.8	6
2	200P	[bdmim]BF_4	5	71 (13)	2.6	5
3	200M	[bdmim]PF_6	24	73 (8)	0.4	7
4	200P	[bdmim]PF_6	48	71 (14)	0.5	7
5	200M	[bmim]PF_6	5	97 (20)	4.4	80
6	200M	*i*-Pr_2O	48	>99 (22)	0.5	>200

[a] Isolated yield.
[b] Relative rate = conversion (%)/reaction time (h).

The reaction proceeded very smoothly and we were able to use the enzyme repeatedly 10 times while still maintaining perfect enantioselectivity and high reactivity as shown in Table 1 (entries 1–10). The reaction rate in the [bdmim]BF_4 solvent system (entry 1) was superior to that in [bmim]BF_4 (entry 13) and close to that in diisopropyl ether (*i*-Pr_2O) (entry 12). In particular, it should be emphasized that no drop in the reaction rate was observed in [bdmim]BF_4 solvent system after repeating use of enzyme five times (entry 5), while the reaction rate was significantly reduced when the reaction was conducted in [bmim]BF_4 (entry 15) or in [bmim]PF_6 (entry 18) [5]. It was thus found that [bdmim]BF_4 is the best solvent for recycling use of enzyme under normal pressure conditions using vinyl acetate as acyl donor. Curiously, no reaction took place when [bdmim]PF_6 was used as solvent in the lipase-catalyzed reaction (entry 11). Since it was suspected that this might be due to the quality of the commercial ionic liquid we employed, we carefully purified this salt ([bdmim]PF_6) by filtration through a Al_2O_3 (neutral type I, activated) column as an acetone solution and rinsed out the salt with a mixed solvent of hexane and ethyl acetate [7,8]. However, no enzymatic reaction was observed in the [bdmim]PF_6 solvent. Although we still suspect that a trace amount of impurity included in the solvent significantly inhibited the enzyme, no impurity was detected by ^{1}H NMR analysis of [bdmim]PF_6. Because [bdmim]PF_6 was a neutral solvent and there was no accumulation of acetaldehyde oligomer as confirmed by ^{1}H NMR analysis of the reaction mixture after 24 h stirring, this must not have been due to the inhibitory action of an acetaldehyde oligomer formed by the reaction process. It was very interesting that the 2-methyl group of the imidazolium salt determined the enzyme reactivity exactly. We are assuming that the difference in reactivity between [bdmim]PF_4 and [bmim]PF_6 reflects the change of interaction of the counter anion part of the imidazolium salts with the enzyme protein [24]. It has sometimes been suggested that enzymatic reactions in an ionic liquid solvent system were anion dependent [5,21]. However, we believe that the results of an enzymatic reaction might be due to overall solvent properties, because the reaction proceeded smoothly in [bmim]BF_4 (entry 13) or [bmim]PF_6 (entry 16).

It was possible to apply the [bdmim] salts solvent system to the reaction of Toyonite 200M or Toyonite 200P supported lipase PS catalyzed reactions [7] (Eq. (2)). As shown in Table 2, the reaction proceeded smoothly in both [bdmim]BF_4 and [bdmim]PF_6 systems (entries 1–4). However, enantioselectivity of these reactions was insufficient and the *E*-values [23] were inferior to the reactions in [bmim]PF_6 (entry 5) or in *i*-Pr_2O (entry 6). Therefore, it was concluded that enantioselectivity of the lipase-catalyzed reaction was determined by a combination of the cationic part and the anionic part of the ionic liquid.



(2)

3. Conclusion

In summary, we established an excellent lipase-recycling system using vinyl acetate as acyl donor in

the [bdmim]BF_4 solvent system, although the enantioselectivity may be depend on the substrates. Obviously, [bdmim]BF_4 is not a panacea for non-aqueous biocatalysis, however, we do believe that further investigation of the scope and limitations of this reaction, especially optimization of the combination of imizadolium cation and anion, will make it even more beneficial.

4. Experimental

Enantiomeric excess of the products was determined by capillary GC analysis for compounds **2** and **3**, or HPLC analysis using chiral column for compounds **3** and **4**. Chiraldex G-Ta was used for GC analysis: Ø 0.25 mm × 20 m, carrier gas: He 40 ml/min, temperature: 100 °C, inlet pressure: 1.35 kg/cm^2, amount 400 ng, detection: FID, HPLC analysis: Chiralcel OD (δ: 4.6 mm × 250 mm), hexane:2-propanol (10:1–8:1), 35 °C, 1.0 ml/min, 254 nm. Methyl (±)-mandelate (**4**) and methyl (*S*)-(+)-**4** as a reference sample were purchased from Aldrich. Novozym 435 (*Candida antarctica*) is now commercially available as CHIRAZYME L-2, c.-f., C2, Iyo from Roche Molecular Biochemicals. (bdmim]BF_4 is commercially available from several companies, one being Merck KGaA, Frankfurter Strasse, Darmstadt, Germany. Toyonite is a porous ceramic prepared from a kaolinite: Toyodenka Co. Ltd. Tel.: +81-888-31-1241, e-mail: m-kamori@toyodenka.com. Toyonite 200P immobilized lipase PS is now commercially available from Amano Enzyme Ltd.

4.1. Lipase-catalyzed acylation of 5-phenyl-1-penten-3-ol (*1*) [22]

To a mixture of Novozym 435 (25 mg) in the [bdmim]BF_4 (1.5 ml) were added racemic 5-phenyl-1-penten-3-ol ((±)-**1**) (49.0 mg, 0.30 mmol) and vinyl acetate (39.0 mg, 0.45 mmol). The resulting mixture was stirred at 35 °C for 3 h. The reaction course was monitored by GC analysis and the product and unreacted alcohol were extracted with diethyl ether (20 times). The remaining ionic liquid phase was placed under reduced pressure for 5 h to remove the ether and a mixture of (±)-**1** (49.0 mg, 0.30 mmol) and vinyl acetate (39.0 mg, 0.45 mmol) was added to the ionic liquid phase. The acylation reaction took place smoothly, and the product (*S*)-**2** was obtained without any loss in enantioselectivity or reaction rate. Acetate (*S*)-**2**; bp 85 °C/2.5 mmHg (Kugelrohr); R_f 0.53 (hexane/ethyl acetate = 7:1); $[\alpha]_D^{26}$ +4.6° (*c* 1.40, $CHCl_3$); >99% e.e. (Chiraldex G-Ta); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.74–1.99 (2H, m), 2.49–2.66 (2H, m), 5.10–5.23 (3H, m), 5.73 (1H, ddd, J = 6.3, 10.7, 21.6 Hz), 7.08–7.23 (5H, m); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ: 21.2, 31.4, 35.7, 74.2, 116.9, 125.9, 128.3, 128.4, 136.2, 141.3, 170.3; IR (neat, cm^{-1}) 3028, 2934, 1738, 1497, 1371, 1236, 1022, 932, 750, 700. Anal. Calcd.: C, 76.44; H, 7.90. Found: C, 76.60; H, 7.91. Alcohol (*R*)-**3**; $[\alpha]_D^{25}$ + 5.2° (*c* 1.46, $CHCl_3$); 89% e.e. (Chiraldex G-Ta); R_f 0.17 (hexane/ethyl acetate = 7:1); bp 80 °C/2.0 mmHg (Kugelrohr); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.60–1.98 (2H, m), 2.13–2.52 (1H, brd, OH), 2.68 (2H, dt, J = 3.4, 7.9 Hz), 4.08 (1H, dt, 3 = 6.3, 6.4 Hz), 5.00–5.30 (2H, m), 5.86 (1H, ddd, J = 6.4, 10.5, 17.1 Hz), 6.91–7.49 (5H, m); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 31.5, 38.4, 72.3, 114.8, 125.7, 128.3, 128.3, 140.9, 141.8; IR (neat, cm^{-1}) 3379, 3027, 2927, 1604, 1495, 1451, 992, 924, 746, 699. Anal. Calcd.: C, 81.44; H, 8.70. Found: C, 81.21; H, 8.46.

4.2. Optical resolution of methyl (±)-mandelate (*4*)

To a mixture of lipase PS immobilized by Toyonite 200P (Amano) (25 mg) in the [bdmim]BF_4 (1.5 ml) were added (±)-**4** (50.0 mg, 0.30 mmol) and vinyl acetate (39.0 mg, 0.45 mmol). The resulting mixture was incubated at 35 °C for 72 h with agitating at 155 rpm. The reaction course was monitored by silica-gel TLC. To the reaction mixture was added 1.5 ml of diethyl ether to form the biphasic state. The product and unreacted alcohol were extracted from the ether layer. Repeating the same process 10 times, the product and unreacted alcohol were quantitatively extracted. TLC (hexane/ethyl acetate = 4:1) afforded acetate (*S*)-**5** (71% e.e.) (8.1 mg, 0.039 mmol) in 13% yield and alcohol (*R*)-**4** (12.0 mg, 0.24 mmol) in 80% yield. (*S*)-**5**: R_f 0.55 (hexane/ethyl acetate = 4:1); $[\alpha]_D^{21}$ + 124.6° (*c* 0.82, $CHCl_3$), 71% e.e. (Chiralcel OD); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 2.18 (3H, s), 3.70 (3H, s), 5.92 (1H, s), 7.36–7.46 (5H, m); ^{13}C NMR 25.8 MHz, $CDCl_3$) δ: 20.7, 52.6, 76.7, 127.6, 128.8, 129.2, 133.7, 169.3, 170.3; IR (neat, cm^{-1}) 3038, 2955, 1761, 1747,

1497, 1435, 1373, 1177, 978. (*R*)-**4**: $[\alpha]_D^{21}$ − 130.1° (*c* 0.90, $CHCl_3$), 10% e.e. (Chiralcel OD).

Acknowledgements

The authors are grateful to Novo Nordisk Bioindustry Co. Ltd., Meito Sangyo Co. Ltd., and Amano Pharmaceutical Co. Ltd. for providing the lipases. They are also grateful to Mr. Masanobu Kamori of Toyodenka Co. Ltd. for providing Toyonite.

References

[1] T. Welton, Chem. Rev. 99 (1999) 2071.
[2] R.D. Rogers, K.R. Seddon (Eds.), Ionic Liquids Industrial Applications to Green Chemistry, American Chemical Society, ACS Symposium Series 818, Oxford University Press, Oxford, 2002.
[3] P. Wassersceid, T. Welton (Eds.), Ionic Liquids in Synthesis, Wiley-VCH, 2003 and references cited therein.
[4] R.A. Sheldon, R.M. Lau, M.J. Sorgedrager, F. van Rantwijk, K.R. Seddon, Green Chem. 4 (2002) 147.
[5] T. Itoh, E. Akasaki, K. Kudo, S. Shirakami, Chem. Lett. (2001) 262.
[6] T. Itoh, E. Akasaki, Y. Nishimura, Chem. Lett. (2002) 154.
[7] T. Itoh, Y. Nishimura, M. Kashiwagi, M. Onaka, in: R.D. Rogers, K.R. Seddon (Eds.), Ionic Liquids as Green Solvents: Progress and Prospects, ACS Symposium Series, American Chemical Society, Oxford University Press, Oxford, Chapter 21.
[8] T. Itoh, N. Ouchi, S. Hayase, Y. Nishimura, Chem. Lett. 32 (2003) 654.
[9] S.G. Cull, J.D. Holbrey, V. Vargas-More, K.R. Seddon, G.J. Lye, Biotechnol. Bioeng. 69 (2000) 227.
[10] M. Erbeldinger, A.J. Mesiano, A.J. Russell, Biotechnol. Prog. 16 (2000) 1131.
[11] M.R. Lau, F. van Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189.
[12] J.A. Laszlo, D.L. Compton, Biotechnol. Bioeng. 35 (2001) 181.
[13] S.H. Schöfer, N. Kaftzik, P. Wasserscheid, U. Kragl, Chem. Commun. (2001) 425.
[14] K.-W. Kim, B. Song, M.-Y. Choi, M.-J. Kim, Org. Lett. 3 (2001) 1509.
[15] J. Howarth, P. James, J. Dai, Tetrahedron Lett. 42 (2001) 7517.
[16] S. Park, R.J. Kazlauskas, J. Org. Chem. 66 (2001) 8395.
[17] M. Uyama, T. Tetsufumi, S. Kobayashi, Polymer J. 34 (2002) 94.
[18] P. Lozano, T. de Diego, D. Carrié, M. Vaultier, J.L. Iborra, Chem. Commun. (2002) 692.
[19] M. Eckstein, M. Sesing, U. Kragl, P. Adlercreutz, Biotechnol. Lett. 24 (2002) 867.
[20] S.J. Nara, J.R. Harjani, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 2979.
[21] J.L. Kaar, A.M. Jesionowski, J.A. Berberich, R. Moulton, A.J. Russell, J. Am. Chem. Soc. 125 (2003) 4125.
[22] Y. Takagi, R. Ino, H. Kihara, T. Itoh, H. Tsukube, Chem. Lett. (1997) 1247.
[23] C.-S. Chen, Y. Fujimoto, G. Girdauskas, C.J. Sih, J. Am. Chem. Soc. 102 (1982) 7294.
[24] P. Lozano, T. Diego, D. Carrié, J. Am. Chem. Soc. 123 (2003) 692.