

A rational design of phosphonium salt type ionic liquids for ionic liquid coated-lipase catalyzed reaction†

Yoshikazu Abe, Kazuhide Yoshiyama, Yusuke Yagi, Shuichi Hayase, Motoi Kawatsura and Toshiyuki Itoh*

Received 27th May 2010, Accepted 16th September 2010

DOI: 10.1039/c0gc00151a

A rational design of phosphonium ionic liquid for ionic liquid coated-lipase (IL1-PS)-catalyzed reaction has been investigated, and very rapid transesterification of secondary alcohols accomplished when IL1-PS was used as catalyst in 2-methoxyethoxymethyl(tri-n-butyl)-phosphonium bis(trifluoromethanesulfonyl)amide ([P_{444MEM}][NTf₂]) as solvent while perfect enantioselectivity was maintaining. Increased K_{cat} value was suggested to be the most important factor in IL1-PS working the best in [P_{444MEM}][NTf₂] solvent.

Introduction

Lipases are the most widely used enzymes applicable for organic synthesis, however, the reaction rates are significantly dependent on the reaction media and very slow reactions or poor enantioselective reactions are sometimes obtained.¹ Therefore, development of a protocol to improve lipase reaction performance is desirable.¹ Ionic liquids (ILs) are now recognized as suitable for use in organic reactions and as providing potential for improvement in control of product distribution, enhanced reactivity, ease of product recovery, catalyst immobilization, and recycling.² ILs have also emerged as useful non-aqueous reaction media for biochemical reactions, particularly for lipase-catalyzed transesterifications.^{3–5} We have been investigating the use of ILs in asymmetric lipase-catalyzed reactions,^{3c,4d,6–15} and have developed two methodologies for a recyclable use system of lipase in an ionic liquid reaction medium: one is a lipase-catalyzed reaction under conditions of reduced pressure,⁶ and the other is design of an ionic liquid appropriate for biocatalysts.^{7,8,10,14} During our conduct of these studies, we discovered that introduction of alkyl ether moiety in the anionic part of ILs was very effective in improving the lipase reaction performance in the solvent.^{8,14} We then established a powerful means of activating lipase protein by coating it with imidazolium alkyl PEG sulfate ionic liquid: the ionic liquid coated *Burkholderia cepacia* lipase (IL1-PS) displayed excellent reactivity for many substrates in conventional organic solvents.^{11–17} It allows recyclable use of the IL1-PS if the reaction were possible in an ionic liquid solvent; we found that it was essential to choose an appropriate ionic liquid when using IL1-PS in the IL solvent. 2-Methoxyethyl(tri-n-butyl)phosphonium bis(trifluoromethane-sulfonyl)amide ([P_{444ME}][NTf₂])¹⁸ was thus selected as a good candidate for IL1-PS-catalyzed reaction medium from among six types of ionic liquids.¹⁴

Various types of ILs have been used as solvents for biochemical reactions, the most frequent being imidazolium salts.³ Although examples were limited, acceptable results were also obtained in non-imidazolium salts, such as ammonium salts,^{19b–d,20} pyrrolidinium salts,^{19a} alkylguanidinium salts,²¹ pyridinium salts,^{4e,19f–h} and phosphonium salt.^{14,20} It is well recognized that hydrophobic ionic liquids are generally suitable for biochemical reactions.^{3c,19} As mentioned, however, we previously reported that hydrophilic imidazolium salts ILs, which have alkyl ether functionalizing sulfate salts, were appropriate for lipase-catalyzed reaction.⁸ Recently, the groups of Xu,²¹ Kragl,²² Zhao,²³ and Iborra²⁴ have independently reported that ILs that have an alkyl ether moiety as a cationic part acted as good solvents for these reactions. Therefore, with the objective of optimizing the design of ionic liquids for asymmetric transesterification using IL1-PS, we again focused on phosphonium salts that have an alkyl ether group. Because the phosphonium salts moiety is commonly found in living creatures, we anticipated that the salts probably have good affinity with enzyme proteins and may provide a good environment for enzymes. Several types of phosphonium ionic liquids have been prepared as illustrated in Fig. 1, and we attempted to evaluate them as reaction media for IL1-PS-catalyzed reaction.²⁵ We now report that novel developed 2-methoxyethoxymethyl(tri-n-butyl)phosphonium bis(trifluoromethanesulfonyl)amide ([P_{444MEM}][NTf₂]) acts as an excellent solvent for IL1-PS-catalyzed reactions.

Experimental

Materials

For the lipase, commercial Lipase PS (Amano) from *Burkholderia cepacia*, imidazolium alkyl-PEG sulfate ionic liquid coated lipase PS (IL1-PS),¹² lipase QLM (Meito) (*Alcaligenes* sp.), and Novozym 435 (*Candida antarctica*) were employed. IL1-PS was prepared by the reported method¹² or using a commercial one provided by Tokyo Chemical Industry Co., Ltd. (TCI-B3028).¹⁶

Hydrophobic salts are far more preferable from the standpoint of realizing an easy workup process in organic reactions. Since NTf₂ salts or C₅F₈ salts have hydrophobic property with less

Department Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori, 680-8552, Japan.
E-mail: titoh@chem.tottori-u.ac.jp; Fax: +81 857 31 5259; Tel: +81 857 31 5259

† Electronic supplementary information (ESI) available: Preparation of IL1 and IL1-coated lipase PS.¹² Results of IL1-PS-catalyzed reaction of (±)-1a in various solvents (Table S1). See DOI: 10.1039/c0gc00151a

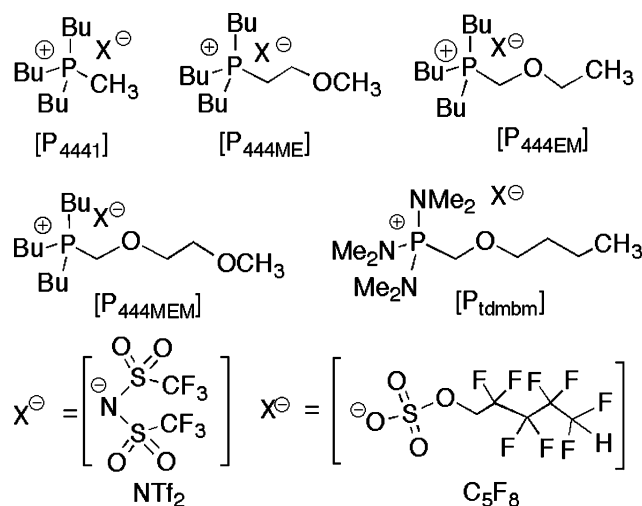


Fig. 1 Rational design of the cationic part of ionic liquids as solvent for lipase-catalyzed reaction.

toxicity,²⁶ they are now considered as a preferred anionic part of ionic liquids. Therefore, we selected NTf₂ or C₅F₈ as anion for ILs. Ionic liquid [bmim][C₅F₈] was prepared by the reported method.¹⁰ [bmim][NTf₂] and [N₂₂₁ME][NTf₂] were purchased from Kanto Reagents Co., Ltd. [P₄₄₄₁][NTf₂] was a gift from Nippon Chemical Co., Ltd. and [P_{tdmbm}][NTf₂] was a gift from Kanto Denka Kogyo Co., Ltd. Water content of the ionic liquids employed was determined by Karl Fischer moisture titrator. The values are listed as follows: [bmim][NTf₂] (Kanto Reagents Co., Ltd.): 170 ppm; [bmim][C₅F₈]: 195 ppm; [N₂₂₁ME][NTf₂] (Kanto Reagents Co., Ltd.): 290 ppm; [P₄₄₄₁][NTf₂] (Nippon Chemical Co., Ltd.): 145 ppm; [P_{444EM}][NTf₂]:¹⁸ 120 ppm; [P_{444ME}][NTf₂] (TCI T2564):¹⁸ 250 ppm; [P_{444MEM}][NTf₂]: 280 ppm; [P_{tdmbm}][NTf₂] (Kanto Denka Kogyo Co., Ltd.): 146 ppm.

Typical enzymatic reaction

The reaction was typically carried out as follows: To a mixture of 5 mg of IL1-PS (0.25 mg of the enzyme protein) in 1.0 mL of solvent was added (±)-**1a** (50 mg, 0.41 mmol) and 55 mg of vinyl acetate (1.5 equiv.) and the resulting mixture was stirred at 35 °C. To evaluate the initial reaction rate, the reaction was conducted in the presence of 0.5 mmol of hexadecane as an internal reference, an aliquot of the reaction mixture was sampled at 30 min. of the reaction and extracted with a mixed solvent of diethyl ether and hexane (1 : 4) and the rate was determined by capillary GC analysis. The reaction course was monitored by silica gel thin layer chromatography (TLC) analysis and the product (*R*)-**2a** and unreacted alcohol (*S*)-**1a** were extracted with a mixed solvent of diethyl ether and hexane (1 : 4) when the spots became the same size, then purified by silica gel TLC. Since it is well recognized that water content of the solvent influences the lipase performance, ionic liquids were dried under reduced pressure at 50 °C at 1 Torr for 3 to 5 h prior to the reaction. Enantiomeric excess of the product acetate and alcohol unreacted were determined by HPLC (Chiralcel OB-H, n-hexane: 2-propanol = 9 : 1 or 20 : 1). The reaction rate was determined by GC analysis at 30 min. of reaction in the presence of an internal reference. Enantioselectivity of the reaction was shown as the *E* value²⁷ which was calculated by %ee of (*R*)-**2**

(ee_p) and %ee of (*S*)-**1** (ee_s). $E = \ln[(1 - c(1 + ee_p))/1 - c(1 - ee_p)]$; here, *c* means conv. which was calculated by the following formula according to ref. 27: $c = ee_s/(ee_p + ee_s)$.

Synthesis of 2-methoxyethoxymethyl(tri-*n*-butyl)phosphonium bis(trifluoromethanesulfonyl)amide ([P_{444MEM}][NTf₂])

To an ethanol (20 mL) solution of 2-methoxyethoxymethyl chloride (MEM Chloride) (4.98 g, 40 mmol) was added tributylphosphine (7.5 g, 37 mmol) and the resulting mixture was stirred for 22 h at 80 °C. After being cooled to room temperature (rt), hexane was added to form a precipitate which was removed by filtration. The resulting filtrate was evaporated under vacuum to give the chloride salt (20.6 g, 36 mmol) in 97% yield. The salt was dissolved in ethanol (18 mL) and lithium bis(trifluoromethanesulfonyl)amide (11.37 g, 40 mmol) powder was added, then the mixture was stirred at rt for 17 h to form lithium bromide as a precipitate. The precipitate was removed by filtration, the filtrate was washed with hexane 3 times and the solvent removed using lyophilization. The resulting oil was dissolved in acetone and treated with active charcoal, and the charcoal was then removed by filtration. The filtrate was passed through active alumina (Type III) and dried under vacuum at 50 °C for 5 h to give [P_{444MEM}][NTf₂] (20.0 g, 35 mmol) as colorless oil in 95% yield. ¹H NMR (500 MHz, CDCl₃) δ 0.97 (9H, t, *J* = 7.6 Hz), 1.48–1.59 (12H, m), 2.15–2.22 (6H, m), 3.36 (3H, s), 3.55 (2H, dt, *J*_{HP} = 4.4, 5.5 Hz), 3.79 (2H, dt, *J*_{HP} = 2.4, 5.5 Hz), 4.39 (2H, d, *J*_{HP} = 4.8 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 12.8, 16.7 (d, *J*_{CP} = 47.0 Hz), 22.9 (d, *J*_{CP} = 3.90 Hz), 23.4 (d, *J*_{CP} = 15.3 Hz), 58.4, 60.2 (d, *J*_{CP} = 65.3 Hz), 71.0, 73.1 (d, *J*_{CP} = 11.4 Hz), 119.6 (q, *J*_{CF} = 321.5 Hz); ³¹P NMR (202.46 MHz, CDCl₃) δ 32.8; ¹⁹F NMR (170.6 MHz, CDCl₃, C₆F₆) δ 78.7; IR (neat) 2966, 1467, 1352, 1193, 1133, 1058, 923, 788, 740, 617, 570, 515 cm⁻¹; HRMS (EI) calcd for C₁₆H₃₆O₂P, 291.2410; found. 291.2408. Viscosity = 80.5 cP s at 25 °C (H₂O = 270 ppm), *T*_g = −84.1 °C (DSC). Since it sometimes colored when [P_{444MEM}][NTf₂] was dried at higher temperature, we usually dried the liquid at 50 °C at 1 Torr for 3 to 5 h and used it for the lipase-catalyzed reaction. However, it was possible to reduce the water content to 90 ppm when it was dried under reduced pressure at 5 Torr at 100 °C for 6 h.

Results and discussion

We initially evaluated IL1-PS-catalyzed reaction using racemic 1-phenylethanol ((±)-**1a**) as a model substrate in nine types of solvents (eqn (1)), and the results are illustrated in Fig. 2 (see Table S1 in the ESI for detailed results†). Diisopropyl ether (*i*-Pr₂O) was chosen as the typical non-aqueous organic solvent because we had previously established that the solvent was the best reaction medium for the IL1-catalyzed reactions among conventional organic solvents.¹⁴ We also conducted the enzymatic reactions in three types of hydrophobic ILs, [bmim][NTf₂], [bmim][C₅F₈], and [N₂₂₁ME][NTf₂] to compare the solvent effect of phosphonium ILs. Although none of the enzymes we tested was soluble in the solvents we employed and made only a sluggish mixture, the transesterification proceeded very smoothly. Both the enantioselectivity and reaction rate were significantly dependent on the solvent as illustrated in Fig. 2.

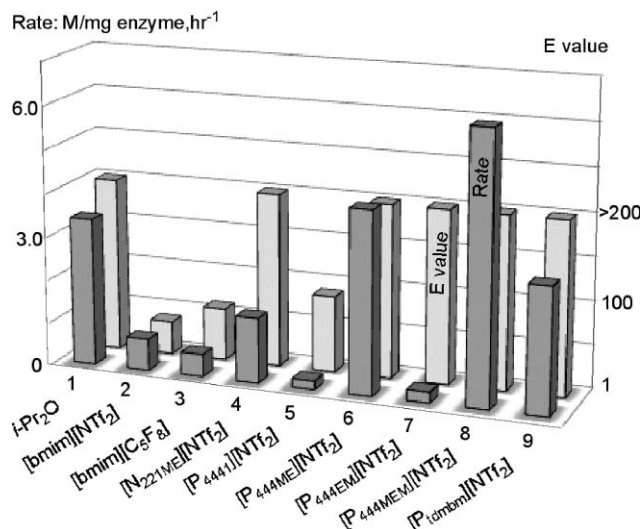
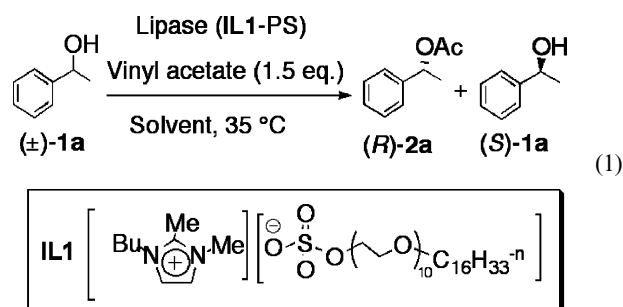


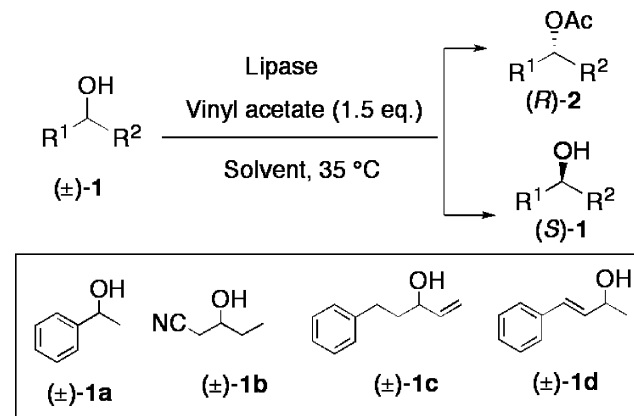
Fig. 2 IL-coated lipase PS-catalyzed reaction of (±)-1-phenylethanol (**1a**) in various ILs.



The reaction rate in [P_{444MEM}][NTf₂] solvent reached a remarkably high level at 6.2 M h⁻¹ per mg of enzyme (entry 8, Table S1), 1.8-fold faster than that recorded in *i*-Pr₂O solvent (entry 1). To the best of our knowledge, this is the record to date the rapid transesterification of 1-phenylethanol using lipase-catalyzed reaction. It should be noted that enantioselectivity also significantly depended on the solvent system; perfect enantioselectivity was obtained for the reactions using four types of phosphonium ILs and ammonium salt that have alkyl ether moiety in the cationic part (entries 4, and 6–9). On the contrary, reduced enantioselectivity was recorded when the IL1-PS-catalyzed reactions were carried out in [bmim][C₂F₈], [bmim][NTf₂], or [P₄₄₄₁][NTf₂] solvent systems (entries 2,3 and 5).

We next evaluated reactions of commercial lipase PS with those of IL1-PS in different solvent systems (Table 1, entries 1–4). As shown, IL1-PS showed drastically improved activity vs. commercial lipase PS in both solvent systems: IL1-PS showed 52-fold acceleration in *i*-Pr₂O (entry 3 vs. entry 1) and 84-fold acceleration in [P_{444MEM}][NTf₂] (entry 4 vs. entry 2) compared to commercial lipase PS.

Because lipase reactivity is generally dependent on the substrates, we next tested IL1-PS-catalyzed reactions using three substrates, 3-hydroxypentanenitrile (**1b**), 5-phenylpent-1-en-3-ol (**1c**), and (*E*)-4-phenylbut-3-en-2-ol (**1d**) in various solvent systems (Scheme 1, and the results are shown in Table 1, entries 5–20). It was found that the best solvent was not ILs but *i*-Pr₂O for the reaction of 3-hydroxypentanenitrile (**1b**) (entry 5). On the



Scheme 1

other hand, the combination of IL1-PS and [P_{444MEM}][NTf₂] again gave an excellent result for compound **1c** (entry 15): 4.8-fold acceleration was recorded compared to that in *i*-Pr₂O (entry 11 vs. entry 15). The IL1-PS-catalyzed reaction of **1d** did not depend on these solvent systems and similar results were obtained except for the reaction in [P₄₄₄₁][NTf₂] solvent; lower enantioselectivity was obtained in this solvent (entry 17).

We next evaluated reactions of two types of commercial enzymes in different solvent systems (Table 1, entries 21–26). As shown, [P_{444ME}][NTf₂] and [P_{444MEM}][NTf₂] worked as good solvents for these enzymes. However, a slightly reduced enantioselectivity was observed when Novozym435 was used in [P_{444MEM}][NTf₂] (entry 23). No significant change was observed when QLM-catalyzed reactions were carried out in these solvent systems (entries 24–26). It has thus been found that [P_{444MEM}][NTf₂] solvent is especially useful for IL1-PS-catalyzed reaction.

Since the viscosity of [P_{444MEM}][NTf₂] or [P_{444ME}][NTf₂] is much higher than that of *i*-Pr₂O,¹⁴ the origin of the high reaction efficiency of IL1-PS in the phosphonium ILs might not be due to the enhanced rate of mass transfer in the solvent system but to improved activity of the enzyme protein in [P_{444MEM}][NTf₂].

To gain more detailed information on what factor would contribute to the acceleration of the present IL1-PS-catalyzed reaction, the kinetic parameters of IL1-PS-catalyzed transesterification of (*R*)-**1c** or (*S*)-**1c** were measured (eqn (2) and (3)) and the results are shown in Table 2. IL1-PS-catalyzed reactions of (*R*)-**1c** and (*S*)-**1c** were accelerated in [P_{444MEM}][NTf₂] with similar magnitude (1.8 to 3-fold). The most interesting result of the kinetic experiments of IL1-PS-catalyzed reaction in [P_{444MEM}][NTf₂] solvent was found in the modified *K*_{cat} values between enantiomers: the *K*_{cat} value of (*S*)-isomer in [P_{444MEM}][NTf₂] (entry 8) was increased ca. 2-fold compared to that of *i*-Pr₂O (entry 5), and 3.6-fold acceleration was recorded for (*R*)-**1c** (entry 4 vs. entry 1). In addition, a slightly increased *K*_m was obtained for the reaction of (*R*)-**1c** (entry 4 vs. entry 1) and (*S*)-**1c** (entry 8 vs. entry 5). These results clearly suggest that solvent provides a certain impact on the reactivity of the enzyme protein of IL1-PS. Increased *K*_{cat} value was suggested to be the most important factor in why IL1-PS worked best in [P_{444MEM}][NTf₂] solvent.

Table 1 Results of lipase-catalyzed transesterification in various solvent systems

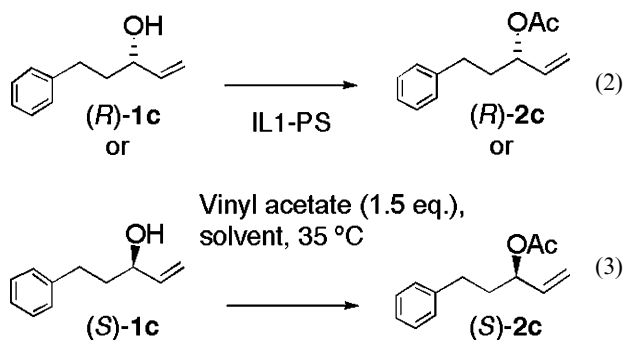
Entry		Enzyme	Solvent	%ee of (<i>R</i>)- 2 ^a (% yield) ^b	%ee of (<i>S</i>)- 1 ^a (% yield) ^b	Rate ^c	Conv. ^d	E value ^d
1	1a	PS ^f	<i>i</i> -Pr ₂ O	83 (21)	38 (61)	65	31	16
2	1a	PS ^f	[P ₄₄₄ MEM][NTf ₂]	99 (28)	45 (58)	74	31	>200
3	1a	IL1-PS ^g	<i>i</i> -Pr ₂ O	>99 (34)	98 (53)	3.4 × 10 ³	50	>200
4	1a	IL1-PS ^g	[P ₄₄₄ MEM][NTf ₂]	>99 (49)	96 (38)	6.2 × 10 ³	47	>200
5	1b	IL1-PS ^g	<i>i</i> -Pr ₂ O	92 (32)	73 (42)	1.1 × 10 ⁴	45	49
6	1b	IL1-PS ^g	[P ₄₄₄][NTf ₂]	80 (36)	27 (63)	8.2 × 10 ³	29	11
7	1b	IL1-PS ^g	[P ₄₄₄ EM][NTf ₂]	71 (4)	1.2 (43)	1.9 × 10 ²	2	6
8	1b	IL1-PS ^g	[P ₄₄₄ ME][NTf ₂]	77 (42)	61 (51)	4.3 × 10 ³	44	15
9	1b	IL1-PS ^g	[P ₄₄₄ MEM][NTf ₂]	73 (29)	51 (34)	6.7 × 10 ³	41	10
10	1b	IL1-PS ^g	[P _{tdmbm}][NTf ₂]	85 (35)	69 (61)	2.3 × 10 ³	45	25
11	1c	IL1-PS ^g	<i>i</i> -Pr ₂ O	>99 (21)	22 (75)	60	18	>200
12	1c	IL1-PS ^g	[P ₄₄₄][NTf ₂]	96 (16)	45 (71)	1.9 × 10 ²	32	68
13	1c	IL1-PS ^g	[P ₄₄₄ EM][NTf ₂]	99 (6)	1.8 (93)	41	2	>200
14	1c	IL1-PS ^g	[P ₄₄₄ ME][NTf ₂]	98 (34)	38 (63)	2.5 × 10 ²	28	137
15	1c	IL1-PS ^g	[P ₄₄₄ MEM][NTf ₂]	>99 (38)	59 (51)	2.9 × 10 ²	37	>200
16	1d	IL1-PS ^g	<i>i</i> -Pr ₂ O	98 (35)	85 (35)	9.0 × 10 ²	47	>200
17	1d	IL1-PS ^g	[P ₄₄₄][NTf ₂]	93 (33)	72 (45)	5.6 × 10 ²	44	62
18	1d	IL1-PS ^g	[P ₄₄₄ ME][NTf ₂]	99 (35)	78 (44)	1.0 × 10 ³	44	>200
19	1d	IL1-PS ^g	[P ₄₄₄ MEM][NTf ₂]	99 (42)	89 (50)	8.5 × 10 ²	47	>200
20	1d	IL1-PS ^g	[P _{tdmbm}][NTf ₂]	>99 (43)	84 (55)	2.6 × 10 ²	46	>200
21	1a	Novozym435 ^f	<i>i</i> -Pr ₂ O	99 (33)	96 (57)	49 (% h ⁻¹) ^e	49	>200
22	1a	Novozym435 ^f	[P ₄₄₄ ME][NTf ₂]	97 (42)	97 (32)	25 (% h ⁻¹) ^e	50	>200
23	1a	Novozym435 ^f	[P ₄₄₄ MEM][NTf ₂]	94 (47)	80 (49)	61 (% h ⁻¹) ^e	46	80
24	1a	QLM ^f	<i>i</i> -Pr ₂ O	97 (47)	93 (47)	65 (% h ⁻¹) ^e	49	194
25	1a	QLM ^f	[P ₄₄₄ ME][NTf ₂]	92 (45)	99 (38)	71 (% h ⁻¹) ^e	52	152
26	1a	QLM ^f	[P ₄₄₄ MEM][NTf ₂]	92 (45)	99 (47)	67 (% h ⁻¹) ^e	50	123

^a Determined by HPLC (Chiralcel OB-H, hexane : 2-Propanol = 20 : 1). ^b Isolated yield. ^c mM h⁻¹ per mg enzyme. The rate was determined by GC analysis at 30 min of reaction. ^d Calculated by %ee of (R)-2 (ee_p) and %ee of (S)-1 (ee_s). $E = \ln[(1 - c(1 + ee_p))/\ln[(1 - c(1 - ee_p))]$, here c means conv. which was calculated by the following formula: $c = ee_s/(ee_p + ee_s)$. See ref. 27. ^e Rate was determined by GC analysis at 30 min of reaction, but the value was shown as %conv h⁻¹, because we have no information on the accurate weight of the enzyme protein included for the corresponding commercial enzyme. ^f The reaction was carried out using 50 wt% (vs. substrate) of the commercial enzyme. ^g The reaction was conducted in the presence of 10 wt% (vs. substrate) of IL1-PS.

Table 2 Results of kinetic experiments of IL-coated PS (IL1-PS)-catalyzed transesterification of 5-phenylpent-1-en-3-ol (1c) in four types of solvents

Entry	Sub.	Solvent	V_{max} ^a	K_m	K_{cat}	K_{cat}/K_m
1	(R)-1c	<i>i</i> -Pr ₂ O	3.0	6.1	12	2.0
2	(R)-1c	[bmim][NTf ₂]	0.27	2.2	1.1	0.50
3	(R)-1c	[P ₄₄₄ ME][NTf ₂]	4.7	5.7	19	3.3
4	(R)-1c	[P ₄₄₄ MEM][NTf ₂]	1.1	9.3	44	4.8
5	(S)-1c	<i>i</i> -Pr ₂ O	0.041	0.50	0.16	0.32
6	(S)-1c	[bmim][NTf ₂]	2.1 × 10 ⁻³	0.087	8.7 × 10 ⁻³	0.10
7	(S)-1c	[P ₄₄₄ ME][NTf ₂]	0.12	0.79	0.48	0.61
8	(S)-1c	[P ₄₄₄ MEM][NTf ₂]	0.077	0.59	0.31	0.53

^a M min⁻¹ per mg enzyme.



Zhao and co-workers recently reported that dissolution and stabilization of a lipase protein took place in ILs that have a

long alkyloxyalkyl chain in an ammonium cation, and that this might provide improved catalytic efficiency of the corresponding biochemical reactions.²³ Although IL1-PS is still present in a suspension state in [P₄₄₄MEM][NTf₂] solvent, we assume that stronger affinity between the salt with lipase protein might cause preferable modification of enzyme reactivity because the surface of lipase protein would be partially covered with alkyl PEG ionic liquid for IL1-PS.¹² We also speculate that presence of the methoxyethoxymethyl group in the solvent may play an important role in improving flexibility of the enzyme protein in the solvent and contributing to the remarkable acceleration of the present transesterification.

Conclusions

In conclusion, we established that phosphonium ionic liquid [P₄₄₄MEM][NTf₂] becomes an excellent reaction medium for lipases, especially for ionic liquid coated-lipase PS (IL1-PS). In fact, very rapid acetylation of 1-phenylethanol has been accomplished using the combination of IL1-PS and [P₄₄₄MEM][NTf₂] as solvent while maintaining perfect enantioselectivity. It has now been shown that introduction of alkyl ether moiety might be a sure way to design ionic liquids suited for enzymatic reaction. Our phosphonium ionic liquid has a certain advantage over conventional organic solvents, because the solvent makes it possible to use the enzyme repeatedly and has less-volatile and less-flammable properties. After the reaction, we always recover the ILs and use them repeatedly after simple purification; we

have several bottles of ILs which we have used many times over successively for more than ten years. We believe that further investigation of the scope and limitations of the biochemical reaction in ILs will make them even more beneficial.

Acknowledgements

The present work was supported by a Grant-in-Aid for Scientific Research in a Priority Area, "Science of Ionic Liquids", from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors are grateful to Mr Tsuyoshi Tagata of Koei Chemical Co., Ltd. for measurement of physical properties of phosphonium salt ionic liquids.

Notes and references

- For reviews, see: (a) C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Pergamon, Oxford, (1994); (b) U. T. Bornscheuer and R. J. Kazlauskas, *Hydrolases in Organic Synthesis: Regio-And Stereoselective Biotransformation*, John Wiley & Sons Inc., Chichester, (1999); (c) T. Matsuda, Ed., *Future Directions in Biocatalysis*, Elsevier, The Netherlands, (2007).
- For reviews for ILs see: (a) P. Wasserscheid and T. Welton (Eds), *Ionic Liquids in Synthesis*, Wiley-VCH, Verlag, 2008; (b) J. Ranke, S. Stolte, R. Störmann, J. Arning and B. Jastorff, *Chem. Rev.*, 2007, **107**, 2183–2206; (c) N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123.
- Review for biochemical reactions in ILs: (a) H. Zhao, *J. Mol. Catal. B: Enzym.*, 2005, **37**, 16; (b) F. van Rantwijk and R. A. Sheldon, *Chem. Rev.*, 2007, **107**, 2757; (c) T. Itoh, in *Future Directions in Biocatalysis* (Ed. T. Matsuda), Elsevier Bioscience, (The Netherlands), 2007, Chapter 1, (pp. 3–20); (d) P. Lozano, *Green Chem.*, 2010, **12**, 555; (e) M. Moniruzzaman, K. Nakashima, N. Kamiya and M. Goto, *Biochem. Eng. J.*, 2010, **48**, 295; (f) H. Zhao, *J. Chem. Technol. Biotechnol.*, 2010, **85**, 891.
- Early examples of biochemical reactions in ILs: (a) S. G. Cull, J. D. Holbrey, V. V. Mora, K. R. Seddon and G. J. Lye, *Biotechnol. Bioeng.*, 2000, **69**, 227; (b) M. Erbeltinger, A. J. Mesiano and A. J. Russell, *Biotechnol. Prog.*, 2000, **16**, 1129; (c) R. M. Lau, F. van Rantwijk, K. R. Seddon and R. A. Sheldon, *Org. Lett.*, 2000, **2**, 4189; (d) T. Itoh, E. Akasaki, K. Kudo and S. Shirakami, *Chem. Lett.*, 2001, 262; (e) S. H. Schöfer, N. Kaftzik, P. Wasserscheid and U. Kragl, *Chem. Commun.*, 2001, 425; (f) P. Lozano, T. De Diego, J. P. Guegan, M. Vaultier and J. L. Iborra, *Biotechnol. Bioeng.*, 2001, **75**, 563; (g) K.-W. Kim, B. Song, M.-Y. Choi and M.-J. Kim, *Org. Lett.*, 2001, **3**, 1507; (h) S. Park and R. J. Kazlauskas, *J. Org. Chem.*, 2001, **66**, 8395; (i) H. Zhao and S. V. Malhotra, *Biotechnol. Lett.*, 2002, **24**, 1257.
- Recent selected examples of lipase-catalyzed reactions in ILs: (a) N. M. T. Lourenço and C. A. M. Afonso, *Angew. Chem., Int. Ed.*, 2007, **46**, 8178; (b) S. H. Lee, T. T. N. Doan, S. H. Ha and Y.-M. Koo, *J. Mol. Catal. B: Enzym.*, 2007, **45**, 57; (c) P. Lozano, E. García-Verdugo, R. Piamtongkam, N. Karbass, T. De Diego, M. I. Burguete, S. V. Luis and J. L. Iborra, *Adv. Synth. Catal.*, 2007, **349**, 1077; (d) M. Moniruzzaman, N. Kamiya, K. Nakashima and M. Goto, *Green Chem.*, 2008, **10**, 497; (e) T. Ema, T. Doi and T. Sakai, *Chem. Lett.*, 2008, **37**, 90; (f) H. Shan, Z. Li, M. Li, G. Ren and Y. Fang, *J. Chem. Technol. Biotechnol.*, 2008, **83**, 886.
- (a) T. Itoh, E. Akasaki and Y. Nishimura, *Chem. Lett.*, 2002, 154; (b) T. Itoh, Y. Nishimura and M. Kashiwagi, M. Onaka, *Ionic Liquids as Green Solvents: Progress and Prospects*, ACS Symposium Series 856, R. D. Rogers and K. R. Seddon, ed. American Chemical Society, Washington DC, Chapter 21, pp. 251–261, (2003).
- T. Itoh, Y. Nishimura, N. Ouchi and S. Hayase, *J. Mol. Catal. B: Enzym.*, 2003, **26**, 41.
- T. Itoh, N. Ouchi, S. Hayase and Y. Nishimura, *Chem. Lett.*, 2003, **32**, 654.
- T. Itoh, N. Ouchi, Y. Nishimura, S.-H. Han, N. Katada, M. Niwa and M. Onaka, *Green Chem.*, 2003, **5**, 494.
- Y. Tsukada, K. Iwamoto, H. Furutani, Y. Matsushita, Y. Abe, K. Matsumoto, K. Monda, S. Hayase, M. Kawatsura and T. Itoh, *Tetrahedron Lett.*, 2006, **47**, 1801.
- T. Itoh, S.-H. Han, Y. Matsushita and S. Hayase, *Green Chem.*, 2004, **6**, 437.
- T. Itoh, Y. Matsushita, Y. Abe, S.-H. Han, S. Wada, S. Hayase, M. Kawatsura, S. Takai, M. Morimoto and Y. Hirose, *Chem.-Eur. J.*, 2006, **12**, 9228.
- S.-H. Han, T. Hirakawa, T. Fukuba, S. Hayase, M. Kawatsura and T. Itoh, *Tetrahedron: Asymmetry*, 2007, **18**, 2484.
- Y. Abe, K. Kude, S. Hayase, M. Kawatsura, K. Tsunashima and T. Itoh, *J. Mol. Catal. B: Enzym.*, 2008, **51**, 81.
- Y. Abe, T. Hirakawa, S. Nakajima, N. Okano, S. Hayase, M. Kawatsura, Y. Hirose and T. Itoh, *Adv. Synth. Catal.*, 2008, **350**, 1954.
- IL1-PS is commercially available from Tokyo Chemical Industry Co., Ltd. (TCI-B3028).
- M.-J. Kim *et al.* first reported the ionic liquid coated lipase, though no acceleration was obtained for the enzyme because the authors prepared it by just mixing the lipase protein with an ionic liquid: J. K. Lee and M.-J. Kim, *J. Org. Chem.*, 2002, **67**, 6845.
- T. Itoh, K. Kude, S. Hayase and M. Kawatsura, *Tetrahedron Lett.*, 2007, **48**, 7774: [P₄₄₄ME][NTf₂] is now commercially available from Tokyo Chemical Industry Co., Ltd. (TCI-T2564).
- (a) J. L. Kaar, A. M. Jesionowski, J. A. Berberich, R. Moulton and A. J. Russell, *J. Am. Chem. Soc.*, 2003, **125**, 4125; (b) T. De Diego, P. Lozano, S. Gmouh, M. Vaultier and J. L. Iborra, *Biomacromolecules*, 2005, **6**, 1457; (c) R. M. Lau, M. J. Sorgedrag, G. Carrea, F. van Rantwijk, F. Secundo and R. A. Sheldon, *Green Chem.*, 2004, **6**, 483; (d) C. Chiappe, L. Neri and D. Pieraccini, *Tetrahedron Lett.*, 2006, **47**, 5089; (e) L. Zheng, S. Zhang, X. Yu, L. Zhao, G. Gao, X. Yang, H. Duan and S. Cao, *J. Mol. Catal. B: Enzym.*, 2006, **38**, 17; (f) H. Zhao, R. G. Luo and S. V. Malhotra, *Biotechnol. Prog.*, 2003, **19**, 1016; (g) S. V. Malhotra and H. Zhao, *Chirality*, 2005, **17**, S240–S242; (h) H. Zhao, O. Olubajo, Z. Song, A. L. Sims, T. E. Person, R. A. Lawal and L. A. Holley, *Bioorg. Chem.*, 2006, **34**, 15.
- H. Yu, J. Wu and C. C. Bun, *Chirality*, 2005, **17**, 16.
- Z. Guo, B. Chen, R. L. Murillo, T. Tan and X. Xu, *Org. Biomol. Chem.*, 2006, **4**, 2772.
- S. Dreyer and U. Kragl, *Biotechnol. Bioeng.*, 2008, **99**, 1416.
- (a) H. Zhao, G. A. Baker, Z. Song, O. Olubajo, T. Crittle and D. Peters, *Green Chem.*, 2008, **10**, 696; (b) H. Zhao, C. L. Jones and J. V. Cowins, *Green Chem.*, 2009, **11**, 1128; (c) H. Zhao, Z. Song and O. Olubajo, *Biotechnol. Lett.*, 2010, **32**, 1109.
- T. De Diego, P. Lozano, M. A. Abad, K. Steffensky, M. Vaultier and J. L. Iborra, *J. Biotechnol.*, 2009, **140**, 234.
- We attempted to prepare several types of methoxy poly(ethoxy)ethyl-substituted phosphonium ILs, however, it was found that these salts were not appropriate as solvents because they were too viscous to conduct the lipase-catalyzed reactions.
- For a review for toxicity of ILs see: T. P. T. Pham, C.-W. Cho and Y.-S. Yun, *Water Res.*, 2010, **44**, 352.
- C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.