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Supported ionic liquids in *Burkholderia cepacia* lipase-catalyzed asymmetric acylation

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ABSTRACT

Lipase PS from *Burkholderia cepacia* was successfully immobilized on Kynol™ ACC 507-15 active carbon cloth with and without ionic liquids as SILE catalysts. Activity, enantioselectivity and reuse of the catalysts were evaluated in the acylation of 1-phenylethanol with vinyl acetate in toluene and in hexane over the temperature range 25–60 °C. The presence of [EMIM][NTf_2] clearly stabilized the enzyme against inactivation and preserved enantioselectivity in reuse in a process which is affected by the nature of the IL, solvent and substrate structure.

1. Introduction

Room temperature ionic liquids (RTILs), or simply ionic liquids (ILs), are salts which are often formed from an organic cation like *N*-alkylated imidazolium cation and from an inorganic anion like BF_4^- or NTf_2^-. As non-volatile liquids, ILs have gained increasing interest in the place of organic solvents in various applications, biocatalytic applications being in our interest herein. The relation between the structure of an IL and enzyme stability and activity is still not well understood being covered with contradictions and exceptions. It has been suggested that ILs as salts may stabilize enzymes by protecting the hydration layer surrounding the enzyme, and/or by the permanent activating conformational change [1–3]. MALDI-TOF mass spectrometric analysis has shown that the cationic part of an IL can bind with the lipase protein [4]. The use of ILs or their mixtures together with organic solvents has been excellently reviewed for many biocatalytic reactions where ILs have been shown to improve enzymatic stability, enantioselectivity and activity [5,6]. Unfortunately, ILs as reaction media are often costly and for enzymatic reactions they need to be of high purity. Moreover, ecotoxicity, synthetic procedures and purification methods, especially those of imidazolium-based ILs, have rendered their green properties questionable and put them more or less into the same category with most organic solvents [7–9].

Lipase PS from *Burkholderia cepacia* (former *Pseudomonas cepacia*) in its various immobilized forms is one of the most versatile lipases for enantioselective acyl-transfer reactions in nonaqueous media. Previously we used the enzyme immobilized on Toynite (lipase PS-C II), encapsulated as sol–gels (highly porous silica materials) and cross-linked as CLEAs in ILs [10–12]. These works revealed that ILs may introduce new kinds of demands for enzyme immobilization. Thus, sol–gels and CLEAs resulted in hampered performance probably due to diffusion and mass-transfer limitations associated with high viscosity of ILs [12]. We also learnt that adsorption forces (for instance lipase PS adsorbed on celite in the presence of sucrose [10,13]), are not necessarily enough to keep the lipase on its support in ILs as solvents.

Interesting non-solvent applications of ILs have been described where the advantages of ILs still exist even when they are present in minor amounts [14,15]. ILs have been used as additives in encapsulating *Candida rugosa* lipase in sol–gel [16], supporting L-proline for an aldol reaction [17] and anchoring substrates to ILs for kinetic resolution with lipases [18]. *Candida antarctica* lipase B has been immobilized on membranes with the assistance of ionic liquids although the membranes prepared simply by adsorption were more reactive than membranes prepared with ILs [19–21]. A remarkable acceleration and/or excellent enantioselectivity have been reported for various substrates when lipase PS was coated with an IL [4,22,23]. The preparation of supported IL catalysts has been introduced for both metal [24] and enzymatic catalysts [19,25,26]. In supported ionic liquid catalysis, a thin IL layer between the enzyme protein on a solid material and, for instance,

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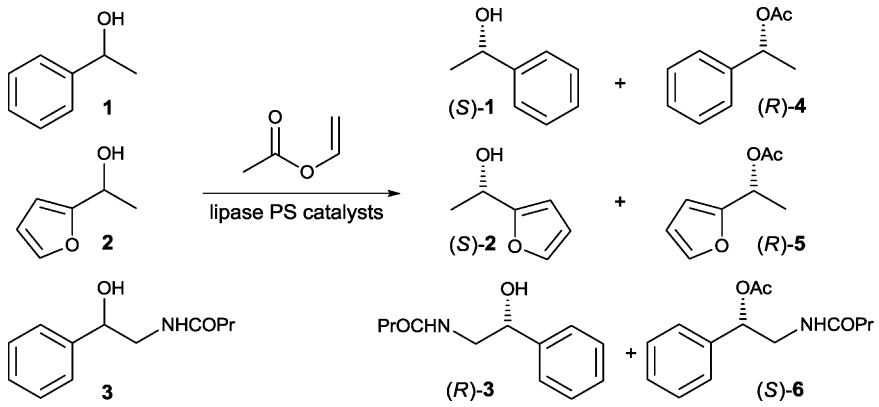

Scheme 1. Kinetic resolution of **1**–**3** catalyzed by lipase PS catalysts.

an organic reaction medium has proved to be enough in retaining similar characteristics in lipases as the IL has when used as a bulk solvent. Thus, while organic solvents cause restrictions in the flexibility of proteins, a thin IL layer or water molecules in it may enhance flexibility [10,6,27]. Thin ionic liquid layers can also facilitate a more rapid diffusion and mass-transfer compared to catalysis where IL is used as a reaction medium.

We found the concept of supported ionic liquid enzymes (support/IL/enzyme, SILE) highly fascinating, and herein describe the preparation and characters of immobilized lipase PS as SILE catalysts. The behavior of four different SILEs has been evaluated in the acylation of racemic 1-phenylethanol (**1**) with vinyl acetate in toluene and in hexane (Scheme 1). Under optimized conditions, the usability of the best SILE has been applied also to the enantioselective acylation of racemic **2** and **3**. The acylation results are compared to the catalysts prepared simply by adsorbing commercial free lipase PS powder on a solid support and to the results obtained with free lipase PS powder.

2. Experimental

2.1. General remarks

Lipase PS "Amano" (from *B. cepacia*) as a powder containing 10% free enzyme in essence celite was purchased from Amano Pharmaceuticals Co., Ltd. (Nagoya, Japan). The supports applied in this study were active carbon (AC), activated carbon cloth (ACC 507-15, 1500 m^2/g) and activated carbon paper (STV 505, 700 m^2/g) from Nippon Kynol, Japan and alumina (Versal VGL-25, 63–100 μm). 1-Phenylethanol (98%) was obtained from Aldrich and 1-(2-furyl)ethanol (>97%) from Fluka. Amide **3** was prepared by the reaction of 2-amino-1-phenylethanol (Aldrich, 98%) with butanoic anhydride (0.95 eqv.). Vinyl acetate and the solvents were of the highest grade from Aldrich, J.T. Baker and Lab-Scan Ltd. 1-Ethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([EMIM][NTf_2]) and tetrafluoroborate ([EMIM][BF_4]) were prepared by the methods described in literature [10,28,29], methyl trioctylammonium trifluoroacetate ([MTOA][TFA]), 1-butyl-4-methylpyridinium tetrafluoroborate ([4MBPy][BF_4]) and 1-butyl-3-methylimidazolium trifluoromethanesulfonate [BMIM][TfO] were obtained from Merck KGaA and used as received.

The progress of enzymatic reactions was followed and initial rates were determined by taking samples (50 μL, calculated for the first 15–20 min) at intervals. The samples were derivatized with propionic anhydride in the presence of 4,4-dimethylaminopyridine (DMAP, 1% in pyridine) to achieve a good baseline separation when analyzed by GC equipped with a Chrompack CP-Chirasil-DEX CB column (25 m × 0.25 mm) or a Chrompack CP-Chirasil-L-valine column. The determination of E was based on equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ with $c = ee_S/(ee_S + ee_P)$ using linear regression (E as the slope of the line $\ln[(1-c)(1-ee_S)]$ vs. $\ln[(1-c)(1+ee_S)$ at conversions less than 40%) [30]. Minor experimental errors in ee or conversion can result in huge distortion in E around 200 or higher. Therefore, $E > 200$ is given rather than more exact values. Possible leaching of an ionic liquid into the enzymatic reaction mixture was followed by HPLC equipped with a Zorbax eclipse XDB-C8 column at 215 nm.

2.2. Immobilization

Support/IL/lipase PS (SILE) catalysts were prepared by following the methods developed for support/IL/metal catalysis (active carbon cloth as a support) [24] and for immobilizing *C. antarctica* lipase B onto the ceramic membranes via ionic liquids [19]. Thus, the pre-dried (105 °C, 1 h) support was wetted with a solution containing lipase PS in a phosphate buffer (0.02 M NaH_2PO_4, pH = 7.8), an IL and a dilutant (Table 1). The SILEs prepared were dried in a rotary evaporator at 60 °C and stored at room temperature in vacuum. These catalysts were characterized by means of nitrogen physisorption (B.E.T. and Dubinin equations were used) with Carlo Erba Instruments Sorptomatic 1900-sorptometer. The original protein content of lipase PS powder in the buffer/IL mixture and the content remained after the SILE catalyst was removed from the original solution were determined using bicinchoninic acid assay and bovine serum albumin as the standard protein. The difference gave the amount of the protein on the catalyst.

2.3. Enzymatic acylation

For enzymatic acylation, vinyl acetate (0.2 M) was dissolved in an organic solvent (30 mL) and the mixture was stirred (300 rpm) with tailor-made stirrer shaft where the matt-structured SILE cloth was attached as suitable, rectangular pieces, or the catalyst was dispersed with a stirring blade in the case of AC and alumina powders. The amount of SILE was 50–100 mg/mL in the cases of AC and alumina powders, 8.5–16 mg/mL in the cases of ACC catalysts and 4–5 mg/mL in the cases of STV-505 catalysts. The addition of one of the substrates **1**–**3** (0.1 M) initiated the reaction. Prior to the addition of the substrate the system was preheated to the desired temperature. In the recycling tests, the catalyst was recycled by transferring the cloth catalyst pieces from a resolved mixture to a fresh solution of a racemate in toluene or in hexane without washing or otherwise purifying the catalyst between reuses.

Table 1
Properties of the prepared catalysts.

Entry	Support	IL	Dilutant (mL)	mg (lipase PS)[a]/g (cat.)	%(PS) on catalyst	%(IL) on catalyst	Fresh catalyst		Used catalyst	
							Specific surface area[b] (m^2/g)	Micropore volume[b] (cm^3/g)	Specific surface area[b] (m^2/g)	Micropore volume[b] (cm^3/g)
1	AC	[EMIM][NTf_2]	B[c]/10	37	3.7	69	331	0.12	234	0.08
2	AC	[EMIM][BF_4]	B[c]/10	25	2.5	50	58	0.02	32	0.01
3	AC	[EMIM][NTf_2]	A[c]/10	26	2.6	54	55	0.02	27	0.01
4	AC	[EMIM][BF_4]	A[c]/10	23	2.3	54	69	0.02	63	0.02
5	AC	[EMIM][BF_4]	EtOH/2	14	1.4	3.4	747	0.26	554	0.20
6	AC	[EMIM][NTf_2]	EtOH/3	18	2.0	5.5	824	0.29	484	0.17
7	Alumina	[MTOA][FAc]	A:B[c] (1:1)/10	39	3.9	82	339	0.12	344	0.12
8	Alumina	[4MBPy][BF_4]	A:B[c] (1:1)/10	26	2.6	50	61	0.02	36	0.01
9	Alumina	[EMIM][NTf_2]	acetone/10	26	2.6	51	97	0.03	57	0.02
10	Alumina	[BMIM][TfO]	A:B[c] 1:1/10	26	2.6	50	59	0.02	104	0.04
11	Alumina	[EMIM][BF_4]	water/6	19	2.0	6.2	336	0.12	410	1.45
12	Alumina	[EMIM][NTf_2]	EtOH/4	20	2.0	5.7	340	0.12	329	0.12
13	ACC 507-15	[EMIM][NTf_2]	EtOH/5	40	4.0	8.9	980	0.35	745	0.26
14	ACC 507-15	[EMIM][BF_4]	EtOH/4	40	4.0	8.4	1644	0.58	1160	0.41
15	ACC 507-15	–	EtOH/4	41	4.0	-	1207	0.43	1069	0.38
16	STV 505	[EMIM][NTf_2]	EtOH/2	67	7.0	13.2	35	0.01	66	0.02
17	STV 505	[EMIM][BF_4]	EtOH/2	57	6.0	11.7	72	0.03	55	0.02

[a] Amount of lipase PS protein (bicinchoninic acid assay) as a difference between the initial amount and the amount left after the catalyst was removed.
[b] Dubinin surface area, ACC 507-15 pure: 1680 m^2/g, 0.60 cm^3/g; STV 505 pure: 1223 m^2/g, 0.43 cm^3/g.
[c] A = 0.02 M NaH_2PO_4 buffer pH = 7.8, B = 0.02 M Tris buffer pH = 7.8.

3. Results and discussion

3.1. Catalyst preparation and characterization

SILEs were prepared using Kynol™ active carbon (AC), Versal alumina, Kynol™ active carbon cloth (ACC 507-15) and Kynol™ active carbon paper (STV 505) as support materials. Supports like ACC and active carbon paper were chosen since the catalysts, when cut into pieces with known amounts of the enzyme, can conveniently be inserted onto the reaction mixture or attached to a stirring device [24] allowing an easy separation of the catalyst from the reaction products. This is an advantage in reuse over more traditional catalyst preparations where the enzyme is immobilized in the form of granules or balls and is removed by filtration or centrifugation. Thoroughly purified [EMIM][NTf_2] and [EMIM][BF_4] were selected as ILs as they are commonly used solvents for lipase PS-catalyzed acylations [10,12,28]. Some other ILs were also used with alumina. To start the catalyst preparation, commercial lipase PS powder (in essence celite containing 10% free enzyme) was mixed in a buffer at pH = 7.8 (pH optimum is 7–8), since it has been reported that lipases have a pH memory effect in ILs [31] as they have in organic solvents [32]. The bicinchoninic acid assay gave the protein content of the clear solution obtained after centrifugation. Buffers, ethanol, acetone and water were used to dilute the enzyme–IL mixture, thus rendering the solution suitable for wetting the support material. When the liquid was not totally absorbed into the support, the absolute amount on the support was obtained as a difference by repeating protein determination after the support/IL/lipase PS was removed. Immobilization resulted in a seemingly dry SILE catalyst with uniform and thin IL–enzyme layer on the support material. The properties of the catalysts are given in Table 1. SEM images revealed the morphology of the active carbon cloth ACC 507-15 (Fig. 1) and active carbon paper STV 505 (Fig. 2) catalysts. The lipase loading was 1.4–7 wt.% and the IL loading 3.4–82 wt.%. As a comparison, the catalyst without IL (entry 15) was prepared using the same method except without an IL.

The prepared catalysts were analyzed by means of nitrogen physisorption before and after the acylation of **1** with vinyl acetate in toluene. As the support materials are microporous, the specific surface areas and pore volumes were calculated by using the Dubinin method. The specific surface area was low with the catalysts immobilized on active carbon coal, alumina and STV 505 active

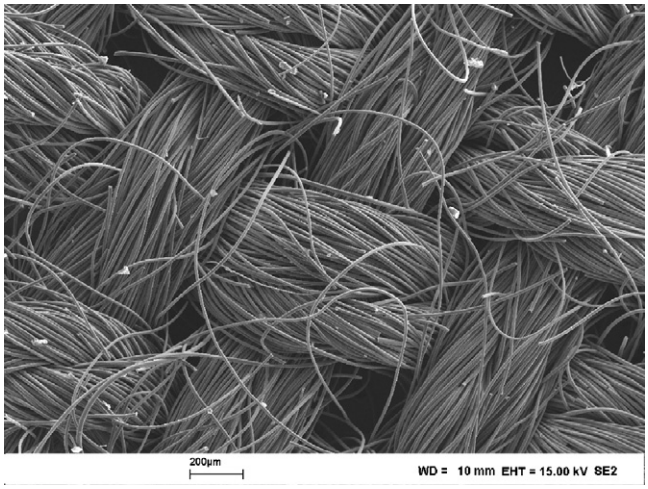

Fig. 1. SEM image of the Kynol™ active carbon cloth support.

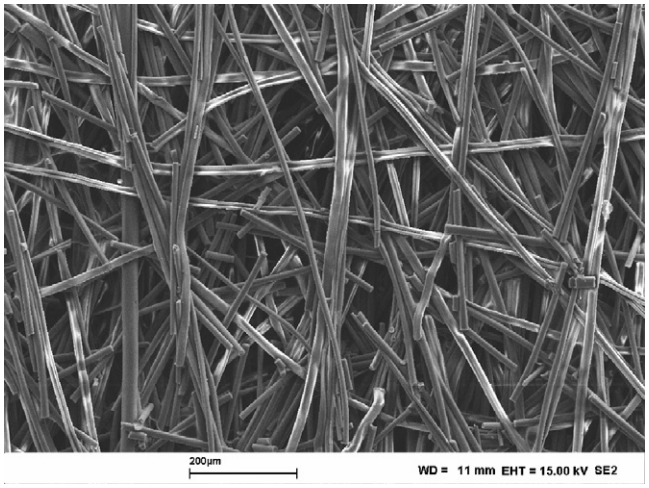

Fig. 2. SEM image of the Kynol™ active carbon paper support.

Table 2
Acylation of **1** (0.1 M) with vinyl acetate (0.2 M) in toluene in the presence of SILE catalysts at room temperature.

Entry	Support	IL	Dilution[a] (mL)	m (enzyme) (mg/mL)	Conversion[b] (%)	*E*
1	AC	[EMIM][NTf_2]	B[b]/10	100	1	7
2	AC	[EMIM][BF_4]	B[b]/10	100	4	32
3	AC	[EMIM][NTf_2]	A[b]/10	100	7	56
4	AC	[EMIM][BF_4]	A[b]/10	100	14	96
5	AC	[EMIM][BF_4]	EtOH/2	50	2	7
6	AC	[EMIM][NTf_2]	EtOH/3	50	1	3
7	Alumina	[MTOA][FAc]	A:B[b] 1:1/10	100	3	7
8	Alumina	[4MBPy][BF_4]	A:B[b] 1:1/10	100	1	>200
9	Alumina	[EMIM][NTf_2]	acetone/10	100	10	78
10	Alumina	[BMIM][TfO]	A:B[b] 1:1/10	100	1	15
11	Alumina	[EMIM][BF_4]	water/6	50	7	35
12	Alumina	[EMIM][NTf_2]	EtOH/4	50	10	29
13	ACC 507-15	[EMIM][NTf_2]	EtOH/5	14	50	>200
14	ACC 507-15	[EMIM][BF_4]	EtOH/4	13	50	>200
15	ACC 507-15	–	EtOH/4	16	49	>200
16	STV 505	[EMIM][NTf_2]	EtOH/2	4	24	70
17	STV 505	[EMIM][BF_4]	EtOH/2	5	43	>200
18	–	–	–	16[c]	30	112

[a] A = 0.02 M NaH_2PO_4 buffer pH = 7.8, B = 0.02 M Tris buffer pH = 7.8.
[b] Reaction time 6 h (entries 13–15), otherwise 24 h.
[c] 160 mg/mL commercial lipase PS powder, containing 10% enzyme.

carbon paper (Table 1, entries 1–4, 7–12, 16 and 17) while it was high with catalysts immobilized on ACC 507-15 active carbon cloth (entries 5, 6 and 13–15). Compared to a fresh catalyst, the reduction of specific surface areas and micropore volumes were observed after the catalyst was used, indicating pore blocking. With some alumina and STV 505 catalysts the specific surface areas and micropore volumes were higher after using the catalyst (entries 10, 11 and 16), indicating that either the enzyme or IL or both are leaching from the catalyst.

The efficiency of the SILE catalysts defined as conversion after 24 h (Table 2, entries 1–12 and 16–17) or after 6 h (entries 13–15) was evaluated using the enzymatic acylation of **1** with vinyl acetate in toluene as a model reaction. As usual, enantiomer ratio, *E*, was used as a measure of enantioselectivity. The catalyst systems on AC and alumina generally gave slow reactions with low enantioselectivity, and it was not possible to improve the situation by varying ILs or dilutants (entries 1–12). Reactivities were negligible also when compared to the reaction with commercial free enzyme powder where much lower enzyme content (16 mg/mL) gave 2–30 times faster reactions (entry 18). This indicates that AC and alumina might not be suitable supports for lipase PS with this method. On the other hand, the catalyst preparations on ACC 507-15 and STV 505 were highly active (entries 13–17). Enantioselectivity was excellent ($E > 200$) with all catalytic systems on ACC 507-15 (entries 13–15) and with STV 505/[EMIM][BF_4]/lipase PS (entry 17). As shown by the kinetic curves in Fig. 3, catalyzing power of the ACC 507-15/[EMIM][NTf_2]/lipase PS (■) and ACC 507-15/[EMIM][BF_4]/lipase PS (◊) catalysts in toluene is similar and better than that of commercial lipase PS powder (□) or ACC 507-15/lipase PS (♦). For STV 505-based catalysts the amount of lipase PS absorbed was small due to very poor wettability. Finally, our focus was turned to the ACC 507-15-based catalysts due to their high specific surface area and micropore volume as well as to excellent wetting properties.

3.2. Effect of solvent, temperature and reuse on acylation

It is essential for our SILE catalysts that the IL is insoluble in hydrophobic organic solvents used as reaction media. HPLC analysis revealed some [EMIM][NTf_2] leaching from the catalyst into toluene during the acylation of **1**. For that reason, the reaction was studies also in hexane where no leaching took place (Fig. 3, Tables 3 and 4). Although the acylation with free lipase PS powder proceeded considerably faster in hexane (○) than in toluene (□), the opposite effect on conversion with time was seen when ACC 507-15/[EMIM][NTf_2]/lipase PS was submerged in toluene (■) and hexane (●).

In enzymatic applications, one of the most important features is that the catalyst is stable and preserves activity and enantioselectivity in reuse. To study the stability of ACC 507-15 SILEs in reuse, the same catalyst was subjected to the acylation of **1** (0.1 M) with vinyl acetate (0.2 M) in toluene and in hexane at eight different temperatures over the range 25–60 °C before applying the SILE catalyst again at 25 °C (Tables 3 and 4). The temperature program rather than repeating the use at the same temperature was used to see how well the catalyst stands harsh reaction conditions. Initial rates were determined as μmol min^{-1} against the amount of the enzyme protein (Table 3). For initial rates, samples were taken within the first 10–20 min. The 6 h sample was always taken as well (see the results in Table 4). Beyond that, each reaction proceeded 24 h in all before the catalyst was subjected to a subsequent reaction with a new batch of reactants at the next temperature. In general, initial rates for ACC 507-15/[EMIM][NTf_2]/lipase PS in toluene were higher than with ACC 507-15/lipase PS in the absence of the IL and

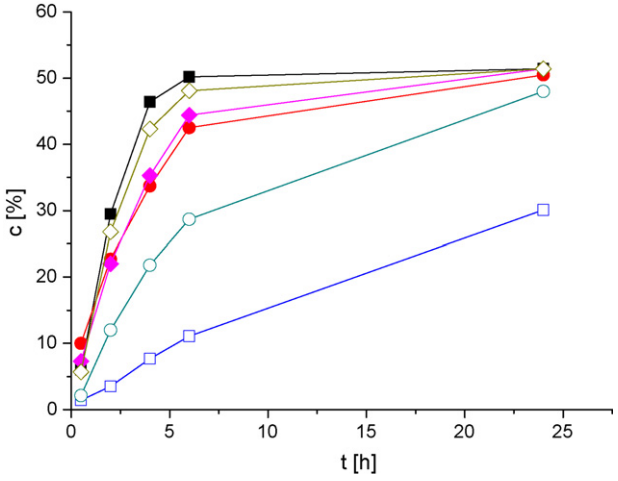

Fig. 3. Kinetic curves for the acylation of **1** (0.1 M) with vinyl acetate (0.2 M) in the presence of commercial free lipase PS powder (□, 16 mg/mL), ACC 507-15/[EMIM][NTf_2]/lipase PS (■, 13 mg/mL), ACC 507-15/[EMIM][BF_4]/lipase PS (◊, 16 mg/mL) and ACC 507-15/lipase PS (♦, 14 mg/mL) in toluene as well as in the presence of commercial free lipase PS powder (○, 16 mg/mL) and ACC 507-15/[EMIM][NTf_2]/lipase PS (●, 11 mg/mL) in hexane.

Table 3
Temperature effects on reuse: initial rates for the acylation of **1** (0.1 M) with vinyl acetate (0.2 M) in toluene in the presence of Kynol™ ACC 507-15-based SILEs.

Number of use[a]	T (°C)	v_o [μmol min^{-1} g^{-1}]			
		ACC 507-15/lipase PS (13.6 mg/mL)	ACC 507-15/[EMIM][NTf_2]/lipase PS (13 mg/mL)	ACC 507-15/[EMIM][BF_4]/lipase PS (16 mg/mL)	ACC 507-15/[EMIM][NTf_2]/lipase PS[b] (11 mg/mL)
1	25	15.7 ± 0.6	19.5 ± 0.9	12.0 ± 0.6	18.6 ± 1.1
2	30	24.9 ± 0.2	36.5 ± 1.6	13.6 ± 0.9	32.4 ± 3.5
3	35	20.8 ± 0.9	45.5 ± 3.5	24.5 ± 1.0	22.5 ± 0.7
4	40	31.3 ± 2.9	42.1 ± 1.4	23.0 ± 0.7	39.0 ± 1.4
5	45	32.3 ± 0.2	47.6 ± 1.9	24.8 ± 0.6	18.6 ± 1.1
6	50	36.9 ± 1.2	33.9 ± 0.7	21.8 ± 0.2	21.4 ± 1.2
7	55	31.0 ± 0.8	32.9 ± 1.0	13.9 ± 0.3	3.7 ± 0.8
8	60	23.8 ± 0.7	36.7 ± 0.9	9.2 ± 0.4	4.6 ± 0.7
9	25	6.4 ± 0.3	18.8 ± 1.3	3.2 ± 0.2	No reaction

[a] The same catalyst worked 24 h in every use before transferred to catalyze the reaction in the next temperature.
[b] Reaction performed in hexane instead of toluene.

Table 4
Temperature effects on reuse: conversion and enantioselectivity for the reaction of **1** (0.1 M) with vinyl acetate (0.2 M) in toluene in the presence of Kynol™ ACC 507-15/lipase PS catalyst. Reaction time 6 h.

Number of use[a]	T (°C)	Conversion (%)/E			
		ACC 507-15/lipase PS (14 mg/mL)	ACC 507-15/[EMIM][NTf_2]/lipase PS (13 mg/mL)	ACC 507-15/[EMIM][BF_4]/lipase PS (16 mg/mL)	ACC 507-15/[EMIM][NTf_2]/lipase PS[b] (11 mg/mL)
1	25	44/>200	50/>200	48/152	43/>200
2	30	50/>200	51/>200	50/136	46/>200
3	35	49/>200	51/>200	50/>200	43/>200
4	40	51/>200	51/>200	50/>200	46/>200
5	45	51/>200	51/>200	50/>200	41/>200
6	50	51/>200	51/>200	50/>200	23/>200
7	55	51/>200	51/>200	45/>200	8/>200
8	60	50/>200	51/>200	38/>200	4/>200
9	25	40/>200	47/>200	15/117	–

[a] The same catalyst worked 24 h in every use before transferred to catalyze the reaction in the next temperature.
[b] Reaction performed in hexane instead of toluene.

toluene clearly more favorable as a solvent than hexane (Table 3). Thus, the initial rates in toluene at 25 °C with the fresh catalyst and after the reuses were practically unchanged (19.5 μmol min^{-1} g^{-1}, number of use 1 and 18.8 μmol min^{-1} g^{-1}, number of use 9) indicating that the IL protects the enzyme from inactivation throughout the temperature cycle. On the other hand, only some 40% of the initial activity was left with ACC 507-15/lipase PS as a catalyst, and the presence of [EMIM][NTf_2] in hexane resulted in the total inactivation of the enzyme. [EMIM][NTf_2] as an IL stabilized the catalyst better than [EMIM][BF_4]. The efficiency of acylations was maximal at around 50 °C for ACC 507-15/lipase PS and at 35–45 °C with the catalysts containing one of the ILs. This is in accordance with the temperature optimum 43 °C of lipase PS as reported by Amano [33].

For the acylation of **1** in toluene, 50% conversion was reached in 6 h at 25 °C with fresh ACC 507-15 SILEs (Table 2, entries 13–15). For this reason, the 6 h samples were analyzed carefully in every reuse. The results in Table 4 confirm clear differences in the behavior of [EMIM][NTf_2] and [EMIM][BF_4] in our ACC 507-15-based SILEs. With ACC 507-15/[EMIM][BF_4]/lipase PS in toluene, enantioselectivity tended to be more favorable at higher temperatures (entries 3–8). In addition, there was a drop in E when the same catalyst was repeatedly applied at 25 °C after completing the temperature cycle (compare entry 1–9). Moreover, the catalyst was considerably inactivated. Inactivation and a drop in E reflect conformational changes in the protein structure and possibly increased water content in hydrophilic [EMIM][BF_4] [34]. When ACC 507-15/[EMIM][NTf_2]/lipase PS catalyzed the reaction in hexane, reactivity started to drop already at temperatures over 40 °C, and finally the enzyme had practically lost its activity at 50 °C (entries 5–9). Note that hexane better dissolves the substrate than toluene as shown by mixing racemic **1** in the (1:2) mixtures of [EMIM][NTf_2]/toluene (60% of racemic **1** was detected in the toluene phase) and [EMIM][NTf_2]/hexane (**1** was practically all in the hexane phase). Thus in hexane, the substrate concentration in the vicinity of the enzyme is evidently so low that the stabilizing effect of the substrate binding is lost.

Finally, the effect of molecular structure and solvent on the acylation of **1–3** with ACC 507-15/lipase PS and ACC 507-

Table 5
Acylation of **1**–**3** (0.1 M) with vinyl acetate (0.2 M) in the presence of Kynol™ ACC 507-15/lipase PS preparations at 25 °C.

Entry	Comp.	Catalyst	Solvent	v_o (μmol min^{-1} g^{-1})	t (h)/Conv. (%)/E	ee (substr.) (%)	ee (prod.) (%)
1	**1**	ACC 507-15/lipase PS (14 mg/mL)	Toluene	15.7 ± 0.6	6/44/>200	79 (*S*)	99 (*R*)
2	**1**	ACC 507-15/[EMIM][NTf_2]/lipase PS (13 mg/mL)	Toluene	19.5 ± 0.9	6/50/>200	98 (*S*)	97 (*R*)
3	**2**	ACC 507-15/lipase PS (8.5 mg/mL)	DIPE	27.1 ± 0.01	6/46/25	72 (*S*)	86 (*R*)
4	**2**	ACC 507-15/[EMIM][NTf_2]/lipase PS (9.9 mg/mL)	DIPE	40.0 ± 0.01	6/51/33	89 (*S*)	84 (*R*)
5	**3**	ACC 507-15/lipase PS (8.8 mg/mL)	TBME[a]	23.5 ± 1.5	24/32/>200	46 (*R*)	>99 (*S*)
6	**3**	ACC 507-15/[EMIM][NTf_2]/lipase PS (12 mg/mL)	TBME[a]	35.1 ± 2.1	24/36/>200	56 (*R*)	>99 (*S*)

[a] Reaction temperature 45 °C.

15/[EMIM][NTf_2]/lipase PS catalysts was studied (Table 5). Based on our earlier results [11,12], diisopropyl ether (DIPE) was chosen as a solvent for the acylation of **2** at 25 °C and *tert*-butyl methyl ether (TBME) for the acylation of **3** at 45 °C. The initial rates for **1**–**3** were all clearly enhanced when [EMIM][NTf_2] was present in the catalyst (compare entries 1–2, 3–4 and 5–6). The acylation of **2** proceeded to 51% conversion in 6 h with poor enantioselectivity in the presence of ACC 507-15/[EMIM][NTf_2]/lipase PS and to 46% conversion the presence of ACC 507-15/lipase PS (entries 3 and 4). The acylation of **3** started to retard significantly already after 4 h and the reaction stopped at around 36% conversion. When the catalyst was removed, clear substrate and product crystals were detected on the surface of the catalyst explaining the inactivation. In spite of this, the reaction proceeded with excellent enantioselectivity as shown by $E > 200$ and ee > 99% for the product enantiomer (entries 5 and 6).

4. Conclusions

Lipase PS was immobilized on active carbon coal, alumina, ACC 507-15 active carbon cloth and STV 505 active carbon paper with and without ionic liquids. These catalysts were evaluated in the acylation of **1** with vinyl acetate in toluene. ACC 507-15/[EMIM][NTf_2]/lipase PS catalyst in toluene was shown to be excellently reusable and temperature stable when the same SILE catalyst was studied for reuse at eight temperatures over the temperature range 25–60 °C before reusing the catalyst again at 25 °C. The same study with ACC 507-15/lipase PS catalyst showed activity loss, indicating that [EMIM][NTf_2] in the SILE activates and stabilizes lipase PS. Another benefit of the catalyst is that it can be cut into pieces with known amounts of the enzyme and the catalyst can be picked up with tweezers instead of using filtration or centrifugation. It was also shown that the catalyst in hexane looses its activity already at 50 °C and that another SILE, ACC 507-15/[EMIM][BF_4]/lipase PS, in toluene shows similar although less pronounced inactivation trend with temperature. Encouraging trends with the ACC 507-15/[EMIM][NTf_2]/lipase PS catalyst were also observed when the acylation of two secondary alcohols **2** and **3** in addition of **1** were studied.

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