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Sol–gels and cross-linked aggregates of lipase PS from *Burkholderia cepacia* and their application in dry organic solvents

Piia Hara [a,b], Ulf Hanefeld [b], Liisa T. Kanerva [a,*]

[a] *Department of Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, Lemminkäisenkatu 5C, FIN-20520 Turku, Finland*
[b] *Biocatalysis and Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands*

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Abstract

Lipase PS from *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) was successfully immobilized in sol–gels under low methanol conditions using lyophilization in order to dry the gel. The enzyme was also cross-linked with glutaraldehyde to CLEAs without any additives. These immobilized enzyme preparations were employed for the highly enantioselective acylations of 1-phenylethanol (**1**), 1-(2-furyl)ethanol (**2**) and *N*-acylated 1-amino-2-phenylethanol (**3**) with vinyl acetate in organic solvents. Enzymatic hydrolysis of the obtained ester product was observed as a side reaction of the acylation of **3** in the presence of lipase PS powder. Hydrolysis was suppressed when the immobilized preparations of lipase PS were used.

Keywords: Lipase catalysis; Kinetic resolution; Immobilization; Sol–gel processes; CLEA

1. Introduction

In recent years enzymes have become increasingly popular catalysts, both in academic research and in fine chemical production [1,2]. By far the most popular type of enzymes are lipases. Lipases (EC 3.1.1.3) are so well accepted since they can readily be exploited as chiral catalysts in various types of acyl-transfer reactions for the preparation of enantiopure compounds. What makes them particularly attractive is that they are especially suited for reactions in organic solvents. In this respect, *Burkholderia cepacia* lipase (former *Pseudomonas cepacia* lipase, also known under its commercial trade name as lipase PS) has proven to be among the most versatile lipases. In order to enhance reactivity and enantioselectivity of the *Burkholderia cepacia* lipase/lipase PS we have generally used the enzyme adsorbed on celite in the presence of sucrose rather than the native lipase [3]. It has recently become clear that this simple adsorption method is less usable in polar environments such as in the presence of ionic liquids [4]. In polar environments, lipase PS immobilized on ceramic particles (lipase PS-SC II) has been preferable. *Candida antarctica* lipase B (CAL-B, Novozym 435) is another highly versatile lipase. This enzyme preparation contains the enzyme on a methacrylate polymer, which can introduce considerable amounts of water into dry organic solvents. This presence of water previously prevented the dynamic kinetic resolution of mandelonitrile by enantioselective acylation in dry toluene in the presence of a base because the acid produced by the undesired hydrolysis neutralized the base [5]. Lipase-catalyzed hydrolysis of the product or the acyl donor as a side reaction of enzymatic acylations can play a role more often than realized [6]. Thus, the tendency for an acylation to stop before the reactive enantiomer is consumed has been usually interpreted as a reaction of the produced ester with the alcohol (liberated from an achiral ester) rather than as its hydrolysis by the water in the system [7]. These and similar examples [8] indicate the importance of immobilization as well as the importance of the material on which the catalyst is immobilized [9,10].

Sol–gel and CLEA techniques are attractive and effective immobilization methods that are well-established [11–18]. In the sol–gel method the enzyme is immobilized by building the porous gel network around each enzyme macromolecule through hydrolysis of tetraalkoxysilanes and alkyltrialkoxysilanes, giving inert glasses with high porosity and high thermal and mechanical resistance [12]. The synthesis of efficient biocatalysts by encapsulation of lipase PS has been well documented

* Corresponding author. Tel.: +358 2 333 6773; fax: +358 2 333 7955.
E-mail address: lkanerva@utu.fi (L.T. Kanerva).

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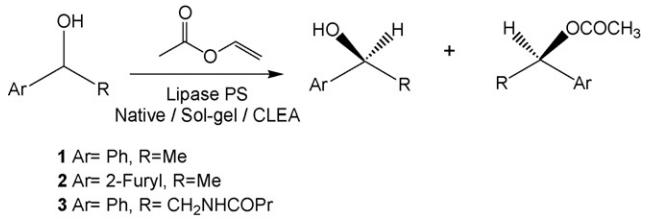

Scheme 1. Acylation reactions performed with *Burkholderia cepacia* preparations.

[13,19–24]. However, no attention has been paid to the methanol released during the hydrolysis of alkoxysilanes and its harmful effects for lipase PS. Previously the low methanol immobilization in sol–gels was reported for hydroxynitrile lyases [18,25], avoiding denaturation during the sol–gel formation.

Cross-linked enzyme aggregates (CLEA) are strictly speaking not immobilised enzymes. Their straightforward preparation combines protein precipitation followed by cross-linking with a suitable dialdehyde, usually with glutaraldehyde, without the need for expensive protein purification [14,15,26,27]. Thus a CLEA is not an immobilized enzyme but stabilized and almost pure enzyme. The synthesis of CLEAs for several lipases has been reported [14,16,17]. In the case of lipase PS, bovine serum albumin (BSA) as additive has been used to facilitate the formation of CLEAs from samples of low protein content and to protect enzymatic activity against the presence of glutaraldehyde [17]. The disadvantage of this approach is the further dilution of the enzyme.

In this study, the preparations of the sol–gel and the CLEA of lipase PS are presented. Lipase PS "Amano" SD powder (in 2006 the production line was changed by Amano, and the former lipase PS "Amano" is now sold as lipase PS "Amano" SD; not only the name was changed but also the diluent, dextrin being now in the place of earlier used diatomaceous earth; the previous works [17,23] are based on the use of lipase PS "Amano") has served as the source of the enzyme. From here on we call this commercial *Burkholderia cepacia* lipase (lipase PS Amano SD) "lipase PS". The two newly prepared immobilized enzyme preparations and lipase PS powder have been used for the acylation of secondary alcohols **1**–**3** with vinyl acetate in organic solvents (Scheme 1). The results with immobilized lipase PS are compared to those obtained with the lipase PS powder. Attention is given to the reuse of the new lipase PS preparations.

2. Experimental

2.1. General remarks

Lipase PS "Amano" SD (from *Burkholderia cepacia*, lipase PS powder) was purchased from Amano Pharmaceuticals Co., Ltd. (Nagoya, Japan). 1-Phenylethanol (98%) and 2-amino-1-phenylethanol (98%) were products of Aldrich and 1-(2-furyl)ethanol (>97%) was from Fluka. Amide **3** was prepared by the reaction of 2-amino-1-phenylethanol with butanoic anhydride (0.95 eqv.). Methyltrimethoxysilane (MTMS, Aldrich, >99%), tetramethoxysilane (TMOS, Fluka, >99%), glutaraldehyde (Fluka, 25% in water) and 1,2-dimethoxy ethane (DME, Aldrich, >99%) were used as supplied. 4-Nitrophenyl acetate (>99%) was from Fluka. Vinyl acetate and the solvents were of the highest grade from Aldrich, J.T. Baker and Lab-Scan Ltd.

The progress of the reactions was followed by taking samples (50 μL) at intervals and analyzing them by GC equipped with a Chrompack CP-Chirasil-DEX CB column (25 m × 0.25 mm) and a Chrompack CP-Chirasil-L-valine column. The samples were derivatized with propionic anhydride in the presence of 4,4-dimethylaminopyridine (DMAP, 1% in pyridine) to achieve a good baseline separation. The determination of E was based on equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ with $c = ee_S/(ee_S + ee_P)$ [28]. UV measurements were carried out at 25 °C on Perkin Elmer Lambda 650 UV–vis spectrophotometer. The protein content of lipase PS powder was determined using bicinchonic acid assay using bovine serum albumin as the standard protein [29,30].

2.2. Encapsulation of lipase PS in a sol–gel matrix

The sol–gel precursor was prepared according to a literature method [25]. Acidic water (1.38 mL, pH 2.85 by HCl) was added to a mixture of MTMS (2.1 g, 15.4 mmol), TMOS (9.08 g, 58.5 mmol) and distilled water (10.4 mL) and the mixture was stirred until it was homogenous. The formed methanol was removed by evaporation in a rotary evaporator until the odours of MTMS, TMOS and MeOH were not detectable any longer. The mixture was cooled to 0 °C and water was added until the total volume corresponded to the initial MTMS/TMOS volume. The sol precursor was used immediately for the encapsulation of lipase PS.

For encapsulation, lipase PS powder (containing 3% protein according to the bicinchonic acid assay, 100 mg) was dissolved in KH_2PO_4-buffer (200 μL, 0.1 M, pH 7.0). The sol precursor mixture (200 μL) was added and the mixture was stirred magnetically until homogenous, followed by the removal of the stirring bar. The mixture gelled (1–2 min) and the gel was aged at 4 °C for 24 h followed by lyophilization at 0.2 atm for 4 h. The formed xerogel was stored at 4 °C and used as a pellet.

2.3. Preparation of lipase PS-CLEA

The preparation of CLEA was based on a literature method [14]. Lipase PS powder (containing 3% protein according to the bicinchonic acid assay, 50 mg) in KH_2PO_4 buffer (1 mL, 0.1 M, pH 7) was added dropwise to a saturated $(NH_4)_2SO_4$ solution (9 mL) at 4 °C. Glutaraldehyde (377 μL, 100 mM, 25% in water) was added and the mixture was stirred at 4 °C for 5 h. The suspension was diluted with 3 mL of the buffer and centrifugated. The pellet was washed two times with the buffer (3 mL) and once with acetonitrile (3 mL). After centrifugation, the obtained CLEA was dried in vacuum and stored at 4 °C.

2.4. Enzymatic reactions

The standard activity test [14,16] in water was performed with *p*-nitrophenyl acetate as a substrate. Enzyme solution (lipase

PS powder (50 mg) in KH_2PO_4 buffer (1 mL, 0.1 M, pH 7) or CLEA/enzyme aggregate (suspended in 1 mL KH_2PO_4 buffer) was added to KH_2PO_4 buffer (2.9 mL, 0.1 M, pH 4.5) containing *p*-nitrophenyl acetate in EtOH (50 μL, 0.1 M). The reaction was monitored at 400 nm.

For enzymatic acylation, an organic solvent (1 mL) and vinyl acetate (0.2 M) were added to the lipase and the addition of a substrate (0.1 M) started the reaction. The reactions were shaken at room temperature or at 48 °C (temperature optimum for lipase PS is 50 °C). Results are given in Tables 3 and 4. For enzymatic hydrolysis by residual water the lipase PS powder and the sol–gel preparation, an organic solvent, lipase and one of the enantiopure esters of **1**–**3** were shaken at room temperature or at 48 °C (for results see Table 5).

2.5. Reuse of the lipase PS sol–gels and lipase PS CLEA

Reuse of the enzyme preparations were studied using the enzymatic acylation of 2 as a model. Each reaction cycle took 24 h. The lipase preparation was washed with DIPE before a new cycle.

3. Results and discussion

3.1. Immobilized enzymes

Lipase PS sol–gels were prepared following the reported low methanol condition procedure where methanol is removed by evaporation from the sol before mixing with the enzyme [25]. This procedure differs from those previously described for lipases [19–23]. The obtained gel was aged in a fridge for 24 h before drying it by lyophilization to form a xerogel. Various lyophilization times were tested, and it became clear that lyophilization times longer than 5 h started to be harmful for enzymatic activity. In addition, xerogels were prepared by evaporation at normal pressure at room temperature or at elevated temperature. These latter lipase PS xerogels showed low reactivity and enantioselectivity for the acylation of 1-phenylethanol (**1**) with vinyl acetate in toluene as a model reaction (Table 1, entries 1 and 2). Interestingly, in spite of considerable shrinking during drying by lyophilization, the lyophilized lipase PS xerogel catalyzed the reaction in a highly enantioselective and smooth way (entry 3) with activities of the same order of magnitude as those observed with the lipase PS powder (Table 3, entries 1 and 2). Obviously the capillary forces that cause the gel to shrink, in all of the xerogels, have not a negative effect on the enzyme. Instead it seems that the water content remaining in the air-dried xerogels is relatively high and causes the hydrolyses of the produced ester and the acyl donor used. Thus, we have developed a low methanol procedure for the preparation of lipase PS in a dry sol gel by lyophilization. The method is straightforward and needs no specialist equipment. It yields an active lipase for acylations in dry organic solvents. This preparation will from hereon be called lipase PS lyo sol–gel.

Table 1
Acylation of **1** (0.1 M) with vinyl acetate (0.2 M) in toluene at room temperature in the presence of dried sol–gel preparation (corresponds to 100 mg of the lipase PS powder), reaction time 24 h

Entry	Drying method	ee_S (%)	ee_P (%)	Conversion (%)	*E*
1	Evaporation in air, room temperature	3	85	3	13
2	Evaporation in air, at 40 °C	23	95	19	53
3	Lyophilization for 5 h	>99	>99	50	>200

Table 2
Activity yield of lipase PS (corresponds to 50 mg of lipase the lipase PS powder) for the hydrolysis of *p*-nitrophenyl acetate after precipitation with optimized precipitant volume and after cross-linking with glutaraldehyde (100 mM) at 4 °C for 5 h

Entry	Precipitant	*V* (mL)	After precipitation[a,b] (%)	After cross-linking[a,b] (%)
1	Sat. $(NH_4)_2SO_4$	9	446	174
2	1,2-Dimethoxyethane	4	65	39
3	Acetone	4	52	n.d.[c]
4	Ethanol	2	39	n.d.

[a] Activity yield (units found/units used) in %. 100% activity corresponds with the activity of the lipase PS powder in KH_2PO_4 buffer (0.1 M, pH 7.0).
[b] Activity test: see Section 2.1., enzyme activity measurements in water.
[c] Not determined.

The CLEA of lipase PS was first prepared by following the reported procedure for lipase CLEAs [14]. Lipase PS powder was used without prepurification in spite of the large amount of dextrin present (3% protein content according to bicinchonic acid assay). The first step of preparing CLEA was to study the precipitation of the lipase and to optimize the activity recovery of the aggregated enzyme. Common precipitants (saturated ammonium sulphate, 1,2-dimethoxyethane (DME), ethanol and acetone) for lipases were used [14,16,17]. Activity yields for the hydrolysis of *p*-nitrophenyl acetate in phosphate buffer (0.1 M, pH 7) after precipitation and after cross-linking with glutaraldehyde are shown in Table 2. Saturated ammonium sulphate was the precipitant of choice since it gave 100% precipitation in addition to the highest activity in the case of both the precipitated and cross-linked enzyme (174%, entry 1 compared to entries 2–4). This result differs from the previous CLEA preparation where acetone was a precipitant [17]. In those studies a very significant loss of activity (>95%) was described, when lipase PS was cross-linked without an additive. With an additive (BSA) the activity of the resulting CLEA showed activities significantly above 100% (up to 12-fold) [17]. The difference may be due to the presence of dextrin in our lipase PS powder compared to the diatomaceous earth in the older commercial *Burkholderia cepacia* lipase samples. Various amounts (25–150 mM) of glutaraldehyde were tested for cross-linking, 100 mM concentration and 5 h cross-linking time leading to the best result. Lower concentrations or shorter times gave leaching of the enzyme into the washing buffer. At higher glutaraldehyde concentrations, with longer reaction times or with organic solvents as precipitants, the dextrin in the enzyme powder also precipitated as a sticky substance. No additives were necessary for the preparation of the

Table 3
Initial rates (μmol min^{-1} g^{-1}) in the acylation of **1–3** (0.1 M) with vinyl acetate (0.2 M) in an organic solvent in the presence of lipase PS preparations at room temperature

Entry	Substrate	Solvent (1 mL)	Powder (100 mg mL^{-1})	Sol–gel[a]	Powder (50 mg mL^{-1})	CLEA[b]
1	**1**	Toluene	2.7 ± 0.3	1.42 ± 0.02	3.4 ± 0.2	7.0 ± 0.9
2	**1**	TBME	2.5 ± 0.2	1.27 ± 0.04	3.2 ± 0.3	8.5 ± 0.3
3	**1**[c]	Toluene	3.8 ± 0.2	2.05 ± 0.06	5.9 ± 0.2	32.3 ± 0.1
4	**1**[c]	TBME	4.4 ± 0.1	1.69 ± 0.02	5.7 ± 0.1	19.7 ± 0.9
5	**2**	DIPE	3.5 ± 0.2	1.47 ± 0.02	3.8 ± 0.2	30.1 ± 0.4
6	**2**	TBME	3.9 ± 0.1	0.94 ± 0.03	3.6 ± 0.1	18.6 ± 0.2
7	**3**[c]	TBME	1.69 ± 0.05	1.54 ± 0.03	2.38 ± 0.03	6.7 ± 0.3

[a] lyo sol–gel based on 100 mg mL^{-1} of lipase PS powder.
[b] Based on 50 mg mL^{-1} of lipase PS powder.
[c] Reaction temperature 48 °C.

present CLEA preparation, thus keeping the system as simple as possible.

3.2. Acylation with lipase PS lyo sol–gel and CLEA

Activity and selectivity of the lyo sol–gel and CLEA preparations were studied in the acylation of **1–3** in organic solvents (Scheme 1). Solvents [toluene, diisopropyl ether (DIPE) and *tert*-butyl methyl ether (TBME)] were selected on the basis of solvent screening with the lipase PS powder in the acylation of **1**. The amount of the protein was kept the same for the reactions with the lyo sol–gel preparation (100 mg mL^{-1} of the lipase PS) and the lipase PS powder, on one hand, and with the CLEA preparation (50 mg mL^{-1} of the original lipase PS) and the lipase PS powder, on the other hand. The results as initial rates measured by μmol min^{-1} g^{-1} of the original enzyme (activities) are shown in Table 3 and the progression curves for the acylation reactions in the most suitable solvents (toluene for **1**, DIPE for **2** and TBME for **3**) in Figs. 1–3. As seen from Table 3, enzymatic activities of the lyo sol–gel system are systematically lower than (entries 1–6) or similar to (entry 7) the activities of lipase PS powder while the CLEA system is considerably more active than the lipase PS powder system. But while the activities differ, all three follow the same trends for the solvents. Minor losses in activity for the lyo sol–gel of lipase PS might be due to diffusion limitations. The acylations of **1–3** in TBME catalyzed by lipase PS powder tend to proceed sluggishly and often stop at low conversions (Fig. 3, Table 4, entries 2, 4, 6 and 7). The same is true for the reactions catalyzed by the lyo

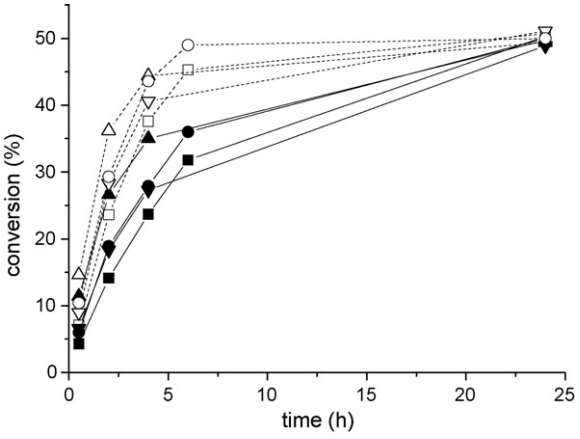

Fig. 1. Acylation of 1 (0.1 M) with vinyl acetate (0.2 M) in toluene at room temperature: lipase PS powder100 mg mL^{-1} (▲), lipase PS powder 50 mg mL^{-1} (▼), lyo sol–gel from 100 mg lipase PS (■), CLEA from 50 mg lipase PS (●). Acylation at 48 °C: lipase PS powder 100 mg mL^{-1} (△), lipase PS powder 50 mg mL^{-1} (▽), lyo sol–gel from 100 mg lipase PS (□), CLEA from 50 mg lipase PS (◯).

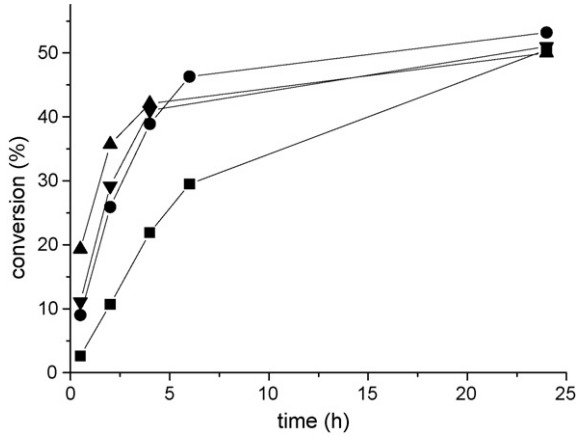

Fig. 2. Acylation of 2 (0.1 M) with vinyl acetate (0.2 M) in dry DIPE. Lipase PS powder 100 mg mL^{-1} (▲), lipase PS powder 50 mg mL^{-1} (▼), lyo sol–gel from 100 mg lipase PS (■), CLEA from 50 mg lipase PS (●).

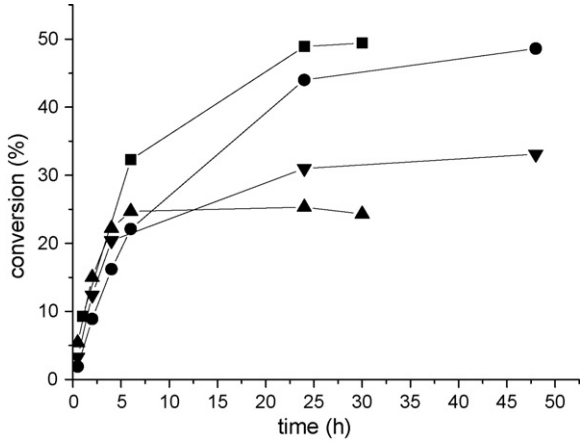

Fig. 3. Acylation of 3 (0.1 M) with vinyl acetate (0.2 M) in TBME at 48 °C. Lipase PS powder 100 mg mL^{-1} (▲), lipase PS powder 50 mg mL^{-1} (▼), lyo sol–gel from 100 mg lipase PS (■), CLEA from 50 mg lipase PS (●).

Table 4
Conversion and enantioselectivities in the acylation of **1**–**3** (0.1 M) with vinyl acetate (0.2 M) in an organic solvent in the precence of lipase PS at room temperature

Entry	Substrate	Solvent (1 mL)	Powder[a] (100 mg mL^{-1})	sol–gel[a,b]	Powder[a] (50 mg mL^{-1})	CLEA[a,c]
1	**1**	Toluene	24/50/>200	24/50/>200	24/49/>200	24/50/>200
2	**1**	TBME	27/36/56	24/47/>200	27/36/53	24/51/>200
3	**1**[d]	Toluene	24/49/>200	24/51/>200	24/51/198	24/50/>200
4	**1**[d]	TBME	24/40/77	24/40/174	24/46/112	30/47/>200
5	**2**	DIPE	24/50/115	24/51/139	24/51/131	24/53/58
6	**2**	TBME	27/42/50	30/40/78	27/46/30	24/56/35
7	**3**[d]	TBME	24/25/30	30/49/>200	48/33/14	48/49/>200
8	**3**[d]	Toluene	–	30/46/>200	–	–

[a] *t* (h)/conversion (%)/*E*.
[b] lyo sol–gel based on 100 mg mL^{-1} of lipase PS powder.
[c] Based on 50 mg mL^{-1} of lipase PS powder.
[d] Reaction temperature 48 °C.

sol–gel preparation in the case of **2**. However, the acylations of **1** and **3**, which smoothly proceed until the reactive enantiomers have reacted (entries 2 and 7, Fig. 1 (■) and Fig. 3 (■)) prove that the immobilisation has stabilized the lipase, so that it can be used in TBME. Even more remarkable is, that the acylations catalyzed by the CLEA preparation proceed smoothly, independent of the solvent (Table 4, entries 1–8, Figs. 1–3). However, there is a reason to pay attention to the substrate structure and reactivity over the entire progression of the reaction rather than to initial rates and enzymatic activities alone (Figs. 1–3). This is due for instance to diffusional differences in lyo sol–gels and CLEAs. Thus, the initial rates of **3** in TBME are faster in the case of the lipase PS CLEA than in that of lipase PS lyo sol–gel (Table 3, entry 7) while the progress of the reaction shows an opposite course (Fig. 3, ● for CLEA compared to ■ sol–gel). As is shown in Figs. 1 and 2 (● for CLEA compared to ■ sol–gel) the acylations of **1** and **2** proceeded faster in the CLEA preparation than in the lyo sol–gel preparation in accordance with the initial rates (Table 3, entries 1 and 5).

Lipase PS-catalyzed kinetic resolutions of **1**–**3** through acylation in organic solvents have been well-described for years. They lead to the *R*-enantiopreference in the case of alcohols **1** and **2** and to the *S*-enantiopreference in the case of *N*-acylated amino alcohol 3 as shown in the Scheme, in line with Kazlauskas rule [4,6,31,32]. The acylation of **1** ($E > 200$) and **2** ($E = 88$) with isopropenyl acetate in toluene has been described in the presence of a sol–gel preparation where lipase PS was co-lyophilized with peracetylated β-cyclodextrin [23]. The present results in Table 4 clearly demonstrate that a simple entrapment of the lipase into the sol–gel followed by drying through lyophilization is enough, leading to a highly enantioselective kinetic resolution catalyst from the lipase PS powder. Excellent enantioselectivities ($E > 200$) are also evident in the presence of the CLEA preparation except for the acylation of **2** (entries 5 and 6). This can be taken to indicate the stabilization of the protein as compared to the lipase PS powder. For the lipase PS powder, and also in the case of substrate **2** in the presence of the lyo sol–gel, TBME seems to be unsuitable as solvent because the acylation reactions tend to stop before the 50% conversion. This tendency is extremely clear for the acylation of **3** where the reaction stopped earlier and sharply in the case of the higher lipase PS powder content (with more water) (Fig. 3, ▲ and ▼), and from the slight concentration increase of (*S*)-**3** at the expense of the corresponding *S*-ester at longer reaction times. As an explanation to this TBME (being the most hydrophilic of the used solvents) was shown to favour the hydrolysis of the produced *S*-esters of **1** and **2** and the *R*-ester of **3** by the water in the lipase PS powder while the hydrolysis was considerably reduced for the same reactions when the sol–gel preparation was used (Table 5). Also the fact that lipase PS lyo sol–gel at higher temperatures has difficulties to fully acylate **1** in TBME, sustains this (Fig. 1 and Table 4, entry 4 vs. entry 2). Obviously the competing hydrolysis is faster at higher temperatures, causing a faster degradation of the acyl donor and/or product.

3.3. Reuse of immobilized enzymes

The recycling of the lipase PS lyo sol–gels and CLEAs for the acylation of **2** with vinyl acetate in DIPE was studied as a model reaction. For this purpose, the enzyme preparation was washed with the dry solvent between the cycles and the enzymatic acylation was repeated. The lyo sol–gels were reusable at least up to eight times at room temperature (Fig. 4). No significant loss of activity was observed while the *E* values randomly varied between 65 and 139. This variation is acceptable because it is well-known that the accuracy of *E* is low in highly enantioselective reactions where minor experimental errors can cause

Table 5
Conversion (%) after 24 h in the hydrolysis of enantiopure **1**–**3** esters (0.05 M) by the residual water in the enzyme preparation in an organic solvent at room temperature

Entry	Substrate	Solvent	log *P*	Conv. (%) by enzyme powder[a]	Conv. (%) by sol–gel[b]
1	(*S*)-**1**-ester	Toluene	2.8	4	1
2	(*S*)-**1**-ester	TBME	1.35	18	3
3	(*S*)-**2**-ester	DIPE	1.9	20	10
4	(*S*)-**2**-ester	TBME	1.35	12	2
5	(*R*)-**3**-ester[c]	TBME	1.35	86	16

[a] 100 mg mL^{-1} of lipase PS powder.
[b] lyo sol–gel based on 100 mg mL^{-1} of lipase PS powder.
[c] Butanoate instead of acetate; reaction temperature 48 °C.

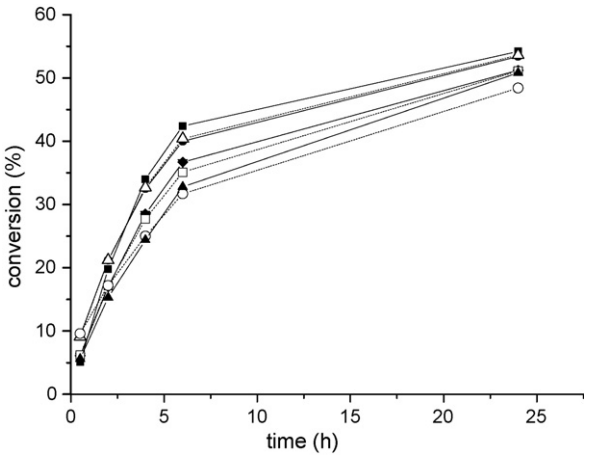

Fig. 4. Recycling of the lipase PS lyo sol–gel in the acylation of **2** with vinyl acetate in dry DIPE. Cycle 1 (■) ($E = 139$), cycle 2 (●) ($E = 77$), cycle 3 (△) ($E = 65$), cycle 4 (▼) ($E = 80$), cycle 5 (♦) ($E = 85$), cycle 6 (□) ($E = 90$), cycle 7 (◯) ($E = 80$), cycle 8 (▲) ($E = 90$).

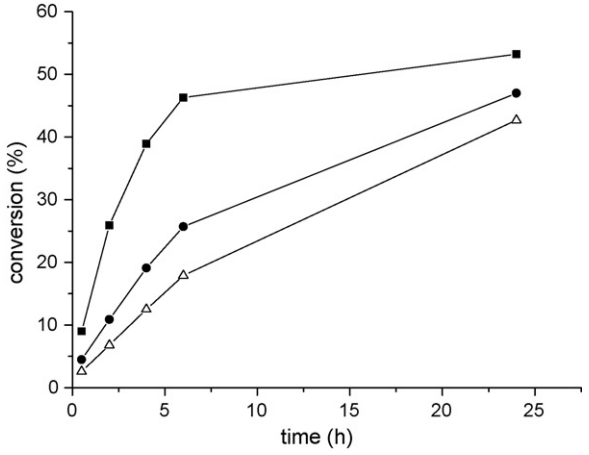

Fig. 5. Recycling of the lipase PS CLEA in the acylation of **2** with vinyl acetate in dry DIPE. Cycle 1 (■) ($E = 58$), cycle 2 (●) ($E = 80$), cycle 3 (△) ($E = 59$).

huge variations in E. At 48 °C the sol–gel structure was visibly breaking down already during the second cycle. The recycling of the CLEAs was less successful (Fig. 5). The loss of activity was 28% already after the second cycle. As a positive sign, however, the reuse had no effect on enantioselectivity.

4. Conclusions

In this study, the straightforward, additive free preparations of the lyo sol–gel and the CLEA of lipase PS are presented and their application in dry organic solvents is demonstrated. The enzyme preparations were successfully used for the enantioselective acylation of secondary alcohols **1–3** with vinyl acetate. The results show that a dry sol–gel preparation can be effectively prepared from the lipase PS powder simply by encapsulating it inside a sol–gel and drying the preparation by lyophilization. This preparation is highly reusable at room temperature and is an excellent catalyst also for cases where the produced ester is activated and accordingly susceptible to hydrolysis as a side reaction.

In the case of the CLEA of lipase PS a significant increase in activity upon immobilization was observed (174%) and the lipase PS CLEA is highly enantioselective in acylation reactions. However, the recyclability of this preparation is limited.

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