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Synthesis of glycidyl esters catalyzed by lipases in ionic liquids and supercritical carbon dioxide

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Abstract

Free and immobilized commercial lipases from *Candida antarctica* (CALA and CALB) and *Mucor miehei* (MML) were assayed as catalysts in the synthesis of glycidyl esters from *rac*-glycidol in non-aqueous conditions. Four different ionic liquids (ILs), based on dialkylimidazolium or quaternary ammonium cations associated with hexafluorophosphate or bis(trifluoromethane)sulfonyl amide anions, and supercritical carbon dioxide (40–50 °C and 100–150 bar) were used as reaction media. All lipases were able to catalyze glycidyl ester synthesis. Also, their activity was greatly enhanced (up 95-times) by both the use of ILs media in comparison with a classical organic solvent (toluene), and the increase in the alkyl chain length of the acyl donor ester. The activity and enantioselectivity exhibited by each lipase were practically independent of the assayed ILs. *R*-Glycidyl esters were preferentially obtained by both CALA and MML biocatalysts, while *S*-glycidyl ester synthesis was favoured by CALB, which showed the highest degree of activity in all cases. Continuous processes for glycidyl butyrate synthesis in supercritical carbon dioxide, catalyzed by each assayed lipase (free and immobilized) suspended in 1-ethyl-3-methylimidazolium bis[(trifluoromethyl) sulfonyl]amide, were also studied leading to excellent results. Supercritical CO_2/IL biphasic systems slightly reduced the synthetic activity of lipases, while the enantioselectivity of the process remained unchanged.

Keywords: Ionic liquids; Lipases; Glycidol; Applied biocatalysis; Supercritical CO_2

1. Introduction

The use of biocatalysts in non-aqueous environments has received growing attention during the last two decades due to advantages they offer, such as increasing the solubility of organic substrates, the possibility of carrying out processes which are thermodynamically unfavourable in water (e.g. transesterification), and facilitating enzyme and products recovery to be applied in fine chemistry [1]. Among these non-conventional media, ionic liquids (ILs) and supercritical fluids (SCFs) have recently been appeared as interesting clean alternatives to classical organic solvents [2].

ILs are compounds that consist only of ions and are liquids at room temperature. Unlike classical organic solvents, ILs possess no vapour pressure and high thermal stability, are able to dissolve many kinds of compounds and can be used to form two-phase systems with many solvents [3]. Also, ILs have widely tuneable properties with regard to polarity, hydrophobicity and solvent miscibility behaviour through the appropriate selection of the cation (e.g. 1-alkyl-3-methylimidazolium or tetraalkylammonium) and anion (e.g. tetrafluoroborate, $[BF_4^-]$; hexafluorophosphate, $[PF_6^-]$; bis[(trifluoromethyl)sulfonyl]amide, $[NTf_2^-]$) [4,5]. These properties have increasingly attracted attention as green and high-tech reaction media for potential use in the near future. In the same way, SCFs, i.e. fluids at temperatures and pressures slightly above the critical points (e.g. 31 °C and 7.38 MPa for CO_2), exhibit unique combinations of properties: liquid-like density (and hence solvent power) and high compressibility, very low viscosity and high diffusivity. The first two properties make the solvent power of SCFs easily controllable simply by changing pressure and/or temperature, while low viscosity and high diffusivity markedly enhance mass-transport phenomena and hence the kinetics of the process [6].

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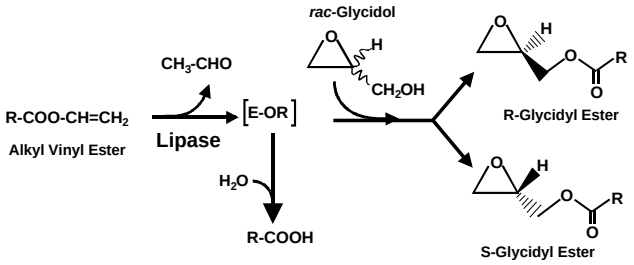

Fig. 1. Schematic representation of lipase-catalyzed kinetic resolution of *rac*-glycidol.

Biocatalytic transformations in these neoteric solvents appear to be a promising way of developing integral green chemical processes because of their physical and chemical characteristics of these solvents and the catalytic properties of enzymes [7]. For enzymes to be completely successful as catalysts at the industrial level it is important that they have high catalytic efficiency, a feature difficult to obtain in the case of non-aqueous media where enzymes usually have lower activity than that seen in aqueous buffers [8]. Nevertheless, ILs have been shown to be by far the best non-aqueous reaction media for enzyme-catalyzed reactions, not only because enzymes displayed high level of activity and stereoselectivity for many different chemical transformations (esterifications, transesterifications, kinetic resolutions, etc.) [9–12], but mainly because of an over-stabilization effect on biocatalysts (up 2300-folds half-life time respect to classical organic solvents) [13,14]. Furthermore, recent research has demonstrated the possibility to carry out integral green biocatalytic processes by combining these two completely different neoteric solvents (ILs and $scCO_2$) with enzymes [15,16], because their different miscibilities produce to two-phase systems that show an exceptional ability to carry out both the biotransformation and the products extraction steps simultaneously [2], even under extremely harsh conditions (e.g. 150 °C and 100 bar) [17].

In this paper, we report the lipase-catalyzed kinetic resolution of glycidol (2,3-epoxy-1-propanol) by transesterification with vinyl alkyl esters (see Fig. 1) in four different ionic liquids—(i) 1-ethyl-3-methylimidazolium bis[(trifluoromethyl) sulfonyl]amide or triflimide, [emim][NTf_2]; (ii) 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim][PF_6]; (iii) 1-butyl-3-methylimidazolium triflimide, [bmim][NTf_2]; and (iv) trioctylmethylammonium triflimide [troma][NTf_2]. Chiral glycidol is one of the most important building blocks for the synthesis of various enantiopure pharmaceuticals, including beta-blockers, chiral lactones and antiviral substances. The enzymatic resolution of *rac*-glycidol has been studied by several authors in both organic [18–20] and supercritical [21] media. The aim of this paper is to analyze the activity and stereoselectivity of three different lipases (*Candida antarctica* lipase A, CALA; *Candida antartica* lipase B, CALB; and *Mucor miehei* lipase, MML), free or immobilized, for glycidyl ester synthesis in both ILs and ILs/$scCO_2$ reaction systems.

2. Experimental

2.1. Materials

Five different commercial lipase (EC 3.1.1.3.) preparations were used as catalysts: free CALA (Novozym 868L), free CALB (Novozym 525L), free MML (Novozym 388L), immobilized CALB (Novozym 435) and immobilized MML (Lipozyme RM). All were from Novo España S.A. The immobilized preparations were used without further purification, while the free ones were ultrafiltered to eliminate all the low molecular weight additives, as follows: 25 ml of each free lipase preparation were diluted in 225 ml of water, and the resulting solutions were concentrated 10-fold by ultrafiltration at 4 °C, using a Minitan (Millipore) system equipped with polysulphone membranes (5 kDa. cut-off). For each enzyme preparations, the process was repeated 3-times resulting in CALA, CAB and MML solutions of 24.5, 14.9 and 9.9 mg ml^{-1}, respectively, as determined by Lowry's method. Substrates, solvents and other chemicals were purchased from Sigma-Aldrich-Fluka Chemical Co, and were of the highest purity available.

2.2. Synthesis of ionic liquids

2.2.1. Synthesis of 1-ethyl-3-methylimidazolium triflimide, [emim][NTf_2]

According to Bonhôte et al. [22], the procedure was as follows—13.16 g (68.89 mmol) of [emim][Br] and 20.5 g of $LiNTf_2$ (68.89 mmol) were mixed in 50 ml of hot water (70 °C). Extraction with CH_2Cl_2 (2 × 50 ml), concentration under reduce pressure and drying under high vacuum (10^{-2} Torr) at 100 °C for 24 h led to 23.9 g of [emim][Tf_2N] (89% yield).

2.2.2. Synthesis of 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim][PF_6]

This ionic liquid was prepared according to Huddleston et al. [23], as follows: hexafluorophosphoric acid (1.3 mol) was slowly added to a solution of 1-*n*-butyl-3-methylimidazolium chloride (176.5 g, 1 mol) in 500 ml of water. After stirring for 12 h, the upper acidic aqueous layer was decanted. The lower ionic liquid portion was washed with water until the washings were no longer acidic. The ionic liquid was then dried under high vacuum (10^{-2} Torr) at 70 °C for 24 h leading to 252.8 g of [bmim][PF_6] as a colourless clear oil (89% yield).

2.2.3. Synthesis of 1-butyl-3-methylimidazolium triflimide, [bmim][NTf_2]

The following experimental procedure was carried out according to [22]: 8.72 g (50 mmol) [bmim][Cl] and 14.35 g

$LiNTf_2$ (50 mmol) were dissolved in 50 ml acetone at room temperature. After 6 h, elimination of acetone under vacuum left a residue, which was taken up in 100 ml dichlomethane. This solution was washed with water (3 × 25 ml). Concentration under reduce pressure and drying under high vacuum (10^{-2} Torr) at 100 °C for 24 h led to 17.8 g [bmim][NTf_2] (85% molar yield).

2.2.4. Synthesis of trioctylmethylammonium triflimide, [troma][Tf_2N]

This ionic liquid was obtained according to the method described by Suarez et al. [24], as follows: 10 mmol of Aliquat 336 (4.04 g) and 10 mmol of $LiNTf_2$ (1.54 g) were dissolved in 50 ml of acetone at room temperature. After 24 h, the reaction mixture was filtered through a plug of celite. The solvent was removed under reduced pressure and the ionic liquid dried under high vacuum (10^{-2} Torr) at 70 °C for 24 h, giving 4.37 g of a clear oil (85%). ^{1}H NMR (Acetone d_6, 300 MHz, δ ppm/TMS)°: 3.49 (m, 6H), 3.21 (s, 3H), 1.90 (brs, 6H), 1.38 (m, 36H), 0.88 (t, $J = 6.6$ Hz, 9H). ^{13}C NMR (Acetone, 50 MHz, δ ppm/TMS)°: 123.51, 118.52, 92.48, 62.52, 32.59, 32.41, 26.48, 23.30, 23.23, 22.78, 14.32. Positive-ion LSIMS (*m*/*z*): 1014.8, 1042.9, 1070.8, 1098.9, 1126.9, 1154.8.

2.3. Enzymatic synthesis of glycidyl esters in ionic liquids

Two hundred microlitres of each ionic liquid, e.g. [emim][NTf_2]; [bmim][PF_6]; [bmim][NTf_2] and [troma][NTf_2], or toluene, 360 μmol (25 μl) of *rac*-glycidol and 720 μmol of vinyl ester (67 μl of vinyl acetate or 92 μl of vinyl butyrate), were added to screw-capped vials of 1 ml total capacity. The reaction was started by adding 5 μl of the ultrafiltered lipase solution (CALA, CALB or MML, respectively) two-fold diluted in 10 mM phosphate buffer at pH 7.0, and run at 35 or 50 °C in an oil bath with shaking for 1 h. At regular time intervals, 25 μl aliquots were withdrawn and suspended in 375 μl of toluene. The biphasic mixture was strongly shaken for 3 min to extract all the substrates and product into the toluene phase. For toluene reaction media, aliquots were dissolved in a mixture acetone:HCl (99:1 (v/v)) to quench the reaction. Then, 300 μl of the toluene or acetone extracts were added to 50 μl of 100 mM ethyl hexanoate (internal standard) solution in toluene, and 1 μl of the resultant solution was analyzed by GC. Analyses were performed with a Shimadzu GC-17A instrument equipped with FID detector and a Chiraldex™ gamma cyclodextrin trifluoroacetyl column (40 m × 0.32 mm, Astec, USA), using the following conditions: carrier gas (helium) at 51 kPa (147 ml min^{-1} total flow); temperature program: 50 °C, 15 min, 5 °C min^{-1}, 70 °C, 60 min, 70 °C, split ratio, 131:1; detector, 230 °C. Retention times of peaks were as follows: acetic acid: 8.8 min; vinyl acetate: 10.3 min; *R*-glycidol: 10.9 min; *S*-glycidol: 11.3 min; ethyl hexanoate: 17.1 min.; *S*-glycidyl acetate: 29.1 min; *R*-glycidyl acetate: 32 min; butyric acid: 30.3 min; *S*-glycidyl butyrate: 68.5 min; *R*-glycidyl butyrate: 70.2 min. One unit of synthetic activity was defined as the amount of enzyme that produces 1 μmol of glycidyl ester per min.

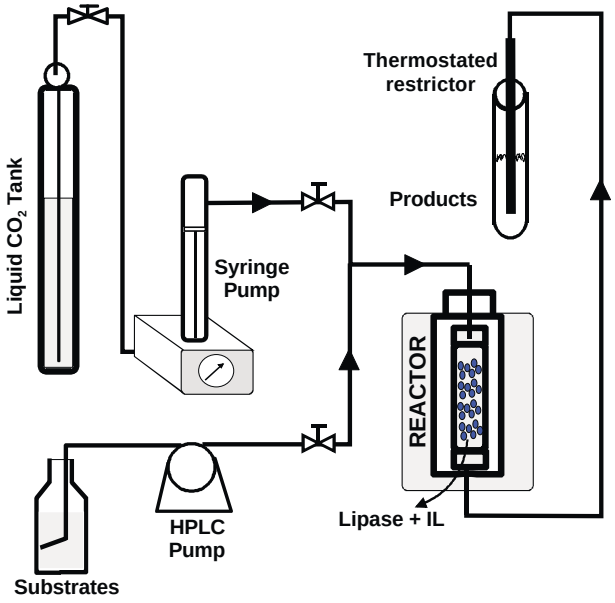

Fig. 2. Experimental set-up of the enzymatic reactor with IL-$scCO_2$ for continuous kinetic resolution of *rac*-glycidol.

2.4. Enzymatic synthesis of glycidyl esters in IL-$scCO_2$

Fifty microlitres of the ultrafiltered lipase solution (CALA, CALB or MML, respectively), or 100 mg of commercial immobilized lipase preparation (Novozym 435 or Lipozyme RM), were mixed with 2 ml of [emim][NTf_2] in a test-tube, and then 3 g of dry Celite were added to absorb the enzyme-IL mixture. The final mixture was placed in the cartridge of an ISCO 220SX high pressure extraction apparatus of 10 ml total capacity. Glycidyl ester synthesis was carried out by continuous pumping of a substrate solution (200 mM *rac*-glycidol and 400 mM vinyl acetate or vinyl butyrate in toluene) at 0.1 ml min^{-1}, and mixed with the $scCO_2$ flow of the system at different temperatures and 10 MPa (Fig. 2). The reactor was continuously operated for 4 h. Substrates and products were fully soluble in $scCO_2$, and the reaction mixtures were recovered by continuous depressurizing through a calibrated heated restrictor (1.5 ml min^{-1}, 50 °C) for 30 min steps, and placed in a controlled amount of cold-toluene. Samples were analyzed by GC as described above.

3. Results and discussion

3.1. Influence of reaction media on the synthetic reaction

Fig. 1 depicts the kinetic mechanism for glycidyl ester synthesis catalyzed by serine hydrolases, such as lipases. In

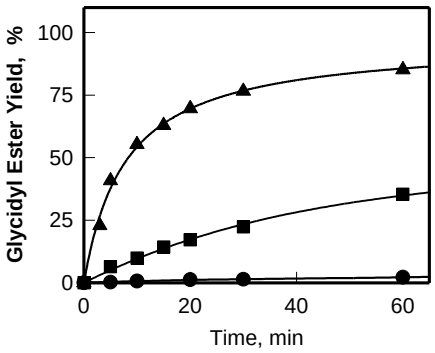

Fig. 3. Time-course of glycidyl ester yield synthesized by *M. miehei* lipase in toluene (●) and [emim][NTf_2] (■, ▲) using vinyl acetate (●, ■) or vinyl butyrate (■) as acyl donor, at 2% (v/v) water content and 35 °C.

these reactions, a covalently linked acyl-enzyme intermediate is formed, and the nucleophilic attack by water results in ester hydrolysis, although the presence of another nucleophile (e.g. glycidol) might involve the formation of the transesterification product. This latter synthetic pathway can be regarded as a kinetically controlled process, where the rapid accumulation of the acyl-enzyme intermediate and the preferential nucleophilic attack by the alcohol are essential. The first condition is enhanced by the use of activated acid acyl-donors such as vinyl esters, because the vinyl alcohol released in the degradation of the vinyl ester tautomerizes to acetaldehyde, which cannot act as a substrate for the enzyme [25]. The second condition may arise from using reaction media with a very low water content and a high nucleophile (e.g. *rac*-glycidol) concentration. In this context, the efficiency in lipase-catalyzed reactions can mainly be expressed by two parameters, the synthetic rate, and the ratio between this and the acyl-donor consumption rate (selectivity).

Fig. 3 shows the time-course of glycidyl ester yield catalyzed by free MML in [emim][NTf_2] or toluene at 35 °C and at 2% water content. As can be seen, the enzyme was able to synthesize glycidyl acetate in toluene, but the catalytic efficiency displayed was poor. However, the glycidyl acetate profile with time in the ionic liquid was clearly higher than in the organic solvent. Indeed, this catalytic activity in the IL medium was greatly increased by using vinyl butyrate as acyl donor, clearly showing the highest efficiency of this ionic liquid as reaction medium.

In order to quantify the catalytic efficiency of this biotransformation in ILs, three different soluble lipases (CALA, CALB and MML) were assayed as catalysts. Table 1 shows the selectivity and synthetic activity of these enzymes to synthesize glycidyl esters in both toluene and [emim][NTf_2] reaction media, using vinyl acetate and vinyl butyrate as acyl donor. Additionally, in the case of chiral acyl acceptor (e.g. *rac*-glycidol), the enantioselectivity of the enzyme towards one of the *R*- or *S*-isomers is one of the most interesting properties of processes catalysed by lipases, which involves the kinetic resolution of the racemic mixture. The synthetic activity was determined for each stereomeric product, as well as, the enantioselective parameter (*R*/*S* rates ratio). As can be seen, all the assayed lipases were able to synthesize glycidyl esters in both assayed non-aqueous media. Furthermore, the low water content (2% (v/v)) allowed to reach a degree of selectivity greater than 90% to be attained in all cases for the synthetic reaction. The use of water-immiscible reaction media involved a low ability to sequester free-water molecules, which therefore act as nucleophiles yielding the hydrolytic product. Nevertheless, all the enzymes increased their synthetic activity by using both the IL as reaction medium and vinyl butyrate as acyl donor. For all assayed conditions, CALB showed the best catalytic properties, while CALA and MML exhibited the highest enhancement of the overall synthetic activity (44- and 95-fold, respectively, for $R + S$ synthetic rates) when [emim][NTf_2] and vinyl butyrate were assayed. The enantiomeric excess obtained by CALB was close to 100% of *S*-glycidyl butyrate at low conversion percentages, but was 52 ± 3% for 35–40% conversion, and fell progressively for higher conversions. Additionally, it is important to point out how the

Table 1
Influence of reaction media and acyl donor on the transesterification synthetic activity and selectivity of different free lipases for the preparation of *R*- and *S*-glycidyl esters from *rac*-glycidol at 35 °C

Lipase	Medium	Acyl donor	Select. (%)	Synthetic activity (U/mg Prot)		*R*/*S* ratio
				R-Product	*S*-Product	
MML	Toluene	VA	92	4.4	1.7	2.6
	[emim][NTf_2]	VA	94	109.3	41.8	2.6
	[emim][NTf_2]	VB	94	421.1	155.6	2.7
CALA	Toluene	VA	94	3.3	1.9	1.7
	[emim][NTf_2]	VA	96	6.1	3.6	1.7
	[emim][NTf_2]	VB	96	138.9	90.2	1.6
CALB	Toluene	VA	96	138.2	403.2	2.9[a]
	[emim][NTf_2]	VA	97	232.2	673.3	2.9[a]
	[emim][NTf_2]	VB	97	323.7	906.8	2.8[a]

VA: vinyl acetate; VB: vinyl butyrate.
[a] *S*/*R* ratio.

assayed lipases showed a different preference for each stereomer of glycidol. Thus, while both MML and CALA displayed a preference for the *R*-stereomer, the preference of CALB was for the *S*-stereomer, which is of particular interest because the lipases described in the literature generally present *R*-enantioselectivity for these kinds of substrate [20]. It is also of note that each lipase maintained its enantioselectivity parameter, independently of the reaction medium or acyl donor assayed. The best results were obtained with CALB, which showed the highest synthetic activity and the *R*/*S* ratio. At this point, Martins et al. [18] reported the effect of several organic solvents on the kinetics of porcine pancreas lipase (PPL) for the resolution of *rac*-glycidol through the esterification with butyric acid. These authors described toluene as one of the best organic solvents, with regards to the initial esterification rate of *rac*-glycidol (1.5 g product $dm^{-3}\,h^{-1}$) at 35 °C, which was comparable to what we have obtained for the same reaction medium, and concluded that the enzyme enantioselectivity did not depend on enzyme hydration level. On the other hand, Cárdenas et al. [20] reported the ability of lipases from 13 different microbial sources to catalyze the kinetic resolution of *rac*-glycidol with vinyl acetate in di-isopropyl ether as reaction medium. The best results given by these authors were obtained from *F. oxysporum* lipase with values of *R*- and *S*-glycidyl acetate synthetic rates of 41 and 34 U mg^{-1}, respectively, while the best *R*/*S* ratio (2.5) was exhibited by *F. Poae* lipase. In our case, the use of ILs as reaction media does not improve the enantiolectivity of lipases towards anyone of the glycidol stereomer, which remains as a specific property of each individual biocatalyst, although these media greatly enhance the synthetic activity of the enzymes. In agreement with this fact, Martins et al. [18] also reported that PPL enantioselectivity does not correlate with solvent polarity but mainly depends on specific enzyme-solvent interactions. The increase in lipase synthetic activity by using ILs as reaction media was previously reported for CALB-catalyzed butyl butyrate, where the use of [emim][NTf_2] allowed a four-fold improvement in the synthetic activity compared to that obtained with a classical organic solvent, such as hexane [14].

3.2. Influence of ILs as reaction media on the synthetic reaction

Four different ILs were used as reaction media for lipase-catalyzed kinetic resolution of *rac*-glycidol with vinyl butyrate as acyl donor. Fig. 4 shows the synthetic activity of MML, CALA and CALB, respectively, for *R*- and *S*-glycidyl butyrate synthesis in different ionic liquids ([emim][NTf_2]; [bmim][PF_6]; [bmim][NTf_2] and [troma][NTf_2]) at 2% (v/v) water content and 50 °C. As can be seen, all the ILs used were suitable media for lipase-catalyzed glycidyl butyrate synthesis, except for the [troma][NTf_2] case when the lowest active enzymes (MML and CALA) were assayed. The synthetic activity level exhibited by assayed lipases on each stereomer product was in the same order for all the

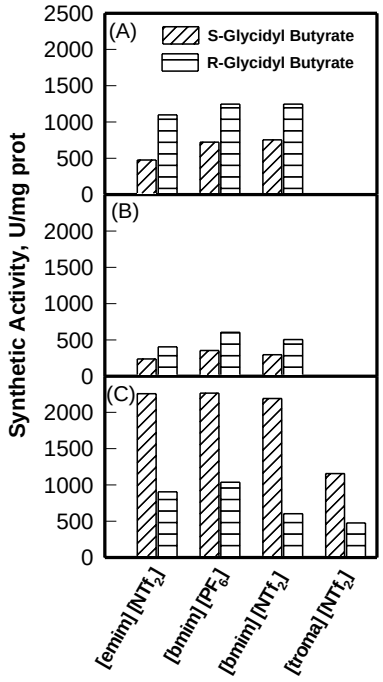

Fig. 4. Synthetic activity of MML (A), CALA (B) and CALB (C) for *R*- and *S*-glycidyl butyrate synthesis in different ionic liquids at 2% (v/v) water content and 50 °C.

three alkylimidazolium ILs, being in agreement with previous results for CALB-catalyzed butyl butyrate synthesis [14]. The synthetic activity of CALB was 2220 U mg^{-1} prot. in [bmim][NTf_2] when the stereomer *S*-glycidol was used as substrate, a similar result to that obtained in presence of *rac*-glycidol. The low activity shown by CALB in [troma][NTf_2] could be related with the enhanced viscosity (574 cP, 10-times higher than that of the assayed alkylimidazolium ILs) exhibited by this IL [13,22]. In the case of [emim][NTf_2], all the enzymes enhanced their synthetic activities three-fold when the temperature was increased from 35 to 50 °C. Schöfer et al. [11] also described the efficiency of ILs as reaction media for several lipase-catalyzed kinetic resolution of *rac*-1-phenylethanol compared with a classical organic solvent, such as methyl *tert*-butyl ether. In this work, CALA, CALB and MML also exhibited an enhancement of their synthetic activity when [bmim][PF_6]; [bmim][NTf_2] were assayed as reaction media, showing an excellent enantioselectivity.

3.3. Synthetic activity of free and immobilized lipases in IL-$scCO_2$ systems

Glycidyl ester synthesis was carried out in a continuous biphasic enzymatic reactor (see Fig. 2), which was designed to take advantage of the excellent properties of ILs at maintaining the catalytic properties of enzymes in a non-aqueous environment, as well as, the ability of $scCO_2$ to transport hydrophobic substrates [2,15–17]. As it was described, $scCO_2$ is highly soluble in certain ILs, while ILs are insoluble in

Table 2
Synthetic activities of different free and immobilized lipases suspended in [emim][NTf_2], to produce glycidyl esters from *rac*-glycidol in supercritical carbon dioxide

Lipase	Temp (°C)	Press. (bar)	Acyl donor	Synthetic activity[a]		*R*/*S* ratio
				R-Product	*S*-Product	
SE						
MML	40	100	VA	13.5	5.2	2.6
CALB	40	100	VA	51.2	153.7	3.0[b]
IME						
MML	50	R.P.	VB	161.6	62.1	2.6
MML	50	150	VB	17.5	8.2	2.6
CALB	50	R.P.	VB	64.6	192.8	3.0[b]
CALB	50	150	VB	17.3	50.1	2.9[b]

SE, soluble enzyme; IME, immobilized enzyme; R.P., room pressure.
[a] The unit of synthetic activity for soluble enzyme (SE) is U mg^{-1} Prot and for immobilized enzyme (IME) is U g^{-1} support.
[b] *S*/*R* ratio.

$scCO_2$ [3]. Thus, a phase separable biocatalytic reactor can be used, where free or immobilized enzyme can be dissolved or suspended, respectively, in the IL phase (catalytic phase) while substrates and/or products reside largely in the supercritical phase (extractive phase). The solubility of *rac*-glycidol, the least hydrophobic substrate, in $scCO_2$ at 35 °C and pressures in the range 7–18 MPa was proportional to the applied pressure, showing the ability of these media to perform synthetic reactions at substrate concentration similar to those used in organic media [21].

Table 2 shows the synthetic activity of both free and immobilized CALB and MML suspended in [emim][NTf_2] to synthesize *R*- and *S*-stereomers of glycidyl esters in $scCO_2$ at different conditions. The enzyme reaction did not occur when CALA was assayed as biocatalyst under the same conditions. As it can be seen, both free CALB and MML were able to catalyze the kinetic resolution of *rac*-glycidol in $scCO_2$, although the corresponding activities were clearly lower than in this IL without $scCO_2$ (see Table 1). However, another catalytic property, such as the enantioselectivity (*R*/*S* ratio) was maintained unchanged in supercritical conditions with respect to the IL media. The decrease in the synthetic activity of lipases when $scCO_2$ was used as reaction media as compared to non-aqueous liquid media (e.g. organic solvents, ILs) has been previously described [15,21]. Martins et al. [21] described how porcine pancreas lipase exhibits a low activity for glycidyl ester synthesis (about one-half of the lowest rates obtained in organic solvents under similar conditions). The above authors explain these results as resulting from covalent modifications of the enzyme induced by CO_2 under these conditions, which induce a loss of its catalytic activity. In our case, it is also necessary to take into account that process occurs into a biphasic system, where enzymes are suspended in ILs, and ILs are not soluble in $scCO_2$. In this way, the microscopic solvent properties of IL/$scCO_2$ systems have been spectroscopically studied by using fluorescence probes [26,27]. These works described how the presence of $scCO_2$ involves changes in three physical parameters of the IL, clearly related with the mass-transfer phenomena, such as volume, viscosity and polarity. The solubilization of $scCO_2$ in ILs produced a volume expansion (up 1.5-fold for [bmim][PF_6] at 20 MPa and 35 °C), as well as a decrease in viscosity (up five-fold for the same case, respect to the absence of $scCO_2$) of this liquid phase, which could be related with an improvement of the mass-transfer rates of compounds, favouring the catalytic efficiency of the process. However, Baker et al. [27] reported how the dissolution of CO_2 greatly decreases the polarity of the ILs, a phenomenon which clearly affects negatively the efficiency of the transport of polar compounds, such as glycidol, from the extractive phase to the catalytic phase. Thus, the observed activity loss in the IL/$scCO_2$ biphasic systems should be regarded as being limitations in the mass-transfer phenomena across the IL-layer around the biocatalyst, rather than an enzyme deactivation phenomena. Indeed, the protective effect of ILs on enzymes towards both non-aqueous environments and $scCO_2$ at extreme conditions (e.g. 150 °C, 100 bar) has been reported [14,17].

On the other hand, two commercial immobilized enzyme preparations of MML and CALB were also assayed in this IL-$scCO_2$ system to carry out the kinetic resolution of *rac*-glycidol. As it can be seen from Table 2, both immobilized derivatives were also able to catalyze the transesterification reaction in both [emim][NTf_2] and [emim][NTf_2]/$scCO_2$ media, but the enzyme activity was again reduced in the latter reaction medium with respect to the former. In addition, both immobilized enzymes showed the same stereomeric preference and enantioselectivity as in the free configuration. These results show again how ILs provided an adequate microenvironment for the catalytic action of the enzyme, where the decrease in activity could also be related with mass-transfer limitations in the IL-$scCO_2$ biphasic systems [26,27].

4. Conclusions

ILs are shown to act as excellent non-aqueous reaction media for the lipase-catalyzed kinetic resolution of *rac*-glycidol by using vinyl ester as acyl donor. For three

different lipases (CALA, CALB and MML), the use of ILs improved the synthetic activity to the same level for each stereomer with respect to that observed in a selected organic solvent, such as toluene. In the case of MML, its synthetic activity was increased 95-times by both the use of [emim][NTf_2] as reaction media, and vinyl butyrate as acyl donor. For the assayed ILs, all enzyme synthetic activites were practically independent of the nature of the individual ILs, with the exception of [troma][NTf_2] where CALA and MML were inactive. CALB in [emim][NTf_2] was shown as the best IL-enzyme systems for catalyzing the proposed biotransformation. The use of enzyme-ILs-$scCO_2$ system permitted the proposed kinetic resolution in continuous operation as a clean process. The efficiency of the system was dependent on both the specificity of the enzyme towards the catalyzed reaction and the mass-transfer phenomena between ILs and $scCO_2$ immiscible phases. These results show how clean chemical biotransformations can be by using appropriate enzyme-ILs systems in $scCO_2$, opening the door for a green chemical industry in the near future.

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