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Short communication

Ionic liquids: efficient additives for *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate

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Abstract

The *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** has been carried out in aqueous buffer with ionic liquid as co-solvent. The influence of ionic liquid on the catalytic efficiency and selectivity has been studied, using both hydrophobic and hydrophilic ionic liquids. The markedly enhanced enantioselectivity towards the *R* enantiomer of substrate **1** is observed under optimum additive conditions (1:1 composition of ionic liquid and buffer). Hydrophobic ionic liquids offered almost quantitative conversions with ee $\geq$ 99%.

Keywords: *Candida rugosa* lipase; Butyl 2-(4-chlorophenoxy)propionate; Enantioselective hydrolysis; Ionic liquid; Co-solvent

1. Introduction

Enantiopure compounds have undoubtedly gained a vital role in the development of modern chemical technology. Enzymes as biocatalysts have proved their capability to achieve the speed and co-ordination of multiple transformations involved in the preparation of enantiomerically pure compounds such as pharmaceutical and agricultural chemicals [1]. In particular, lipases are a unique class of hydrolases, which have been exploited the most because they display relatively higher enantioselectivity, possesses broad substrate specificity and are commercially available [2]. Lipase activity and selectivity are strongly influenced by the medium used for the desired reaction [3,4]. They exhibit high catalytic activity in water and even higher activity in two-phase systems such as water and hydrophobic organic solvents [5–7]. However with all the known advantages of organic solvents as reaction media for biotransformations they suffer from degradation in value when the question of environmental concern arises.

The groundbreaking success of ionic liquids has paved the way for convenient, efficient and environmentally friendly methodologies for a wide array of chemical reactions having significant synthetic value. The negligible vapour pressure of ionic liquids and their recyclability satisfies the safety and financial necessity for any industrial application. The excellent reviews by Welton [8], Wasserscheid and Wilhelm [9] and Dupont et al. [10] have benchmarked the advantages associated with the ionic liquid based systems. Enzyme catalysis in ionic liquids has established completely green solution towards development of environmentally benign procedures [11–13].

Recently, *Candida rugosa* lipase-catalysed hydrolysis of butyl 2-(4-chlorophenoxy)propionate in aqueous buffer containing dimethyl sulfoxide (DMSO) as co-solvent was studied by Watanabe and Ueji [14]. Wherein, they reported a markedly enhanced enantioselectivity of lipase in presence of DMSO as compared to no-additive conditions. Here presence of DMSO again poses the question of environment factors as well as recyclability of the solvent. The process lipase-catalysed asymmetric hydrolysis has been judged to be superior from the standpoint of productivity, ease of product separation and the number of steps required for the practical resolution of racemic acids [15]. All these things encouraged us to carry out an applied study on the

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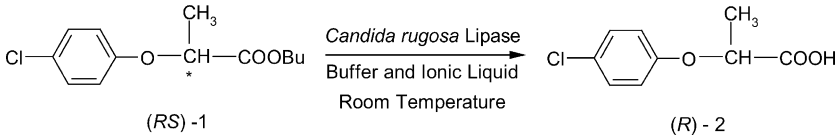

Scheme 1. *Candida rugosa* lipase-catalysed hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** in aqueous buffer with ionic liquid as co-solvent.

effect of ionic liquid as co-solvent on the lipase-catalysed enantioselective hydrolysis.

As a part of our continuing interest to build up newer strategies for various biotransformations [16–19] and to study the role of ionic liquids as designer solvents, [20–22] we herein report for the first time, a simple alternative procedure for *Candida rugosa* lipase-catalysed hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** in aqueous buffer with ionic liquid as co-solvent (Scheme 1). The lipase preferentially hydrolyses the *R* enantiomer of the substrate **1** which is a well known herbicide and also has biological activities [23]. The hypocholesterolemic and hypolipidemic effects of the same have been the subjects of numerous publications [24].

2. Experimental

2.1. Material

Candida rugosa lipase, gifted by Amano Pharmaceuticals (Japan) was used as received. Ionic liquids were prepared by the procedures given in literature and purified by the modifications suggested by Park and Kazlauskas [25]. All other chemicals and reagents were of analytical grade and used as obtained.

2.2. General procedure for the Candida rugosa lipase-catalysed hydrolysis of butyl 2-(4-chlorophenoxy)propionate *1* in aqueous buffer with ionic liquid as co-solvent

In a typical experimental procedure, to the aqueous buffer of pH 7 containing ionic liquid (0–50 vol.%) 2 ml, racemic butyl 2-(4-chlorophenoxy)propionate **1** (0.05 mmol) and *Candida rugosa* lipase (30 mg) were added. The reaction mixture was stirred at room temperature for an appropriate time interval (ca. 50% hydrolysis of **1**). The products were extracted using diethyl ether (3 × 10 ml). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was then diluted and the hydrolysed isomer *R* was separated by preparative T.L.C. (using pet ether–ethyl acetate 95:5 (v/v)).

The isolated products were dried and weighed. Optical rotations of the isolated compounds were measured on the digital polarimeter (Jasco-390 digital polarimeter). The enantiomeric excess of the products were calculated by comparing the results with the value given in the literature [19].

The extent of hydrolysis in all cases was monitored on GC. A Nucon 5700 chromatograph equipped with FID was employed for the analysis. The detector temperature was maintained at 270 °C. The column was programmed with an initial temperature of 100 °C and was increased thereafter to 270 °C at the rate of 10 °C min^{-1}. The column used was liquid phase ov-17 (length 6 ft.).

2.3. General procedure for recycling of the ionic liquid

After separation of the product, the ionic liquid was extracted from the reaction mixture by using dichloromethane. The dichloromethane layer was washed with saturated sodium carbonate solution, the solvent was evaporated. The ionic liquid was purified for further use by subjecting it to consecutive extractions with diethyl ether to remove the organic impurities, if any, followed by drying under vacuum. The recycled ionic liquid (with 3–5% loss in each cycle due to handling) was used for the reaction following the experimental procedure as mentioned in Section 2.2.

3. Results and discussions

Initially, we employed ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6, as co-solvent with 0.1 M phosphate buffer of pH 7, for the hydrolysis of **1**. The ionic liquid being hydrophobic in nature provides biphasic reaction media for the enantioselective hydrolysis. The effect of this novel medium on the enantioselectivity of lipase was monitored by varying the amount of ionic liquid in combination with aqueous buffer. Table 1 summarizes the results of the variation of the enantioselectivity of lipase at ca. 50% conversion of **1**.

A tremendous enhancement in enantioselectivity of lipase is observed with increasing amount of ionic liquid as additive as compared to the *Candida rugosa* lipase mediated hydrolysis in neat aqueous buffer. As evident from the results, under

Table 1
Effect of addition of ionic liquid [bmim]PF_6 on the *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** in aqueous buffer

Ionic liquid (vol.%)	Time (min)	Conversion (%)	ee (%)
0	20	43	47
25	90	37	78
50	240	48	>99
60	720	29	>99

Table 2
Effect of addition of ionic liquids [bmim]BF_4 and [hmim]BF_4, on the *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** in aqueous buffer

Ionic liquid (vol.%)	[bmim]BF_4			[hmim]BF_4		
	Time (min)	Conversion (%)	ee (%)	Time (min)	Conversion (%)	ee (%)
0	20	43	47	20	43	47
25	210	32	67	150	35	73
50	600	37	99	360	46	99
60	2 days	22	99	1440	28	99

optimum additive condition, i.e. 1:1 composition of buffer and ionic liquid, the lipase displayed excellent ee value (>99%) at 48% conversion of **1** to *R* form of its acid **2**. However, the rate of the reaction retarded significantly by addition of excess amount of ionic liquid [bmim]PF_6, (60 vol.%).

Furthermore, in order to investigate the effect of other ionic liquids on the lipase activity and selectivity, we studied the hydrolysis in ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4, which is hydrophilic in nature, offers monophasic environment for the reaction. Also we used the hydrophobic ionic liquid 1-hexyl-3-methylimidazolium tetrafluoroborate, [hmim]BF_4. From the data summarized in Table 2, it is clear that, in presence of both [bmim]BF_4 and [hmim]BF_4 ionic liquids, the enantioselectivity of lipase improved dramatically, accompanying a decrease in catalytic efficiency of lipase with excess addition of ionic liquid, as observed in case of ionic liquid [bmim]PF_6. At 50% concentration of ionic liquids [bmim]BF_4 and [hmim]BF_4, the lipase exhibited very good enantioselectivity, the values are 99% ee at 37% conversion and 99% ee at 46% conversion respectively.

To compare the extent of conversion in three different reaction media as co-solvent, we carried out the hydrolysis with same 1:1 composition of ionic liquid and aqueous buffer, as it is observed to be the optimum condition. We have monitored the conversion of **1** at fixed time intervals of respective reactions (Fig. 1). It is clearly seen from Fig. 1 that lipase show good activity for hydrolysis under biphasic conditions, i.e. with hydrophobic ionic liquids ([bmim]PF_6 and [hmim]BF_4) as compared to hydrophilic ionic liquid ([bmim]BF_4) which provides monophasic environment.

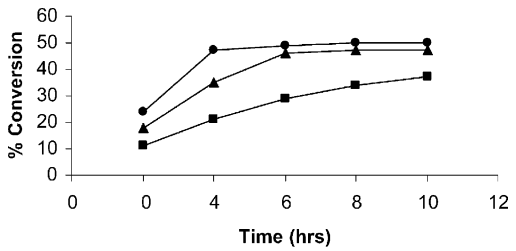

Fig. 1. *Candida rugosa* lipase-catalysed hydrolysis of butyl 2-(4-chlorophenoxy)propionate **1** in aqueous buffer with different ionic liquids viz. [bmim]PF_6 (●), [bmim]BF_4 (■) and [hmim]BF_4 (▲) as co-solvent.

Table 3
Recyclability of the ionic liquids [bmim]PF_6 and [hmim]BF_4

Run no.	[bmim]PF_6		[hmim]BF_4	
	Conversion (%)	ee (%)	Conversion (%)	ee (%)
1	48	>99	46	99
2	48	>99	46	99
3	46	>99	45	99
4	45	99	45	99

As compared to DMSO promoted hydrolysis [14], where, in particular, upon the addition of optimum amount of DMSO (55 vol.%), 40% conversion of enantiopure product (ee = 100%) was obtained, the noteworthy feature of present protocol is high yield (48%) of enantiopure product (ee = 99%) under optimum additive conditions of ionic liquid (50 vol.%) and also the recyclability of these innovative ionic media. As we obtained highest possible yields of enantiopure product **2** with ionic liquids [bmim]PF_6 and [hmim]BF_4, we have examined their recyclability (Table 3). We observed no substantial diminution in % conversion and enantioselectivity, even after recycling them for four consecutive runs. Further investigations about the reusability of *Candida rugosa* lipase in hydrolysis reaction are currently in progress.

All the three ionic liquids were prepared by the method suggested by Park and Kazlauskas [25], to avoid the possibility of enzyme inactivation by acid traces present in ionic liquid. The method ensures the removal of a known impurity in these ionic liquids viz. 3-alkyl-1 methylimidazolium halide as well as the acidic impurities.

4. Conclusions

We have demonstrated the *Candida rugosa* lipase-catalysed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy)propionate in aqueous buffer with ionic liquid as co-solvent. In view of both lipase activity and selectivity, hydrophobic ionic liquids [bmim]PF_6 and [hmim]BF_4 are observed to be the efficient co-solvents than the hydrophilic ionic liquid [bmim]BF_4. The addition of ionic liquid offers markedly enhanced enantioselectivity of lipase and its recyclability adds to the development of alternative greener procedures.

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