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Pseudomonas cepacia lipase-catalysed resolution of racemic alcohols in ionic liquid using succinic anhydride: role of triethylamine in enhancement of catalytic activity

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Abstract

Racemic secondary alcohols were resolved via enantioselective acylation using succinic anhydride as acyl donor catalysed by lipase from *Pseudomonas cepacia* supported on celite (PS-C) in ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate [bmim]PF_6. Organic base, namely triethylamine as an additive in ionic liquid has been found to enhance the rate of the reaction.

Keywords: Lipase PS-C; Kinetic resolution; Succinic anhydride; Ionic liquid; Additive

1. Introduction

The increasing awareness of the importance of chirality in the context of biological activity has stimulated a growing demand for efficient methods for the industrial synthesis of pure enantiomers. Enzymes have a strong foothold in organic synthesis when it comes to resolution of racemates or asymmetric induction. Enzymes offer the advantages of chemical specificity as well as stereospecificity. Enzyme-catalysed transformations carried out in organic media have several advantages over hydrolytic reactions [1,2]. The biotransformations such as aminolysis and alcoholysis are suppressed by hydrolysis, if carried out in aqueous media [3]. Despite some obvious advantages of organic solvents, such as good solubility of substrates and better yields, they suffer from some disadvantages. They are potential environment hazard and toxic to process operators. Consequently, the need for chemical processes, which have negligible impact on the environment, has intensified. One potential solution would be to employ an alternative medium for carrying out enzyme-catalysed reactions.

Recently, room temperature ionic liquids have emerged as a potential replacement for organic solvents in various synthetic processes, on both laboratory and industrial scale [4–6]. Typically consisting of nitrogen containing cation and inorganic anion, these ionic liquids exhibit vanishingly small vapor pressure and can be recycled and reused. They are promising solvents for a wide range of organic, organo-metallic and inorganic compounds. Amongst several unique properties, the one that attracts much attention is the ease with which the properties of the ionic liquids can be engineered, by altering the structure of the cation or anion or both to suit the requirements of a particular process. Hence, the replacement of organic solvents with these ionic liquids would enable major process design and overcome the constraints associated with the toxicity and flammability of organic solvents. Enzyme catalysis in ionic liquids therefore appears as a unique process, combining the advantages of the two, leading to an environmentally benign chemistry. The pioneering work by Lau et al. [7] has triggered an exploration of potential benefits of lipase catalysis in ionic liquids [8]. Itoh et al. demonstrated lipase-catalysed enantioselective acylation for the first time [9]. This was immediately followed by a report by Schöfer et al. showing improved enantioselectivity in various ionic liquids [10]. Lipase-catalysed reactions in ionic liquids have been identified to have some potential advantages besides environmental ones. The hydrolytic enzymes have been shown to be more stable in these neoteric solvents when compared with conventional organic

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solvents and offer much better enantioselectivity [11]. The use of supercritical CO_2 in combination with ionic liquids has been shown to influence the overall performance of α-chymotrypsin-catalysed transesterification [12]. As a part of our continuous efforts to explore the potential of enzyme catalyzed reactions in ionic liquids [13,14], we present here the kinetic resolution of secondary racemic alcohols in ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate, [bmim]PF_6, via acylation using succinic anhydride, catalysed by *Pseudomonas cepacia* supported on Celite (PS-C).

The role of an organic base namely triethylamine as an additive in rate enhancement has been studied.

Optically active hydroxy compounds and their derivatives are a versatile class of molecules, which have been extensively exploited as chiral building blocks and reagents in asymmetric synthesis.

Anhydrides [15] are superior to other acyl donors, as the alcohol is not produced as a by-product of the reaction making the reaction irreversible. This increases the optical purity of the desired product. The distinct advantage of using succinic anhydride lies in the easy separation of products by employing simple chemical methods [16].

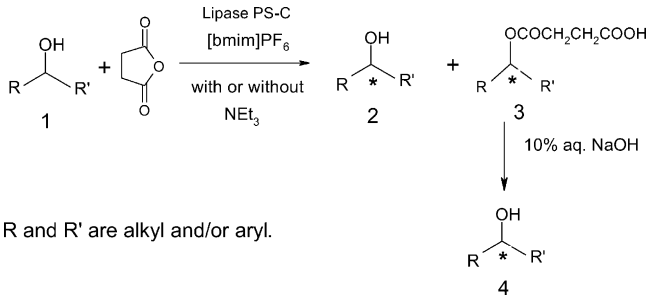
2. Experimental

2.1. Material

P. cepacia lipase supported on Celite (lipase PS-C), 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 [24]. All other chemicals and reagents were of analytical grade and used as obtained.

2.2. General procedure for the lipase-catalysed acylation of secondary alcohols in ionic liquids

To a homogeneous mixture of ionic liquid (4 ml) and alcohol (**1**), (2 mmol), PS-C (44 mg, 1300 units) and succinic anhydride (2 mmol) were added. The reaction mixture was stirred at room temperature for specified time (approximately 50% conversion). The reaction was quenched by adding 1 M Na_2CO_3 solution. The resultant biphasic system was stirred for 10 min and aqueous layer was separated. This was repeated thrice. The ionic liquid was then extracted with diethyl ether (thrice with 10 ml portions) and the combined ether extracts were evaporated to obtain the isomer (**2**) (refer Scheme 1). The aqueous layer was then shaken vigorously with 10% NaOH solution for about 10 min to carry out the hydrolysis of ester group. It was then extracted with dichloromethane. The organic extracts were combined and solvent was evaporated to afford the corresponding isomer (**4**).



Scheme 1. Lipase PS-C catalysed resolution of racemic alcohols using succinic anhydride in ionic liquid, [bmim]PF_6.

2.3. General procedure for lipase-catalysed acylation in ionic liquids in presence of catalytic amount of additive

The reaction was carried out according to the same procedure as mentioned above, in presence of 10 mol% of triethylamine. The work up was exactly the same as given above.

The isolated products were dried and weighed. Optical rotations of the isolated compounds were measured on digital polarimeter (Jasco-390 digital polarimeter). The enantiomeric excesses were calculated according to the values given in the literature [25–28].

2.4. General procedure for recycling of the ionic liquid and supported lipase

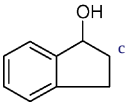
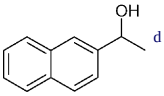
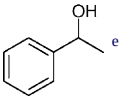
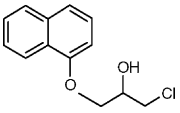
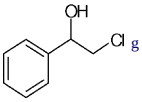
The ionic liquid containing the lipase (PS-C) was purified for further use by subjecting it to consecutive extractions with diethyl ether to remove the organic impurities, if any. To this dichloromethane was added and lipase PS-C was filtered off. The lipase could be dried and used repeatedly. The dichloromethane layer was then washed with saturated sodium carbonate solution, the solvent was evaporated and the ionic liquid was dried under vacuum for subsequent reactions.

3. Results and discussions

Racemic secondary alcohols have been found to be enantioselectively acylated with succinic anhydride as the acyl donor catalyzed by lipase (PS-C) in ionic liquid, [bmim]PF_6, leading to the formation of monoester of succinic acid, which could be easily separated using an alkaline solution. The reaction proceeds with good enantioselectivity, as the enantiomeric excesses were quite high. Ionic liquid [bmim]PF_6 was selected owing to its hydrophobic nature, which facilitates biphasic separation of the products and ease in handling.

Interestingly, additives have great potential for fine-tuning the reaction conditions for lipase-catalysed reactions [17–19]. Certain organic bases like triethylamine [20,21], pyrrolidine [22] and inorganic substances such as NaCl, Amberlite have been shown to be highly beneficial, as

Table 1
Comparative results of the kinetic resolution of racemic alcohols using succinic anhydride in ionic liquid [bmim]PF_6 catalysed by lipase PS-C with or without NEt_3

Substrate	Reaction without additive					Reaction with additive[a]				
	Time (h)	Isomer (**4**)		Isomer (**2**)		Time (h)	Isomer (**4**)		Isomer (**2**)	
		Yield[b] (%)	e.e. (%)	Yield[b] (%)	e.e. (%)		Yield[b] (%)	e.e. (%)	Yield[b] (%)	e.e. (%)
 [c]	26	45	96	42	94	15	46	94	43	93
 [d]	31	44	95	46	93	21	47	92	44	92
 [e]	33	43	96	45	94	20	47	92	45	93
 [f]	29	42	95	41	92	18	46	92	44	91
 [g]	31	43	92	45	91	20	45	91	43	90

[a] 10 mol% additive.
[b] Isolated yields.
[c] *R* isomer: $[\alpha]_D^{30} - 29^\circ$ (c 2, $CHCl_3$); *S* isomer: $[\alpha]_D^{20} + 30^\circ$ (c 2, $CHCl_3$) [25].
[d] *R* isomer: $[\alpha]_D^{20} + 38^\circ$ (c 5, C_2H_5OH); *S* isomer: $[\alpha]_D^{20} - 40^\circ$ (c 5, C_2H_5OH) [25].
[e] *R* isomer: $[\alpha]_D^{20} + 54.8^\circ$ (c 0.9, $CHCl_3$); *S* isomer: $[\alpha]_D^{20} - 41.6^\circ$ (c 0.75, CH_3OH) [26].
[f] R isomer: $[\alpha]_D^{25} - 8.7^\circ$ (c 1–5, EtOH) [27].
[g] *R* isomer: $[\alpha]_D^{25} - 51.5^\circ$ (c 2.0, cyclohexane); *S* isomer: $[\alpha]_D^{25} - 53.3^\circ$ (c 2.0, cyclohexane) [28].

the overall catalytic activity and enantioselectivity of enzymes in organic solvents improves dramatically. We were, therefore, prompted to investigate the effect of an organic base, namely triethylamine (Et_3N) on the activity as well as the enantioselectivity of lipase in [bmim]PF_6. The use of triethylamine has been particularly shown to be more advantageous over other bases [23]. The addition of Et_3N resulted in a significant increase in the catalytic activity of the lipase in ionic liquid. At the same time, enantioselectivity was not affected appreciably as the enantiomeric excesses of the products were similar to those obtained in reactions carried out without an additive. The reaction rates were increased by more than 1.5 times as compared to original rates, and the results are presented in Table 1.

To ensure that no chemical acylation took place (which is a prerequisite for a high optical purity of products), control experiments with additive were set up, in which no enzyme was used for the acylation of the alcohols. The results showed no significant product formation over a period of 24 h.

In some cases [17], the effect of an additive has been attributed to the formation of an ion pair between the base and any acidic by-product. As one of the products in the present study is an acid, we believe that the rate enhancement in ionic liquid might have occurred because of the removal of the acid by the added organic base in the form of an ion pair. On the other hand, the presence of acidic impurities in ionic liquids may also cause inactivation of the enzyme leading to slower reaction rates and thereby the addition of a base might act as a scavenger for the acid traces. Thus, the addition of an organic base would restore the activity of the enzyme and the rate would get enhanced. However, the possibility of enzyme inactivation by acid traces present in ionic liquid can be ruled out, since in the present study, we have used the ionic liquid prepared by method suggested by Park and Kazlauskas [24]. The method ensures the removal of a known impurity in these ionic liquids, viz. 3-alkyl-1-methylimidazolium halide as well as acidic impurities. Table 1 gives the comparison between the rates of reactions carried out in ionic liquid with and without triethylamine. Following this train of thought, the ion-pair formation can therefore be considered as the most likely explanation for the rate enhancement.

After the reaction, ionic liquid and enzyme was purified as per the procedure given and reused for consecutive two cycles without significant loss in their activity.

4. Conclusion

From this study we can conclude that, ionic liquid serves as a recyclable medium for enzymatic reactions that is so well suited for present day's demand for environmentally beneficial processes. The use of non-reactive organic base proves to be worthwhile as the amount of the time required for enzymatic reaction reduces considerably, thereby making the process more efficient.

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