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SOLID-PHASE IMMOBILIZED BIOCATALYSTS FOR OPTICAL RESOLUTION OF SECONDARY ALCOHOLS

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SUMMARY

Chirality emerged as a key issue for the efficiency of many pharmaceutical products. Optical resolution of synthetic racemic intermediates by biocatalysis was intensively studied in the last decades. Secondary alcohols are important intermediates in several pharmaceutical processes and their optical resolution by lipases could be used to introduce chirality in a specific reaction sequence. Immobilization of the enzyme represents an important advantage, allowing multiple use of a robust solid-phase catalyst. We carried out sol-gel entrapment of *Candida antarctica* B lipase in matrices derived from ternary silane precursor systems and then adsorbed on Celite. Several ionic liquids have been employed as structure-directing additives and stabilizers against lipase inactivation during the sol-gel immobilization process. The obtained biocatalysts were investigated in the kinetic resolution of model secondary alcohols. The addition of an ionic liquid during the immobilization process resulted in preparations with higher activities and better enantioselectivity, compared to the native lipase. The proper selection of the ionic liquid cation and/or anion was essential to obtain robust biocatalysts with adequate properties. Lipase catalysts will be employed for optical resolution of heterocyclic intermediates of pharmacologically active compounds.

Keywords: sol-gel immobilization; lipase; additive; ionic liquid; enantioselectivity; optical resolution.

INTRODUCTION

Biocatalysis has emerged as an important tool in the industrial synthesis of bulk chemicals, pharmaceutical and agrochemical intermediates, active pharmaceuticals, and food ingredients. However, the number and diversity of the applications are still limited, perhaps in part because of the limitations of the biocatalysts, such as limited enzyme availability and operational stability.

The use of biocatalysis for industrial synthetic chemistry is on the way of significant growth. Biocatalytic processes can be carried out in organic solvents as well as aqueous environments. As the use of biocatalysis for industrial chemical synthesis becomes easier, several chemical companies have begun to increase significantly the number and complexity of the biocatalytic processes used in their synthesis operations [1].

The application of biocatalysts offers a remarkable arsenal of highly selective transformations for modern preparative organic chemistry. Kinetic resolution of 2-hydroxy-methyl-1,4-benzodioxane derivatives, valuable building blocks for compounds with hepatoprotective activity, has been achieved using *Pseudomonas fluorescens* lipase [2].

Apart from new trends, the older fashioned techniques such as immobilization (within physical, chemical modification area) were improved a lot as well in creating properly designed robust biocatalysts. For industrial applications, immobilization of biomolecules or microorganisms became a key issue in the development of biotechnology. The primary advantage of immobilization is to increase the stability of the enzyme, as well as easier separation, recovery, and reuse of the enzyme from the product mixture [3, 4]. Thus, immobilization of enzymes has been an active research topic in enzyme technology to enhance their activity, thermal and operational stability, and reusability [5, 6].

MATERIALS AND METHODS

Lipase from *Candida antarctica* B was produced by C-Lecta (Leipzig, Germany). The silane precursors methyl- (MeTMOS) and phenyl-trimethoxysilane (PhTMOS) were purchased from Merck and tetramethoxysilane (TMOS) from Fluka. Other materials used: tris-(hydroxymethyl)-aminomethane (Loba Chemie), HCl 1N (Chimopar), 2-propanol (Merck), sodium fluoride (Fluka), Celite 545 (Merck), 2-hexanol (Fluka), 2-octanol (Merck), vinyl acetate (Merck), *n*-hexane (98%, Merck), were of analytical grade and have been used as purchased. Decane (>99%, Aldrich) and dodecane (99%, Merck) were used as internal standards for quantitative gas-chromatographic analysis. Ionic liquids 1-ethyl-3-methyl-imidazolium tetrafluoroborate [Emim]BF_4 (Merck), 1-hexyl-3-methyl-imidazolium

tetrafluoroborate [Hmim]BF_4 (Merck), 1-octyl-3-methyl-imidazolium tetrafluoroborate [Omim]BF_4 (Merck), 1-butyl-3-methyl-imidazolium hexafluorophosphate [Bmim]PF_6 (Merck), 1-ethyl-3-methyl-imidazolium acetate [Emim]$COOCH_3$ (Aldrich), 1-ethyl-3-methyl-imidazolium trifluoroacetate [Emim]$COOCF_3$ (Aldrich) and 1-butyl-3-methyl-imidazolium bis(trifluoromethylsulfonyl)imide [Bmim]Tf_2N (Aldrich) were purchased at the highest available purity.

General procedure for immobilization by sol-gel entrapment and adsorption

A typical sol–gel immobilization process was used, which involves acid- or base-catalyzed hydrolysis, then polycondensation of alkoxysilane precursors [$Si(OR)_4$] and organically modified silanes of the type R'-$Si(OR)_3$ (6 mmoles) in the presence of additives to form a matrix in which the enzyme is encapsulated [7]. When the gelation process started, the solid support (Celite 545) was blended with the gelling mixture. The obtained gel was kept for 24 h at room temperature to complete polymerization. The bulk gel was washed with isopropyl alcohol, distilled water and finally hexane, filtered and dried at room temperature. Finally, it was crushed in a mortar and kept in refrigerator.

General procedure for the transesterification studies

Acylations were performed in 4 mL capacity glass vials, charged with a mixture of 2-hexanol or 2-octanol (0.5 mmole), vinyl acetate (1.5 mmole), reaction medium (organic solvent, 1 mL) and free (5 mg) or immobilized lipase (25 mg). The mixture was incubated using an orbital shaker (MIR-S100, Sanyo, Japan) at 300 strokes/min and 40ºC (ILW 115 STD incubator, Pol-Eko-Aparatura, Poland). The conversion and enantiomeric excess of the product were assayed by gas-chromatography, on a Varian 450 instrument (Varian Inc., USA) equipped with flame ionization detector, using a 30 m x 0,25 mm Elite-Cyclosil B chiral column with 0.25 mm film thickness (Perkin-Elmer, USA). The analysis conditions were: oven temperature: 50º to 120ºC with 10ºC/min heating rate, injector temperature 240ºC, detector temperature 280ºC, carrier gas (hydrogen) flow 1.2 mL/min. The reactions were usually run for 24 hrs. Conversions have been calculated based on the internal standard method.

Transesterification activities were calculated at 24 hrs reaction time and expressed as the average 2-octyl-acetate amount (in micromole) synthesized per hour by 1 mg of free or immobilized enzyme. The control reaction without enzyme did not give any product in the same conditions. To characterize the overall efficiency of the immobilization process, total activity yield was calculated as % of the total enzymatic activity recovered following immobilization, divided by the total activity of the lipase subjected to immobilization. The enantiomeric excess of the resulted ester product ($e.e._p$) was determined from the two enantiomers peak area, and the enantiomeric ratio (*E*) values were calculated based on conversion and $e.e._p$ values [8].

RESULTS

In this study, lipase from *Candida antarctica* B (CalB, C-Lecta) was immobilized by the combined method as described, and the activities of the obtained preparates were tested in the kinetic resolution of two secondary alcohols (2-hexanol and 2-octanol) in *n*-hexane, at 40°C. The sol-gel matrix was obtained using a ternary silane precursors sistem (PhTMOS, MeTMOS and TMOS) at 1.6/0.4/1 molar ratio and then adsorbed on Celite 545. As additives we tested several ionic liquids ($EmimBF_4$, $HmimBF_4$, $OmimBF_4$, $BmimPF_6$, $EmimCOOCH_3$, $EmimCOOCF_3$ and $BmimTf_2N$). The obtained biocatalysts were used for the acylation of two secondary alcohols, 2-hexanol and 2-octanol at 40°C in n-hexane as solvent (Table I, Table II and Figure 1).

Table I. Influence of the additive on the catalytic efficiency of the double immobilized *Candida antarctica* lipase B tested in the enantioselective acylation of 2-hexanol in n-hexane

Additive	Conversion (%)	Activity ($\mu mole \cdot h^{-1} \cdot mg^{-1}$)	Total activity yield (%)	$e.e._P$ (%)	E
Native lipase	20	0.852	100	93	35
$EmimBF_4$	49	0.411	528	96	163
$HmimBF_4$	47	0.402	501	96	134
$OmimBF_4$	46	0.304	398	97	170
$BmimPF_6$	51	0.432	529	96	380
$[Emim]COOCH_3$	49	0.417	516	94	101
$[Emim]COOCF_3$	48	0.396	477	95	113
$BmimTf_2N$	49	0.413	504	95	125

Table II. Influence of the additive on the catalytic efficiency of the double immobilized *Candida antarctica* lipase B tested in the enantioselective acylation of 2-octanol in n-hexane

Additive	Conversion (%)	Activity ($\mu mole \cdot h^{-1} \cdot mg^{-1}$)	Total activity yield (%)	$e.e._P$ (%)	E
Native lipase	20	0.842	-	89	21
$EmimBF_4$	51	0.433	563	94	146
$HmimBF_4$	51	0.434	547	95	201
$OmimBF_4$	51	0.434	482	95	201
$BmimPF_6$	51	0.459	569	95	201
$[Emim]COOCH_3$	51	0.465	582	94	146
$[Emim]COOCF_3$	51	0.437	533	95	201
$BmimTf_2N$	51	0.420	518	95	201

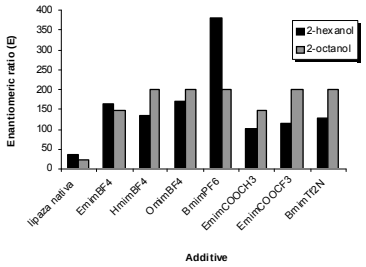

Figure 1. Influence of the ionic liquid used as immobilization additive on the enantiomeric ratio of the obtained preparates tested in the acylation of 2-hexanol and 2-octanol.

DISCUSSION

Ionic liquids with 1,3-dialkylimidazolium cation are widely used for biocatalytic applications. Their physical properties cover a broad range of values, but their catalytic properties are strongly influenced by polarity, hydrophobicity and miscibility with the solvent. The double immobilized preparates showed higher values of the total recovered activity and enantioselectivity in comparison with those obtained with the native lipase.

Ionic liquids with the same anion, tetrafluoroborate, led to high values of the activity with the decrease of the alkyl chain in the cationic part. Among ionic liquids with the same cation, 1-ethyl-3-methylimidazolium, the total recovered activity and enantioselectivity of the immobilized preparates decreased if the anion was bulky (trifluoroacetate, acetate).

The ionic liquid which showed the best results as immobilization additive in the enantioselective acylation of 2-hexanol was $BmimPF_6$, with a 5-fold increase of the total activity in comparison with the native lipase and with an enantiomeric ratio of 380 (Table I).

When the biocatalysts were tested in the enantioselective acylation of 2-octanol, we obtained better values of the enzymatic activity, but there were not significant differences between the nature of the ionic liquids used as immobilization additives. In this case, the ionic liquid which showed the best results as immobilization additive was $EmimCOOCH_3$, with a 5-fold increase of the total activity compared to the native lipase.

Using ionic liquida as immobilization additives, excellent values of the enantioselectivity were observed (compared to the native lipase) in the tested reactions. The obtained enantiomeric ratio values were between 100 and 380 (Figure 1). For both tested substrates the ionic liquid with the best results was $BmimPF_6$ (Table I, II and Figure 1).

CONCLUSION

The combined method of sol-gel entrapment and adsorbtion determined an increase in the stability and activity of the tested biocatalysts. The enantioselectivity of the obtained enzymatic preparates was significantly modified compared to the native enzyme.

The use of ionic liquids as immobilization additives at the sol-gel entrapment of lipases resulted in high values of the recovered total activity and of enantioselectivity compared to the native lipase.

When the biocatalysts were tested in the enantioselective acylation of secondary alcohols, the ionic liquid which showed the best results as immobilization additive was $BmimPF_6$, with a 5-fold increase of the total activity related to the native lipase and with enantiomeric ratio values between 201 and 380.

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