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Enantioselective resolution of racemic ibuprofen esters using different lipases immobilized on epoxy-functionalized silica

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ABSTRACT

Here we report the stereoselective hydrolysis of racemic ibuprofen esters catalyzed by *Candida rugosa* lipase (CRL), *Rhizomucor miehei* lipase (RML) and *Candida antarctica* lipase B (CALB) immobilized on epoxy-functionalized silica particles via covalent attachment. The performance and yield of the reaction were evaluated as a function of the critical reaction parameters such as enzyme to substrate ratio and organic co-solvent. The hydrolysis reactions were carried out in presence of two organic solvents; *n*-hexane and isooctane. High enantioselective hydrolysis of the racemic esters (yielding S (+) ibuprofen) can be achieved using the immobilized CRL, RML and CALB. Among various esters the kinetic resolution of ibuprofen isopentyl and octyl ester yielded the best results. By considering both conversion and enantioselectivity the best condition is obtained by using 10 mg of RML immobilized on epoxy-functionalized silica in hydrolysis of octyl ibuprofen ester (*E* value 66.3). For CRL-epoxy catalyzed reaction the highest enantioselectivity is achieved for 50 mg of biocatalyst in hydrolysis of isoamyl ester (*E* value 419) and finally CALB-epoxy catalyzed reaction in hydrolysis of isoamyl ibuprofen ester resulted in *E* value 30.5.

1. Introduction

In pharmaceutical and chemical industries, producing optically pure active compounds becomes a central issue, especially in the development of new drugs (Muñoz Solano et al., 2012). The reason for this fact is prevention from any side effects followed by consumption of racemic mixtures. Only one of the enantiomers of a racemic chiral drug has medicinal effect while the second enantiomer does not or may be less effective, totally ineffective, or in the worst case even toxic (Adams et al., 1976). Accordingly, a great deal of effort has been developed over the years to find new strategies for obtaining single enantiomer of a chiral compound. Among all of the strategies, biotechnology presents more environmentally and economically attractive approach to obtain bioactive and valuable compounds (Siódmiak et al., 2013).

Ibuprofen is one of the commercially successful and important member of non-steroidal anti-inflammatory drugs (NSAIDs) that belongs to the family of propionic acid which is widely used to treat rheumatoid arthritis, headache, muscular strain, cephalalgia, and muscular strain (Fazlena et al., 2006). This drug is a chiral molecule and employed as racemate in pharmaceutical formulations but has its main pharmacological activity in (*S*)-enantiomer which is 160 times more active in therapeutics effects despite of (*R*)-ibuprofen which causes serious side effect such as gastrointestinal pain, normal lipids metabolism and membrane function (Madhav and Ching, 2001; Shanbhag et al., 1992). The enantioselective resolutions of (*R*) and (*S*)-profen were reported using gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) (Evans, 2001). In recent years, preparation of optically pure compound has been done by enzymes as an alternative to chemical synthesis. Enzyme mediated kinetic resolution is the most precious way of obtaining a pure enantiomer due to its ability to discriminate between enantiomers (Lau et al., 2013; Carvalho et al., 2006; Ghanem, 2007).

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are very suitable enzymes for organic synthesis because of accepting wide range of

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substrates, stability and activity in organic solvents, availability from several (micro) organisms. Lipase-catalyzed transformations play an important role in organic synthesis to fulfill the rapidly growing demand for enantiomerically pure compounds such as pharmaceuticals, agrochemicals, and natural products (Nishigaki et al., 2008). Enantioselectivity is one of the key features of lipases making them attractive for biocatalysis, no requirement to cofactors and also capacity of catalyzing different reactions such as asymmetric esterification, asymmetric transesterification and asymmetric hydrolysis (Contesini and de Oliveira Carvalho, 2006). For this reason, an applied study of the enantioselectivity enhancement of lipase is highly desirable. In general, the enantioselectivity of lipase may be determined by a special relationship between the substrate structure and the origin of lipase, that is, the stereochemical environment of its active site. A traditional approach for this problem is to screen a large number of commercial lipases to find a more enantioselective one. This procedure, however, seems to be somewhat time consuming for organic chemists.

Beside of all the usages of lipases in organic synthesis, their application is limited in the industry because of prohibitive cost and also stability in organic solvents. There are a lot of different immobilization methods for overcoming this problem. For selecting a strategy for immobilization of enzymes some factors including good catalytic activity, stability and reusability of the enzymes should be taken into account. There are lots of published reports on the immobilization of lipase techniques onto different supports so far (Nasratun et al., 2009; da Silva et al., 2009; Bayramoglu et al., 2011; Temoçin, 2013; Romdhane et al., 2011; Dhake et al., 2012).

In our previous work we have reported immobilization of *Rhizomucor miehei* lipase (RML), *Candida rugosa* lipase (CRL) and *Rhizopus oryzae* lipase (ROL) via different protocols such as physical adsorption and covalent attachment to catalyze hydrolysis of various ibuprofen esters (Habibi et al., 2013; Yousefi et al., 2014). The aim of the present investigation is development of an enzymatic method for the production of the *S*-enantiomer of ibuprofen. For this purpose, CRL, CALB and RML were immobilized on epoxy-functionalized silica (silica-epoxy) via covalent attachment and their application was examined in resolution of various (*R*,*S*)-ibuprofen esters by hydrolysis. The optimization of hydrolysis reaction was performed regarding to the amount of the immobilized lipase.

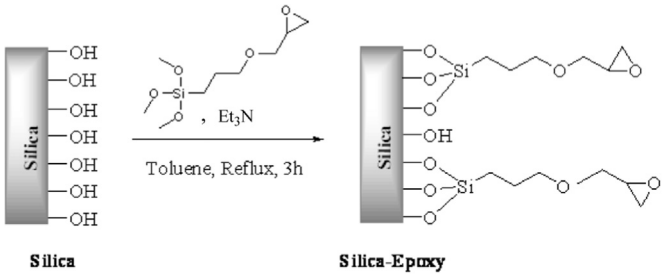
2. Materials and methods

2.1. Materials

Ibuprofen was extracted from the readily marketed tablets according to literature procedure (Lakin et al., 1994). *p*-Nitrophenyl butyrate (*p*-NPB), silica gel, molecular sieves (4 Å, 4–8 mesh), (*S*)-(+)-ibuprofen (purity 99%), lipase from *C. rugosa* and lipase from *R. miehei* were obtained from Sigma (Steinhiem, Germany). Lipase B from *Candida antarctica* was kindly donated by Novozymes (Bagsvaerd, Denmark). Other reagents and solvents were of analytical or HPLC grade.

2.2. Functionalization of silica particles

One gram of dry silica gel was mixed in a dry toluene solution (30 ml) containing 3-glycidyloxypropyl trimethoxysilane (3-GPTMS) (1 ml) and triethyamine Et_3N (0.15 ml) (Scheme 1). The resulting mixture was refluxed under argon atmosphere and constant stirring for 4 h. The silica gel was then washed thoroughly with $CHCl_3$ and dried at 60 °C for 2 h. The presence of



Scheme 1. A description of the silica gel treatment used for immobilization. The surface was then reacted directly with lipases as described in Section 2.

epoxy groups on the support was confirmed in our previous study by IR spectroscopy and TGA–DTA analysis (Mohammadi et al., 2014b). Quantification of the epoxy groups on the support was carried out as follows: 200 mg of the support was added to 1.5 ml of 1.3 M sodium thiosulphate solution and the solution was titrated by addition of 0.1 M hydrochloric acid until neutralization. The amount of epoxy groups was calculated from the amount of hydrochloric acid needed in order to maintain neutrality of the mixture (Sundberg and Porath, 1974). The same reaction was performed using unmodified particles as blank.

2.3. Immobilization of lipase

Epoxy functionalized silica (1 g) was mixed with 10 mg lipase in 10 ml phosphate buffer 25 mM (pH 7) followed by incubation at 25 °C for 24 h. Immobilized lipase was recovered by filtration, washed thoroughly with distilled water, and then dried overnight at room temperature.

2.4. Determination of the amount of enzyme bound to the silica particles

The amount of protein was determined by the Bradford method (Bradford, 1976). The amount of lipase bound to the carrier was determined as the difference between the initial and residual protein concentration. The yield of bound enzyme was calculated as the ratio of the amount bound on silica gels to the initial amount.

2.5. Enzyme activity assay

Hydrolytic activity of enzyme derivatives was determined by measuring the increase in absorbance at 348 nm produced by the release of *p*-nitrophenol during the hydrolysis of *p*-nitrophenyl butyrate (dissolved in acetonitrile) in 25 mM potassium phosphate buffer (pH 7.0) at room temperature. To start the reaction, 20–80 μl of the lipase suspension or solution was added to 1 ml of the reaction mixture (0.8 mM). Hydrolysis was followed for 2 min.

2.6. General method for the chemical synthesis of ibuprofen esters (**1–8**)

To a solution of 0.1 mol of the racemic acid in 100 ml toluene, 0.5 mol of corresponding alcohol (methanol, ethanol, propanol, isopropanol, *n*-butanol, isobutanol, isopentanol and octanol) was added followed by few drops of sulfuric acid (98%) (Ghanem et al., 2010). The mixture was stirred under reflux over night and the solvent was evaporated under vacuum and the residue was neutralized with 10% sodium hydrogen carbonate. The ester was extracted twice with 50 ml chloroform and then dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under vacuum to afford the racemic ibuprofen esters.