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New Biosilified Pd-lipase hybrid biocatalysts for dynamic resolution of amines

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ABSTRACT

In this work lipase CaLB was immobilized on functionalized Pd-SiO_2 nanoparticles in order to simplify the DKR of α-methylbenzylamine. Hybrid biocatalysts showed immobilization efficiencies of 82%, 80% and 76% when containing 1, 5 and 10% of Pd respectively. On DKR reaction values of ee > 99% and conversion of 82% were found with only 1% of Pd, generating a productivity of 2.21 mg of product h^{-1} mg of support^{-1} against 0.76 found by N435®. Compared to commercial N435®, the novel biocatalysts showed protein loads about 15-fold lower and higher activity, demonstrating competitive performances and high industrial applications.

Introduction

During the past years much attention has been given to the development of new strategies for production of chiral compounds and biocatalytic methods have played an important role.[1a–c] Among the many different biocatalysts used for the production of chiral building blocks, enzymes which are able to produce chiral amines are in evidence mainly due to their biodegradability, ability to work under mild and environmentally benign conditions as well as to present high chemo-, regio- and stereoselectivity, leading to cleaner reactions.[2] However, these catalysts can also present kind disadvantages like limited substrate scope and reaction conditions or the access to only one enantiomeric form. Besides, many groups of enzymes require co-factors and their applications outside the whole cell environment are still a challenge.[3]

Although new strategies have been recently developed by the use of transaminases, imine reductases or monoamine oxidases, lipases (E.C. 3.1.1.3) have shown to be overly promising enzymes all over the years.[4] These enzymes are carboxyl-ester esterases which in living organisms catalyze the hydrolysis of fatty acids esters.[5] In several bioprocesses, lipases have found to be versatile biocatalysts of a range of reactions under bland conditions with high stability, broad substrate range, and non-requirement of co-factors. In addition, many lipases present good to excellent stereoselectivity, are readily available and can be immobilized and reused, being suitable for industrial purposes.[6]

Among many reactions catalyzed by lipases, hydrolysis and esterification applied to asymmetric synthesis are the most important since the high enantioselectivity displayed by many of these enzymes allows the preparation of enantiopure compounds through kinetic resolution (KR) and dynamic kinetic resolution (DKR) of racemates.[5] Kinetic resolutions are by far the well-studied reaction for the production of chiral intermediates mediated by lipases. Unfortunately, this process can lead to a maximum yield of 50%. In order to overcome this limitation, dynamic resolution (DKR) can be used in combination to a metallic-catalyst to racemize the remaining free amine (Scheme 1) achieving theoretically at 100% yield.[7] Several metallic catalysts have been developed for this transformation, including ruthenium,[8] iridium,[9] and palladium.[10–12] Lipases have been shown to be more tolerant to the organic chemistry reaction environment and maintain the activity in organic solvents. Because of these characteristics they are often

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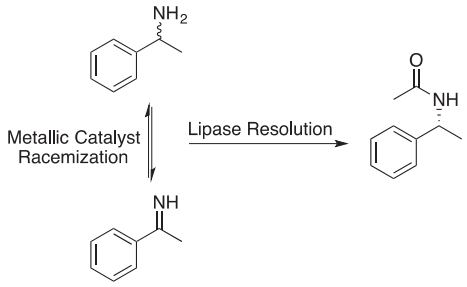

Scheme 1. Dynamic resolution mediated by lipases with metallic catalyst for racemization.

included in the DKR as the biocatalysts.[7,13,14] However, to perform a robust lipase-catalyzed DKR, some aspects should be taken into account: the KR must display a sufficient enantioselectivity (E value = $k_{fast}/k_{slow} \geq 20$), the metallic racemization catalyst must be compatible with lipase, the rate of racemization must be at least $10k_{slow}$, and metallic catalyst must be compatible with product.[15]

Amines are strong coordinating ligands, and normally deactivate metallic catalyst in the racemization step. Therefore high temperatures are required for disruption of coordination; nevertheless these conditions decrease the number of lipases that can be employed in the progress.[16] Thus, an efficient racemization catalyst is essential to the success of the process.

In general lipases and metallic catalysts are used in different phases where one is homogeneous and the other is heterogeneous or both are heterogeneous on different supports. Lately several groups have been working on the development of hybrid biocatalysts where the enzyme is covalently bounded to a matrix where the metallic catalyst is already supported, enhancing in this way the recovery of the catalyst.[15]

Following our recent publications on the immobilization and development of DKR procedures,[16] here in we presented a solid protocol for the immobilization of lipase B from *Candida Antarctica* (CaLB) by covalent bond on functionalized silica containing palladium, in order to simplify the reactions of dynamic kinetic resolution of amines, where the racemization and acetylation steps proceeded simultaneously catalyzed by our new hybrid catalyst.

Materials and methods

Materials

Lipase B from *Candida antarctica* (Lipozyme CaLB, soluble form) and Novozyme 435® (immobilized form) were purchased from Novozymes® (Brazil). α-methylbenzylamine, TEOS (Tetraethyl orthosilicate), APTES ((3-Aminopropyl) triethoxysilane), H_2PdCl_4, $NaBH_4$, bovine serum albumin (BSA), *p*-nitrophenyl palmitate (pNPP) and glutaraldehyde were purchased by Sigma Aldrich (Brazil). All other reagents used were of analytical grade.

Silica preparation

10 ml $NH_3{\cdot}H_2O$ (25%), 25 ml H_2O and 300 ml ethanol were added into a flask at room temperature under constant stirring for 5 min. Then, the 45 ml TEOS was added in a constant rate into the mixture. The reaction was performed under vigorous stirring for 3 h in water bath under 40 °C followed by a constant stirring for 21 h at room temperature. The SiO_2 nanoparticles were centrifuged at 6000 rpm and washed 3 times with 10 ml of absolute ethanol each time.

Silica nanoparticles functionalization

Around 0.55 g of SiO_2 nanoparticles were dissolved in 200 ml of absolute ethanol under constant stirring followed by the addition of 450 μl of APTES at room temperature. The system was maintained under constant stirring during 12 h. The functionalized SiO_2 nanoparticles were centrifuged at 6000 rpm and washed by 10 ml absolute ethanol for 3 times. The support was named as SiO_2@APTES.

Mettalic catalyst production

H_2PdCl_4 (0.15 M) aqueous solution was first prepared by mixing $PdCl_2$ and HCl (1:2 molecular ratio) in water at 50 °C with vigorous stirring. 500 mg of SiO2@APTES was dissolved into 200 ml water. 1 ml of H_2PdCl_4 was added into the SiO_2 solution followed by the addition of $NaBH_4$ (10%, 400 μl). 10 min later, 2 ml of ammonia was added into the solution and the system was stirred during 1 h. After condensation, catalyst was precipitated and washed with 10 ml of ethanol followed by centrifugation at 6000 rpm three times. The final catalyst was named as Pd-SiO_2@APTES.

Functionalization of Pd-SiO2@APTES

5 mL of 0.025 M phosphate buffer pH 7 and 1 mL of glutaraldehyde were added to 250 mg of Pd-SiO2@APTES. The reaction was stirred at room temperature for 24 h. The product was filtered and washed repeatedly with water to eliminate unreacted residues. The functionalized support was named as Glt-Pd-SiO_2@APTES.

Immobilization conditions

In all experiments, immobilization efficiency and yields were followed by measuring the hydrolytic activities and the protein concentrations in the supernatant solution. Immobilization yields (Eq. (1)) were calculated after determining the amount of protein and enzyme units which disappeared from the supernatant and comparing with the initial protein and enzyme concentrations offered to reaction (units per gram of support). Efficiency (Eq. (2)) was calculated after determining the activity of the immobilized enzyme and comparing with the number of enzyme units that disappeared from the supernatant (theoretically immobilized). Soluble protein was determined by the Bradford method using bovine serum albumin (BSA) as protein standard.[17]

$$Y = \frac{Sa - Ra}{Sa} \cdot 100 \quad (1)$$

Eq. (1), where:
Y = Immobilization yield
Sa = total starting activity
Ra = total residual activity

$$E = \frac{Oa}{Sa - Ra} \cdot 100 \quad (2)$$

Eq. (2), where:
E = Immobilization Efficiency
Oa = Observed activity
Sa = total starting activity
Ra = total residual activity

The catalyst Glt-Pd-SiO2@APTES was applied as support for CaLB immobilization. For this purpose, 1 mL of commercial CalB solution (0.62 U. mL-1 of specific activity) was diluted in 5 mL of 0.025 M phosphate buffer pH 7.0, and 100 mg of Glt-Pd-SiO2@APTES was added. The mixture was stirred for 4 h at 40 °C using a flask shaker. The final biocatalyst was filtered under