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Kinetic resolution of a drug precursor by *Burkholderia cepacia* lipase immobilized by different methodologies on superparamagnetic nanoparticles

Leandro H. Andrade[a,*], Lya P. Rebelo[a], Caterina G.C.M. Netto[b], Henrique E. Toma[b]

[a] *Laboratory of Fine Chemistry and Biocatalysis, Institute of Chemistry, University of São Paulo, CEP 05508-900, São Paulo, SP, Brazil*
[b] *Supramolecular NanotechLab, Institute of Chemistry, University of São Paulo, CEP 05508-900, São Paulo, SP, Brazil*

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ABSTRACT

Burkholderia cepacia lipase was immobilized on superparamagnetic nanoparticles using three different methodologies (adsorption, chemisorption with carboxibenzaldehyde and chemisorption with glutaraldehyde) and employed in the kinetic resolution of a chiral drug precursor, (*RS*)-2-bromo-1-(phenyl)ethanol, *via* enantioselective acetylation reaction. An excellent improvement of lipase catalytical performance was observed. Free *B. cepacia* lipase gave the ester (*S*)-**2** with poor *E*-value <30, and after its immobilization to magnetic nanoparticles the *E*-value was up to >200. The effect of several reaction parameters in the kinetic resolution was studied. The best results for kinetic resolution were obtained using vinyl acetate as acetyl donor and toluene as solvent, typically yielding the ester in high enantiomeric excess (>99%) and *E*-value (*E* > 200). Of the three tested immobilization methods, chemisorption with glutaraldehyde was the best one in terms of temperature stability and yield product.

1. Introduction

The use of lipases as catalysts is contributing to the rise of the exciting and rapidly growing area of chiral organic synthesis [1–10]. As a matter of fact, research in this area is pursuing the discovery of new efficient enzymes, new target compounds, and also of new convenient solid supports, capable of sustaining the enzymatic activity in organic media with minimum loss. In particular, the immobilization of enzymes on magnetic particles is viewed as a very attractive strategy, for allowing their separation and recovery from the reaction media by the simple use of a magnet [11–15]. On the other hand, the reusability of the immobilized enzymes represents an outstanding green-chemistry approach, reducing the cost and amount of such expensive biocatalysts. It should be noticed that superparamagnetic nanoparticles exhibit a single magnetic domain in their nanocrystalline structures, and this is responsible for their exceptional magnetization properties in the presence of a magnetic field. A suitable superparamagnetic species is based on magnetite (γ-Fe_3O_4). Large surface area, high mass transference and rapid response to external magnetic fields are remarkable characteristics ennobling this type of nanometric support or carrier. In addition, enzyme immobilization on superparamagnetic nanoparticles can permit the recovery of the biocatalyst without spending energy, by using permanent magnets.

Recently, *Burkholderia cepacia* lipase (BCL, also known as *Pseudomonas cepacia* lipase) has been employed as biocatalyst in a variety of synthetic applications, performing reactions, such as hydrolysis [16–18], enantioselective acylation [19] and transesterification [20–23]. In view of the wide usefulness of *P. cepacia* lipase, the possibility of exploring its recyclability using superparamagnetic nanoparticles may be quite rewarding. For this reason, a detailed study focusing on the immobilization of this lipase on superparamagnetic nanoparticles was carried out, aiming its application in the kinetic resolution of the chiral drug precursor, (*RS*)-2-bromo-1-(phenyl)ethanol. This compound is envisaged as a common building block to the chiral compounds Fluoxetine, Tomoxetine and Nisoxetine [24–26], which are among the most important pharmaceuticals for the treatment of psychiatric disorders (depression, anxiety, alcoholism) and also metabolic problems (obesity and bulimia).

2. Results and discussion

Immobilization of enzymes at superparamagnetic nanoparticles involves specific molecular interactions at the surface, and can induce some structural reorganization, thus changing the behavior of the catalytic sites. Therefore, a comparative study was carried out in this work, by immobilizing the lipase according to three different procedures. Initially, magnetite (Fe_3O_4) nanoparticles (MagNP) were prepared by the co-precipitation method, mixing Fe^{3+} and

* Corresponding author.
E-mail addresses: leandroh@iq.usp.br (L.H. Andrade), henetoma@iq.usp.br (H.E. Toma).

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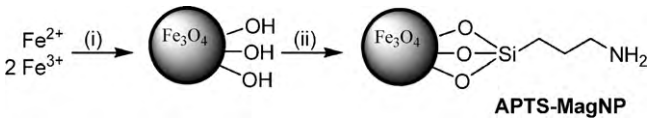


Scheme 1. (i) Co-precipitation of Fe^{2+} and Fe^{3+} oxides in alkaline solution; (ii) silanization of the Fe_3O_4 nanoparticles with APTS.

Fe^{2+} ions in NaOH solution, under an argon atmosphere. In order to eliminate any possible interference from the iron-oxide/hydroxide sites in the catalytic process, and to promote a better nanoparticles protection against air oxidation, a silanization treatment was applied, using γ-aminopropyltriethoxysilane (APTS), as shown in Scheme 1. The resulting black material exhibited strong magnetization in the presence of a magnetic field. Transmission electron microscopy revealed the presence of nearly cubic particles, exhibiting an average core size of 10 nm [27]. The silanization treatment provides a stable silicate coating by forming strong Fe–O–Si and cross-linked Si–O–Si bonds, leaving the γ-aminopropyl groups available for interacting with external species, protons, metal ions and solvent molecules. At neutral or slightly acidic solutions, the APTS amino groups are protonated ($pK_a = 9$), yielding a net positive charge responsible for the stabilization of the colloidal solutions.

The first immobilization procedure consisted in the direct interaction of lipase with the APTS modified superparamagnetic nanoparticles (APTS-MagNP). In spite of its rather simple nature, the direct interaction of the enzyme with the functionalized nanoparticles surface is driven by adsorption, proceeding according to a Langmuir isotherm which depends on many parameters, including the relative amounts of the interacting species, temperature and time. In this work, by using the same concentration of *B. cepacia* lipase (0.55 mg/mL) at 32 °C, the amount of APTS-MagNP was varied from 10 to 30 mg. The extent of enzyme binding was quantified using Bradford's method [28] after 3 successive washings. A typical analysis indicated the presence of 0.21 mg of protein bound to 20 mg of APTS-MagNP, a result consistent with a relatively strong enzyme–nanoparticle interaction. It can involve hydrogen bonding formation and electrostatic interactions, since at pH 7, *B. cepacia* lipase has a negative charge (isoelectric point = 5.2) [29] and the magnetic nanoparticles exhibit a positive charge due to the protonation of the aliphatic amino group [30]. In addition, there are also many amide and aminoacid residues in this lipase available for interacting with the proton.

In the second procedure, immobilization of *B. cepacia* lipase on superparamagnetic nanoparticles was carried out chemically through the formation of a covalent link, after the modification of APTS-MagNP with 4-carboxybenzaldehyde (Scheme 2). In this procedure, the amino group of APTS reacts with aldehyde, forming an imine intermediate, which is then reduced with $NaBH_4$, along with the remaining aldehyde groups, to give a carboxy-substituted species (Carboxy-APTS-MagNP). Thus, the lipase can be covalently bound to the carboxy-substituted species *via* carbodiimide activation. This step involves the conversion of the carboxyl group into an active ester by reacting with EDC (1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride), allowing the binding of the lipase to afford the biocatalyst named as BCL-Carboxy-APTS-MagNP.

By using this methodology, it was possible to bind 0.25 mg of protein to 20 mg of Carboxy-APTS-MagNP, quantified by Bradford method.

In the third procedure, *B. cepacia* lipase was immobilized onto the superparamagnetic nanoparticles using glutaraldehyde to afford the biocatalyst named as BCL-Glu-APTS-MagNP (Scheme 3). The role of glutaraldehyde is to react with the amino group of APTS, forming an imine bond and leaving another terminal aldehyde group for reacting with the amino residues of the enzyme, as shown in Scheme 3.

A typical amount of lipase immobilized by this methodology was 0.28 mg of protein to 20 mg of Glu-APTS-MagNP, quantified by Bradford method.

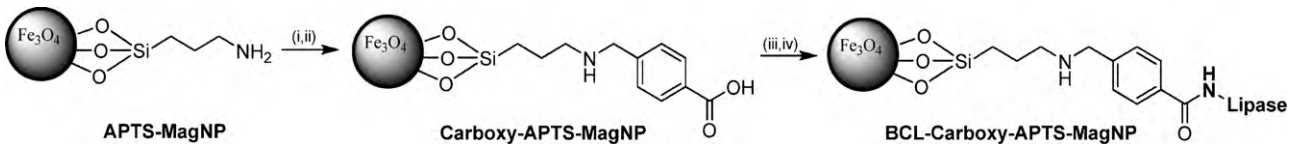
2.1. Topographic analysis

B. cepacia lipase immobilized onto APTS-MagNPs, seems to form extensive conglomerates when it was deposited at a flat mica surface, as can be seen in the AFM topographic and phase contrast imaging shown in Fig. 1. The conglomerate images are reproduced in the magnetic force microscopy (Fig. 1C) confirming their magnetic nature. Similar images were observed for the immobilized enzymes prepared by the several methods.

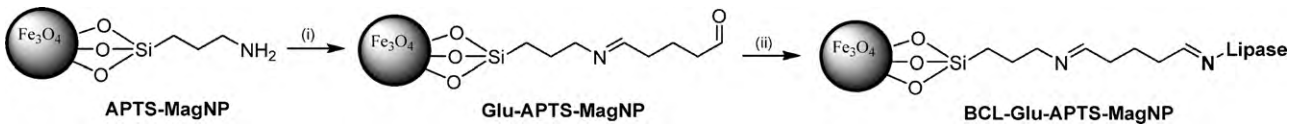
The conglomerate profiles are better seen in the 3D and cross-diagonal scan images, shown in Fig. 2. They actually appear like islands about 500 nm wide, composed by agglomerated nanoparticles. However, it should be noticed that the typical AFM images of APTS-MagNP exhibit a rather uniform distribution of nanoparticles all over the flat mica surface (Fig. 3). Therefore, the formation of such conglomerates, instead of a random distribution of the particles, indicates that the enzyme is facilitating their agglomeration onto the flat mica surface, during the drying process.

2.2. FTIR vibrational analysis

Typical FTIR spectra of the magnetic nanoparticles (MagNP) and their APTS modified forms (Fig. 4), as well as of the free *B. cepacia* lipase and their immobilized forms were superimposed in Fig. 5, for comparison purposes. The strong peaks at 585 and 632 cm^{-1} in the magnetic nanoparticles correspond to the ν(Fe–O) vibrational mode characteristic of bulk magnetite [31–34]. The silica network is adsorbed on the magnetite surface by Fe–O–Si bonds, and the corresponding infrared signals are usually overlapped with



Scheme 2. (i) 4-Carboxybenzaldehyde, 4 h; (ii) $NaBH_4$, 1 h; (iii) EDC, 20 min; (iv) *Burkholderia cepacia* lipase, 1 h.



Scheme 3. (i) Glutaraldehyde; (ii) *B. cepacia* lipase.