Journal of Molecular Catalysis B: Enzymatic 134 (2016) 43–50

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Enantioselective acetylation of (*R*,*S*)-atenolol: The use of *Candida rugosa* lipases immobilized onto magnetic chitosan nanoparticles in enzyme-catalyzed biotransformation

Adam Sikora[a], Dorota Chełminiak-Dudkiewicz[b], Tomasz Siódmiak[a], Agata Tarczykowska[a], Wiktor Dariusz Sroka[a], Marta Ziegler-Borowska[b], Michał Piotr Marszałł[a,*]

[a] *Department of Medicinal Chemistry, Collegium Medicum in Bydgoszcz, Faculty of Pharmacy, Nicolaus Copernicus University in Toruń, Dr. A. Jurasza 2, 85-089 Bydgoszcz, Poland*

[b] *Chair of Chemistry and Photochemistry of Polymers, Faculty of Chemistry, Nicolaus Copernicus University in Toruń, Gagarina 7, 87-100 Toruń, Poland*

ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form
15 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
Enantioselective acetylation
(*R*,*S*)-atenolol
Magnetic nanoparticles
Lipase

ABSTRACT

This paper describes the enzyme immobilization protocol as well as the enzymatic method for the direct resolution of (*R*,*S*)-atenolol. The used magnetic enzyme carriers possess on their surface new-synthetized chitosan derivatives with free amine groups distanced by ethyl or butyl chain. Additionally the catalytic activity of two types of commercially available lipases from *Candida rugosa* immobilized onto two different magnetic nanoparticles were compared. The highest values of enantioselectivity (E = 66.9), enantiomeric excess of product (ee_p = 94.1%) and conversion (c = 41.84%) were obtained by using lipase from *Candida rugosa* OF immobilized onto Fe_3O_4-CS-EtNH_2. The study confirmed that even after 5 reaction cycles the immobilized lipase maintain its high catalytic activity.

1. Introduction

Nowadays, the use of biotechnology is an alternative approach, which offers more environmentally and economically attractive ways to obtain bioactive compounds [1–3]. Lipases (E.C. 3.1.1.3) are ubiquitous enzymes, widely used in several industrial applications because of their ability to catalyse enantioselective biotransformation [2,4]. In recent years, these enzymes have been useful in organic synthesis and kinetic resolution of racemic compounds, which are well described in numerous papers [1,5–11]. However, lipases as proteins are extremely sensitive to reaction media as well as temperature, which may influence their enantioselectivity and catalytic activity. Additionally, the utilization of a native enzyme from a reaction mixture is another serious issue, particularly in industrial processes, because of the difficulty in its separation, recycling and reuse, which is directly related to high total cost of biotransformation [12,13]. Thus, the application of lipases in the industry is limited. Nevertheless, the process of immobilization of enzyme on the surface of nanoparticles could provide many additional advantages over the use of native lipases, because it does not only allow to reuse the bounded enzyme in another catalytic system but also increases the catalytic activity and operational stability of biocatalysts [14,15], and therefore many immobilization techniques have been investigated.

Magnetic nanoparticles are promising materials for many applications: biomedical, catalytic, analytical and industrial [14,16–18]. The immobilization of enzymes onto a support gives the opportunity for its reusability [19–21]. The application of magnetic nanoparticles as enzyme carriers allows to easily separate the bounded biocatalyst from reaction mixture by attracting with an external magnetic field. It gives the possibility to reuse the enzyme in another catalytic system, since the removal of enzymes from the reaction mixture is easy, it allows for simple product purification [12,13,22,23]. Additionally, magnetic supports based on Fe_3O_4 are usually nontoxic [16]. Due to the simplicity of their synthesis and modification of commonly large surfaces, magnetite (Fe_3O_4) nanoparticles are very popular and have gained a great attention in materials science.

* Corresponding author at: Collegium Medicum in Bydgoszcz, Jurasza 2, 85-089 Bydgoszcz, Poland.
E-mail address: mmars@cm.umk.pl (M.P. Marszałł).

http://dx.doi.org/10.1016/j.molcatb.2016.09.017
1381-1177/

Atenolol chemically known as 2-(4-(2-hydroxy-3-(propan-2-ylamino)propoxy)phenyl)acetamide is one of the most important β-adrenolytic drug widely used in treatment of hypertension and cardiovascular disorders [24]. Due to the fact, β-blockers possess asymmetric carbon atom in their structure, they are presented in two enantiomeric forms [25,26]. It was reported by many studies, only the *S*-enantiomers of these drugs possess the desired therapeutic effect, whereas the administration of the racemate may cause dangerous side effects such as bronchoconstriction or diabetes [6,27,28]. Nevertheless, β-blockers are still commercially available drugs mainly used in medicine as racemates.

The main aim of the study presented herein was to perform the kinetic resolution of (*R*,*S*)-atenolol with the use of two lipases from *Candida rugosa* (OF and MY) immobilized onto two types of not commercially available magnetic nanoparticles, which were *de novo* synthesised. Additionally, the reusability of immobilized enzyme was investigated, and the high catalytic activity of enzyme after five reaction cycles was confirmed. What is more, the new analytical method with the use of chiral stationary phases and UPLC system coupled with mass spectrometry was suggested in order to determine the quality and quantity of the atenolol enantiomers and its derivatives.

2. Materials and methods

2.1. Chemicals

(*R*,*S*)-atenolol, (*R*)-atenolol, toluene, isopropenyl acetate, 2-propanol, acetonitrile, Iron(II) chloride tetrahydrate, iron(III) chloride hexahydrate, chitosan (low molecular weight), glutaraldehyde, epichlorohydrine, sodium periodate, ethylenediamine, acetic acid, sodium hydroxide, EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride), sulfo-NHS (*N*-hydroxy-sulfosuccinimide sodium salt), glycine, acetic acid, ethanol, diethylamine, 1,4-diaminobutane, acetyl chloride were purchased from Sigma-Aldrich Co. (Stainhaim, Germany). Sodium sulphate anhydrous, sodium sulphate decahydrate, molecular sieves 4 Å, ethanol were purchased from POCH S.A. (Gliwice, Poland). Lipases OF and MY from *Candida rugosa* were a gift from Meito Sangyo Co., Ltd. (Japan). Water used in the study was prepared using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). All incubations were performed at adjusted temperature and number of rotation.

2.2. Instrumentation

The Refrigerated CentriVap Concentrator was purchased from Labconco, the Inkubator 1000 and Unimax 1010 were purchased from Heidolph, the FT-IR Spectrometer model Spectrum Two was purchased from Perkin Elmer, the Shimadzu UPLC–MS/MS system (Japan) equipped with solvent delivery two pumps LC-30AD combined with gradient systems, degasser model DGU-20A5, an autosampler model SIL-30AC, a column oven model CTO-20AC, UV detector model SPD-M20A and triple quadrupole mass spectrometer detector LCMS-8030. Lux Cellulose-2 (LC-2) column with cellulose tris(3-chloro-4-methylphenylcarbamate) stationary phase and Guard Cartridge System model KJO-4282 were purchased from Phenomenex Co.

2.3. Chromatographic conditions

The most appropriate chromatographic conditions for the resolution of racemic atenolol and its acetylated forms were optimized with Lux Cellulose-2 (4.6 mm × 250 mm × 3 μm) HPLC column, which was chosen for the chromatographic separation in polar/organic phase mode (Fig. 1). The mobile phase consisted of acetonitrile/2-propanol/diethylamine in ratio 98/2/0.1 (v/v/v). The chromatographic process was operated at 30 °C. The detection was made using triple quadrupole mass spectrometer in multiple reaction monitoring mode (MRM). The transitions of MRM for atenolol were 267.20 > 256.05; 267.20 > 190.05; 267.20 > 116.10; whereas for atenolol acetate were 309.2 > 158.10; 309.20 > 145.15; 309.20 > 116.10. In order to determine optical purity and enantioselectivity of enantioselective acetylation, the equations were used basing on peak areas from chromatogram achieved in chromatographic separation of (*R*,*S*)-atenolol and its acetylated forms. The percentage enantiomeric excesses of the substrate (ee_s) and product (ee_p), conversion (c) as well as enantioselectivity (E) were calculated, as below [11]:

$$ee_s = \frac{|R-S|}{R+S} \times 100\%$$

$$ee_p = \frac{|R-S|}{R+S} \times 100\%$$

$$c = \frac{ee_s}{ee_s + ee_p} \times 100\%$$

$$E = \frac{\ln\left[(1-c)\left(1+ee_p\right)\right]}{\ln\left[(1-c)\left(1-ee_p\right)\right]}$$

where R was values of peak areas for (*R*)-atenolol and its ester, whereas S was values of peak areas for (*S*)-atenolol and its ester.

2.4. Synthesis of magnetic chitosan nanoparticles: Fe_3O_4-CS-$EtNH_2$ and Fe_3O_4-CS-$BuNH_2$

Chitosan (0.2 g) was added into 20 mL of 1% acetic acid solution and mechanically stirred at room temperature for 20 min. After that, iron(II) chloride tetrahydrate (0.74 g, 3.75 mmol) and iron(III) chloride hexahydrate (2.02 g, 7.5 mmol) were added (1:2 molar ratio) and the resulting solution was chemically precipitated at room temperature by adding dropwise 30% solution of NaOH (7 mL). The black mixture was formed, separated by filtration and washed by deionized water for five times. Next, 10 mL of bicarbonate buffer pH – 10 and 10 mL of 5% glutaraldehyde solution were added and the composed mixture was mechanically stirred at room temperature for 1 h. In order to differentiate the synthesis between the Fe_3O_4-CS-$EtNH_2$ and Fe_3O_4-CS-$BuNH_2$ nanoparticles, the 20 mL of aqueous solution of ethylenediamine (2.4 g, 40 mmol) or 1,4-diaminobutane (3.53 g, 40 mmol), respectively, was added and the mixtures were stirred at room temperature for 2 h. The obtained magnetic materials were recovered from the suspension by applying a magnet, washed five times with deionized water and dried under vacuum at 50 °C for 24 h (Fig. 2).

2.5. Lipase immobilization onto chitosan magnetic nanoparticles with the use of EDC and sulfo-NHS

The covalent coupling of *Candida rugosa* lipase onto the surface of chitosan magnetic nanoparticles was performed by the formation of an amide bond between the carboxyl group of lipase and the primary amine group of the nanoparticle. The preparation procedure was performed according to the previously reported methodology [16,19], with few modifications (Fig. 3). In brief, the 36.5 mg of lipase OF and MY were suspended separately in 1.0 mL of 50 mM phosphate buffer (pH 6.4). After that, 2 mg of EDC in 50 μL of phosphate buffer was added to each tube with lipase suspension. The solutions were incubated at 21 °C and shaken for 1 h. After that time, 2.4 mg of sulfo-NHS was dissolved in 50 μL of phosphate buffer (50 mM, pH = 6.4) and added to each of the