ELSEVIER

Process Biochemistry 41 (2006) 1455–1460

Process Biochemistry

www.elsevier.com/locate/procbio

Short communication

Resolution of (*R*,*S*)-2-octanol by *Penicillium expansum* PED-03 lipase immobilized on modified ultrastable-Y molecular sieve in microaqueous media

Da-Zhang Dai, Li-Ming Xia *

Department of Chemical Engineering and Bioengineering, Zhejiang University, Hangzhou 310027, PR China

Received 30 August 2005; received in revised form 15 January 2006; accepted 23 January 2006

Abstract

The resolution of (*R*,*S*)-2-octanol was performed in microaqueous media by the lipase from *Penicillium expansum* PED-03 lipase (PEL) immobilized on modified ultrastable-Y (USY) molecular sieve. It was found that the conversion, enantiomeric excess (ee), and enantioselectivity (*E*) of the resolution catalyzed by PEL immobilized on modified USY molecular sieve were much higher than that of the reaction catalyzed by free PEL and PEL immobilized on other supports. Media type, ''memorial'' pH value and water content were of great importance in the resolution of (*R*,*S*)-2-octanol by immobilized PEL, and the conversion of the reaction catalyzed by PEL immobilized on modified USY molecular sieve reached 48.84% with excellent enantioselectivity (average *E* of eight batches > 460) in *n*-hexane with 0.8% (v/v) water at ''memorial'' pH 9.5, 50 °C for 24 h, which showed a good application potential in the production of optically pure (*R*,*S*)-2-octanol.

Keywords: Modified ultrastable-Y molecular sieve; Immobilized lipase; *Penicillium expansum* PED-03; Resolution; 2-Octanol; Microaqueous media

1. Introduction

Lipase (triacylglycerol acylhydrolases, E.C. 3.1.1.3) is one of the most important industrial enzymes, which catalyze the hydrolysis and the synthesis of triglycerides or acyl and aryl esters at an oil–water interface [1]. There has been a growing interest in the use of lipases for kinetic resolution of racemic compounds through esterification [2–5], hydrolysis [6–8] and transesterification [9,10] reactions because of its wide substrate specificity and ability to recognize chirality. Owing to the poor solubility in water of most racemic compounds, the enzymatic resolution is usually performed in nonaqueous media. However, a certain amount of water is necessary to keep the enzyme in catalytically active conformation and the lipase will be inactivated under absolutely anhydrous condition [11–13].

Chiral 2-octanol is one of the most important building blocks for the preparation of liquid crystal materials as well as an important intermediate of many optically active pharmaceuticals. Interest in the enzymatic resolution of (*R*,*S*)-2-octanol has increased markedly due to the rapid progress in the technique of enzyme-catalyzed reaction in nonaqueous media in the last two decades. Kinetic resolution of (*R*,*S*)-2-octanol by enantioselective lipase has been reported in the literature [14–16]. However, these reports mainly focus on the selection of free lipase and the optimization of reaction condition. As can be seen, few researches are exclusively directed towards the lipase immobilization on molecular sieve or related materials and the effect of immobilization on the conversion, enantiomeric excesses (ee) and enantioselectivity (*E*).

Recently, we have isolated and identified a strain of *Penicillium expansum*, *P. expansum* PED-03 from a rap oil manufactory in China, and found that it could produce an alkaline lipase (PEL) at a high level [17] with fine enantioselectivity in the resolution of (*R*,*S*)-2-octanol. Since free lipase is sensitive to the water in nonaqueous media and easy to agglomerate, which character has a negative influence on the resolution reaction, it is necessary for the lipase to be immobilized on a certain support. In this work, we found that modified ultrastable-Y (USY) molecular sieve was very suitable to be

* Corresponding author. Tel.: +86 571 87951840; fax: +86 571 88478437.
E-mail address: xialm@zju.edu.cn (L.-M. Xia).

1359-5113/$ – see front matter
doi:10.1016/j.procbio.2006.01.015

used as support for the immobilization of PEL, which not only could immobilize the lipase commendably, but also could maintain the required microenvironment of PEL for the resolution reaction in nonaqueous media. The enantioselectivity and the activity of PEL immobilized on modified USY molecular sieve were much higher than that of free PEL and PEL immobilized on other supports in the resolution of (*R*,*S*)-2-octanol through transesterification in nonaqueous media with a stable catalytic performance. This paper describes the new method to immobilize PEL using modified USY molecular sieve as support and its evaluation for the biocatalytic resolution of (*R*,*S*)-2-octanol in microaqueous media.

2. Materials and methods

2.1. Materials

The lipase powder (80,000 U/g) was produced in submerged fermentation by *P. expansum* PED-03 in our laboratory according to the method we have reported [17]. The enzyme was lyophilized and used for enzymatic reaction without further purification after lyophilization.

(*S*)-2-octanol, (*R*)-2-octanol and (*R*,*S*)-2-octanol were purchased from Shijiazhuang Jida Fine Chemicals Co., Hebei, China. USY molecular sieve was obtained from Wenzhou Huahua Co., Zhejiang, China. Other reagents used for the enzymatic resolution were of analytical grade and were obtained commercially. All nonaqueous solvents were dried with anhydrous Na_2SO_4 before being used for resolution.

2.2. Molecular sieve modification

Ten grams of Al_2O_3 was added as binder to 50 g USY molecular sieve in a 100 ml beaker. Then the mixture was kneaded and extruded after being agitated completely, and then dried at room temperature. The dried mixture was baked in a muffle furnace at 600 °C for 3 h and was ground to pass a 40-mesh sieve to be used as modified USY molecular sieve to immobilize PEL.

2.3. PEL immobilization

PEL immobilization on modified USY molecular sieve: 1 g PEL was dissolved in 50 ml 0.1 M Na_2CO_3–$NaHCO_3$ buffer (pH 9.5) in a 250 ml Erlenmeyer flask. Then 30 g modified USY molecular sieve was added to the PEL solution, and then the flask was put into a 25 °C homeothermia water bath shaker at 150 rpm for the adsorption process of PEL onto modified USY molecular sieve for 12 h. After the adsorption at equilibrium, the modified USY molecular sieve was collected by centrifugation (4000 rpm, 5 min), then washed several times with the same buffer, lyophilized and used as immobilized lipase for the resolution of (*R*,*S*)-2-octanol.

Lipase immobilization on sodium alginate: 100 mg PEL was dissolved in 3 ml 0.1 M Na_2CO_3–$NaHCO_3$ buffer (pH 9.5) and mixed with 3 ml 2% sodium alginate. Then the mixture was magnetically stirred for 6 h. When it was homogenized, the mixture was injected into 0.1 M $CaCl_2$ with a small injector. As they were hardened, the spherules were take out and washed several times with the same buffer, then lyophilized and used as immobilized lipase for the resolution of (*R*,*S*)-2-octanol.

Lipase immobilization on silica gel, macroporous resin and diatomite: the method was the same with that of PEL immobilization on modified USY molecular sieve.

2.4. Enzymatic resolution

The resolution was performed in a bioreactor contained 5 ml (*R*,*S*)-2-octanol, 5.8 ml vinyl acetate, 50 ml *n*-hexane, immobilized lipase (1000 U) and water (0.8%) at 50 °C for 24 h. The resolution process was monitored by high performance liquid chromatography (HPLC), and the conversion (*c*), enantiomeric excesses (ee) and enantioselectivity (*E*) are defined as follows [18–20]:

$$\text{enantiomeric excesses, ee}\,(\%) = \frac{[S-R]}{[S+R]} \times 100$$

$$\text{conversion, } c\,(\%) = \frac{\text{ee}_s}{\text{ee}_s + \text{ee}_p} \times 100$$

$$\text{enantioselectivity, } E = \frac{\ln[(1-c)(1-\text{ee}_s)]}{\ln[(1-c)(1+\text{ee}_s)]} = \frac{\ln[1-c(1+\text{ee}_p)]}{\ln[1-c(1-\text{ee}_p)]}$$

where *S* and *R* represent the concentrations of the (*S*)-enantiomer and (*R*)-enantiomer, respectively; ee_s and ee_P represent the enantiomeric excesses for the substrate (ee_s) and enantiomeric excesses for the product (ee_p) respectively.

2.5. Lipase activity analysis

Activity of free and immobilized lipase was determined according to a slightly modified olive oil emulsion method [21,22], and all reactions were performed at 35 °C unless otherwise indicated. The assay mixture contained 5 ml of olive oil, 4 ml of 0.2 M glycin–NaOH (pH 9.5), 0.2 ml of 1 M $CaCl_2$ and 200 mg of immobilized lipase. The reaction was stopped by the addition of 15 ml of 95% ethanol. Fatty acids released were determined by titration with potassium hydrogen phthalate standardized with 50 mM NaOH. One unit of lipase activity was defined as the lipase quantity that librated 1 μ mol of fatty acid per minute under assay condition.

2.6. HPLC analysis

The conversion, enantiomeric excesses (ee) and enantioselectivity (*E*) were determined by HPLC according to a published method with a slight modification [16]. The chromatographic conditions were as follows: column, SYL-TECH-YMC (150 mm × 4.6 mm); detector, UV; detection wavelength, 220 nm; flow rate, 1.0 ml/min; mobile phase, 0.01 mM H_3PO_4–CH_3CN (37:63); injection volume, 20 μl.

2.7. Statistical analysis

Analyses of variance (ANOVA) were done with Statistical Analysis System (SAS) for windows version 6.12. All experiments were performed in triplicate. The maximum difference among the three values was less than 5% of the mean. Fisher's least significant difference (LSD) test was used to determine the significant differences among the means.

3. Results and discussions

3.1. Immobilization of PEL

It was reported that the support used for lipase immobilization played an important role in the enzymatic reaction in nonaqueous media, and the lipase activity could be much higher when immobilized on a suitable support [23,24]. The resolution of (*R*,*S*)-2-octanol by PEL immobilized on different supports was carried out to investigate the effect of support on the enzymatic reaction (Table 1). As shown in Table 1, the performance of PEL immobilized on modified USY molecular sieve was much better than that of free PEL and PEL immobilized on other supports. Conversion of the reaction catalyzed by PEL immobilized on modified USY molecular sieve reached 48.84%, which was much higher than that of the reaction catalyzed by free PEL (15.91%) and PEL immobilized on sodium alginate (1.35%). In addition, the *E* value of the reaction catalyzed by PEL immobilized on modified USY molecular sieve reached 560.32, which was also much