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Glutaraldehyde modification of lipases adsorbed on aminated supports: A simple way to improve their behaviour as enantioselective biocatalyst

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Abstract

A new lipase from porcine pancreas was adsorbed on PEI-coated support and utilized in the resolution of (±)-glycidyl butyrate, reaching a moderate *E* value of 6. The treatment of the adsorbed lipase with glutaraldehyde permitted to increase this value by a 10-fold factor (to $E = 61$). Similar improvement of enantioselectivity promoted by the glutaraldehyde treatment was obtained employing immobilized preparations of other lipases. Moreover, enzyme activity in some instances can be even improved by this treatment, and the effect of the experimental conditions on the enzyme activity was completely altered. Thus, this simple treatment, in many cases necessary to stabilize the enzyme preparations, may be also a powerful tool to alter the enzyme properties.

Keywords: Crosslinking; Glutaraldehyde; Lipase modulation; Lipase immobilization; Enantioselectivity

1. Introduction

Lipases (EC 3.1.1.3) are among the most used enzymes in synthetic organic chemistry, catalyzing the kinetic resolution of different racemates (e.g. racemic secondary alcohols, racemic carboxylic acids, etc.) with a high regio- and enantioselectivity [1,2]. They are structurally characterized by the existence of a polypeptide chain called "lid" that secludes the active centre from the reaction medium [3]. The lipases suffer a conformational change from an inactive state (closed form) to an active state (open form), exposing the active site to the reaction medium and increasing its catalytic activity, the presence of hydrophobic surfaces promotes the stabilization of this open form (the so-called interfacial activation) [4].

It may be assumed that these two structures are not rigid. In aqueous homogeneous medium, equilibrium exists between both of them, very likely with different shapes of the open forms. Thus, it has been shown that using different methods of immobilization, that permit to alter the rigidity, orientation or microenvironment of the lipases, it is possible to dramatically alter their functional properties, e.g., its enantioselectivity [5,6].

Furthermore, the treatment with glutaraldehyde of previously immobilized enzymes has been reported to be a useful method to get an intense multi-point crosslink between the enzyme and the support, promoting an increase in the enzyme stability [7–10].

Herein, we study the glutaraldehyde treatment of enzymes adsorbed on aminated supports as a new tool to modulate the properties of lipases. This treatment may modulate the enzyme mobility, and, in this way, it may alter the final properties of the enzyme as the immobilization on different supports. The hydrolytic kinetic resolution of (±)-glycidyl butyrate [(±)-**1**] (Scheme 1) was used as model reaction, because both enantiomers of glycidol **2** have become widely used as starting materials for the synthesis of many interesting compounds, such as anticancer drugs, protein synthesis inhibitors as well as a 2-oxazolidinone derivative used against depression (Scheme 2) [11–13].

2. Materials and methods

2.1. General

The lipase from *Alcaligenes QL* (AQL) was kindly donated by Prof. Illanes (Universidad Católica de Valparaiso) (Valparaiso, Chile). Polyethyleneimine (PEI) (Mr 600,000), 1,4-dioxane, glutaraldehyde, *Porcine pancreatic* lipase

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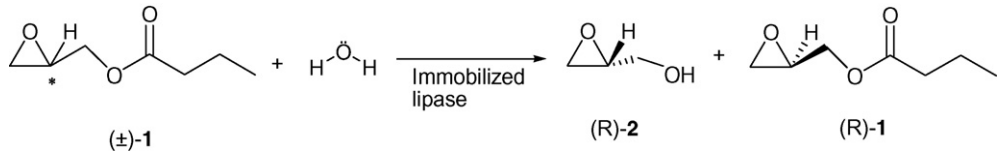
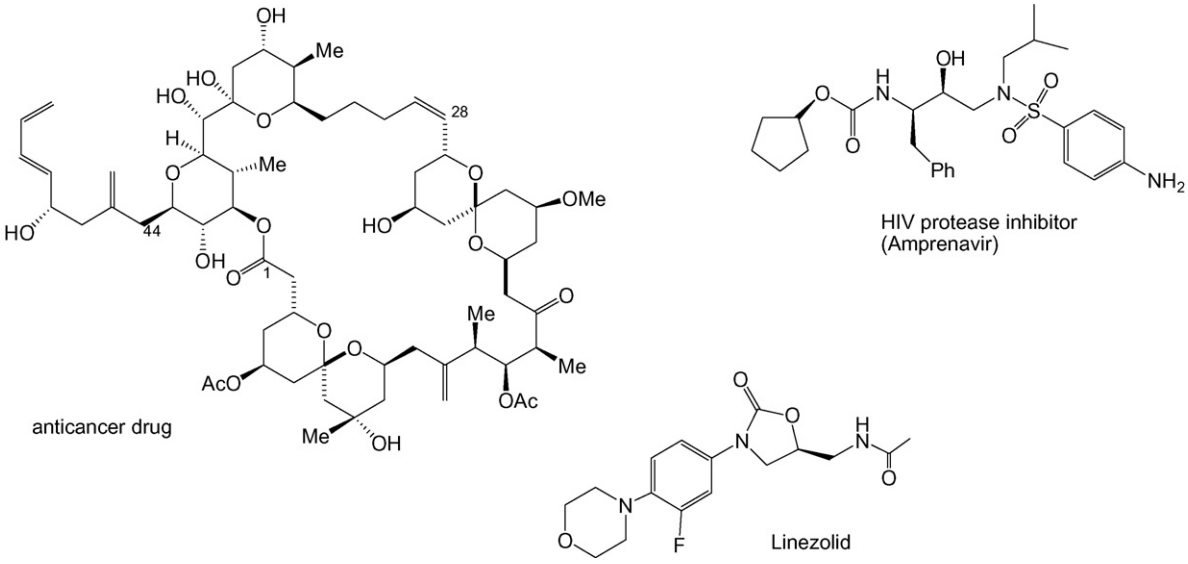


Scheme 1. Enzymatic resolution of glycidyl butyrate.



Scheme 2. Different drugs synthesizes from glycidyl derivatives.

(type II) (PPL), Triton X-100 were obtained from Sigma–Aldrich (St. Louis, EEUU). Purified 25 kDa lipase from PPL crude was prepared as previously described [14–16]. Glyoxyl-agarose 4BCL was kindly donated by the company Hispanagar SA (Burgos, Spain). The PEI-agarose support was prepared as previously described [17,18]. (±)-Glycidyl butyrate [(±)-**1**] was kindly donated by Dr. Terreni (University of Pavia) (Milan, Italy). Protein concentration was determined by the Bradford's method [19]. pH-stat Mettler Toledo DL50 graphic was used to maintain constant the pH value during the reactions. Conversion was analyzed by RP-HPLC (Spectra Physic SP 100 coupled with an UV detector Spectra Physic SP 8450) using a kromasil C_{18} (25 cm × 0.4 cm) column. Products were eluted at flow rate of 1.5 mL/min using acetonitrile-10 mM ammonium phosphate buffer at pH 2.95 (35:65, v/v) and UV detection performed at 225 nm. The enantiomeric excess (e.e.) of the remaining ester was determined by Chiral Phase HPLC analysis. The column was a Chiracel OD, mobile phase an isocratic mixture of isopropanol and hexane (2:98, v/v) at a flow of 0.4 mL/min and UV detection was performed at 225 nm. The retention times of the enantiómeros were: *S*-**1** (17.32 min), *R*-**1** (18.60 min).

2.2. Enzymatic activity assay determination

This assay was performed by measuring the increase in the absorbance at 348 nm produced by the release of *p*-nitrophenol in the hydrolysis of 0.4 mM *p*NPP in 25 mM sodium phosphate buffer at pH 7 and 25 °C. To initialize the reaction, 0.05 mL of lipase solution or suspension was added to 2.5 mL of substrate solution. One international unit of *p*NPP activity was defined as the amount of enzyme that is necessary to hydrolyze 1 μmol of *p*NPP per minute (IU) under the conditions described above.

2.3. Immobilization of lipases on PEI-agarose support

Ten grams of gel agarose coated with polyethyleneimine (PEI) [17,18] was added to 100 mL of 5 mM sodium phosphate buffer lipase solution at pH 7. The mixture was then shaken at 4 °C and 250 rpm for 4 h. After that, the solution was removed by filtration and the supported lipase washed several times with distilled water. In all cases, the enzyme loading was 1 mg protein/g of support and more than 95% of the lipase became immobilized. The immobilization was followed by the assay described above. Protein concentration was determined by the Bradford method [19].

2.4. Crosslinking of immobilized lipases by glutaraldehyde

One gram of PEI-lipase immobilized preparation was added to a 20 mL solution of 1% glutaraldehyde. The mixture was shaken at 25 °C and 250 rpm for 1 h, washed with distilled water, and then stored at 4 °C during – at least – 24 h. After that, a 100 mL solution of $NaBH_4$ (10 mg) in 100 mM sodium bicarbonate buffer at pH 10 was added to the solid preparation for 30 min. Then, the solution was removed by filtration and the supported lipase washed several times with distilled water and stored at 4 °C.

2.5. Enzymatic hydrolysis of (±)-glycidyl butyrate [(±)-*1*]

(±)-**1** (1 mmol) was dissolved in 25 mM sodium phosphate buffer (10 mL) at pH 7 or 25 mM sodium acetate buffer (10 mL) at pH 5 with different percentage of 1,4-dioxane from 0 to 20% (v/v) and biocatalyst (100 mg) was added. The mixture was then shaken at 25 °C and 250 rpm. The biocatalyst was filtered and the conversion was analyzed by RP-HPLC. Enantiomeric excesses were determined using a chiral phase-column previous extraction 0.2 mL of aqueous phase with hexane in HPLC (4× 0.2 mL). After that, the enantiomeric ratio (*E*) was calculated in all cases using the equation reported by Chen et al. [20].

3. Results and discussion

3.1. Effect of the glutaraldehyde treatment on the enzymatic activity

A lipase with a molecular weight of 25 kDa (purified from commercial *Porcine pancreatic* lipase) (25L) [11–13] and the