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Asymmetric hydrolysis of dimethyl 3-phenylglutarate catalyzed by Lecitase Ultra®
Effect of the immobilization protocol on its catalytic properties

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ABSTRACT

The asymmetric hydrolysis of dimethyl 3-phenylglutarate (**1**) by different immobilized preparations of a phospholipase A_1 (Lecitase Ultra (LECI)) at pH 7 and 25 °C has been studied. Agarose beads coated with octyl, cyanogen bromide (CNBr), polyethylenimine (PEI) or glyoxyl groups were used as supports for the immobilization of LECI. The different derivatives behaved very differently in terms of activity, discrimination between **1** and methyl 3-phenylglutarate (**2**) resulting from the hydrolysis of **1**, enantioselectivity (in the hydrolysis of **1** to produce R or S-**2**) and enantiospecificity in the hydrolysis of R-**2** and S-**2**. Using 1 mM of **1**, CNBr-LECI showed the highest activity (13×10^{-3} μmol/min mg protein) while octyl-LECI was about 20 times less active. All the enzyme preparations mainly produced (S)-**2**, but with different enantioselectivity. CNBr-Lecitase was the most enantioselective, producing the S-**2** 10 fold more rapidly than the R-**2**, while octyl-Lecitase gave only half of that difference.

LECI adsorbed on octyl-agarose allowed to get a yield up to 99% of S-**2** (ee was 66%). The reaction stopped in the monoester and no isomer of this compound was further hydrolyzed by the enzyme. However, when the reaction was catalyzed by the other immobilized LECI preparations, the enzyme was able to hydrolyze mainly the minority isomer, permitting to improve the ee of the remaining S-**2**. The best results were obtained using CNBr-LECI, which gave (*S*)-methyl-3-phenylglutarate with a yield of 80% and an ee exceeding 99%.

1. Introduction

Chiral 3-arylglutaric acid derivatives are important building blocks for the synthesis of several biologically active compounds that possess a chiral glutaric fragment in their structures, which is essential for their activity [1]. Among these, some may be (Scheme 1): (i) (−)-paroxetine hydrochloride, a serotonine receptor antagonists [2,3], (ii) (*R*)-Baclofen: a $GABA_B$ receptor agonist, which is used clinically in the treatment of spasticity [4–6], (iii) spiro-substituted piperidines, antiasthmatic drugs [7], (iv) 1-amino-2-phenyl-4-(piperidin-1-yl)butanes, an antagonist of receptor CCR5 involved in HIV-1 transmission to cells [8] and (v) SCH 54016, a potent cholesterol inhibitor [9]. Enantiomerically pure 3-arylglutaric acid monoesters have been prepared using chymotrypsin, pig liver esterase, pig pancreas lipase, lipases, and other enzymes in the hydrolysis of different prochiral glutarate derivatives [10–17].

In these reactions, an ideal biocatalyst needs to be fully enantioselective, producing the desired monoester, and without hydrolyzing this first product of the reaction. In this ideal situation, 100% of the desired isomer of the monoester could be produced with an ee of 100%.

If the enantioselectivity of the enzyme was not perfect, other possibility to get a enantiomerically pure monoester might be to use an enzyme with a good enantioselecivity which produced mainly one of the enantioisomers and, in a second step, use another enzyme able to enantiospecifically hydrolyze the minority isomer. This could give a very high ee, although with a yield under 100%. The final yield will depend on the initial percentage of the minority isomer (resulting from the enantioselectivity of the enzyme in the hydrolysis of the diester) and the enantiospecificity of the second enzyme (the discrimination of the two enantiomers of the monoester by this enzyme).

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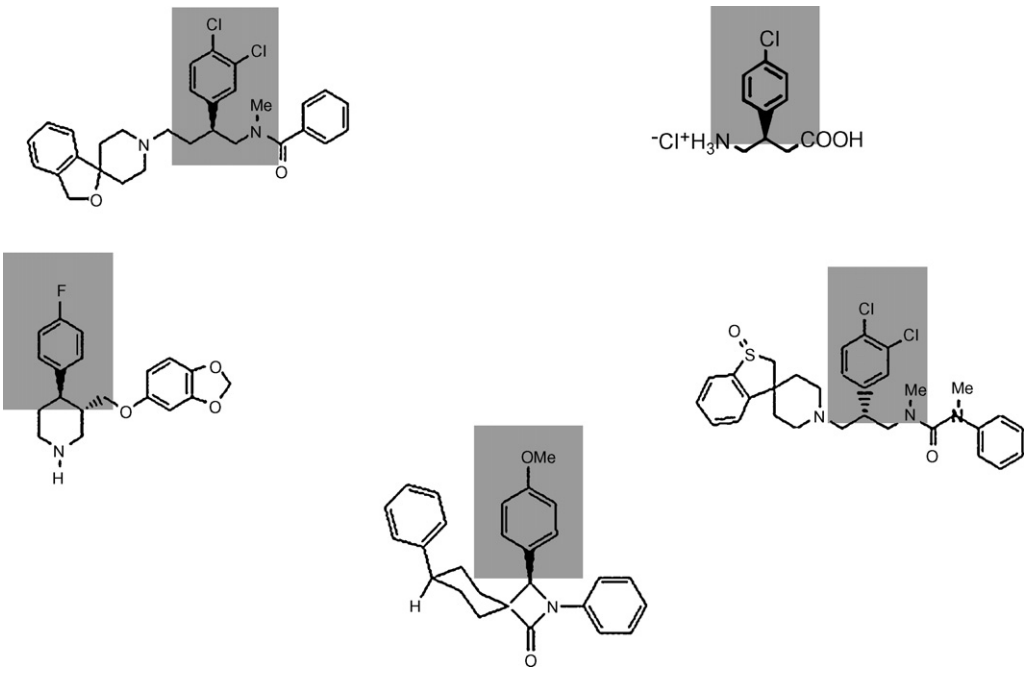


Scheme 1. Biologically active compounds containing a 3-arylglutaric building block.

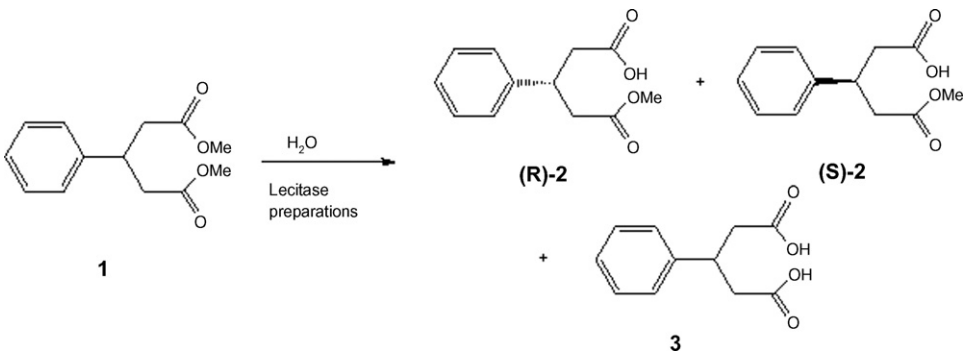
In some cases, it might be possible that the same enzyme catalyzed both processes. Thus, the enantioselectivity (to produce mainly one enantiomer of the monoester) and the enantiospecificity (to hydrolyze the minority and non-desired enantiomer of the monoester) of an enzyme could be used.

Phospholipases A_1 are enzymes having some features similar to lipases: they suffer interfacial activation [18–20], they recognize a wide range of structurally different substrates [21,22] and their properties may be dramatically altered by the immobilization technique employed, by altering the changes produced in the opening of the active center [22]. Phospholipases A_1 are employed to produce 2-acyl-lysophospholipids with interesting fatty acid composition (eicosapentaenoic acid, conjugated linoleic acid and docosahexaenoic acid) and degumming process of oils [23–29]. The interest on finding new phospholipases is shown by the high number of sources for this enzyme discovered in recent years [30–34]. Lecitases are enzymatic commercial products containing phospholipase A_1 developed by Novozymes mainly for degumming of oils [35–38]. There are very few examples of using these commercially available enzyme preparations in fine chemistry, but those few trials have been quite successful [21,22].

Here, a commercial preparation of phospholipase, Lecitase Ultra, immobilized on different supports, has been assayed to produce (*S*)-methyl 3-phenylglutarate by the asymmetric hydrolysis of dimethyl 3-phenylglutarate (Scheme 2). Lecitase Ultra (LECI) is an enzyme obtained from the fusion of the genes of the lipase from *Thermomyces lanuginosus* and the phospholipase from *Fusarium oxysporum* [39,40]. This new enzyme presented the stability of the lipase from *T. lanuginosus* and the activity of the phospholipase from *F. oxysporum*.

In this paper, different immobilization protocols have been assayed, trying to obtain a biocatalyst with the optimal features for this asymmetric hydrolysis.

The first one has been the interfacial activation of LECI by octyl-agarose [41]. Recently, it has been described that the immobilization of lipases *via* interfacial activation on hydrophobic supports at low ionic strength (via the hydrophobic surroundings of their active centre) may permit to modulate the enzyme specificity of the lipases in hydrolysis of diesters [42]. The first product of the reaction, at neutral pH values, will have a charged moiety, being quite hydrophilic. By this reason, it becomes partitioned from the hydrophobic environment surrounding the active center of the enzyme and this may stop the hydrolysis at the monoester.



Scheme 2. Hydrolysis of dimethyl 3-phenylglutarate (**1**) catalyzed by immobilized preparations of Lecitase Ultra.