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Novozym 435 displays very different selectivity compared to lipase from *Candida antarctica* B adsorbed on other hydrophobic supports

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ABSTRACT

This paper shows that the properties of lipase B from *Candida antarctica* (CAL-B) may be easily modulated using different hydrophobic supports to immobilize it (octyl and butyl-agarose, octadecyl-Sepabeads or Lewatit). CAL-B could be fully desorbed from the supports by just incubating the biocatalyst with Triton X-100, although the concentration of detergent necessary was to fully desorb the enzyme varied with the support employed (from 1% for butyl-agarose to 4% for octadecyl-Sepabeads), suggesting that in all cases, the main reason for the enzyme immobilization was hydrophobic interactions. Lewatit VP OC 1600 yielded very different results in terms of activity, selectivity or enantioselectivity in the hydrolysis of rac-2-*O*-butyryl-2-phenylacetic acid (**1**) and 3-phenylglutaric acid dimethyl diester (**3**) compared to the other preparations. For example, in the hydrolysis of **1**, Novozym 435 preferred the S-isomer (with an *E* value higher than 100) whereas all the other preparations preferred the R isomer (e.g. octyl-agarose-CAL-B with *E* value of 50). In the hydrolysis of **3**, Novozym 435 gave S-3-phenylglutaric acid methyl ester with an ee higher than 99%, by coupling the first asymmetric hydrolysis to the enantiospecific hydrolysis of the monoester. CAL-B immobilized on Lewatit at low ionic strength not only behaved similarly to Novozym 435, but also presented some differences that should be due to the exact protocol of the enzyme immobilization in Novozym 435.

1. Introduction

Lipases are among the most used enzymes in Biocatalysis. The major advantages of using lipases are their wide range of substrates in many instances with high chemo-, regio- and stereoselectivity as well as the very mild reaction conditions that can be used [1,2].

Lipases may exist in two different structural forms [3]. In one of them, the active site of the lipase is isolated from the medium by a helical oligopeptide chain called "lid" or "flap". The other structure presents the lid displaced and the active site exposed to the reaction medium, and it is considered to be the lipase in an active (open) form. In homogeneous aqueous media, the lipase is in equilibrium between these two structures. Upon interaction with a hydrophobic surface such as drops of oils [4], hydrophobic proteins [5], other lipases [6] or hydrophobic supports [7], the open form of the lipase becomes adsorbed to it and this equilibrium shifts towards the open form [3]. This makes that the immobilization of lipases by their interfacial activation on hydrophobic supports may be a very suitable and simple method for lipase purification, immobilization and hyper-activation (at least against small and hydrophobic substrates) [8]. These conformational changes make possible that lipases may be greatly altered by controlled immobilization [9].

The isoform B of the lipase from *Candida antarctica* (CAL-B) has a molecular weight of 33 kDa, with an isoelectric point of 6.0; the 3D structure and aminoacid sequence has been resolved by Uppenberg et al. [10]. CAL-B is one of the most used lipases in biotransformations in many different applications (kinetic resolution of racemic mixtures, desymmetrization reactions, aminolysis, etc.) [11–14]. Novozyme 435 is CAL-B immobilized onto a macroporous acrylic polymer resin (Lewatit VP OC 1600, Bayer) by Novozymes [15], following an unknown protocol. CAL-B had been desorbed from Lewatit VP OC 1600 by incubation with detergents and organic solvents, suggesting that the lipase may be adsorbed on the support mainly through hydrophobic interactions [15c].

We have compared Novozym 435 and CAL-B immobilized on Lewatit VP OC 1600, with CAL-B immobilized in other hydrophobic

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supports of well described properties (agarose coated with butyl or octyl groups and Sepabeads coated with octadecyl groups).

In order to check the specificity and selectivity of these different CAL-B preparations, the effect of these modifications in the kinetic resolutions of a racemic ester: 2-*O*-butyryl-2-phenylacetic acid [(±)-**1**] – precursor of both isomers of mandelic acid (**2**) (Scheme 1) – and the asymmetric hydrolysis of 3-phenylglutaric dimethyl diester (**3**) – precursor of (R)-phenyl glutaric monomethyl ester, key intermediate in the synthesis of different drugs such as a potent HIV inhibitor (Scheme 2) [16] – has been studied.

2. Experimental

2.1. General

Soluble lipase B from *C. antarctica* (CAL-B) and its immobilized form (Novozym 435) were kindly donated by Novozymes (Denmark). Octyl-agarose, and butyl-agarose 4BCL were from Pharmacia Biotech (Uppsala, Sweden). Octadecyl-Sepabeads was from Resindion rsl (Italy). Lewatit VP OC1600 was purchased by Bayer (Leverkusen, Germany). *p*-Nitrophenyl butyrate (*p*NPB) and 3-phenylglutaric acid dimethyl diester were from Sigma. 2-*O*-butyryl-2-phenylacetic acid [(±)-**1**] was synthesized as previously described [17]. NMR data were recorded on a Bruker AC-300 (^{1}H-400 MHz) spectrometer at room temperature.

2.2. Lipase activity determination

The standard activity assay was performed by measuring the increase in absorbance at 348 nm (isobastic point of *p*-nitrophenol) produced by the releasing of *p*-nitrophenol in the hydrolysis of 0.4 mM *p*-nitrophenyl butyrate in 25 mM sodium phosphate at pH 7 and 25 °C, using a thermostatized spectrophotomer with magnetic stirring. To initialize the reaction, 0.1 mL of lipase solution or suspension was added to 2.5 mL of substrate solution. An international unit of *p*NPB activity is defined as the amount of enzyme necessary to hydrolyze 1 μmol of *p*NPB/min (IU) under the conditions described above.

2.3. Lipase desorption

One gram of immobilized lipase preparation was re-suspended in 10 mL of 5 mM sodium phosphate and Triton X-100 was progressively added. The immobilized enzyme was gently stirred for 30 min before measuring the enzyme activities in the supernatant and in the suspension. Then, if necessary, new additions of detergent were performed. References with soluble enzyme submitted to identical treatment were used to determinate the effect of the detergent on the enzyme activity.

2.3.1. Proteins removing from the support from Novozym 435

One gram of Novozym 435 was added to 50 mL of 25 mM phosphate pH 7 containing 2% (v/v) Triton X-100 and incubated for 4 h. Under these conditions, 100% of CAL-B was desorbed from the support, checked by the activity assay described in Section 2.2. The suspension was vacuum dried filtrated and the support was abundantly washed with 2% (v/v) Triton X-100 (5 × 50) and finally with distilled water (10 × 200 mL). This support (called NOVO) was used to immobilize fresh CAL-B in some instances. This support was used as a reference to check if the support was modified by the immobilization procedure utilized by Novozymes.

2.4. Lipase immobilization

Ten grams of support was added to 300 mL of lipase solution (1 mg_{lipase}/mL in 10 mM sodium phosphate) at 25 °C and pH 7 under very mild stirring. The activities of the supernatants and immobilization suspensions were periodically checked by the method described above. After 4 h, the immobilized enzyme was vacuum dried filtrated [18].

2.5. Enzymatic hydrolysis of esters

The activities of the different CAL-B preparations in the hydrolysis of different esters were determined by adding 0.08 g of catalyst in 2 mL solution of 1 mM (±)-**1** in 25 mM sodium acetate at pH 5 and 25 °C; or 0.42 g of catalyst in 6 mL solution of 2 mM of **3** in 25 mM sodium acetate at pH 5 and 25 °C.

During the reaction, the pH value was maintained constant by automatic titration using a pH-stat Mettler Toledo DL50 graphic. The degree of hydrolysis was followed by reverse-phase HPLC (Spectra Physic SP 100 coupled with an UV detector Spectra Physic SP 8450) on a Kromasil C18 (15 cm × 0.4 cm) column supplied by Analisis Vinicos (Spain). At least triplicates of each assay were made. The elution was performed with a mobile phase of acetonitrile (35%, v/v) and 10 mM amonium phosphate (65%, v/v) at pH 2.95. The flow rate was 1 mL/min. The elution was monitored by recording the absorbance at 225 nm (substrate **1**) or 205 nm (substrate **3**).

2.6. Determination of enantiomeric excess

The enantiomeric excess (ee) of the produced acid (**2**) or the formed monoester (**4**) was analyzed by Chiral Reverse Phase HPLC. The column was a Chiracel OD-R. In the case of **1**, the mobile phase was an isocratic mixture of 5% acetonitrile and 95% $NaClO_4$/$HClO_4$ 0.5 M at pH 2.3 and the analyses were performed at a flow of 0.5 mL/min by recording the absorbance at 225 nm. For **3**, the mobile phase was acetonitrile (25%, v/v) and 10 mM ammonium phosphate (75%, v/v) at pH 2.95 and the analyses were performed at a flow of 0.7 mL/min by recording the absorbance at 205 nm.

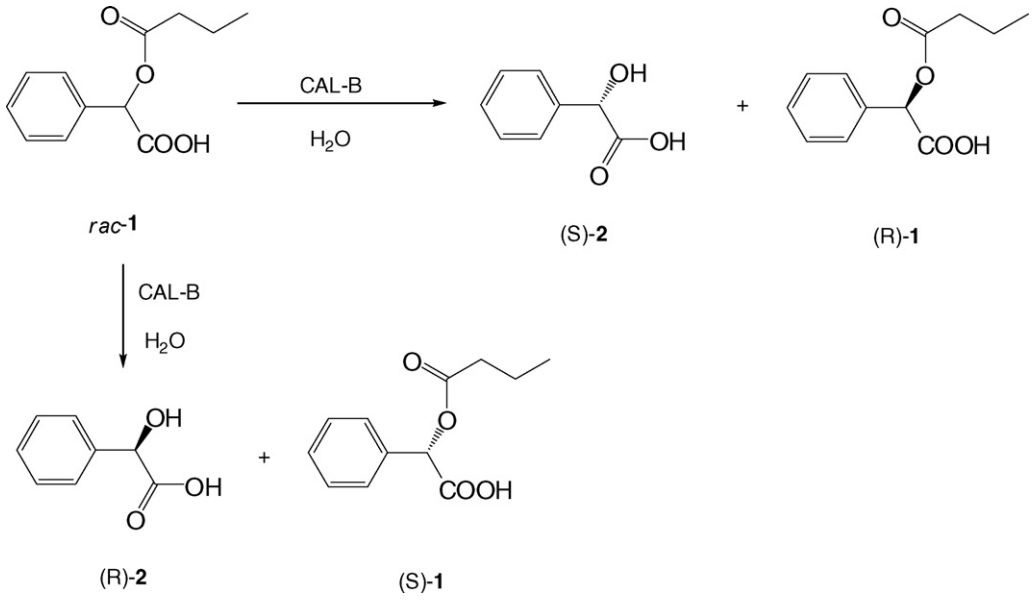
2.7. Calculation of E-value

The enantiomeric ratio (*E*) was defined as the ratio between the percentage of hydrolyzed R and S isomers (from racemic mixture) at hydrolysis degrees between 15 and 20%, where the reaction kinetic is in first order. R-**2** and S-**2** were used as standard enantiomerically pure products.

Also the *E* value was calculated from the enantiomeric excess of the release acid (ee_p) and the conversion degree (*c*) using the equation $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$ described by Chen et al. [19]. Also a third method was applied using the computer program created by Faber [20] and in all cases the results were similar.

2.8. Production of (R)-monomethyl-phenylglutarate [(R)-(+)-**4**]

The dimethyl diester **3** was hydrolyzed using Novozym 435. Fifteen grams of catalyst was added to 50 mL of 10 mM sodium phosphate at pH 5 and 25 °C containing **3** (1.25 mmol, 332.5 mg). After 10 days of reaction, the aqueous solution was filtrated and washed twice with 15 mL of 100 mM sodium phosphate and extracted with ethyl acetate (5 × 50 mL). The collected organic layers were dried over anhydrous Na_2SO_4, which was removed by filtration and concentrated under vacuum (117 mg, 35%). $[\alpha]_D^{20} = +23.0$ (c = 1.5, MeOH). ^{1}H-RMN (400 MHz $CDCl_3$): δ = 7.33–7.20 (m, 5H, Ph); 3.90–3.72 (m, 1H, CH), 3.60 (s, 3H, CH_3), 2.67–2.41 (m, 4H,



Scheme 1. Kinetic resolution of (±)-**1** by different CAL-B immobilized preparations.

$2 \times \alpha CH_2$). The absolute configuration was assigned in agreement with the results of Ostaszewski and co-workers [21].

3. Results and discussion

3.1. Immobilization of CAL-B on different supports

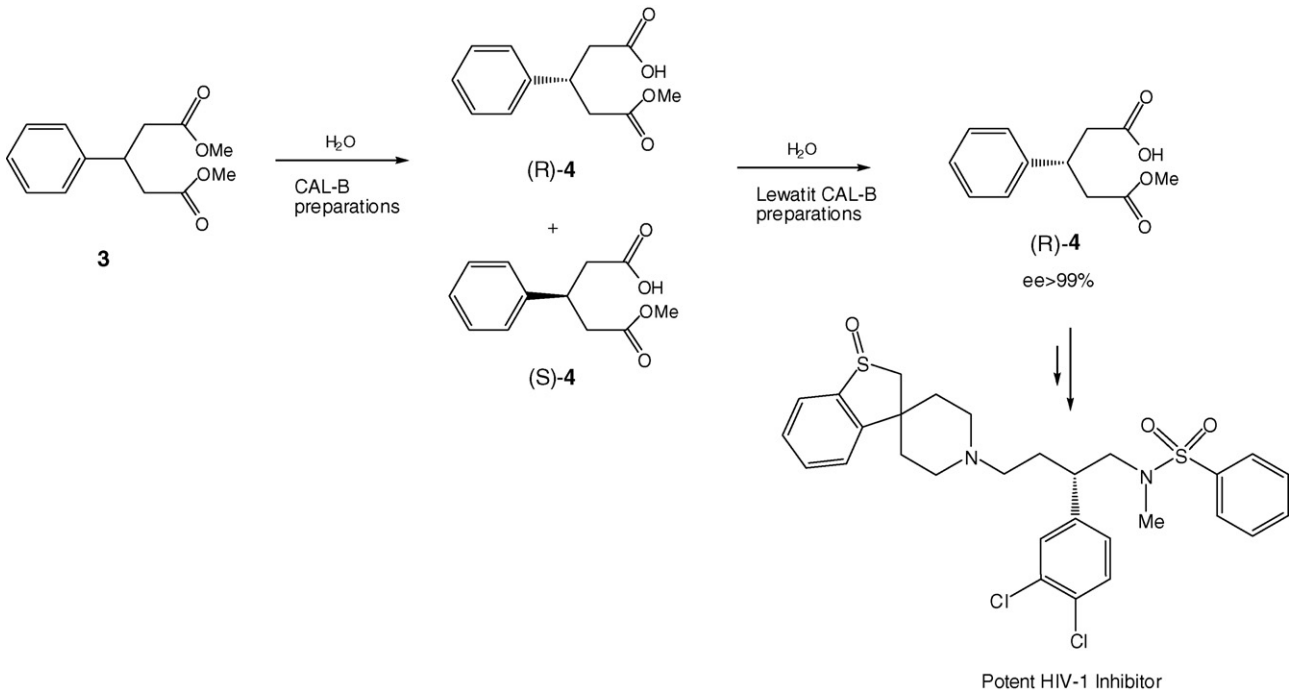
CAL-B was immobilized at low ionic strength on different supports looking for the highest loading of enzyme per gram of support (Table 1).

Using Lewatit or the support NOVO (obtained after desorption of the lipase, see Section 2), 30 mg lipase per gram of support was immobilized.

Table 1
Maximum loading of CAL-B on different supports

Enzyme	Maximum loading[a]
NOVO	30 ± 2
Lewatit	30 ± 2
Octyl-agarose	23 ± 2
Octadecyl-Sepabeads	20 ± 1.5
Butyl-agarose	22 ± 2

[a] mg of lipase/g of support.



Scheme 2. Asymmetric hydrolysis of **3** by different CAL-B immobilized preparations.

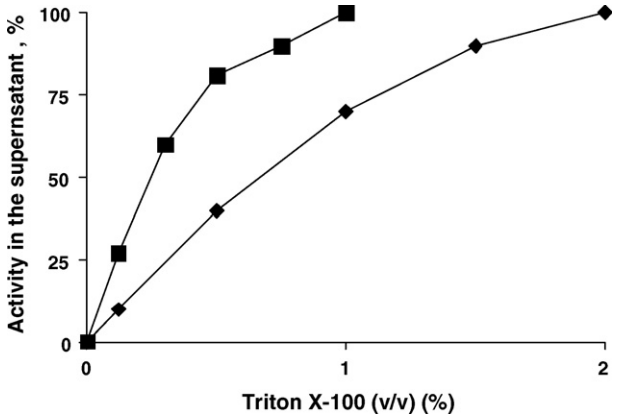


Fig. 1. CAL-B desorption profile using Triton X-100. Octyl-agarose-CAL-B (squares) and Novozym 435 (rhombus).

When butyl-agarose, octyl-agarose or octadedyl-Sepabeads were employed as supports, the maximum enzyme loading in these cases was of 20–23 mg lipase/g support.

3.2. Lipase desorption from the supports

The different CAL-B immobilized preparations were incubated in the presence of Triton X-100, a non-ionic detergent.

The amount of desorbed enzyme increased with the concentration of detergent until all the enzyme became desorbed (Fig. 1 shows the comparison of CAL-B immobilized on octyl-agarose and Novozym 435). CAL-B was completed released (Table 2) from butyl or octyl-agarose using 1% (v/v) of Triton X-100, from Novozym 435 or from Lewatit or the NOVO support using 2% (v/v) and from octadecyl-Sepabeads requiring 4% (v/v) of detergent. Thus, although each support adsorbed the lipase with different strength, this experiment suggested that CAL-B remains immobilized on Lewatit mainly throughout hydrophobic interactions. That is, the enzyme immobilization on this support (at least, in the home-made preparations) could be mainly due to interfacial activation as when using octyl-agarose, butyl-agarose or octadecyl-Sepabeads [22]. Although the immobilization protocol used by Novozymes is unknown, it seems that when enzyme–support hydrophobic interactions were annulled in Novozym 435, the enzyme become desorbed exactly as when Lewatit was used to immobilize the lipase at low ionic strength.

Using all these immobilization protocols, it was possible to purify the lipase [18]. The only protein released from Novozym 435 was also CAL-B (Fig. 2).

Table 2
Concentration of detergent that is necessary to fully release CAL-B from the different supports

Biocatalyst	Triton X-100 (%)
Novozym 435	2
NOVO-CAL-B	2
Lewatit-CAL-B	2
Octyl-agarose-CAL-B	1
Butyl-agaose-CAL-B	1
Octadecyl-Sepabeads-CAL-B	4

Experimental conditions are described in Section 2.

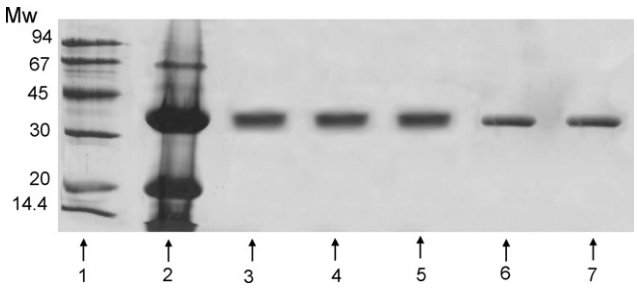


Fig. 2. SDS–PAGE of the CAL-B adsorbed on different hydrophobic supports. Lane 1: molecular weight markers; lane 2: commercial extract crude of CAL-B; lane 3: protein in Novozym 435; lane 4: Lewatit 1600; lane 5: butyl-agarose; lane 6: octyl-agarose; lane 7: octadecyl-Sepabeads.

3.3. Hydrolytic resolution of (±)-2-O-butyryl-2-phenylacetic acid [(±)-*1*] catalyzed by different CAL-B immobilized preparations

The activities and the enantiospecificities of the different CAL-B immobilized preparations in the hydrolysis of **1** at pH 5 and 25 °C are shown in Table 3. The catalytic activity of CAL-B was quite different depending on the support.

Novozym 435 was the most active catalyst, slightly more active than the Lewatit-CAL-B or NOVO-CAL-B preparation, which presented similar activities. The octyl-CAL-B or butyl-CAL-B preparations exhibited six to times lower activity. It should be considered that the CAL-B loading using these last preparations were only around 1.3 lower than using Lewatit, suggesting a different specific activity of the immobilized lipase molecules depending on the support.

The immobilization of CAL-B on the different hydrophobic supports presented in this paper yielded enzymes with very different enantiospecificity (Table 3 and Scheme 1), from an *E* value of around 8 (butyl-CAL-B) up to an *E* value of 49 (octyl-CAL-B). In these cases, the enzyme enantiopreference was towards the R isomer (Scheme 1). Novozym 435 was enantiospecific in the hydrolysis of **1**, with an *E* value >100 (ee > 99%), but surprisingly with an inverse enantiopreference, hydrolyzing exclusively the S-isomer. The same behavior was observed for CAL-B after the immobilization on Lewatit or NOVO support, indicating that Lewatit was the likeliest responsible of this effect.

Thus, depending on the selected support, it was possible to obtain biocatalysts of the same lipase with a high enantiospecificity for one or the other enantiomer of **1** (Scheme 1), being Lewatit the support that gave the most different properties; highest activity and opposite specificity.

The enzyme desorbed from Novozym 435 was diluted (to decrease the detergent concentration) and immobilized on

Table 3
Performance of different CAL-B preparations in the hydrolytic resolution of rac-**1**

Biocatalyst	Activity[a]	*c*[b] (%)	ee_p[c] (%)	SP[d]	*E*
Novozym 435	0.75	12	>99	S	>100
NOVO-CAL-B[e]	0.56	10	>99	S	>100
Lewatit-CAL-B	0.55	10	>99	S	>100
Octyl-agarose-CAL-B	0.19	20	95	R	49
Octadecyl-Sepabeads-CAL-B	0.35	18	90	R	23
Butyl-agarose-CAL-B	0.16	23	72	R	7.5

Experiments were performed at pH 5 and 25 °C.
[a] Activity in μmol g_{cat}^{-1} h^{-1}.
[b] Conversion.
[c] Enantiomeric excess of product.
[d] Stereochemical preference.
[e] Novozym 435 support (Lewatit) after full enzyme desorption was used to immobilize CAL-B at low ionic strength.

Table 4
Asymmetric hydrolysis of **3** catalyzed by different CAL-B preparations

Biocatalyst	Activity[a]	SP[b]	ee[c] (%)	ee[d] (%)
Novozym 435	0.70	R	77	>99
NOVO-CAL-B[e]	0.28	R	80	>99
Lewatit-CAL-B	0.27	R	80	>99
Octyl-agarose-CAL-B	0.5	R	77	77
Octadecyl-Sepabeads-CAL-B	0.1	R	61	61
Butyl-agarose-CAL-B	0.3	R	63	80

Experiments were performed at pH 5 and 25 °C.
[a] Activity in $\mu mol\, g_{cat}^{-1}\, min^{-1}$ calculated at 10–20% conversion.
[b] Stereochemical preference.
[c] Enantiomeric excess of monoester R-**4** calculated at 10–20% conversion.
[d] Enantiomeric excess of monoester R-**4** calculated at 100% conversion (achieved in 1 day) after 10 days of reaction.
[e] Novozym 435 support (Lewatit) after full enzyme desorption was used to immobilize CAL-B at low ionic strength.

octadecyl-Sepabeads. This desorbed and immobilized enzyme gave the same results than the preparation produced using soluble commercial CAL-B. Thus, CAL-B was not modified by the immobilization procedure used by Novozymes in its immobilization, and the changes detected were due to the immobilization protocol (support or conditions).

*3.4. Asymmetric hydrolysis of dimethyl 3-phenylglutarate **3** catalyzed by different CAL-B immobilized preparations*

The hydrolytic activities displayed by the six CAL-B immobilized preparations in the hydrolysis of dimethyl 3-phenylglutarate (**3**) at pH 5 and 25 °C are shown in Table 4.

Novozym 435 was the most active catalyst with 0.70 UI/$g_{catalyst}$ whereas the Lewatit-CAL-B or NOVO-CAL-B preparations were more than 2.5 times less active (Table 4). In the hydrolysis of **1** these three biocatalysts exhibited similar activities.

The octyl-CAL-B preparation was slightly lower active than Novozym 435 while octadecyl-Sepabeads-CAL-B exhibited seven times less activity value (Table 4). Again, there were large differences compared to the results obtained in the hydrolysis of **1** (Table 3).

On the other hand, the enantioselectivity of the different CAL-B preparations were different (Table 4). The Lewatit and NOVO-CAL-B preparations showed an initial enantiomeric excess of 80% towards the monoester R-**4** catalyzing the desymmetrization of **3** (Table 2). Using Novozym 435 or the octyl-agarose-CAL-B preparation, the ee values were quite similar (77%). When the other hydrophobic preparations were used, the observed ee value was lower (61–63%) (Table 4).

Moreover, very interesting results were achieved when the enantiospecificity of the enzyme towards the different isomers of **4** was analyzed. After 100% conversion of **3** to **4** (achieved in 24 h), the hydrolytic reaction was maintained for 10 days following the evolution in the concentration and enantiomeric excess of the monoester.

Novozym 435 and CAL-B immobilized on Lewatit hydrolyzed all S-**4**, and much more slowly R-**4**, to phenylglutaric acid being possible to get R-**4** in ee > 99% with a 55% yield. The reaction course in the asymmetric desymmetrization using Novozym 435 to produce enantiomerically pure product is shown in Fig. 3.

However, using CAL-B immobilized on octyl-agarose or octadecyl-Sepabeads the enantiomeric excess of **4** did not change after this long incubation times, showing that the immobilized enzyme was unable to hydrolyze any of the isomers of the monoester **4**. In opposition, using butyl-CAL-B preparation, the ee of R-**4** increased from 63% up to 80% by using longer reaction times (Table 4).

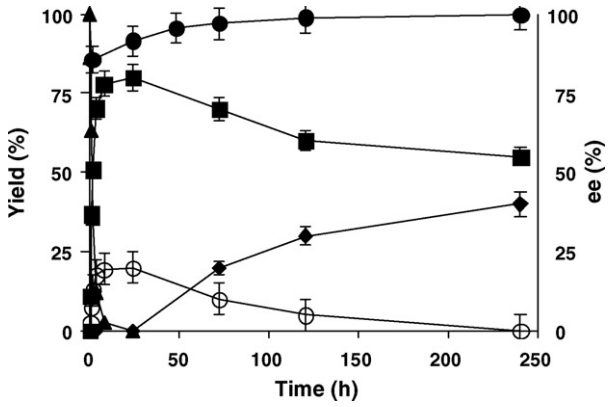


Fig. 3. Reaction course of the enantioselective and enantiospecific production of (R)-**4** catalyzed by Novozym 435. The reaction was performed at pH 5 and 25 °C. Yield of **3** (triangles), (R)-**4** (squares), (S)-**4** (empty squares) and 3-phenylglutaric acid (rhombus). Enantiomeric excess of (R)-**4** (circles).

4. Conclusions

The results presented in this paper show how the immobilization of a lipase on different supports may alter its catalytic features, even if using the same mechanism of immobilization.

Very interestingly, Novozym 435 seems to be prepared using a hydrophobic support that offers some specific properties for the immobilized lipases. CAL-B may be desorbed from Novozym 435 just by incubation in the presence of Triton X-100, pointing that the main mechanism of immobilization of CAL-B in this support may be via hydrophobic interactions. Considering the features of lipases, this means very likely via interfacial activation against the hydrophobic surface of the support. However, the final properties of the immobilized enzyme suggest that the immobilization may involve some other kind of enzyme–support interactions that could not be enough to keep the enzyme immobilized but could be enough to produce changes in the enzyme features. Some properties of Novozym 435 are very different to those of CAL-B immobilized on other hydrophobic supports where only hydrophobic moieties were present in their surface (octyl, butyl or octadecyl groups). Large changes in activity and a spectacular change in enantiopreference in the hydrolysis of **1** are clear examples of these alterations. Immobilization of CAL-B on Lewatit or NOVO support at low ionic strength (via interfacial activation) gave biocatalysts with similar properties suggesting that the support was not modified by Novozymes during the immobilization. Moreover, they behave quite similar to Novozym 435. This suggested that the main reason for the behavior of Novozym 435 is the support and not to the immobilization protocol. In fact, the enzyme desorbed from Novozym 435 gave the same results that the free enzyme.

Although Novozym 435 gave the best results in terms of activity and specificity in the examples shown in this paper, this should not be considered a general rule. Previously, in the hydrolytic resolution of a precursor of Zoplicone, CAL-B immobilized on octadecyl-Sepabeads gave an *E* higher than 100, while Novozym 435 was not selective at all [23]. In this way, it may be stated that the immobilization of lipases on different supports may greatly alter the enzyme properties, but it is difficult that there are an immobilized lipase that have the optimal properties in all the processes and conditions.

On the other hand, some differences between Novozym 435 and CAL-B immobilized on the same support suggest some disparities in the immobilization procedure when Novozym 435 is produced compared to the protocols used in this paper. Results (activities, enantiospecificities or enantiosselectivities) were almost identical comparing commercial Lewatit and the support obtained after CAL-

B desorption (confirming the no modification of the support by Novozymes), but somehow different when compared to Novo 435. Thus, while using compound **1** the activities of the home-made preparations were around 75% of that found using Novozyme 435, using compound **3** the activity was only 40%, suggesting a different specificity of CAL-B in both cases. Moreover, the initial ee of **4** was slightly lower using Novozym 435. Previously, it has been shown that the immobilization conditions, even using the same lipase and the same support, may determine the final catalytic properties of the immobilized lipase, mainly if the immobilization protocol may stabilize the enzyme structure induced by these conditions [24]. The present case could be another example. As long as the exact protocol to produce Novozym 435 is not fully public, it is hard to speculate on the exact protocol of immobilization used in the production of Novozym 435 and the reasons for the difference catalytic properties reached; mainly when the enzyme may be fully desorbed from the support in a similar way that from the other supports.

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References

[1] (a) Q. Jing, R.J. Kazlauskas, Chirality 20 (2008) 724–735;
(b) A. Liljeblad, L.T. Kanerva, Tetrahedron 62 (2006) 5831–5854;
(c) P. Gadler, K. Faber, Trends Biotechnol. 25 (2007) 83–88;
(d) E.E. Jacobsen, L.S. Andresen, T. Anthonsen, Tetrahedron: Asymmetry 16 (2005) 847–850.
[2] (a) K. Faber, Biotransformations in Organic Chemistry, 5th ed., Springer-Verlag, Berlin, 2004;
(b) A. Liese, Andreas, K. Seelbach, C. Wandrey, Industrial Biotransformations, Wiley–VCH, Weinheim, 2006.
[3] L. Brady, A.M. Brzozowski, Z.S. Derewenda, E. Dodson, G. Dodson, S. Tolley, J.P. Turkenburg, L. Christiansen, B. Huge-Jensen, L. Norskov, L. Thim, U. Menge, Nature 43 (1990) 767–770.
[4] (a) L. Sarda, P. Desnuelle, Biochim. Biophys. Acta 30 (1958) 513–521;
(b) A. Aloulou, J.A. Rodriguez, S. Fernandez, D. van Oosterhout, D. Puccinelli, F. Carrière, Biochim. Biophys Acta 1761 (2006) 995–1013.
[5] J.M. Palomo, M.M. Peñas, G. Fernandez-Lorente, C. Mateo, A.G. Pisabarro, R. Fernandez-Lafuente, L. Ramirez, J.M. Guisan, Biomacromolecules 4 (2003) 204–210.
[6] (a) J.M. Palomo, M. Fuentes, G. Fernandez-Lorente, C. Mateo, J.M. Guisan, R. Fernandez-Lafuente, Biomacromolecules 4 (2003) 1–6;
(b) J.M. Palomo, C. Ortiz, M. Fuentes, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, J. Chromatogr. A 1038 (2004) 267–273.
[7] R. Fernández-Lafuente, P. Armisen, P. Sabuquillo, G. Fernández-Lorente, J.M. Guisán, Chem. Phys. Lipids 93 (1998) 185–197.
[8] G. Fernández-Lorente, J.M. Palomo, Z. Cabrera, J.M. Guisán, R. Fernández-Lafuente, Enzyme Microb. Technol. 41 (2007) 565–569.
[9] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisán, R. Fernandez-Lafuente, Enzyme Microb. Technol. 40 (2007) 1451–1463.
[10] J. Uppenberg, M.T. Hansen, S. Patkar, T.A. Jones, Structure 2 (1994) 293–308.
[11] (a) O. Torre, E. Busto, V. Gotor-Fernández, V. Gotor, Adv. Synth. Catal. 349 (2007) 1481–1488;
(b) V. Gotor-Fernández, E. Busto, V. Gotor, Adv. Synth. Catal. 348 (2006) 797–812;
(c) N.M.T. Lourenco, C.A.M. Afonso, Angew. Chem. Int. Ed. 46 (2007) 8178–8181;
(d) K. Bogár, P.H. Vidal, A.R. Alcántara León, J.-E. Backvall, Org. Lett. 9 (2007) 3401–3404;
(e) A.R. Moen, K. Ruud, T. Anthonsen, J. Mol. Catal. B: Enzym. 50 (2008) 74–79;
(f) J.M. Palomo, R.L. Segura, C. Mateo, M. Terreni, J.M. Guisán, R. Fernández-Lafuente, Tetrahedron: Asymmetry 16 (2005) 869–874.
[12] (a) T. Miyazawa, M. Hamada, R. Morimoto, T. Murashima, T. Yamada, Tetrahedron Lett. 49 (2008) 175–178;
(b) A. Baba, T. Yoshioka, J. Org. Chem. 72 (2007) 9541–9549;
(c) A.T. Omori, L.F. Assis, L.H. Andrade, J.V. Comasseto, A.L.M. Porto, Tetrahedron: Asymmetry 18 (2007) 1048–1053.
[13] (a) I. Pérez-Victoria, J.C. Morales, J. Mol. Catal. B: Enzym. 48 (2007) 8–15;
(b) D.E. Stevenson, S.G. Parkar, J. Zhang, R.A. Stanley, D.J. Jensen, J.M. Cooney, Enzyme Microb. Technol. 40 (2007) 1078–1086.
[14] (a) C. Orellana-Coca, J.M. Billakanti, B. Mattiasson, R. Hatti-Kaul, J. Mol. Catal. B: Enzym. 44 (2007) 133–137;
(b) M. Marzorati, K. Hult, S. Riva, B. Danieli, Adv. Synth. Catal. 349 (2007) 1963–1968.
[15] (a) B. Chen, J. Hu, E.M. Miller, W. Xie, M. Cai, R.A. Gross, Biomacromolecules 2 (2008) 463–471;
(b) B. Chen, E.M. Miller, R.A. Gross, Langmuir 23 (2007) 6467–6474;
(c) Y. Mei, L. Miller, W. Gao, R.A. Gross, Biomacromolecules 4 (2003) 70–74.
[16] (a) J.-P. Surivet, J.M. Vatèle, Tetrahedron Lett. 39 (1998) 9681–9682;
(b) P.C. Dorn, P.E. Finke, B. Oates, R.J. Budhu, S.G. Mills, M. MacCoss, Bioorg. Med. Chem. Lett. 11 (2001) 259–264.
[17] J.M. Palomo, G. Fernández-Lorente, J.M. Guisán, R. Fernández-Lafuente, Adv. Synth. Catal. 349 (2007) 1119–1127.
[18] G. Fernandez-Lorente, Z. Cabrera, C. Godoy, R. Fernandez-Lafuente, J.M. Palomo, J.M. Guisan, Process Biochem. 43 (2008) 1061–1067.
[19] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294–7299.
[20] The computer program for calculation of the Enantiomeric ratio (*E*) was obtained in the ftp-server at ftp://borg185.kfunigraz.ac.at/.
[21] A. Fryszkowska, M. Komar, D. Koszelewski, R. Ostaszewski, Tetrahedron: Asymmetry 16 (2005) 2475–2485.
[22] R.L. Segura, L. Betancor, J.M. Palomo, A. Hidalgo, G. Fernandez-Lorente, M. Terreni, C. Mateo, A. Cortes, R. Fernandez-Lafuente, J.M. Guisán, Enzyme Microb. Technol. 39 (2006) 817–823.
[23] J.M. Palomo, C. Mateo, G. Fernández-Lorente, L.F. Solares, M. Diaz, V.M. Sánchez, M. Bayod, V. Gotor, J.M. Guisan, R. Fernandez-Lafuente, Tetrahedron: Asymmetry 14 (2003) 429–438.
[24] R. Torres, C. Ortiz, B.C.C. Pessela, J.M. Palomo, C. Mateo, J.M. Guisan, R. Fernandez-Lafuente, Enzyme Microb. Technol. 39 (2006) 167–171.