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On the inactivity of *Candida antartica* lipase B towards strong acids

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ABSTRACT

Candida antarctica lipase B (CalB, Novozyme 435) was evaluated as catalyst for the conversion of so-called edible acids (e.g. malic and tartaric acid). While transesterification using these acyl donors proceeds smoothly, albeit with low regioselectivity, esterification is hardly catalyzed.

As major reason for CalB inactivation the high acidity of edible acids was identified leading to irreversible inactivation of the biocatalyst. Furthermore, indication exist that all acids exhibiting a pK_a value below 4.8 cause irreversible inactivation of CalB.

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1. Introduction

Enzyme catalysis has become a standard technique within organic chemistry [1–4] with lipases representing the class of biocatalysts being most widely applied [5].

Advantages of enzyme-catalyzed reactions over 'classical' chemical routes not only derive from often high enantioselectivity [4] but also from the mild reaction conditions leading to purer products in more environmentally benign processes [6–8]. We became interested in using immobilized lipase B (CalB) from *Candida antarctica* (Novozyme 435) as catalyst for the preparation of esters of malic and tartaric acid. Such products are used for various purposes, for example as emulsifiers in food (e.g. di-acetyl tartaric ester of monoglyceride (DATEM) and corresponding citric acid analogs) as low-irritating humectants and exfoliants in cosmetics [9,10], as lubricants, and as building blocks for polymers. Compared to classical Lewis or Brønsted acid-catalyzed reactions we envisioned various advantages of enzyme catalysis. On the one hand, reaction temperatures well below those required for efficient chemocatalysis (140–180 °C) should circumvent undesired thermal side-reactions such has dehydratation and self-condensation of the acid. On the other hand, putative regioselectivity of the enzymatic reaction could give access to new products with unprecedented properties (Scheme 1).

To date, regioselective hydrolysis of the sterically less hindered carboxylate group in triethyl citrate [11] as well as regioselective esterification [12] and transesterification [13] of malic acid (esters) has been reported.

2. Experimental

Novozyme 435 was purchased from Novozymes (Bagsvaerd, Denmark), all other chemicals were purchased from Sigma–Aldrich (Munich, Germany) in the highest quality available and used without further purification.

Enzyme-free carrier material was prepared by using Novozyme 435 and desorption of CalB from the carrier: Novozyme 435 was incubated in a 10-fold excess (w/w) of a mixture water/acetonitrile (1:1, v/v) at 60 °C for 1 h, filtrated, washed with water, and dried. The resulting enzyme-free resin exhibited no significant esterification activity using lauric acid and 1-octanol.

2.1. Enzymatic reactions

In all cases mechanic stirring was performed using stirring blades instead of magnetic stirrer bars in order to circumvent enzyme grinding. At intervals, samples were withdrawn and analyzed.

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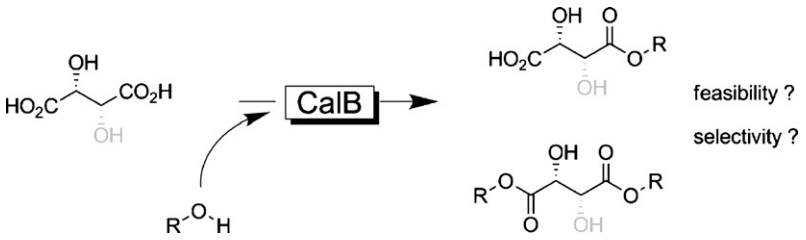

Scheme 1. Use of immobilized CalB (Novozyme 435) as selective catalyst for the conversion of edible acids (tartaric acid and malic acid).

Table 1
'CalB-catalyzed' condensative polymerization of tartaric acid with glycerol using Novozyme 435 and enzyme-free carrier compared to literature reported reactions.

Enzyme preparation	Activity (U g^{-1})	Yield (%)	Oligomerweight (Mn)
Novo 435	10,300	100	1045
Leached Novo 435	54	100	1296
Novo 435 [14,15]	–	100	1100

2.2. Esterification of tartaric acid with glycerol according to the literature [14,15] (Table 1)

Equimolar amounts of tartaric acid, glycerol, and ethanol were heated to 80 °C until a homogeneous solution was obtained. After addition of 5% (w/w) Novo 435 (native (entry 1), or enzyme-free carrier (entry 2)) the pressure was reduced to 250 mbar. After 24 h, pressure was further reduced to 50 mbar followed by an additional stirring for 24 h. Afterwards the reaction mixture was filtered and analyzed by GPC.

2.3. CalB-activity on substituted valeric acids (Fig. 1)

Equimolar mixtures of acid with 1-octanol were stirred at 60 °C in the presence of 0.5% (w/w) Novozyme 435 at ambient pressure and the initial activity was determined as described above.

2.4. CalB-catalyzed transesterifications (Fig. 2)

0.118 mol of acyl donor were dissolved in 90 mL 1-octanol (five-fold molar excess), supplemented with 5% (w/w$_{\text{total mass}}$) Novozyme 435, T = 60 °C, p = 50 mbar, and the initial activity was determined as described above.

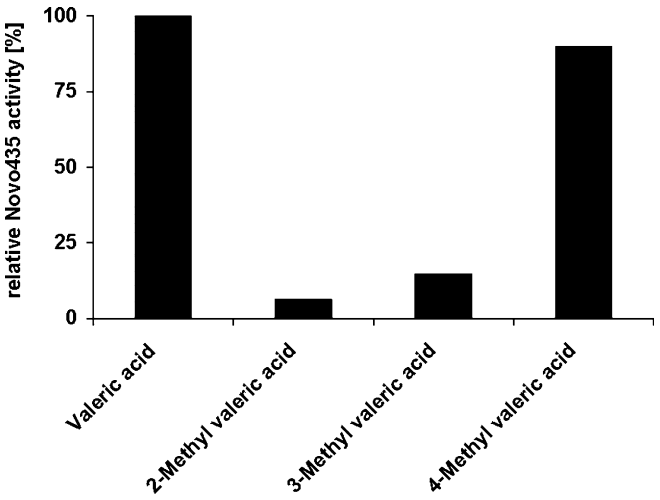

Fig. 1. Influence of the relative position of a methyl substituent in valeric acid on the rate of CalB-catalyzed esterification with 1-octanol. 100% corresponds to a specific Novozyme 435 activity of 4.34 U mg^{-1}.

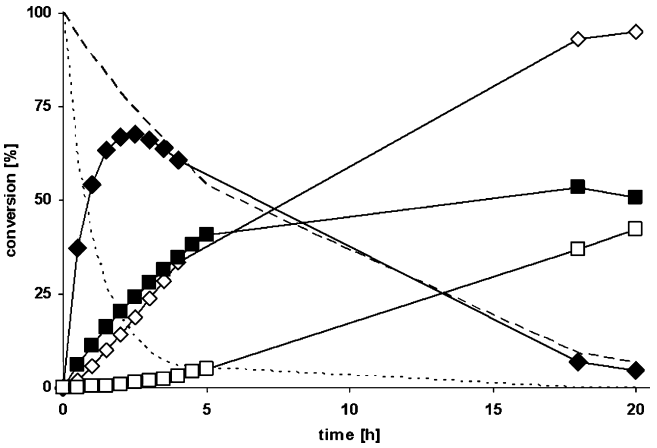

Fig. 2. Transesterification of diethylmalate (diamonds) and diethyltartrate (squares) with 1-octanol using Novozyme 435. Starting materials (– – –, . . .), mono-transesterification products (filled symbols), di-transesterification products (open symbols).

2.5. Influence of tartaric acid concentration on CalB-activity (Fig. 3)

Tartaric acid was dissolved in a mixture containing 288 mmol of lauric acid and 1-octanol each supplemented with 5% (w/w) DMSO at 80 °C. Reactions were started by the addition of 0.722 g Novozyme 435 and the initial activity was determined as described above.

2.6. CalB-activity with various acids (Fig. 4)

Equimolar solutions of the respective acid and 1-octanol were incubated at 65 °C in the presence of 5% (w/w) Novozyme 435 (exception: tartaric and malic acid which were dissolved 0.1 M in

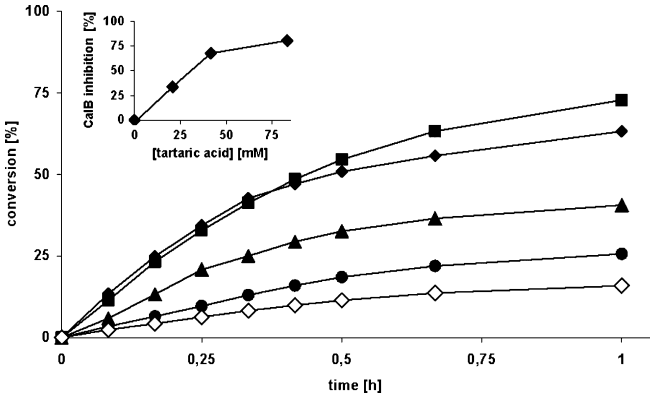

Fig. 3. Influence of tartaric acid (0 mM (♦), 0.4 mM (■), 20.8 mM (▲), 41.6 mM (●), and 83.3 mM (◊)) on the rate of Novozyme 435-catalyzed esterification of lauric acid with 1-octanol. Inset: Extend of CalB-inhibition as determined from initial rates.

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