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Immobilization does not influence the enantioselectivity of CAL-B catalyzed kinetic resolution of secondary alcohols

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Abstract—Decreasing enantioselectivity (*E*-value) by increasing conversion has been observed in transesterification reactions of secondary alcohols catalyzed by a pure protein formulation of lipase B from *Candida antarctica* (Novozym 525 F). Addition of a range of enantiopure alcohols caused a temporary increase in selectivity of the transesterification reaction of 3-chloro-1-phenoxy-2-propanol with vinyl butanoate. The corresponding immobilized lipase B, (Novozym 435) showed a similar relationship between the *E*-value and degree of conversion.

1. Introduction

Previously we have reported that the enantioselectivity (*E*) decreased during esterifications of a range of secondary alcohols **1**–**4** catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435) and that addition of enantiopure (*R*)-alcohols, (*R*)-**1**, (*R*)-**2**, (*R*)-**5**, (*R*)-**6** and (*R*)-**7**, induced an increase in the *E*-value of the esterification of 3-chloro-1-phenoxy-2-propanol **4**.[1] We suggested that the increase in enantioselectivity was caused by inhibition of the slower reacting enantiomer due to an allosteric binding of the enantiopure additive. Enantioselective inhibition of *Candida rugosa* (*cylindracea*) by dextromethorphan and levomethorphan resulting in an enhanced enantioselectivity has been reported.[2] Inhibition experiments revealed that the action of the base was non-competitive inhibition, that is, binding of the base to an allosteric site in the lipase. This caused inhibition of the transformation of one enantiomer leading to increased selectivity. Hydrolysis of 3-acetoxy nitriles catalyzed by lipase PS (*Pseudomonas* sp.) with the addition of L-methioninol showed an increasing hydrolysis rate of the (*R*)-enantiomer and a decreasing hydrolysis rate of the (*S*)-enantiomer. It was suggested that the substrate and L-methioninol were bound to the enzyme at different sites and consequently that conformational changes provided a change of reaction rates.[3] This observation was first reported in 1930 when it was found that strychnine enhanced the human liver esterase catalyzed hydrolysis of methyl L-mandelate but not the D-isomer. These results indicated an allosteric binding of the enantiopure additive.[4,5]

Recently it was reported that the changing *E*-value in esterifications of 4-methyloctanoic acid catalyzed by Novozym 435 was due to substrate sorption into the polymer matrix of the immobilized enzyme.[6] Hence it was interesting to investigate whether immobilization was the reason for the changing *E*-value in our experiments. In addition to the immobilized CAL-B, Novozym 435, we used lipase B from *C. antarctica*, Novozym 525 F that was not immobilized, for comparison.

2. Results and discussion

Esterifications of the alcohols 1-phenoxy-2-butanol **1**, 1-phenoxy-2-pentanol **2**, 3-bromo-1-phenoxy-2-propanol **3** and 3-chloro-1-phenoxy-2-propanol **4** catalyzed by a freeze dried pure preparation of lipase B from *C. antarctica*, Novozym 525 F, with vinyl butanoate as acyl donor, were performed in hexane. The predominantly formed esters and remaining unreacted alcohols are shown in Scheme 1.

As for the reactions with the immobilized CAL-B, Novozym 435[1] the Novozym 525 F that was not immobilized showed a decrease in *E* with conversion (Fig. 1).

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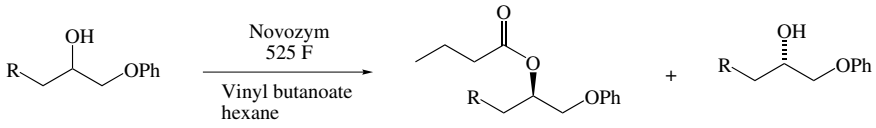


1 R = CH_3
2 R = CH_2CH_3
3 R = Br
4 R = Cl

(*R*)-1a and **(*R*)-2a**
(*S*)-3a and **(*S*)-4a**

(*S*)-1 and **(*S*)-2**
(*R*)-3 and **(*R*)-4**

Scheme 1.

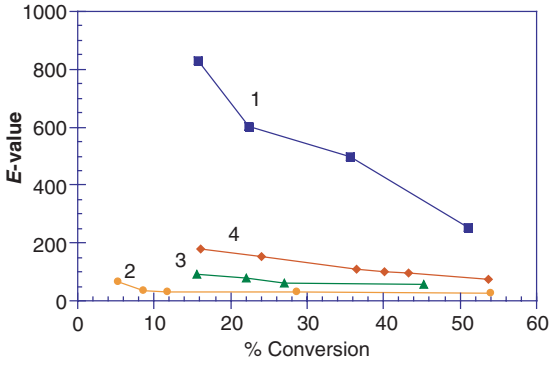


Figure 1. Decrease of enantioselectivity with conversion in the transesterification reactions of **1**–**4** with vinyl butanoate catalyzed by CAL-B Novozym 525 F. (■) = **1**, (●) = **2**, (▲) = **3**, (◆) = **4**.

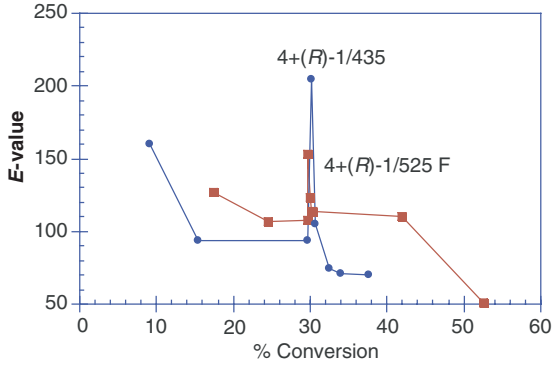


Figure 3. Transesterification of 3-chloro-1-phenoxy-2-propanol **4** catalyzed by CAL-B 435 (●) and CAL-B 525 F (■) in hexane with vinyl butanoate as acyl donor and with addition of (*R*)-**1** at 30% conversion.

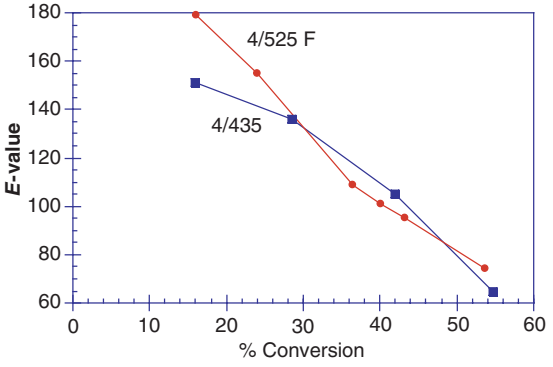


Figure 2. Comparison of the decreasing enantioselectivity (*E*-value) in the transesterification reactions of **4** catalyzed by CAL-B Novozym 525 F (●) and CAL-B Novozym 435 (■).

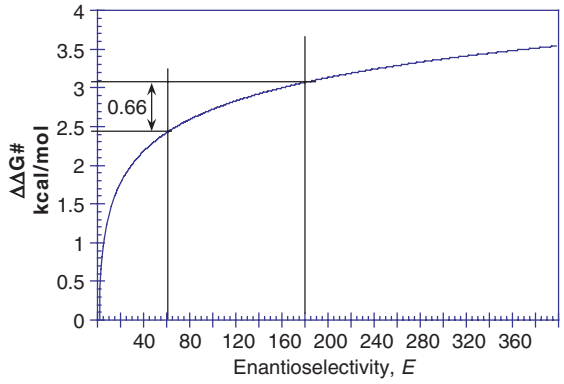
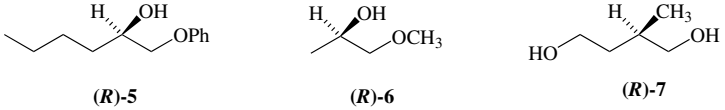


Figure 4. The relationship between the *E*-value and the difference in free energy of activation, $\Delta\Delta G^{\#}$, for the reaction with the two enantiomers ($\Delta\Delta G^{\#} = -RT\ln E$). The exemplified reaction is the transesterification of **4** catalyzed by CAL-B Novozym 525 F (see Fig. 2). The difference in the *E*-value of 180 at an early stage of the reaction (15% conversion) and at the end of the reaction ($E = 62$, 55% conversion) corresponds to the difference in free energy of activation of 0.66 kcal/mol.

The largest effect was observed with **1**, however, it is difficult to measure extremely large values of *E* accurately, hence we chose **4** as the candidate for comparative studies. The results of the esterification of **4** with the two different catalysts are shown in Figure 2. In fact, the graphs relating the *E*-values with conversion were virtually identical for the immobilized and the not immobilized preparations of CAL-B.

Moreover, when enantiopure (*R*)-**1** was added to the Novozym 525 F catalyzed esterification of **4** at 30% conversion, a similar burst of increase in *E*-value was observed (Fig. 3). Also when (*R*)-**2**, (*R*)-**5**, (*R*)-**6** and (*R*)-**7** were used as additives, similar results were obtained.

The drop in enantioselectivity in these reactions corresponds to a change in free energy of activation, $\Delta\Delta G^{\#}$,



(*R*)-5 **(*R*)-6** **(*R*)-7**

for the reaction with the two enantiomers of 0.66 kcal/mol (Fig. 4). The significance of this relatively small number is uncertain. However, it indicates that a small change in enzyme conformation may lead to considerable effects on the selectivity of the enzyme.

3. Conclusions

Resolutions of 1-phenoxy-2-butanol **1**, 1-phenoxy-2-propanol **2**, 3-bromo-1-phenoxy-2-propanol **3** and 3-chloro-1-phenoxy-2-propanol **4** catalyzed by Novozym CAL-B 435 and Novozym CAL-B 525 F both showed a significant decrease in *E*-values by increasing conversion. Addition of the (*R*)-alcohols (*R*)-**1**, (*R*)-**2**, (*R*)-**5**, (*R*)-**6** and (*R*)-**7** at 30% conversion to the resolution of **4** with both enzymes induced a temporary increase in the enantioselectivity of the reactions. It can be concluded that the decrease in *E*-value by increasing conversion in resolutions of **1**–**4** is not due to the immobilization preparation of the lipase B from *C. antarctica* as in Novozym 435 as reported by Heinzman et al. for the esterification of 4-methyloctanoic acid catalyzed by Novozym 435.

4. Experimental

4.1. General

Immobilized lipase B from *C. antarctica* (CAL-B Novozym 435) had an activity of 10 PLU/mg and a water content of 2% w/w. The pure enzyme preparation of lipase B from *C. antarctica* (CAL-B Novozym 525 F) was a water solution with 1–10% protein content. Both enzyme preparations were gifts from Novozymes, Bagsværd, Denmark. Chemicals were purchased from Fluka. Column and flash chromatography were performed using silica gel 60 from Fluka, with pore size 0.0663–0.2000 mm and 0.035–0.070 mm, respectively. Optical rotations were determined using an Optical Activity Ltd. AA-10 automatic polarimeter, concentrations are given in g/100 mL. Chiral analyses were performed using a Varian 3400 gas chromatograph equipped with CP-Chirasil-Dex CB columns from Chrompack (25 m, 0.25 or 0.32 mm i.d., 0.25 μm film density). For syntheses of racemic substrates with NMR data and chromatographic parameters of the resolution products see Refs. 7 and 8. Enantiomeric ratios, *E*, were calculated based on ping-pong bi-bi kinetics using the computer program E&K Calculator 2.1b0 PPC.[9]

4.2. Enzymatic reactions

4.2.1. Transesterification reactions. Substrates **1**–**4** (1.31×10^{-4} mol) and an acyl donor (6.55×10^{-4} mol) were added to hexane (3 mL). The reactions were started by the addition of Novozym 525 F (30 mg) and performed in an Infors shaker incubator at 30 °C. Chiral GLC analyses gave ee_s- and ee_p-values from which the degree of conversion was determined according to $c = ee_s/(ee_s + ee_p)$. In controlled experiments under the reaction conditions without an enzyme, no acylation was observed.

4.2.2. Transesterification reactions with the addition of enantiopure alcohols. Substrate **4** (1.31×10^{-4} mol), an acyl donor (6.55×10^{-4} mol) and Novozym 525 F (30 mg) were added to hexane (3 mL) and performed in the same way as the original reaction of **4** but with the addition of (*R*)-**1** (0.0099 g, 5.96×10^{-5} mol), (*R*)-**2** (0.0097 g, 5.38×10^{-5} mol), (*R*)-1-phenoxy-2-hexanol, (*R*)-**5**, (0.0045 g, 2.33×10^{-5} mol), (*R*)-1-methoxy-2-propanol, (*R*)-**6**, (0.0117 g, 1.30×10^{-4} mol) and (*R*)-2-methyl-1,4-butanediol, (*R*)-**7**, (0.0083 g, 7.97×10^{-5} mol) at approximately 30% conversion.

4.3. Synthesis of enantiopure alcohols

(*R*)-1-Phenoxy-2-butanol (*R*)-**1**: The butanoate of 1-phenoxy-2-butanol **1a**, (0.8691 g, 3.68 mmol) was hydrolyzed by addition of CAL-B Novozym 435 (0.105 g) in phosphate buffer (0.1 M, 183.5 mL). The enantiopure alcohol (*R*)-**1** was separated from the remaining butanoate on silica with acetone/hexane, 2:8, as eluent with a yield of 0.137 g (15.75%), purity 100% (GLC), and an ee of 96%, $[\alpha]_D^{25} = -6.6$ (*c* 1.369, $CHCl_3$).

(*R*)-1-Phenoxy-2-pentanol (*R*)-**2**: The butanoate of 1-phenoxy-2-pentanol **2a**, (1.47 g, 5.89 mmol) was hydrolyzed by addition of CAL-B Novozym 435 (0.20 g) in phosphate buffer (0.05 M, 100 mL). The enantiopure alcohol (*R*)-**2** was separated from the remaining butanoate on silica with acetone/hexane, 3:7, as eluent with a yield of 0.279 g (19%), purity 95% (GLC) and an ee of 99.3%, $[\alpha]_D^{30} = -12.25$ (*c* 1.142, $CHCl_3$).

(*R*)-1-Phenoxy-2-hexanol (*R*)-**5** was synthesized from (*R*)-phenyl glycidyl ether as described in Ref. 7. The yield was 0.630 g (65.5%) with a purity of 100% (GLC) and an ee higher than 99% $[\alpha]_D^{25} = -5.55$ (*c* 0.90, $CHCl_3$).

(*R*)-1-Methoxy-2-propanol (*R*)-**6** and (*R*)-2-methyl-1,4-butanediol (*R*)-**7** were purchased from Fluka.

4.4. Determination of absolute configurations

The absolute configurations of the faster reacting enantiomers of **1**–**3** were determined by comparisons of the specific rotation and of the retention times on GLC with (*R*)-**1**, (*R*)-**2** and (*S*)-**3** synthesized by a two-step procedure from (*R*)-phenyl glycidyl ether made from (*S*)-epichlorohydrin and phenol.[10,11] The synthesized enantiopure alcohols had the following properties: (*R*)-**1** ee >99%, $[\alpha]_D^{25} = -6.4$ (*c* 1.40, $CHCl_3$), (*R*)-**2**: ee >99%, $[\alpha]_D^{20} = -6.9$ (*c* 1.17, $CHCl_3$) and (*S*)-**3** ee = 96%, $[\alpha]_D^{22} = +5.3$ (*c* 1.71, EtOH). The absolute configuration of **4** was not determined directly, but assigned by comparing relative retention times on chiral GLC supported by the known enantiopreference of CAL-B.

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