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Enhanced selectivity in Novozym 435 catalyzed kinetic resolution of secondary alcohols and butanoates caused by the (*R*)-alcohols

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Abstract—In esterifications of secondary alcohols catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435) the *E*-values decreased during the reaction. Hydrolysis of the corresponding butanoates showed the opposite effect. When an enantiopure (*R*)-alcohol, related but different, was added to the transesterification reaction, the *E*-value was significantly enhanced.

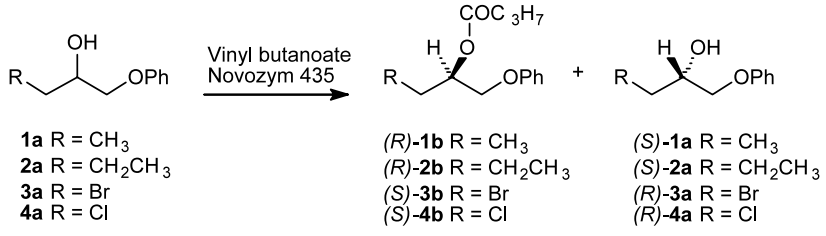
The enantiomeric ratio *E*, or often termed the enantioselectivity in kinetic resolutions, is the relative rate of reaction of the two enantiomers. It is related to the difference in free energy of activation $\Delta\Delta G^{\#}$ and to the ratio of the specificity constants k_{cat}/K_M for the enantiomers in enzyme catalyzed reactions.

$$\Delta\Delta G^{\#} = -RT\ln E \qquad E = \frac{\left(\frac{k_{cat}}{K_M}\right)_R}{\left(\frac{k_{cat}}{K_M}\right)_S}$$

The *E*-value can be calculated by measuring the enantiomeric excess of the product fraction ee_p, and the remaining substrate fraction ee_s, at a certain degree of conversion.[1,2]

$$+E = \frac{\ln\frac{[ee_p(1-ee_s)]}{(ee_p+ee_s)}}{\ln\frac{[ee_p(1+ee_s)]}{(ee_p+ee_s)}}$$

Provided that $\Delta\Delta G^{\#}$ is not influenced during the reaction, *E* will remain constant.[3] We have discovered that the *E*-value can increase by increasing degree of conversion in lipase catalyzed kinetic resolutions by hydrolysis of secondary esters. We suggested that the deviation from the theory might be due to change of the reaction medium during the reaction. The medium, taken in a wider sense, not only consists of the solvent, but also of the reactants and products present in the solvent. During lipase catalyzed hydrolysis the concentration of the



Scheme 1. Kinetic resolutions of (**1a**–**4a**) by transesterification of vinyl butanoate catalyzed by Novozym 435, lipase B from *Candida antarctica* (CAL-B). The faster reacting enantiomer is the same with respect to relative size of the groups at the stereocenter, however, the (*R*)- and (*S*)-notation changes when going from **1a** and **2a** to **3a** and **4a** and the corresponding esters.

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faster reacting enantiomer, usually the (*R*)-ester, is decreasing and the (*R*)-alcohol is formed and its concentration is increasing.[4,5] Consequently the medium is changing. The significance of the solvent for the *E*-value has been discussed.[6] Similar effects in esterifications catalyzed by *Pseudomonas cepacia* have been observed in chlorinated solvents.[7] Since each single product was shown not to cause the effect, it was suggested that complex interactions in the medium or on the enzyme between substrate and solvent and two or more products, improve enzyme selectivity. Inhibition of CAL-B by different primary alcohols has also been reported.[8] It has been reported that enantioselectivity increased when acetone was added to the medium when CAL-B was used to hydrolyse 3-chloro-1-phenylmethoxy-2-propyl butanoate. Addition of co-solvent solvates the liberated (*S*)-alcohol thus preventing the effect of inhibition.[9] It has been reported that strychnine enhances the hydrolysis of methyl L-mandelate catalyzed by human liver esterase and not the D-isomer. The results indicate an apparent allosteric activation.[10] Enantioselective inhibition of *Candida rugosa* (*cylindracea*) by dextromethorphan and levomethorphan has also been reported.[11]

We have now expanded the range of substrates in order to elucidate the nature of the effect of the changing *E*-value. Esterification of the secondary alcohols (**1a**–**4a**) (Scheme 1) and hydrolysis of their corresponding butanoates (**1b**–**4b**) catalyzed by Novozym 435, have been carried out. Synthesis, analysis and spectroscopic data of **1a**, **2a**, **1b** and **2b** have been described,[12] this also applies to **3a**, **4a**, **3b** and **4b**.[13]

E-values for each degree of conversion were determined using the method devised by Rakels et al.[2] The results for esterification are shown in Figure 1 and for hydrolysis in Figure 2. The esterifications of the alcohols **1a**–**4a** applying a transesterification reaction with vinyl butanoate as acyl donor all showed a decrease of selectivity ranging from 27 to 57%, when the reactions were monitored up to 65% conversion.

When the butanoates **1b**–**4b** were hydrolyzed in phosphate buffer up to 50% conversion, an increase of *E*-value ranging from 18% (**4b**) to 90% (**3b**) was observed (Fig. 2).

Our first hypothesis concerning these observations, that the *E*-value was decreasing during esterification and increasing during hydrolysis, was that the effect was connected to the changing concentration of the enantiomeric esters. When enantiopure (*R*)-**1b**, the faster reacting enantiomer, was added to the transesterification reaction of 1-phenoxy-2-pentanol (**2a**), no effect on *E* was observed. The same negative result was obtained when enantiopure (*R*)-**4b**, the slower reacting enantiomer, was added to the esterification of 1-phenoxy-2-butanol (**1a**). We therefore concluded that the effect of the changing *E*-value had to be connected to the changing concentration of the alcohol. In order to verify this we planned the following experiment: (a) monitor an esterification reaction of a racemic alcohol; (b) at a certain degree of conversion, add enantiopure (*R*)-alcohol, produced in a

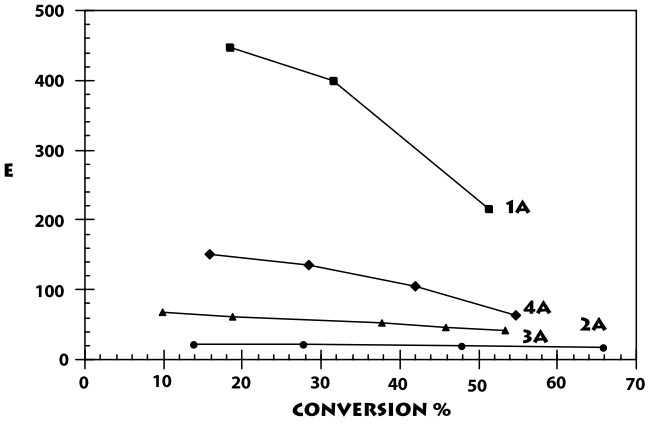

Figure 1. *E*-values at different degrees of conversion in esterifications of **1a**–**4a**.

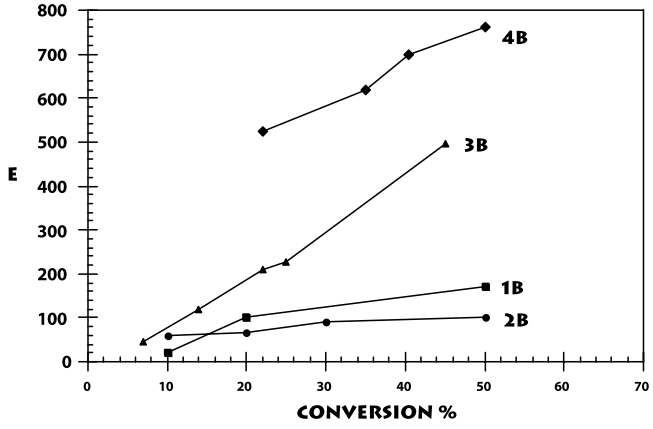

Figure 2. *E*-values at different degrees of conversion in hydrolysis of **1b**–**4b**.

different resolution reaction, and observe an increase of the *E*-value. However, analysis and *E*-value calculations would be influenced by the added (*R*)-alcohol and we therefore decided to add a different, but structurally related (*R*)-alcohol. We monitored carefully the esterification of **4a** and observed a decrease of *E* from 160 at 9% conversion to 94 at 30% conversion (Fig. 3). How-

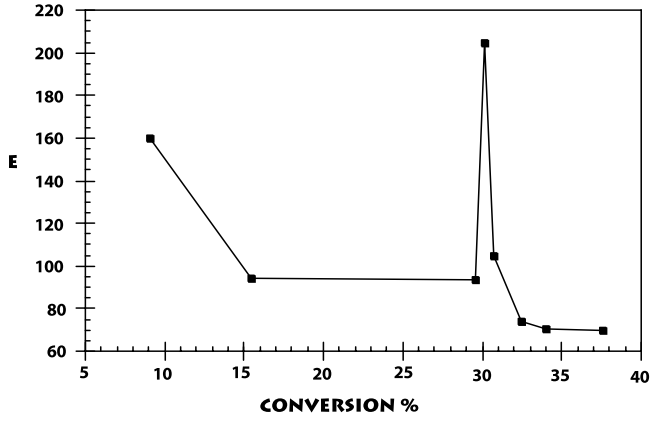

Figure 3. *E*-values at different degrees of conversion during transesterification of **4a**, (*R*)-**1a** was added at 30% conversion and the *E*-value increased from 94 to 205.

ever, when (*R*)-**1a** was added to the transesterification reaction at 30% conversion the *E*-value increased from 94 to 205 (Fig. 3). It is remarkable that the *E*-value dropped quickly.[14] We assumed that the reduction of the effect was due to (*R*)-**1a** being esterified under these conditions to give (*R*)-**1b**.

Addition of (*R*)-**2a** and (*R*)-phenyl ethanol gave similar results, however with smaller increase of *E*. In a final experiment we added (*R*)-1-phenoxy-2-hexanol as a possible selectivity enhancer. The results were almost identical to what is shown in Figure 3. However, it was surprising, that the *E*-value also in this case dropped to the low level quickly after addition. We have previously shown that 1-phenoxy-2-hexanol is esterified by vinyl butanoate and CAL-B very slowly and with almost no selectivity.[12] Hence, it seems unlikely that the disappearance of the selectivity enhancement, was due to esterification of the alcohol. It is more reasonable to believe that (*R*)-1-phenoxy-2-hexanol immediately after addition is bound to the enzyme, and after a short while brought into the bulk solution by the solvent. The observed quick drop of effect after addition may therefore be a combination of removal of (*R*)-alcohol by esterification or by solvation.

Since addition of the enantiopure (*R*)-alcohols increased the selectivity of CAL-B, it is likely that it is causing a conformational change in the enzyme, possibly due to an allosteric effect. Further studies towards the mechanism of this activation effect on various CAL-B preparations are in progress.

Acknowledgements

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