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Water Activity Influences Enantioselectivity in a Lipase-Catalysed Resolution by Esterification in an Organic Solvent

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Abstract: The enantioselectivity of *Candida rugosa* lipase-mediated esterification of 2-methylalkanoic acids with *n*-alcohols in cyclohexane is dependent on water activity.

Lipase-catalysed resolutions in organic solvents has emerged as one of the most versatile methods for the preparation of enantiomerically pure compounds.[1] We have studied the enzyme-catalysed resolution of 2-methylalkanoic acids by esterification (see *Fig. 1.*) in alkane solvents using commercial *Candida rugosa* lipase (*CRL*).[2]

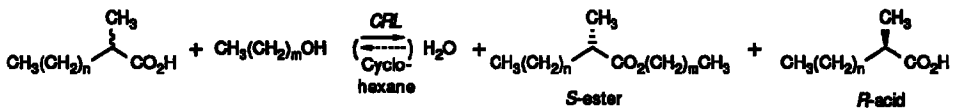

Fig. 1. Resolution of 2-methylalkanoic acids by esterification in cyclohexane catalysed by *Candida rugosa* lipase.

Recently it has been established that the reaction rates of some enzyme-catalysed reactions in organic solvents are influenced by the water content in the reaction medium.[3] A constant water activity (a_w) is desirable for good reproducibility.[4] The simplest way to maintain a constant water activity throughout the reaction is to perform the esterification in an organic solvent in the presence of a binary inorganic salt mixture containing, for example, an anhydrous salt and the corresponding hydrated salt.[5]

How the water content of the reaction medium influences the enantioselectivity of the enzyme (expressed as the enantiomeric ratio, *E*) has been documented in only a few cases.[3c,6] The *E*-values registered when using, for example, lipases from *Pseudomonas* seem to be independent of water activity.[6f,h] However, when 2-haloacids were esterified using *CRL* in organic solvents different enantioselectivities were obtained if varying amounts of water were added to the reaction mixtures.[3c,6c]

When studying *CRL*-catalysis, we were puzzled by the fact that after extended storage of the enzyme, the enantioselectivity seemed to decrease in esterifications of 2-methylalkanoic acids in apolar solvents, whereas it remained constant in hydrolysis reactions of the corresponding esters in water. Thus, the use of enzyme powder that had been stored for some months at 4° and in the original container in a desiccator over dried silica gel, led to considerably lowered reaction rates as well as enantiomeric ratios, *E*, in esterification reactions in cyclohexane as compared with the use of freshly delivered enzyme. An obvious explanation for this observation is that the stored enzyme was too dry.

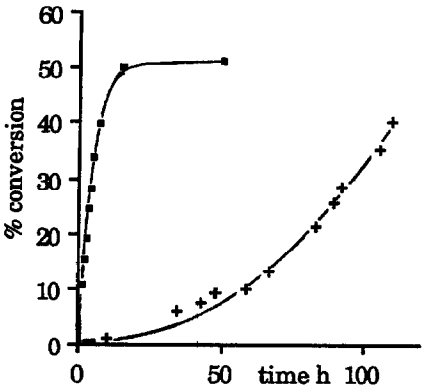

Fig. 2. Rate profile for the *CRL*-catalysed esterification of *rac*-2-methyloctanoic acid with *n*-tetradecanol ▪ : with added Na_2SO_4 / $Na_2SO_4 \cdot 10\ H_2O$ and + : without added salts.

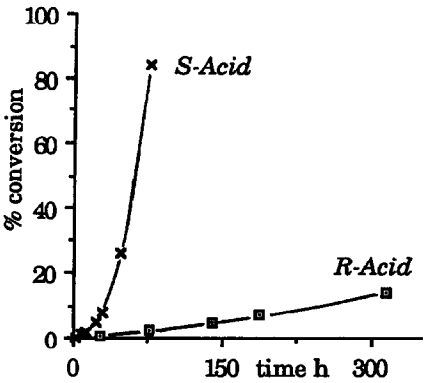

Fig. 3. *CRL*-catalysed esterification of × : (*S*)-(87.3 % *ee*) and □ : (*R*)-2-methyloctanoic acid (97.4 % *ee*) with *n*-dodecanol in cyclohexane without added salts.

We now wish to report our results obtained from the esterification of 2-methyloctanoic acid with an excess of *n*-do- and *n*-tetradecanols, respectively, using the same batch of stored *CRL* as the catalyst in cyclohexane at 25° without or with either added anhydrous sodium hydrogen phosphate / sodium hydrogen phosphate dihydrate or anhydrous sodium sulphate / sodium sulphate decahydrate mixture. These salt mixtures are known to maintain water activities of a_w=0.15 and 0.76 respectively.[7]

The resolutions were performed as follows: the acid (3.75 mmol) and the alcohol (22.5 mmol) and an internal standard were dissolved in cyclohexane (25 ml). In some experiments salt mixtures of either Na_2HPO_4 / $Na_2HPO_4 \cdot 2H_2O$ (equimolar amounts, 1.3 g) or Na_2SO_4 / $Na_2SO_4 \cdot 10\,H_2O$ (equimolar amounts, 1.8 g) was present. After stirring (10 min) the crude enzyme powder (Sigma, stored as mentioned above, 1.69 g, corresponding to 1.5×10^6 units) was added. The mixture was stirred in a closed flask at ambient temperature and samples were withdrawn at intervals. Conversions were determined by gas chromatography. The enantiomeric excesses of the esters produced were determined by reduction ($LiALH_4$) to the 2-methyl-1-alkanol followed by oxidation to the acid and conversion to the amide with (*S*)-1-phenylethylamine and GC-analyses of the diastereomeric mixtures on a Carbowax-type column.[2b] Contrary to direct alkaline hydrolysis - acidification of the esters, the reduction - oxidation protocol does not lead to any detectable racemization of the resultant acid.[2b]

The esterification reaction of racemic 2-methyloctanoic acid and *n*-tetradecanol catalysed by a stored sample of *CRL* without added salt shows a rate profile (see *Fig. 2.*) with an induction period before the reaction rate increases. The apparent *E*-values[8] measured in two separate experiments from *ee* of the product esters were 23 ± 2 at < 35 % conversion. However, if the salt mixture of sodium sulphates was present, the rate profile is much more linear up to close to 40 % conversion (see *Fig. 2.*). In this case the *E*-values determined at <35 % conversion were 91 ± 10 in two separate experiments.

The ratios between the initial rates for the pure enantiomers determined separately may in some cases differ from the *E*-values determined from the racemate using the same conditions.[9] However, below we have used these ratios as rough approximations of the *E*-values and assumed a linear relationship between these and the enantiomeric composition of the substrate.

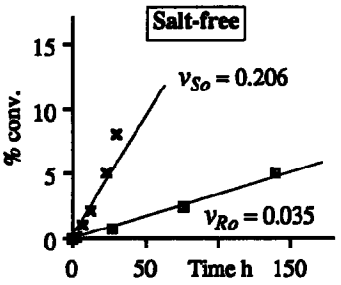

Fig. 4 Initial stages of *CRL*-catalysed esterifications of × : (*S*)- (87.3 % *ee*) and □: (*R*)-2-methyloctanoic acid (97.4 % *ee*) with *n*-dodecanol in cyclohexane without added salts.

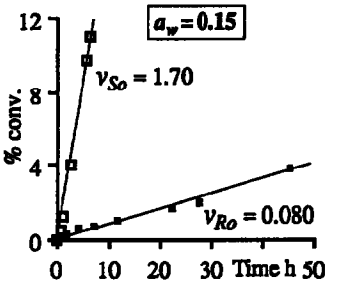

Fig. 5 Initial stages of *CRL*-catalysed esterifications of □ : (*S*)- (87.3 % *ee*) and ▪ : (*R*)-2-methyloctanoic acid (99.8 % *ee*) with *n*-dodecanol in cyclohexane with added $Na_2HPO_4/Na_2HPO_4{\cdot}2H_2O$.

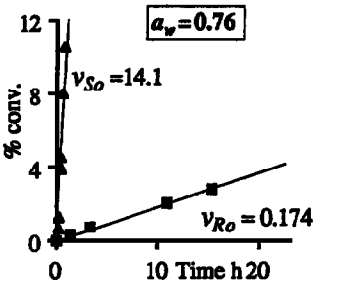

Fig. 6 Initial stages of *CRL*-catalysed esterifications of ▲ : (*S*)- (73.1 % *ee*) and ■ : (*R*)-2-methyloctanoic acid (99.8 % *ee*) with *n*-dodecanol in cyclohexane with added $Na_2SO_4/Na_2SO_4{\cdot}10H_2O$.

Using stored enzyme and salt-free conditions, the individual enantiomers of 2-methyloctanoic acid were esterified with *n*-dodecanol and the rate profiles obtained are described in *Figs. 3* and *4*. At conversions below 6 %, straight lines were observed which enabled calculation of the initial rates v_{So} and v_{Ro} (% *conversion / h*). From v_{So} and v_{Ro} the estimated rates $v^{100}{}_{So}$ and $v^{100}{}_{Ro}$ for (*S*)- or (*R*)-2-methyloctanoic acid of 100 % *ee* respectively can be calculated assuming that $v_o = v^{100}{}_{So}\cdot(1\text{-}x)+v^{100}{}_{Ro}\cdot x$, where x represents the initial proportion of the *R*-acid. The enantiomeric ratio at the starting point, E_0, was then roughly approximated by the initial rate ratio $v^{100}{}_{So} / v^{100}{}_{Ro} = 7 \approx E_0$. As seen in *Fig. 3*. not only do the rates for both enantiomers above 6 - 10 % conversion increase, but also the rate ratio v_S / v_R; *i. e.* the *E*-value increases as the salt-free reactions proceed, probably due to increased a_w since water is produced in the esterification. When esterifying racemic acid using salt-free conditions and fresh enzyme, an apparent E = 40 at ≈ 40 % conv. (from *ee* of the product[8]) is registered, which should be the integrated result of a lower *E* at the start compensated by a higher *E* at 40 % conversion.

In contrast to the salt-free case, experiments with the same stored enzyme batch performed under identical conditions except for the presence of salt mixtures gave linear plots over wide conversion ranges (for *S*-acids up to at least 50 %; the reactions with *R*-acids were linear at least up to the point of interruption ≈ 10 %). Thus the phosphate salt mixture of low water activity (a_w = 0.15; *Fig. 5.*) led to $v^{100}{}_{So} / v^{100}{}_{Ro} = 23 \approx E_0$ whereas the sulphate salt mixture of high water activity (a_w = 0.76; *Fig. 6.*) resulted in a $v^{100}{}_{So} / v^{100}{}_{Ro} \approx 95 \approx E_0$. (both E_0-values calculated as described above). A similar *E*-value (83 ± 10) was determined[2b] for the esterification of dodecanol and *rac*-2-methyloctanoic acid by end point determination from *ee* of the product[8] at ≈ 30 % conversion using freshly delivered enzyme, without prior storage.

Even if the true *E*-values for esterification of the racemic acid should differ from the initial rate ratios between the individual enantiomers measured under otherwise identical conditions, we expect the dependance on water activity to be very similar for the rate ratios and the *E*-values. Thus, we have demonstrated that, at least for the *CRL*-enzyme system studied here it is very important to control the water activity not only, as earlier noted, for obtaining high reaction rates but also for maximum enantioselectivity. Further studies are under way.

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