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Enantioselectivity of lipases: effects of water activity

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Abstract

The enantioselectivity (E) of lipases in esterifications of secondary alcohols with decanoic acid was studied in organic media. The enantioselectivity of 2-octanol differed greatly among the lipases used. *Candida antarctica* lipase was extremely selective ($E = 9\ 000$) while *Candida rugosa* lipase was much less selective ($E = 1.7$). Other enzymes (Lipozyme and lipases from *Pseudomonas* and *Rhizopus arrhizus*) had intermediate selectivities. In all cases the enantioselectivity for an enzyme was unaffected by changes in water activity. Different methods of determining the enantioselectivity was used: reactions using single enantiomers as well as racemic mixtures. The effect of water activity on enantioselectivity and the enanatioselectivity values themselves were similar irrespective of the method used. The enantioselectivity of other alcohols were also found to be unaffected by the water activity. The enantioselectivity of *Pseudomonas* lipase was influenced by the organic solvent. The E decreased with increasing hydrophobicity, from 62 in acetonitrile to 40 in toluene and 33 in hexane. In none of these cases was the enantioselectivity affected by the water activity. However, for Lipozyme and *Candida rugosa* lipase in toluene a trend of increased E with increasing water activity was observed. In summary it can be stated that the water activity does not generally affect the enantioselectivity of the five lipases tested.

Keywords: Lipases; Water activity; Enantioselective esterification; Alcohol resolution

1. Introduction

Enzyme catalysis in organic media is greatly influenced by the environment of the enzyme. The organic solvent as well as the water content in the reaction medium affect the rate of catalysis. Much work has been devoted to investigations of how enzymes and enzyme activities are influenced by these parameters [1]. After the enzyme activity the enantioselectivity is one of the most important characteristics of enzymes. The biocatalyst itself and the reaction conditions can influence the measured enantioselectivity. The effect of solvents on enantioselectivity has been described in several reports and sometimes good correlations have been achieved between the obtained results and physico-chemical parameters of the solvent. However in some cases the enantioselectivity is shown to increase with, for instance, the log P [2] and in others the relationship is inverse [3,4]. Nevertheless, adequate explanations to the results can be obtained in each separate case. The observations are therefore quite limited to the particular enzymatic reaction studied. In a recent review Carrea and coworkers point out the lack in the

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literature of correlation of E to any solvent parameter [5].

The water activity will determine the amount of water associated with the enzyme. The hydration level of the enzyme is greatly influencing the flexibility of the protein and thereby the catalytic activity. Low hydration leads to a less flexible enzyme, which is normally less active. How this fact will influence the enantioselectivity of the enzymes is not completely known. Some reports say that a flexible enzyme has less possibility to select between the different enantiomers. An enzyme with more conformational flexibility, has relaxed discrimination of the two enantiomers [6]. However, a stiffer enzyme molecule might be hindered to accommodate the fast reacting enantiomer more than the slow reacting enantiomer, thereby the enzyme would loose enantioselectivity at low hydration. These arguments are similar to those used in order to explain the effect of different solvents on the enantioselectivity. In the literature there are reports showing that increasing water activity (content) will increase the enantioselectivity [7,8] but in some cases the enantioselectivity decreases [9,10] or is unaffected by additions of water [11]. However one should bare in mind that these investigations were performed with different types of enzymes and substrates (alcohols, acids). The reported discrepancies can therefore be expected if for instance the effect on the selectivity of the acid or alcohol is studied in a lipase catalyzed reaction. An effect on the acid substrate does not have to be similar when the alcohol is studied. There are reports that show that the concentration or structure of the other (nonchiral) substrate influence the measured enantioselectivity [12,13].

The methods of measuring the enantioselectivity differ in the literature. The most commonly used technique is to use a racemic substrate and measure the reaction yield and enantiomeric excess of substrate and/or product after certain reaction times. The enantioselectivity (E) can be determined from the equations of Chen and coworkers [14,15]. However, these determinations are based on two single, separate measurements and the errors in the estimation of E can be assumed to be high if not multiple samples/analyses are conducted. The E value is assumed to be constant throughout the progress of the reaction. There are indications that this is not always true [16,17]. The main reasons for this behaviour are effects of reversibility of the reactions which decrease the enantioselectivity with time. Another method of determining the enantioselectivity is to calculate the ratio of the specificity constants (k_{cat}/K_m) of the two enantiomers in two separate reactions [14]. A third method is to measure the enzymatic activity in reactions with substrates of varying enantiomeric ratios [16]. The two last techniques require a least one of the two enantiomers in a purer form.

The present study deals with the influence of water activity on the enantioselectivity of different lipases in an esterification reaction of chiral alcohols. A similar study has been reported where the enantioselectivity of *Pseudomonas* lipase in a transesterification reaction was shown to be independent of the water activity, between 0.1 and 0.5 [11]. The aim of our study is to systematically document the effect of different parameters such as method of analysis, reaction conditions and lipase source on the water activity dependence of the enantioselectivity of enzymes.

2. Materials and methods

2.1. Chemicals

2-(+)Octanol, 2-(−)octanol and n-decanoic acid (capric acid) (99%) were purchased from Sigma Chemical Co. (St. Louis, USA). Racemic 2-octanol was obtained from Kodak Co. (New York, USA). DL-sec-phenethyl alcohol (98%) came from Janssen Chimica (Beerse, Belgium) and acetonitrile from Labscan (Dublin, Ireland). Toluene, n-hexane and racemic 2-nonanol (99%) were obtained from Merck (Darmstadt, Ger-

many). $R(+)$-1-phenylethyl isocyanate was purchased from Fluka Chemie AG (Buchs, Switzerland). Polypropylene powder EP100 (200–400 μm), was a gift from Akzo (Obernburg, Germany). Other chemicals were of analytical grade.

2.2. Lipases (E.C. 3.1.1.3)

Lipase from *Candida rugosa* (formerly *Candida cylindracea*) was obtained from Sigma Chemical Co. (St. Louis, USA). Immobilized lipase SP 435 (Novozyme) from *Candida antartica* and Lipozyme IM 20 were donated by Novo Nordisk A/S (Bagsværd, Denmark). Lipase PS from *Pseudomonas sp.* came from Amano Pharmaceutical Co. (Nagoya, Japan). Lipase from *Rhizopus arrhizus* (lipase 80,000) was a gift of Gist-Brocades S.A. (Delft, The Netherlands).

2.3. Enzyme adsorption

Solid support, polypropylene powder, (1 g) was mixed with 3 ml ethanol. Lipase (1 g) (from *Candida rugosa, Pseudomonas sp.* or *Rhizopus arrhizus*) was dissolved in 10 ml sodium phosphate buffer (20 mM, pH 6.0), and undissolved material was removed by centrifugation. The enzyme solution was added to the support material and incubated at room temperature on a head over head incubator for 24 h. The preparation was filtered and washed with water. One ml phosphate buffer (200 mM, pH 7.0) was added to the preparation, followed by vacuum drying at 10 mm Hg overnight.

2.4. Water activity equilibration

Enzyme preparations and reaction media were separately equilibrated through vapour phase in sealed containers with saturated salt solutions of known water activity. The salts used were LiBr (water activity, a_w 0.06), LiCl (a_w 0.11), $MgCl_2.6H_2O$ (a_w 0.33), $Mg(NO_3)_2.6H_2O$ (a_w 0.53), NaCl (a_w 0.75) and K_2SO_4 (a_w 0.97). Equilibration was performed overnight (at least 16 h) at room temperature.

2.5. Enzyme reactions

The lipase-catalyzed esterification of decanoic acid (40–400 mM) and alcohols (10–100 mM) in different organic solvents was performed in 5 ml containers with screw caps and teflon-lined septa. The reaction was started when the enzyme preparation (5–100 mg) was added to the pre-equilibrated reaction medium (4 ml). The reactions were performed at 25°C on a shaker, 180 rpm. The reactors were placed horizontally and shaken along their length to fully suspend the enzyme preparations. Samples (50 μl) were withdrawn at different stages of conversion. The samples were analyzed by gas chromatography and the initial rates in the experiments were calculated.

2.6. Gas chromatography

The ester formation was followed using a Shimadzu gas chromatograph (GC-14A) equipped with a flame ionization detector and a column (i.d. 2.6 mm, length 2.1 m) packed with GP10% SP-216-PS on Supelcoport (Supelco, Bellefonte, USA). Helium was used as carrier gas (35 ml/min). The temperature of the injector was 200 and 240°C of the detector. The initial temperature of the column was 110°C for 2 min then the temperature increased at a rate of 20°C/min up to 170°C. Standard curves of substrates and product were obtained to calculate response factors. The conversion was calculated from the amounts of the ester and alcohol peaks.

The enantiomeric excess (ee) of the remaining alcohol was determined using the same gas chromatograph equipped with a fused silica capillary column DB-210 (0.326 mm, 30 m) from J&W (Folsom, USA). The temperature of the injector was 100°C, the detector temperature was 250°C and the column temperature was 210°C. The alcohol was derivatized with an

optically pure isocyanate. The samples (50 μl) from the reactions were mixed with $R(+)$-1-phenylethyl isocyanate (4 μl) and dry toluene (100 μl) and left overnight at room temperature. Dry ethanol (10 μl) was added to react (30 min) with the excess of reagent. The samples were diluted with 200 μl hexane and analyzed by GC.

2.7. Determination of E (enantioselectivity)

2.7.1. By measurement of apparent kinetic parameters

The alcohol concentration was varied between 10 and 100 (400) mM (in some cases) and the acid concentration was kept constant at 100 (400) mM. The apparent kinetic constants, V_{max} and K_m, were deduced by fitting the experimental data of initial activity directly to the Michaelis–Menten equation by using non-linear regression calculated by Kaleidagraph® (for Macintosh). E was calculated from the apparent specificity constants for the two separate enantiomeric alcohols.

$$E = \frac{(V_{max}^{-}/K_m^{-})}{(V_{max}^{+}/K_m^{+})} \tag{1}$$

2.7.2. By measurement of initial reaction rate of single enantiomers

The specificity constants from Eq. (1) can be assumed to be proportional to the initial reaction rates. This is valid if the apparent K_m of the two enantiomers are equal or if the K_m are much higher than the substrate concentration. E was calculated from the ratio of the initial reaction rates of two separate reactions with the two alcohol enantiomers.

$$E = \frac{\text{rate of 2-}(-)\text{octanol}}{\text{rate of 2-}(+)\text{octanol}} \tag{2}$$

2.7.3. By measurements of reactions of racemic mixtures:

The method described by Chen et al. was used [14]. Eq. (3) was rearranged with conversion (c) as an explicit function of enantiomeric excess (ee_s) with the enantioselectivity (E) as a parameter. The data of conversion and enantiomeric excess (6 to 10 points) were fitted to this new equation and E was determined by a computer software, Kaleidagraph® (for Macintosh).

$$E = \frac{\ln((1-c)(1-ee_s))}{\ln((1-c)(1+ee_s))} \tag{3}$$

3. Results and discussion

3.1. Enantioselectivity measured with single enantiomers

In a first set of experiments the enantioselectivity was determined by performing experiments with single enantiomers and calculating the enantioselectivity (E) from the ratio of the reaction rates. The reaction rate of Lipozyme decreases as the water activity is increased (Fig. 1a). The shape of the profiles for the two enantiomers are similar, though the absolute values differ greatly. A racemic mixture of 40 mM 2-octanol (20 mM each of (+) and (−) 2-octanol) was also used and the reaction rate was the same as the sum of the reactions of single enantiomers. This indicates that there is no negative influence of the slow reacting enantiomer on the rate of the other enantiomer. The ratio of the reaction rates (equal to E) from the two single enantiomer experiments was independent of the water activity (Fig. 1b). The E value was around 90–110 for the water activities studied.

Similar experiments were performed with *Candida rugosa* lipase (CRL) using single enantiomers (Fig. 2a). The shape of the activity profiles are different from the ones of Lipozyme but similar for the three different experiments with CRL. This shows that the water activity profiles can be very different for different enzymes. However this difference depends mainly on effects of apparent V_{max} and K_m of the systems and not on effects on the enzyme itself

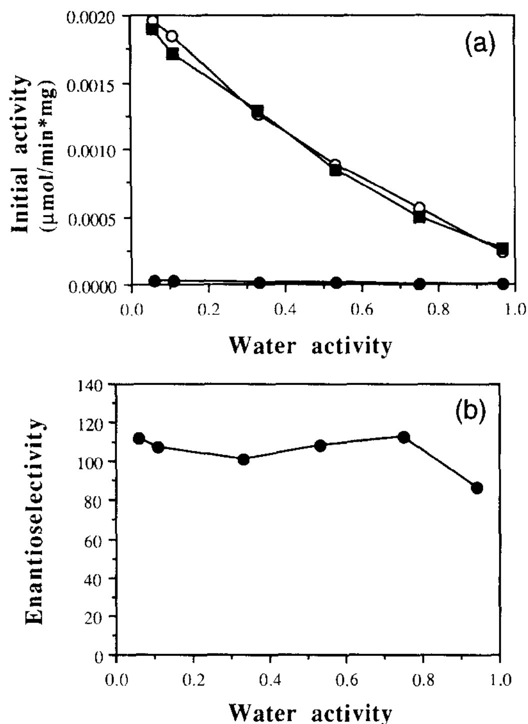

Fig. 1. (a) Lipozyme catalyzed esterification of 2-octanol in hexane. The reaction rate was measured using 100 mM decanoic acid and 20 mM 2-(−) octanol (○), 20 mM 2-(+) octanol (●) or 40 mM of a racemic mixture (■). (b) Enantioselectivity of Lipozyme as a function of water activity. The enantioselectivity was calculated as the ratio of the reaction rates of 2-(−) octanol and 2-(+) octanol in (a), according to Eq. (2).

[18]. The absolute reaction rates are more similar in the experiments with CRL indicating that the two enantiomers are more equal in being accepted as substrates to the enzyme. The E value, calculated as the reaction rate ratio, is approximately 1.4–1.7 and independent of water activity (Fig. 2b). The rate of the racemic mixture (total 40 mM) became approximately 26% less than the rate of the sum of the 20 mM 2-(+)-octanol and 20 mM 2-(−)-octanol reactions. This can be expected if the substrate concentration is in the range of the apparent K_m, which in the case of CRL is approximately 24 mM (as will be shown later in Table 1). When the reactions were studied at 5 mM of the enantiomers the reduction in rate for the racemic (total 10 mM) compared to the sum of the enantiomers were less, 11%. The rates of single enantiomers are only equal to the rate of racemic mixture if the total concentration is below the apparent K_m, if not reduction in rate is observed due to increased substrate saturation of the enzyme, that is the rate is approaching V_{max}.

The enantioselectivity of lipases from *Pseudomonas* and *Candida antarctica* was also shown to be unaffected by the water activity as shown for the first two tested lipases (Fig. 3). The *Pseudomonas* lipase in this reaction had an average enantioselectivity value of 33. The *Candida antarctica* lipase is extremely selective, E was approximately 9000. The rate of the 2-(+)-octanol was low and difficult to measure (yet reliable results were obtained). Because of these practical difficulties this enzyme preparation was not used as much in the following

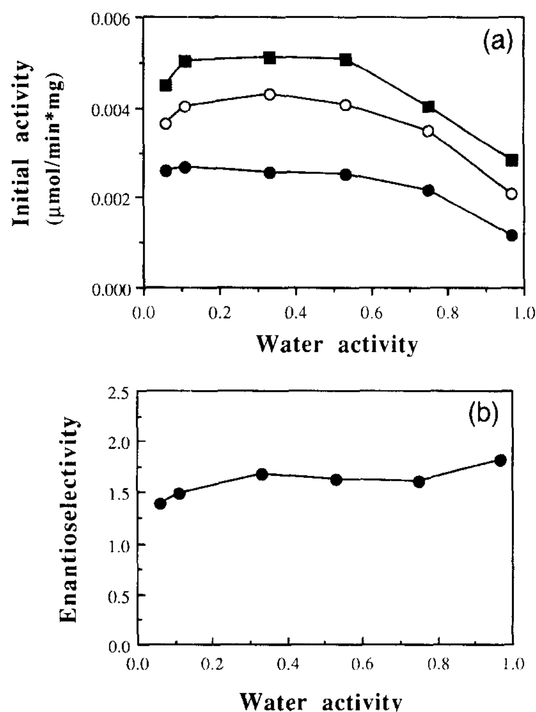

Fig. 2. (a) *Candida rugosa* lipase catalyzed esterification of 2-octanol in hexane. The reaction rate was measured using 100 mM decanoic acid and 20 mM 2-(−) octanol (○), 20 mM 2-(+) octanol (●) or 40 mM of a racemic mixture (■). (b) Enantioselectivity of *Candida rugosa* lipase as a function of water activity. The enantioselectivity was calculated as the ratio of the reaction rates of 2-(−) octanol and 2-(+) octanol in (a), according to Eq. (2).

Table 1
Apparent kinetic parameters for 2-octanol in esterification with decanoic acid using different lipase preparations. The reactions were performed in hexane at water activity 0.33. The enantioselectivity was calculated from the specificity constants, Eq. (1)

Enzyme	Acid conc. (mM)	2(−)-Octanol		2(+)-Octanol		E
		V_{max} (μmol/min × mg)	K_m (mM)	V_{max} (μmol/min × mg)	K_m (mM)	
Lipozyme	400	8.3×10^{-3}	210	8.7×10^{-5}	250	115
Candida rugosa	400	1.2×10^{-2}	70	6.2×10^{-3}	90	2.4
Candida rugosa	100	2.2×10^{-3}	24	1.4×10^{-3}	24	1.6
Pseudomonas	100	1.0×10^{-2}	80	4.1×10^{-4}	100	32

experiments. For practical purposes, such as synthesis of products of high optical purity this enzyme is ideal.

3.2. Reactions using mixtures of enantiomers

The reaction rate in experiments performed with various ratios of the two enantiomers were determined. If the apparent K_m for the enantiomers is similar the obtained rate should be proportional to the molar fraction of the enantiomers, provided no other interfering effect is present. For Lipozyme one can see in Fig. 1a that the two enantiomers don"t seem to influence the rate of each other. In these new experiments the total concentration of 2-octanol was kept constant at 20 mM and the ratio of the enantiomers was varied (Fig. 4). The dependence for Lipozyme is almost a straight line and this fits with the earlier observations. The rate of the 2-(+)-octanol is very low, since the enantioselectivity is around 100. A similar dependence was found for *Candida rugosa* lipase (Fig. 4). In this case the reaction rates for the enantiomers are similar because the enantioselectivity was around 1.6. *Pseudomonas* lipase also showed a similar behaviour. The data for these three preparations were plotted as described by Jongejan and coworkers; $(1/V_S - V_R)$ as a function of the inverse of the molar ratio $(1/X)$ [16]. The enantioselectivity can be calculated from the slope and the results gave E's of 1.49, 35 and 78 for *Candida rugosa* lipase, *Pseudomonas* lipase and Lipozyme, respectively. These values correlate well with the

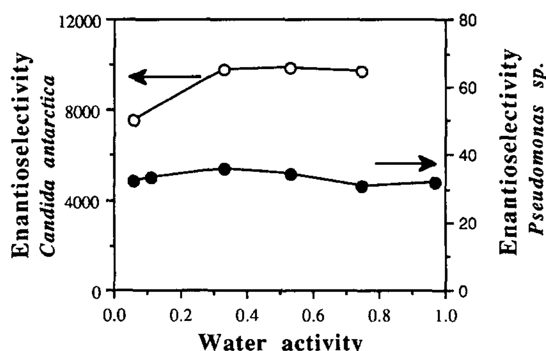

Fig. 3. Enantioselectivity of lipases as a function of water activity in hexane. The enantioselectivity of *Pseudomonas* (●) and *Candida antarctica* lipases (○) was calculated from the ratio of reaction rates for 20 mM 2-(−) octanol and 20 mM 2-(+) octanol, according to Eq. (2).

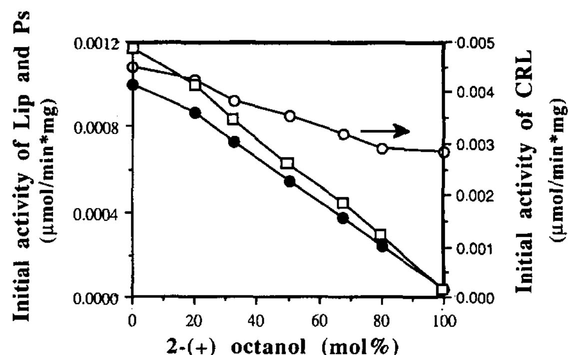

Fig. 4. Reaction rates in mixtures of enantiomers of 2-(−) octanol and 2-(+) octanol. Lipozyme (●), *Pseudomonas* lipase (□) and *Candida rugosa* lipase (○) were studied in hexane at water activity 0.33. The total concentration of alcohol was kept constant at 20 mM and the concentration of decanoic acid was 100 mM.

values determined in previous experiments. There seems to be no negative influence of the enantiomers on the reaction rates. This demonstrates the generality of this phenomenon.

3.3. Effect of substrate concentration on enantioselectivity

The influence of the substrate concentrations on the enantioselectivity of lipases were studied. The alcohol concentration was varied from 10 to 100 mM and in this case the acid concentration was kept constant at 100 mM. The reaction rates for the two different enantiomers increase with increasing alcohol concentrations according to Michaelis–Menten kinetics. However, the enantioselectivity (ratio of reaction rates) for *Candida rugosa* lipase was approximately 1.6 and unaffected by the concentration used. Similar effect was noticed for *Pseudomonas* lipase yielding an E of 30–31. On the other hand, the acid concentration influences not only the reaction rate but also the enantioselectivity of *Candida rugosa* lipase (Fig. 5). Increasing acid concentration (from 10 to 400 mM), with a constant alcohol concentration of 100 mM gave an increasing enantioselectivity, from 1.1 to 2.2. There are reports of enantioselective inhibition by nonchiral substrates on the action of lipases [13]. However in that study it was the concentration of the nonchiral alcohol which influenced the enantioselectivity for the acid moiety and the E decreased with increased concentration. Another reason might be an effect on the pH in the reaction system at increasing acid concentrations. The reaction rate profiles show a dependence on substrate concentration which looks like a substrate inhibition profile with a relatively larger effect on the 2-(+)-octanol.

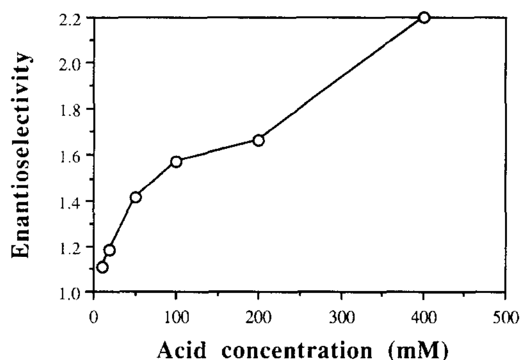

Fig. 5. Enantioselectivity of *Candida rugosa* lipase as a function of decanoic acid concentration. The alcohol concentration was 20 mM. The enantioselectivity was calculated from the reaction rates of 2-(−) octanol and 2-(+) octanol according to Eq. (2).

3.4. Calculation of enantioselectivity from apparent V_{max}/K_m data

The kinetic parameters were determined for the two enantiomers at water activity 0.33. The reaction follows Michaelis–Menten kinetics and the kinetic constants are presented in Table 1. Because of the ping-pong bi-bi mechanism of these enzymes the absolute values of the kinetic constants will be dependent on the concentration of the other substrate, lower concentrations will give reduced apparent V_{max} and K_m [18,19]. The apparent K_m for the two enantiomers are almost identical for the enzymes used. This has indirectly been reported previously [13]. In another study 2–3 fold differences in the apparent K_m between the enantiomers have been observed, being higher for the slow reacting species [20]. The apparent V_{max} differs significantly in most cases. Estimations of the enantioselectivity (E) from the ratio of specificity constants (V_{max}/K_m) are very similar to the one determined in the single enantiomer experiments, for Lipozyme 115, to be compared with 90–110. The results show that it is the V_{max} (catalytic rate) and not the K_m (binding) which predominantly influences the enantioselectivity [20]. Other reports have also verified that the apparent K_m for two enantiomers is similar though the enantioselectivity is high [13].

3.5. Enantioselectivity determined in racemic mixtures

In most studies in the literature the enantioselectivity is determined from reactions using

racemic mixtures of the enantiomers by the methods described by Chen et al. [14,15]. Most substrates are not available as pure enantiomers so this method is more commonly used than the single enantiomers method. Often the enantioselectivity is determined by Eq. (3) using single determination at one conversion. However there is a risk of errors in the determinations and comparison of data is difficult. To be able to compare results from different reactions and reaction conditions these single point determinations of the enantioselectivity must be representative for the reaction. The esterification reaction studied is reversible and therefore the second method described by Chen et al. should be used [15]. The equilibrium conversion using 20 mM 2-octanol and 100 mM decanoic acid is 97.3%, giving a K of 0.028, which according to the results of Chen et al. give plots which are similar to those of irreversible reactions, except at very high conversions. The first published, simpler, Eq. (3) was used in the determinations, since the conversions were not that high.

The enantioselectivity for *Candida rugosa* lipase was determined at six water activities. The yield of ester and the optical purity of the substrate were measured in a series of samples from the reactions and the results were modelled by a computer program. As in the case of single enantiomer reaction there was no influence of the water activity on the enantioselectivity of *Candida rugosa* lipase. The enantioselectivity was between 1.6 and 1.8 in all reactions

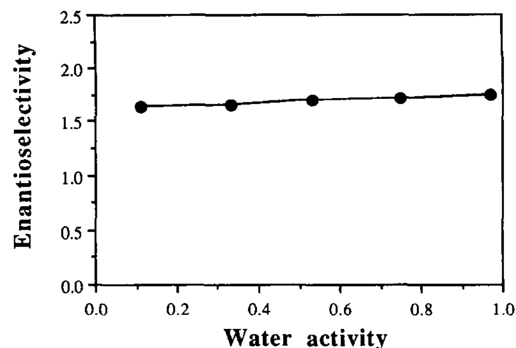

Fig. 6. Enantioselectivity of *Candida rugosa* lipase in hexane at different water activities. The enantioselectivity, studied in reactions using 20 mM racemic 2-octanol and 100 mM decanoic acid, was determined from data of conversion and enantiomeric excess of the remaining alcohol according to the Chen et al. method, Eq. (3) [14]. The standard deviations in the determinations were between 0.01 and 0.02.

(Fig. 6). This is similar to the enantioselectivity determined earlier (Fig. 2b, Table 1). The same method was used to study the enantioselectivity of different lipases at two water activities (0.33 and 0.75) (Table 2). For all lipases studied the water activity did not affect the measured enantioselectivity. The values of E are lower than the values determined by the single enantiomer method for all enzymes except for CRL. This is especially obvious for *Candida antarctica* lipase, the E decrease from 9000 to 40. The enantioselectivity for Lipozyme decreased from 100 to 20 and for *Pseudomonas* the values were 33 and 15, respectively. In these cases the method of measuring the enantioselectivity

Table 2
Activity and enantioselectivity of lipases in esterification of 40 mM racemic 2-octanol and 100 mM decanoic acid in hexane at two water activities, 0.33 and 0.75. The results were analyzed according to the Chen et al. method, Eq. (3) [14]. The standard deviations in the determinations of E are given in parantheses

Enzyme	Enzyme activity		Enantioselectivity	
	a_w 0.33 (μmol/ min × mg prep.)	a_w 0.75 (μmol/ min × mg prep.)	a_w 0.33	a_w 0.75
Candida rugosa	2.6×10^{-3}	1.7×10^{-3}	1.74 (0.04)	1.83 (0.02)
Rhizopus arrhizus	1.3×10^{-4}	0.8×10^{-4}	8.9 (0.6)	7.7 (0.6)
Pseudomonas sp.	7.0×10^{-4}	8.9×10^{-4}	15.2 (1.2)	14.9 (0.8)
Lipozyme	6.3×10^{-4}	2.7×10^{-4}	20.6 (1.7)	22.0 (1.5)
Novozyme	1.2×10^{-1}	1.1×10^{-1}	40.8 (2.6)	39.2 (2.5)

seems to influence the E value, why this happens is not known. However, the important conclusion is that the E is not changed with varying water activity.

3.6. Effects of solvent on enantioselectivity

All the preceding experiments were conducted in hexane. To test the generality in the findings other solvents were tested. Three lipase preparations were tested in toluene by the single enantiomer method: Lipozyme, *Candida rugosa* and *Pseudomonas* lipases. The reaction rates were approximately 3–4 times lower than in hexane. The enantioselectivity values were similar to the ones obtained in hexane (Fig. 7a). There is a minor tendency that the values were slightly higher in toluene than in hexane. There is also a trend that the enantioselectivity for Lipozyme and *Candida rugosa* lipase increase with increasing water activity. This effect was not seen for *Pseudomonas* lipase, whose enantioselectivity was unaffected by the water activity as was seen for all lipases in hexane.

The reaction was also studied in a water miscible solvent, acetonitrile. Of the enzymes tested only the *Pseudomonas* lipase expressed sufficient activity in this solvent. The enantioselectivity of this lipase was measured in acetonitrile at four different water contents (Fig. 7b). The water contents used were 0.5, 2, 5 and 10% (by vol.) which correspond to water activities of 0.10, 0.35, 0.52 and 0.65, respectively. The enzyme activity was slightly lower than the activity in hexane. The enantioselectivity was

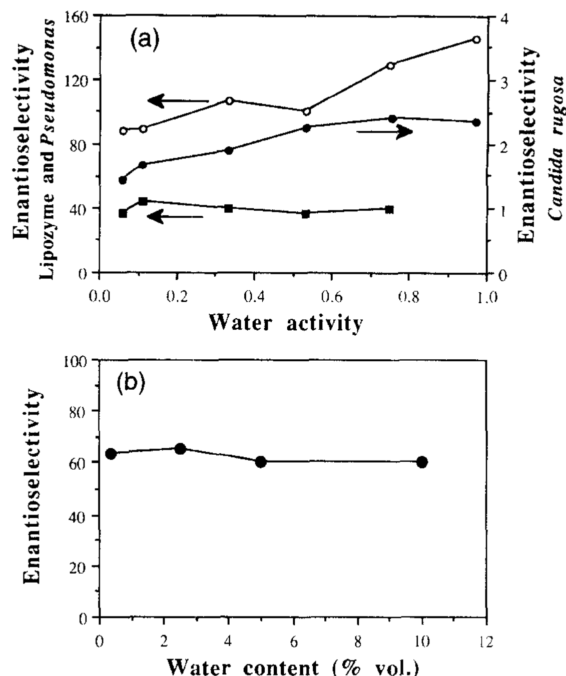

Fig. 7. (a) Enantioselectivity of Lipozyme (○), *Candida rugosa* (●) and *Pseudomonas* (□) lipases in toluene at different water activities. (b) Enantioselectivity of *Pseudomonas* lipase in acetonitrile at different water contents.
The enantioselectivity was calculated from the ratio of reaction rates for 20 mM 2-(−) octanol and 20 mM 2-(+) octanol using 100 mM decanoic acid, according to Eq. (2).

constant at the different water activities. The E value was around 60, which is higher than the one obtained in hexane and toluene. For comparison to other reports one can say that in the present study the enantioselectivity decreased with increasing hydrophobicity of the solvent (acetonitrile, toluene and hexane, gave E values of 62, 40 and 33 respectively). The number of

Table 3
Activity and enantioselectivity for other alcohols in hexane at two water activities, 0.33 and 0.75. The results were analyzed according to the Chen et al. method, Eq. (3) [14]. The standard deviations in the determinations of E are given in parantheses

Enzyme	Alcohol	Enzyme activity		Enantioselectivity	
		a_w 0.33 (μmol/ min × mg prep.)	a_w 0.75 (μmol/ min × mg prep)	a_w 0.33	a_w 0.75
Lipozyme	Phenethylalc.	2.7×10^{-3}	1.4×10^{-3}	6.2 (0.1)	6.1 (0.3)
Candida rugosa	Phenethylalc.	2.9×10^{-3}	2.0×10^{-3}	8.4 (0.4)	7.8 (0.3)
Pseudomonas sp.	Phenethylalc.	1.1×10^{-2}	1.2×10^{-2}	12.5 (0.7)	11.6 (0.4)
Candida rugosa	2 – Nonanol	2.8×10^{-3}	2.2×10^{-3}	1.70 (0.03)	1.74 (0.04)

solvents is too low to be able to draw any further conclusions from this. Previously reported studies have also shown that more hydrophobic solvents (higher log *P* or lower dielectric constant) gave decreased enantioselectivity [3,4]. However, the opposite effect (increased enantioselectivity) has also been presented [2,21]. This is an indication that correlations between enantioselectivity and solvent parameters have to be looked upon from case to case.

3.7. Enantioselectivity of other racemic alcohols

The esterification of other alcohols with decanoic acid was also studied. Racemic phenethyl alcohol was used with Lipozyme, *Candida rugosa* and *Pseudomonas* lipases (Table 3). The activity and enantioselectivity were studied at water activity 0.33 and 0.75. As shown for 2-octanol the enantioselectivity was not affected by the water activity. The enantioselectivity for the enzymes seem to be different to the values for 2-octanol. Lipozyme and *Pseudomonas* lipase are not as selective as for 2-octanol and *Candida rugosa* lipase shows a higher selectivity.

The enantioselectivity of *Candida rugosa* lipase for 2-nonanol was very similar to the enantioselectivity of 2-octanol. The structural resemblance of the two substrates is obvious.

4. Conclusions

Even though the hydration level of enzymes greatly affects the catalytic activity this seems not to influence the enantioselectivity. There is a striking similarity in the experiments performed and the conclusion is that irrespective of lipase source, alcohol substrate, method of determination or solvent used the enantioselectivity of lipases in the studied reaction is not affected by the water activity.

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