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ORIGINAL CONTRIBUTION

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Stereoselectivity and competing reactions as studied by lipase-catalyzed esterifications in aqueous lecithin-based gelatin gels

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Abstract The enzyme catalyzed conversion of R/S-(±)-2-octanol with hexanoic acid to R/S-(±)-2-octyl hexanoate has been studied in different microenvironments and in the presence of the competing substrate ethanol. The reactions were performed in various gels made from aqueous gelatin solutions and liposome dispersions or isotropic liquid solutions, with or without oil and ethanol. The lipase *Candida* sp. (SP 525) was dissolved in the dispersions or solutions stabilized by the naturally occurring zwitterionic surfactant soybean lecithin. The sectioned porous gel was immersed in hexane containing 0.33 mol dm^{-3} of racemic 2-octanol and hexanoic acid. Since ethanol acts both as a substrate and as a part of the gel it is of fundamental interest to know the phase behaviour of the used systems. Partial phase diagrams for the systems ethanol–water–soybean lecithin and ethanol/water (7:3)–oil–soybean lecithin were determined at 298.2 K. The oil was either castor oil or hexadecane. The conversion of R-2-octyl hexanoate was about 0.45 when no or small amounts of ethanol was present, but decreased considerably with high amounts of ethanol present and ethyl hexanoate became the main product. Hydrolysis of R-2-octyl hexanoate was favoured in the latter systems and hexanoic acid formed was immediately esterified to ethyl hexanoate. The conversion of R-2-octyl hexanoate and ethyl hexanoate depends only on the ethanol content present in the systems and is thus independent of the environment of the enzyme. However, the chiral esters synthesized from racemic 2-octanol and hexanoic acid showed high optical purities regardless of the ethanol content.

Key words Phase equilibria – soybean lecithin-based gels – lipase – esterification – stereoselectivity – competing reactions

Introduction

During the last decade enzymes have become an interesting tool for organic stereoselective synthesis. These reactions are very attractive in pharmacological chemistry since they can be carried out under mild conditions and without toxic solvents. Lipases are hydrolytic enzymes acting on ester bonds. Most lipases are water soluble, while their substrates usually are water insoluble. Therefore, many reactions have been performed in oil/water two-phase systems. However, it has been observed that the organic solvent influences the catalytic ability of the lipase [1–3]. To overcome this inconvenience the reactions can be performed in a microemulsion, where most of the lipase is in the aqueous phase separated from the oil phase by a surfactant film. Furthermore, maximal enzyme activity has been observed in the presence of an interface [4, 5]. For this reason synthesis of organic compounds using enzymes immobilized in microemulsions or microemulsion-based gels is a suitable method in biotechnology.

Surfactants are amphiphilic molecules which aggregate in water to form spherical, disc- or rodlike micelles, various mesophase structures such as three-dimensional cubic phases, hexagonally coordinated rods, or lamellae. These structures can be related to the molecular architecture of the surfactant. One of the earliest ways of understanding qualitatively the different geometric structures was the "packing ratio", V/a_0l, introduced by Israelachvili et al. [6] based on an old idea by Hartley [7]. The repulsive steric interactions between the hydrocarbon chains determine their final volume V and extended length l, while the repulsive hydrophilic forces between the head groups balanced by the attractive hydrophobic forces at the hydrocarbon/water interface determine the final area of head groups, a_0 [8]. For spherical micelles the values of V/a_0l are $<1/3$, for disc- and rodlike micelles the values lie in the range 1/3 to 1/2. At some value close to 1/2 a spherical vesicle becomes viable as regards packing with an aggregation number much smaller than that of a long rodlike micelle [6]. As the packing ratio increases above 1/2 the vesicle will grow gradually becoming a lamella as V/a_0l reaches 1. The naturally occurring zwitterionic surfactant soybean lecithin used in this work does not form micelles in water, but a lamellar liquid crystalline phase appears at high lecithin contents and room temperature [9]. In the region between water and lecithin liquid crystals a vesicle or liposome dispersion exists which may be stable for years [10–12], the lower the content of liposomes the slower the flocculation rate [13]. The different structures mentioned above are referred to as normal structures when the packing parameter is less than 1 and as inverted structures when V/a_0l is greater than 1.

The packing ratio can also be applied to microemulsions [14]. Addition of oil to a surfactant–water system gives rise to similar aggregate structures, as discussed above, but the oil can both restrict and derestrict the characteristics of the surfactant molecules by changing the packing parameters [8]. By careful choice of surfactant, oil and their mutual contents oil droplets-in-water microemulsions, water droplets-in-oil microemulsions or microemulsions with a bicontinuous structure will be formed at a defined temperature [15]. Microemulsions are isotropic solutions with good solubility properties for both nonpolar organic molecules and polar inorganic ones. They are therefore involved in many applications [6, 16, 17] and in recent years microemulsions have widely been used as reaction media for enzyme catalysis [4, 5, 18–45]. By immobilizing the enzyme in microemulsion-based gelatin gels Rees and co-workers showed that the concentration of the substrates in the reaction medium could be increased considerably and the enzyme re-used [46–49]. They also observed a stereoselective esterification of 2-octanol with high enantiomeric excess values [47]. However, there have been only a few reports of enzyme catalyzed synthesis in normal [50–54] and reverse [55, 56] vesicles and, to our knowledge, none in vesicle-based gelatin gels. Phospholipid vesicles or liposomes are microscopic and submicroscopic colloidal particles with sizes ranging from 20 to 10 000 nm [57]. They are composed of one or several lipid bilayers enclosing aqueous or oil compartments. Liposomes are usually used as model membranes for transport phenomena and their biocompatibility makes them useful for delivery of drugs.

In oil–water systems soybean lecithin is slightly too lipophilic to form microemulsions spontaneously, but addition of a short-chain alcohol to the system results in thermodynamically stable microemulsions [58, 59]. This gives a novel method to study the effect of a 1-alcohol on the resolution of another racemic alcohol using alkanoic acids as acyl donors. In a previous work [43] we investigated an enzymatic esterification where the alcohol acted both as a substrate and as a cosurfactant stabilizing the microemulsion. In this kind of study it is of great fundamental interest to know the phase behaviour of the used multi-component systems. Some used phase diagrams are known from the literature [10–12, 60] and some have been determined in this work.

In previous works we have investigated the catalytic behaviour of different lipases on enantioselective syntheses of ibuprofen esters in water-in-oil microemulsions [36] and R-(−)-octyl alkanoates using microemulsion-based gels [61, 62]. In this study, we investigate the activity of the commercial lipase from *Candida* sp. (SP 525) in liposome-based gelatin gels and in lecithin solution-based gelatin gels. The liposome dispersions were prepared with ethanol as the good solvent for the lipid soybean lecithin and water as the poor solvent according to the phase diagram by Söderberg [10]. Ishii et al. [63] used aqueous solutions of short-chain alcohols in preparing liposomes stabilized by egg lecithin with different size and bilayer structure. Methanol resulted in large and uni-bilayer liposomes, ethanol in multi-bilayer liposomes and finally 1-propanol in homogeneous and uni-bilayer liposomes. Furthermore, the enzyme in this work has been dissolved in the aqueous lecithin containing isotropic liquid phase at high ethanol contents with and without oil. The lecithin-based gels were prepared by mixing the liposome dispersion or the isotropic solution containing the enzyme with an aqueous gelatin solution as previously described for microemulsion-based gels [47–49, 64, 65]. The competing esterifications were carried out at 298.2 K according to the following reactions:

$$(\pm)\text{-2-octanol} + \text{hexanoic acid} \Leftrightarrow (\pm)\text{-2-octyl hexanoate} + \text{water} \quad (1)$$

$$\text{ethanol} + \text{hexanoic acid} \Leftrightarrow \text{ethyl hexanoate} + \text{water} \quad (2)$$

$$\text{ethyl hexanoate} + (\pm)\text{-2-octanol} \Leftrightarrow (\pm)\text{-2-octyl hexanoate} + \text{ethanol} \quad (3)$$

with the sectioned gels and the substrates in hexane. The reactions were studied by analyzing the concentrations and the optical purities of the substrates and products in the reaction medium as a function of time. Soybean lecithin contains several different esterified fatty acids [58] and is also a proper substrate for lipases. Some recent papers [66–72] demonstrate how lipases can be used for transesterification and hydrolysis of phospholipids, however, these reactions were not studied in the present work. Thus, the aim of this work was to elucidate how different ethanol contents in the lecithin-based gelatin gels affect the conversion and enantioselectivity of the esterification of (±)-2-octanol with hexanoic acid.

Experimental

Chemicals

Soybean lecithin (Epikuron 200) was obtained from Lucas Meyer Co., FRG. Hexanoic acid (>98% purity) and (±)-2-octanol (>98%) were from Fluka, Switzerland. Castor oil was a commercially available product of pharmaceutical purity. Hexane was purchased from Merck, FRG, hexadecane from Sigma Chemicals, USA and ethyl hexanoate (>99%) from Aldrich, FRG. Acetic anhydride of 97% purity was supplied by Merck and ethanol by Oy Alko Ab, Finland. Pyridine with a purity of 99% and the catalyst 4-dimethylaminopyridine were purchased from Sigma Chemicals. The water was distilled and deionized immediately before use. All other chemicals were used without further purification. Racemic 2-octyl hexanoate (95% purity) was synthesized using well-known procedures. Gelatin (Bloom 231) was obtained from YA, Finland. The *Candida* sp. lipase, SP 525 (225 U/mg), was kindly supplied by Novo Nordisk, Denmark.

Phase behaviour

The partial phase diagrams for ethanol–water–soybean lecithin and for ethanol/water–hexadecane–soybean lecithin have been determined at 298.2 K, and for ethanol/water–castor oil–soybean lecithin at both 298.2 and 323.2 K. The mass ratio between ethanol and water in the last two phase diagrams was 7:3. Samples with fixed compositions were thoroughly shaken, allowed to equilibrate in a thermostated bath at the defined temperature and analyzed visually between polarizing glasses. The structure of the liquid crystalline phases were identified by polarizing microscopy. Anisotropic liquid crystalline phases are birefringent and the lamellar D phase is identified by the mosaic pattern, while the hexagonal phases E and F usually show a non-geometric texture.

Characterization of liposomes

The two-phase system (D phase and water) consists of particles (liposomes or vesicles) of the lamellar phase in water [73]. These large (>500 nm) particles are regarded as multilamellar vesicles (MLVs). By using ultrasonic irradiation the particles become smaller (<100 nm) and unilamellar and are called small unilamellar vesicles (SUVs) [74]. We made dispersions of lecithin in water, with or without ethanol. The mass fractions of lecithin were 0.03 and of ethanol 0, 0.05 and 0.30, respectively. After vortexing, the milky dispersion was used as such, MLV, or sonicated in an ultrasound water bath until a clear and blueish dispersion was obtained, SUV. The liposomes were studied by the negative staining method. Liposomes on carbon coated formvar grids were stained with either 1% ammonium molybdate, pH 7.0 or 1% phosphotungstic acid, pH 7.4. Micrographs were obtained with a JEOL 100 SX transmission electron microscope (60 kV).

Preparation of gel

The gelation procedure has previously been described [61, 62]. Usually the soybean lecithin-based gels were prepared by adding a liposome dispersion or a liquid phase solution of 328 K, with or without oil, to an aqueous gelatin solution of the same temperature. The liposome gels were made of MLVs or SUVs, in which the lipase was solubilized. Hard gels were prepared of 1.8 g dispersion and 1.4 g gelatin and soft gels of 2.662 g dispersion and 0.538 g gelatin. The final mass fraction of gelatin was 0.438 in the hard gels and 0.168 in the soft gels. The liquid phase solution, used for gelation, consisted of lecithin and ethanol/water (7:3) at mass fractions of 0.25 and 0.75, respectively. The oil-containing liquid phases consisted of mass fractions of 0.35 lecithin, 0.50 ethanol/water (7:3) and 0.15 oil (hexadecane or castor oil). The lipase was solubilized in 4.53 g of these solutions, which were gelatinized with 1.4 g gelatin and 2.4 g water. The enzyme content was 8.8 mg in all gels, i.e., 1980 U/gel.

Enzymatic reactions

The reactions were performed with the sectioned gels in 30 cm^3 hexane containing the substrates, as previously described [61, 62]. In the esterification reactions the concentrations were 0.33 $\mathrm{mol\,dm}^{-3}$ for both ($\pm$)-2-octanol and hexanoic acid and in the hydrolysis reactions the concentration of racemic ($\pm$)-2-octyl hexanoate was also 0.33 $\mathrm{mol\,dm}^{-3}$. The ethanol concentration varied with the gel used, since no additional ethanol was added to the reaction medium. The enzyme catalyzed reactions took place in the pores of the gel after spontaneous imbibition of the reaction medium. The temperature was 298.2 K. Samples withdrawn from the reaction medium were acetylated with acetic anhydride in the presence of pyridine and 4-dimethylaminopyridine prior to gas chromatography analysis on a Cyclodex-B chiral column (J&W Scientific) [62]. The enantiomeric excess (e.e.) values for both product (p) and substrate (s) were calculated from the peak areas by the formula:

$$\text{e.e.} = (A_1 - A_2)/(A_1 + A_2) \tag{4}$$

where A_1 is the area for the dominating enantiomer and A_2 is the area for the other enantiomer. The conversion of the ester formed, c, was determined using a calibration curve with ester standards or by the formula:

$$\text{c} = \text{e.e.(s)}/(\text{e.e.(s)} + \text{e.e.(p)}) \tag{5}$$

according to Chen et al. [75].

Results and discussion

Phase diagrams

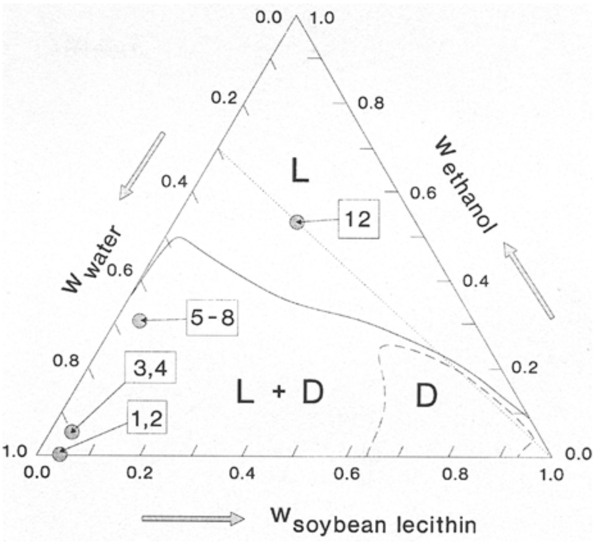

Fig. 1 Phase diagram at 298.2 K for the system ethanol–water–soybean lecithin. The isotropic liquid phase is denoted by L and the lamellar liquid crystalline phase by D. The extent of the D phase is estimated according to Söderberg [10]. L +D is the two-phase region (dispersion). The dotted line corresponds to the ethanol/water ratio 7:3. The numbered points represent the compositions of the dispersions and liquid used in the preparation of the gels. They are also indicated in Table 1

Soybean lecithin has very low solubility in water and packs at high contents into lamellar structures, i.e., a D phase will form [9]. It does not spontaneously form liposomes in water, but by dissipating energy into the two-phase water–liquid crystalline system, liposomes can be produced. The solubility of lecithin can be increased considerably by addition of a short-chain alcohol to the water. 1-Propanol clearly has the greatest effect, while methanol is the least effective. This has been illustrated as phase diagrams for egg yolk lecithin by Small [60]. A similar phase diagram at 294 K has been estimated by Söderberg [10] for the system water–ethanol–soybean lecithin. An extensive isotropic liquid phase and a lamellar phase (D phase) was found. At low lecithin fractions the liquid phase consists of a micellar, L_1 type of solution and at high lecithin fractions of a reversed micellar, L_2 type of solution. Between these two regions, the major part of the liquid phase exists. The dispersed lamellar phase in almost pure water was considered as liposomes. With an increasing ethanol content, the life-time of the liposomes was strongly reduced [10, 12]. We have redetermined the phase boundary between the liquid phase, L, and the two-phase region (dispersion of D and L) at 298.2 K (Fig. 1), since the enzyme reactions, described below, were performed at this temperature. The liquid phase at 298.2 K was extended to a somewhat lower ethanol content than at 294 K. The extent of the lamellar phase, D, was estimated according to Söderberg [10]. Electron micrographs from the two-phase dispersion, with 0.03 mass fraction of lecithin and 0, 0.05 and 0.30 mass fractions of ethanol, showed a heterogeneous population of multibilayer liposomes of sizes between about 200 and 1000 nm. By ultrasound water-bath sonication the dispersions became quite clear and shifted in blue, indicating that the liposomes had become much smaller. The electron microscopy revealed unibilayer liposomes of sizes between 20 and 200 nm. The ethanol content did not markedly affect the size ranges of the heterogeneous liposome populations. Samples from the isotropic liquid phase as well as different

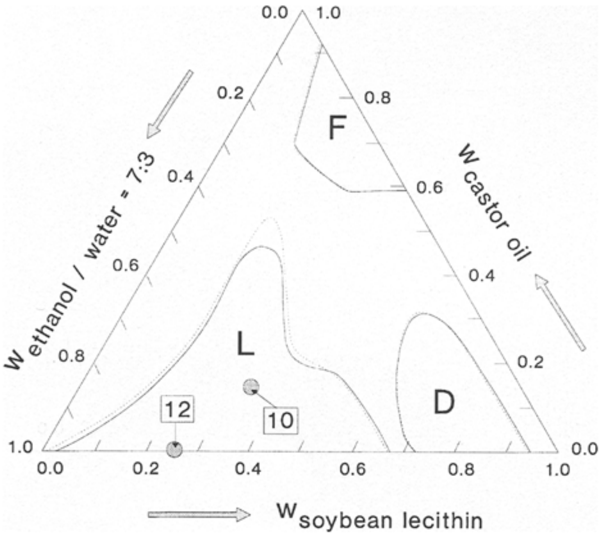

Fig. 2 Partial phase diagram at 298.2 K (lines) and 323.2 K (dotted lines) for the system ethanol/water (7:3)-castor oil-soybean lecithin. L is the isotropic liquid phase, D the lamellar phase and F the inverted hexagonal liquid crystalline phase. The numbers refer to Table 1

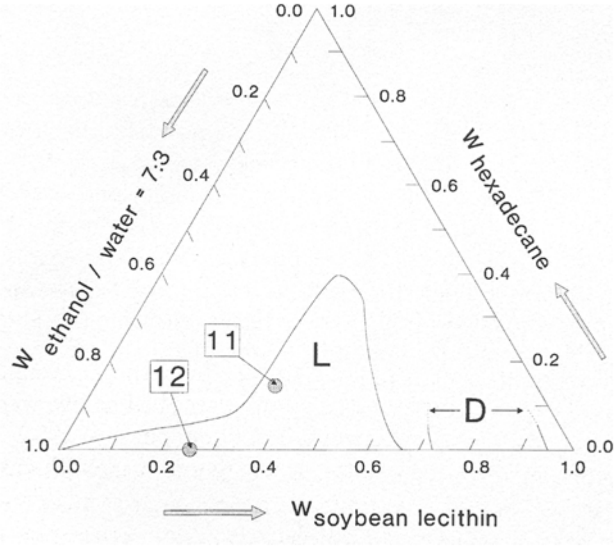

Fig. 3 Partial phase diagram at 298.2 K for the system ethanol/water (7:3)-hexadecane-soybean lecithin. L is the isotropic liquid phase and D the lamellar phase. The numbers refer to Table 1

MLV- and SUV-dispersions were used in preparing the gels for the enzymatic reactions.

Phase diagrams of the four component systems containing lecithin, ethanol/water (7:3) and castor oil or hexadecane are shown in Figs. 2 and 3, respectively. As can be seen, considerable amounts of castor oil and hexadecane can be dissolved in the liquid phase as well as in the lamellar phase. The oils are accomodated in the hydrocarbon part of the bilayers in the lamellar phase. However, the structures, if any, of the oil-containing liquid phase, L, is still unresolved. Enzymatic reactions were for comparison performed also in these systems.

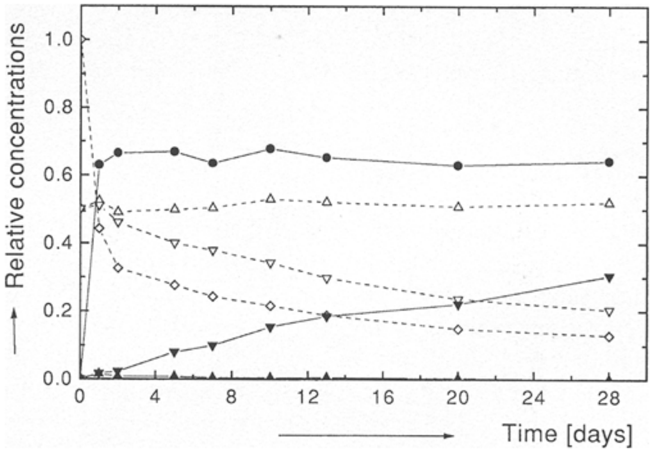

Fig. 4 Progress of reaction No. 5 in Table 1 at 298.2 K as a function of time. The concentrations of products are relative to the initial concentration of hexanoic acid. The symbols represent: S-2-octanol (△), R-2-octanol (▽), hexanoic acid (◇), S-2-octyl hexanoate (▲), R-2-octyl hexanoate (▼) and ethyl hexanoate (●). Ethanol is not included in the figure

Competing esterifications

The reactions (1), (2) and (3) were studied regarding conversions, stereoselectivity, reaction times and reversibility in different lecithin-based gelatin gels. A typical progress of a reaction is shown in Fig. 4. The changes in the relative reactant and product concentrations in the reaction medium are shown as a function of time for the system consisting of multilamellar liposomes at 0.30 mass fraction of ethanol (No. 5 in Table 1). The concentration of ethanol as calculated on the volume of hexane was 0.39 mol dm^{-3}. The concentrations of products were calculated relative to the initial concentration of hexanoic acid. The fast initial decrease in hexanoic acid concentration was clearly a consequence of the formation of ethyl hexanoate. The slow decrease of the R-enantiomer of 2-octanol was dependent on the formation of R-(−)-2-octyl hexanoate. The relative concentration of S-(+)-2-octanol remained constant throughout the reaction, i.e., no reaction occurred. Furthermore, the sum of the relative concentrations in Fig. 4 of R-(−)-2-octyl hexanoate, ethyl hexanoate and hexanoic acid is at any time equal to 1.0, indicating that no transesterification between hexanoic acid and soybean

Table 1 The conversions of 2-octyl hexanoate and ethyl hexanoate using different lecithin-based gelatin gels. The initial concentration of (±)-2-octanol and hexanoic acid were 0.33 mol dm^{-3}, while the concentration of ethanol varied from gel to gel

Experiment No.	Type of gel	c^{a}_{ethanol} mol dm^{-3}	conversion 2-octyl hexanoate	e.e.(p) (−)-2-octyl hexanoate	e.e.(s) (+)-2-octanol	conversion[b] ethyl hexanoate
1	MLV, hard	0	0.46	0.95	0.80	0
2	SUV, hard	0	0.46	0.95	0.81	0
3	MLV, hard	0.065	0.46	0.96	0.80	0.12
4	SUV, hard	0.065	0.46	0.95	0.78	0.12
5	MLV, hard	0.392	0.32	0.99	0.43	0.64
6	SUV, hard	0.392	0.29	0.99	0.41	0.64
7	MLV, soft	0.579	0.25	0.99	0.32	0.70
8	SUV, soft	0.579	0.23	0.99	0.29	0.69
9	Bicontinuous μe[c] [62]	0.906	0.24	0.99	0.32	nd
10	Liquid + castor oil	1.15	0.16	0.99	0.21	0.68
11	Liquid + hexadecane	1.15	0.16	0.99	0.20	0.62
12	Liquid	1.723	0.02	0.99	0.02	nd

[a] Total concentration of ethanol as calculated on the volume of hexane.
[b] Conversion of ethyl hexanoate as calculated on the initial concentration of hexanoic acid.
[c] μe denotes microemulsion.

lecithin takes place. The esterification of ethanol proceeded with a high reaction rate as compared to R-2-octanol. In all experiments with ethanol present the reaction between ethanol and hexanoic acid reached equilibrium before 2-octanol became the substrate. It has previously been pointed out that the reaction rate for primary alcohols is much higher than the reaction rates for secondary alcohols [47]. The final conversion of ethyl hexanoate was only about 0.64 of the acid (Fig. 4), although the ethanol content in this case was in excess over the acid and might have approached 1. However, ethanol was initially only in the gel and not dissolved in the reaction medium and compared to the initial ethanol content, only half of it was esterified with hexanoic acid. Thus, there is an opportunity for any free fatty acid, released from a possible hydrolysis of lecithin [66–72], to be esterified with the rest of ethanol. However, no such ethyl alkanoates nor any hydrolyzed lecithin fractions could be detected by the analysis methods used. This is in agreement with the results by Scartazzini and Luisi [76]. The same pattern as in Fig. 4 was also obtained for the reactions with gels from sonicated liposome dispersions, SUVs.

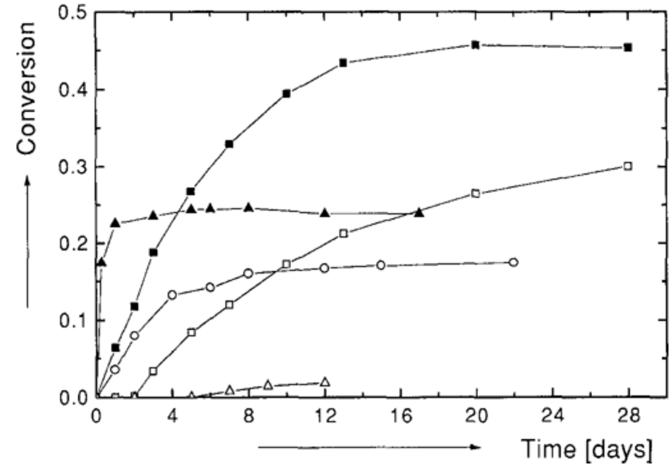

Fig. 5 Conversion of R/S-2-octyl hexanoate at 298.2 K in different systems as a function of time. The symbols represent the experiment No. 3 (■), No. 5 (□), No. 9 (▲), No. 10 (○) and No. 12 (△) explained in Table 1

Conversions versus time for the esterification of (±)-2-octanol in five different systems (No. 3, 5, 9, 10 and 12 in Table 1) are shown in Fig. 5. An almost constant conversion value was reached with time for all gels studied. This equilibrium value in Table 1 compares the conversion of R-2-octyl hexanoate in twelve systems with different microstructures. The conversion of R-2-octyl hexanoate was about 0.46 when no or only small amounts of ethanol was present and the conversion of ethyl hexanoate was smaller than 0.5, i.e., there was still enough acid for further conversion of R-2-octanol to the theoretical value of 0.5. Thus, the conversions in Table 1 and Fig. 4 confirm that the reactions are reversible, as already was indicated in schemes (1)–(3). The variations in conversion between the different systems correlate with the ethanol concentration as illustrated in Fig. 6. As the ethanol concentration in the gel increases, the equilibrium conversion of R-2-octyl hexanoate decreases almost linearly. In a previous paper [62] we compared the same esterification reaction in a sodium 1,4-bis(2-ethylhexyl) sulfosuccinate (AOT) – stabilized W/O microemulsion gel with a lecithin-stabilized and ethanol-containing bicontinuous microemulsion gel and found conversions of 0.45 and 0.24, respectively. The lower conversion in the bicontinuous microemulsion gel can now be understood in the light of the competing esterification of hexanoic acid with ethanol in the gel

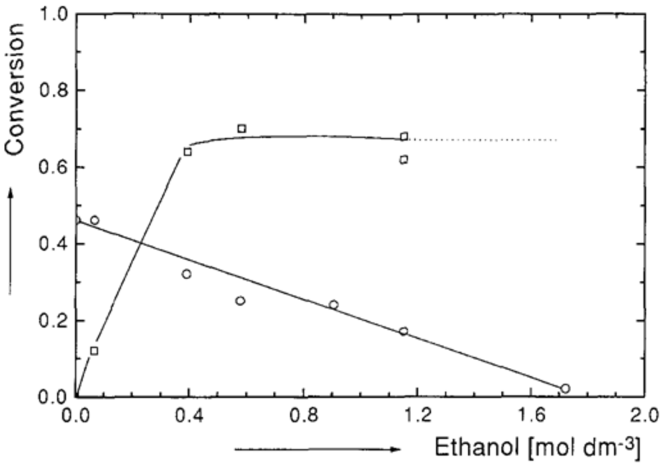

Fig. 6 Equilibrium conversion values at 298.2 K of R-2-octyl hexanoate (○) and ethyl hexanoate (□) as a function of ethanol concentration, as calculated on the volume of hexane

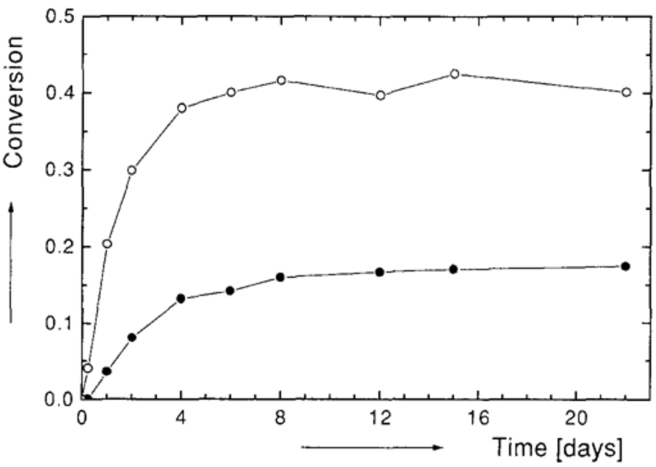

Fig. 7 The synthesis and hydrolysis of R/S-2-octyl hexanoate at 298.2 K as a function of time, using a gel made from the liquid phase containing castor oil (No. 10, Table 1). The symbols represent synthesis of R-2-octyl hexanoate (●) and hydrolysis producing R-2-octanol (○)

(No. 9 in Table 1). Contrary to R-(−)-2-octyl hexanoate, the conversion of ethyl hexanoate in Fig. 6 increases with the ethanol concentration in the gel. The hydroxyl group on the triglyceride castor oil has little or no effect on the conversions (No. 10 in Table 1), since castor oil is present at a very low concentration in the gel. Furthermore, the corresponding gel with hexadecane gives almost the same result (No. 11 in Table 1). Thus, the ethanol concentration in the gel is the determining factor for the conversion and not the environment of the enzyme, i.e., not the microstructure of the gelatinized solutions.

Hydrolysis and alcoholysis

As the ethanol concentration in the reaction medium was higher than the concentration of R/S-(±)-2-octanol, ethyl hexanoate was the main product (Fig. 4). The equilibrium for reaction (1) is now directed towards hydrolysis, as can be seen from Fig. 7, and reaction (2) dominates. The hydrolysis of R/S-(±)-2-octyl hexanoate was studied with a gel made from the liquid phase containing castor oil (No. 10 in Table 1). Conversion values in Fig. 7 are about 0.39 for R-(−)-2-octanol and 0.16 for R-(−)-2-octyl hexanoate and were reached after 8 days of reaction. In comparison, the hydrolysis in an AOT-based gel reached a conversion of only 0.10 (data not shown), while the conversion of the synthesis reaction was about 0.45 [62]. The main products of the hydrolysis reaction were R-(−)-2-octanol and ethyl hexanoate, at about equal concentrations. Only very small amounts of hexanoic acid were detected in the reaction medium. This fact shows that the hexanoic acid released from R/S-(±)-2-octyl hexanoate was simultaneously esterified with ethanol to form ethyl hexanoate. Although we did not study the hydrolysis of ethyl hexanoate, we might assume that since the conversion of ethyl hexanoate never exceeded 0.70, the hydrolysis occurred, but the synthesis dominated and the equilibrium was reached at this point. The transesterification or alcoholysis, i.e., reaction (3) did not take place, since the concentration of the ethyl hexanoate neither increased nor decreased in the reaction system with time after the equilibrium had been reached.

Optical purity

The e.e.(p) values for R-(−)-2-octyl hexanoate were very high in all systems (> 0.95). This confirms the high enantioselectivity of the lipase SP 525 from *Candida* sp. found in a previous study [62], and also pointed out by others [77, 78]. The e.e.(s) values correlated with the conversion values. The e.e.(s) for S-(+)-2-octanol reached 0.80 when the conversions were high, but remained low when the reaction reached equilibrium at a low conversion. Also the e.e.(p) for the product, R-(−)-2-octanol, of the hydrolysis of (±)-2-octyl hexanoate reached 0.99.

Conclusions

The conversion of R-2-octyl hexanoate and ethyl hexanoate depends on the ethanol content and not on the environment of the enzyme. The stereoselectivity of the lipase remained very high in all systems studied, e.e.(p)

>0.95, regardless of the ethanol content or the direction of the reversible reactions. The reaction times for reaching equilibrium were quite long. The exception to this, among the lecithin-based gels, was the gel from a bicontinuous microemulsion, studied previously [62]. The environment might thus have an influence on the reaction times, although not on the conversions nor stereoselectivities. Studies of efficient biotechnical methods for synthesis of optically pure compounds or their separation from racemates are an important issue in production of fine chemicals, pharmaceuticals and agrochemicals. Since the surfactants in these applications are undesired in the final product the gel technique is a new and effective method from a technical point of view. Furthermore, the re-use of the enzyme using this technique has economical consequences.

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