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The effect of the migrating group structure on enantioselectivity in lipase-catalyzed kinetic resolution of 1-phenylethanol

Effet de la structure du groupe partant sur l'énantiosélectivité des lipases lors de la résolution cinétique du 1-phényl éthanol

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ABSTRACT

We have studied the effects of the acyl moiety on the enantioselectivity of three lipases: *Candida antarctica B*, *Pseudomonas cepacia* and *Candida cylindracea*, frequently used in kinetic resolutions by acylation or hydrolysis. The size of the acyl group was examined using various enol esters during the transesterification of 1-phenylethanol and the hydrolysis of the corresponding phenylethylesters. *C. antarctica-B* lipase showed the highest selectivity in the transesterification of 1-phenylethanol with isopropenyl and vinyl acetate, vinyl decanoate, vinyl laurate, ($E > 200$). The esters 1-phenyl -ethyl-acetate, decanoate and laurate are also hydrolyzed with high selectivities ($E > 150$) with CAL-B. The results can be correlated to the three-dimensional form of each lipase. The effect of the migrating group on the reactivity and selectivity of the lipases are discussed for both reactions.

Mots-clés:
Acylation
Hydrolyse
Lipases
Groupe partant
Esters de vinyle
Esters de phényléthyle
Énantiosélectivité

RÉSUMÉ

Nous avons étudié les effets du fragment acyle sur l'énantiosélectivité de trois lipases : *Candida antarctica B*, *Pseudomonas cepacia* et *Candida cylindracea*, fréquemment utilisées dans les dédoublements cinétiques par acylation ou hydrolyse. La taille du groupe acyle a été examinée en utilisant divers esters d'énols dans l'acylation du 1-phényléthanol et par hydrolyse des phenylethylesters correspondants. La lipase de *C. antarctica-B* a montré une haute sélectivité ($E > 200$) lors de la transestérification du 1-phényléthanol avec de l'acétate d'isopropényle, l'acétate de vinyle, le décanoate de vinyle, le laurate de vinyle. Avec cette lipase (*CAL-B*), les esters 1-phényl éthyl-acétate, décanoate et laurate sont hydrolysés avec des sélectivités élevées ($E > 150$). Les résultats peuvent être corrélés à la forme tridimensionnelle de chaque lipase. Pour les deux réactions, l'effet de la nature du groupe partant sur la réactivité et la sélectivité des lipases est discuté.

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1. Introduction

Biocatalysis is now an essential tool for chemical industry and is the core of industrial biotechnology. Biocatalysts such as enzymes or whole cells are currently employed in chemical processes as an alternative to chemical catalysts [1] and applications of biocatalysis to produce fine chemicals especially for the pharmaceutical industry are now developed [2] as green chemistry processes [3] for the preparation of non racemic compounds. Hydrolytic enzymes, which usually display high efficiencies and functional specificities, are among some of the most exploited enzymatic catalysts used for biotransformations [4]. The most important method to produce a single enantiomer in industrial synthesis remains the resolution of racemates [5]. Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are considered to be particularly efficient due to a broad substrate scope, excellent stability, and by displaying high regio and stereoselectivity in various media. They have been used in a wide range of industrial applications under mild and environment friendly conditions [6].

Lipases offer the advantage of accessing a wide range of possible reactions [7] and are thus convenient for obtaining optically enriched compounds from their racemic form by kinetic resolution [8]. In lipase kinetic resolution involving an enzyme, various parameters have to be taken into account for controlling both reactivity and enantioselectivity. A variation of the reaction parameters can induce a large change, depending on the microenvironment of the enzyme. For instance, in the kinetic resolution of a racemic alcohol with a lipase, the parameters such as the nature of the enzyme [9], additives [10], residual water in the reaction media [11], solvent [12], amount of lipase [13] and nature of acylating agent [14] are often examined for the optimization of selectivity.

Alcohols are often conveniently resolved through lipase-catalyzed acylations through an irreversible reaction [15] by the use of suitable acylating agents such as enol esters [16] or anhydrides [14b,17]. The choice of the acyl donor allows to improve the enantioselection in the production of optically active compounds. The chain-length of substrate and its effect on the selectivity of various lipases has been studied in a number of works [18]. The effect of alcohol chain length on enzymatic esterification is the most studied. However, there are still a few investigations regarding the structure of the acyl group in both transesterification and acylation reactions [19].

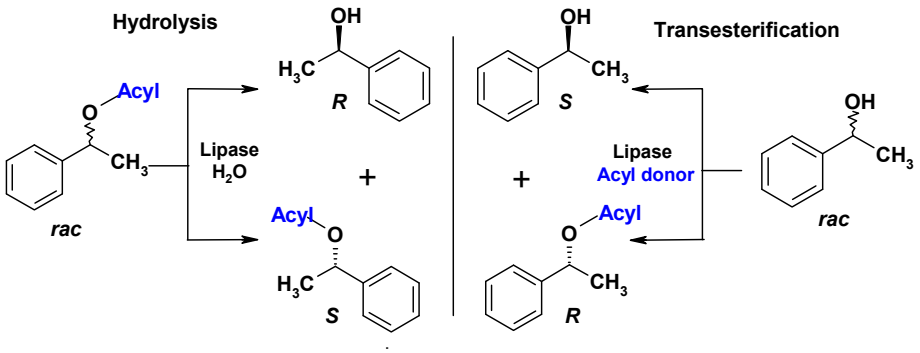
Kazlauskas' rule predicts which enantiomer will react faster in the acylation of secondary racemic alcohols. The stereo-preference for the (*R*)-enantiomer is observed for the hydrolysis of esters or acylation of alcohols [20]. Enzymatic hydrolysis and transesterification reactions can thus be complementary processes for the resolution of secondary alcohols, and when the selectivities of the transesterification and hydrolysis are high ($E > 100$), alcohols and acetates enantiomers are obtained with high enantiopurity [21]. However, the influence of the migrating group on enantioselectivity in hydrolysis and acylation reaction catalyzed by lipases has been rarely studied in the literature. We have thus investigated the selectivity of three lipases according to the acyl moiety of the acyl donor using: (i) the enzymatic acylation of 1-phenylethanol by six various enol esters (ii) the hydrolysis of the corresponding phenylethylesters with the same lipases (Scheme 1).

The acyl donor can influence the enantioselectivity of the lipase by its acyl moiety or the alkyl moiety. Some studies justified this behavior on the basis that the enzyme would be imprinted by the migrating group during the formation of the acyl-enzyme intermediate and influencing thus the selectivity of the enzyme [22]. This substrate matching strategy is described as a new approach for enhancing effectively the lipase enantioselectivity in organic solvents [22a]. A combination of molecular modeling and kinetic studies allowed to build models that explain and predict the enantioselectivity of lipases [23], and structural factors improving affinity enzyme-substrate and influencing enantioselectivity have been identified [24].

We report here a study of the effect of the acyl moiety of enol esters **2a**–**f** on the selectivity of the lipase in the acylation of 1-phenyl ethanol **1** as a model substrate. We compare these effects to those observed for the hydrolysis reaction of the corresponding ester **3a**–**f** with the same lipase (Scheme 2).

2. Results and discussion

In this study, activities and enantioselectivities of three different microbial lipases frequently used in enzymatic resolution were screened: immobilized *Candida antartica*



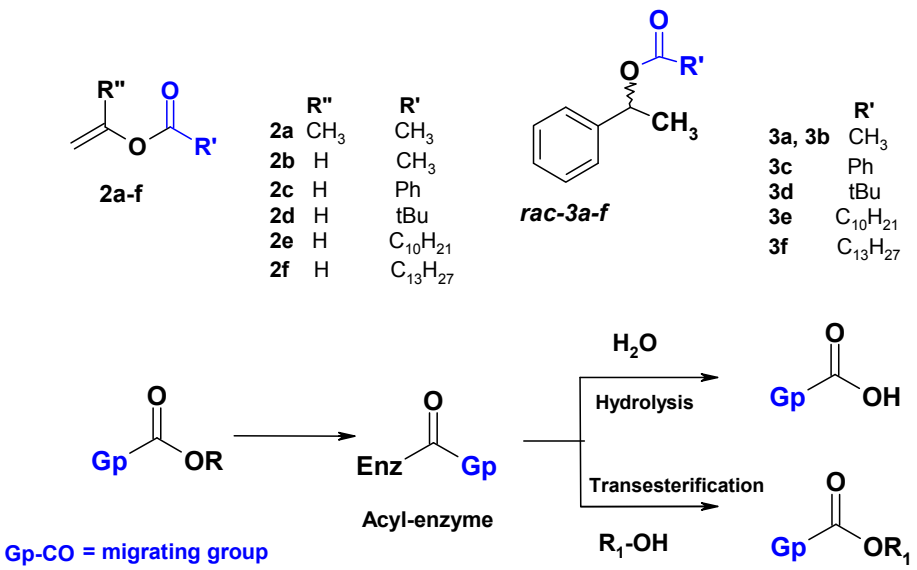
Scheme 1. Enantiocomplementarity of hydrolysis and transesterification reactions.

lipase-type B *(CAL-B)*, *Pseudomonas cepacia* lipase *(PCL)*, and *Candida cylindracea* lipase *(CCL)*. Six enol esters of various structures (isopropenyl acetate, vinyl acetate, vinyl pivalate, vinyl benzoate, vinyl decanoate, and vinyl laurate) were selected as acylating agents of (*R,S*) 1-phenylethanol. Phenylethylesters **3a**–**3f** are hydrolyzed with the same lipases. The goal is to determine their affinity to the substrate relative to the acyl moiety in the acyl donor or in the racemic esters. It is interesting to determine the influence on the selectivity and reactivity of lipase and the impact on both acylation and hydrolysis reactions.

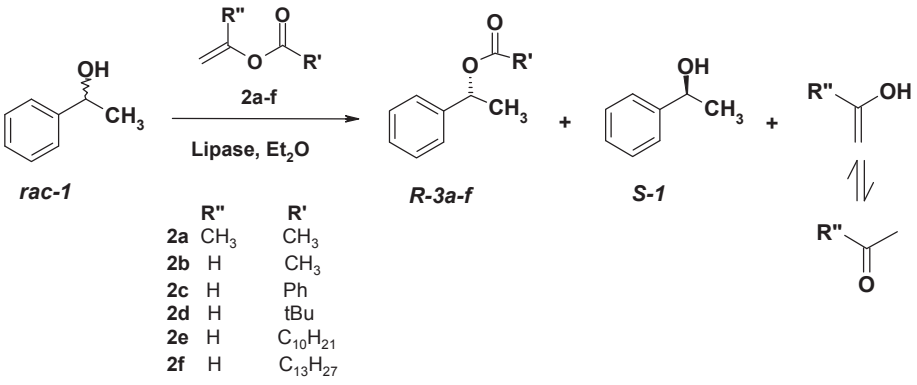
*2.1. Lipase-catalyzed transesterification of 1-phenylethanol with enol esters **2a**–**f***

We have examined the effect of enol esters **2a**–**f** on the activity and selectivity of three lipases on 1-phenylethanol (Scheme 3). The enzymatic-acylation is carried out with 1 mmol of 1-phenylethanol, 3 mmol of enol ester **2a**–**f** in 6 mL of diethyl ether at 40 °C for 24 h. The unreacted alcohol and the acetate were separated by silica-gel flash chromatography. The ee's of both compounds are measured before separation through analysis by GC or HPLC on a chiral phase. Results of transesterification of 1-phenylethanol by vinyl esters are collected in Table 1.

Results of Table 1 show that the conversion and enantioselectivity of the kinetic resolution of 1-phenylethanol with vinyl esters **2a**–**f** depends on the lipase and the enol ester. High selectivities are observed with *CAL-B* and *PCL* for the vinyl decanoate and vinyl laurate ($E > 200$, $C = 50\%$) (entries 5,6 and 11,12). With these acylating agents, *CCL* shows low activity ($9.4\% < C < 12.2\%$) and selectivity ($9 < E < 11$) (entry 17,18). With isopropenyl acetate and vinyl acetate, the selectivities are very high $E > 100$ (entries 1,2 and 7,8) but with different reactivities. The conversion is $C = 50\%$ for *CAL-B* but decreases with *PCL* ($10\% < C < 31\%$). *CCL* is active $15\% < C < 20\%$ and nonselective ($E < 5$) with these enol esters (entry 13,14). The vinyl pivalate is inactive with *CAL-B* lipase (entry 4), while low activity but selectivity are observed with *PCL* ($C = 2\%$ $E = 111$, entry 10). *CCL* is



Scheme 2. Lipase-resolution by acylation by **2a**–**f** and by hydrolysis of **3a**–**f**.

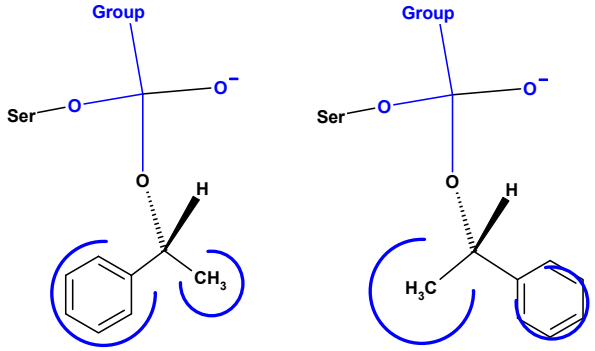


Scheme 3. Vinyl ester reagents for the lipase-catalyzed resolution of ***rac*-1**.

neither active nor selective ($C = 2\%$, $E = 1$) with this acylating agent (entry 16). No reaction was observed with vinyl benzoate using *CAL-B* (entry 3) and *PCL* (entry 9), but with *CCL*, we obtained a good conversion $C = 40\%$ with moderate selectivity $E = 16$ (entry 15).

According to Kazlauskas' rule [20a], the *R*-enantiomer reacts faster in the kinetic resolution of secondary alcohols by lipases for substrates with a small and a large substituent (Scheme 4). In all cases, the *R*-enantiomer was acylated faster by the three studied lipases.

These results show that the structure of vinyl esters significantly affects the reactivity and the selectivity of the lipases. In the case of vinyl pivalate and vinyl benzoate, steric hindrance of the esters probably inhibits the formation of the acyl enzyme. The origin of the enantioselectivity depends on the stereo-pocket structure whose shape can be different according to the lipase [26]. The stereo-preference of the lipase from *P. cepacia* is determined predominantly by the substrate structure and the stereoselectivity depends on atomic details of interactions between substrate and lipase. A simple model has been found by Schulz et al. [27] to predict the enantioselectivity of *P. cepacia* lipase toward a broad range of secondary alcohols. This model is based on the $H_{N\varepsilon}$—$O_{alcohol}$ hydrogen bond distance in an average minimized structure for the slow enantiomer. Another explanation would be that the energy curve of enzyme/substrate interaction is different between *R* and *S*, showing a different accessibility.



Scheme 4. Enantio-preference for lipases with secondary alcohols.

2.2. Enzymatic hydrolysis of racemic esters *3a–f*

The hydrolysis of ester bond is catalyzed by lipases [28]. This reaction is part of a complementary process of acylation in the kinetic resolution of secondary alcohols [13b]. We investigated the hydrolysis of phenylethylesters **3a**–**f** (1-phenyl-ethyl acetate **3a**, 1-phenyl-ethyl benzoate **3c**, 1-phenyl-ethyl pivaloate **3d** 1-phenyl-ethyl decanoate **3e**, 1-phenyl-ethyl laurate **3f**) to examine the influence of the acyl part in the hydrolysis with the same lipases we used in the enzymatic acylation *CAL-B*, *PCL* and *CCL* (Scheme 5). The hydrolysis reaction was performed with 1 mmol racemic ester dissolved in 2 mL solvent, which was added to a buffer solution (pH = 7) with the appropriate amount of lipase.

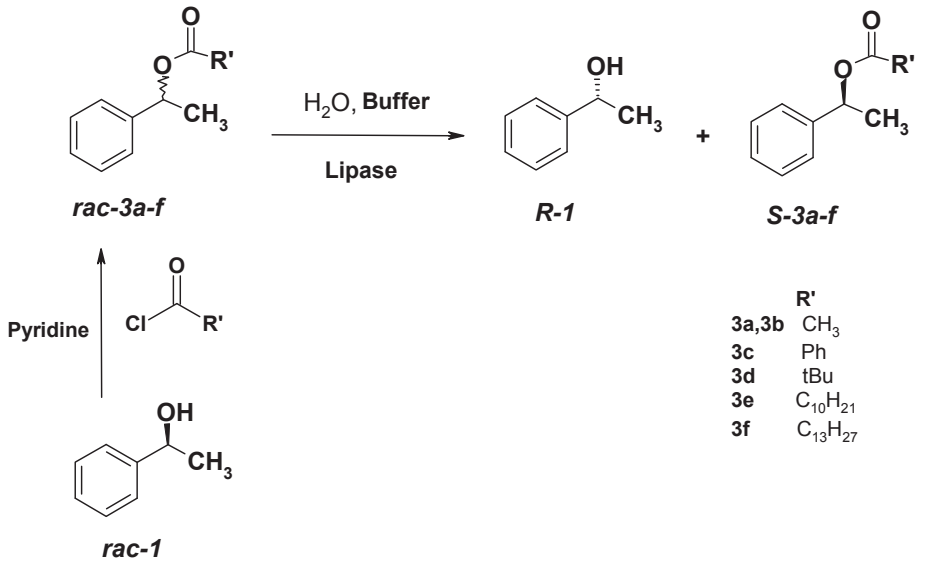
The results are reported in Table 2.

Analysis of the results in Table 2 shows that the activity and selectivity of the *CAL-B* lipase in hydrolysis of racemic esters are highly comparable to those observed in the acylation reaction, except **3d** where reactivity increased in hydrolysis (Table 1). The results in Table 2 show that the activities and selectivities of the three lipases in the hydrolysis of racemic esters are related to the effect of the migrating group structure. With *CAL-B* and *PCL*, the derivatives **3a**, **3e** and **3f** are hydrolyzed with high selectivities ($E > 150$) but *PCL* is less active than CAL-B for the hydrolysis of the three substrates; the conversion decreases respectively from $C = 49\%$ to $C = 40\%$ for **3a** (entry 1 vs. 6), from $C = 30\%$ to $C = 19\%$ for **3e** (entry 4 vs. 9) and from $C = 51\%$ to $C = 20\%$ for **3f** (entry 4 vs. 9). *CCL* is active but not

Table 1
Lipase-catalyzed transesterification of 1-phenylethanol with vinyl esters **2a**–**f**.

Entry[a]	Lipase (mg)	Acyl donor	ee_s[b] (*S*)[d] (yield)[c]	ee_p[b] (*R*)[d] (yield)[c]	*C*(%)[b]	*E*[b]
1	*Candida antarctica* lipase B (20)	**2a (Isopropenyl acetate)**	**99 (44)**	**99 (48)**	**50**	**>200**
2		**2b (Vinyl acetate)**	**99 (43)**	**98.7 (50)**	**50**	**>200**
3		**2c (Benzoate)**	—	—	—	—
4		**2d (Pivalate)**	—	—	—	—
5		**2e (Decanoate)**	**99 (42)**	**95.6 (44)**	**50.8**	**>200**
6		**2f (Laurate)**	**99 (45)**	**96.3 (43)**	**50.7**	**>200**
7	*Pseudomonas cepacia* lipase (20)	**2a (Isopropenyl acetate)**	**11.4 (85)**	**99 (09)**	**10.3**	**>200**
8		**2b (Vinyl acetate)**	**44 (58)**	**98.5 (28)**	**31**	**148**
9		**2c** (Benzoate)	—	—	—	—
10		**2d** (Pivalate)	2.2 (95)	98.7 (2)	2.2	111
11		**2e (Decanoate)**	**99 (49)**	**99 (48)**	**50**	**>200**
12		**2f (Laurate)**	**99 (42)**	**98.7 (48)**	**50**	**>200**
13	*Candida cylindracea* lipase (80)	**2a** (Isopropenyl acetate)	8.9 (57)	50 (10)	15	3
14		**2b** (Vinyl acetate)	13 (76)	51.3 (19)	20.2	5
15		**2c** (Benzoate)	52.4 (55)	78.8 (38)	40	16
16		**2d** (Pivalate)	0.04 (95)	1.5 (2)	2	1
17		**2e** (Decanoate)	10.8 (75)	77.8 (11)	12.2	9
18		**2f** (Laurate)	8.5 (72)	81.4 (6)	9.4	11

The bold characters showcase the best results.
[a] Each experiment was carried out with 1 mmol of racemic 1-phenylethanol and 3 mmol of acylating agent in diethyl ether (6 mL) at 40 °C for 24 h.
[b] Measured by chiral chromatography, GC or HPLC. Conversion: $C = ee_S/ee_P + ee_S$; selectivity: $E = \ln[(1 - C)(1 - eeS)]/\ln[(1 - C)(1 + ee_S)]$ [25].
[c] Isolated yields after silica gel chromatography, hexane/ethyl acetate 90:10.
[d] Absolute configuration of the products was assigned through comparison of the sign of the optical rotation with the literature data.



Scheme 5. Lipase-catalyzed hydrolysis of racemic esters **3a**–**f**.

very selective for the hydrolysis of **3a** ($C = 21\%$, $E = 4$) (entry 11), **3e** ($C = 45\%$, $E = 6$) (entry 14) and **3f** ($C = 29\%$ $E = 19$) (entry 15).

In the hydrolysis of 1-phenyl-ethyl benzoate **3c** with *CCL*, the selectivity obtained is $E = 24$ for a conversion of $C = 13\%$ (entry 12), *CAL-B* and *PCL* are inactive with this ester derivative (entries 2 and 7). The three lipases are very active without any selectivity for the hydrolysis of 1-phenyl-ethyl pivaloate **3d**, $C = 52\%$ and $E = 1$ for *CAL-B* (entry 3), $C = 58\%$ and $E = 1$ for *PCL* (entry 8), $C = 98\%$ without selectivity ($E = 1$) for *CCL* (entry 13).

The reactivity of *CCL* is better over the two other lipases for the hydrolysis of all substrates, albeit much less selective. For an irreversible hydrolysis procedure, the fast reacting enantiomer is (a) and the slow reacting one is (b) in the active side model for lipases derived from Kazlauskas' rule (Scheme 4). It is noted that the three lipases exhibit *R*-enantiopreference esters in accordance with the literature [29].

The results of this study can be explained by taking into account the structure of lipase binding sites. It is established that the length, the form and the binding site of the lipases vary considerably according to their nature. The lipases from *Candida antarctica*, *P. cepacia* have a funnel-like binding site, larger funnel for *PCL* and narrower for *CAL-B*, but for *CCL*, it is a tunnel-like binding site [30]. Thus, the enantioselective pocket of *CAL-B* and *PCL* are tailored to accept linear substituents, such as decanoate or laurate, which can easily penetrate the funnel. Moreover, these two lipases can adapt to accept a variety of similar ester forms. According to form, size and substitution, the insertion of such substrates is more difficult and activity of the lipase is

Table 2
Lipase-catalyzed hydrolysis of racemic esters **3a**–**f**.

Entry[a]	Lipase (mg)	Substrate	ee_s[b] (Yield)[c]	ee_p[b] (Yield)[c]	C[b] (%)	E[b]
1	*Candida antarctica* lipase B (20)	**3a (Acetate)**	**96** (36)	**99** (42)	**49**	**>200**
2		**3c** (Benzoate)	6 (78)	—	—	—
3		**3d** (Pivalate)	0.6 (45)	0.6 (50)	52	1
4		**3e (Decanoate)**	**43 (43)**	**98 (21)**	**30**	**150**
5		**3f (Laurate)**	**99 (33)**	**97 (12)**	**51**	**>200**
6	*Pseudomonas cepacia* lipase (20)	**3a (Acetate)**	**67 (30)**	**99 (28)**	**40**	**>200**
7		**3c** (Benzoate)	7.4 (78)	—	—	—
8		**3d** (Pivalate)	6 (23)	4.6 (56)	58	1
9		**3e (Decanoate)**	**23 (67)**	**99 (14)**	**19**	**>200**
10		**3f (Laurate)**	**24 (76)**	**99 (15)**	**20**	**>200**
11	*Candida cylindracea* lipase (80)	**3a** (Acetate)	14 (78)	54 (20)	21	4
12		**3c** (Benzoate)	14 (64)	91 (9)	13	24
13		**3d** (Pivalate)	6.5 (2)	0.1 (67)	98	1
14		**3e** (Decanoate)	47 (51)	57 (43)	45	6
15		**3f** (Laurate)	35 (60)	86 (24)	29	19

[a] Each experiment was carried out with 1 mmol of racemic esters **3a**–**f** in buffer solution and diethyl ether at 40 °C for 24 h.
[b] Measured by chiral chromatography, GC or HPLC. Conversion: $C = eeS/eeP + eeS$; selectivity: $E = \ln[(1 - C)(1 - eeS)]/\ln[(1 - C)(1 + ee_S)]$ [25].
[c] Isolated yields after silica gel chromatography.

lowered in acylation as well as in hydrolysis. For *CCL*, the long and narrow site will affect the insertion of the substrate at the active site.

We observed the affinity of three lipases relative to the impact of the migrating group of the ester derivatives on vinyl esters in acylation or on the corresponding racemic phenylethylesters in hydrolysis. It is interesting to note that the lipases studied display different selectivities and reactivities in these two complementary reactions (acylation/hydrolysis). The results can be correlated to the three-dimensional shape of each lipase and conditions of each reaction.

3. Conclusion

We compared the structural and environmental parameters governing the enantioselectivity of three lipases *CAL-B*, *CCL* and *PCL* for resolving chiral secondary alcohols by acylation with vinyl esters of different lengths and shapes, and by hydrolysis of the corresponding racemic esters for a better understanding of the phenomena involved in both reactions.

The results show the strong influence of the migrating group of the acyl donor. There is a significant difference in reactivity and selectivity of the lipases according to the structure of the acylating agents. This can be attributed to their respective paths to the active site. *CAL-B* is highly selective in the transesterification of 1-phenyl ethanol with IA, VA, VD, VL ($E > 200$), while vinyl benzoate and vinyl pivalate do not react. With *CAL-B*, 1-phenyl-ethyl acetate, 1-phenyl-ethyl decanoate and 1-phenyl -ethyl laurate are hydrolyzed with high selectivities ($E > 150$).

Comparison of the reactivity and selectivity of lipases for acylation and hydrolysis shows, for the first time, many similarities for the two reactions, which depend on the structure of the migrating group. *C. antarctica* lipase B is by far the most effective in both reactions. This enantiocomplementarity offers a very interesting additional catalysis tool; the strategy can be adapted to the desired enantiomer.

4. Experimental section

4.1. General

NMR spectra were performed with Bruker spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C). Chemical shifts were reported in d ppm from tetramethylsilane with the solvent resonance as internal standard for ^{1}H NMR and chloroform-d (d 77.0 ppm) for ^{13}C NMR. The enantiomeric excesses (ees) were determined by gas chromatography (Thermo-Finnigan Trace GC) equipped with an automatic autosampler and using a CHIRALSIL-DEX CB column (25 m; 0.25 mm; 0.25 μm), or by a chiral stationary phase HPLC on Chiralcel-ODH column using racemic compounds as references. Retention times are reported in minutes. *C. antarctica* lipase fraction B immobilized on acrylic resin (***CAL-B***; LA > 10,000 U/g), *P. cepacia* lipase (***PCL***; LA > 30,000 U/mg), and *C. cylindracea* lipase (***CCL***; LA = 3.85 U/mg) were from Sigma-Aldrich. Vinyl esters **2a**–**f** and both enantiomers of 1-phenylethanol **1** are commercially available (Aldrich). The organic solvents were dried over molecular sieves prior to use.

4.2. General procedure for the synthesis of racemic acetates *3a–3f*

The acetates **3a**–**b** were synthesized by chemical acetylation via the corresponding racemic alcohol (1 mmol), using 1.5 mmol of anhydride acetic, 1.2 mmol of Et_3N, and a catalytic amount of 4 dimethylaminopyridine (0.1 mmol) in 4 mL of ether. The alkyl esters **3c**–**f** were prepared from 1-phenylethanol (10 mmol) and pyridine (12 mmol) in dry diethyl ether (10 mL) and cooled (0 °C) in an ice bath, the acyl chloride (12 mmol) is added dropwise and the mixture was stirred at room temperature for 24 h. Next, the reaction mixture was diluted with diethyl ether and washed with 0.1 M aqueous HCl, saturated $NaHCO_3$, and finally with brine. The ether solution was dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate, 9:1). The ester derivatives were obtained with good yields (72% < yields < 99%). The ^{1}H and ^{13}C NMR spectra of these products were in good agreement with the literature.

4.3. General procedure for enzymatic acylations

The selected enzyme was added to a solution of racemic 1-phenylethanol (1 mmol) and the vinyl ester **2a**–**f** (3 mmol) in diethyl ether (5 mL), and the mixture was stirred at 40 °C for 24 h. The reaction mixture was filtered on Celite and concentrated in vacuo. The (*S*)-1 phenyl-ethanol and the corresponding (*R*)-ester were separated by flash chromatography on silica gel (petroleum ether/ethyl acetate: 90/10) and analysed by chiral HPLC or GC.

4.4. General procedure for enzymatic hydrolysis of racemic esters

One mmol of racemic esters of 1-phenylethanol **3a**–**f** was dissolved in 2 mL of ether and added to 4 mL of phosphate buffer pH 7. The reaction was initiated by the addition of lipase and the mixture was shaken at 300 rpm at 40 °C for 24 h. The reaction mixture was filtered on Celite. The alcohol formed and the remaining ester were separated by aqueous base-organic solvent liquid–liquid extraction and finely by flash chromatography on silica gel (petroleum ether/ethyl acetate: 90/10) and analysed by chiral HPLC or GC.

4.5. Chiral GC analysis and/or Chiral HPLC analysis

Retention times are reported in minutes. The conditions for the analysis of alcohols **(*R*)-1**–**9** and acetates **(*S*)-1a**–**9a** are reported below.

(*R,S*)-1-Phenylethanol 1: GC (Chiralsil-Dex CB): $t_R = 3.9$ min, $t_S = 4.1$ min ($T_{column} = 140$ °C, flow 1.2 mL/min).

(*R,S*)-1-Phenylethyl acetate 3a,b: GC (Chiralsil-Dex CB), $t_S = 2.9$ min, $t_R = 3.2$ min ($T_{column} = 140$ °C, flow 1.2 mL/min). Eluent: petroleum ether–AcOEt: 8/2.

(*R,S*)-1-Phenylethyl benzoate 3c: HPLC (Chiralcel OD-H, hexane/EtOH: 95/5, flow 1.0 mL/min): $t_S = 4.69$ min, $t_R = 5.26$ min. Eluent: petroleum ether–AcOEt: 9/1.

(*R,S*)-1-Phenylethyl pivaloate 3d: HPLC (Chiralcel OD-H, Isohexane/*i*PrOH: 90/10, flow 1.0 mL/min): $t_S = 7.68$ min, $t_R = 9.37$ min. Eluent: petroleum ether–AcOEt: 9/1.

(*R,S*)-1-Phenylethyl decanoate 3e: HPLC (Chiralcel AD-H, isohexane/iPrOH: 98/2, flow 1.0 mL/min): $t_S = 4.23$ min, $t_R = 5.06$ min. Eluent: petroleum ether–AcOEt: 9/1.

(*R,S*)-1-Phenylethyl laurate 3f: HPLC (Chiralcel AD-H, isohexane/*i*PrOH: 98/2, flow 1.0 mL/min): $t_S = 3.98$ min, $t_R = 4.57$ min. Eluent: petroleum ether–AcOEt: 9/1.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.crci.2016.05.002.

References

[1] P. Grunwald (Ed.), Industrial Biocatalysis, Pan Stanford Publishing, 2014, pp. 1–232.
[2] J. González, P. Poechlauer, Q.B. Broxterman, B.S. Yang, D. Ende, J. Baird, C. Bertsch, R.E. Hannah, P. Dell'Orco, H. Noorman, S. Yee, R. Reintjens, A. Wells, V. Massonneau, J. Manley, Org. Pro. Res. Dev. 15 (2011) 900.
[3] (a) P.T. Anastas, J.C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, New York, 2000, p. 132;
(b) B.M. Trost, Science 254 (1991) 1471;
(c) R.A. Sheldon, Chem. Ind. (London) (1992) 903–906.
[4] (a) A.M. Klibanov, ChemTech 16 (1986) 354;
(b) G. Kirchner, M.P. Scollar, A.M. Klibanov, J. Am. Chem. Soc. 107 (1985) 7072;
(c) A. Zaks, A. Klibanov, Proc. Natl. Acad. Sci. USA 82 (1985) 3192.
[5] (a) J.M. Hawkins, T.J. Watson, Angew. Chem., Int. Ed. 43 (2004) 3224;
(b) R.A. Sheldon, The industrial synthesis of optically active compounds, in: B. Pearson (Ed.), Specialty Chemicals - Innovations in Industrial Synthesis and Applications, Elsevier, Amsterdam, 1991, pp. 473–497.
[6] (a) K. Faber, Biotransformations in Organic Chemistry. A Textbook, 5th ed., Springer-Verlag, Berlin, Heidelberg, 2004;
(b) J.B. Jones, C.J. Sih, D. Perlman, Application of Biochemical Systems in Organic Chemistry, Wiley, New York, 1976.
[7] (a) D.A. MacManus, E.N. Vulfson, Enzyme Microb. Technol. 20 (1997) 225;
(b) E. Rubio, A. Fernandez- Mayorales, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 695.
[8] C.H. Wong, G.M. Whitesides, Enzymes in Synthetic Organic Chemistry, Pergamon, Oxford, 1994.
[9] (a) C. Bidjou, L. Aribi-Zouioueche, J.-Y. Legros, J.-C. Fiaud, J. Soc. Alger. Chim. 9 (1999) 261;
(b) A. Ghanem, H.Y. Aboul-Enein, Chirality 17 (2005) 1.
[10] M. Merabet-Khelassi, L. Aribi-Zouioueche, O. Riant, Tetrahedron: Asymmetry 19 (2008) 2378.
[11] (a) A. Zaks, A. Klibanov, Proc. Natl. Acad. Sci. USA 82 (1985) 3192;
(b) L. Chua, S.M.R. Sarmidi, Enzyme Microb. Technol. 38 (2006) 551.
[12] (a) A.M. Klibanov, Acc. Chem. Res. 23 (1990) 114;
(b) P.A. Fitzpatrick, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 3166;
(c) Y. Kitamoto, Y. Kuruma, K. Suzuki, T. Hattori, J. Org. Chem. 80 (2015) 521.
[13] (a) M. Merabet-Khelassi, N. Bouzemi, J.-C. Fiaud, O. Riant, L. Aribi-Zouioueche, C. R. Chimie. 14 (2011) 978;
(b) N. Bouzemi, I. Grib, Z. Houiene, L. Aribi-Zouioueche, Catalysts 4 (2014) 215.
[14] (a) M. Kawasaki, M. Goto, S. Kawabata, T. Kometani, Tetrahedron: Asymmetry 12 (2001) 585;
(b) N. Bouzemi, H. Debbeche, L. Aribi-Zouioueche, J.-C. Fiaud, Tetrahedron Lett. 45 (2004) 627;
(c) T. Miyazawa, E. Kaito, T. Yukawa, T. Murashima, T. Yamada, J. Mol. Catal. B Enzym. 37 (2005) 63.
[15] U. Hanefeld, Org. Biomol. Chem. 1 (2003) 2405.
[16] (a) Y.F. Wang, J.-J. Lalonde, M. Momongan, D.E. Bergbreiter, C.H. Wong, J. Am. Chem. Soc. 110 (1988) 7200;
(b) R. Chenevert, N. Pelchat, P. Morin, Tetrahedron: Asymmetry 20 (2009) 1191.
[17] D. Bianchi, P. Cesti, E. Battistel, J. Org. Chem. 53 (1988) 5531.
[18] (a) L. Vaysse, A. Ly, G. Moulin, E. Dubreucq, Enzyme Microb. Technol. 31 (2002) 648;
(b) Q.L. Chang, C.H. Lee, K.L. Parkin, Enzyme Microb. Tech. 25 (1999) 290;
(c) G. Aggelis, M. Komaitis, M. Pina, J. Graille, Grasas Aceites 44 (1993) 331;
(d) M. Berger, M.P. Schneider, Biotechnol. Lett. 13 (1991) 641.
[19] (a) Y. Pan, K.W. Tang, C.Q. He, W. Yi, W. Zhu, Y.N. Liu, Biotechnol. Appl. Biochem 61 (2014) 274;
(b) H. Zhong, Z. Fang, B. Zou, X. Lia, P. Ouyang, K. Guo, J. Mol. Catal. B Enzym. 90 (2013) 114;
(c) H.Y. Zhang, X. Wang, C.B. Ching, J.C. Wu, Biotechnol. Appl. Biochem. 42 (2005) 67.
[20] (a) R.J. Kazlauskas, A.N. Weissfloch, A.T. Rappaport, L.A. Cuccia, J. Org. Chem. 56 (1991) 2656;
(b) V. Gotor-Fernández, R. Brieva, V. Gotor, J. Mol. Catal. B Enzym. 40 (2006) 111.
[21] L. Aribi-Zouioueche, J.-C. Fiaud, Tetrahedron Lett. 41 (2000) 4085.
[22] (a) D. Lee, Y.K. Choi, M.J. Kim, Org. Lett. 2 (2000) 2553;
(b) B.H. Hoff, H.W. Anthonsen, T. Anthonsen, Tetrahedron: Asymmetry 7 (1996) 3187;
(c) J. Gonzalez-Sabın, V. Gotor, F. Rebolledo, Tetrahedron: Asymmetry 15 (2004) 481.
[23] F. Haffner, T. Norin, Chem. Pharm. Bull. Tokyo 47 (1999) 591.
[24] (a) J. Ottosson, K. Hult, J. Mol. Cat. B Enzym. 11 (2001) 1025;
(b) M. Ardhaoui, A. Falcimaigne, S. Ognier, J.M. Engasser, P. Moussou, G. Pauly, M. Ghoul, J. Biotechnol. 110 (2004) 265.
[25] (a) C.S. Chen, Y. Fujimoto, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294;
(b) H.B. Kagan, J.-C. Fiaud, in: E. Eliel, S.H. Wilen (Eds.), Topics in Stereochemistry, Wiley-Interscience, 1988, p. 249.
[26] (a) J. Uppenberg, M.T. Hansen, S. Patkar, T. Alwyn Jones, Structure 2 (1994) 293;
(b) E.M. Anderson, K.M. Larsson, O. Kirk, Biocatal. Biotransform 16 (1998) 181.
[27] T. Schulz, J. Pleiss, R.D. Schmid, Protein Sci. 9 (2000) 1053.
[28] S. Koul, S.C. Taneja, R. Parshad, G.N. Qazi, Tetrahedron: Asymmetry 9 (1998) 3395.
[29] (a) T. Hayashi, Y. Matsumoto, Tetrahedron: Asymmetry 2 (1991) 601;
(b) K. Naemura, M. Murata, R. Tanaka, M. Yano, K. Hirose, Y. Tobe, Tetrahedron: Asymmetry 7 (1996) 1581;
(c) K. Naemura, M. Murata, R. Tanaka, M. Yano, K. Hirose, Y. Tobe, Tetrahedron: Asymmetry 7 (1996) 3285.
[30] J. Pleiss, M. Fischer, D. Rolf, R.D. Schmid, Chem. Phys. Lipids 93 (1998) 67.