

Controlling lipase enantioselectivity for organic synthesis

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Abstract

Lipases are used frequently as chiral catalysts in the synthesis of various fine chemicals and intermediates. The increasing need of compounds with high stereochemical purity requires catalysts with an improved and controlled performance. This overview emphasizes some important aspects for the control of lipase enantioselectivity and some examples where the enantioselectivity has been altered or reversed are highlighted. However, in several of these cases the complete explanation for the altered or reversed enantioselectivity remains unclear and needs to be solved. Three different strategies (engineering of the reaction medium, the substrate molecule, and the enzyme) for exploring lipase enantioselectivity at a molecular level are discussed and summarized. These three different approaches represent powerful tools for understanding the molecular basis for lipase enantioselective catalysis and can guide the rational improvement and tailoring of catalyst performance. By combining approaches from chemistry and biology much is learnt about the most important parameters controlling lipase enantioselectivity for organic synthesis. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The explosive growth of modern drug discovery in recent years and the high-throughput development of new or modified potent pharmaceuticals have led to an increasing demand for new efficient stereoselective synthetic methods and the interest for enzyme catalysis in this respect has increased [1]. The number of drugs in single-enantiomer form is growing annually and reached the total sales of \$115 billion worldwide in 1999 [2]. However, some of the synthetically produced biologically active chiral substances in the market are still being sold in a racemic mixture, with the situation for pesticides being the worst with 75% of the chiral products sold as racemates [3]. Another indication of the growing need for new efficient selective processes for synthesis of stereochemically pure drugs is the currently increasing use of racemic switches in industry, the redevelopment in single-enantiomer form of a drug previously approved as the racemate [4].

The field of enzyme catalysis in organic synthesis began in the late 19th century with many useful reactions explored. Two articles on historical perspectives in the biocatalysis area have appeared recently [5,6]. Enzyme catalysis is now an established tool in stereoselective synthetic organic chemistry with several textbooks [7–11] and numerous reviews, exemplified by some published in the recent two years [12–17], available in the field. The term ‘asymmetric synthesis’, a frequently used term in organic chemistry for the selective synthesis of one of several stereoisomers from a substrate molecule where a new stereocenter is created, was in fact first used in 1908 for the enzyme-catalyzed stereoselective addition of HCN to aldehydes [18].

The need for compounds with high stereochemical purity, for example, in many cases enantiomeric excess of more than 99.5% ee, requires catalysts with optimized performance and maximized enantioselectivity. Several techniques for modifying enzymes, both on a gene level and using chemical modification, are available. However, rational tailoring of enzyme enantioselectivity by engineering of the enzyme molecule is complicated and is often a matter of trial and error. To facilitate this, a more detailed molecular-level under-

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standing of the substrate–enzyme interactions needs to be obtained for a complete control of the structural factors controlling enzyme stereoselectivity. This overview discusses three different aspects and approaches for exploring the control of lipase enantioselectivity. A combination of all three is a successful way towards the rational tailoring of lipase enantioselectivity for organic synthesis.

1.1. Target compounds and processes for lipase catalysis

Lipases (EC 3.1.1.3) are ubiquitous enzymes belonging to the esterase class of hydrolases. They belong to the α/β -fold family of enzymes [19], recently reviewed [20], and they follow the reaction mechanism used by all serine hydrolases, involving a catalytic triad of serine, histidine, and glutamate or aspartate. Lipases catalyze the hydrolytic cleavage of triglycerides into fatty acids and glycerol, or into fatty acid and mono- or diglyceride, at a water–oil interface in nature. Nevertheless, lipases constitute a rather diverse group of enzymes in terms of substrate specificity for unnatural substrates. They are found in most organisms and many lipases are available commercially today from recombinant bacteria and yeast. The substrates for lipases can be esters, like the natural triglyceride substrates in hydrolytic reactions, or, if the reaction is reversed in an organic solvent, carboxylic acids, alcohols, amines, or esters in various esterification, alcoholysis, aminolysis or transesterification reactions. One feature of lipases important for organic synthesis is their recognition of enantiomeric molecules or enantiotopic groups on prostereogenic molecules with high enantioselectivity. This important property can be used either in the case of a racemic chiral compound to achieve kinetic resolution with a theoretical yield of 50% or in the case of a prochiral or *meso* compound to achieve asymmetrization in quantitative yield. Lipase-catalyzed kinetic resolution and asymmetrization processes are well documented in the literature [10].

The E value is the selectivity factor and a measure of the enantioselectivity in kinetic resolution and is defined as the ratio of the specificity constants k_{cat}/K_M for the two competing enantiomeric substrates. It is independent of the reaction conversion and should be constant over time in a true kinetic resolution reaction. The E value [21] is therefore a convenient and often-used parameter for describing the efficiency of enzymatic resolution reactions and many aspects of its determination and utility have been previously reviewed [22–25]. The difficulty of finding enantiomerically pure unnatural compounds as substrates has led to alternative ways for determination of an E value from a racemic substrate. It can be calculated from the enantiomeric excess of the substrate (ee_S) and of the product (ee_P) at a certain extent of conversion (ξ) [26],

$$E = \frac{\ln\left(\frac{1 - ee_S}{1 + ee_S/ee_P}\right)}{\ln\left(\frac{1 + ee_S}{1 + ee_S/ee_P}\right)} \quad (1)$$

or, alternatively, from multiple or single data points of the enantiomeric excess of the product and the conversion [21].

$$E = \frac{\ln[1 - \xi(1 + ee_P)]}{\ln[1 - \xi(1 - ee_P)]} \quad (2)$$

In recent years, lipase catalysis has been used in combined processes where the slow-reacting enantiomer is racemized [27] continuously by a second, usually not a protein, catalyst. The yield of the product in such dynamic resolution processes can be close to 100%. Since a racemic compound usually is much easier to synthesize chemically than only one of its enantiomers, dynamic resolution processes have attracted much attention recently and many review articles have appeared [28–35].

2. Parameters controlling lipase enantioselectivity

2.1. Reaction medium

Lipase activity and selectivity are influenced strongly by many factors related to the reaction medium. Numerous reports on lipase reactions in various solvents, substrate concentrations, and water content illustrate this. Another important parameter to control is the pH in the micro-aqueous phase by salts [36,37]. Enhancement of lipase selectivity by various kinds of additives, such as chiral [38,39] or achiral [40] amines, crown ethers [41], and various salts [42] have also been reported to have a dramatic effect. This has been reviewed recently [43] and will not be discussed further here.

2.1.1. Organic solvents

Many lipase-catalysis applications in organic solvents are well established in laboratories worldwide. Some recent review articles illustrate this [44–46]. The first lipase used in a non-aqueous solvent was already described in 1900 [47], and after a review by Singer in the 1960s [48] the field exploded in the middle of the 1980s with numerous papers published on various aspects of enzyme-catalyzed reactions of chiral compounds in organic solvents [49–51]. The nature of the solvent is known to be of importance for a high activity in lipase reactions. A direct correlation between solvent hydrophobicity and enzyme activity is found in some cases and a solvent having a $\log P$ value, where P is the partition coefficient of a given solvent between *n*-octanol and water, above 2 has been suggested as the best

[52]. However, many reports find no satisfactory correlation between $\log P$ of a solvent and enantioselectivity [53,54]. Probably the water content, or, more correct, the water activity (a_w) of the solvent plays a more important role, as discussed below.

One example from the early literature where a variation of solvent induces a striking effect on lipase enantioselectivity is shown in Fig. 1 [55]. By changing the solvent from water-saturated cyclohexane to water-saturated isopropyl ether a reversed enantiomer preference is observed in a *Pseudomonas* lipase-catalyzed (lipase AH) hydrolysis reaction of a prochiral diester. A similar reversal of enantioselectivity for other substrates by changing the solvent is also reported for *Candida rugosa* lipase [56], for *Pseudomonas cepacia* lipase [57] and for a protease [58]. It has been suggested from molecular modeling studies that enantiomers bind in different orientations in the enzyme active site [59]. The reason for the reversal of enantioselectivity is that solvation of, for instance, a phenyl group exposed to the solvent is thermodynamically more favorable in solvents of a high $\log P$ [53]. Another explanation suggested is that solvent molecules coordinated to the active site interfere differently with the substrate enantiomers [55].

2.1.2. Water activity

The water activity, a_w , plays an important role in lipase-catalyzed reactions. Halling suggests the use of water activity instead of water content to describe the effect of water in an organic solvent, and demonstrates its usefulness, control, and impact on lipase performance in several review articles [60–63]. The water activity describes the mass action of water on equilibrium and is therefore a better measure of the effect of water than the water content. A polar solvent requires higher water content to reach a certain water activity than a non-polar solvent. Consequently, in order to compare the enzyme reactions in different media it is of fundamental importance to do this at an equal and constant water activity.

The rates of enzyme-catalyzed reactions are in many cases strongly dependent on the water activity of the reaction medium. The picture is more complex regarding the effect on enzyme enantioselectivity. Results appearing to be contradictory on how lipase enantioselectivity is influenced by a_w can be found in the literature. On lowering the water activity (or water content), both decreased [64–69], increased [57,70–75], as well as unaffected [68,70,76–78] E values are reported. Some of these observations can be rationalized as a kinetic effect by taking into account if the stereocenter resides in the alcohol part of the substrate molecule or in the acyl part [16]. Since water is a competitive nucleophile for the acyl enzyme intermediate, it is participating in an enantioselective step of the reaction if the acyl part of the acyl enzyme is chiral [16]. This suggests that an altered water activity leads to altered enantioselectivity only in the cases of a substrate with a chiral acyl part. Many of the effects of engineering of the reaction system, sometimes referred to as ‘medium engineering’ [53], on enzyme enantioselectivity is, however, unclear and it has been speculated that several different synergetic mechanisms might be present [46].

2.1.3. Substrate concentration

Another aspect of ‘medium engineering’ is the concentration of the substrate employed in the reaction. Since an esterification reaction catalyzed by a lipase in an organic solvent is reversible, it is common to use an excess of one of the substrates in order to displace the equilibrium position towards product formation. Due to the preference of the enzyme for the same enantiomer both in the forward and reverse reactions, shifting the equilibrium will influence the enantiomeric excess of the product. In the case of an esterification reaction in an organic solvent the backward reaction, hydrolysis, is of importance at high extents of conversion close to the equilibrium position. Displacing the equilibrium towards product formation would therefore

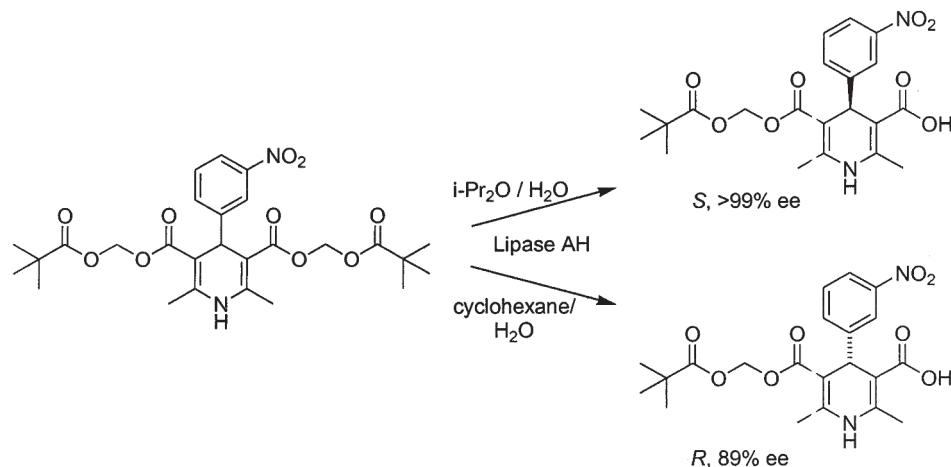
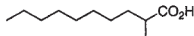
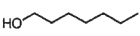
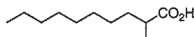
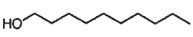
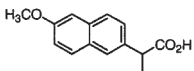


Fig. 1. Lipase AH-catalyzed hydrolysis of a prochiral diester shows opposite enantiomer preference in isopropyl ether than in cyclohexane [55]

Table 1

Influence of alcohol concentration on enantioselectivity in *C. rugosa* lipase-catalyzed esterification of chiral acids

Acid	Acid conc. (mM)	Alcohol	Alcohol conc. (mM)	<i>E</i>
	150		900	37 ^a
	150		150	70 ^a
	150		90	83 ^a
	150		900	67 ^a
	150		150	90 ^a
	150		90	100 ^a
	50		100	50 ^b
	50		50	90 ^b
	50		10	375 ^b
	50		1	350 ^b

^a Reaction run in cyclohexane. Data from Ref. [79]^b Reaction run in isooctane. Data from Ref. [81]

increase both the yield and the enantiomeric excess of the product at a high extent of conversion. However, in contradiction to the expected result, a molar excess of a simple primary alcohol leads to low enantiomeric excess of the formed ester product in *C. rugosa* lipase-catalyzed kinetic resolution of chiral acids [68,79]. This phenomenon of enantioselective inhibition by the alcohol has been observed recently and independently also by others [80,81]. In all cases, higher enantioselectivity is obtained when a lower amount of alcohol is employed in the reactions (Table 1).

The data in Table 1 are obtained using *C. rugosa* lipase. This lipase has a substrate-binding site that extends from the site of reaction through the interior of the enzyme similar to a tunnel [82]. This tunnel has been suggested, based on X-ray structures of enzyme-inhibitor complexes, as the binding site for an acyl part of a substrate [82]. However, in order to give a molecular-level explanation to the data on the influence of alcohol concentration on enantioselectivity (Table 1) [79], a molecular modeling study reveals two possible ways of binding a 2-methyldecanoic acid ester to the active site of *C. rugosa* lipase [83]. This work has been reviewed recently [84,85]. In contrast to the fast-reacting enantiomer, the slow-reacting *R*-enantiomer leaves the tunnel empty. It is thus suggested that the alcohol coordinates to the tunnel and inhibits the fast-reacting enantiomer and not the slow reacting one [83]. An increased alcohol concentration would therefore decrease the enantioselectivity in agreement with the data in Table 1. The generality of this behavior for other lipases is still unknown. However, as will be discussed below, enantiomeric substrate molecules are proposed to bind in different conformations in several enzymes. It can therefore be expected that their individual reaction rates would be influenced differently by a second non-chiral substrate and that the phenomenon of enantioselective inhibition by the alcohol therefore is not

limited only to enzymes having a tunnel-shaped binding site like the *C. rugosa* lipase.

2.2. Substrate structure

Lipase-catalyzed resolution of chiral carboxylic acids is kinetically more complex than the resolution of racemic alcohols since two diastereomeric acyl enzymes are formed in the former case. Both the formation and the breakdown of the acyl enzymes formed from a chiral acid pass through transition states that might be involved in defining the enantioselectivity [22,86]. In the cases of enzyme-catalyzed resolution of racemic chiral carboxylic acids or esters, a small change of the substrate can result in a complete switch in the enantiomer preference. This has been explored in our laboratories based on molecular modeling data from the study on *C. rugosa* lipase suggesting different modes of binding enantiomeric substrate molecules [83]. Different ways of binding enantiomers to an enzyme active site has been confirmed before. For instance, for the serine protease subtilisin, enantiomeric boronic acid inhibitors in complex with the enzyme and subjected to X-ray analysis confirms the presence of different orientations of the enantiomers in the active site [87]. Similarly, for compounds with a remote stereocenter, such as 4-methyl-branched acids, molecular modeling shows that the enantiomers orient differently in the active site of *C. antarctica* lipase B [88]. This is also the case for chiral secondary alcohols with *C. antarctica* lipase B [89].

The fact that the enantiomers of a chiral substrate may orient and bind differently to a lipase active site allows for the tailoring of enantioselectivity, and even of the enantiomer preference of the lipase. This can be achieved by engineering of the substrate molecule based on molecular modeling experiments. We have used this approach in the resolution of chiral carboxylic acids with two lipases and developed a strategy in each case.

In the case of *C. rugosa* lipase, which is a large lipase with a molecular weight of 60 kDa, molecular modeling suggests two different conformations, or modes, for each enantiomer in the enzyme active site [83], as schematically outlined in Fig. 2. One of the modes for the slow-reacting *R*-enantiomer is non-productive since the methyl group of the substrate distorts the active-site histidine residue. The fast-reacting *S*-enantiomer in complex with the enzyme is predicted by the modeling to be the lowest energy complex, in agreement with the experimental data. This fact suggests that if a substrate-engineering approach is used to block it from coordinating to the active-site tunnel, the lipase might invert its enantiomer preference since the *S*-enantiomer is changing from its fast to slow binding mode.

A diagnostic substrate analog has been designed and synthesized in order to experimentally test this. The analog has a thienyl group as a masked four-carbon unit at the end of the acyl chain [90]. The thienyl group is expected to be too sterically demanding for the acyl chain to be accommodated by the active-site tunnel. Hydrolyses reactions of the corresponding ethyl esters indeed show the predicted enantioreversal (Fig. 3 [90]). This represents the first kinetic evidence supporting the existence of two different modes of binding the enan-

tiomers to a lipase active site. A similar situation has been evidenced also in an organic solvent with the same lipase [91].

In the second case, a similar approach on the different and smaller lipase from *Humicola lanuginosa* (recently reclassified as *Thermomyces lanuginosa*), with a molecular weight of 30 kDa, was used. In contrast to *C. rugosa* lipase, *H. lanuginosa* lipase has a superficial active site with a hydrophobic crevice where an acyl chain of a substrate can bind [92]. Molecular modeling performed on both enantiomers of 2-phenoxy alkanolic acid ethyl esters [93] suggests two different modes of binding (Fig. 4). For the short substrate ($R = \text{CH}_3$), the mode where the phenoxy substituent is bound in the hydrophobic active-site crevice is the lower-energy complex. This is in agreement with the experimental data, which shows *R*-enantiomer preference by the lipase.

Molecular modeling indicates that an engineered substrate having a more hydrophobic substituent than a methyl group on the stereocenter alters the situation. We have designed and synthesized a number of substrate analogs that support our hypothesis by showing a reversed enantiomer preference (Fig. 5) [93].

For the shortest substrate containing a methyl substituent, the product is produced with *R* configuration

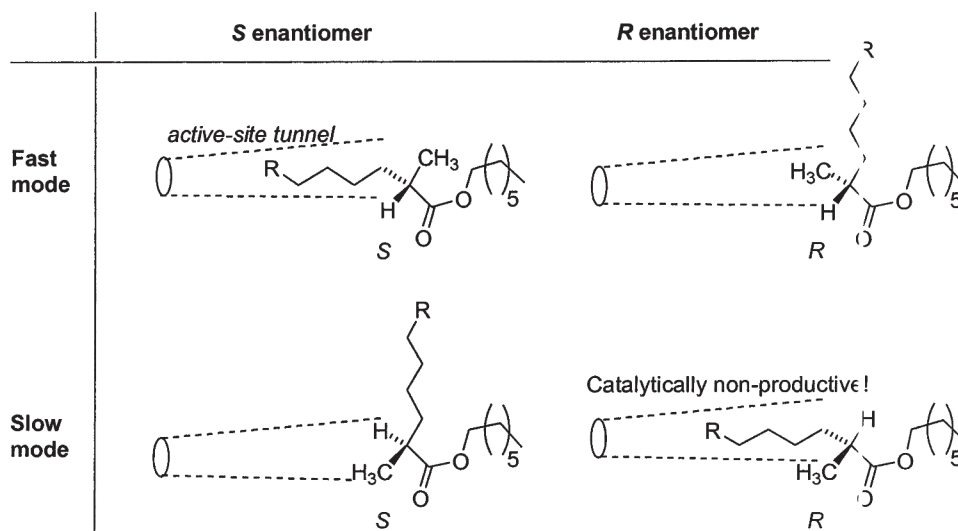


Fig. 2. Modes of the enantiomers of 1-heptyl 2-methyldecanoate ($R = n$ -butyl) in the active site of *C. rugosa* lipase suggested from molecular modeling [83]. The active-site tunnel is shown schematically. The strategy developed for obtaining a reversed enantiomer preference involves employing a more bulky acyl chain, R , which imposes the *S*-enantiomer to change from the fast to the slow binding mode.

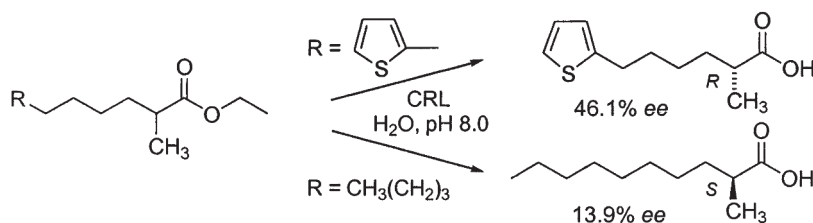


Fig. 3. Hydrolyses of the ethyl esters of the analogous substrates catalyzed by *C. rugosa* lipase (CRL) illustrating the reversed enantiomer preference induced by the bulky thienyl group. Data from Ref. [90].

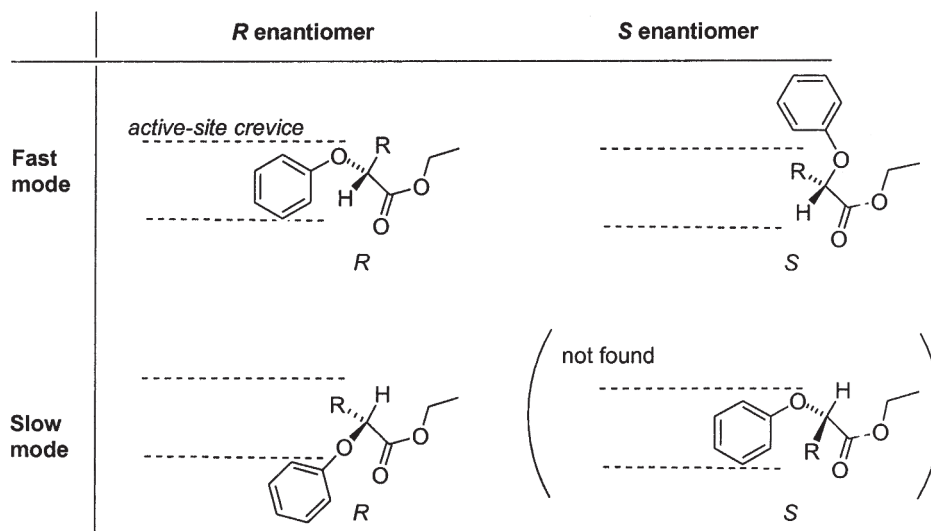


Fig. 4. Modes of the enantiomers of ethyl 2-phenoxypropanoate ($R = \text{CH}_3$) in the active site of *H. lanuginosa* lipase suggested from molecular modeling [93]. The strategy developed for obtaining a reversed enantiomer preference involves employing a more hydrophobic alkyl group, R , which is advantageous in terms of binding energy provided by the hydrophobic active-site crevice.

with an E value of 13, and for a substrate with a longer alkyl chain on the stereocenter the reaction with an E value of 56 produces the product acid with S configuration.

2.3. The lipase

2.3.1. Chemical modification

Chemical modification processes for improving the enantioselectivity of lipases have been successful in many cases. Many of these involve processes such as crosslinking and crystallization of the enzyme [94,95], organic solvent pre-treatment with 2-propanol [96] or acetone [97], lipid coating [98], entrapment in sol–gel matrices [99] and various membranes [100]. Chemical modifications of lipases has been summarized in a recent review article and will not be discussed further here [101].

2.3.2. DNA shuffling and directed evolution

Much of what we know today of enzyme structure–function relationships is based on rational enzyme redesign by site-directed mutagenesis. Several studies on altering enzyme activity and substrate selectivity through site-directed mutagenesis are summarized by Villeneuve et al. [101]. The process of site-directed mutagenesis for creating enzymes with improved properties is normally slow and too labor-intensive and allows for the production of a limited number of enzyme variants. Furthermore, the prediction of candidate sites for a site-directed mutagenesis study requires detailed knowledge of the three-dimensional structure of the enzyme, its catalytic mechanism, and some knowledge about structure–function relations. Instead, using nature's own approach of enzyme evolution is more feasible for a fast optimization of enzyme function.

Several strategies for generating enantioselective biocatalysts using combinatorial approaches have been explored recently. These involve proper molecular biological methods such as random mutagenesis or DNA shuffling [102,103] for generating large diverse DNA libraries, combined with gene expression [104] and coupled with high-throughput screening [105–107] for identification of mutant enzymes with improved biocatalytic properties. DNA shuffling involves in vitro homologous recombination of related genes. If repeated cycles of recombination are combined with error-prone PCR to introduce point mutations, this technique is then an efficient protein evolution strategy in vitro for generating enzymes with improved properties. From the outcome of various directed evolutionary strategies, much is learned regarding the factors and the structural elements controlling enzyme performance that can guide in more rational strategies for enzyme design/modification [108].

Directed evolution of enzymes involves the combination of mutagenesis and gene expression with high-throughput screening systems for identification of candidate enzymes with the desired improved proper-

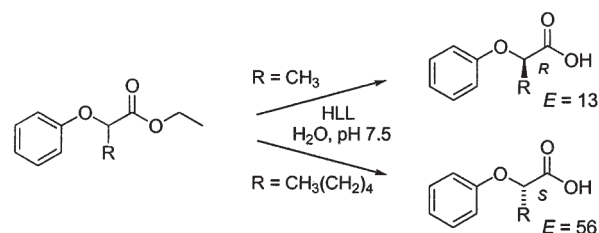


Fig. 5. Hydrolyses of the ethyl esters of the analogous substrates catalyzed by *H. lanuginosa* lipase illustrating the reversed enantiomer preference induced by the hydrophobic alkyl chain. Data from Ref. [93].

Table 2

Lipase-catalyzed resolution of 2-methyldecanoic acid esters by hydrolysis; literature data on improved enantioselectivity (E value)

Lipase	Enantiomeric ratio (E)
<i>Candida rugosa</i>	35 ^a
<i>Humicola lanuginosa</i>	
Wild type	10 ^b
E87A single mutant	17 ^b
<i>Pseudomonas aeruginosa</i>	
Wild type	1 ^{c,d}
Random mutagenesis	11 ^c
Random and saturation mutagenesis	26 ^d
<i>Geotrichum candidum</i>	
Wild type I	32 ^e
Wild type II	10 ^e
Hybrid	54 ^e

^a Ref. [125].

^b Ref. [126].

^c Ref. [120].

^d Ref. [127].

^e Ref. [128].

ties. Directed evolution of improved enzyme function for applications in chemistry is a powerful strategy that does not need any knowledge of enzyme structure and mechanism. It has been summarized in several review articles during the recent two years [109–118]. Normally, enzyme variants with moderate enantioselectivity factors (E values) of 10–20 are obtained after a few generations of mutagenesis from a wild-type enzyme showing no or very low enantioselection ($E = 1$) [119,120].

A random mutagenesis approach followed by screening for enzymes with improved biocatalytic properties has been pioneered by Arnold [121]. This directed evolution strategy is used to improve the enantioselectivity of *P. aeruginosa* lipase in the hydrolysis of a racemic 2-methyldecanoate ester [120]. The enantioselectivity of the lipase increases from $E = 1$ to 11.3 after four generations of mutagenesis (Table 2). Methyl-branched carboxylic acids of high enantiomeric purity are important building blocks in the synthesis of insect pheromones [122], chiral drugs [123], and liquid crystals [124]. 2-Methyldecanoic acid esters are also used as substrates in several other lipase-engineering studies for obtaining high enantioselectivity lipases (Table 2). The lipases are in these cases used in hydrolysis producing an enantiomerically enriched acid of S -configuration in all but one case (Fig. 6).

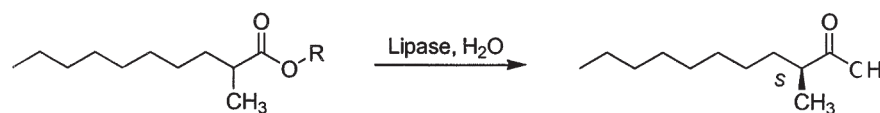


Fig. 6. Lipase-catalyzed resolution of racemic 2-methyldecanoic acid esters by hydrolysis. R = ethyl, n -heptyl, or p -nitrophenyl. R = phenyl produced an acid with R configuration. Data presented in Table 2.

The enantioselectivity of *C. rugosa* lipase in hydrolysis varies with the chain length of the alcohol part of the ester and the highest value is obtained for heptanol ester [125] (Table 2). This value serves as a reference for the engineering approaches with the other lipases shown in Table 2. The enantioselectivity of *H. lanuginosa* lipase toward the heptyl ester of 2-methyldecanoic acid improves from 10 to 17 by a single mutation (Glu87Ala) in the flexible lid region of the lipase [126]. The same mutant causes a minor decrease for the phenyl ester from $E = 2.9$ of the wild type to $E = 2.5$. However, the enantiomer preference is reversed with the phenyl ester. This study shows that targeting a flexible region in the enzyme has an impact on enantioselectivity.

Reetz and Jaeger use p -nitrophenyl ester of 2-methyldecanoic acid in a study of improving the enantioselectivity of *P. aeruginosa* lipase by a random mutagenesis approach [120,127]. The E value improves to 26 by a combination of error-prone PCR in three generations followed by saturation mutagenesis at identified key positions (Table 2). After sequencing and graphical analysis of the new enzyme variants, it is concluded that four out of five mutations in the variant with the highest enantioselectivity involves the introduction of a glycine residue. This can be expected to increase conformational flexibility of the lipase, a property that previously has been identified as an important factor for substrate selectivity [84]. Furthermore, all substitutions are far from the stereocenter of the substrate and are located directly or close to loops involved in the transition from the closed to the open form of the lipase [127]. It is thus evident that mobile regions are crucial high-enantioselectivity targets. Interestingly, the wild-type lipase shows virtually no stereoselection at all which allows for the evolution of R selective lipase mutants as well [117].

The fungus *Geotrichum candidum* produces two lipase isozymes with 86% sequence identity but with different enantioselectivity towards ethyl 2-methyldecanoate [128] (Table 2). In order to identify structural regions responsible for this change we have explored a method, recently reviewed [129], of swapping homologous regions between these related enzymes. By focusing on the mobile lid region of the lipase and on another flexible region known to influence fatty acid specificity in this lipase [130], exchange of these regions results in hybrid lipases among which one shows an enantioselectivity surpassing that of the best wild-type parent

isozyme [128]. Further site-directed mutagenesis studies within this hybrid identify residues that independently control enantioselectivity and total activity of the enzyme. Amino acid residues at the active-site entrance control enantioselectivity with maintained activity and residues at the active-site bottom control the total activity with maintained enantioselectivity [128]. This study illustrates that a rational approach, such as fractional isozyme recombination, can be successful when sufficient knowledge exist on which structural regions to be targeted. This version of DNA shuffling is then fast and straightforward and only a limited number of hybrid enzymes have to be investigated, which does not have to involve a high-throughput screening protocol.

3. Conclusions and outlook

One obstacle for synthetic chemists using biocatalysts is to identify the best commercially available wild-type enzyme with the highest enantioselectivity for a given transformation. This is sometimes difficult and time consuming and therefore, for example in the case of lipases, to guide in this process empirical rules predicting the preferred enantiomer have been developed for primary alcohols [131], secondary alkanols [132], and carboxylic acids [133]. These rules, or models, are of great value when synthetic routes are being planned but have limited value in the understanding of the enantioselective process and design of more selective systems. Usually only the preferred enantiomer can be predicted and not the extent of enantioselectivity. A quantitative estimation of the enantioselectivity is very difficult to achieve also by computational methods today [134–136] but several groups are working actively toward this important goal and useful predictions can be expected in the near future.

The major conclusion that can be drawn from this overview is that for a rational control of lipase enantioselectivity many different aspects should be taken into account. Engineering of the enzyme itself is fundamental but a rational approach is difficult. A random modification approach followed by efficient selection or screening can, after careful analysis, give important information on crucial structural changes necessary for obtaining high enantioselectivity enzymes. Parameters such as the reaction medium and substrate structure also play a major role and can markedly alter enantioselectivity, particularly due to differential binding modes of the substrate enantiomers. Chemistry in close symbiosis with molecular biology and modeling can be expected to be a successful strategy in many areas of catalysis in the future, especially for the tailoring and improvement of biocatalysts for applied chemistry.

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