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# Kinetic and modelling studies on the lipase catalysed enantioselective esterification of (±)-perillyl alcohol

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**Abstract**

Several lipases were kinetically studied with the aim to exploit their enantioselectivity in the esterification of (*S*)-(−) and (*R*)-(+)-perillyl alcohol with decanoic acid. Most of the lipases studied exhibited stereopreference towards the *R*-enantiomer with apparent *E*-values from 3.8 to 0.6, calculated as the initial esterification rates ratio for the individual enantiomers. In an attempt to interpret the structural basis of enantioselectivity, modelling studies were performed with two of these lipases, *Candida cylindracea* lipase (CcL) and *Pseudomonas cepacia* lipase (PcL) based on their previously determined X-ray crystal structures. The results derived from modelling studies confirm their stereopreferences towards the *R*-enantiomer, since increased conformational energy of the *S*-ester was found compared to the *R*-ester.


*Keywords:* Biocatalysis; Lipase; Enantioselectivity; Molecular modelling

## 1. Introduction

The use of biocatalysis by employing hydrolytic enzymes in non-aqueous media for the synthesis of compounds of biological interest has gained particular interest during the last years. Among the enzymes used lipases are the most common, partly because of the enantioselectivity they present towards a variety of substrates [1,2]. Chirality is a key feature in the efficiency of many drug products and agrochemicals, and consequently the production of single enantiomers of chiral intermediates has become increasingly significant in the pharmaceutical industry [3,4]. For this reason, understanding the molecular recognition of alcohols by lipases is essential in order to achieve the desirable biotransformation. This issue is rather complicated especially when dealing with primary alcohols, since most lipases show low enantioselectivity towards them.

In our previous report, we showed that *Candida antarctica* lipase B (CALB) discriminates the two enantiomers of (±)-perillyl alcohol in esterification reactions in favour of the *R*-enantiomer [5]. In this report, we present the results from kinetic experiments of the enantioselective esterification of (*S*)-(−)- and (*R*)-(+)-perillyl alcohol with decanoic acid catalysed by 11 lipases. Perillyl alcohol is a constituent of plant essential oils and has been reported to possess very interesting chemopreventive and chemotherapeutic activity against malignancies [6,7]. The esterification of the individual enantiomers of this monoterpene with fatty acids catalyzed by lipases is expected to increase their lipophilic properties and facilitate the research on their biological activity since the active enantiomer has not been specified yet.

*Abbreviations:* AoL, *Aspergillus oryzae* lipase; CALB, *Candida antarctica* lipase B; CcL, *Candida cylindracea* lipase; ClL, *Candida lipolytica* lipase; MjL, *Mucor javanicus* lipase; RmL, *Rhizomucor miehei* lipase; PrL, *Penicillium roqueforti* lipase; PPL, porcine pancreas lipase; PcL, *Pseudomonas cepacia* lipase; PfL, *Pseudomonas fluorescens* lipase; RaL, *Rhizopus arrhizus* lipase; *S*-POH, (*S*)-(−)-perillyl alcohol; *R*-POH, (*R*)-(+)-perillyl alcohol; *S*-ester, (*S*)-(−)-perillyl decanoate; *R*-ester, (*R*)-(+)-perillyl decanoate

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In an attempt to provide a structural basis of enantioselectivity, modelling studies were performed with CcL and PcL. Comparison of the kinetic and modelling results suggest that the conformational energy of the tetrahedral intermediate is an important determinant for enantioselectivity.

## 2. Experimental

### 2.1. Materials

Lipases from *Aspergillus oryzae*, *Candida cylindracea*, *Candida lipolytica*, *Mucor javanicus*, *Rhizomucor miehei*, *Penicillium roqueforti*, porcine pancreas, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and *Rhizopus arrhizus* (0.05, 0.002, 1, 5, 0.001, 2, 0.002, 0.05, 0.04 and 2 U/g, respectively) were kindly offered by Fluka Chemie GmbH (Buchs, Switzerland). Immobilized lipase B from *Candida antarctica* (Novozyme 435, 7 PLU/mg) was offered by Novo Nordisk (Baegsvaerd, Denmark). (*S*)-(−)- and (*R*)-(+)-perillyl alcohol were purchased from Fluka Chemie AG (Buchs, Switzerland) and decanoic acid from Sigma (Steinheim, Germany). Hexane was of analytical grade and was stored over dry 3 Å molecular sieves (Fluka Chemie GmbH, Buchs, Switzerland) for at least 48 h prior to use.

### 2.2. Esterification reactions

The esterification of the enantiomers of perillyl alcohol (*S*-POH or *R*-POH, 83.5 mM) with decanoic acid (83.5 mM) was conducted at 50 °C and 150 rpm in separate vials with dry hexane (5 ml), using the appropriate amount of lipase. Gas chromatography analyses with a chiral a-DEX 120 column (Supelco, Bellefonte, PA, USA) were carried out as previously reported [5]. The enantioselectivity of the lipases was expressed as an apparent *E*-value, calculated as the initial rates ratio for the esterification of the individual enantiomers, $E = v_0^R/v_0^S$, as shown in Table 1. The data reported in this paper represent the mean value from triplicate experiments (S.D. ≤ 8%).

Table 1
Calculation of the apparent enantioselectivity of various lipases in the esterification of (±)-perillyl alcohol with decanoic acid

| Lipase | Initial esterification rate (mM/h) | | Apparent enantioselectivity ($v_0^R/v_0^S$) |
|---|---|---|---|
| | (*S*)-(−)-perillyl alcohol | (*R*)-(+)-perillyl alcohol | |
| CcL | 142.9 | 550.1 | 3.8 |
| PrL | 22.8 | 48.7 | 2.1 |
| CALB | 210.6 | 427.8 | 2.0 |
| PcL | 5.4 | 8.4 | 1.6 |
| MjL | 27.6 | 36.6 | 1.3 |
| PPL | 15.6 | 18.0 | 1.2 |
| RaL | 49.2 | 56.5 | 1.1 |
| AoL | $25.4 \times 10^{-3}$ | $23.7 \times 10^{-3}$ | 0.9 |
| ClL | 4.8 | 3.4 | 0.7 |
| PfL | $254.9 \times 10^{-3}$ | $190.3 \times 10^{-3}$ | 0.7 |
| RmL | $155.3 \times 10^{-3}$ | $95.3 \times 10^{-3}$ | 0.6 |

### 2.3. Modelling

The atomic coordinates of CcL and PcL crystal structures used for modelling were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/) (1CRL and 3LIP, respectively). The model of the tetrahedral intermediate formed during the esterification of perillyl alcohol with decanoic acid was generated using the program *SYBYL* [Tripos Associates Inc. (1992), SYBYL Molecular Modelling Software, St. Louis, Missouri, USA]. Conformational energy calculations were also performed with the same program using the Powell minimizer and default parameters. *S*-ester and *R*-ester were fitted manually into the catalytic site of each enzyme with the molecular graphics program O [8]. Modelling studies were performed with *CNS* Version 1.1 using 40 000 steps of conjugate gradient minimisation with no experimental energy terms, as implemented by the program [9]. Superposition of the *S*-ester and *R*-ester coordinates before and after binding to the active site was done by the program *LSQMAN* [10]. The figures were prepared with *MOLSCRIPT* [11] and rendered with *RASTER3D* [12].

## 3. Results and discussion

A preliminary study on lipase enantioselectivity on the esterification of (±)-perillyl alcohol with decanoic acid reported previously, showed that CALB exhibits selectivity for *R*-POH; the esterification of *R*-POH proceeded two times faster than the esterification of *S*-POH [5]. With the aim to explore further lipase enantioselectivity, other lipases were also used: 1 pancreatic and 10 microbial lipases, all in their free form, except CALB, which was used immobilized. CcL showed the highest selectivity in favour of *R*-POH with an apparent *E*-value 3.8 (Table 1). The other lipases exhibited lower enantioselectivity towards the same enantiomer with the exception of AoL, PfL, ClL and RmL which showed a minor preference in favour of *S*-POH.

In an attempt to interpret the results obtained from the kinetic experiments, a theoretical approach was employed to mimic the tetrahedral intermediate (enzyme–ester complex) formed during the reaction. Conformation energy calculations were performed before docking the ligands (*S*-ester and *R*-ester) to the substrate binding site. Two lipases were used for this purpose (CcL which showed the highest selectivity and PcL with moderate selectivity, both towards the *R*-enantiomer) for which the crystal structures in the free form and complexes with inhibitors or transition state analogues were available.

The mode of binding of *S*-ester and *R*-ester to the crystal structure of CcL as determined by Grochulski et al. [13] was first explored. The orientation of the enantiomers of the perillyl decanoate was adjusted to fit in the catalytic site of the

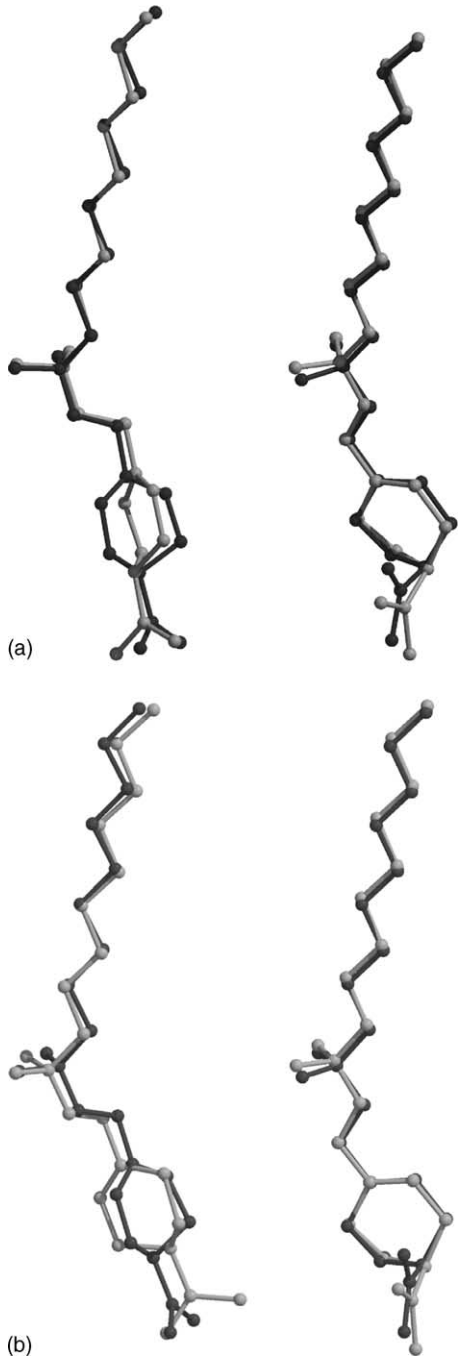


Fig. 1. Superimposed structures of the enantiomers of perillyl decanoate modelled in (a) CcL and (b) PcL onto the conformation of the enantiomers of the ester before fitting in the binding site (shown in light and dark grey, respectively): (left) *S*-enantiomer; (right) *R*-enantiomer.

lipase taking into account that a covalent bond with Ser209 located at the catalytic site is formed. The crystal structures of CcL complexed with phosphonates covalently linked to the catalytic site serine [14] were also superimposed onto the native structure [8] to guide fitting of enantiomers. Comparison of the ligand conformation on binding to CcL as calculated by positional refinement, with the computed (by SYBYL) conformation shows that the r.m.s. deviation for

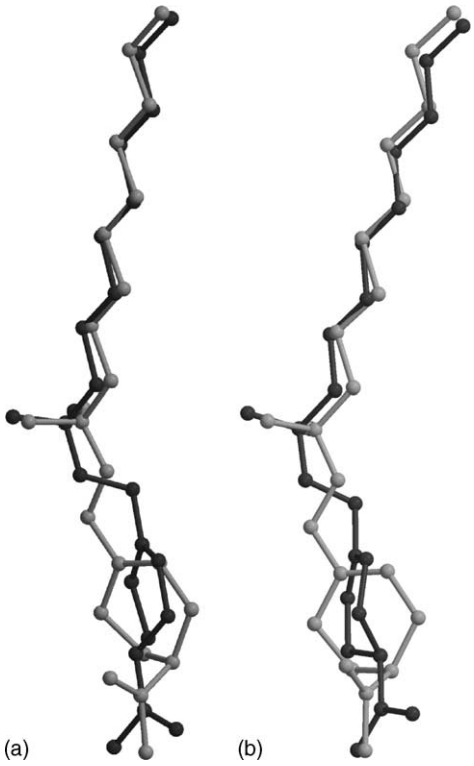


Fig. 2. Superposition of the modelled structures of the *S*-ester and *R*-ester (shown in dark and light grey, respectively) of perillyl decanoate in the (a) CcL and (b) PcL structures.

all atoms of the *S*-ester is 0.844 Å and for the *R*-ester is 0.410 Å (Fig. 1a). The superimposed structures of the two enantiomers of perillyl decanoate after their binding in the catalytic site of CcL are shown in Fig. 2a. The difference observed suggests that the increase in conformational energy of the *S*-ester might account for its low binding energy (compared to the *R*-ester), in agreement with the results obtained from the enantioselectivity measurements (Table 1).

In the case of PcL [15–17], a plausible model of the complex structure with the tetrahedral intermediate also derived with both enantiomers to be covalently bonded to Ser87 of the catalytic site. Superposition of the *S*-ester and *R*-ester before and after docking is shown in Fig. 1b and the r.m.s. deviation for all atoms is 1.043 and 0.406 Å, respectively. The superimposed structures of *S*-ester and *R*-ester after their binding in the active site of PcL are shown in Fig. 2b. In conformational energy terms, the *R*-ester binding is likely to be more favourable against *S*-ester in agreement with the apparent *E*-value calculated for both enantiomers (Table 1).

## 4. Conclusions

Eleven lipases were used in kinetic experiments in order to explore their ability to discriminate the two enantiomers of (±)-perillyl alcohol in esterification reactions with decanoic acid. Most of the lipases tested showed a stereopreference towards the *R*-enantiomer with low apparent *E*-values (high-

est selectivity was found for CcL lipase), while some of them showed a minor preference towards the *S*-enantiomer. In an effort to comprehend the results obtained, modelling studies using conjugate gradient minimization with no experimental energy terms were performed with two lipases (CcL and PcL). Conformational analysis showed that the bound conformation of the enantiomers of perillyl decanoate is different from the computed minimum free energy conformation in agreement with the kinetic results. A systematic study of molecular dynamics free energy simulations is required in order to calculate the energetic contributions more accurately and take into account the contribution of the solvent. X-ray crystallographic studies are also absolutely essential to map the key interactions at the catalytic site that would enhance our understanding on the recognition pattern these molecules follow and give new insights in protein engineering.

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