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A molecular modelling approach to rationalize the stereochemical outcome of the *Burkholderia cepacia* lipase-catalyzed transesterification of aromatic primary alcohols with vinyl esters with different chain lengths in chloroform

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ABSTRACT

The *Burkholderia cepacia* lipase-catalyzed transesterification of 2-methyl-3-phenyl-1-propanol with vinyl esters proceeds with high enantioselectivity independently of the acyl chain length and the low enantioselectivity of the same reaction with 2-phenyl-1-propanol is not affected by chain length of the vinyl esters. A molecular modelling approach has been developed in order to rationalize the enzymatic results.
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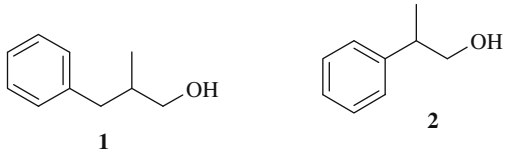
1. Introduction

The *Burkholderia cepacia* lipase (BCL), formerly known as *Pseudomonas cepacia* lipase (PCL), is a highly selective catalyst for a broad range of substrates, catalyzing various reactions in water and non-polar solvents under mild conditions.[1] This includes the kinetic resolution of racemic secondary alcohols by hydrolysis in water or esterification in organic solvents.[2–4] The stereochemical outcome of the lipase-catalyzed reactions can be interpreted according to various models of the enzyme active site.[5] In particular, the enantiospecificity of the lipase from *P. cepacia* (PCL)[6] has been extensively investigated and a model can be adopted as a predictive rule for secondary alcohols.[7] This model has been extended to primary alcohols with a lower degree of confidence, since, in this case, it is more difficult to predict the stereopreference of the enzymatic reaction.[8] In the case of a primary alcohol, the enantioselectivity is frequently from low to moderate and can be increased by optimizing the reaction conditions. For instance, we were able to achieve a high enantioselectivity in the acetylation of the primary alcohol 2-methyl-3-phenyl-1-propanol **1**.[9] The reaction was stopped at approximately 40% conversion and the (*S*)-acetate was obtained in enantiomerically pure form. The unreacted alcohol could be recovered at the highest enantiomeric excess (ee) when the reaction was carried out at 60% conversion.[10] The alcohol **1** has frequently been used as a model for studies on the mechanism and stereochemistry of PCL-catalyzed hydrolysis of esters of primary alcohols.[11,12] From a stereochemical point of view, the same enzymatic reaction proceeds in an unsatisfactory manner with 2-phenyl-1-propanol (compound **2**, Fig. 1).[13] This relevant difference in the stereoselective outcome of the enzymatic reaction for the alcohol **2** is most probably related to the subtle structural difference that exists between the two homologous alcohols **1** and **2**.



1 **2**

Figure 1. Structure of the two aromatic primary alcohols 2-methyl-3-phenyl-1-propanol **1** and 2-phenyl-1-propanol **2**.

Recently, we have shown that the lipase-catalyzed resolution of the esters (*R,S*)-**3a–d** affords (*S*)-**1**, with the ee depending on the ester chain length.[14] In fact, the excellent enantioselectivity of the PCL-catalyzed hydrolysis of esters **3a** and **3b** dramatically fell when the chain of the fatty acid was lengthened to esters **3c** and **3d** (Scheme 1).

Using a two-step MM-based modelling approach, it was proposed that the drop in enantioselectivity was determined by an overflowing conformational rearrangement due to the refolding of the fatty acid chain inside the hydrophobic binding pocket. Consequently, the compulsory increase in the activation energy of the rate-determining step leading to the first tetrahedral intermediate becomes a relevant factor. This effect was confirmed by calculating the free energy differences between the complexes of PCL with the (*R*)- and (*S*)-enantiomers.[14]

The foregoing results prompted us to undertake research aimed at verifying whether similar conformational effects could be evidenced in an organic solvent. Therefore, we have extended the

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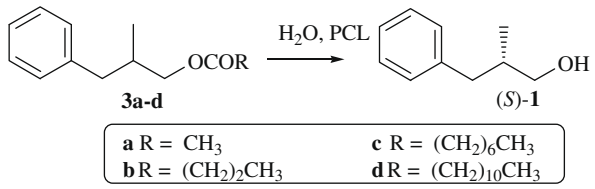
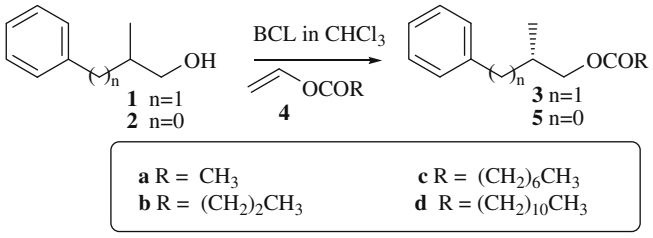


Scheme 1. The stereochemical outcome of the PCL-catalyzed hydrolysis of esters **3a–d**.

study of the chain length effect to the BCL-catalyzed transesterification of alcohols **1** and **2** using vinyl esters of different chain lengths.

2. Results and discussion of the enzymatic results

The transesterification reactions of alcohols **1** and **2** were carried out at room temperature in chloroform with commercially available vinyl esters **4a–d** (see Scheme 2).[15]



Scheme 2. BCL-catalyzed transesterification of alcohols **1** and **2** with vinyl esters **4a–d**.

We have found that, independent of the chain length of the vinyl ester used, the enantioselectivity of the reaction with the alcohol **1** at about 30% conversion (expressed as enantiomeric ratio E[16]) remained considerably high. The enzymatic transesterification of 2-phenyl-1-propanol **2** with vinyl esters **4a–d** was slower than that of the alcohol **1** (20–30 h for 25–35% conversion) and low enantiomeric ratio values (ca. 2) were obtained, irrespective of the chain length of vinyl esters. All the results obtained from the transesterifications are collected in Table 1.

Table 1
BCL-catalyzed transesterification of alcohols **1** and **2** with vinyl esters **4a–d**

Product	Acyl donor	Time (h)	Conversion[a] (%)	ee[b]	E[c]
3a	**4a**	3	31	>98	>100
3b	**4b**	4	33	>98	>100
3c	**4c**	3	32	>98	>100
3d	**4d**	5	31	>98	>100
5a	**4a**	24	40	39	2.9
5b	**4b**	20	21	35	2.3
5c	**4c**	20	33	15	1.6
5d	**4d**	30	35	22	1.4

[a] Determined by GC analysis.[15]
[b] Enantiomeric excess evaluated by ^{1}H NMR analysis of the MTPA esters[17] of alcohols **1** and **2** obtained from the hydrolysis of the esters **3a–d** and **4a–d**.[18]
[c] Enantiomeric ratio calculated according to Chen et al.[16]

Also the result obtained with the alcohol **2** is interesting, because in many other examples the change of the acyl group was sufficient to increase the enantioselectivity of this reaction.[19] However, it has also been observed that the enzymatic resolution of alcohol **2** showed a negligible enantioselectivity (E <2) under several conditions such as transesterification,[20–22] alcoholysis[23] or acylation.[24,25] In order to rationalize the above BCL-catalyzed enzymatic transesterification of alcohols **1** and **2**, molecular modeling studies were carried out.

3. Molecular modelling results and discussion

Molecular modelling studies involved a preliminary MD simulation of the enzyme in chloroform with a view to revealing possible conformational shifts induced by the apolar medium. The so-obtained BCL structure was then used in docking analyses to account for the different enantioselectivity in the transesterification of most significant esters. In order to rationalize the transesterification data in silico, docking simulations for the aromatic primary alcohols **1** and **2** were performed considering the Ser87 residue covalently bound to the acyl group that should lead to the formation of the esters **3a–d** and **5a–d**. The resolved structure of BCL was retrieved from PDB (Pdb Id: 3LIP).[26] The structure was completed by adding the hydrogen atoms. According to the physiological pH, glutamate, aspartate, lysine, and arginine residues were considered ionized while histidine and cysteine residues were simulated as neutral by default. The calcium ion which plays a structural role in BCL was maintained in the simulation, while the crystallization water molecules were removed to better enlighten the effects of apolar medium. The structure underwent a minimization keeping the backbone atoms fixed to preserve the experimental folding, and then it was inserted in a 70 Å side cubic box of chloroform solvents.[27] The simulations were carried out in two phases: an initial period of heating from 0 K to 300 K over 6000 iterations (6 ps, i.e., 1 K/20 iterations), and a monitored phase of simulation of 1 ns. Only the frames memorized during this last phase were considered. The duration of an MD run was suitably chosen to reveal significant conformational shifts induced by an apolar solvent without affecting the folding stability. The final structure was then minimized and used to model the covalently modified enzyme after deleting the solvent molecules. In particular, Ser87 was acylated to simulate the first step of the transesterification process**.** Among the acyl groups, calculations were limited to the acetyl group that should produce the acetates **3a** and **5a** in the classical procedure that uses vinyl acetate as an acyl transfer agent. The octanoyl group was selected as a typical medium-long chain example of an acyl group that yields the octanoates **3c** and **5a**. The two covalently modified enzyme structures were finally minimized and exploited in docking calculations. The simulated aromatic primary alcohols (**1** and **2**, considering for both substrates the two possible enantiomers) were built by VEGAZZ[28] and optimized by MOPAC2009. The modified structures of BCL were prepared by AutoDock Tools as implemented in VEGAZZ software. The 3D affinity grid box was designed to include the catalytic pocket around the modified Ser87 residue. Docking searches were performed by AutoDock version 4 with the Lamarckian genetic algorithm.[29] The lowest energy pose was further minimized to optimize the mutual complementarity between the enzyme and substrate. All minimizations as well as the described MD run were carried out by NAMD 2.6.[30]

A detailed description of the conformational changes that the BCL enzyme experiences during the MD run in chloroform goes beyond the objectives of this study, but the analysis of the trajectory produced allows some relevant considerations. Firstly, the performed simulation does not vastly alter the experimental folding as assessed by the percentage of residues falling in the allowed regions of the Ramachandran plot, which remained constantly around 75% during the MD run. Globally, such a result suggests that the structural differences described below are mainly due to the dynamic response of the enzyme to the apolar medium and not to random structural distortions. Secondly, the analysis of surface profiles discloses a divergent behavior since the SAS surface slightly increases during the simulation suggesting a further opening of the lid domain which exposes its apolar side chains, as recently evidenced by longer simulations in apolar solvents.

Conversely, the polar surface shows a marked diminution suggesting that the apolar solvent induces a hydrophilic collapse in which the polar residues minimize their unfavorable contacts with the medium. Lastly, such a hydrophilic collapse reflects on the catalytic center which significantly decreases its wideness. This is probably due to the approaching of polar residues as clearly assessed by the volume of catalytic cavity which almost halves during the simulation shifting from 1533 Å^3 to 871 Å^3 (as computed by CASTP server). It should be noted that during the MD simulation the catalytic cavity includes several solvent molecules that should prevent an unrealistic collapse of the cavity despite the removal of water molecules. These results are in line with those reported by previous studies[31,32] and suggest a contrasting situation since the enzyme in chloroform should possess a more accessible, but less wide, catalytic cavity.

As a preamble, it should be noted that the two acyl groups are conveniently accommodated in the catalytic cavity. In both structures the carbonyl group interacts with Tyr23 and to a minor extent with Tyr29. The acetyl group realizes hydrophobic contacts with Tyr29, His86, and Ile290, while the octanoyl chain is inserted in a region lined by apolar residues (Ile33, His86, Ile110, Pro304, Val305, Val307, and Ile308) with which it can stabilize many hydrophobic interactions. Figure 2A depicts the putative complex between (*S*)-**1** and the BCL enzyme covalently bound to octanoyl

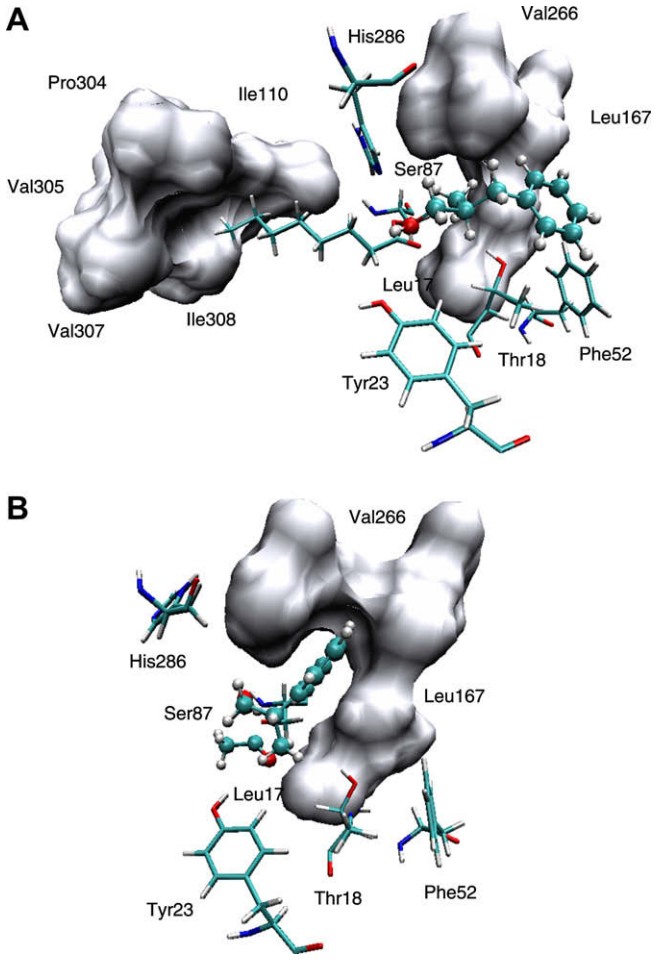


Figure 2. Putative complexes between octanoyl-Ser87 BCL and (*S*)-**1** substrate (A) as well as acetyl-Ser87 BCL and (*S*)-**2** (B). The hydrophobic residues are represented by gray surfaces. In both complexes the alcohol approaches the catalytic residues and the main differences concern the hydrophobic contacts since (*S*)-**1** suitably inserts the methyl in the hydrophobic pocket lined by Leu 17, Leu167, and Val266, while (*S*)-**2** accommodates the phenyl ring into the apolar cavity losing the pivotal stacking with Phe52 and bumping the methyl against Thr18.

to yield (*S*)-**3c** ester. It reveals that the substrate can be conveniently accommodated in the catalytic center since the hydroxyl group approaches Ser87 and interacts with Tyr23 and Thr18. The methyl group is inserted in a suitable subpocket lined by aliphatic residues (e.g., Leu17, Leu167, and Val266), while the phenyl ring interacts with the apolar residues already mentioned for the methyl group and stabilizes π-π stacking with Phe52 and Phe119 (not shown in Fig. 2 for clarity). Such a precise interaction pattern is likewise also found with the acylated enzyme and can explain well the enantioselectivity in the transesterification of (*S*)-**1**. In fact, the (*R*)-**1** alcohol can be harboured in the BCL catalytic cavity, whereas the methyl group clashes against Leu17 moving the ester function away from Ser87.

Docking results can also explain the lack of enantioselectivity for the transesterification of the alcohol **2,** since the methyl group detrimentally clashes against catalytic residues in both isomers. In particular, the methyl group bumps against the hydroxy function of Thr18 in (*R*)-**1** isomer (as seen in Fig. 2B) and approaches Ser87 hampering the catalytic mechanism in the (*S*)-**1** isomer. Furthermore, both enantiomers of alcohol **2** lose the π–π stacking with Phe52 and Phe119 which characterizes the complexes of **1** isomers.

Table 2 shows the docking scores for the optimized complexes as defined by electrostatic energies. Such energy values can well rationalize the experimental data, since the electrostatic energies for the two enantiomers of **1** are markedly different. This justifies the reported enantioselectivity for this substrate, while the difference between the isomers of the alcohol **2** is clearly reduced. Furthermore, the calculated energy scores are almost independent of the length of the acyl chains. Although these substrates are substantially apolar molecules, the score functions were focused on polar interactions since they play a key role in triggering the catalytic mechanism as recently confirmed by us.[33]

4. Conclusions

Our results show that in the transesterification of primary alcohols **1** and **2** with various vinyl esters, the length of the acyl chain does not influence the stereochemical outcome of the process. In fact, the enantioselectivity remains high or low, depending significantly on the specific structure of the substrates **1** and **2** that present a phenyl ring that is close to the stereocenter or part of it. Thus, π–π interactions of the substrate with aromatic residues present in the active site might become significant. Computational studies were desired for an explanation of the reported observations. However, different to the lipase-catalyzed hydrolytic process of esters,[12,32] only a few molecular modeling studies have been proposed to explain the results of transesterification of primary alcohols catalyzed by a lipase in an organic solvent.[34]

The computational studies performed suggest that the BCL structure, as derived by MD simulation in chloroform, can account for the different enantioselectivity observed in the resolution of

Table 2
Electrostatic energies and differences between enantiomeric esters obtained by docking simulations

Alcohol	Acyl	Ester produced	Electrostatic energies (kcal/mol)	Isomeric difference
(*S*)-**1**	Acetyl	(*S*)-**3a**	−21.28	8.51
(*R*)-**1**		(*R*)-**3a**	−12.77	
(*S*)-**2**		(*S*)-**5a**	−14.03	1.14
(*R*)-**2**		(*R*)-**5a**	−12.89	
(*S*)-**1**	Octanoyl	(*S*)-**3c**	−18.29	7.19
(*R*)-**1**		(*R*)-**3c**	−11.10	
(*S*)-**2**		(*S*)-**5c**	−12.33	1.21
(*R*)-**2**		(*R*)-**5c**	−11.12	

substrates **1** and **2.** In fact, using appropriate docking simulations, an insightful rationalization of the enzymatic data is possible and docking results are able to explain the experimental results in terms of specific interactions of the substrates with aminoacid residues of the catalytic site. Finally, our approach opens the opportunity of exploiting such simple but successful modeling analyses to predict the stereochemical outcome of the resolution of other substrates.

Acknowledgement

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18. The ee of the enzymatic transesterification was established by ^{1}H NMR analysis of the MTPA esters of alcohols **1** and **2** obtained by hydrolysis of the enzymatically formed esters **3a**–**d** and **5a**–**d**. This hydrolysis was effected most satisfactorily with lithium aluminum hydride, since after the work-up, the alcohol was obtained practically pure and was used as such for specific rotation measurement or evaluation of ee. Thus, to a solution of the ester (1 mmol) in dry diethyl ether (2 mL), lithium aluminum hydride (0.38 g, 10 mmol) was added and the mixture was stirred for the time required to complete hydrolysis. To the above mixture, water (0.4 mL), 15% sodium hydroxide (0.4 mL), and water (1.2 mL) were added sequentially. The solid was removed by filtration on a Celite pad and washed with diethyl ether (2 × 1 mL) and evaporation of the solvent afforded the pure alcohol.
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