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Molecular recognition of *sec*-alcohol enantiomers by *Candida antarctica* lipase B

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Abstract

A model to explain the enantioselectivity of *Candida antarctica* lipase B towards *sec*-alcohols based on structure activity and molecular modelling is presented. The origin of the enantioselectivity was found to be due to different modes of binding for the enantiomers. The fast enantiomer places its medium substituent in a site of limited size, the stereoselectivity pocket, whereas the slow enantiomer has to position the large substituent in that same pocket. Our model is in agreement with the 24 different substrates tested. Only substituents smaller than *n*-propyl can be accommodated by the stereoselectivity pocket. Moreover, important unfavourable electrostatic interactions are involved between this region and halogenated substituents. The former requirement entails a high enantiomeric ratio (E) for *sec*-alcohols with a medium group smaller than *n*-propyl and a large group larger than *n*-propyl. The latter requirement allows high E only for short chain *vic*-halogenated alcohols.

Keywords: Kinetic resolution; Molecular modeling; Transesterification; Enzyme–substrate interaction

1. Introduction

Lipases are among the most attractive catalysts for asymmetric synthesis due to their broad substrate specificity, their ease of use and, often, high enantioselectivity [1,2].

The catalytic mechanism has been elucidated [3] and simple rules to predict the fast enantiomer defined [for example, Ref. [4]]. However, our knowledge of the factors which determine the stereospecificity is still limited.

We have investigated the enantioselectivity of *Candida antarctica* lipase B (CALB) towards acyclic *sec*-alcohols in order to delineate the substrate structure requirements and understand how CALB discriminates between enantiomers. A molecular explanation of the sub-

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strate specificity has been proposed based on structure activity studies and molecular modelling.

2. Experimental and results

2.1. Kinetic resolution

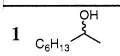
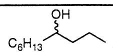
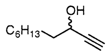
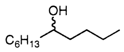
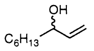
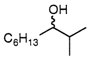
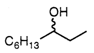
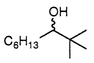
Enantioselective transesterifications of substrates **1–17** (Tables 1 and 2) and substrates **4**, **11**, **19–24** (Table 3) mediated by CALB were reported earlier [5,6]. Data concerning substrate **18** were gathered from the literature [7]. Transesterification for substrate **21** was carried out as described in reference [6] and the absolute configuration was assigned by comparison of the specific rotation with literature data. All experiments reported in Tables 1 and 2 were carried out under identical conditions (*S*-ethyl thiooctanoate, 39°C, neat) in order to compare the enantioselectivity. Different acyl donors were used for the alcohols reported in Table 3 (vinyl alkanoate, 18–23°C, C_6H_{14} except for substrate **22**: CH_2Cl_2) in order to make possible a direct analysis of the enantiomeric excess of the products by GC.

In a first set of experiments, we were interested in probing the size limitation for the medium sized (*M*) substituent (Table 1). High enantiomeric ratios (*E*) were found for long chain alcohols with an *M* substituent smaller than *n*-propyl. Further extension of the *M* group entailed a drop in activity and enantioselectivity. Thus, we concluded that the enzyme provides space enough to accommodate an *M* substituent smaller than an *n*-propyl group.

All substrates tested with CALB followed the empirical rule proposed by Kazlauskas et al. [4] for the prediction of the fast reacting enantiomer. When the *M* group was kept fixed, *E* increased while increasing the size of the large (*L*) substituent (Table 2). The only exception to this trend was 3,3-dimethyl-2-butanol, for which a much lower reaction rate than all the other substrates listed in Table 2 was noted. A drastic difference in selectivity between 2-butanol and 2-pentanol or 3-methyl-2-butanol was also noted even though their large groups differ only by one methyl. An explanation is presented in Section 3.

Substrates presented in Tables 1 and 2 differ mainly in size. Therefore, *E* varies for those substrates as a function of the van der Waals interactions between the enzyme and the substrate. In order to determine if other interactions are important for the enantioselectivity, we

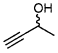
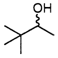
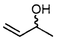
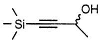
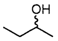
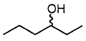
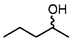
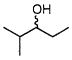
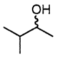
Table 1
Enantioselectivity of CALB towards long chain alcohols

Substrate	*E*[a]	*f.e.*[b]	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})	**Substrate**	*E*[a]	*f.e.*[b]	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})
1 	>340	*R*-(-)	>3.6	**5** 	10	*n.d.*	1.4
2 	160	*S*-(-)	3.1	**6** 	3	*n.d.*	0.7
3 	160	*S*-(-)	3.1	**7** 		*n.r*	
4 	340	*R*-(-)	3.6	**8** 		*n.r*	

[a] Enantiomeric ratio calculated according to Rakels et al. [8].

[b] Fast reacting enantiomer, *n.d.* not determined, *n.r.* no reaction.

Table 2
Enantioselectivity of CALB towards short chain alcohols

Substrate		E^a	*f.e.*^{b}	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})	Substrate		E^a	*f.e.*^{b}	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})
9		1	*n.d.*	0.2	**14**		350	*R*-(-)	3.6
10		7	*R*-(-)	1.2	**15**		>800	*R*-(+)	>4.1
11		9	*R*-(-)	1.4	**16**		97	*R*-(-)	2.8
12		390	*R*-(+)	3.7	**17**		109	*R*-(+)	2.9
13		705	*R*-(-)	4.1					

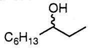
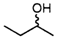
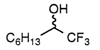
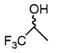
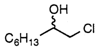
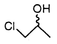
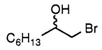
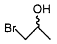
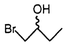
[ab] As described in Table 1.

tested several halogenated *sec*-alcohols. The difference in enantioselectivity was surprisingly high between aliphatic and *vic*-chlorinated or *vic*-brominated *sec*-alcohols of similar size. Substrates with a halogen atom on the *M* substituent (entries **18**, **19** and **20**) had a lower enantioselectivity than their aliphatic analogue **4**. The opposite effect occurred when the halogen atom was placed on the *L* substituent (entries **21**, **22** and **23**). Enantioselectivity remains high for **24** which has two substituents of similar size, thereby showing the importance of electrostatic interactions for *vic*-halogenated compounds.

2.2. Molecular modelling

Serine 105 in the crystal structure of CALB was covalently connected to the tetrahedral intermediate, consisting of the acyl donor and the

Table 3
Comparison of CALB enantioselectivity towards aliphatic and *vic*-halogenated alcohols

Substrate		E^a	*f.e.*^{b}	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})	Substrate		E^a	*f.e.*^{b}	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})
4		340	*R*-(-)	3.4	**11**		9	*R*-(-)	1.4
18^c		>274	*S*	>3.3	**21**		16	*R*-(+)	1.6
19		14	*S*-(-)	1.5	**22**		164	*R*-(-)	3.0
20		7	*S*-(-)	1.2	**23**		371	*R*-(-)	3.5
					24		81	*R*-(-)	2.6

[ab] As described in Table 1.
[c] Refer to Hamada et al. [7].

alcohol moiety, in order to simulate the transition state formed in the deacylating step [5]. Enantiomers of substrates **10**, **11**, **12**, **13** and **14** were manually docked in two different starting conformations in the alcohol site and subjected to molecular dynamics and subsequent energy minimisation. Analysis of the resulting potential energies and visual inspection of the different minimised structures provided information about plausible orientations of the enantiomers. In a first set of simulations, the correct fast reacting enantiomer was predicted for four of the five substrates tested [5]. In a somewhat different calculation scheme, simulations predicted all the fast reacting enantiomers correctly [9]. In both studies, the alcohol moiety was found to be oriented differently for the fast and slow reacting enantiomers. Fast reacting enantiomers bind with the *M* substituent in a small pocket situated at the bottom of the active site and the *L* substituent pointing out towards the surface of the enzyme. This pocket will be referred to as the stereoselectivity pocket and this orientation of the substrate as docking mode I. Docking mode I was found to be a non-productive mode for slow reacting enantiomers. Instead these enantiomers bound with the *L* substituent in the pocket and the *M* substituent pointing towards the entrance of the active site (docking mode II).

3. Discussion

The dramatic increase in enantioselectivity for 2-pentanol ($E = 390$) compared to 2-butanol ($E = 9$) could be rationalised with the help of molecular modelling. *R* enantiomers bind with the *M* substituent in the stereospecificity pocket. The pocket provides enough space to accommodate each *M* substituent in docking mode I. In order to be catalysed, *S* enantiomers have to place the large substituent in the stereospecificity pocket. In the latter docking mode II, the pocket provides room enough to fit the *L* group of (*S*)-2-butanol. Modes I and II can thus operate simultaneously on the *R* and *S* enantiomers respectively, resulting in a low *E*. On the other hand, the *n*-propyl substituent of (*S*)-2-pentanol can hardly fit in the pocket thereby preventing it from being hydrolysed and thus inducing a high *E* (Fig. 1).

The docking mode concept can also be used to explain the results in Table 3. Even though **4** and **20** are of similar size, they show a large difference in *E*. These results could be understood from their different electrostatic potentials. Unfavourable electrostatic interactions could be present when the halogenated *M* group is placed in the stereoselectivity pocket. The *M* group is placed in this region when the fast enantiomer reacts. If so, the opposite effect

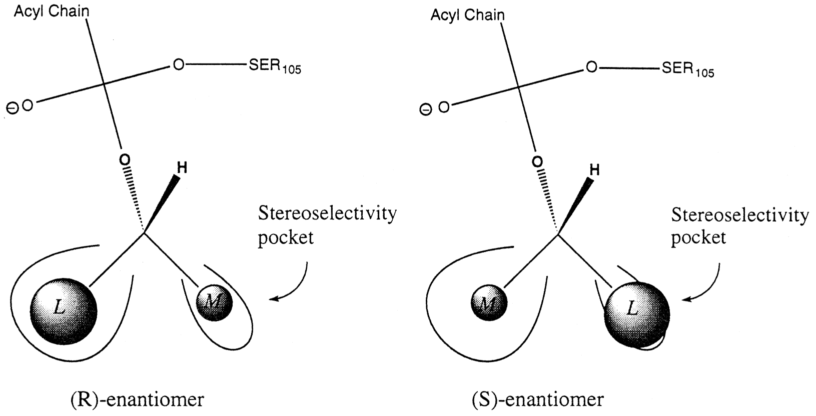


Fig. 1. Schematic representation of CALB’s productive binding modes for alcohol enantiomers.

should be expected for substrates with a halogen atom placed on the *L* group because the slow enantiomer orients the *L* substituent in the pocket (docking mode II). Such a difference was found experimentally. Thus, we concluded that the high enantioselectivity for substrates **22**, **23** and **24** is caused by unfavourable electrostatic interactions between slow enantiomers and the stereospecificity pocket whereas **19** and **20** have a low *E* because of unfavourable interactions for the fast enantiomers.

1-Bromo-2-butanol has two substituents of approximately the same size therefore *E* reflects the electrostatic contribution to the enantioselectivity for *vic*-brominated substrates which is quantitatively more than 2 kcal/mol.

Regarding **18** and **21**, it was found by ab initio calculations that tetrafluoromethane has a less negative minimum in the electrostatic potential than fluoromethane, bromomethane and chloromethane [10]. Thus, the electrostatic potentials of **18** and **21** are likely to be between those of alkanols and monohalogenated alcohols and consequently, according to the discussion above, their *E* as well. This was also found experimentally.

It is interesting to note that the origin of the selectivity seems to be different among lipases. It was found from X-ray crystal structures of *Candida rugosa* lipase-complexes that enantiomers of menthol dock in similar manner except that an important hydrogen bond is missing for the slow reacting enantiomer [11]. Similar results were obtained from the simulations with CALB when the slow enantiomer was docked in the mode I. However, in our simulations, these conformations were often higher in energy than those operating in docking mode II and had a disrupted hydrogen bond network.

4. Conclusion

It has been shown that CALB is a useful catalyst for the kinetic resolution of *sec*-alcohols with an *M* substituent smaller than *n*-propyl and an *L* substituent larger than *n*-propyl or short chain *vic*-halogenated alcohols. Molecular modelling provided indications of the origin of the enantioselectivity. It was found that the enantiomers bind in two different docking-modes. The slow reacting enantiomer has to position the *L* substituent in a stereoselectivity pocket of limited size whereas the fast reacting enantiomer positions the *M* substituent. Experimental data fully support this model.

Electrostatic interactions are greatly involved in the chiral recognition of *vic*-halogenated compounds. Such interactions are suspected between the stereoselectivity pocket and the substrate. In the case of long chain *vic*-halogenated alkanols those interactions ruined the enantioselectivity. Future site directed-mutagenesis of this region may allow us to prepare such long chain precursors to chiral epoxides.

Acknowledgements

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