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On the applicability of the Jones active site model of pig liver esterase to *S*-chiral and prochiral sulfinyl substrates

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Abstract

A series of racemic methyl sulfinylacetates was hydrolyzed in the presence of pig liver esterase (PLE) under kinetic resolution conditions to give the corresponding *S*-chiral sulfinylacetic acids and recovered esters in moderate enantiomeric purity. The Jones active site model was found to be suitable for explaining the enantioselectivity of the above reaction and for the PLE-mediated desymmetrization of prochiral sulfinyldicarboxylates.

1. Introduction

Chemoenzymatic methodologies have recently become a powerful tool for the synthesis of chiral non-racemic compounds.[1–3] Of several enzymes used for such purposes hydrolases, especially esterases and lipases, are of particular importance due to their stability, commercial availability at low cost and lack of a need for cofactors. One such hydrolytic enzyme that has considerable synthetic potential is pig liver esterase (PLE, E.C. 3.1.1.1). Pig liver esterase has been used as a catalyst in the synthesis of a large number of optically active compounds via hydrolysis of chiral, racemic esters or prochiral diesters.[4]

The observed enantioselectivity exerted by PLE prompted several groups to develop models which would account for the stereochemical results obtained. As no X-ray structure of PLE has been determined to date, empirical models had to be created. The first one, proposed by Tamm et al.,[5] formulated an optimum substrate structure. Some further attempts at the description of the PLE active site were subsequently published.[6,7] The most versatile PLE active site model, which is capable of interpreting the enzyme specificity and predicting stereoselectivity for new substrates, was proposed by Jones et al. in 1990[8] and complemented in 1994.[9] This model is a purely empirical one. It was developed by analyzing the results of over 100 PLE-mediated hydrolyses of *C*-chiral or prochiral methyl esters with broadly representative structures, which had been reported in the literature. It explains and predicts the stereochemical outcome of both kinetic resolutions of racemates of type **1** and asymmetric syntheses using prochiral substrates **2**.

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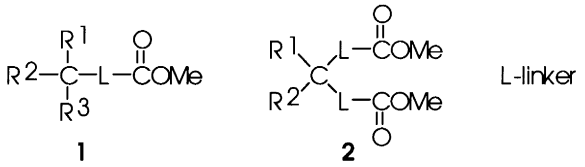
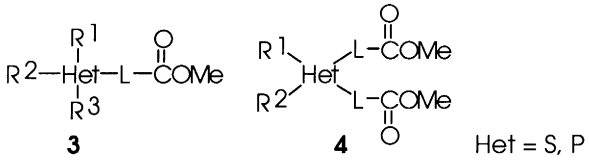


1 2

However, this model was constructed exclusively on the basis of the substrates in which the stereogenic or prostereogenic centre was located on carbon.

In recent years we have been involved in the investigations of the application of enzymes to the synthesis of chiral non-racemic heteroorganic compounds. As a matter of fact, our substrates **3** and **4** closely resemble the esters **1** and **2**, the only difference being the location of a stereogenic or prostereogenic centre on the heteroatom.[10]

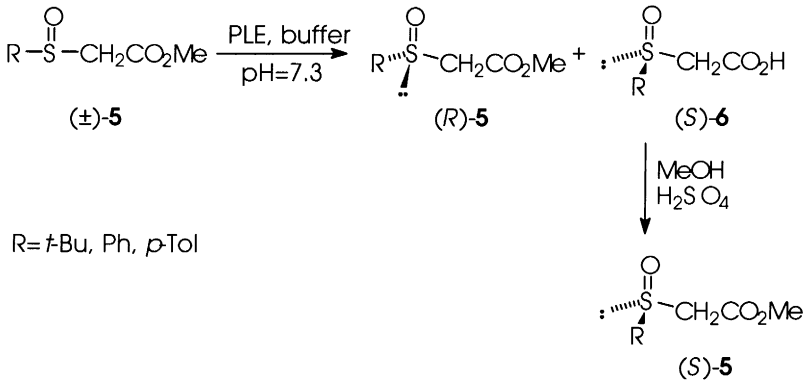


3 4

Since PLE has been the most frequently used enzymatic catalyst, we decided to check whether Jones' model can also be applied to such substrates. In our previous papers we were able to prove that it can satisfactorily account for the enantiodiscrimination observed in the PLE-mediated kinetic resolution of a diversity of racemic phosphoroacetates.[11,12] The aim of the present study was to determine the stereoselectivity of the PLE-promoted hydrolysis of various sulfinylacetates and to check whether it can also be explained in terms of the Jones model.

2. Results and discussion

Three representatives of racemic, *S*-chiral sulfinylacetates, i.e. **5a**–**c**, were subjected to hydrolysis in the presence of PLE at 30°C in a phosphate buffer using an automatic titrator to keep the appropriate pH value. The reaction was performed under kinetic resolution conditions, i.e. it was stopped after 50% conversion was reached. The reaction was then quenched, the unreacted esters and resulting acids **6** were separated (see Experimental) and their enantiomeric excess values were determined by means of ^{1}H NMR spectra of their complexes with (+)-(*R*)-*t*-butylphenylphosphinothioic acid as a chiral solvating agent.[13] To obtain comparable data, the acids formed were re-esterified with an excess of methanol in the presence of a catalytic amount of H_2SO_4 to give the enantiomeric esters. The data are collected in Table 1.



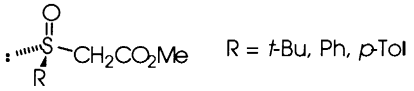
(±)-5 (*R*)-5 (*S*)-6 (*S*)-5 (1)

Table 1
Enzymatic hydrolysis of sulfinylacetates **5**

Substrate		Recovered ester **5**				Acid **6** (Ester **5**)				E
No	R	Yield (%)	$[\alpha]_D^{20}$ ($CHCl_3$)[a]	ee (%)	Abs. conf.	Yield (%)	$[\alpha]_D^{20}$ ($CHCl_3$)[a]	ee (%)	Abs. conf.	
a	*t*-Bu	53	+48	48	*R*	38	-19.1	38	*S*	3.5
b	Ph	52	+37	21	*R*	40	-58 (-51.8)	34	*S*	2.5
c	*p*-Tol	32	+145	80	*R*	58	-82.8 (-84)	46	*S*	6.1

[a]c=1

As the absolute configurations of all the products were known from the literature to be (+)-(*R*),[14–16] it was very easy to recognize that in all cases the (*S*)-enantiomers of the substrates were recognized by the enzyme and hence were preferentially hydrolyzed.



(*S*)-**5**

The regularity observed allows the application of the Jones model. According to the model, the enantiomers that undergo faster hydrolysis to give the corresponding acids should be accommodated in the appropriate pockets of the enzyme active site as follows (Fig. 1).

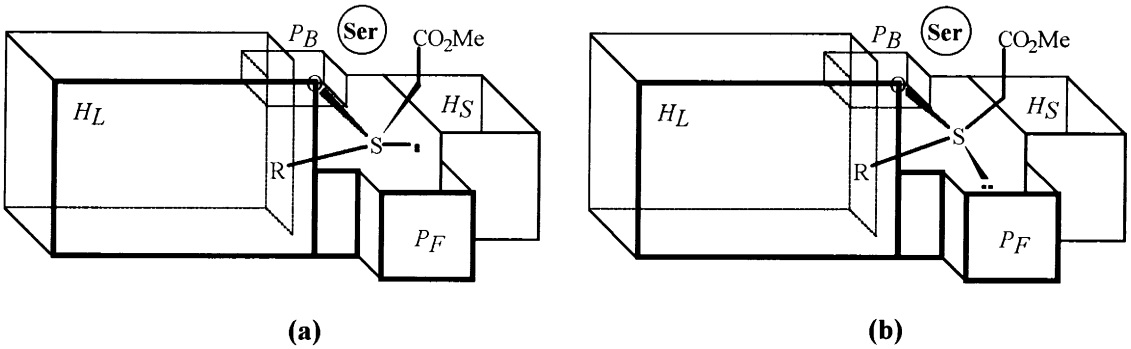
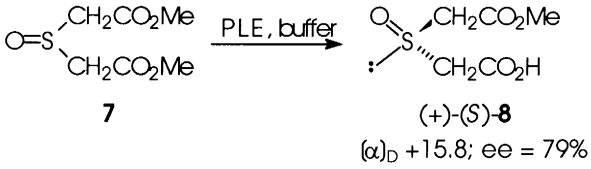


Fig. 1. Binding orientations in the PLE active site of the preferably hydrolyzed (*S*)-enantiomers of sulfinylacetates **5**

The methoxycarbonyl group to be hydrolyzed should be located within the spherical locus of the catalytically active serine function, the large organic group (*t*-Bu, Ph or *p*-Tol) in the large hydrophobic pocket (H_L) and the oxygen atom of the strongly polar sulfinyl group in the back polar pocket (P_B). The remaining lone electron pair should be treated here as the smallest substituent and located in the small hydrophobic pocket (Fig. 1a). However, as the lone electron pair is known to possess a highly polar character, an alternative location, namely in the front polar pocket (P_F), seems more likely (Fig. 1b). Nevertheless, in both cases the model predicts preferential accommodation of the same enantiomer, i.e. (*S*), which is in full agreement with the results obtained. A relatively moderate enantioselectivity of the hydrolysis may be explained in terms of a competition of a reverse accommodation of both polar groups (i.e. S=O in P_F and the lone electron pair in P_B), leading to preferential hydrolysis of the opposite enantiomer.

In a similar way Jones' model may be used to explain the stereoselectivity of the PLE-mediated hydrolysis of prochiral sulfinyldiacetate **7** which was previously found by us[17,18] to produce the monoester (+)-(*S*)-**8** (Fig. 2).



(2)

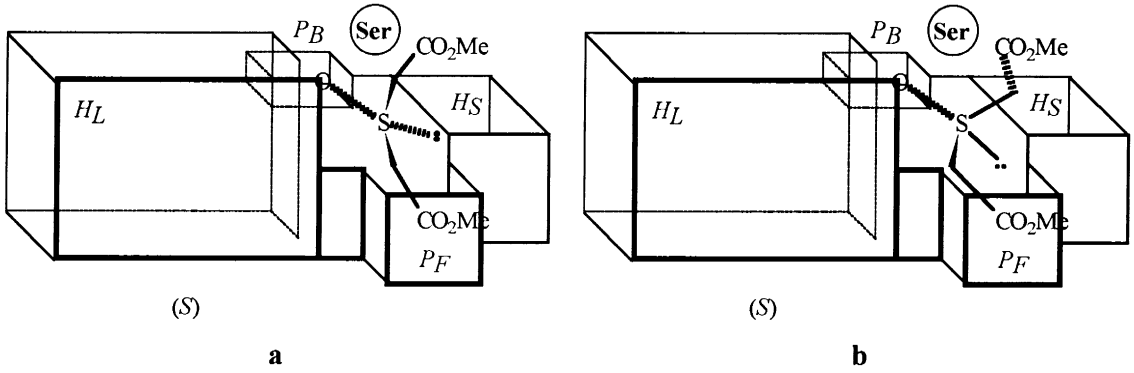


Fig. 2. Preferred binding orientations of sulfinyldiacetate **7** in the active site of PLE leading to (+)-(*S*)-**8**

This model requires that the ester group which undergoes hydrolysis be located in the serine sphere and the second one in the front polar pocket (P_F). Location of the sulfinyl oxygen atom in the back polar pocket (P_B) and the lone electron pair (again treated here as the smallest substituent) either in the small hydrophobic pocket (H_S, Fig. 2a), or rather in the front polar pocket (P_F) together with the non-reacting ester group (Fig. 2b), leads to the unambiguous conclusion that the (*S*)-enantiomer of **8** should be preferentially produced, which is in agreement with the experiment.

Finally, it should be mentioned that we have recently used Jones' model to explain different stereochemical outcomes of the PLE-mediated hydrolyses of various types of sulfoximino-carboxylates,[19] compounds closely related to sulfinylacetates.

In conclusion, the successful application of Jones' model to heteroatomic substrates may be considered as further confirmation of its highly predictive value. The new examples presented broaden the scope of its applicability and serve the purpose of refining the sizes and shapes of the active site pockets.

3. Experimental

3.1. General

Phosphate buffer solutions were purchased from Aldrich. An ammonium sulfate suspension of pig liver esterase was purchased from Sigma. A 0.2 M solution of NaOH was used in an automatic titrator to maintain pH. NMR spectra were recorded at 200 MHz with $CDCl_3$ as a solvent. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. All the substrates were prepared by oxidation of the corresponding sulfides using H_2O_2 in the presence of *i*-PrOH and H_2SO_4 as catalyst[20] and had spectral data identical to those reported in the literature.[14–16]

3.2. General procedure for the enzymatic hydrolysis of **5**

To a stirred solution of ester **5** in a phosphate buffer (ca. 15 mL), at 30°C, PLE (15 μL) was added. The pH was maintained by continuous addition of 0.2 M aqueous NaOH using an automatic titrator. After the desired conversion was reached, the reaction was quenched by adding acetone (ca. 100 mL) and freezing for ca. 2 h. The mixture was filtered through Celite® and the acetone was evaporated. The residual aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated to give the crude product which was purified by silica gel chromatography (column or TLC), using AcOEt/hexane as solvent, to give pure unreacted esters (*R*)-**5**. The remaining aqueous layer was acidified with H_2SO_4 (pH ca. 3.0) and lyophilized. The residue was extracted with CH_2Cl_2, the solution was dried and the solvent evaporated to give acids **6**. In some cases, the acids were dissolved in excess methanol, a drop of concentrated H_2SO_4 was added and the solution was left overnight, during which time the acids underwent re-esterification. After the reaction was completed (TLC control), the methanol was evaporated. The residue was extracted with CH_2Cl_2, washed with a small amount of water and dried over $MgSO_4$. After evaporation of the solvent the crude product was purified by chromatography (as above) to give the esters (*S*)-**5** (see Table 1).

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