Lipase Catalysed Regio- and Enantio-selective Hydrolysis: Molecular Recognition Phenomenon and Synthesis of (*R*)-Dimorphecolic Acid†

U. T. Bhalerao,* L. Dasaradhi, P. Neelakantan and N. W. Fadnavis

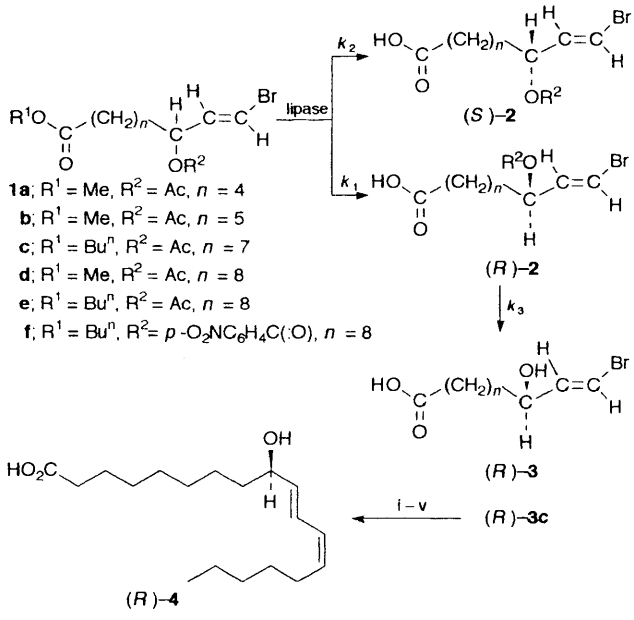
Organic Division II, Indian Institute of Chemical Technology, Hyderabad 500 007, India

Molecular recognition has been observed in hydrolysis of racemic esters of (*E*)-9-acetoxy-11-bromoundec-10-enoic acid by a lipase of *Candida cylindracea* (E.C. 3.1.1.3) where optically active (*R*-(*E*)-9-acetoxy-11-bromoundec-10-enoic acid [enantiomeric excess (e.e.) > 99%] was obtained which was used in synthesis of (*R*)-dimorphecolic acid.

It is now well known that lipases can accept a broad range of artificial substrates in both aqueous and non-aqueous media and have been employed in the enantiospecific synthesis of various chiral molecules.[1] In most cases the reaction is carried out at a functional group situated very close to the chiral centre, usually one or two carbon atoms away. For meaningful applications of lipases in synthesis, it is important to know how far one can move away from the chiral centre and still get useful enantioselectivity. Here, we report a molecular recognition phenomenon observed in the hydrolysis of racemic (ω − 2)-acetoxy-ω-bromoalkenoates **1a–f** catalysed by a lipase of *Candida cylindracea* (EC 3.1.1.3). These substrates‡ were designed so as to possess essential functionalities like a chiral hydroxy group, a carboxylic group situated at a distance (4 to 8 carbon atoms) from the chiral centre, and a bromovinyl group for facile synthesis of a variety of bioactive natural products. The enzymatic hydrolysis was found to be not only regiospecific but also enantioselective.

The hydrolysis of **1a–f** catalysed by a lipase of *C. cylindracea* proceeds in the order **1**→**2**→**3** (Scheme 1). For the hydrolysis of the ester **1f**, the initial rate of hydrolysis of **1f** to **2f** was more than 15 times faster than the initial rate of hydrolysis of **2f** to **3f** ($k_1 > 15k_3$).§ Ester **1f** in fact hydrolyses to acid **2f** in two stages; a rapid hydrolysis with zero-order kinetics (k_1) up to 40 to 45% reaction and a slower hydrolysis, again with zero-order kinetics (k_2), between 55 and 80% reaction with $k_1 = 5.3k_2$.¶ Table 1 shows the enantioselectivity observed in lipase catalysed hydrolysis of **1a–f** to acid **2a–f** at the 40% hydrolysis stage.‖ It is seen that the enantioselectivity increases as the distance between the hydroxy and carboxy ends increases from 4 to 8 carbon atoms and this improves dramatically when an n-butyl ester instead of methyl ester is used as the substrate. Optically pure product **3c** (isolated yield 35%, e.e. > 99%) thus obtained was esterified with diazomethane (quantitative) and coupled with hept-1-yne.[2b] The product (52%) on partial hydrogenation furnished methyl 9-hydroxy-octadeca-10(*E*),12(*Z*)-dioenoate (65%), which was hydrolysed to dimorphecolic acid** (83%), a protecting agent for rice plants.[3]

By comparison of the sign of optical rotation of the benzoyl derivative of methyl dimorphecolate with that reported for its (*S*)-enantiomer,[2b] the configuration of the product, was found to be (*R*): $[\alpha]_D^{25}$ −68.9° (*c* 1, chloroform), lit.[2b] $[\alpha]_D^{25}$ for (*S*) + 69.2° (*c* 1, chloroform). Thus the enzyme shows (*R*)-



Scheme 1 *Reagents*: i, CH_2N_2–diethyl ether; ii, H–C≡C[CH_2]$_4$Me, $Pd(PPh_3)_4$, CuI, Pr^nNH_2; iii, H_2–Pd, $BaCO_3$; iv, K_2CO_3–MeOH; v, H^+

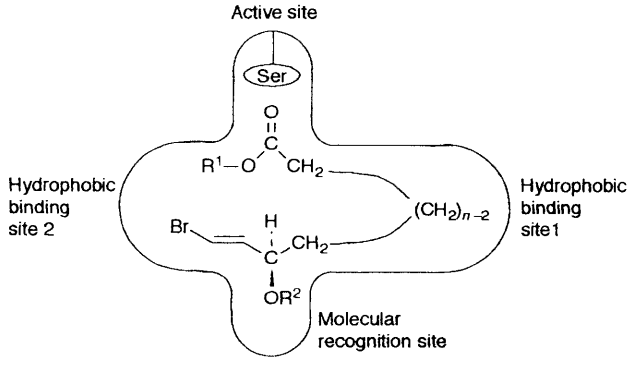


Fig. 1 A schematic representation of the active site model for *C. cylindracea* lipase

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‡ Compound **1** was synthesized by reaction of the corresponding tribromide [ref. 2(*a*)] with KOAc (2 equiv.)–18-crown-6 in dimethylformamide.

§ *Reaction conditions*: [ester] = 1 mmol dm^{-3}, enzyme (Sigma Chemical Co., USA) 0.1 mg ml^{-1}, 0.02 mol dm^{-3} tris buffer, pH 7.5, 0.5 mol dm^{-3} NaCl in 10% (v/v) methanol–water. The reactions were followed by reverse phase HPLC using Zorbax RP-18 column and 80% acetonitrile–water as the eluent.

¶ At high buffer concentration (0.1 mol dm^{-3}) considerable non-enzymatic general base catalysed formation of *p*-nitrobenzoic acid was observed.

‖ *Typical reaction conditions were*: [ester] = 0.2 mol dm^{-3}, enzyme 2.5 mg ml^{-1}, 0.01 mol dm^{-3} tris, 0.5 mol dm^{-3} NaCl, pH 7.5 in 10% (v/v) methanol–water. pH was maintained at 7.5 by addition of 0.2 mol dm^{-3} NaOH. The reactions generally proceeded to the 40% hydrolysis stage in 4 to 6 h. Enantiomeric purity of the acids (**2a–f**) was determined after converting them to corresponding hydroxy esters (i, K_2CO_3–MeOH; ii, diazomethane) by ^1H NMR spectroscopy using $Eu(hfc)_3$ as the chiral shift reagent.

** The structure was confirmed by spectral and analytical data. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.91 (t, 3H, CH_3), 1.25 (br s, 18H, 9 × CH_2), 1.91–2.13 (m, 2H, CH_2–CH=CH), 2.32 (t, 2H, CH_2–CO_2H), 4.07 (m, 1H, CHOH), 4.71 (br s, 1H, –OH), 5.03–6.12 (m, 3H, CH=CH–CH=CH–CHOH), 6.37 (dd, 1H, CH=CH–CHOH, *J* 9.8 and 15.1 Hz), 9.81 (br s, 1H, CO_2H).

Table 1 Enantioselective hydrolysis of racemic alkenoates **1** catalysed by lipase of *C. cylindracea*

Compd.	R^1	R^2	*n*	E.e. (%) of the acid recovered after 40% hydrolysis of **1**
1a	Me	Ac	4	28
b	Me	Ac	5	50
c	Bu^n	Ac	7	>99
d	Me	Ac	8	68
e	Bu^n	Ac	8	>99
f	Bu^n	*p*-Nitrobenzoyl	8	>99

stereochemical preference providing (*R*)-acids **2** and (*S*)-unhydrolysed esters. By carrying out the hydrolysis to the 60% stage, optically pure (*S*)-unhydrolysed ester is obtained which is similarly converted to (*S*)-dimorphecolic acid. Hence it can be concluded that the initial rapid reaction observed during enzymatic hydrolysis of **1f** (k_1) corresponds to the hydrolysis of the (*R*)-ester and the slower reaction (k_2) corresponds to the hydrolysis of the (*S*)-ester (Scheme 1). This is consistent with our observation that no such distinct regions of zero-order reactions were observed for the hydrolysis of the racemic acid **2f** to the hydroxy acid **3f** and no enantioselectivity was observed in the product analysis at 40% hydrolysis.†† Also, no enantioselectivity was observed in the hydrolysis of the hydroxy ester **1** (R^1 = n-butyl, R^2 = H, n = 8) to hydroxy acid **3d**.

These observations suggest that the lipase of *C. cylindracea* posseses two hydrophobic binding sites along with the active site (Fig. 1). The hydrocarbon chain between the two functional groups can be visualized to be bound to the hydrophobic binding site 1 and the hydrophobic parts of the molecule at both ends can be visualized as being bound at the other hydrophobic binding site 2. This model is similar to that proposed recently by Jones and coworkers for the active site of pig liver esterase,[4] but differs somewhat from the active site models proposed for other lipases where a single hydrophobic binding site has been proposed.[5] The low velocity ratio k_1/k_2 = 5.3 for the hydrolysis of **1f** to **2f** is intriguing in view of the high enantioselectivity observed (e.e. > 99%) for the reaction. It appears to us that the reaction is not a simple hydrolysis with (*R*)- and (*S*)-esters hydrolysing at vastly different rates but involves a molecular recognition phenomenon where (*R*)-ester is readily recognized, efficiently bound at the binding site and is rapidly hydrolysed, while no reaction with (*S*)-ester occurs till most of the (*R*)-ester is hydrolysed to its corresponding acid. In other words, the (*R*)-ester is also acting as a competitive inhibitor to the hydrolysis of the (*S*)-ester. This molecular recognition is expressed through the enantioselectivity in the hydrolysis reaction: the better the fit of the substrate, the better is the enantioselectivity as demonstrated by our results in Table 1.

Our results thus provide an insight into the nature of the active site of the lipase of *C. cylindracea*. The enzyme can 'recognize' a substrate and provide chiral products even if the chiral centre is far removed from the reaction centre. A preliminary rate study with closely resembling reporter molecule can help in optimization of reaction conditions for better enantio- and regio-selectivity in enzymatic reactions. Naturally occurring dimorphecolic acid is mostly a racemic mixture with a slight excess of the (*S*)-enantiomer.[2b,3] Our methodology provides a simple synthetic route to both the enantiomers. Work is in progress for a detailed description of substrate binding pattern and enantioselectivity for reaction catalysed by lipases.

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†† The enzymatic nature of this hydrolysis was determined independently using racemic **2f** in a control run without the addition of enzyme. Compared to enzymatic hydrolysis, the non-enzymatic hydrolysis was only 8 to 10% under identical conditions.

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