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Enantioselectivity of the PPL-catalysed hydrolysis of racemic esters: some cases implying a conformational substrate model

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The dependence of the PPL-mediated optical resolution of eighteen racemic esters on the substrate's structure (the sense and magnitude of enantioselectivity, or the inertness of certain substrates) appears to be best explained by a mechanistic model involving a W-shaped active conformation of the substrate laying in a diastereodiscriminating plane.

Porcine pancreatic lipase (PPL) is a biocatalyst widely used in asymmetric synthesis. Several mechanistic models have been advanced to explain and predict the enantioselectivity of a PPL-catalysed reaction,[1–4] but none of them takes into account, at least explicitly, the possibility of conformational effects.

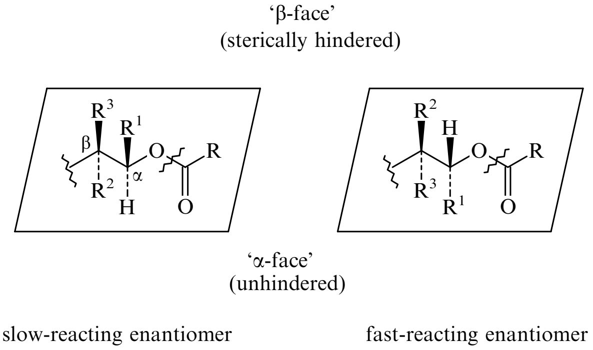
In the context of our studies towards the lipase-supported synthesis of chiral insect pheromones,[5,6] hypolipidaemics,[7,8] polycyclic terpenoids[9] and asymmetry-inducing ligands, 18 esters with various chiral alcohol moieties were submitted to partial enzymatic hydrolysis in the presence of porcine pancreatic lipase (PPL) (Table 1). The sense of the racemate hydrolysis enantioselectivity was determined either from the sign of $[\alpha]_D$ (for the known products) or by stereochemical correlations (see refs. 6, 7), and the magnitude of enantioselectivity was characterized by enantiomeric ratios, *E*, calculated by using the Chen–Sih's equations for irreversible reactions.[10] Resolutions were carried out under nearly standard conditions to afford, in sixteen cases, products of various enantiomeric purity; in two cases no reaction took place.[†]

Thus, the ability of PPL to discriminate between the enantiomers of pantolactone acetate and isobornyl acetate, as well as between those of 3,7-dimethyloct-1-yl carboxylates (Table 1, entries 1, 2 and 8–10, respectively) was extremely poor. The acetates of 1-phenylethanol, α-cyclogeraniol and 2-methyl-3-(4-methoxycarbonyl)phenylpropan-1-ol are also hydrolysed with rather low enantioselectivity (entries 3, 11 and 13, respectively). Their close analogues, in which the same chiral moieties are combined with considerably bulkier outlying groups, behave differently. Racemic 11-acetoxydrim-7-ene (*RS*)-**12** and the racemic arene(tricarbonyl)chromium complex (*RS*)-**14** are hydrolysed with markedly higher enantioselectivity than their less encumbered relatives (*RS*)-**11** and (*RS*)-**13**; moreover, upon the transition from the latter to (*RS*)-**14** the enantioselectivity is reversed. Conversely, the transformation of (*RS*)-**3** into the respective arene(tricarbonyl)chromium complex (*RS*)-**4** results in complete inertness towards hydrolysis. Amido acetate (*RS*)-**5**, another sterically crowded substrate, is also stable to hydrolysis.

Of the two secondary alkenyl acetates (*RS*)-**6** and (*RS*)-**7** the latter is hydrolysed with much higher enantioselectivity, which may be due to the presence of an (*E*)-configurated double bond in the γ-position with respect to the chiral reaction centre (*cf.* ref. 2). 1-*O*-Acetyl-2,4:3,5-di-*O*-methylidene-DL-xylitol (*RS*)-**15** reacts with excellent enantioselectivity.

Pseudoracemic mixtures of epimeric (2*RS*,6*R*)- or (2*RS*,6*S*)-2,6-dimethyloct-1-yl formates undergo hydrolysis with differing degrees of diastereoselectivity (*cf.* entries 16, 17). The formation of (2*S*,6*R*)-2,6-dimethyloctan-1-ol from (2*RS*,6*R*)-**16** occurs with noticeably higher diastereoselectivity than that observed for the liberation of the (2*S*,6*S*)-alcohol from (2*RS*,6*S*)-**17**.[‡] When the latter is replaced by acetate (2*RS*,6*S*)-**18**, the selectivity of the hydrolysis increases.

Deviations from the typical conditions of PPL-catalysed hydrolysis (*i.e.*, from the heterogeneous 'oil–water' system) may strongly affect the course of the reaction. Thus, the enhancement and reversal of enantioselectivity in the hydrolysis of acetate (*RS*)-**9**, accompanied by a sharp deceleration, occurs upon replacing the phosphate buffer by its mixture with Bu^nOH–hexane (entry 9a); these phenomena might be associated with the preponderance of the dissolved ('molecular') form of the substrate in this medium (*cf.* ref. 13) and/or by the involvement of Bu^nOH in the parallel transesterification reaction.



(R = H or Alk; among R^1, R^2, R^3 at least one of the substituents is distinct from hydrogen)

Figure 1

Attempts to rationalize the data of Table 1 by the known mechanistic models of PPL[1–4] resulted in a few controversial cases (*e.g.*, for substrates **3**, **4**, and **13**, **14**). This prompted us to look for a more consistent alternative, which would take into account present-day knowledge of the structure of PPL and its mode of action at the interface.[14]

The following assumptions were made: (1) The formation of the pivotal complex EnzOH·RC(O)OR* and/or its reorganization to EnzOC(O)R·R*OH takes place only *via* a specific energy-rich conformation of the substrate molecule. (2) In the latter no less than four contiguous σ bonds, including the breaking O–CO bond and the flanking bonds, have a planar W-shaped disposition. (3) The plane accomodating this zig-zag array is diastereofaceous: one of its faces corresponds to the

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[†] Porcine pancreatic lipase (47.8 U mg^{-1}) purchased from 'Olainfarm' (Latvia) was used throughout. In all runs the substrates [oils or crystalline powders, substrate/enzyme ratios ranging from 2 : 1 to 1 : 1 (w/w), normally ≈ 100 mg of PPL per 1 mmol of a substrate] were dispersed in 0.1 M phosphate buffer (pH 6.5–7.0; normally, 0.5–7.5 ml per 1 mmol of a substrate) by vigorous magnetic stirring. Then PPL (powder) was added, and the stirring was continued at 22–24 or 37 °C with regular sampling and readjustment of pH. The progress of the reaction was monitored by GC and/or TLC. The extent of substrate conversions was determined by gas chromatography (reproducibility ±3%), and, in experiments 1–3, 9a, 12, 14, 15, additionally from the parameter $ee_S/(ee_S + ee_P)$; for the complexes (*RS*)-**4** and (*RS*)-**14** the conversion was measured by weighing the integral alcohol and ester fractions after their quantitative separation by column chromatography on SiO_2 (*cf.* ref. 8). Except when noted, the bulk of PPL was removed by filtering the reaction mass through a pad of Celite which was subsequently washed with Et_2O. The aqueous phase was extracted with Et_2O, the combined organic phase was washed with aq. Na_2CO_3 and brine, dried (Na_2SO_4) and then concentrated *in vacuo*. The products were separated by column chromatography on SiO_2.

The values of ee_p and ee_s were determined both polarimetrically and from the integral intensity ratios of the signals in the ^{19}F NMR spectra of the (*S*)-MTPA esters formed by the (*S*)-(−) Mosher acid (Fluka AG, ee ⩾ 97%) with the enzymic hydrolysis-derived alcohols or with their counterparts obtained upon saponifying the unconverted ester fractions with NaOMe–MeOH or aq. KOH; the reproducibility of the ee values obtained from the ^{19}F NMR spectra was within ±3%. The products resulting from partial hydrolyses of esters (*RS*)-**1**, (*RS*)-**2**, (*RS*)-**8** and (*RS*)-**9** were additionally checked by gas chromatography on a capillary column with a β-cyclodextrin-derived stationary phase. All three methods gave comparable values of ee_P and *E*. The values of the de for the alcohols obtained from (2*RS*)-**17** and (2*RS*)-**18** were also corroborated by their ^{13}C NMR spectra (see ref. 6). Since in the range 95–99% ee the enantiomeric purity of a chiral derivatizing agent does not seriously affect the measurement of *E* for moderately enantioselective processes,[11] the values of *E* found in this work appear to be good enough to allow comparisons to be made of substrates with 'low', 'moderate' and 'high' hydrolysis enantioselectivity. The values of *E*, marked by 'p' or 's', in Table 1, were obtained by replacing the parameter $ee_s/(ee_p + ee_s)$ in the Chen–Sih's equations by experimentally determined values of conversion; otherwise, they are averages of the *E* values calculated both from ee_P and ee_S.

[‡] The low stereoselectivity in the case of formate **18** may be due in part to the competing non-enzymatic hydrolysis (*cf.* ref. 12). A similar situation ($ee_p \ll ee_s$) in the case of acetoxy lactone **1** was shown to result from partial artefact contamination of the liberated pantolactone upon chromatography.

[§] Thus, the reversal of enantioselectivity upon the transition from (*RS*)-**13** to (*RS*)-**14** may be attributed to the failure of (*R*)-**14** to adopt the *gauche*-like planar W-conformation because of the large size of the three-dimensional arene(tricarbonyl)chromium moiety, which impedes the rotation around the C(1)-C(2) bond necessary to transform the 'unfitting' staggered conformation of (*R*)-**14** into the 'fitting' *gauche* conformation. As a result, the staggered conformation of (*S*)-**14**, which is a fitting one, can bind to PPL uncompeted. In the case of (*R*)-**13** a similar rotation is possible, and the resultant *gauche* conformation successfully competes with the staggered conformation of (*S*)-**13**. In both enantiomers of (*RS*)-**4** the rotation around C(1)–C(1′) is impeded by the proximity of the $Cr(CO)_3$ group, so the necessary planar W-conformations cannot take place, and the hydrolysis is totally blocked. The 'fitting' planar conformation of the (*S*)-enantiomer of acetoxy lactone **1**, when the O–CO–Me grouping lies in the same plane as atoms C(2) and C(3) and only one of the two geminal methyl groups intrudes in the β-side, differs very little in energy from the 'fitting' conformation of (*R*)-**1**, in which the O–CO–Me grouping is coplanar with C(2) and C(1) while the β-side is occupied only by the oxygen atom of the lactone carbonyl group; as a result, enzymatic hydrolysis of both enantiomers occurs at nearly the same rate and gives, practically, a racemate. Due to annelation, the energy difference between the planar conformations of *S* and *R* enantiomers of acetate **12** markedly increases with respect to the case of its monocyclic analogue (*RS*)-**11**, and so the hydrolysis enantioselectivity is increased.

Table 1 Partial PPL-catalysed hydrolysis of various racemic esters in 0.1 M aqueous phosphate buffer (pH 6.5–7.0).

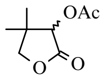
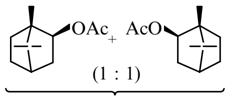
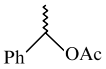
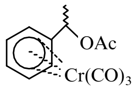
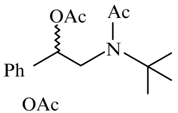
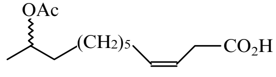
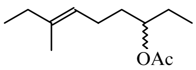
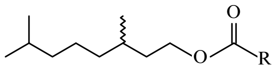
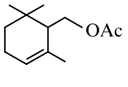
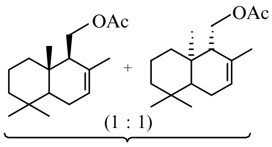
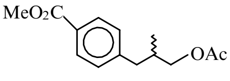
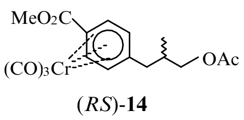
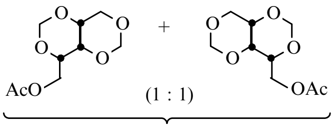
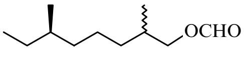
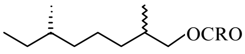
Entry	Substrate	Time (h)	Conversion (%)	Abs. configuration and ee: Alcohol	Abs. configuration and ee: Ester	E
	Secondary alkyl carboxylates					
1	 (*RS*)-**1**	157[a]	45	*S*(0.012)	*R*(0.022)	1.05
2	 (*RS*)-**2**	59	50	*1R*,*2R*(0.104)	*1S*,*2S*(0.108)	1.36
3	 (*RS*)-**3**	48	48	*R*(0.45)	*S*(0.43)	3.93
4	 (*RS*)-**4**	72	≈0	No products		
5	 (*RS*)-**5**	72	≈0	No products		
6	 (*RS*)-**6**, ref. 5	48	25	*S*(0.66)	*R*(n.d.)	6.04_p
7	 (*RS*)-**7**, ref. 5	69	50	*R*(0.93)	*S*(⩾50)[b]	94.2_p
	Primary alkyl carboxylates					
						
8	(*RS*)-**8** R = H	7	35	*S*(0.05)	*R*(n.d.)	1.13
9	(*RS*)-**9** R = Me	13	28	*S*(0.05)	*R*(n.d.)	1.13
9a	(*RS*)-**9**[c]	192[c]	35[c]	*R*(0.824)	*S*(0.359)	13.2[c]
10	(*RS*)-**10** R = Hept	20	12	*S*(0.05)	*R*(n.d.)	1.10
11	 (*RS*)-**11**	30	16	*R*(0.28)	*S*(n.d.)	1.42_p
12	 (*RS*)-**12**	22	30	9*R*,10*R*(0.785)	9*S*,10*S*(0.299)	11.2
13	 (*RS*)-**13**, ref. 7	3.5	50	*R*(0.222)	*S*(0.23)	1.93
14	 (*RS*)-**14**	19 27 34	30(ref.8) 55 70(ref.8)	*S*(0.98) *S*(0.496) *S*(n.d.)	*R*(n.d.) *R*(0.524) *R*(⩾0.98)	150.2_p 4.9 9.82s
15	 (*RS*)-**15**[d]	25[d]	45[d]	2*S*(0.98)	2*R*(0.896)	> 150[d]
	Pseudo-racemic epimeric carboxylates					
16	 (2*RS*)-**17**, ref. 6	20[a]	50[a]	2*S*,6*R*(0.424)[e]	*R*,*R*(0.414)[e]	3.72

Table 1 (continued).

Entry	Substrate	Time (h)	Conversion (%)	Abs. configuration and ee		E
				Alcohol	Ester	
						
17	(2*RS*)-**18** R = H, ref. 6	20[a]	50	*S*,*S*(0.012)[e]	2*R*,6*S*(0.16)[e]	1.31
18	(2*RS*)-**19** R = Me, ref. 6	18[a]	35	*S*,*S*(0.626)[e]	2*R*,6*S*(n.d.)[e]	6.0p

[a] At 37 °C. [b] This estimate relates to a specimen of the (*R*)-alcohol obtained from the crude residual acetate (*S*)-**7** by consecutive saponification, mesylation, S_N2 substitution (KOAc–DMF), and resaponification (*cf.* ref. 5). [c] The substrate (6.25 mmol) and PPL (1.25 g) were dispersed in a mixture of phosphate buffer (2.5 ml) with *n*-butanol (0.86 ml, 9.37 mmol) and hexane (3 ml). [d] Hydrolysis in suspension; substrate : enzyme = 1 : 1 (w/w). The products were separated by fractional dissolution in $CHCl_3$ and subsequent recrystallization. [e] Diastereomeric excess (de).

less hindered side ('α-side'), and the other borders on the sterically congested side ('β-side'). This diastereodiscriminating plane represents a rough approximation for the surface-activated catalytic locus of PPL at the interface (*cf.* refs. 13, 14). Enantiomeric substrates, once fixed at this plane, as shown by Figure 1, differ in energy and thus are hydrolysed at different rates. (4) Each enantiomer of a substrate can adopt a planar W-shaped array either from the staggered or a low-energy *gauche* conformation; the adoption of such an array from the *gauche* conformation depends on whether a bulky substituent at the essential chiral centre can be turned to the less hindered side of the plane without strongly increasing the energy of repulsive interactions. The stereochemical result of the reaction reflects the energy difference between the two planar W-shaped conformations providing the best fit of each enantiomer and the enzyme.

By applying these principles to the comparison of both the staggered-like and *gauche*-like planar W-conformations of each enantiomer (or epimer) we succeeded in finding a consistent explanation of all the results presented in Table 1, *i.e.* the sense and magnitude of the hydrolysis enantioselectivity, as well as the inertness of substrates (*RS*)-**4** and (*RS*)-**5** towards hydrolysis.[§] The technique of comparing the possible effects of various factors (*e.g.* C=C and NH–CO isosterism), and the extension of this approach to the literature data will be the subject of a separate publication.

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