Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 13, pp. 2227–2230, 1997
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Printed in Great Britain
0957-4166/97 $17.00 + 0.00

PII: S0957-4166(97)00226-7

Enantioselectivity of lipase-catalysed transesterification of 2-ethyl-1,3-propanediol: comparison of lipases from bacterial, fungal and animal sources

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Abstract: Lipase preparations from four bacteria, *Pseudomomas fluorescens*, *Pseudomonas aeruginosa*, *Chromobacterium viscosum* and *Corynebacterium oxydans*, from a fungus, *Candida cylindracae* and from pig pancreas were contrasted for use in the transesterification of prochiral 2-ethyl-1,3-propanediol. The results showed that the enzyme in a whole-cell preparation from *C. oxydans* was the most effective with vinyl acetate as acyl donor. © 1997 Elsevier Science Ltd

Lipases are established catalysts for the stereoselective resolution of synthetic or semi-synthetic chiral alcohols[1–4]. A limitation to the use of lipases in several cases is that the maximum theoretical yield of a desired enantiomer is 50%: however the use of a prochiral substrate presents the opportunity for a theoretical yield of 100% of chiral product. In such a case, the enzyme distinguishes between enantiotopic faces or groups on the prochiral molecule. The enantiomer formed from a prochiral substrate can, in principle, be transformed into its mirror image by functional group inversion.

From of the enantiomeric selectivity of lipases for chemically related alcohols[5,6] the generalisation is that the enantiomeric purity is, typically, enhanced if there are bulky groups near the stereogenic centre. In the absence of detailed structures for lipase–substrate complexes, the selection and design of lipase reagents for specific purposes is largely empirical. In this paper we report on a screen of enzymes which examines the relatively difficult transesterification of 2-ethyl-1,3-propanediol. The enantiomerically pure acetate had previously been prepared[7].

In principle, lipases can be used to catalyse hydrolysis/acylation reactions and transacylation reactions. Transesterification with a vinyl ester[8] is irreversible as one product of transesterification is an aldehyde and not a substrate for the enzyme.

Results and discussion

2-Ethyl-1,3-propanediol was used as a substrate for several lipases in the reaction of Scheme 1.

The results are summarised in Table 1. The findings confirm that an empirical approach to the screening of lipases for a stereospecific transformation of substrate is still required. The fungal enzyme from *C. cylindracae* which is a popular off-the-shelf enzyme for resolutions[6] has no selectivity in the present case even though very high yields are possible. Of the remaining enzymes, the preparation from *C. oxydans* is different from the remainder in two significant ways: the selectivity is for the different enantiomer (rotation values) and the e.e is highest in this case. Our comment that empirical methods are required in the absence of a 3-d structure for the enzyme–substrate complex is particularly valid in this case because the enzyme has never been isolated let alone crystallised. It is presumably a component of the cell envelope of *C. oxydans*, an acid-fast bacterium of uncertain taxonomic position that presumably contains mycolic acid and other cell wall components typical of the coryneform

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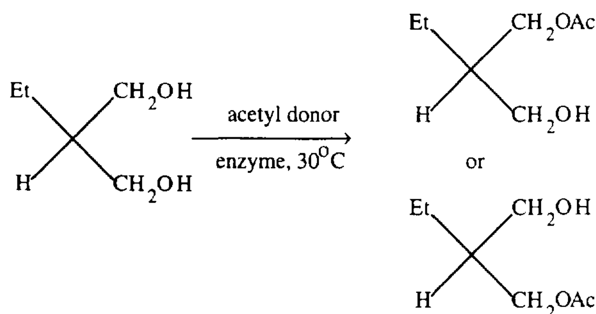

Scheme 1.

Table 1. Enantiomeric excess (e.e.) of transacetylation reactions catalysed by various lipases. *The references are to the conditions of incubation

Enzyme Source*	Acyl Donor	e.e. (%)	$[\alpha]_D$	Yield(%)
P. fluorescens[9]	vinyl acetate	19	+ 3.5	88
P. aeruginosa[9]	vinyl acetate	38	+ 7.0	88
C. viscosum[10]	vinyl acetate	64	+ 9.1	92
C. oxydans[11]	i. ethyl acetate	62	- 8.7	84
	ii. vinyl acetate	90	- 12.3	94
Pig pancreas[10]	i. ethyl acetate	22	+ 3.5	79
	ii. vinyl acetate	48	+ 7.2	89
C. cylindracae[9]	ethyl acetate	0	0	93

group of organisms. We note that the use of vinyl acetate (as opposed to ethyl acetate) leads to greater stereoselectivity in the three cases studied. We interpret this as meaning that the relative selectivities of the enzyme for its prochiral substrates are different for the forward and reverse reactions: in the case of the vinyl acetate reaction, the half-ester products of 2-ethyl-1,3-propanediol must have binding site(s) on the enzyme but cannot react as there is no cognate alcohol.

The *P. aeruginosa* lipase described here has not previously been reported. Its N-terminal amino acid sequence was determined and found to be:

STYTQSKYPIVLASGM....

The sequence was aligned with all known protein sequences and the four highest scoring proteins were all lipases from other *Pseudomonas* strains. The amino acids shown in bold in the sequence were conserved.

Experimental

2-Ethyl-1,3-propanediol was prepared[12] from diethyl ethylmalonate in 90% yield IR ν_{max}: 3600–3050 cm^{-1} (OH stretch); ^{1}H-NMR δ_H (300 MHz, $CDCl_3$,δ): 3.8 (2H, dd, H a/b, J=3.9 Hz), 3.6 (2H, dd, H a/b, J=7.2 Hz), 1.65 (1H, m, CH), 1.3 (2H, qn, CH_2, J=7.3 Hz), 0.95 (3H, t, J=7.5 Hz, CH_3); Mass m/z, (%) 103 (M^+-1); 86 (8), 71 (26), 56 (100), 43 (44).

Racemic 2-ethyl-1,3-propanediol monoacetate was synthesised[12] from 2-ethyl-1,3-propanediol dissolved in and acetic anhydride in 78% yield. IR ν_{max}: 3600–3100 cm^{-1} (OH stretch), 1740 cm^{-1} (C=O stretch ester); ^{1}H-NMR: δ_H (300 MHz, $CDCl_3$) 4.1–4.25 (2H, dd, J=6.4 and 4.8 Hz, CH_2OAc), 3.5–3.7 (2H, dd, J=6.5 and 4.7 Hz, CH_2OH), 2.1 (3H, s, $OCOCH_3$), 1.75 (1H, m, CH), 1.3–1.45 (2H, qn, J=7.2 Hz, CH_2), 0.95 (3H, t, J=7.4 Hz, CH_3) ppm; Mass m/z, (%) 147 (M^++1); 129 (8), 103 (11), 87 (16), 56 (76), 43 (100), 31 (29).

Lipase preparations. Bacterial lipases from *P. fluorescens* and *C. viscosum*, a fungal lipase from *C. cylindracae* and pig pancreatic lipase were obtained from Sigma.

Lipase from *P. aeruginosa* ATCC 12718 was obtained from the culture supernatant of 500 ml cultures following methods and media[13] developed for a different strain.. After 30 h growth in the defined, production medium, the culture medium was clarified by centrifugation (9000g_{av}., 25 min). The cell-free supernatant (0°C) was dripped into 3 vol. acetone pre-cooled to −20°C. The preparation was centrifuged (9000g_{av}., 1 h) and the resulting pellet was re-suspended in distilled water (25 ml). Subsequent ammonium sulphate fractionation and chromatography on Octyl-Sepharose CL-4B followed the literature[13]. Denatured polypeptides were fractionated by SDS-PAGE[14] on 15% acrylamide gels, electroblotted on to a PVDF membrane and the excised band was placed (according to the manufacturers' protocol in the Blott® reaction cartridge of an Applied Biosystems 477A automated protein sequencer[15]. Sequence comparison and analysis used the OWL database and associated interrogatory software mounted in Leeds[16].

C. oxydans ATCC 21245 was grown (100 ml cultures in defined medium) and the cells were harvested by centrifugation and the enzyme preparation comprised freeze-dried cell pellet[9].

All transesterifications were conducted at 30°C with stirring and the progress of the reactions was monitored by using GLC. For the *C. oxydans* system, 2-ethyl-1,3-propanediol (0.5 g; 4.8 mmol) was dissolved in either ethyl acetate (22 g; 0.25 mol) or vinyl acetate (4.7 g; 54 mmol) and freeze-dried cells (0.2 g) were suspended in 2.0 ml water and added[11]. The same conditions (ethyl acetate only) were used in the case of the lipase from *C. cylindricae*. For the pig pancreatic lipase, 2-ethyl-1,3-propanediol (255 mg; 2.45 mmol) was dissolved in either ethyl acetate (10 g; 0.114 mol) or vinyl acetate (9.3 g; 0.108 mol) and enzyme (75 mg) was added[10]. In the case of the enzymes from *Pseudomonas* spp. , 2-ethyl-1,3-propanediol (0.104 g; 1 mmol) was dissolved in chloroform (2 ml) and vinyl acetate (0.34 g; 4 mmol) and enzyme (11 mg) were added[9]. In the case of the *C. viscosum* enzyme, 2-ethyl-1,3-propanediol (0.2 g; 1.92 mmol) was dissolved in vinyl acetate (9.3 g; 0.108 mol) and enzyme (1 mg) was added[10].

Analysis of products from transacetylations. Monoacetate product[12] was purified by column chromatography (silica gel) and the e.e. was determined by ^{1}H-NMR using the chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium (III)[17]. Yield was measured by gas chromatography.

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(Received in UK 29 April 1997)