Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 2, pp. 337–339, 1997

Printed in Great Britain
PII: S0957-4166(96)00519-8
0957-4166/97 $17.00 + 0.00

Remote control of stereoselectivity: lipase catalyzed enantioselective esterification of racemic α-lipoic acid †

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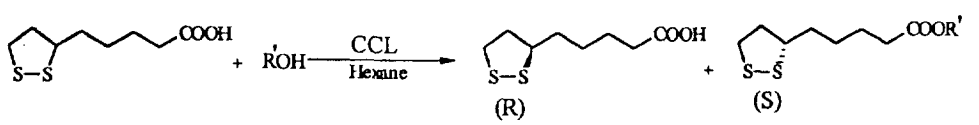
Abstract: Lipase of *Candida rugosa* (E.C.3.1.1.3) catalyzes the enantioselective esterification of racemic 6,8-dithioctic acid (α-lipoic acid) with aliphatic alcohols in hexane. Although the reaction centre is four carbon atoms away from the stereogenic centre, (S)-esters with enantioselectivity dependent upon the chain length of alcohol are obtained. A model for the active site of the enzyme is suggested. © 1997 Elsevier Science Ltd. All rights reserved.

(R)-(+)-α-Lipoic acid **1** is a known growth factor for many bacteria and protozoa, and acts as a coenzyme in many enzyme catalyzed reactions, particularly in oxidative decarboxylations[1]. Several syntheses of the racemic mixture and asymmetric syntheses of the (R)- and (S)-enantiomers have been reported[2–4] but there has been no report on enzymatic resolution of the racemic acid. Here we present our results on the enantioselective esterification of lipoic acid with various alcohols in hexane using *Candida rugosa* lipase (E.C.3.1.1.3) (Scheme 1).

It is interesting to note that the stereogenic centre and the carboxylic group (where the actual enzymatic reaction takes place) are four carbon atoms away and yet the enzyme shows enantioselectivity towards the S-enantiomer. This enantioselectivity is found to be strongly dependent on the number of carbon atoms in the alcohol moiety.

Table 1 shows the effect of the number of carbon atoms in the alcohol chain on the enantioselectivity of the lipase catalyzed esterification reaction in hexane at 30% conversion. It can be seen that lipase catalyzed esterification with methanol and ethanol yields only racemic esters. When n-propanol is used, a low enantioselectivity is observed and (S)-ester is produced with 12.5% e.e.. The enantioselectivity improves as the number of carbon atoms in the alcohol chain increases. An optimum is reached with n-hexanol yielding (S)-ester with 72% e.e. but the enantioselectivity decreases to 58% during esterification with n-octanol.

It was also observed that all esterification reactions under identical reaction conditions (temperature 30°C, lipoic acid 0.2 M and alcohol 1 M in hexane, enzyme 50 mg, reaction volume 60 ml) proceeded at identical rates. The plots of time vs % conversion were linear (up to at least 50% conversion) with identical slopes (6.6 h^{-1} within experimental error of ±5%) indicating that the esterification reaction



R^1 = Me, Et, n-Pr, n-Bu, n-Hex, n-Oct

Scheme 1.

† IICT Communication No. 3744

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Table 1. Enantioselective esterification of lipoic acid by lipase of *Candida rugosa* in hexane[$6].

Alcohol	%e.e. acid	%e.e. ester	E**
Methanol	0	0	0
Ethanol	0	0	0
n-Propanol	2 (R)	12.5 (S)	1.3
n-Butanol	15.5 (R)	49 (S)	3.4
n-Hexanol	20.1 (R)	72 (S)	7.5
n-Octanol	23.8 (R)	58 (S)	4.7

[$] Reaction stopped at 30% conversion ** Calculated from the enantiomeric excess according to Sih *et al*[6].

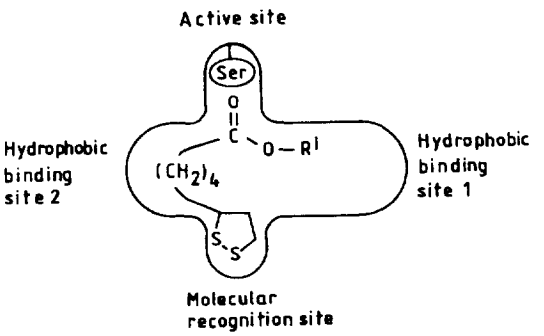

Figure 1. A schematic representation of the active site model for *Candida rugosa* lipase.

proceeds through a rate limiting formation of an acyl-enzyme intermediate between lipoic acid and the enzyme; and the attack of the nucleophile (alcohol) to produce the corresponding ester is fast.

Our results provide further proof of our earlier suggestion[5] that the lipase of *Candida rugosa* possesses two hydrophobic binding sites along with the active site (Figure 1). The hydrocarbon chain of four carbon atoms between the carboxylic group and the five membered dithiolane ring can be visualized to be bound to the hydrophobic binding site 2 (small cavity) and the alcohol attacking the acyl-enzyme intermediate at binding site 1 (large cavity).

The origin of enantioselectivity lies in the correct 'fit' of the nucleophile (alcohol) which attacks the acyl-enzyme intermediate to produce the ester. The 'fit' is very bad for small chain alcohols, gets better as the alcohol chain length increases and goes through an optimum fit for n-hexanol.

Experimental

Varian Gemini 200 MHz spectrometer was used to obtain ^{1}H NMR with $CDCl_3$ as a solvent (TMS as internal standard), mass spectra were recorded on VD-micromass 7070H spectrometer, IR spectra were recorded on Perkin–Elmer model 283B spectrophotometer (values are given in cm^{-1}) and optical rotations were measured on JASCO DIP-270 digital polarimeter.

HPLC grade solvents (Spectrochem, India) were used in all experiments. Racemic lipoic acid and lipase from *Candida rugosa* (type VII) were obtained from Sigma, USA and were used as received.

Reactions were followed by HPLC on LC-9A Shimadazu, Japan. Enantiomeric excess of unreacted

α-lipoic acid was determined by using Chiralcel O.D. column (25×0.46 cm, Diacel, Japan; solvent system 2% isopropanol and 0.1% trifluoroacetic acid in n-hexane; flow rate 0.9 ml/min; retention times (S)-acid 17.8 min, (R)-acid 18.5 min.; detection at 330 nm).Enantiomeric excess of the ester was determined on Chiralcel OJ column (25×0.46 cm, Diacel, Japan; solvent system 0.4% isopropanol in hexane; flow rate 0.9 ml/min). Retention times varied with esters (13 to 22 min) but the (S) and (R) esters were well resolved. The configurations were checked by isolating the esters by column chromatography, and measuring their optical rotation.

In a typical experiment, lipoic acid (206 mg, 1 mmole), n-hexane (60 ml), alcohol (5 mmoles) and lipase (50 mg) were placed in conical flask (250 ml) and the contents were shaken at 200 rpm in a constant temperature orbital shaker. After 30% conversion, the enzyme powder was removed by filtration. Unreacted lipoic acid was extracted with sodium bicarbonate and recovered after acidification and extraction with dichloromethane.

(S)-n-Propyl lipoate [propyl(5S)-5-(1,2-ditholane-3-yl-pentanoate)]

$[\alpha]_D^{23}=-13.0$ (c 0.5, $CHCl_3$; e.e 12.5%); IR (neat) 3000, 2950, 1745, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3H, CH_2CH_3), 1.4–2.8 (m, 12H), 3.2 (t, 2H, H-8,8′), 3.7 (m, 1H, H-6), 4.05 (t, 2H, OCH_2), Mass spectrum: m/z 248 ($M^{+\cdot}$).

(S)-n-Butyl lipoate [butyl(5S)-5-(1,2-ditholane-3-yl-pentanoate)]

$[\alpha]_D^{23}=-18.2$ (c 0.5, $CHCl_3$; e.e. 49%); IR (neat) 3000, 2950, 1750, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3H, CH_2CH_3), 1.3–2.8 (m, 14H), 3.18 (t, 2H, H-8,8′), 3.68 (m, 1H, H-6), 4.13 (t, 2H, OCH_2CH_2). Mass spectrum: m/z 262 ($M^{+\cdot}$).

(S)-n-Hexyl lipoate [hexyl(5S)-5-(1,2-ditholane-3-yl-pentanoate)]

$[\alpha]_D^{23}=-29.6$ (c 0.5 $CHCl_3$; e.e. 72%); IR: (neat) 3000, 2950, 1750, 1200 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.9 (t, 3H, CH_2CH_3), 1.4–2.8 (m, 18H), 3.18 (t, 2H, H-8,8′), 3.68 (m, 1H, H-6), 4.13 (t, 2H, OCH_2CH_2). Mass spectrum: m/z 290 ($M^{+\cdot}$).

(S)-n-Octyl lipoate [octyl(5S)-5-(1,2-ditholane-3-yl-pentanoate)]

$[\alpha]_D^{23}=-33.3$ (c 0.5 $CHCl_3$; e.e. 58%). IR (neat) 3000, 2940, 1745, 1155 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, CH_2CH_3), 1.4–2.9 (m, 22H), 3.1 (t, 2H, H-8,8), 3.7 (m, 1H, H-6), 4.15 (t, 2H, OCH_2CH_2). Mass spectrum: m/z 318 ($M^{+\cdot}$).

Acknowledgements

We thank U.G.C. New Delhi for grant of award of Junior Research Fellowship to K. Koteshwar and C.S.I.R. New Delhi for financial support.

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(Received in UK 18 November 1996)