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Lipase catalyzed regio- and stereospecific hydrolysis: chemoenzymatic synthesis of both (*R*)- and (*S*)-enantiomers of α-lipoic acid †

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Abstract

Native lipase of *Candida rugosa* (EC 3.1.1.3) enantioselectively and regiospecifically hydrolyses the n-butyl ester of 2,4-dithioacetyl butanoic acid either at the carboxylic acid terminus or at the α-thioacetate to provide enantiomerically pure (*R*)-2,4-dithioacetyl butyric acid and (*S*)-butyl 2-thio-4-thioacetyl butyrate (ee >98%) while the lipase modified by treatment with diethyl p-nitrophenyl phosphate attacks only the α-thioacetate giving enantiomerically pure (*S*)-butyl 2-thio-4-thioacetyl butyrate. These enantiomerically pure intermediates can be used as chiral building blocks to obtain both (*S*)- and (*R*)-enantiomers of α-lipoic acid and their analogues.

Lipases are now routinely being used in resolution of racemic alcohols and carboxylic acids.[1] These versatile reagents are not only enantioselective but also display diastereoselectivity[2] and regiospecificity[3] in several cases. Butyl 2,4-dithioacetyl butyrate **1** possesses three hydrolysable groups, namely an ester of α-carboxylic acid, an acetate of a thiol group attached to a stereogenic carbon atom and an acetate of thiol group placed two carbon atoms away from the stereogenic centre. It was interesting to determine whether the enzyme binding site discriminates between the carboxylic acid and the thiol functionality, and whether this leads to enantiomerically pure products that can be converted to enantiomerically pure α-lipoic acid, an important growth factor for a variety of micro-organisms, and also a co-factor for the multi-enzyme complex that catalyses oxidative decarboxylation of α-keto acids.[4]

Thus **1** (2 mmol), prepared by the method of Claeson,[5] was hydrolysed in 0.05 M phosphate buffer (100 ml) at pH 7.5 in the presence of lipase from *Candida rugosa* (Sigma, USA; 50 mg) at room temperature. The reaction stopped at 50% conversion after 3 h and the recovered products were identified as 2,4-dithioacetyl butyric acid **2** and butyl 2-thio-4-thioacetyl butyrate **3**. The enantiomeric purity of **2** and

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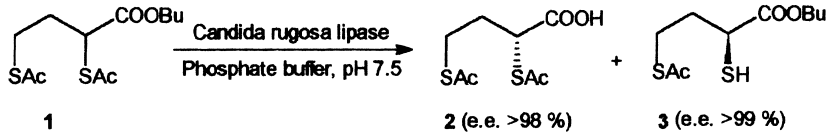
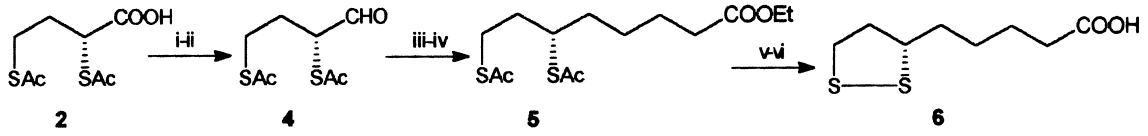
3 was determined by chiral HPLC and both the products were found to have ee >98% with opposite configuration[6] (Scheme 1).



Scheme 1.

The presence of an intact ester group, the disappearance of a singlet in the ^{1}H NMR spectra of **3** at δ 2.2 ppm and its failure to form a thiolactone when treated with NaH in THF confirmed that the primary thioacetate was not hydrolysed. The stereochemistry of **2** and **3** was established as (*R*)-**2** and (*S*)-**3** by conversion to the corresponding enantiomer of α-lipoic acid (Scheme 2). Enantiomerically pure 2,4-dithioacetyl butyric acid **2** was reduced with $BH_3 \cdot DMS$ to the alcohol which was oxidised with PCC to the aldehyde **4**. Wittig reaction of **4** with the triphenylphosphonium salt of ethyl 4-bromobutyrate at −78°C in THF and subsequent hydrogenation of the olefin with Wilkinson's catalyst gave the ethyl ester **5**. The ethyl and thioacetyl groups were removed by hydrolysis with non-enantiospecific enzyme wheat germ lipase in phosphate buffer. Treatment of the hydrolysed product with oxidative enzyme mushroom tyrosinase[7] (EC 1.14.18.1) in the same pot gave α-lipoic acid **6** which was isolated after acidification and extraction with n-hexane (Scheme 2). Comparison of optical rotation[8] and the retention time from chiral HPLC of the final product with the literature[9] showed that the (*S*)-lipoic acid was obtained from **2**. (*R*)-Lipoic acid was also obtained in a similar fashion, starting with **3**, after acetylation. Hence the absolute configuration of **2** was assigned as (*R*) and that of **3** as (*S*).[10]



Scheme 2. (i) $BH_3 \cdot DMS$, 0°C; (ii) PCC; (iii) $Br^- PPh_3^+(CH_2)_3COOEt$, NaHMDS, −78°C; (iv) $(PPh_3)_3RhCl$, H_2; (v) wheatgerm lipase, pH 7.0; (vi) tyrosinase

Lipase catalysed esterification of (*S*)-3-(acetylthio)-2-methyl propanoic acid with n-butanol in organic solvent leads to the formation of the butyl ester in the first step and deacylation of the thioacetate in the second step to give the butyl ester of (*S*)-2-methyl-3-thio-propanoic acid.[11,12] By analogy, hydrolysis of **1** could lead to **2** (reaction stopping at ester hydrolysis stage), but formation of **3** with an intact ester group is surprising since the reaction is being carried out in an aqueous buffer. The formation of **3** suggested the possibility of the presence of a contaminating esterase or protease which selectively deacetylates the thiol function but does not attack the ester function.

SDS-PAGE experiments with commercial lipase showed a major enzyme band at 55–60 kD and some low molecular weight (15–20 kD) protein fractions. The enzyme was purified by cross-flow membrane filtration using a membrane with a molecular weight cut-off of 30 kD. This effectively removed the impurities present in the enzyme. This purified enzyme preparation was again used to hydrolyze the racemic **1**. It was observed that both the product distribution and optical purities were unchanged, indicating that the formation of **3** is not due to a contaminating enzyme, otherwise a change in product distribution ratio would have been observed. When the purified lipase of *Candida rugosa* was treated with diethyl p-nitrophenyl phosphate by the method described for pancreatic lipase,[13,14] the lipase preparation so obtained (DPNP-lipase) was found to be active towards **1** in phosphate buffer at pH 7.5. Although formation of **3** was observed in optical and chemical yields comparable to that obtained with the native lipase, **2** was not formed. The unreacted (*R*)-enantiomer of **1** (ee >99%) was recovered instead of **2**.

Similar results were obtained when a methyl ester instead of butyl ester of **1** was used, indicating that small changes in hydrophobicity of the substrate were not important. Interestingly, DPNP-lipase was found to be inactive towards not only the hydrophobic substrates such as olive oil and tributyrin, but also towards water soluble p-nitrophenyl acetate, indicating that chemical treatment of the enzyme brings about significant changes not only in regiospecificity but also in its substrate specificity.

A change in enzyme enantioselectivity has been observed on treatment of *Candida rugosa* lipase with isopropanol.[15] Changes in the enzyme conformation were suggested to be responsible for this. However, neither the regiospecificity nor the enantioselectivity was affected when a lipase preparation after similar isopropanol treatment was used for hydrolysis of **1**.

Crystal structure studies[16,17] have shown the presence of a 'lid' which can adopt an 'open' or 'closed' conformation and oxyanion-stabilizing residues which orient correctly in the active site. The substrate binds in a narrow hydrophobic tunnel and must adopt a tuning fork conformation. It appears to us that in the present case, two enantiomers of **1** can bind in different ways and the molecular recognition for the substrate is such that the carboxylic group of only the (*R*) substrate and the thioacetyl group of only the (*S*) substrate are recognised by the enzyme. Chemical modification of the amino acid residues which are responsible for opening or closing of the lid and stabilising the oxyanion by treatment with diethyl p-nitrophenyl phosphate can affect substrate binding and cause significant changes in regiospecificity/substrate selectivity of the enzyme which is reflected in the results obtained in the present case. Further work is in progress to study this aspect in detail.

Acknowledgements

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References

1. Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Baldwin, J. E.; Magnes, P. D., Eds.; Tetrahedron Organic Chemistry Series; Pergamon: Oxford, 1994; Vol. 12.
2. Schoffers, E.; Golebiowshi, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769.
3. Bhalerao, U. T.; Dasarathi, L.; Neelakantan, P.; Fadnavis, N. W. *J. Chem. Soc., Chem. Commun*. **1991**, 1197.
4. Yadav, J. S.; Mysorekar, S. V.; Garyali, K. *J. Sci. Ind. Res.* **1990**, *49*, 400 and references cited therein.
5. Claeson, G. *Acta. Chimica. Scand.* **1955**, *9*, 178.
6. HPLC conditions: Column Chiralcel OJ™, Daicel, Japan; 5×250 mm, λ 232 nm; **1**: solvent 10% isopropanol in hexane; flow rate 0.5 ml/min; retention times (*S*)-**1**: 26.9 and (*R*)-**1**: 29.1 min; **2**: $[\alpha]_D^{20}$ −117.4 (c 1, methanol); ee 98.5% determined after converting into corresponding butyl ester (*R*)-**1**. **3**: $[\alpha]_D^{20}$ −35.2 (c 1, methanol); ee 99.2% determined after acetylation to (*S*)-**1**. Optical purity of **3** can also be measured directly by chiral HPLC: solvent 12% isopropanol in hexane; flow rate 0.6 ml/min; retention times (*S*)-**3**: 23.3 and (*R*)-**3**: 24.3 min; ee 99.2%.
7. Sridhar, M.; Vadivel, S. K.; Bhalerao, U. T. *Synth. Commun.* **1998**, *28*, 1499.
8. Merck Index, pp. 1591, 9462.
9. Fadnavis, N. W.; Koteshwar, K. *Tetrahedron: Asymmetry* **1997**, *8*, 337.
10. HPLC conditions **6**: Chiralcel OD™, Daicel, Japan; 5×250 mm, λ 330 nm; 1% isopropanol in hexane; flow rate 0.6 ml/min; retention times: (*R*)-**6**: 17.8 and (*S*)-**6**: 18.3 min; ee 98.3%. $[\alpha]_D^{20}$ −103.0 (c 1.9, benzene), lit.[12] $[\alpha]_D^{20}$ −113 (c 1.88, benzene). Selected ^{1}H NMR ($CDCl_3$ 200 MHz) δ **1**: 0.9 (t, 3H, J=7.5 Hz), 1.3 (m, 2H), 1.6 (m, 2H), 1.9–2.3 (m, 2H), 2.2 (s, 3H), 2.3 (s, 3H), 2.8 (t, 2H, 6.1 Hz), 4.0 (t, 2H, J=5.6 Hz), 4.1 (t, 1H, 7.0 Hz). **2**: 1.85–2.2 (m, 2H), 2.21 (s, 3H), 2.26 (s, 3H), 2.9 (m, 2H), 4.3 (t, 1H, 7.1 Hz). **3**: 1.0 (t, 3H, J=7.2 Hz), 1.3 (m, 2H), 1.6 (m, 2H), 1.9–2.3 (m, 2H), 2.3 (s, 3H), 2.9 (t, 2H, J=5.8 Hz), 4.0 (t, 2H, J=5.9 Hz), 4.3 (t, 1H, 7.2 Hz). **4**: 1.5–2.0 (m, 6H), 2.4 (t, 2H, J=7.1 Hz), 2.5 (m, 2H), 3.15 (m, 2H), 3.6 (m, 1H).

11. Bianchi, D.; Cesti, P. *J. Org. Chem*, **1990**, *55*, 5657.
12. Sproull, K. C.; Bowman, G. T.; Carta, G.; Gainer, J. L. *Biotechnol. Prog*. **1997**, *13*, 71.
13. Antonov, V. K.; Dyakov, V. L.; Mishin, A. A.; Rotanov, T. V. *Biochimie* **1988**, 1235.
14. Chapus, C.; Semeriva, M. *Biochemistry* **1976**, *15*, 4988.
15. Colton, I. J.; Ahmed, S. N.; Kazlaukskas, R. J. *J. Org. Chem*. **1995**, *60*, 212.
16. Grochulski, P.; Schrag, J. D.; Cygler, M. *Prot. Sci*. **1994**, *3*, 82.
17. Grochulski, P.; Bouthillier, F.; Kazlaukskas, R.; Senreqi, A. N.; Schrag, J. D.; Ziomek, E.; Cygler, M. *Biochemistry* **1994**, *33*, 3494.