Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 177–179

TETRAHEDRON: *ASYMMETRY*

Enhancement of the enantioselectivity of lipase OF catalyzed hydrolysis

Yuan-Fung Chang and Dar-Fu Tai*

Department of Chemistry, National Dong-Hwa University, Hualien, Taiwan

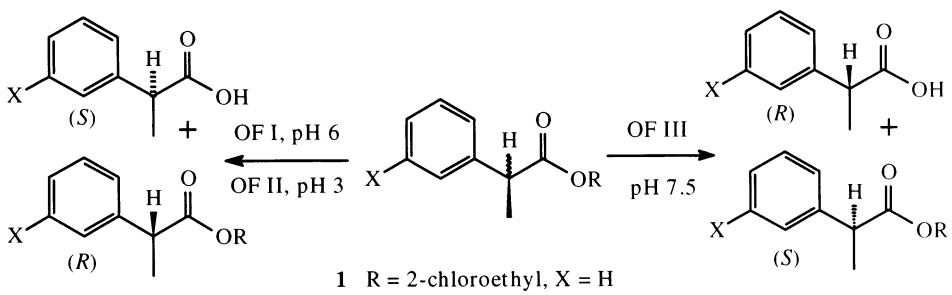
Received 6 December 2000; accepted 26 December 2000

Abstract—By rational purification of lipase OF on a mercurial affinity column three fractions were identified as responsible for the improved enantioselectivity without compromising the total activity of the crude enzyme. These three portions of lipase OF have remarkably different abilities to differentiate between the enantiomers of α-arylpropionic acids in the lipase catalyzed hydrolysis of the corresponding esters.

1. Introduction

Lipase catalysis is a well established method to obtain enantiomerically pure building blocks for the synthesis of structurally complex molecules with controlled stereochemistry.[1] Since most of the commercial lipases contain several competing enzymes and additives,[2] the enantiorecognition of lipase has not been as accurate as it should be. In fact, the enantioselectivity can be boosted by increasing the purity of the enzyme.[3] Some examples of improved lipase/esterase enantioselectivity have been reported in the literature.[4] However, these purification protocols are often laborious and involve multiple steps resulting in low enzyme recovery. Previously, subtilisin and thiosubtilisin, with only one amino acid difference, were easily separated[5] using a convenient method based on an agarose mercurial column.[6] This method was therefore selected as a tool for the fast separation of a crude lipase. As (*S*)-ibuprofen and (*S*)-ketoprofen are non-steroidal anti-inflammatory drugs, many kinetic resolutions of related compounds were reported.[3,4,7] Recently, we observed that crude lipase OF[8] possessed significantly higher enantioselectivity at pH 3 than pH 6 upon hydrolysis of ibuprofen esters.[9]

Herein, we report the purification of lipase OF isozymes on an agarose mercurial column and an improved resolution procedure for the hydrolysis of 2-chloroethyl 2-phenylpropionate **1**, ibuprofen 2-chloroethyl ester **2** and ketoprofen 2-chloroethyl ester **3** by utilizing these isolated isozymes (Scheme 1).



1 R = 2-chloroethyl, X = H
2 R = 2-chloroethyl, X = Isobuty
3 R = 2-chloroethyl, X = Benzoyl

Scheme 1.

* Corresponding author. E-mail: dftai@mail.ndhu.edu.tw

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00017-9

2. Results and discussion

An initial screening of crude lipase OF character was performed on the hydrolysis of 2-chloroethyl α-arylpropionates (Table 1). It was thought that the low enantioselectivity could be due to contamination by other hydrolases. It was therefore decided to use a purified hydrolyze to facilitate the resolution process.

For the purification, a method based on an agarose mercurial column for the separation of mercaptoprotein and non-mercaptoprotein[6] was explored. Consequently, crude lipase OF was purified and a schematic representation of the chromatogram is shown in Fig. 1. Three fractions were obtained. The first fraction is named OF I, followed by OF II and OF III, respectively. OF III was bound to the mercurial column and eluted with 0.15 mmol of cysteine buffer at pH 7. Each fraction was combined and lyophilized. Various portions of these lyophilized powders (1.5 mg/mL) were used directly in the same manner as above to examine the hydrolysis of 2-chloroethyl esters of 2-phenylpropionic acid, ibuprofen and ketoprofen. The best hydrolysis results of these esters by lipase OF I was found at pH 6 (E>100). In fact, OF I was also able to boost the enantioselectivity for ketoprofen at pH 6 with an e.e. of 99.4% and showed an E value of 397 (entry 10), which resembles the results obtained from 2-propanol-treated CRL[4d] or CRL-CLECs (E=64).[3] At pH 3, lipase OF II demonstrated a similar character to crude lipase OF. Moreover, both the e.e. and E values were improved. As shown in entry 12 of Table 2, an e.e. of 99.5% was observed.

The lipase OF III has a preference for the (R)-enantiomer. The hydrolysis of ketoprofen 2-chloroethyl ester by lipase OF III was found to have the opposite stereoselectivity[7] with good enantioselectivity at pH 8 (entry 21) (Table 3). It is quite possible that OF III is still a mixture of several hydrolases. Further purification of OF III could improve its enantioselectivity in using it for other resolution processes.

3. Conclusion

We have shown that the lipase OF contains at least three isozymes with different enantioselectivity. We have discovered that separation on a mercurial column can differentiate these lipases with a superior enantiose-

Table 1. Lipase OF catalyzed the hydrolysis of 2-chloroethyl α-arylpropionates[a]

Entry	Enzyme	Ester	Ph	Duration (h)	E.e.$_s$ (%)	E.e.$_p$ (%)	Conversion (%)	E_p[10]
1	Crude lipase OF	**1**	3	34	89.7(R)	87.9(S)[b]	51	47
2	Crude lipase OF	**1**	6	8	16.7(R)	4.7(S)[b]	31	1
3	Crude lipase OF	**1**	9	6	1.9(R)	3.5(S)[b]	36	1
4	Crude lipase OF	**2**	6	8	28.4(R)	50.2(S)[c]	46	5
5	Crude lipase OF	**3**	2.6	48	23.9(R)	91.9(S)[d]	21	30
6	Crude lipase OF	**3**	8	16	1.2(S)	42.7(R)[d]	12	3

[a] Racemic 2-chloroethyl ester (0.05 mmol), 0.1 M phosphate buffer (2 ml) and crude lipase OF (1 mg) at 37°C.
[b] Determined by HPLC analysis on a chiralcel OD column with hexane:isopropanol:acetic acid=99:1:0.03.
[c] Determined by HPLC analysis on a chiralcel OD column with hexane:isopropanol:acetic acid=99:1:0.05.
[d] Determined by HPLC analysis on a chiralcel OJ column with hexane:isopropanol:acetic acid=92:8:0.3.

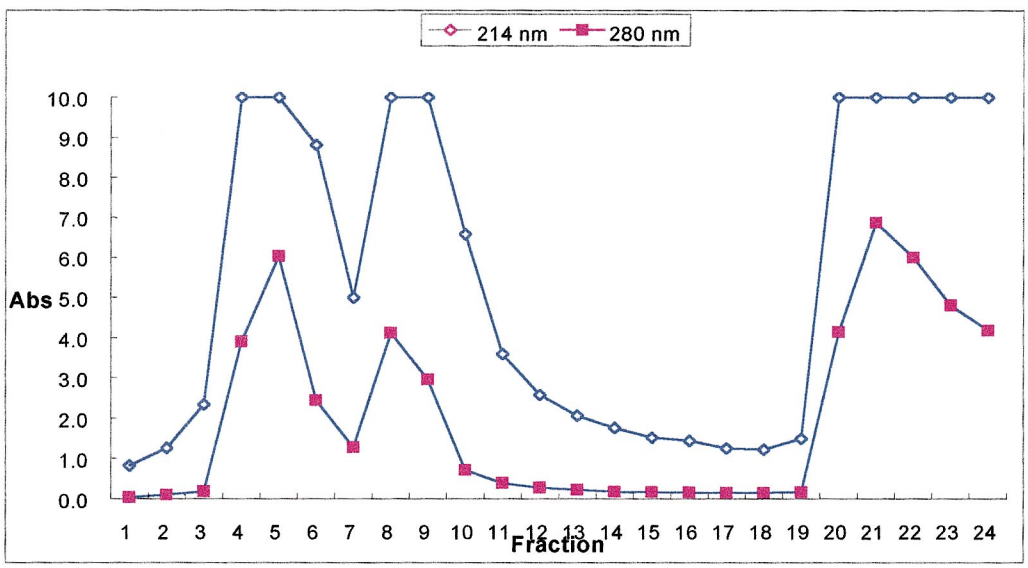


Figure 1. Chromatogram of purification of lipase OF on agarose mercurial affinity column.

Table 2. Comparison of the enantioselectivity of lipase OF I and lipase OF II on the hydrolysis of 2-chloroethyl α-arylpropionates at various pH

Entry	Enzyme	Ester	pH	Duration (h)	E.e.$_s$ (%)	E.e.$_p$ (%)	Conversion (%)	E_p[10]
7	Lipase OF I	**1**	3	48	0	0	0	0
8	Lipase OF I	**1**	6	104	20.4(*R*)	97.7(*S*)	24	104
9	Lipase OF I	**2**	6	20	46.6(*R*)	97.2(*S*)	40	137
10	Lipase OF I	**3**	6	72	29.3(*R*)	99.4(*S*)	19	397
11	Lipase OF I	**3**	8	72	8.6(*R*)	73.3(*S*)	7	7
12	Lipase OF II	**1**	3	36	30.7(*R*)	99.5(*S*)	24	576
13	Lipase OF II	**1**	6	40	88.6(*R*)	45.9(*S*)	40	7
14	Lipase OF II	**2**	6	20	39.8(*R*)	42.2(*S*)	51	4
15	Lipase OF II	**3**	3	56	19.1(*R*)	93.6(*S*)	20	36
16	Lipase OF II	**3**	8	20	42.7(*R*)	71.0(*S*)	45	9

Table 3. Enantioselectivity of lipase OF III in the resolution of 2-chloroethyl esters

Entry	Enzyme	Ester	pH	Duration (h)	E.e.$_s$ (%)	E.e.$_p$ (%)	Conversion (%)	E_p[10]
17	Lipase OF III	**1**	3	48	0	0	0	0
18	Lipase OF III	**1**	7.5	18	0.5(*S*)	10.5(*R*)	42	1
19	Lipase OF III	**2**	6	20	23.4(*R*)	59.5(*S*)	33	5
20	Lipase OF III	**3**	2.6	72	0	0	0	0
21	Lipase OF III	**3**	8	96	92.3(*S*)	75.1(*R*)	58	23

lectivity compared to the crude enzyme. We have furthermore identified the isozymes at different pH that control, independently, the enantioselectivity and the overall catalytic activity of the lipase.

Acknowledgements

This work was financially supported by the National Science Council of Taiwan (NSC 86-2113-M-259-007).

References

1. (a) Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: New York, 1994; (b) Schmid, R. D.; Verger, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1608–1633; (c) Berglund, P.; Hult, K. In *Stereoselective Biocatalysis*; Patel, R., Ed.; Marcel Dekker: New York, 1999; pp. 633–657.
2. Chang, R. C.; Chou, S. J.; Shaw, J. F. *Biotechnol. Appl. Biochem.* **1994**, *19*, 93–97.
3. Lalonde, J. J.; Govardhan, C. P.; Khalaf, N. K.; Martinez, A. G.; Visuri, K.; Margolin, A. L. *J. Am. Chem. Soc.* **1995**, *117*, 6845–6852.
4. (a) Wu, S.-H.; Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990–1995; (b) Brahimi-Horn, M. C.; Guglielmino, M. L.; Elling, L.; Sparrow, L. G. *Biochim. Biophys. Acta.* **1990**, *1042*, 51–54; (c) Hernaiz, M. J.; Sanchez-Montero, J. M.; Sinisterra, J. V. *Tetrahedron* **1994**, *50*, 10749–10760; (d) Colton, I. J.; Ahmed, S. N.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 212–217; (e) Persichetti, R. A.; Lalonde, J. J.; Govardhan, C. P.; Khalaf, N. K.; Margolin, A. L. *Tetrahedron Lett.* **1996**, *37*, 6507–6510; (f) Cittadini, M.; Fringuelli, F. *Tetrahedron* **1998**, *54*, 7883–7890.
5. Sluyterman, L. A.; Wijdenes, J. *Biochim. Biophys. Acta.* **1970**, *200*, 593–595.
6. Polgar, L. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1976**, *11*, 81–86.
7. (a) Goto, M.; Noda, S.; Kamiya, N.; Nakashio, F. *Biotechnol. Lett.* **1996**, 839–844; (b) Tsai, S. W.; Lin, J. J.; Chang, C. S.; Chen, J. P. *Biotechnol. Prog.* **1997**, *13*, 82–88; (c) Cleij, M.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1999**, *64*, 5029–5035.
8. Wirz, B.; Spurr, P. *Tetrahedron: Asymmetry* **1995**, *6*, 669–670.
9. Tai, D.-F.; Chao, Y.-H.; Huang, C.-Y.; Luo, J.-M.; Lin, Y.-T.; Wu, S.-H. *J. Chin. Chem. Soc.* **1995**, *42*, 801–807.
10. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.