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Resolution of non-protein amino acids via *Carica papaya* lipase-catalyzed enantioselective transesterification

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Abstract—*Carica papaya* lipase-catalyzed transesterification of the 2,2,2-trifluoroethyl esters of *N*-benzyloxycarbonylated DL-amino acids carrying aliphatic side chains proceeded smoothly and, in almost all the cases, enantiospecifically ($E = >200$), affording the L-methyl esters and leaving the D-trifluoroethyl esters intact.

1. Introduction

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) have been used as stereoselective catalysts for the preparation of optically active forms of a wide variety of alcohols and carboxylic acids.[1–3] The enzymes utilized for such a purpose are those from mammalian and microbial sources.[4–7] On the other hand, plant lipases have rarely been employed for synthetic purposes until recently. Some lipases from plant latexes, however, have become available in large quantities and are now applied to lipid conversions.[8–10] Quite recently, *Carica papaya* lipase (CPL), stored in the crude papain, was exploited for the kinetic resolutions of (*RS*)-2-(4-chlorophenoxy)propanoic acid via enantioselective esterification.[11] The highest enzyme activity and enantioselectivity were attained with trimethylsilylmethanol as the acyl acceptor in cyclohexane. CPL was more active, enantioselective, and thermally more stable than *Candida rugosa* lipase, which is known to show high enantioselectivity toward carboxylic acids.[12,13] This plant lipase was also exploited for the enantioselective hydrolysis of (*RS*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated organic solvents.[14] An excellent enantioselectivity was observed in water-saturated isooctane at 60 °C. The hydrolytic resolution of this acid with CPL was also investigated as its 2,2,2-trifluoroethyl thioester in water-saturated organic solvents.[15] These successful results have prompted us to examine CPL as a catalyst for the resolution of *N*-protected amino acids as chiral carboxylic acids. We herein report the CPL-catalyzed highly enantioselective transesterification of *N*-benzyloxycarbonyl (Z)-derivatives of non-protein amino acid esters in organic solvents.

Homochiral non-protein amino acids are useful as building blocks for the synthesis of analogues of biologically active peptides[16] and as versatile chiral starting materials or chiral auxiliaries for other synthetic purposes.[17] We have already reported on the resolution of non-protein amino acids via the enantioselective hydrolysis of 2-chloroethyl or 2,2,2-trifluoroethyl esters of their *N*-Z derivatives catalyzed by lipases from *Aspergillus niger* and porcine pancreas.[18] We have also proposed a transesterification procedure for their resolution mediated by *Pseudomonas cepacia* (*Burkholderia cepacia*) lipase.[19]

The results reported herein together with those reported earlier clearly suggest that CPL must be a very promising enzyme for the resolution of carboxylic acids via enantioselective esterification, transesterification, or hydrolysis.

2. Results and discussion

In a general experimental procedure, the 2,2,2-trifluoroethyl ester of an *N*-Z-DL-amino acid DL-**1** was allowed

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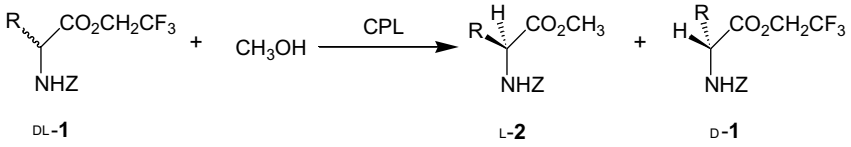

Scheme 1. CPL-catalyzed enantioselective transesterification between an *N*-Z-DL-amino acid 2,2,2-trifluoroethyl ester DL-**1** and methanol. See Table 3 for R.

to react with an alcohol (4 M equiv) in an organic solvent in the presence of CPL at a constant temperature (Scheme 1). The trifluoroethyl ester was employed because of the tardiness of transesterification of other activated esters, such as the 2,2,2-trichloroethyl ester as well as the conventional alkyl esters. The reaction was monitored (conversion and enantiomeric excess values) by HPLC on a chiral column. Initially, norleucine (2-aminohexanoic acid, Nle), an isomer of the naturally occurring leucine, was chosen as a model amino acid and the effects of the reaction parameters investigated on the enzymatic transesterification of the 2,2,2-trifluoroethyl ester of its *N*-Z-derivative DL-**1d**. As temperature has been recognized as one of the major potential factors, which can affect the rate and enantioselectivity of enzymatic reactions,[20] the transesterification between DL-**1d** and propanol in cyclohexane was investigated at different temperatures (25–45 °C). Lowering the temperature resulted in a large decrease in the reaction rate [temperature (°C), % conversion after 24 h of incubation: 25, 11.3; 35, 21.4; 45, 43.4]. On the other hand, temperature did not affect the enantioselectivity of the transesterification at all: it was almost completely enantioselective at each temperature (vide infra). Thus, subsequent experiments were carried out at 45 °C.

Next, the effect of the alcohol nucleophile was investigated on the transesterification of DL-**1d** in cyclohexane. The results are summarized in Table 1. The time course of the transesterification between DL-**1d** and methanol is depicted in Figure 1. The reaction proceeded in an almost completely enantioselective manner: the L-methyl ester L-**2d** was solely formed until the reaction reached 50% conversion, while D-trifluoroethyl ester D-**1d** remained nearly intact.[22] The enantiomeric ratio, *E*,[21] in this case was evaluated to be >200.[23] There was almost no difference found between methanol and ethanol as the reacting nucleophile with regard to the reaction rate and enantioselectivity.[24] When the chain length of the alcohol became longer, the conversion rate decreased severely, with hexanol serving as the poorest nucleophile, although the enantioselectivity was not affected at all.

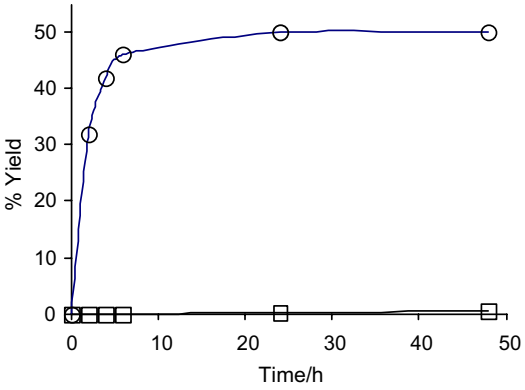

Figure 1. Reaction profile in the CPL-catalyzed transesterification between Z-DL-Nle-OCH_2CF_3 DL-**1d** and methanol in cyclohexane. Symbols: ○, Z-L-Nle-OCH_3 L-**2d**; □, Z-D-Nle-OCH_3 D-**2d**.

Recently, much attention has been focused on the solvent effect on the enantioselectivity of enzymatic reactions.[25] Table 2 shows the effect of solvents on the transesterification between DL-**1d** and methanol. Both the reaction rate and enantioselectivity were affected largely by the solvent employed. The use of ethers, such as isopropyl ether resulted in a marked decrease in enantioselectivity. The reactions were slowed down to a great extent in solvents such as acetonitrile and chloroform, although they proceeded in an almost completely enantioselective manner. Thus, cyclohexane proved to be the best one among the solvents examined.

Based on the above results, the transesterification with methanol of the 2,2,2-trifluoroethyl esters of *N*-Z-DL-

Table 1. CPL-catalyzed transesterification of Z-DL-Nle 2,2,2-trifluoroethyl ester DL-**1d** in cyclohexane[a,b]

Alcohol	% Convn.	% ee_P[c]	*E*
Methanol	50.2	99.2	>200
Ethanol	50.0	>99.8	>200
Propanol	43.4	>99.8	>200
Hexanol	20.5	>99.8	>200

[a] Nle = 2-aminohexanoic acid.
[b] Conditions: 0.1 mmol of Z-DL-Nle-OCH_2CF_3, 0.4 mmol of an alcohol, and 10 mg of CPL in 0.8 ml of anhydrous cyclohexane at 45 °C. The results shown are those obtained after 24 h of incubation.
[c] Enantiomeric excess of the newly formed ester.

Table 2. CPL-catalyzed transesterification between Z-DL-Nle 2,2,2-trifluoroethyl ester DL-**1d** and methanol in different solvents[a]

Solvent	% Convn.	% ee_P[b]	*E*
Cyclohexane	50.2	99.2	>200
Isooctane	13.7	94.2	39
Isopropyl ether	45.1	73.4	12
tert-Butyl methyl ether	44.8	71.4	11
Chloroform	11.9	>99.8	>200
Acetonitrile	17.0	>99.8	>200

[a] Conditions: 0.1 mmol of DL-**1d**, 0.4 mmol of methanol, and 10 mg of CPL in 0.8 ml of an anhydrous organic solvent at 45 °C. The results shown are those obtained after 24 h of incubation.
[b] Enantiomeric excess of the newly formed methyl ester **2d**.

Table 3. CPL-catalyzed transesterification between *N*-Z-DL-amino acid 2,2,2-trifluoroethyl esters DL-**1** and methanol in cyclohexane[a]

Compound	R	% Convn.	% ee_P[b]	*E*	Cf. *E*[c]
1a	CH_3	55.6	78.4	38	8.7
1b	CH_3CH_2	50.8	95.6	>200	42
1c	$CH_3(CH_2)_2$	46.3	>99.8	>200	37
1d	$CH_3(CH_2)_3$	50.2	99.2	>200	30
1e	$(CH_3)_2CHCH_2$	43.6	>99.8	>200	
1f	$CH_3(CH_2)_4$	46.8	>99.8	>200	23
1g	$(CH_3)_2CH(CH_2)_2$	48.0	>99.8	>200	
1h	*c*-$C_6H_{11}CH_2$	26.0	>99.8	>200	
1i	$CH_3SCH_2CH_2$	50.2	99.2	>200	
1j	$CH_3CH_2SCH_2CH_2$	47.7	>99.8	>200	

[a] Conditions: 0.1 mmol of DL-**1**, 0.4 mmol of methanol, and 10 mg of CPL in 0.8 ml of anhydrous cyclohexane at 45 °C. The results shown are those obtained after 24 h of incubation.
[b] Enantiomeric excess of the newly formed methyl ester **2**.
[c] *P. cepacia* (*B. cepacia*) lipase-catalyzed transesterification between DL-**1** and methanol in isopropyl ether.[19]

amino acids DL-**1** carrying a number of aliphatic side chains was examined in cyclohexane at 45 °C. The results are summarized in Table 3, which also includes those with some proteinogenic amino acids for the purpose of comparison. The CPL-catalyzed transesterifications proceeded smoothly, especially with amino acids bearing smaller side chains. In almost all cases examined, the reactions were almost completely enantioselective ($E = >200$),[23] affording the L-methyl esters L-**2** and leaving the D-trifluoroethyl esters D-**1** intact. One exception is the result with the alanine derivative **1a**. Even in this case, though, the *E* value (38) was high enough, although it was smaller than those with other cases. In the last column of Table 3 the *E* values observed in the *P. cepacia* (*B. cepacia*) lipase-catalyzed transesterification between DL-**1** and methanol in isopropyl ether are included.[19] With this lipase, little difference was observed in the solvents employed, isopropyl ether and cyclohexane. The enantioselectivities as judged from the *E* values were good to tolerable with the exception of that obtained with alanine. The *E* values, however, are smaller in one order of magnitude than those obtained in the present study with CPL.

In all the cases mentioned above, the preferential reaction of the L-enantiomers was confirmed by comparison with authentic samples prepared from the optically active amino acids, if available, on HPLC or suggested from the regularity of elution order of the enantiomers on HPLC.[26,27] This stereochemical preference is the same as that observed in the hydrolysis of *N*-Z-DL-amino acid 2-chloroethyl or 2,2,2-trifluoroethyl esters[18] and the transesterification of *N*-Z-DL-amino acid 2,2,2-trifluoroethyl esters[19] mediated by other lipases.

3. Conclusion

The results reported herein show that CPL can act as an enantioselective catalyst in the transesterification of *N*-protected aliphatic amino acid activated esters in an organic solvent such as cyclohexane.[28] This together with the results reported earlier clearly demonstrate that CPL must be an enzyme of great promise for the resolution of carboxylic acids via enantioselective transesterification as well as via enantioselective esterification or hydrolysis, taking into account that the lipase preparation is available easily and inexpensive. We have also started an investigation on the resolution of non-protein amino acids via the enantioselective hydrolysis of their esters utilizing CPL.

4. Experimental

4.1. General

^{1}H NMR spectra were obtained at 300 MHz on a Varian Unity 300 spectrometer using chloroform-*d* as a solvent with TMS as the internal standard. All organic solvents were distilled following standard protocols and dried over molecular sieves prior to use.

4.2. Preparation of substrates

DL-2-Aminoheptanoic acid, DL-2-amino-5-methylhexanoic acid, and DL-2-amino-3-cyclohexylpropanoic acid were prepared from diethyl acetamidomalonate and the corresponding alkyl bromide by adopting essentially the same procedure described in the literature.[29] Other racemic amino acids were purchased from Tokyo Chemical Industry, Wako Pure Chemical Industries, or Aldrich Chemical Co. The amino acids were benzyloxycarbonylated using benzyloxycarbonyl chloride (Z-Cl) under the usual Schotten–Baumann conditions[30] to give *N*-Z-DL-amino acids.[18] These were purified by recrystallization from an appropriate solvent (e.g., EtOAc–petroleum ether) and converted to the 2,2,2-trifluoroethyl esters by the EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]-DMAP (4-dimethylaminopyridine) method[31] using 2,2,2-trifluoroethanol. ^{1}H NMR data ($CDCl_3$) of the *N*-Z-DL-amino acid 2,2,2-trifluoroethyl esters thus prepared are as follows.[32]

4.2.1. Compound 1b. δ_H 0.97 (3H, t, $J = 7.5$ Hz), 1.69–2.00 (2H, m), 4.36–4.50 (2H, m), 4.56–4.69 (1H, m), 5.09 (2H, s), 5.21 (1H, br d), 7.29–7.37 (5H, m).

4.2.2. Compound 1c. δ_H 0.95 (3H, t, $J = 7.3$ Hz), 1.31–1.48 (2H, m), 1.61–1.90 (2H, m), 4.36–4.49 (2H, m), 4.57–4.70 (1H, m), 5.12 (2H, s), 5.17 (1H, br d, $J = 8$ Hz), 7.25–7.39 (5H, m).

4.2.3. Compound 1d. δ_H 0.89 (3H, t, $J = 6.9$ Hz), 1.25–1.40 (4H, m), 1.63–1.91 (2H, m), 4.34–4.48 (2H, m), 4.56–4.69 (1H, m), 5.11 (2H, ABq, $J = 12$ Hz), 5.20 (1H, br d, $J = 8$ Hz), 7.29–7.40 (5H, m).

4.2.4. Compound 1e. δ_H 0.93–0.97 (6H, m), 1.56–1.76 (3H, m), 4.35–4.50 (2H, m), 4.53–4.66 (1H, m), 5.07 (1H, br d, $J = 6$ Hz), 5.10 (2H, s), 5.22 (1H, br d, $J = 8$ Hz), 7.36–7.40 (5H, m).

4.2.5. Compound 1f. δ_H 0.87 (3H, apparent t), 1.23–1.41 (6H, m), 1.62–1.93 (2H, m), 4.34–4.48 (2H, m), 4.56–4.68 (1H, m), 5.10 (2H, ABq, $J = 12$ Hz), 5.16 (1H, br d, $J = 8$ Hz), 7.28–7.38 (5H, m).

4.2.6. Compound 1g. δ_H 0.80–0.93 (6H, m), 1.11–1.32 (2H, m), 1.46–1.61 (1H, m), 1.61–1.92 (2H, m), 4.35–4.50 (2H, m), 4.57–4.70 (1H, m), 5.12 (2H, ABq, $J = 12$ Hz), 5.22 (1H, br d, $J = 8$ Hz), 7.26–7.37 (5H, m).

4.2.7. Compound 1h. δ_H 0.86–1.80 (13H, m), 4.34–4.52 (2H, m), 4.54–4.67 (1H, m), 5.07 (1H, br d, $J = 6$ Hz), 5.05 (1H, br d, $J = 8$ Hz), 5.10 (2H, ABq, $J = 13.5$ Hz), 7.28–7.38 (5H, m).

4.2.8. Compound 1i. δ_H 1.94–2.23 (2H, m), 2.06 (3H, s), 2.53 (2H, t, $J = 7.2$ Hz), 4.36–4.48 (1H, m), 4.53–4.66 (2H, m), 5.10 (2H, s), 5.43 (1H, br d, $J = 8$ Hz), 7.28–7.34 (5H, m).

4.2.9. Compound 1j. δ_H 1.22 (3H, t, $J = 7.4$ Hz), 1.92–2.22 (2H, m), 2.45–2.61 (4H, m), 4.37–4.49 (1H, m), 4.53–4.66 (2H, m), 5.10 (2H, s), 5.38 (1H, br d), 7.24–7.35 (5H, m).

4.3. Preparation of the enzyme[11]

The crude papain (Sigma P-3375, 5.0 g) was dissolved in distilled water (25 ml), stirred for 30 min at 4 °C, and then centrifuged (12,000 rpm, 12 min) at the same temperature. After the supernatant was removed, the above procedure was repeated on the residue. The precipitate was collected and lyophilized to afford the crude CPL (0.75 g).

4.4. HPLC analysis

Transesterification reactions were monitored by chiral HPLC on a Chiralpak AS column (4.6 mm id × 250 mm) or a Chiralcel OD column[27] (4.6 mm id × 250 mm) (Daicel Chemical Industries) using hexane–2-propanol as an eluent. The liquid chromatograph employed was a Shimadzu LC-10AS instrument, equipped with a Rheodyne 7125 sample injector and a Shimadzu SPD-10A variable wavelength UV monitor. The temperature of the column was maintained using a thermostated bath. A Shimadzu C-R8A data processor was used for data acquisition and processing. The enantiomers of the methyl esters **2** of *N*-Z-amino acids were separated well enough for the accurate determination of the ee values on either of the columns by choosing an appropriate proportion of hexane/2-propanol for each compound. In general, the enantiomeric separations of the corresponding 2,2,2-trifluoroethyl esters **1** were inferior to those of the methyl esters on either column. The limit of detection on the HPLC analysis was estimated as ca. 0.1% enantiomer. The separation of the enantiomers of *N*-Z-amino acid 2,2,2-trifluoroethyl esters **1** and methyl esters **2** on the Chiralpak AS column is shown in Table 4.

4.5. General procedure for the CPL-catalyzed transesterification

A solution of an *N*-Z-amino acid 2,2,2-trifluoroethyl ester (0.1 mmol) and an alcohol (0.4 mmol) in an organic solvent (0.8 ml) was stirred with the CPL preparation (10 mg) in a 1-ml screw-capped vessel in a thermostated incubator. Monitoring the reaction and assessing the ee value of the newly formed ester were conducted simultaneously by HPLC analysis on the chiral columns mentioned above. Aliquots (ca. 10 μl) of the reaction mixture were withdrawn at frequent intervals, diluted with ether (200 μl), filtered through a PTFE membrane filter, and then injected (ca. 5 μl) onto the column. The results obtained after 24 h of incubation are shown in Tables 1–3.

Table 4. HPLC separation of the enantiomers of *N*-Z-amino acid 2,2,2-trifluoroethyl esters **1** and methyl esters **2**[a]

R	2,2,2-Trifluoroethyl ester			Methyl ester		
		k_1'[b]	α[c]		k_1'[b]	α[c]
CH_3	**1a**	2.50	1.16	**2a**	3.83	1.17
CH_3CH_2	**1b**	2.00	1.00	**2b**	3.07	1.18
$CH_3(CH_2)_2$	**1c**	1.77	1.13	**2c**	3.27	1.12
$CH_3(CH_2)_3$	**1d**	1.43	1.12	**2d**	2.47	1.18
$(CH_3)_2CHCH_2$	**1e**	1.43	1.23	**2e**	2.57	1.30
$CH_3(CH_2)_4$	**1f**	1.17	1.06	**2f**	2.03	1.07
$(CH_3)_2CH(CH_2)_2$	**1g**	1.20	1.19	**2g**	2.03	1.31
c-$C_6H_{11}CH_2$	**1h**	1.57	1.15	**2h**	3.57	1.00
$CH_3SCH_2CH_2$	**1i**	4.07	1.05	**2i**	7.50	1.09
$CH_3CH_2SCH_2CH_2$	**1j**	3.27	1.07	**2j**	5.93	1.11

[a] HPLC conditions: column, Chiralpak AS; mobile phase, hexane–2-propanol (95:5, v/v); flow rate 1.0 ml min^{-1}; column temperature, 30 °C.
[b] Capacity factor: $k' = (t_R - t_0)/t_0$ where t_0 = void time. The void time was estimated to be 3.0 min using 1,3,5-tri-*tert*-butylbenzene. The suffix 1 denotes for the faster eluting enantiomer.
[c] Separation factor: $\alpha = k_2'/k_1'$. The suffix 2 denotes for the slower eluting enantiomer.

Acknowledgments

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