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ORIGINAL RESEARCH PAPER

Long-chain ethers as solvents can amplify the enantioselectivity of the *Carica papaya* lipase-catalyzed transesterification of 2-(substituted phenoxy)propanoic acid esters

Toshifumi Miyazawa · Wakana Iguchi

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Abstract The enantioselectivity of the transesterification of the 2,2,2-trifluoroethyl esters of 2-(substituted phenoxy)propanoic acids, as catalyzed by the lipase from *Carica papaya*, was greatly improved by using long-chain ethers, such as di-*n*-hexyl ether, as solvents instead of the conventional diisopropyl ether. Thus, for example, the *E* value was enhanced from 21 [in diisopropyl ether (0.8 ml)] to 57 [in di-*n*-hexyl ether (0.8 ml)] in the reaction of 2,2,2-trifluoroethyl(*RS*)-2-phenoxypropanoate (0.1 mmol) with methanol (0.4 mmol) in the presence of the plant lipase preparation (10 mg); it was also improved from 13 (in diisopropyl ether) to 44 (in di-*n*-hexyl ether) in the reaction of 2,2,2-trifluoroethyl(*RS*)-2-(2-chlorophenoxy)propanoate with methanol under the same reaction conditions.

Keywords *Carica papaya* lipase · Enantioselectivity · Long-chain ethers · 2-(Substituted phenoxy)propanoic acids · Transesterification

Introduction

Lipases are a very attractive group of enzymes for synthetic purposes (Faber 2011; Paravidino et al. 2012). They have successfully been employed for the preparation of optically active forms of a wide range of alcohols and carboxylic acids via hydrolysis, esterification, or transesterification. Organic media are ordinarily employed for such transformations and they can affect not only the activity but also the selectivity of enzymes (Koskinen and Klibanov 1996). The most reliable measure for the compatibility of an organic solvent with high enzyme activity has been obtained by using log *P* describing the hydrophobicity of the solvent (Laane et al. 1987). In general, solvents with log *P* values >2 cause negligible enzyme distortion. Those with log *P* values between 1.5 and 2 cause some distortion, but they can be used with caution. Therefore, such solvents as diisopropyl ether [log $P = 1.52$ (Sangster 1989)] are often employed.

Lipases for synthetic purposes have been limited to those from mammalian and microbial sources. *Carica papaya* lipase (CPL), stored in crude papain, was exploited for the kinetic resolutions of chiral acids (Cheng and Tsai 2004; Chen and Tsai 2005; Ng and Tsai 2005). We also reported the kinetic resolution of amino acids (Miyazawa et al. 2005) and, more recently, that of secondary alcohols. Chang and Ho (2011) also reported the enantioselective esterification of 2-methylalkanoic acids.

2-Phenoxypropanoic acids are well-known herbicides but also have other biological activities (Witiak et al. 1968; Kawashima et al. 1984). Their resolution has been tried by employing lipases, α-chymotrypsin and pig liver esterase (Cambou and Klibanov 1984;

T. Miyazawa (✉) · W. Iguchi
Department of Chemistry, Faculty of Science and Engineering, Konan University, Higashinada-ku, Kobe 658-8501, Japan
e-mail: miyazawa@konan-u.ac.jp

Dernoncour and Azerad 1987; Chen et al. 1987; Pan et al. 1990; Yasufuku and Ueji 1997). We also reported their resolution using *Aspergillus niger* lipase (Miyazawa et al. 1999). We have investigated their resolution by employing CPL, and we found a marked improvement of enantioselectivity in transesterification by changing solvents.

Materials and methods

Materials

(*RS*)-2-(Substituted phenoxy)propanoic acids were converted to the 2,2,2-trifluoroethyl esters by the EDC[1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]-DMAP(4-dimethylaminopyridine) method (Dhaon et al. 1982) using 2,2,2-trifluoroethanol. Methyl esters were prepared by treatment of the corresponding carboxylic acids with an ethereal solution of diazomethane. Lipase: crude papain (5 g) was dissolved in distilled water (25 ml), centrifuged ($\sim$10,000$\times g$, 12 min) at 4 °C to collect the precipitate and lyophilized to afford the crude *C. papaya* lipase (CPL 0.75 g) (Miyazawa et al. 2005).

General procedure for the CPL-catalyzed transesterification

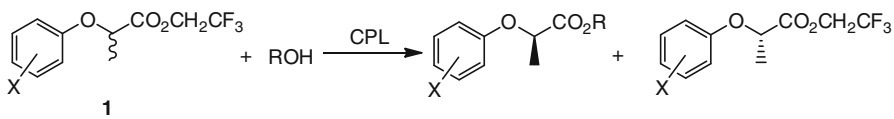
CPL-catalyzed transesterification with methanol of 2,2,2-trifluoroethyl(*RS*)-2-phenoxypropanoate (**1a**) is described as a typical example. A solution of **1a** (0.1 mmol) and methanol (0.4 mmol) in an organic solvent (0.8 ml) was stirred with the lipase preparation (10 mg) in a thermostated bath. The progress of the reaction and the enantiomeric excess (e.e.) value of the newly formed methyl ester were determined directly by chiral HPLC analysis on a Chiralpak AS column (4.6 mm id × 250 mm) or a Chiralcel OD column (4.6 mm id × 250 mm) (Daicel Chemical Industries) using hexane/2-propanol as an eluent. The enantiomers of the methyl esters were separated sufficiently for the accurate determination of the e.e. 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 were inferior to those of the methyl esters.

Results and discussion

In a general experimental procedure, the 2,2,2-trifluoroethyl ester of an 2-(substituted phenoxy)propanoic acid (**1**) was reacted with an alcohol (4 molar equiv.) in an organic solvent in the presence of CPL at a constant temperature (Scheme 1). The reaction was monitored (conversion and e.e. values) by HPLC on a chiral column. Initially, the parent 2-phenoxypropanoic acid 2,2,2-trifluoroethyl ester (**1a**) was chosen as a model compound and the effects of reaction parameters were investigated (Table 1).

According to Faber (2011), the values of enantiomeric ratios, *E*'s (Chen et al. 1982), below 15 are unacceptable, those in the range of 15–30 are regarded as moderate to good and above this value they are excellent. The reaction of **1a** with methanol was very slow in cyclohexane at 25 °C and even at 45 °C. In diisopropyl ether, the reaction was fast enough and afforded a 'moderate' enantioselectivity ($E = 21$) at 25 °C. At 45 °C the reaction became faster at the expense of enantioselectivity. The reactions were retarded in such polar solvents as acetonitrile and acetone, moreover giving poor enantioselectivity. Concerning the effect of alcohol nucleophiles, there was almost no difference between methanol and propanol with regard to the reaction rate but the enantioselectivity was appreciably decreased with the latter alcohol. With hexanol both the conversion rate and enantioselectivity were slightly worse when compared with those with methanol.

Based on the above results, the transesterification with methanol of the 2,2,2-trifluoroethyl esters of 2-(substituted phenoxy)propanoic acid (**1**) carrying a



Scheme 1 CPL-catalyzed enantioselective transesterification of a 2-(substituted phenoxy)propanoic acid 2,2,2-trifluoroethyl ester (**1**) with an alcohol. See Table 2 for the substituent X

number of substituents on the benzene ring was examined in diisopropyl ether at 25 °C. The results are compiled in Table 2. The CPL-catalyzed transesterifications proceeded smoothly except the compounds bearing an *ortho*-chloro substituent (**1c** and **1f**). Among the compounds examined the 2,2,2-trifluoroethyl esters of the parent 2-phenoxypropanoic acid (**1a**) and its alkyl-substituted compounds (**1h** and **1i**) gave 'tolerable' degree of enantioselectivities as judged from the *E* values (20–22). With the halogen-substituted compounds enantioselectivities were generally low and they were negligible with the compounds **1d** and **1f**. In the middle column of Table 2 are also included the *E* values observed in the *A. niger* lipase-catalyzed irreversible transesterification of 2-phenoxypropanoic acid vinyl esters with methanol in diisopropyl ether (Miyazawa et al. 1999). The enantioselectivities observed in the CPL-catalyzed transesterification were almost of the same magnitude as those observed in the *A. niger* lipase-catalyzed transesterification. The substituent effect on the enantioselectivity was similar in the two kinds of transesterifications mediated by these different lipases. In all the CPL-catalyzed transesterifications mentioned above, the preferential reaction of the (*R*)-enantiomers was confirmed by comparison on HPLC with authentic samples prepared form the *A. niger* lipase-catalyzed transesterification of 2-phenoxypropanoic acid vinyl esters or suggested from the regularity of elution order of the enantiomers on HPLC. This stereochemical preference is the reverse as that observed in the *A. niger* lipase-catalyzed transesterification of the vinyl esters.

As 'poor' to 'tolerable' level of enantioselectivities were observed in diisopropyl ether, we envisaged that

Table 1 *Carica papaya* lipase-catalyzed transesterification of 2-phenoxypropanoic acid 2,2,2-trifluoroethyl ester (**1a**)

Solvent	Temp. (°C)	Alcohol	% Convn.	% e.e.$_P$[a]	*E*
Cyclohexane	25	Methanol	1.6	–	–
Cyclohexane	45	Methanol	8.7	–	–
Diisopropyl ether	25	Methanol	46.0	82.3	21
Diisopropyl ether	25	Propanol	45.1	74	12
Diisopropyl ether	25	Hexanol	39.2	82.9	18
Diisopropyl ether	45	Methanol	63.9	52.1	9.7
Acetonitrile	25	Methanol	10.1	74.2	7.3
Acetone	25	Methanol	16.6	76.2	8.6

Reactions were conducted using 0.1 mmol of **1a**, 0.4 mmol of an alcohol and 10 mg of *Carica papaya* lipase in 0.8 ml of an organic solvent for 1 h

[a] Enantiomeric excess of the newly formed ester

Table 2 *Carica papaya* lipase-catalyzed transesterification of 2-(substituted phenoxy)propanoic acid 2,2,2-trifluoroethyl esters (**1**) with methanol in diisopropyl ether or di-*n*-hexyl ether

Compound	X	In diisopropyl ether				Di-*n*-hexyl ether		
		% Convn.	% e.e.$_P$[a]	*E*	Cf. *E*[b]	% Convn.	% e.e.$_P$[a]	*E*
1a	H	46	82.3	21	14	45.9	92.0	57
1b	4-F	42	71.8	10		47[c]	81.0	20
1c	2-Cl	18.1	83.7	13	15	43.4[d]	91.0	44
1d	3-Cl	60.9	26.1	2.4	7.7	45.7	58.7	6.2
1e	4-Cl	49.2	74.2	14	10	46.7	82.2	23
1f	2,4-diCl	18.4	26.7	1.8		–	–	–
1g	4-Br	59.7	63.5	15		42.7	89.8	37
1h	4-Me	52	78.4	22		46.2	91.8	56
1i	4-Et	55.8	73.2	20		47.5	91.6	58

Reactions were conducted using 0.1 mmol of **1**, 0.4 mmol of methanol and 10 mg of *Carica papaya* lipase in 0.8 ml of diisopropyl ether at 25 °C for 1 h

[a] Enantiomeric excess of the methyl ester formed

[b] *Aspergillus niger* lipase-catalyzed transesterification of 2-phenoxypropanoic acid vinyl esters with methanol in diisopropyl ether

[c] Reaction time: 2 h

[d] Reaction time: 6 h

Table 3 *Carica papaya* lipase-catalyzed transesterification of 2-phenoxypropanoic acid 2,2,2-trifluoroethyl ester (**1a**) with methanol in ethereal solvents

Solvent	Log *P*	% Convn.	% e.e.$_P^a$	*E*
Diisopropyl ether	1.52[b]	46	82.3	21
t-Butyl methyl ether	0.94[b]	56.5	70.5	18
Di-*n*-hexyl ether	4.24[c]	45.9	92	57
Di-*n*-octyl ether	5.91[c]	45.2	91.3	50

Reactions were conducted using 0.1 mmol of **1a**, 0.4 mmol of methanol and 10 mg of *Carica papaya* lipase in 0.8 ml of an organic solvent at 25 °C for 1 h

[a] Enantiomeric excess of the newly formed ester

[b] Sangster (1989)

[c] Calculated value using ChemBioDraw Ultra 12.0 from CambridgeSoft

the use of an ethereal solvent with hydrophobicity similar to or greater than that of cyclohexane could ameliorate them. Table 3 shows the results obtained in such ethereal solvents. The enantioselectivity was slightly worse in a less hydrophobic ether, *t*-butyl methyl ether. In contrast, it was gratifying to find that when a more hydrophobic ether, di-*n*-hexyl ether or di-*n*-octyl ether, was employed the reaction rate was not retarded in any degree and the enantioselectivity was profoundly improved, reaching the 'excellent' level. Accordingly, the CPL-catalyzed transesterification with methanol of **1** was reinvestigated in di-*n*-hexyl ether as a solvent. The results are summarized also in Table 2. The reaction rate was not very much affected by the change of solvents to the long-chain ether. On the other hand, the enantioselectivity was profoundly improved by this change. The level of enantioselectivity was elevated by at least one rank: 'low' to 'tolerable' or 'tolerable' to 'good', except **1d**. Even in the latter case, however, the *E* value became 2.6 times larger. Thus, in all the cases examined, the *E* values became 1.6–3.4 times larger by the use of di-*n*-hexyl ether as the solvent.

Conclusion

CPL is a promising enzyme for the resolution of 2-(substituted phenoxy)propanoic acids by changing solvents from diisopropyl ether to long-chain ethers which have larger hydrophobicity. This possibility should be considered when the enantioselectivity is inadequate in diisopropyl ether most frequently employed in lipase-catalyzed transesterifications and esterifications. The general applicability of this approach is now under investigation in our laboratory.

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