PERKIN 1

Dimethyl sulfoxide as a co-solvent dramatically enhances the enantioselectivity in lipase-catalysed resolutions of 2-phenoxypropionic acyl derivatives

Keiichi Watanabe [a] **and Shin-ichi Ueji** *[,b]

[a] *The Graduate School of Science and Technology, Kobe University, Nada, Kobe 657-8501, Japan*
[b] *Division of Natural Environment and Bioorganic Chemistry, Faculty of Human Development and Sciences, Kobe University, Nada, Kobe 657-8501, Japan. Fax: +81-78-803-7761, E-mail: ueji@kobe-u.ac.jp*

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We recently reported that the enantioselectivity for subtilisin-catalysed hydrolysis of ethyl 2-(4-substituted phenoxy)-propionates in aqueous buffer is found to be dramatically enhanced by addition of dimethyl sulfoxide (DMSO). In our present work, as one of the useful methods for improving the enzyme's enantioselectivity, this approach using DMSO is tested for both hydrolysis and transesterification catalysed by various lipases. For instance, for *Candida rugosa* lipase-catalysed hydrolysis in aqueous buffer containing DMSO, the optimum additive conditions (50–65 vol% DMSO) markedly enhance the enantioselectivity toward the substrates used, as compared with that for no-additive conditions, in spite of a decrease in the enzymatic activity. On the other hand, for *Pseudomonas cepacia* lipase-catalysed hydrolysis, the addition of DMSO to the reaction medium enhances the enantioselectivity with an increase in the enzymatic activity. Also, the DMSO effect on the enantioselectivity can apply to the lipase-catalysed transesterification in organic solvent. A mechanism for the DMSO-induced enhancement of the lipase's enantioselectivity is briefly discussed on the basis of the values of the initial rates obtained for each enantiomer of the substrate used.

Introduction

For the preparation of enantiomerically pure compounds such as pharmaceutical and agricultural chemicals, the need for new chemical transformations with high enantioselectivity has increased, because their biological activity is usually confined to only one of the enantiomers.[1] As one of the strategies explored with this goal in mind, biocatalysis is recognized as a useful method for the resolution of enantiomers under mild reaction conditions, which are highly recommended from the viewpoint of environmental factors. Thus, the use of enzymes as enantioselective transformation catalysts is becoming increasingly popular among chemists, and several reaction systems and classes of enzymes have been successfully employed for this synthetic purpose.[2]

The high enantioselectivity of enzymes is always required for effective resolution of racemates. Improvement of an enzyme's enantioselectivity has been achieved by variation of the reaction conditions such as solvents,[3] reaction temperature,[4] or even pressure,[5] use of various additives,[6] protein engineering,[7] modification of enzymes,[8] or formation of ion-paired salts with substrates.[9] Among these approaches, the addition of a compound or a solvent to the reaction medium seems to be advantageous for its simplicity.[10] However, the selection of a common additive for improving the enantioselectivity of a variety of enzymes is lacking, because the additive used to obtain a high enantioselectivity for one enzyme does not always apply to the improvement of another enzyme's enantioselectivity. For instance, it is well known that, for lipase-catalysed reactions in organic solvents, a small amount of water added to the medium improves the lipase's enantioselectivity, as compared with that for no-additive conditions, the origin of which is due to the increase of the lipase's flexibility arising from multiple hydrogen-bond formation with added water,[6] whereas it decreases the enantioselectivity for the subtilisin-catalysed reaction.[11] In particular, lipases are the most popular enzymes in organic synthesis, because they display relatively high enantioselectivity, possess broad substrate specificity, are commercially available, and do not require expensive and unstable coenzymes. For this reason, an applied study of the enantioselectivity enhancement of lipases is highly desirable.

As part of our continuing interest in strategies for improving enzymes' enantioselectivity, we reported that the enantioselectivity for subtilisin-catalysed hydrolysis of ethyl 2-(4-substituted phenoxy)propionates in aqueous buffer was dramatically improved by the addition of dimethyl sulfoxide (DMSO).[12] The high DMSO-induced enantioselectivity is related to a change in the conformation and/or the flexibility of the subtilisin, arising from a partial decrease of its tertiary structure caused by addition of DMSO to the reaction medium. In addition, it is reported that the effect of DMSO showed enantioselectivity enhancement for pig liver esterase-catalysed hydrolysis.[13] In this paper, we report a new and a simple method for markedly improving the enantioselectivity of the hydrolysis of butyl 2-(4-substituted phenoxy)propionates catalysed by various lipases in aqueous buffer containing DMSO. Also, the DMSO effect on the enantioselectivity can apply to the lipase-catalysed transesterification in organic solvents. This is the first example of DMSO as a denaturing solvent of lipases being used to enhance their enantioselectivity. Furthermore, the mechanism of the high DMSO-induced enantioselectivity for lipase-catalysed reactions will be briefly discussed on the basis of the values of the initial rates obtained for each enantiomer of the substrate used here.

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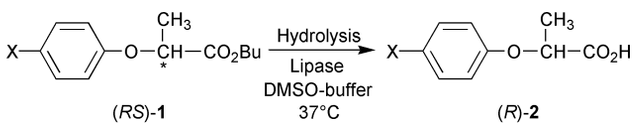
Results and discussion

Improvement of the enantioselectivity by addition of DMSO for lipase-catalysed reactions

For lipase-catalysed hydrolysis of butyl 2-(4-substituted phenoxy)propionates **1** in aqueous buffer, we investigated the behaviour of the enantioselectivity of *Candida rugosa* lipases by addition of DMSO (Scheme 1). For these reactions, all the lipases used catalysed preferentially the *R* enantiomer of the substrates **1**. 2-Phenoxypropionic acids are well-known herbicides and also have other biological activities.[14] Their *R* enantiomers are known to be active in these cases.



a: X = CH_3CH_2, **b**: X = Cl, **c**: X = H

Scheme 1 Lipase-catalysed hydrolysis of butyl 2-(4-substituted phenoxy)propionates **1** in aqueous buffer with addition of DMSO.

Table 1 summarizes the results of the variation of the enantioselectivity (*E*-value estimated from the known method[15]) at *ca*. 40% conversion observed for the hydrolysis of butyl 2-(4-substituted phenoxy)propionates **1** catalysed by lipase MY originating from *Candida rugosa* in aqueous buffer with various amounts of DMSO. As is seen in Table 1, when DMSO was added to the reaction medium for lipase MY-catalysed hydrolysis of **1a** (X = Et), the enantioselectivity was found to be dramatically enhanced, as compared with that for no-additive conditions ($E = 4$). In particular, upon the addition of the optimum amount of DMSO (60–65 vol%), lipase MY displayed excellent enantioselectivity ($E > 200$), thus resulting in the complete resolution of the enantiomers of **1a**. For the hydrolysis of **1a**, however, a steep decrease in the enzymatic activity was produced by addition of an excess of DMSO (70 vol%), probably because of the significant irreversible deformation of lipase MY caused by DMSO. Then, the enhancement effect induced by DMSO was tested using the other substrates, **1b** (X = Cl) and **1c** (X = H), for lipase MY-catalysed hydrolysis (Scheme 1). From the data summarized in Table 1, the optimum additive conditions (60 vol% DMSO) also produced excellent enantioselectivity for both these substrates. Thus, the high DMSO-induced enantioselectivity is useful for the enantioselective improvement of substrates bearing different substituents, although the enantioselectivity for lipase MY-catalysed esterification of acids **2** in organic solvents was affected by the nature of the substituents, such as their steric and/or electronic effects.[16]

Indeed, the effects of DMSO on the enantioselectivity enhancement of lipase MY are consistent with results obtained for subtilisin-catalyzed hydrolysis in aqueous buffer containing DMSO, although the reaction rate as a measure of the enzymatic activity for lipase MY-catalysed hydrolysis of the substrates **1a**–**c** decreased dramatically with an increase in the amount of DMSO added to the medium (55–65 vol%) (Table 1). This observation concerning the enzymatic activity of lipase MY can explain a decrease in the enzymatic activity of the incorrectly binding *S* enantiomer (see also below, for a discussion on the basis of the results of the initial rates for each enantiomer).

In order to investigate the effects of DMSO on the enantioselectivity of the hydrolysis catalysed by other lipases, lipase AY originating from *Candida rugosa* was tested using the model reaction (Scheme 1). Lipase AY is known to differ from lipase MY in its catalytic activity, although both lipases were produced from the same yeast (*Candida rugosa*).[4c,4f] From the data summarised in Table 2, the enantioselectivity of lipase AY for substrates **1a**–**c** was dramatically improved by the addition of DMSO to the reaction medium, accompanying a decrease in enzymatic activity with an increase in the amount of DMSO added, as with lipase MY. Also, the optimum amount of DMSO (55–60 vol%) to maximize the enantioselectivity for lipase AY-catalysed hydrolysis is almost identical to that for the lipase MY-catalysed one.

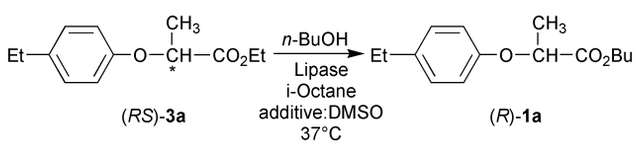
Table 1 Effects of addition of DMSO on the enantioselectivity for lipase MY-catalysed hydrolysis of butyl 2-(4-substituted phenoxy)-propionates **1** in aqueous buffer

X	DMSO (vol%)	Time (*t*/min)	Conversion (%)	ee (%)	*E*-Value
1a Et	0	20	39	49	3.9
	30	20	38	60	5.7
	40	25	42	72	10
	50	30	39	92	44
	55	60	42	96	102
	60	90	41	100	>200
	65	720	37	100	>200
	70	720	<1		
1b Cl	0	20	38	22	1.8
	30	20	43	17	1.6
	55	60	35	95	65
	60	300	40	100	>200
1c H	0	12	42	15	1.5
	30	30	39	44	3.3
	55	360	40	84	20
	60	7 days	41	100	>200

Table 2 Effects of addition of DMSO on the enantioselectivity for lipase AY-catalysed hydrolysis of butyl 2-(4-substituted phenoxy)-propionates **1** in aqueous buffer

X	DMSO (vol%)	Time (*t*/min)	Conversion (%)	ee (%)	*E*-Value
1a Et	0	12	40	26	2.0
	30	12	41	38	2.8
	40	15	36	60	5.5
	50	24	41	95	78
	55	60	43	97	145
	60	80	35	94	54
1b Cl	0	20	43	47	3.9
	30	12	34	69	7.7
	55	90	40	100	>200
	60	7 days	36	100	>200
1c H	0	10	43	26	2.0
	30	20	46	30	2.3
	55	1440	12	89	19
	60	7 days	3		

Furthermore, our approach based on the high DMSO-induced high enantioselectivity was applied to the lipase-catalysed transesterification of ethyl 2-(4-ethylphenoxy)-propionate **3a** with *n*-butyl alcohol in a dry organic solvent (Scheme 2). Unfortunately, lipases except for *Candida rugosa*



Scheme 2 Lipase-catalysed transesterification of ethyl 2-(4-ethylphenoxy)propionate **3a** with *n*-butyl alcohol in isooctane with addition of DMSO.

lipases did not catalyse the transesterification of **3a**. Table 3 summarizes the variation in enantioselectivity (*E*-value) at *ca*. 30–40% conversion caused by the addition of DMSO for lipase MY- or AY-catalysed transesterifications in dry isooctane.† For both lipase-catalysed reactions, the addition of

† Isooctane = 2,2,4-trimethylpentane.

Table 3 Enantioselectivity of lipase MY- or AY-catalysed transesterifications of ethyl 2-(4-ethylphenoxy)propionate **3a** with *n*-butyl alcohol in isooctane with addition of DMSO

Lipase	DMSO (vol%)	Time (*t*/h)	Conversion (%)	ee (%)	*E*-Value
MY	0	6	37	75	11
	0.05	6.5	40	82	21
	0.10	8	40	79	14
	0.15	17	38	100	>200
	0.20	24	2.2		
	0.30	24	<1		
AY	0	6	33	85	19
	0.05	6.5	28	83	15
	0.10	8	26	83	14
	0.15	17	29	91	31
	0.20	24	25	89	23
	0.30	24	8.2	100	>200

a small amount of DMSO enhanced their enantioselectivity, as compared with those for no-additive conditions (Table 3). In particular, upon the optimum additive conditions (0.15 vol% DMSO for lipase MY, 0.3 vol% DMSO for lipase AY), both these lipases displayed excellent selectivity ($E > 200$). Therefore, our approach based on the high DMSO-induced enantioselectivity can be used as a valuable method for improving the enantioselectivity for *Candida rugosa* lipase-catalysed transesterification as well as hydrolysis. A drop in the *E*-value and the enzymatic activity, however, was produced by the addition of a slightly larger amount of DMSO (0.20 vol%) than the optimum additive condition (0.15 vol%), probably because even a small amount of DMSO may cause the deformation of lipases due to the direct interaction between the lipase molecule and DMSO, which results from the low solubility of DMSO in isooctane.

In order to investigate the scope of the DMSO effect on the enantioselectivity of lipases, which was independent of the effect of the origin of the *Candida rugosa* lipases, *Pseudomonas cepacia* lipases were submitted to the model reaction (Scheme 1). Table 4 summarizes the variation in the enantioselectivity for *Pseudomonas cepacia* lipase-catalysed hydrolysis of **1a** (X = Et) caused by the addition of DMSO from 0 to 70 vol%. For lipase AH-catalysed hydrolysis, when DMSO was added to aqueous buffer, the enantioselectivity was found to be largely enhanced, as compared with that for no-additive conditions (Table 4). In particular, upon the addition of 55 vol% DMSO, lipase AH displayed the highest enantioselectivity ($E = 30$), which was 5 times as large as that for no-additive conditions ($E = 6$). Interestingly, the DMSO caused an increase in the reaction rate of the enzyme, although the enzymatic activity for *Candida rugosa* lipase was decreased by the addition of DMSO. A drop in both the *E*-value and the enzymatic activity, however, was produced by the addition of a large amount of DMSO (60–70 vol%). Furthermore, for lipase PS- or lipase AK-catalysed hydrolyses of **1a**, the addition of DMSO to the reaction medium slightly enhanced their enantioselectivity (Table 4). Even an optimum amount of DMSO (55–60 vol%) produced only a moderate enantioselectivity ($E = 8$–9), which would not be sufficient for an effective resolution of **1a**.

Table 5 summarizes the results of the variation in the enantioselectivity for the hydrolysis of **1a** catalysed by lipases except for *Candida rugosa* or *Pseudomonas cepacia* lipases. Unfortunately, *Candida lipolytica*, *Rhizopus niveus*, and Pig pancreas lipases did not catalyse the model reaction, and the enantioselectivity of *Rhizopus delemar* lipase was not improved by the addition of DMSO at all. Thus, when lipases do not catalyse the hydrolysis of **1a** or do not show the relatively moderate enantioselectivity over no-additive conditions, our approach based on the high DMSO-induced enantioselectivity is not appropriate for an improvement in the lipase's enantioselectivity.

Table 4 Effects of addition of DMSO on the enantioselectivity for *Pseudomonas cepacia* lipase-catalysed hydrolysis of butyl 2-(4-ethylphenoxy)propionate **1a** in aqueous buffer

Lipase	DMSO (vol%)	Time (*t*/min)	Conversion (%)	ee (%)	*E*-Value
AH	0	150	39	61	6.0
	30	120	43	79	16
	50	90	37	88	26
	55	80	38	89	30
	60	120	38	85	21
	70	900	41	62	6.4
PS	0	90	43	37	2.8
	30	75	39	51	4.2
	50	70	36	57	5.0
	58	63	33	70	7.9
	60	70	37	58	5.2
	70	70	7	61	4.3
AK	0	270	41	64	7.0
	30	210	41	69	8.7
	50	170	39	70	8.7
	55	120	38	71	9.0
	60	130	41	67	7.9
	70	190	39	57	5.2

Table 5 Lipase-catalysed hydrolysis of butyl 2-(4-ethylphenoxy)-propionate **1a** in aqueous buffer with addition of DMSO

Origin	DMSO (vol%)	Time (*t*/min)	Conversion (%)	ee (%)	*E*-Value
Candida lipolytica	0	60	4	62	4.3
	30	30	0		
	50	30	0		
Rhizopus niveus	0	30	12	9.0	1.2
	30	30	3		
	50	30	1		
Pig pancreas	0	60	9	33	2.0
	30	60	7	86	14
	50	60	1		
Rhizopus delemar	0	30	68	<1	1.0
	30	30	45	<1	1.0
	50	30	2		

Effects of addition of DMSO on the initial rates of each enantiomer of the substrate used for lipase-catalysed reactions

For lipase-catalysed hydrolysis of butyl esters **1** in aqueous buffer with addition of DMSO, we found two types of the enantioselectivity enhancement of lipases caused by this addition of DMSO, *viz*. high DMSO-induced enantioselectivity with a decrease in the enzymatic activity for *Candida rugosa* lipases, and high DMSO-induced enantioselectivity with an increase in the enzymatic activity for *Pseudomonas cepacia* lipases (Tables 1–4). In order to gain insight into the mechanism of the enantioselectivity enhancement by the addition of DMSO, we investigated the initial rates for each enantiomer of **1a** for lipase MY (*Candida rugosa*)- or lipase AH (*Pseudomonas cepacia*)-catalysed hydrolyses. The results of the initial rates affected by the addition of DMSO are summarized in Table 6. There was a marked difference between the behaviour of the initial rate for lipase MY-catalysed hydrolysis and that for the lipase AH-catalysed one. In the former hydrolysis, the initial rate (V_R) for the correctly binding *R* enantiomer remained almost unchanged upon addition of 55 vol% DMSO ($V_R = 2.5$), as compared with that for no-additive conditions ($V_R = 2.9$) (Table 6). However, when 55 vol% DMSO was added to the reaction medium, the hydrolysis of the incorrectly binding *S* enantiomer resulted in the almost complete suppression of its enzymatic activity ($V_S \approx 0$), which was too low to obtain a reproducible value. This observation may be explained by

assuming that the accommodation of the *S* enantiomer into the binding site of lipase MY and the stabilization of the complex between the substrate and the lipase would be decelerated by a change in the conformation and/or the flexibility of lipase MY caused by DMSO. Also, the serious decrease of the initial rate for the *S* enantiomer results in the extension of the reaction time to reach *ca*. 40% conversion for lipase MY-catalysed hydrolysis in 50–65 vol% DMSO (Table 1). Therefore, from the large value of the quotient $V_R/V_S = \infty$, calculated from Table 6, the enantioselectivity enhancement by the addition of DMSO (55 vol%) for lipase MY-catalysed hydrolysis can be mainly ascribed to the serious deceleration of the initial rate for the incorrectly binding *S* enantiomer, as compared with that for the *R* enantiomer.

Furthermore, for lipase MY- and AY-catalysed transesterifications in organic solvents containing a small amount of DMSO, we investigated the initial rates for each enantiomer of **3a** (Table 7). As is seen in Table 7, for both lipase-catalysed transesterifications, the addition of DMSO to isooctane decreased the initial rates for both enantiomers, as compared with those for no-additive conditions. In particular, the increase in the amount of DMSO (to 0.15 vol%) added for lipase MY-catalysed transesterification dramatically decreased the initial rate for the incorrectly binding *S* enantiomer. Thus, the larger value of the quotient of the initial rates, V_R/V_S = 674 calculated from Table 7, arising from the serious inhibition of the initial rate for the *S* enantiomer, is found to be responsible for the significant enhancement of the enantioselectivity for lipase MY. For lipase AY-catalysed transesterification, the smaller decrease in the initial rate for the incorrectly binding *S* enantiomer, as compared with that for lipase MY-catalysed one, does not result in a marked enhancement of enantioselectivity. Thus, the marked enhancement of enantioselectivity for *Candida rugosa* lipase-catalysed hydrolysis and transesterification is attributed to the almost complete inhibition of the initial rate for the incorrectly binding *S* enantiomer.

In contrast, for lipase AH-catalysed hydrolysis, the addition of 55 vol% DMSO was found to bring about an acceleration (2.9-fold) of the initial rate for the *R* enantiomer, whereas that for the *S* enantiomer was decelerated (0.49-fold), as compared with those for no-additive conditions (Table 6). Thus, the high DMSO-induced enantioselectivity of *Pseudomonas cepacia* lipase AH can be characterized by the relative quotient $(V_R/V_S)_{55\ vol\%}/(V_R/V_S)_{0\ vol\%} = 6.0$, arising from the opposing trends of the initial rates for each enantiomer.

Conclusions

The enantioselectivity for various lipase-catalysed hydrolyses in aqueous buffer was significantly enhanced by the addition of DMSO. Also, the addition of DMSO enhanced the enantioselectivity for *Candida rugosa* lipase-catalysed transesterification in an organic solvent. In particular, the high DMSO-induced enantioselectivity of *Candida rugosa* lipases was found to be useful for dramatically improving the enantioselectivity, as compared with that for no-additive conditions. The particularly high DMSO-induced enantioselectivity for *Candida rugosa* lipases and *Pseudomonas cepacia* lipases can be explained following a discussion of the results of the initial rates of each enantiomer of the substrate used. For *Candida rugosa* lipases, the serious deceleration in the initial rate for the incorrectly binding *S* enantiomer upon addition of DMSO, as compared with that for the correctly binding *R* enantiomer, is a main factor in the significant enhancement of the enantioselectivity. On the other hand, for *Pseudomonas cepacia* lipases, the high DMSO-induced enantioselectivity was dependent on the acceleration of the initial rate for the *R* enantiomer and the deceleration of that for the *S* enantiomer, as compared with those for no-additive conditions. Also, the DMSO effect can apply to the enantioselectivity enhancement for *Candida rugosa* lipase-catalysed transesterification in an organic solvent, the origin of which is almost the same as for the hydrolysis. This is the first example of DMSO being used to markedly improve the enantioselectivity, but *via* different mechanisms of both *Candida rugosa* lipases and *Pseudomonas cepacia* lipases. Our findings will form a valuable tool for the improvement of enantioselectivity in various lipase-catalysed reactions in aqueous or non-aqueous media.

Table 6 Initial rates of lipases MY- or AH-catalysed hydrolyses of each enantiomer of butyl 2-(4-ethylphenoxy)propionate **1a** in aqueous buffer with addition of DMSO

Lipase	DMSO (vol%)	V_R (μmol h^{-1} mg^{-1})	V_S (μmol h^{-1} mg^{-1})	V_R/V_S	*E*-Value
MY	0	2.9	0.89	3.3	3.9
	55	2.5	≈0		102
AH	0	0.12	0.049	2.4	6.0
	55	0.35	0.024	15	30

Table 7 Initial rates of lipase MY- or AY-catalysed transesterifications of each enantiomer of ethyl 2-(4-ethylphenoxy)propionate **3a** with *n*-butyl alcohol in isooctane with addition of DMSO

Lipase	DMSO (vol%)	V_R (nmol h^{-1} mg^{-1})	V_S (nmol h^{-1} mg^{-1})	V_R/V_S
MY	0	621	66	9.4
	0.15	357	0.53	674
	0.20	0.2	0.02	
AY	0	434	11	39
	0.15	304	6.5	47
	0.20	191	6.3	30

Experimental

General

Materials

Lipase MY was supplied from Meito Sangyo Co., Ltd., Japan, and lipases AY, AH, PS, AK, *Candida lipolytica*, *Rhizopus niveus*, Pig pancreas, and *Rhizopus delemar* lipases were supplied from Amano Pharmaceutical Co., Ltd., Japan, and used without further purification. Phosphate buffer (pH 6.86) and DMSO (analytical grade) were purchased from Wako Pure Chemical Industries, Ltd., Japan. Racemic butyl 2-(4-substituted phenoxy)propionates **1** were prepared by the reaction of the appropriate 4-substituted phenol and ethyl 2-bromopropionate (Tokyo Kasei Kogyo Co., Ltd., Japan), according to the known method,[17] followed by hydrolysis of the ethyl ester and lipase OF-catalysed esterification of the acid with *n*-butyl alcohol.

Lipase-catalysed hydrolysis of butyl 2-(4-substituted phenoxy)-propionates 1 in aqueous buffer with addition of DMSO

Racemic butyl 2-(4-substituted phenoxy)propionates **1** (0.05 mmol) were added to aqueous buffer containing DMSO (0–70 vol%) (2 ml), followed by ultrasonic dispersion (40000 Hz), and then lipase (30 mg) was added. The reaction mixture was shaken (170 strokes min^{-1}) at 37 °C. At an appropriate time interval, aliquots were withdrawn and the supernatant was analysed by HPLC (using *n*-hexane–isopropyl alcohol 970 : 30 (v/v) as eluent with the addition of 0.1 vol% trifluoroacetic acid) on a chiral column (Chiralcel OK or OJ, from Daicel Chemical Industries Co. Ltd., Japan) to determine the conversion and the enantiomeric excess (ee).

Preparation of enantiomerically pure butyl 2-(4-ethylphenoxy)-propionate 1a

According to our method,[6g] the *R* enantiomer of 2-(4-ethylphenoxy)propionic acid **2a** was obtained by repetition of the enantiomeric esterification of 2-(4-ethylphenoxy)propionic acid **2a** (36 mmol) with *n*-butyl alcohol (108 mmol) in dry diisopropyl ether (200 ml) with 0.75 vol% LiCl-containing water by use of lipase MY (3 g), followed by hydrolysis of the ester. The *S* enantiomer of **2a** containing a small amount of the *R* enantiomer was extracted from the reaction mixture, and the *S* enantiomer of **2a** was obtained by removal of the *R* enantiomer by lipase MY-catalysed esterification. The value of the ee for each enantiomer used in our work was more than 99%.

Initial rate for lipase-catalysed hydrolysis of butyl (*R*)- or (*S*)-2-(4-ethylphenoxy)propionate 1a in aqueous buffer with addition of DMSO

In a typical procedure, butyl (*R*)- or (*S*)-2-(4-ethylphenoxy)-propionate **1a** (0.05 mmol) was added to aqueous buffer containing DMSO (0 or 55 vol%) (2 ml), followed by ultrasonic dispersion, and then lipase (30 mg) was added. The reaction mixture was shaken (170 strokes min^{-1}) at 37 °C. At an appropriate time interval, aliquots were withdrawn and the supernatant was analysed by HPLC (Shimadzu liquid chromatograph LC-10A system, Kyoto, Japan) to obtain the conversion. Five data points (less than 10% conversion) were collected to determine the initial rate. All the experimental data points gave a straight line with correlation coefficient > 0.98.

Lipase-catalysed transesterification of ethyl 2-(4-ethylphenoxy)-propionate 3a with *n*-butyl alcohol in isooctane with addition of DMSO

Racemic ethyl 2-(4-ethylphenoxy)propionate **3a** (0.1 mmol) and *n*-butyl alcohol (0.3 mmol) was added to dry isooctane (2 ml) containing DMSO (0–0.60 vol%), followed by ultrasonic dispersion, and then lipase (30 mg) was added. The reaction mixture was shaken (170 strokes min^{-1}) at 37 °C. At an appropriate time interval, aliquots were withdrawn and the supernatant was analysed by HPLC on a chiral column (Chiralcel OK or OJ, from Daicel Chemical Industries Co. Ltd., Japan) to determine the conversion and the ee.

Initial rate for lipase-catalysed transesterification of ethyl (*R*)- or (*S*)-2-(4-ethylphenoxy)propionate 3a with *n*-butyl alcohol in isooctane with addition of DMSO

(*R*)- or (*S*)-Ethyl 2-(4-ethylphenoxy)propionate **3a** (0.1 mmol) and *n*-butyl alcohol (0.3 mmol) were added to dry isooctane (2 ml) containing DMSO (0, 0.15 or 0.20 vol%), followed by ultrasonic dispersion, and then lipase (30 mg) was added. The reaction mixture was shaken (170 strokes min^{-1}) at 37 °C. At an appropriate time interval, aliquots were withdrawn and the supernatant was analysed by HPLC on a chiral column (Chiralcel OK or OJ, from Daicel Chemical Industries Co. Ltd., Japan) to determine the conversion. Five data points (less than 10% conversion) were collected to determine the initial rate. All the experimental data points gave a straight line with correlation coefficient > 0.96.

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