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Synthetically useful variants of industrial lipases from *Burkholderia cepacia* and *Pseudomonas fluorescens*

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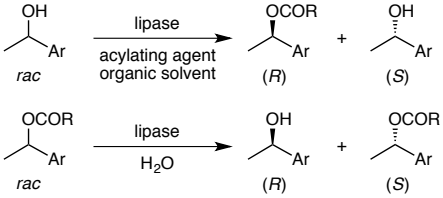
Industrial enzymes called lipase PS (LPS) and lipase AK (LAK), which originate from *Burkholderia cepacia* and *Pseudomonas fluorescens*, respectively, are synthetically useful biocatalysts. To strengthen their catalytic performances, we introduced two mutations into hot spots of the active sites (residues 287 and 290). The LPS_L287F/I290A double mutant showed high catalytic activity and enantioselectivity for poor substrates for which the wild-type enzyme showed very low activity. The LAK_V287F/I290A double mutant was also an excellent biocatalyst with expanded substrate scope, which was comparable to the LPS_L287F/I290A double mutant. Thermodynamic parameters were determined to address the origin of the high enantioselectivity of the double mutant. The $\Delta\Delta H^{\ddagger}$ term, but not the $\Delta\Delta S^{\ddagger}$ term, was predominant, which suggests that the enantioselectivity is driven by a differential energy associated with intermolecular interactions around Phe287 and Ala290. A remarkable solvent effect was observed, giving a bell-shaped profile between the *E* values and the log *P* or ε values of solvents with the highest *E* value in *i*-Pr_2O. This suggests that organic solvent with appropriate hydrophobicity and polarity provides the double mutant with some flexibility that is essential for the excellent catalytic performance.

Introduction

Enzymes show high catalytic activity and stereoselectivity under mild conditions. Industrial enzymatic processes are widely accepted from the viewpoint of environmental harmony and sustainability.[1] Because of the limited diversity of natural enzymes, new technologies have been developed to alter the structure and property of enzymes.[2] The directed evolution method can evolve an enzyme stepwise using random mutagenesis and a high-throughput screening system. Although no information about the enzyme structure and the reaction mechanism is necessary for directed evolution, a large number of variants need to be screened. On the other hand, when the enzyme structure and the reaction mechanism are known, a rational design approach with site-directed mutagenesis is effective and efficient.



Scheme 1. Typical good substrates for lipases, where R and Ar designate the alkyl group and the aromatic/large substituent, respectively.

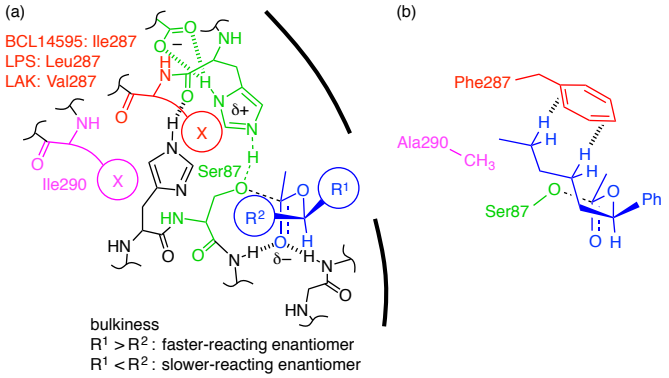


Fig. 1 (a) The transition-state model to explain the enantioselectivity of lipase toward secondary alcohols (residues 287 and 290 are added to the original version). (i) The C–O bond of the substrate takes the *gauche* conformation with respect to the breaking C–O bond, which is due to the stereoelectronic effect. (ii) The H atom attached to the stereocenter of the substrate is *syn*-oriented toward the carbonyl O atom to minimize the torsional strain. Enantioselectivity is explained by the conformational requirements and repulsive interactions and/or strains. Typically, the (*R*)-enantiomer reacts faster because, in this favorable conformation shown in blue, the larger substituent (R^1) can be directed toward external solvent without severe strain and/or steric hindrance. (b) The catalytic activity of the BCL14595_I287F/I290A double mutant for (*R*)-1-phenyl-1-hexanol is enhanced by introducing attractive CH/π interactions and removing steric hindrance.

Lipases are synthetically useful biocatalysts that show high catalytic activity and enantioselectivity for a broad range of unnatural substrates in both water and organic solvent.[1a,3] In particular, they exert high enantioselectivity for various secondary alcohols (Scheme 1). We performed mechanistic studies based on kinetic and thermodynamic analysis, X-ray crystal structures, and MO calculations and proposed a transition-state model (Fig. 1a) to explain the origin of

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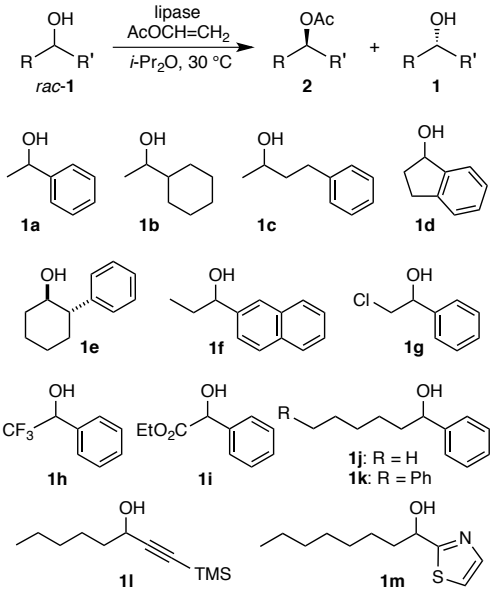
enantioselectivity of lipases for secondary alcohols.[4] The transition-state model essentially represents a mechanism by which the slower-reacting (*S*)-enantiomer is disfavored. We also introduced point mutation(s) into the active site of a lipase to rationally control the enantioselectivity.[5]

We redesigned a *Burkholderia cepacia* NBRC14595 lipase, BCL14595, to create a more useful double mutant, which is herein called BCL14595_I287F/I290A.[5b,c] The wild-type BCL14595 showed very low activity for 1-phenyl-1-hexanol, while the BCL14595_I287F/I290A double mutant showed high activity and enantioselectivity for this secondary alcohol. We have proposed that the reaction is accelerated by the CH/π interactions between Phe287 and the alkyl chain of the (*R*)-enantiomer (Fig. 1b).[5b,c,6] On the other hand, steric repulsion takes place between Phe287 and the benzene ring of the (*S*)-enantiomer (not shown). Phe287 is thus considered to have dual mode interactions with the two enantiomers, improving both catalytic activity and enantioselectivity. In addition, the I290A mutation removes steric hindrance to accelerate the reaction of the (*R*)-enantiomer (Fig. 1b). The wild-type BCL14595 showed very low activity with an *E* value of 5 for 1-phenyl-1-hexanol, while the BCL14595_I287F/I290A double mutant showed much higher activity with a high *E* value of >200.

We employed *E. coli* for the heterologous expression of the BCL14595 gene and successfully converted a denatured protein (inclusion body) into an active enzyme by *in vitro* refolding with a separately overproduced activator (chaperon), which is however unsuitable for large-scale preparation.[5] In contrast, an industrial enzyme called lipase PS (LPS, Amano Enzyme Inc.), which is a homologous protein of BCL14595 with 13 different amino acid residues (96% homology), is produced on a large scale with a *B. cepacia* expression system. Active LPS can be secreted into a culture broth.[7] Here we prepared both the LPS_wild-type enzyme and the LPS_L287F/I290A double mutant using the *B. cepacia* expression system and compared their catalytic properties. We also investigated another industrial enzyme called lipase AK (LAK, Amano Enzyme Inc.), which originates from *Pseudomonas fluorescens*. LAK has 35 different amino acid residues (89% homology) as compared with BCL14595 or LPS. We compared the enzymatic characteristics of the LAK_wild-type enzyme and the LAK_V287F/I290A double mutant. Substrate mapping revealed excellent catalytic performances (expanded substrate scope) of the LPS_L287F/I290A and LAK_V287F/I290A double mutants. The temperature effect and solvent effect were investigated to address the origin of the high enantioselectivity of the double mutant.

Results and discussion

The recombinant enzymes prepared and purified as described in the ESI were immobilized on Toyonite-200M according to the literature.[5] A mixture of secondary alcohol **1**, the immobilized enzyme, and molecular sieves 3A in *i*-Pr_2O was stirred at 30 °C for 30 min, and vinyl acetate was added to start the reaction (Scheme 2). The progress of the reaction was monitored by TLC and NMR, and the reaction was stopped by filtration. Acetate **2** and alcohol **1** were separated by silica gel column chromatography. The enantiomeric purity was determined by GC, HPLC, or NMR, and the *E* value was calculated according to the literature.[8] The results are shown in Tables 1 and 2, where the reaction rates can be compared because the same amounts of enzyme and substrate were used in all cases.



Scheme 2 Lipase-catalyzed kinetic resolution of **1**.

Table 1 Substrate scope of the LPS_L287F/I290A double mutant and the LPS_wild-type enzyme[a]

		Time	L287F/I290A		Wild-type	
Entry	**1**	(h)	*c* (%)[b]	*E*[c]	*c* (%)[b]	*E*[c]
1	**1a**	1	50	>200	40	>200
2	**1b**	4	37	>200	42	119
3	**1c**	2	50	90	35	>200
4	**1d**	1	51	>200	53	>130
5	**1e**	10	40	>200	46	>200
6	**1f**	15	45	>200	36	>200
7	**1g**	2.5	39	>200	24	>200
8	**1h**	96	40	105	19	43
9	**1i**	24	16	31	49	117
10	**1j**	2	50	>200	9[d]	–
11	**1k**	3	49	>200	5[d]	–
12	**1l**	2	49	>200	5[d]	–
13	**1m**	1.5	39	113	10[d]	–

[a] Reaction conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1** (0.50 mmol), vinyl acetate (1.0 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 °C. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [d] Conversion calculated from ^{1}H NMR.

The results of kinetic resolution of **1** with the LPS_wild-type enzyme and the LPS_L287F/I290A double mutant are shown in Table 1. Alcohols **1a**–**e** with a small substituent such as the methyl group were resolved well by the double mutant as well as the wild-type enzyme in most cases (entries 1–5). The double mutant exhibited superior activity for **1a** and a comparable *E* value as compared with the wild-type enzyme (entry 1).

Although the double mutant showed slightly lower activity for **1b** than the wild-type enzyme, the enantioselectivity of the former was improved (entry 2). In the case of **1c**, the catalytic activity of the double mutant was improved but with a drop of the *E* value (entry 3). We consider that the pocket comprising Phe287 and Ala290 (Fig. 1b) attracts the methylene chain of (*S*)-**1c** to enhance the reactivity of (*S*)-**1c**, lowering the enantioselectivity. Alcohols **1f–i** with a substituent that is slightly larger than the methyl group were also examined. The double mutant achieved higher conversions for **1f–h** and higher enantioselectivity for **1h** than the wild-type enzyme (entries 6–8). This outcome for **1h** was unexpected because a fluorine-containing substrate has previously exhibited a dropped enantioselectivity because of the lack of CH/π interactions.[5b,c] The double mutant showed lower activity and enantioselectivity for **1i** than the wild-type enzyme (entry 9). It is likely that the cleft comprising Phe287 and Ala290 (Fig. 1b) cannot accommodate well the ethyl ester group of (*R*)-**1i**. To our delight, the double mutant showed much higher activity and enantioselectivity for **1j–m**, for which the wild-type enzyme showed very low activity (entries 10–13). The trimethylsilyl group or the thiazole ring had a good influence on the outcome (entries 12,13).

Table 2 Substrate scope of the LAK_V287F/I290A double mutant and the LAK_wild-type enzyme[a]

		Time	V287F/I290A		Wild-type	
Entry	**1**	(h)	*c* (%)[b]	*E*[c]	*c* (%)[b]	*E*[c]
1	**1a**	1	50	>200	47	>200
2	**1b**	4	42	>200	50	78
3	**1c**	2.5	49	55	47	>200
4	**1d**	0.25	43	>200	50	>200
5	**1e**	10	25	>200	49	>200
6	**1f**	24	43	>200	35	>200
7	**1g**	3	43	>200	26	>200
8	**1h**	60	48	90	45	4
9	**1i**	12	41	>200	43	30
10	**1j**	1.5	50	>200	4[d]	–
11	**1k**	4	46	>200	6[d]	–
12	**1l**	3	50	>200	6[d]	–
13	**1m**	1.5	48	134	27	8

[a] Reaction conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1** (0.50 mmol), vinyl acetate (1.0 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 °C. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [d] Conversion calculated from ^{1}H NMR.

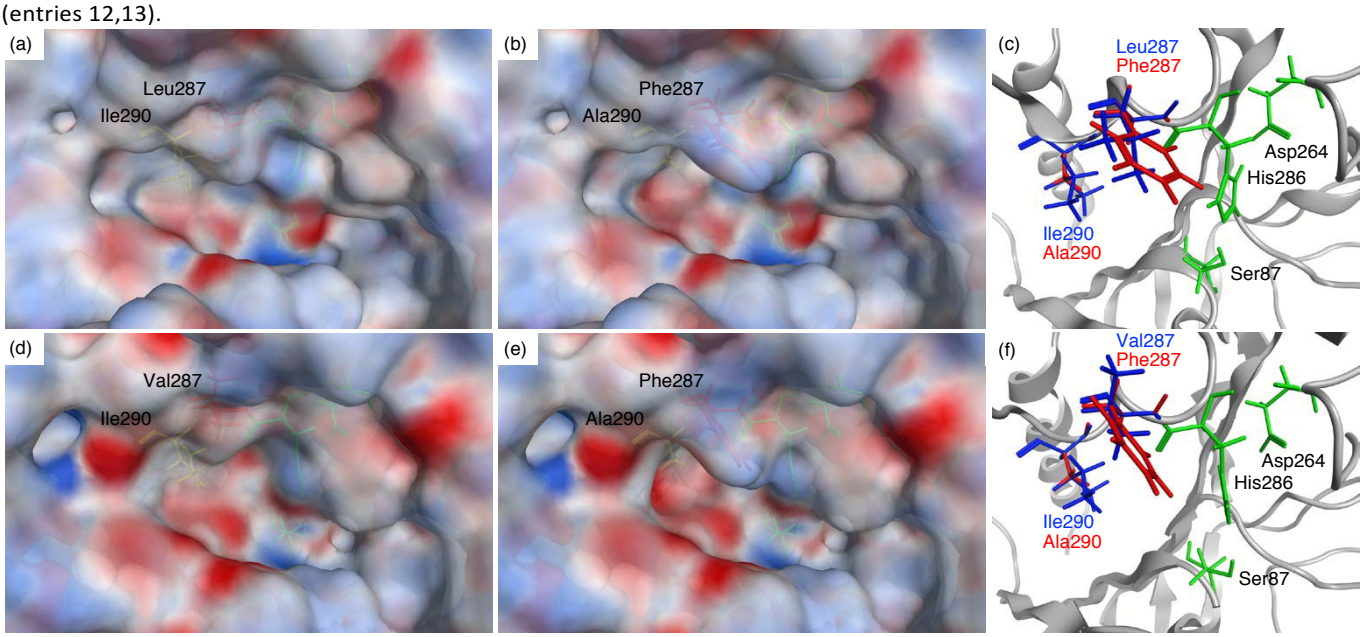


Fig. 2 Electrostatic potential maps of the active sites of (a) the LPS_wild-type enzyme, (b) the LPS_L287F/I290A double mutant, (d) the LAK_wild-type enzyme, and (e) the LAK_V287F/I290A double mutant. Superimposed views of the active sites of (c) the LPS_wild-type enzyme (blue) and the LPS_L287F/I290A double mutant (red) and (f) the LAK_wild-type enzyme (blue) and the LAK_V287F/I290A double mutant (red). Each of (a)–(c) and (d)–(f) is seen from the same direction.

The results of kinetic resolution of **1** with the LAK_wild-type enzyme and the LAK_V287F/I290A double mutant are shown in Table 2. The double mutant and the wild-type enzyme showed comparable enantioselectivities for **1a** (entry 1). The double mutant showed higher enantioselectivity for **1b** than the wild-type enzyme (entry 2), whereas the former gave a lower *E* value for **1c** than the latter (entry 3). The double mutant showed excellent enantioselectivity for **1d–e** as the wild-type enzyme did (entries 4,5). The double mutant showed higher activity for **1f–g** than the wild-type enzyme (entries 6–7). The *E* values of the double mutant for **1h–i** were much improved (entries 8–9). Interestingly, the V287F/I290A double mutations in LAK enhanced enantioselectivity for **1i** (Table 2, entry 9) although the L287F/I290A double mutations in LPS decreased enantioselectivity (Table 1, entry 9). The LAK_V287F/I290A double mutant is a useful biocatalyst because **1h–i** are reported to be poor substrates for a wild-type enzyme.[9,10] Furthermore, the double mutant exhibited high activity and enantioselectivity for **1j–m**, for which the

wild-type enzyme showed poor activity and enantioselectivity (entries 10–13).

We performed molecular modeling (MOE, MOLSYS Inc.) to understand the catalytic behaviors of the wild-type enzymes and the double mutants of LPS and LAK. The structure of LPS was obtained by refining the X-ray crystal structure (PDB: 1OIL), and that of LAK was constructed by homology modeling using LPS as a template. The double mutants of LPS and LAK were then generated from the corresponding wild-type enzymes. The active sites of these lipases are shown in Fig. 2. The LPS_wild-type enzyme with Leu287 has a narrow pocket as compared with the LAK_wild-type enzyme with Val287, which can account for a tendency that LPS is more enantioselective than LAK (Tables 1 and 2). The active-site pockets of the double mutants of LPS and LAK are deeper around residue 290 than those of the corresponding wild-type enzymes, the former of which can accommodate the substituents that are larger than the methyl group. Although it is reasonable that the double mutants of LPS and LAK with similar pockets in size and shape showed similar catalytic properties, the irregular behaviors of LPS and LAK toward **1i** (entry 9 in Tables 1 and 2) may result from the different electrostatic potentials of their active sites (Fig. 2).

The mechanism of enantioselectivity can be inspected by thermodynamic analysis.[4c] Plot of ln E against $1/T$ according to equation 1 gives the $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values.[11]

$$\ln E = -\Delta\Delta H^{\ddagger}/(RT) + \Delta\Delta S^{\ddagger}/R \qquad \text{(eq 1)}$$

The $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values represent the differences in activation enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$), respectively, between the faster-reacting and slower-reacting enantiomers (equations 2,3).

$$\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_{\text{fast}} - \Delta H^{\ddagger}_{\text{slow}} \qquad \text{(eq 2)}$$

$$\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}_{\text{fast}} - \Delta S^{\ddagger}_{\text{slow}} \qquad \text{(eq 3)}$$

The $\Delta H^{\ddagger}$ value involves a change of the energy associated with covalent bonds, strain, and intermolecular interactions, while the $\Delta S^{\ddagger}$ value is associated with a change of the disorder of the system. If the enantioselectivity of the double mutant is enhanced by the additional attractive interaction and steric repulsion (Fig. 1), the $\Delta\Delta H^{\ddagger}$ value for the double mutant should be negatively larger than that for the wild-type enzyme. We selected a combination of LPS (wild-type enzyme and the L287F/I290A double mutant) and **1m** because of the moderate to good E values and determined the thermodynamic values ($\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$) from the E values at 30–50 °C according to equation 1. The results are summarized in Tables 3 and 4 and Fig. 3.

Table 3 Temperature effect in the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant and the LPS_wild-type enzyme[a]

LPS	T (°C)	Time (h)	c (%)[b]	E[c]
L287F/I290A	30	2	50	57
L287F/I290A	35	1	33	46
L287F/I290A	40	1	41	39
L287F/I290A	45	1	44	36
L287F/I290A	50	1	50	26
wild-type	30	7	40	4.9
wild-type	35	5	41	4.1
wild-type	40	4	39	3.9
wild-type	45	4	44	3.6
wild-type	50	4	53	3.3

[a] Reaction conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1m** (0.50 mmol), vinyl acetate (1.0 mmol), dry *i*-Pr_2O (5 mL), molecular sieves 3A (three pieces). [b] Conversion calculated from c = ee(**1m**)/(ee(**1m**) + ee(**2m**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2m}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2m}))]$.

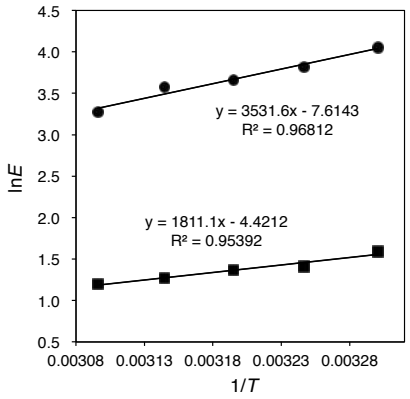


Fig. 3 Temperature effect on the enantioselectivity in the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant (circle) and the LPS_wild-type enzyme (square) in *i*-Pr_2O.

Table 4 Thermodynamic parameters for the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant and the LPS_wild-type enzyme in *i*-Pr_2O

	$\Delta\Delta H^{\ddagger}$ (kcal·mol^{-1})	$\Delta\Delta S^{\ddagger}$ (cal·K^{-1}·mol^{-1})	$\Delta\Delta G^{\ddagger}$ (kcal·mol^{-1})[a]
L287F/I290A	–7.02	–15.1	–2.43
wild-type	–3.60	–8.8	–0.94

[a] Calculated from $\Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - 303\Delta\Delta S^{\ddagger}$.

In both cases, the $\Delta\Delta H^{\ddagger}$ value is a dominant factor in the $\Delta\Delta G^{\ddagger}$ value, which indicates that enantioselectivity is driven by a differential energy associated with covalent bonds, strain, and intermolecular interactions (Table 4). The $\Delta\Delta H^{\ddagger}$ value of the double mutant is two times negatively larger than that of the wild-type enzyme. The attractive interaction between Phe287 and the alkyl chain of (*R*)-**1m** would decrease the $\Delta H^{\ddagger}_{\text{fast}}$ value, and steric repulsion between Phe287 and the thiazole ring of (*S*)-**1m** would increase the $\Delta H^{\ddagger}_{\text{slow}}$ value, both of which give a negatively larger $\Delta\Delta H^{\ddagger}$ value (equation 2). Table 4 also indicates a partial compensation effect; the $\Delta\Delta H^{\ddagger}$ value, which becomes negatively larger, is counterbalanced by the $\Delta\Delta S^{\ddagger}$ value, which also becomes negatively larger.[4c] Steric repulsion between Phe287 and (*S*)-**1m** favors the $\Delta\Delta H^{\ddagger}$ term because the $\Delta H^{\ddagger}_{\text{slow}}$ value becomes larger, whereas it disfavors

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the $\Delta\Delta S^{\ddagger}$ term because the $\Delta S^{\ddagger}_{slow}$ value increases with an increase in the disorder of the system. The CH/π interaction between Phe287 and (*R*)-**1m** as well as the removal of steric hindrance between Ala290 and (*R*)-**1m** also favor the $\Delta\Delta H^{\ddagger}$ term because the $\Delta H^{\ddagger}_{fast}$ value becomes smaller, whereas they disfavor the $\Delta\Delta S^{\ddagger}$ term because the $\Delta S^{\ddagger}_{fast}$ value decreases with a decrease in the disorder of the system. Therefore, the trends observed for the $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values are consistent with the transition-state model (Fig. 1).

Table 5 Solvent effect in the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant[a]

Solvent	log *P*	ε	Time (h)	*c* (%)[b]	*E*[c]
1,4-dioxane	–1.1	2.2	48	38	9
acetone	–0.23	21	48	11	7
THF	0.49	7.5	48	19	11
Et_2O	0.85	4.3	11	37	27
i-Pr_2O	1.9	3.4	2	50	57
toluene	2.5	2.4	5	50	42
cyclohexane	3.2	2.0	1	42	50
hexane	3.5	1.9	0.5	38	24

[a] Reaction conditions: LPS_L287F/I290A double mutant (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1m** (0.50 mmol), vinyl acetate (1.0 mmol), dry organic solvent (5 mL), molecular sieves 3A (three pieces), 30 °C. [b] Conversion calculated from *c* = ee(**1m**)/(ee(**1m**) + ee(**2m**)). [c] Calculated from *E* = ln[1 – *c*(1 + ee(**2m**))]/ln[1 – *c*(1 – ee(**2m**))].

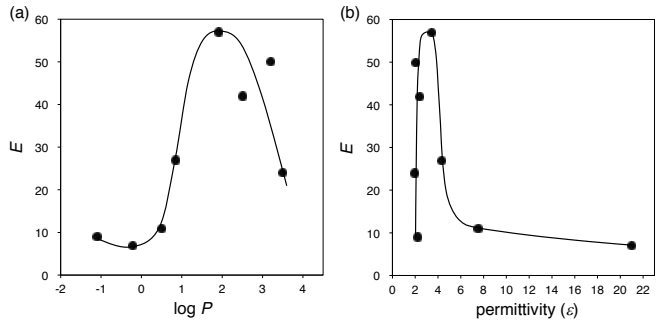

Fig. 4 The solvent effect in the LPS_L287F/I290A-catalyzed kinetic resolution of **1m**. (a) The correlation between the *E* value and the log *P* value of the solvent. (b) The correlation between the *E* value and the permittivity (ε) of the solvent.

The solvent effect is often remarkable and even provides a valuable insight into the mechanism of biocatalysis.[12] We therefore investigated the solvent effect on the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant (Table 5). The best solvent was found to be *i*-Pr_2O. The log *P* value, which is the logarithm of a partition coefficient *P* of a solvent between 1-octanol and water, is a measure of hydrophobicity of the solvent.[13] Table 5 indicates that the *E* value and the reaction rate sharply decreased with a decrease of the log *P* value. The relationships between the *E* value and the log *P* value or permittivity (ε) are plotted in Fig. 4.[14] We speculate that hydrophilic solvent such as 1,4-dioxane deprives the lipase of the essential water, which lowers the protein flexibility that is essential for the catalytic activity.[12a] In contrast, hydrophobic solvent such as hexane enables the lipase to retain the essential water, which keeps the flexibility of the protein. The *E* value was the highest in *i*-Pr_2O, where the lipase is considered to have the most appropriate flexibility (Fig. 4a). A bell-shaped profile with a peak at *i*-Pr_2O is also seen when the *E* values are plotted against the ε values (Fig. 4b). It is likely that the polarity of the solvent also affects the protein flexibility and that the highest *E* value is achieved in the solvent giving the lipase appropriate flexibility.

Conclusions

Industrial enzymes called lipase PS (LPS) and lipase AK (LAK), which originate from *Burkholderia cepacia* and *Pseudomonas fluorescens*, respectively, are synthetically useful biocatalysts. To strengthen their catalytic performances, we introduced two mutations into the hot spots of the active sites (residues 287 and 290). The LPS_L287F/I290A double mutant showed high catalytic activity and enantioselectivity for poor substrates for which the wild-type enzyme showed very low activity. This double mutant also exhibited high catalytic activity and enantioselectivity for good substrates of the wild-type enzyme. Clearly, the substrate scope of the double mutant has been broadened. It should be emphasized again that sterically demanding substrates possessing two bulky substituents on both sides are usually poor substrates.[15] The LAK_V287F/I290A double mutant is also an excellent biocatalyst with expanded substrate scope, which was comparable to the LPS_L287F/I290A double mutant. Although the two double mutants were equally excellent on the whole, some differences were also observed between them. It is therefore recommended that the better one be selected on a case-by-case basis. The enantioselectivity of the LPS_L287F/I290A double mutant was driven by the differential activation enthalpy ($\Delta\Delta H^{\ddagger}$), and this $\Delta\Delta H^{\ddagger}$ value for the double mutant was negatively larger than that for the wild-type enzyme, both of which suggest that attractive interactions and/or steric repulsion are used for chiral discrimination in the transition state. Bell-shaped profiles with a peak at *i*-Pr_2O were obtained when the *E* values for the double mutant were plotted against the log *P* or ε values of organic solvents, which suggests that appropriate protein flexibility is essential for the excellent catalytic performances. The LPS_L287F/I290A and LAK_V287F/I290A double mutants will find many applications in the kinetic resolution and dynamic kinetic resolution of various chiral alcohols.[5c,16]

Conflicts of interest

There are no conflicts of interest to declare.

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Notes and references

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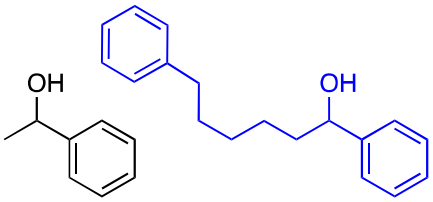

LPS_wild-type	*E* >200	poor activity
LAK_wild-type	*E* >200	poor activity
LPS_L287F/I290A	*E* >200	*E* >200
LAK_V287F/I290A	*E* >200	*E* >200

The double mutants of industrial lipases, LPS_L287F/I290A and LAK_V287F/I290A, exhibited high catalytic activity and enantioselectivity for originally poor substrates.