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Highly enantioselective lipase-catalyzed reactions at high temperatures up to 120°C in organic solvent

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Abstract—Lipase-catalyzed kinetic resolutions of 1,1-diphenyl-2-propanol were performed at high temperatures up to 120°C. *Burkholderia cepacia* lipase immobilized on porous ceramic particles, lipase PS-C II (Amano Enzyme Inc.), gave an enantiopure product at 40–120°C, with the highest conversion (39%) at 80–90°C. The mechanism of high enantioselectivity retained even at 120°C is also described briefly.

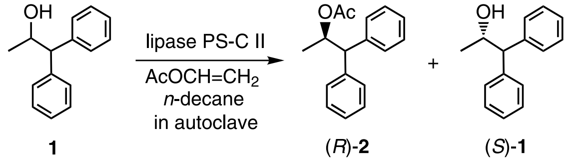
1. Introduction

Potential capabilities of enzymes may be developed by increasing or decreasing the reaction temperature.[1] The fact that the lipase-catalyzed transesterifications proceed even at −60°C is one of the most remarkable examples of the temperature effect.[2] Enantioselectivity was increased dramatically with a decrease in the reaction temperature.[2] Several groups have demonstrated that lipase- and esterase-catalyzed reactions can proceed at temperatures as high as 70–100°C.[3–7] Klibanov et al. have demonstrated that a dry lipase is extremely thermostable and active at 100°C in organic media.[3] Although enantioselectivity is high in some cases,[6] the number of highly enantioselective and efficient biocatalyses at high temperatures is limited. Previously, in a series of mechanistic studies, we encountered several substrates that showed little or no reactivity for lipases. 1,1-Diphenyl-2-propanol **1** is one of them.[8] High temperatures may force **1** to get over the high-energy transition state. Here we report that the lipase-catalyzed transesterifications of **1** proceeded at high temperatures up to 120°C in a completely enantioselective manner.

2. Results and discussion

It is known that when a lipase is immobilized to a porous ceramic support called Toyonite (Toyo Denka Kogyo Co.), the activity and stability of the enzyme are improved.[9,10] We therefore examined the Toyonite-immobilized lipase that is commercially available, lipase PS-C II (Amano Enzyme Inc.), for the high-temperature biocatalysis. We used *n*-decane as solvent, having a high boiling point (174°C), and a stainless autoclave as a reaction vessel to suppress the evaporation of vinyl acetate. The lipase-catalyzed kinetic resolutions of **1** were conducted with vinyl acetate (2 equiv.) at 30–120°C for 3 h (Scheme 1). The results are listed in Table 1 and are plotted in Figure 1. The enantiomeric purities (% ee) of **1** and **2** were determined by means of chiral HPLC, and the *E* values were calculated according to the literature.[11]

Although the conversion was only 3% at 30°C after 3 h, it increased with increasing the reaction temperature to reach 39% at 80 and 90°C. Further increase in the temperature resulted in lower conversions, and a small amount of unknown byproduct was also detected. The absolute configuration of alcohol **1** obtained in the lipase-catalyzed reactions was determined to be (*S*) by comparison of the sign of its specific rotation with the



Scheme 1.

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Table 1. Enantioselective lipase-catalyzed kinetic resolutions of **1** at high temperatures[a]

Temp. (°C)	c (%)[b]	% Yield[c] (% ee[d])		E value[e]
		(R)-**2**	(S)-**1**	
30	3	– (–)	– (–)	–
40	16	13 (>98)	74 (16)	>116
50	23	17 (>98)	70 (27)	>129
60	27	20 (>98)	60 (39)	>145
70	32	27 (>98)	58 (46)	>156
80	39	31 (>98)	52 (58)	>179
90	39	33 (>98)	52 (59)	>181
100	30	29 (>98)	61 (42)	>150
100[f]	0	– (–)	– (–)	–
110	21	25 (>98)	62 (28)	>130
120	12	13 (>98)	71 (14)	>114
120[g]	7	8 (>98)	78 (10)	>109

[a] *Conditions*: Lipase PS-C II (270 mg), **1** (0.82 mmol), vinyl acetate (1.63 mmol), *n*-decane (5 mL), in an autoclave, 3 h.

[b] Conversion determined by ^{1}H NMR for the reaction mixture.

[c] Isolated yield.

[d] Determined by HPLC (Chiralpak AD-H for **2**, Chiralcel OJ-H for **1**).

[e] Calculated from $E = \ln[1-c(1+\mathrm{ee}(\mathbf{2}))]/\ln[1-c(1-\mathrm{ee}(\mathbf{2}))]$, where $c = \mathrm{ee}(\mathbf{1})/(\mathrm{ee}(\mathbf{1})+\mathrm{ee}(\mathbf{2}))$.

[f] Lipase PS, in which Celite is used as a supporting material, was used.

[g] Vinyl hexanoate was used as an acyl donor. The reaction was conducted in a test tube.

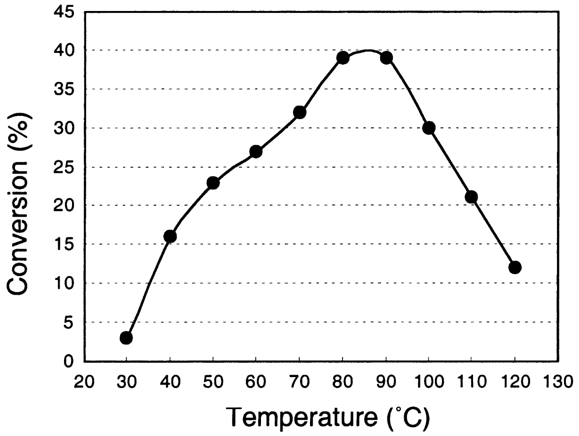
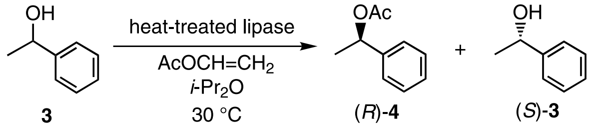


Figure 1. Conversions plotted against the reaction temperature.

reported value.[12] The obtained ester **2** was enantiomerically pure (>98% ee) in all cases measured (except 30°C). It should be noted that the enzymatic catalysis proceeded completely enantioselectively even at 120°C, a sterilization temperature for microorganisms. Although it is known that the enantioselectivity in lipase-catalyzed reactions usually decreases with an increase in temperature[2,8] because of a high racemic temperature,[13] the enantioselectivities remained complete at all the temperatures examined in this study. We also examined vinyl hexanoate as an acyl donor because its boiling point is higher than 120°C. In this case, the reaction was conducted at 120°C in a glass test tube with a rubber septum. As a result, the corresponding ester was obtained in >98% ee and 8% yield.

Thermostability of the lipase in the Toyonite pores (mean pore size: 60 nm) is much higher than that of the same enzyme on Celite (lipase PS, Amano Enzyme Inc.) because the latter showed 0% conversion for **1** at 100°C for 3 h (Table 1). The thermostability of the lipase on Toyonite was examined by the following two methods. After the lipase was heat-treated at either 80, 100, 120, 130, or 140°C for 3 h in *n*-decane in an autoclave in the absence of alcohol and vinyl acetate, the mixture was filtered and dried in vacuo. In method A, it was used in the kinetic resolution of 1-phenylethanol **3** in i-Pr$_2$O at 30°C (Scheme 2), and in method B, it was used for the measurements of the residual activity using Lipase Kit S (Dainippon Pharmaceutical Co., Ltd.). The results are listed in Table 2. In method A, enantiopure ester (R)-**4** was obtained in the kinetic resolution of **3** at 30°C using the lipase that had been heat-treated at 100, 120, and even 130°C, although the conversion decreased gradually. In method B, although the residual activity was reduced with an increase in the heat-treatment temperature, the activity (31%) remained even after the heat treatment at 120°C for 3 h. The activity was lost substantially after the heat treatment at 140°C. These results indicate that although the lipase on Toyonite is denatured gradually at elevated temperatures, it is very robust.



Scheme 2.

The enantioselectivity for **1** was excellent even at high temperatures. The transition-state model that we have previously proposed (Fig. 2)[8,14] suggests that the reactivity of the unfavorable enantiomer, (S)-**1**, is completely suppressed by a severe steric repulsion between the diphenylmethyl moiety of (S)-**1** and the protein wall and/or by a conformational strain which are caused in the transition state, leading to the perfect enantiomeric purity of (R)-**2** even at 120°C. Thus, the configuration of the alcohol and ester was predictable by the mechanism-based enzymatic method.[15] We have previously observed no reactivity of **1** for lipase PS (on Celite) at 30°C,[8] although the transition-state model predicts that lipases will show activity for **1**, having a small substituent (Me group) on one side. The crystal structure of *Burkholderia cepacia* lipase (PDB entry 1OIL)[16] suggested a possibility that even (R)-**1** undergoes a severe steric repulsion between the diphenylmethyl moiety of (R)-**1** and a protein moiety, such as Tyr29, Leu287 and Ile290, which are not shown in the transition-state model. By elevating the temperature, the protein can fluctuate to relieve the steric repulsion, and this fluctuation may also have contributed to the rate acceleration at elevated temperatures. The transition-state model also predicts that both activity and enantioselectivity for **5**, which possesses bulky substituents on both sides, will be very low (Fig. 2(b)), which were actually

Table 2. Effect of heat-treatment on the catalytic activity of the lipase

Heat-treatment temp. (°C)	Method A[a]			Method B
	c (%)[b]	(*R*)-**4** (% ee)[c]	(*S*)-**3** (% ee)[c]	Relative residual activity (%)[d]
80	52	99	>99	66
100	50	>99	>99	63
120	38	>99	61	31
130	8	>99	4	6
140	0	–[e]	2	2

[a] *Conditions*: Lipase PS-C II (heat-treated, 100 mg), **3** (0.82 mmol), vinyl acetate (1.63 mmol), *i*-Pr_2O (5 mL), 30°C, 1 h. The lipase heated at the indicated temperature for 3 h was used.

[b] Conversion determined by ^1H NMR for the reaction mixture.

[c] Determined by chiral GC.

[d] Measured with Lipase Kit S (Dainippon Pharmaceutical Co., Ltd.). The residual activity for the lipase which was treated at 30°C was used as the standard.

[e] Not detected.

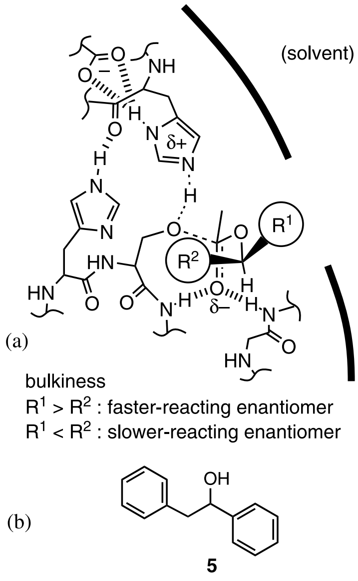


Figure 2. (a) Transition-state model to rationalize the enantioselectivity in the lipase-catalyzed kinetic resolution of secondary alcohols. (i) The C–O bond of the substrate takes the *gauche* conformation with respect to the breaking C–O bond, which is due to the stereoelectronic effect. (ii) The hydrogen atom attached to the stereocenter in the substrate is *syn*-oriented toward the carbonyl oxygen atom. Enantioselectivity is explained by the conformational requirements and repulsive interactions and/or strains caused in the transition state. For details, see Ref. 14. (b) Alcohol **5** having bulky substituents on both sides, whose reactivity is very low as predicted by the transition-state model.

observed. The 0% conversion was observed at 30°C after 3 h. Even the bulky alcohol **5** was allowed to react at a higher temperature but with a low *E* value. The alcohol **5** was converted to the corresponding acetate (*R*)-**6** with 66% ee at 21% conversion ($E=6$) in the reaction at 80°C for 3 h.

3. Conclusion

In summary, lipase PS-C II is a robust biocatalyst useful for organic synthesis. Triggered by the concept represented by the transition-state model, the capabilities of this mesophilic enzyme have been explored, finding that they are comparable to those of (hyper)thermophilic enzymes.[17] The lipase-catalyzed reactions for **1** proceeded at 40–120°C to give enantiopure (*R*)-**2**, with the highest conversion at 80–90°C. Since the lipase-catalyzed enantioselective transesterifications proceed at −60°C using the same enzyme as reported previously,[2,18] it follows that the single enzyme can show the catalytic function at a very wide range of temperatures from −60 to 120°C.

4. Experimental

4.1. General

Silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Lipase PS-C II and lipase PS were purchased from Amano Enzyme Inc. The enantiomeric purities of **1**, **2**, **5**, and **6** were determined by HPLC using Chiralcel OJ-H (**1**), Chiralpak AD-H (**2**), Chiralcel OD-H (**5**), and Chiralpak AS-H (**6**) columns (Daicel Chemical Industries), and that of **3** and **4** was determined by capillary gas chromatography using a CP-cyclodextrin-β-2,3,6-M-19 column (Chrompack, *ϕ* 0.25 mm×25 m). ^1H NMR spectra were measured in $CDCl_3$ at 500 MHz.

4.2. General procedure for lipase-catalyzed kinetic resolution

The relationship between the temperature of the solvent in the reaction vessel (autoclave or test tube) and that of an oil bath was checked in advance to set the reaction temperature correctly. A mixture of alcohol

(0.82 mmol), lipase PS-C II (270 mg) and vinyl acetate (150 μL, 140 mg, 1.63 mmol) in *n*-decane (5 mL) was put in a stainless autoclave. The vessel was then placed in a preheated oil bath. In the case of the reaction using vinyl hexanoate, a mixture of **1** (173 mg, 0.82 mmol) and lipase PS-C II (270 mg) in *n*-decane (5 mL) in a test tube with a rubber septum was heated at 120°C for 15 min. To the mixture was added vinyl hexanoate (260 μL, 230 mg, 1.62 mmol) via syringe to start the reaction. After being stirred for 3 h, the mixture was cooled, filtered and concentrated in vacuo. The alcohol and ester were separated by silica gel column chromatography.

4.3. Lipase-catalyzed kinetic resolution of 1

(*R*)-**2**: 31% yield; >98% ee; $[\alpha]_D^{14}=+44.2$ (*c* 0.5, MeOH), lit.[12] $[\alpha]_D^{25}=-41.3$ (*c* 3.27, MeOH) for (*S*)-**2** with 88% ee; ^{1}H NMR δ 1.20 (d, $J=6.5$ Hz, 3H), 1.84 (s, 3H), 4.02 (d, $J=9.5$ Hz, 1H), 5.71–5.76 (m, 1H), 7.17–7.30 (m, 10H); HPLC: Chiralpak AD-H, hexane/*i*-PrOH = 50: 1, flow rate 0.5 mL/min, detection 254 nm, (*S*) 13.8 min, (*R*) 15.2 min. (*S*)-**1**: 52% yield; 58% ee; $[\alpha]_D^{18}=-28.6$ (*c* 0.5, MeOH), lit.[12] $[\alpha]_D^{25}=+44$ (*c* 0.55, MeOH) for (*R*)-**1** with 96.5% ee; ^{1}H NMR δ 1.20 (d, $J=6.0$ Hz, 3H), 1.56 (br s, 1H), 3.81 (d, $J=9.0$ Hz, 1H), 4.54–4.57 (m, 1H), 7.18–7.40 (m, 10H); HPLC: Chiralcel OJ-H, hexane/*i*-PrOH = 1: 1, flow rate 0.5 mL/min, detection 254 nm, (*S*) 22.6 min, (*R*) 28.0 min.

4.4. Lipase-catalyzed kinetic resolution of 5

(*R*)-**6**: 16% yield; 66% ee; $[\alpha]_D^{15}=+14.0$ (*c* 0.5, $CHCl_3$), lit.[19] $[\alpha]_D=-2.0$ (*c* 3.57, $CHCl_3$) for (*S*)-**6**; ^{1}H NMR δ 2.02 (s, 3H), 3.05 (dd, $J=14.0$, 6.0 Hz, 1H), 3.19 (dd, $J=14.0$, 8.0 Hz, 1H), 5.94 (dd, $J=8.0$, 6.0 Hz, 1H), 7.09–7.33 (m, 10H); HPLC: Chiralpak AS-H, hexane/*i*-PrOH = 9: 1, flow rate 0.5 mL/min, detection 254 nm, (*R*) 9.9 min, (*S*) 11.2 min. (*S*)-**5**: 72% yield; 18% ee; $[\alpha]_D^{15}=+8.2$ (*c* 0.50, MeOH), lit.[20] $[\alpha]_D^{22}=+23.0$ (*c* 1.34, MeOH) for (*S*)-**5** with 41% ee; ^{1}H NMR δ 1.93 (br s, 1H), 2.99 (dd, $J=13.5$, 8.5 Hz, 1H), 3.05 (dd, $J=13.5$, 5.0 Hz, 1H), 4.91 (dd, $J=8.5$, 5.0 Hz, 1H), 7.20–7.38 (m, 10H); HPLC: Chiralcel OD-H, hexane/*i*-PrOH = 9: 1, flow rate 0.5 mL/min, detection 254 nm, (*R*) 20.5 min, (*S*) 25.6 min.

4.5. Measurements of thermostability of lipase

A mixture of lipase PS-C II (200 mg) and *n*-decane (5 mL) was put in an autoclave, and the vessel was then placed in a preheated oil bath. After being heated at a temperature shown in Table 2 for 3 h, the lipase was recovered by filtration and was dried in vacuo.

In method A, a mixture of **3** (0.82 mmol) and the recovered lipase (100 mg) in *i*-Pr_2O (5 mL) was stirred in a test tube with a rubber septum at 30°C. To the mixture was added vinyl acetate (1.63 mmol) via syringe to start the reaction. After being stirred for 1 h, the mixture was filtered and concentrated. The mixture was analyzed directly by chiral GC with a CP-cyclodextrin-β-2,3,6-M-19 column (Chrompack, ϕ 0.25 mm×25 m, Inj. 250°C, Col. 100°C, Det. 220°C) for the determination of the enantiomeric purities of (*R*)-**4** and (*S*)-**3**.[8] In method B, a mixture of the recovered lipase (150 mg) and water (5 mL) was stirred at room temperature for 30 min. After the mixture was filtered, the lipase activity in the filtrate was determined with Lipase Kit S (Dainippon Pharmaceutical Co., Ltd.) according to the manufacturer's instructions. The residual activity for the lipase which was treated at 30°C in the same way was also determined and was used as the standard for the relative residual activity.

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