BIOORGANIC CHEMISTRY **25,** 88–99 (1997)
ARTICLE NO. BH971056

High Temperature-Induced High Enantioselectivity of Lipase for Esterifications of 2-Phenoxypropionic Acids in Organic Solvent[1]

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Received January 10, 1997

The improved enantioselectivities were observed for lipase MY-catalyzed esterifications of 2-phenoxypropionic acids in organic solvent at high reaction temperature. In this paper, we proposed an assumed model for explaining its high temperature-induced high enantioselectivity.

INTRODUCTION

The demand for the enantiomeric purity of chemical compounds has become larger year by year, and the importance of useful catalysts has been also enlarged for enantioselective transformations such as esterifications in organic solvents (*1*). Lipases have been proven as one of the most useful interfacial catalysts for such enantioselective reactions in organic solvents (*2*). For enzymic reactions, it has become apparent that enantioselectivities of enzymes are greatly affected by the reaction conditions such as a nature of reaction medium (*3*). Also, it is known that an addition of small amounts of water to the organic solvents as the reaction medium is necessary for the catalytic function of the enzyme (*4*). Although the reaction temperature is considered to be one of the most important factors to control enzymic functions, definite information is still lacking as to the effect of reaction temperature on the enantioselectivity. In order to obtain the details of the temperature dependence of the lipase's enantioselectivity, especially at high reaction temperature, we have studied the behavior of the lipase's enantioselectivity from 10°C (low temperature) to 57°C (high temperature) for the esterifications of 2-phenoxypropionic acids in isopropyl ether, using lipases originated from *Candida rugosa.*

RESULTS AND DISCUSSION

For the lipase-catalyzed esterifications of the racemic 2-phenoxypropionic acids **1**–**5** with *n*-butyl alcohol in isopropyl ether (Scheme 1), the combined effects of the reaction temperature and an addition of water upon the enantioselectivity (*E*

[1] Parts of this work have been reported as preliminary communications, Refs. (*6*) and (*7*).
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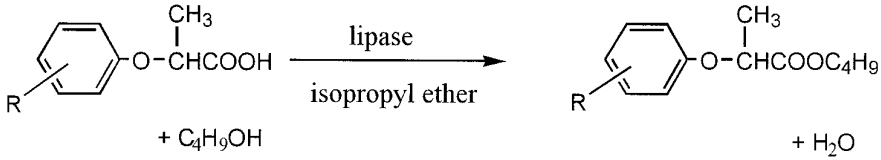
0045-2068/97 $25.00



1 : R= *p*- ethyl
2 : R= *o*- methyl
3 : R= *p*- methyl
4 : R= *p*- *tert*-butyl
5 : R= H

SCHEME 1

value) (*5*) of the lipase were investigated. For these reactions, lipase MY and lipase AY, both lipases originated from *C. rugosa,* produced preferentially the *R* enantiomers of the ester products of 2-phenoxypropionic acids under the ordinary conditions. For the esterification of racemic 2-(4-ethylphenoxy)propionic acid **1**, we have preliminarily reported two types of the lipase's enantioselectivities: high temperature- and low temperature-induced high enantioselectivities (*6*).

As is seen in Figs. 1 and 2, for no water added to isopropyl ether in the esterifications, the enantioselectivities observed for both lipases, lipase MY and lipase AY (at *ca.* 40% conversion), decreased with an increase of the reaction temperature from 10 to 57°C, thus resulting in a loss of the enantioselectivity ($E \sim 1$) at 57°C. This observation could be explained by the assumption in which some of the essential waters maintaining the lipase's native structure are stripped out by the disruption of the lipase–water molecule associations caused by the high reaction temperature. An addition of a suitable amount of water, however, can alter dramatically the behavior of their enantioselectivies as a function of the temperature. The enantioselectivity of lipase MY increased with an increase of the reaction temperature in isopropyl ether containing an optimum amount of water (0.75 vol%)[3] (Fig. 1). Thus, unexpectedly, lipase MY displayed the excellent enantioselectivity ($E = 49$) even at the high reaction temperature (57°C). On the other hand, the reverse temperature dependence of the enantioselectivity was observed for lipase AY in the same reaction (Fig. 2). The enantioselectivity of lipase AY decreased with an increase of the reaction temperature in spite of wide range of an amount of water, in sharply contrast to that of lipase MY. For lipase AY, the highest enantioselectivity ($E = 80$) at the low reaction temperature (10°C) was obtained for the esterification with an addition of an optimum amount of water (0.15 vol%)[3] to the reaction medium.

[3] Lipases MY and AY showed a different optimum amount of water with the highest *E* value studied here, probably depending on the characteristics of the enzyme surface.

In order to elucidate the mechanism of these temperature dependences, we investigated the initial rates for the esterifications of each enantiomer of **1** catalyzed by these lipases in isopropyl ether containing an optimum amount of water, 0.75 vol% for lipase MY and 0.15 vol% for lipase AY. The results of the initial rates affected by the reaction temperature were summarized in Table 1. For lipase MY- and lipase AY-catalyzed esterifications, the initial rates of the *R* enantiomer (V_R) were larger than those of the *S* enantiomer (V_S) at each temperature; this fact is responsible for the *R* preference of both lipases for these esterifications.

There was a marked difference, however, between the temperature dependences of the V_R for the lipase MY-catalyzed esterifications and that for the lipase AY-catalyzed ones. In the former esterifications, the V_R increased smoothly with an increase of reaction temperature; the largest values for the V_R and the relative ratio of the V_R (which defined as the ratio of the initial rates at 37 and 57°C to the initial rate at 10°C) were observed at 57°C (Table 1). In the latter ones, however, the V_R varied irregularly with the reaction temperature. At the high reaction temperature (57°C), the values of V_R and relative ratio of V_R became smaller than those at 37°C, the behavior of which was distinct from the smooth increase of the V_R for lipase MY. In contrast to the behavior of the *R* enantiomer, for both lipases, a smooth

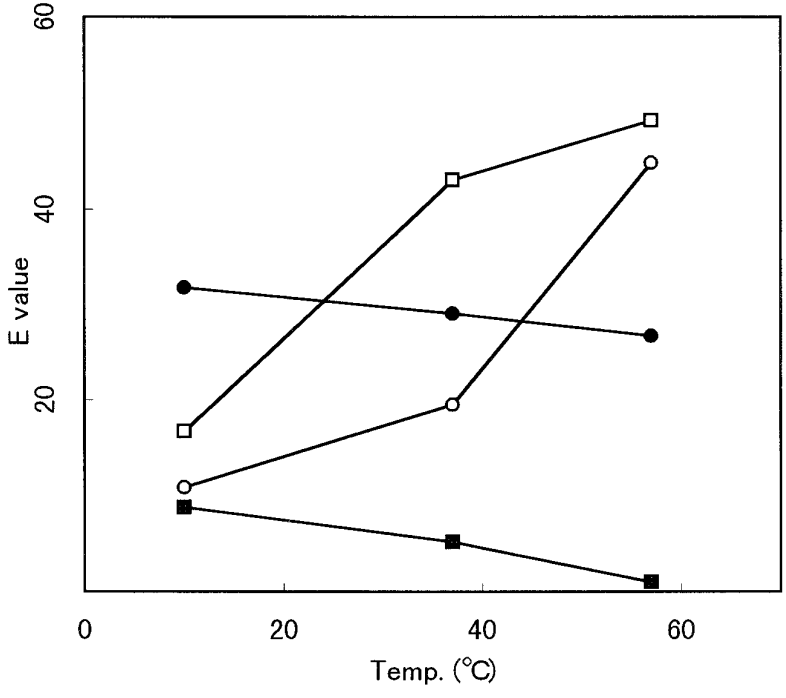


FIG. 1. Temperature dependencies of the enantioselectivities (*E* value) of lipase MY-catalyzed esterifications of **1** with the addition of various amounts of water. ■, No water added; ●, 0.25 vol% water added; □, 0.75 vol% water added; ○, 1.00 vol% water added.

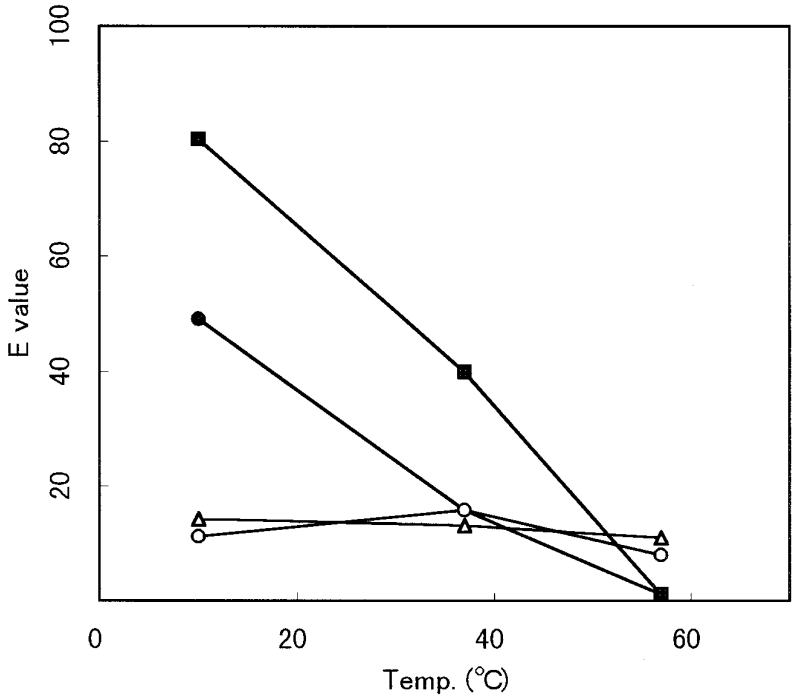


FIG. 2. Temperature dependencies of the enantioselectivities (E value) of lipase AY-catalyzed esterification of **1** with the addition of various amounts of water. ●, No water added; ■, 0.15 vol% water added; △, 0.75 vol% water added; ○, 1.00 vol% water added.

increase of the initial rates for the S enantiomer (V_S) was observed with increasing the reaction temperature.

The high temperature-induced high enantioselectivity of lipase MY can be characterized by the largest value (2.38) of V_R at 57°C and the smooth increase of V_R with the reaction temperature (Table 1). This result suggested that the high temperature-induced high enantioselectivity of lipase MY was probably derived from the flexible conformation of the lipase's molecule. For the lipase MY-catalyzed esterifications, the accommodation of the R enantiomer of **1**, the just fitting substrate, into the lipase MY's active site and the stabilization of the complex between the lipase and the substrate would be accelerated by the combined effects of high reaction temperature and water added, probably because, by the high reaction temperature, the large conformational flexibility might be brought to the lipase's molecule binding water in isopropyl ether. In other words, the active site of lipase MY is found to maintain nearly the native structure even at 57°C. To investigate the scope of the high temperature-induced high enantioselectivity of lipase MY, we tried the esterification of **1** at 67°C, nearly the boiling point of isopropyl ether (68–69°C), and then the higher enantioselectivity of lipase MY was observed; the E value has reached to 84. Also, for trying at the higher temperature, we used n-butyl ether as

the reaction medium, boiling point of which was 142–143°C. In *n*-butyl ether, the high enantioselectivity ($E = 72$) of lipase MY was maintained even at 67°C. However, the loss of the enantioselectivity with the low reactivity was observed at higher temperature than 70°C; the E values were 5.2 and 1.5 at 72 and 77°C, respectively.

On the other hand, a characteristic feature of the low temperature-induced high enantioselectivity of lipase AY is described by the smallest value (0.00068) of V_S at 10°C and the irregular increase of V_R with the temperature. For the lipase AY-catalyzed esterifications containing a small amount of water (0.15 vol%), we speculated that the *R* enantiomer of **1** might not bind correctly to the lipase AY's active site deformed by the high reaction temperature (57°C), because of the loss of the enantioselectivity at 57°C (Fig. 2). The low temperature-induced high enantioselectivity for lipase AY might be due to the highest ratio of V_R/V_S (= 118) at 10°C, calculated from the data in Table 1. The highest enantioselectivity at 10°C could be explained by assuming that the relatively rigid structure of lipase AY arising from lowing the temperature might exclude mainly the *S* enantiomer from its active site. According to this conformational rigidity for lipase AY, we supposed that the phenoxy group of the *S* enantiomer was liable to be excluded from the smaller pocket of the active site, as can be seen from the picture in Fig. 3.

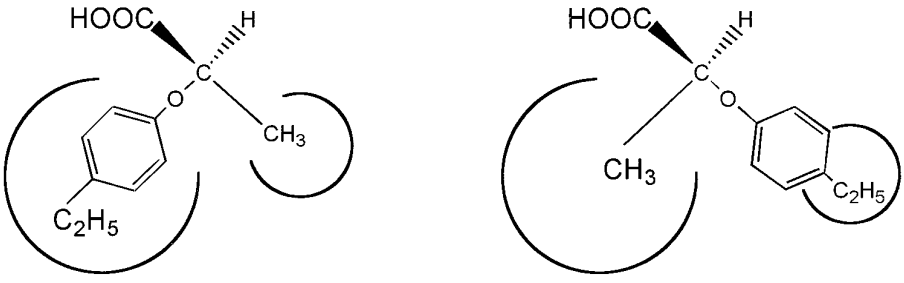
Next, we tried to apply the enantioselective characteristics of lipase MY, high temperature-induced high enantioselectivity, for improving the poor enantioselective behavior of several substrates, bulky ones and a loosely accommodated one.

For the esterifications of the bulky substrates, such as 2-(2-methylphenoxy)propionic acid **2** and 2-(4-*tert*-butylphenoxy)propionic acid **4**, lipase MY displayed the poor enantioselectivity at the ordinary temperatures, 10 and 37°C; for example, the E values of the lipase MY-catalyzed esterifications of **2** and **4** were 1.73 and 1.49, respectively, at 37°C with 1.00 vol% water added (Tables 2 and 3). As is seen from the relative reaction time for ca. 40% conversion at each temperature listed in Table

TABLE 1
Temperature Dependence on the Initial Rates[a] for Lipase MY- and AY-Catalyzed Esterifications Using the Enantiomer of **1**

		Lipase MY-catalyzed, water (0.75 vol%)		Lipase AY-catalyzed, water (0.15 vol%)	
Enantiomer of **1**	Temperature (°C)	Initial rates	Relative ratios[b] of the initial rates	Initial rates	Relative ratios[b] of the initial rates
R	10	0.35	1	0.080	1
R	37	0.91	2.6	0.19	2.4
R	57	2.38	6.8	0.15	1.9
S	10	0.0051	1	0.00068	1
S	37	0.018	3.5	0.0022	3.2
S	57	0.037	7.3	0.0045	6.6

[a] The units of initial rates: μ mol/(h · mg of lipase).
[b] Defined as the ratios of the initial rates of 37 and 57°C to the initial rate at 10°C.



R enantiomer
(correctly binding)

S enantiomer
(incorrectly binding)

FIG. 3. A possible model of the lipase accommodating each enantiomer of **1**.

2, the reactivity of **2** bearing a bulky group (*o*-methylphenyl group) was significantly lowered in comparison with that of **3** bearing a nonbulky group (*p*-methylphenyl group). This result implied that the accommodation of **2** into the lipase's active site was difficult by the steric effect of the methyl group attached to the *ortho*-position in the phenyl ring. Then, we applied the enantioselective characteristics of lipase MY, the high temperature-induced high enantioselectivity, to get over the difficulties about the poor enantioselectivity and the low reactivity arising from the bulky substrate **2** (*7*). The higher reaction temperature (57°C) with a small amount of water (1.00 vol%) was found to produce a remarkable improvement of the lipase's enantioselectivity together with the reactivity, as shown in Table 2. It seemed that the bulky substrate could be accommodated into the lipase's active site by the large flexibility of the lipase molecule arising from the combined effects of high reaction temperature and water added. On the other hand, for the esterifications of **3** bearing

TABLE 2
Enantioselectivity (*E* Value) and Reaction Time for *ca.* 40% Conversion in Lipase MY-Catalyzed Esterification of **2** and **3** at 10, 37, and 57°C with the Addition of 1.00 vol% Water to the Reaction Medium

Substrate	Water added (vol%)	Temperature (°C)	*E* value	Reaction time (h)
2$_{(o\text{-methyl})}$	1.00	10	1.66 (*S* pref.)	941
		37	1.73 (*R* pref.)	222
		57	8.12 (*R* pref.)	166
3$_{(p\text{-methyl})}$	1.00	10	4.94 (*R* pref.)	68
		37	8.03 (*R* pref.)	15
		57	33.3 (*R* pref.)	6