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EFFECT OF TEMPERATURE ON LIPASE-CATALYZED ESTERIFICATION IN ORGANIC SOLVENT

Yoshitaka Yasufuku[a)] and Shin-ichi Ueji*[b)]

[a)]The Graduate School of Science and Technology, Kobe University
[b)]Faculty of Human Development and Sciences, Division of Natural Environment and Bioorganic Chemistry, Kobe University
Nada, Kobe 657, JAPAN

SUMMARY

For the esterification of 2-(4-ethylphenoxy)propionic acid catalyzed by lipase MY (*Candida rugosa*) in isopropyl ether containing a suitable amount of water, the enantioselectivity for the reaction has become higher as the reaction temperature increasing. In contrast, the reverse trend of the temperature effect has been observed for lipase AY (*Candida rugosa*). A model for these temperature dependence has been proposed.

INTRODUCTION

The demand for the enantiomeric purity of chemical compounds is enlarged, and lipases have been proven to be powerful catalysts for enantioselective transformations such as esterifications in organic solvent (Klibanov, 1989; Chen and Sih, 1989; Parida and Dordick, 1991). For these enzymic reactions, it has become apparent that lipase's enantioselectivity is largely affected by the reaction conditions such as the nature of solvents used (Fitzpatrick and Klibanov, 1991; Ueji et al., 1992). The effect of reaction temperature on the enantioselectivity, however, is usually not reported.

Here, we wish to report two types of the temperature dependence of the lipase's enantioselectivity, high temperature- and low temperature-induced high enantioselectivities, and also to discuss their features of the temperature dependence on the basis of a possible model derived from the results of the initial rates obtained for each enantiomer of the substrate, 2-(4-ethylphenoxy)propionic acid, being studied here.

MATERIALS AND METHODS

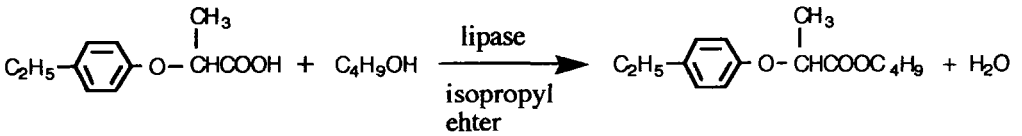
Materials: Racemic 2-(4-ethylphenoxy)propionic acid [1] was prepared from 4-ethylphenol and 2-bromopropionic acid ethyl ester according to the known method (Witiak et al., 1968). Isopropyl ether and *n*-butyl alcohol in this study were of analytical grade, and *n*-butyl alcohol was distilled prior to use. Lipase MY (*Candida rugosa*) and lipase AY (*Candida rugosa*) were supplied from Meito Sangyo Co., Ltd., and Amano Pharmaceutical Co.,Ltd., respectively.

Lipase-catalyzed esterification and determination of the enantiomeric ratio: In a typical experimental procedure, the racemic [1] (70 mg, 0.36 mmole) and a 3-fold molar excess of *n*-butyl alcohol (80 mg, 1.08 mmole) were dissolved in 2 ml of isopropyl ether. To the solution, a small amount of water was added, followed by the ultrasonic dispersion, and then the powdered lipase (30 mg) was added. The suspension was shaken at the settled temperature, and the reaction was monitored by HPLC for the conversion. The enantiomeric ratio (*E* value) was calculated from the enantiomeric excess (*e.e.*) for the butyl ester produced, according to the literature (Chen *et al.*, 1982). The *e.e.* value was determined with HPLC on a chiral column (Chiralcel OK from Daicel Chemical Industries Ltd.,). The initial rates for the esterifications under various reaction conditions were investigated by the use of enantiomerically pure (*R*)- and (*S*)-[1], the *e.e.* values of which were 95% and 96%, respectively.

RESULTS AND DISCUSSION

The esterification of racemic [1] with *n*-butyl alcohol catalyzed by lipase in isopropyl ether was chosen as a model reaction for our study on the combined effects of the reaction temperature and a small amount of water added to the reaction medium (Scheme). In this esterification, the *R* enantiomer of the butyl ester was produced in preference.

Scheme



[1]

As shown in Figs.1 and 2, for no water added to isopropyl ether in the model reaction, the enantioselectivities observed for both lipases, lipase MY (*Candida rugosa*) and lipase AY (*Candida rugosa*), decreased with an increase of the reaction temperature from 10°C to 57°C, thus resulting in a loss of the enantioselectivity ($E \sim 1$) at 57°C. It is logical to suppose that 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 temperature.

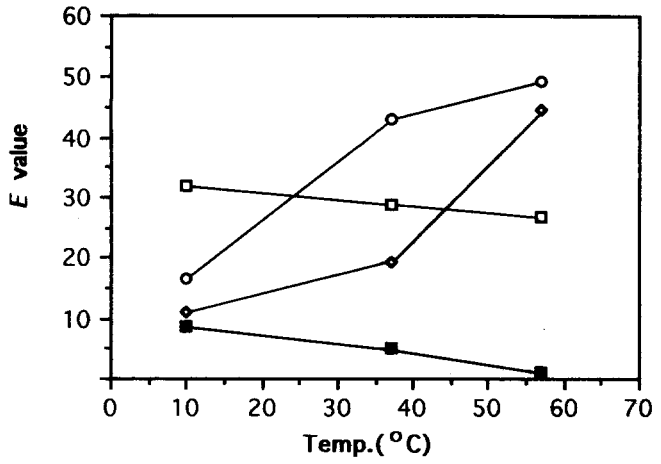


Fig. 1. The *E* value observed for *ca.* 40% of conversion, depending on the combined effects of reaction temperature and water added (vol%) in lipase MY-catalyzed esterification: ■, no water added ; □, 0.25vol% water ; ○, 0.75vol% water ; ◇, 1.00vol% water .

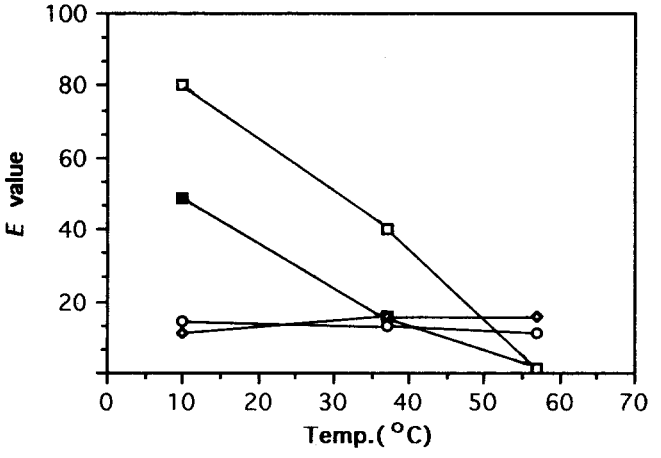


Fig. 2. The *E* value observed for *ca.* 40% of conversion, depending on the combined effects of reaction temperature and water added (vol%) in lipase AY-catalyzed esterification: ■, no water added ; □, 0.15vol% water ; ○, 0.75vol% water ; ◇, 1.00vol% water .

An addition of a suitable amount of water, however, can alter dramatically the behaviour of their enantioselectivities as a function of the temperature. As shown in Fig. 1, for the esterification catalyzed by lipase MY in isopropyl ether containing a suitable amount of water

(0.75 vol%), increasing the temperature led to an increase of its enantioselectivity, contrary to our expectation. Thus, the excellent enantioselectivity (E = 49) for lipase MY was found to emerge even at high temperature (57°C).

On the other hand, using lipase AY in the same esterification, the reverse trend of the temperature effect was observed (Fig. 2). Thus, when a suitable amount of water (0.15 vol%) was added, lipase AY produced the highest enantioselectivity (E = 80) in this study at low temperature (10°C); the enantioselectivity was decreased with an increase of the temperature, as shown in Fig. 2.

In order to gain an insight into a mechanism of the temperature dependence, we investigated the initial rates for the esterifications of each enantiomer of [1] catalyzed by the lipases. As to an optimum amount of water added to the reaction system, we selected 0.75vol% for lipase MY and 0.15vol% for lipase AY. The Table summarized the results of the initial rates affected by the reaction temperatures.

Table. Temperature Dependence on the Initial Rates[a] for Lipase MY- and AY-catalyzed Esterifications using the Enantiomer of [1]

		Lipase MY-catalyzed water (0.75vol%)		Lipase AY-catalyzed water (0.15vol%)	
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 unit of initial rates : μmole / (hours • mg of protein)

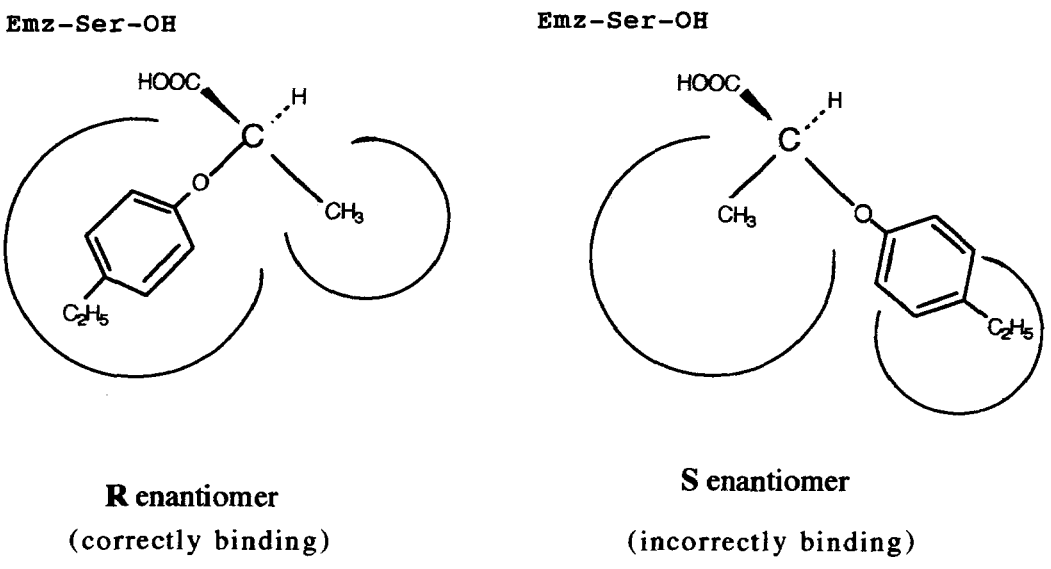
[b]Defined as the ratios of the initial rates at 37°C and 57°C to the initial rate at 10°C.

As shown in the Table, for lipase MY-catalyzed esterification in isopropyl ether containing water (0.75vol%), an increase of the initial rate for the *R* enantiomer (V_R) was remarkable by an increase of the reaction temperature; thus, the largest values for V_R and relative ratio of V_R were observed at 57°C. For lipase AY-catalyzed esterification in isopropyl ether containing water (0.15vol%), however, V_R did not vary smoothly with the reaction temperature. At high temperature (57°C), the values of V_R and relative ratio of V_R became smaller than those at 37°C, which was distinct from the behavior of lipase MY. On the other hand, for both lipases, there was a constant increase of the initial rates for the *S* enantiomer (V_S) with increasing the reaction temperature.

These results suggested that, for lipase MY-catalyzed esterification, the accommodation of the *R* enantiomer to 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 the temperature and water added, probably because the conformational flexibility of the lipase molecule binding water in isopropyl ether may be enhanced by the higher temperature. In other words, the active site of lipase MY is found to maintain nearly the native structure even at 57°C. The presence of a small amount of water in organic solvent is known to enhance the lipase's conformational flexibility, arising from hydrogen bonding effects of water, and to produce an improvement of lipase's enantioselectivity (Kitaguchi *et al.*, 1990; Zaks and Klibanov, 1988).

For lipase AY-catalyzed esterification containing water (0.15vol%), we speculate that the *R* enantiomer, just fitting substrate, may not bind correctly to the lipase AY's active site deformed by high temperature (57°C), although the *S* enantiomer, loose fitting substrate, is much less affected by the change in the lipase structure. Indeed, the loss of the enantioselectivity was observed at 57°C (Fig. 2) .

Lipase AY-catalyzed esterification at 10°C displays the largest *E* value (Fig. 2), which may be ascribed to the largest value (118) for the ratio of V_R/V_S, calculated from the data in the Table. This enhancement of the enantioselectivity at 10°C can be explained by assuming that the relatively rigid structure of lipase AY arising from lowing the temperature may prevent mainly the S enantiomer from the active site. This is probably because the phenoxy group of the *S* enantiomer is liable to be excluded from the smaller pocket of the active site by the lipase's conformational rigidity (Fig. 3).



R enantiomer
(correctly binding)

S enantiomer
(incorrectly binding)

Fig. 3. A possible model of the lipase binding each enantiomer.

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REFERENCES

Chen, C.-S., Fujimoto, Y., Girdaukas, G., and Sih, C. J. (1982). *J. Am. Chem. Soc.* 104, 7294.
Chen, C.-S. and Sih, C. J. (1989). *Angew. Chem., Int. Ed. Engl.* 28, 695.
Fitzpatrick, P. A. and Klibanov, A. M. (1991). *J. Am. Chem. Soc.* 113, 3166.
Kitaguchi, H., Itoh, I., and Ono, M. (1990). *Chemistry Lett.* 1203.
Klibanov, A. M. (1989). *Acc. Chem. Res.* 23, 114.
Parida, S. and Dordick, J. S. *J. Am. Chem. Soc.* (1991). 113, 2253.
Ueji, S., Fujino, R., Okubo, N., Miyazawa, T., Kurita, S., Kitadani, S., and Muromatsu, A. *Biotechnol. Lett.* (1992). 14, 163.
Witiak, D. T., Ho, T. C.-L., Hackney, R. E., and Corner, W. E. (1968). *J. Med. Chem.* 11, 1086.
Zaks, A. and Klibanov, A. M. *J. Biol. Chem.* (1988). 263, 8017.