Effects of Chemical Modification of Lipase on Its Enantioselectivity in Organic Solvents

Shin-ichi Ueji,*† Hiroyuki Tanaka, Takahito Hanaoka, Ai Ueda, Keiichi Watanabe,† Kunihiro Kaihatsu,† and Yasuhito Ebara
Division of Natural Environment and Bioorganic Chemistry, Faculty of Human Development and Sciences, Kobe University, Nada-ku, Kobe 657-8501
†*Graduate School of Science and Technology, Kobe University, Nada-ku, Kobe 657-8501*

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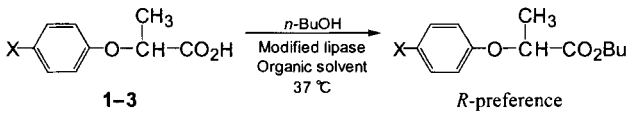
The chemical modification of lipase with the hydrophobic group such as Z (benzyloxycarbonyl) or Z(NO_2) (*p*-nitro-Z) is found to bring about a marked improvement of the enantioselectivity in isopropyl ether and an inversion of the solvent effects on the enantioselectivity.

In enzyme-catalyzed reactions, organic chemists and enzymologists have been dreaming a strategy to rationally control their enantioselectivities by a simple way, because the enantioselectivity is one of the most valuable features of enzymes.[1] Among several strategies proposed for this purpose,[2] the technique of the chemical modification seems to be attractive because of a possible way to radically alter enzyme functions. Much less attention, however, has been paid to the question of whether the chemically modified enzyme is able to change its enantioselectivity.[3]

The elucidation of factors controlling the enantiorecognition of enzymes has recently become one of our interests.[4] Here, we report that the chemical modification of lipase MY from *Candida rugosa* resulted in a marked improvement of its enantioselectivity in isopropyl ether. In particular, the first example of a dramatically different behavior in the solvent effects on the enantioselectivity between the modified lipase and the native one is presented.

Semipurified lipase MY was chemically modified with various hydrophobic groups, such as Z (benzyloxycarbonyl), Z(NO_2) (*p*-nitro-Z), lauroyl, and acetyl, using water-soluble acylating reagents according to the known method.[5] The degree of the modification was determined by measuring the unreacted amino groups of the modified lipase with trinitrobenzenesulfonate.[6] Among the obtained lipases with various degree of the modification, the optimal modified lipase displaying the highest enantioselectivity was selected for this study.



1–3 *R*-preference

1: X = CH_3CH_2, **2**: X = CH_3, **3**: X = $CH_3(CH_2)_2$

Scheme 1. Modified lipase-catalyzed esterification of 2-(4-substituted phenoxy)propanoic acids with *n*-BuOH in organic solvents containing a small amount of water.

In a typical lipase-catalyzed reaction, the substrates **1**–**3** (0.36 mmol) and *n*-BuOH (1.08 mmol, 3 equiv) were dissolved in a dry organic solvent (2 mL). To the solution, a small amount of water (0.9–1.5 vol%) was added, followed by ultrasonic dispersion, and then modified lipase MY (4.5 mg) was added. The suspension was shaken (170 strokes/min) at 37 °C. The enantiomeric ratio (*E* value) was calculated from the enantiomeric excess (ee) for the butyl ester produced, according to the literature.[7] The ee was measured by HPLC on a chiral stationary phase (Daicel Chiralcel OK).

The enantioselectivities of these modified lipases in organic solvents were investigated using the esterification of **1** as a model reaction (Scheme 1). All of the modified lipases and the native one have the *R*-stereochemical preference. One can see from the data in Table 1 that the enantioselectivity (*E* value) is significantly changed not only by the modification group but also by hydrophobicity (log P)[8] of the solvent. In isopropyl ether, the introduction of Z and Z(NO_2) is found to cause the marked enhancement of the enantioselectivity as compared with that of the native one (Table 1), although the modification results in a lowering of the catalytic activity, expressed as the initial rate (Table 2). A similar effect of the Z-modified lipase on the enantioselectivity in isopropyl ether was also observed for **2** and **3**; the native lipase (*E* = 16 for **2** and *E* = 5.2 for **3**) and the Z-lipase (*E* = 28 for **2** and *E* = 21 for **3**). Furthermore, the enantioselectivity observed for each lipase shows some correlation with the solvent hydrophobicity (log P).

Table 1. Relationship between the enantioselectivity (*E* value) and solvent hydrophobicity (log P) for the esterification of **1** catalyzed by the native and modified lipases

Lipase[a]	*E* value			
	i-Pr_2O (1.9)[b]	*n*-Bu_2O (2.9)[b]	Cyclohexane (3.2)[b]	*n*-Hexane (3.5)[b]
N-MY	14	56	124	257
Ac-MY (89)	27	31	16	5.2
La-MY (64)	30	2.2	1.3	1.2
Z-MY (21)	128	31	4.2	4.6
Z(NO_2)-MY (59)	306	23	16	7.3

[a]N-, Ac-, La- Z-, and Z(NO_2)-MY indicate native, acetylated, lauroylated benzyloxycarbonylated, and *p*-nitrobenzyloxycarbonylated lipase MY, respectively. The value in the parentheses represents the degree of the modification. [b]The value in the parentheses represents the solvent hydrophobicity (log P).

Table 2. Initial rates for the esterification of each enantiomer of **1** catalyzed by the native and modified lipases in isopropyl ether.

Lipase	Initial rate[a] / μ mol h^{-1} mg^{-1}		V_R/V_S
	V_R	V_S	
N-MY	14	0.87	16
Z-MY	1.1	0.020	55
Z(NO_2)-MY	1.9	0.016	119

[a]The V_R and V_S values represent the initial rates for *R* and *S* enantiomers, respectively.

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In this correlation, it should be noteworthy that the solvent effects of the modified lipases with Z and Z(NO_2) is opposite to that of the native one. In order to elucidate the origin of the behavior in the enantioselectivity, the solubility of the modified lipases in isopropyl ether and hexane was first checked by the known procedure using protein assay reagent,[9] but they did not show any solubility.

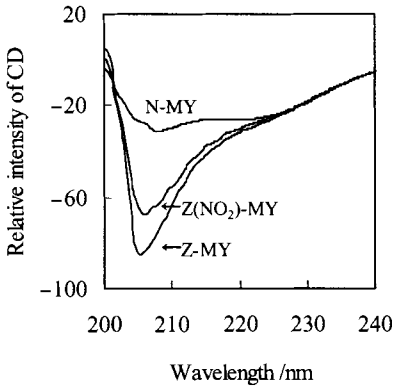

Figure 1. CD spectra of the native and modified lipases in the far-UV region. The measurements were carried out for 0.5 mg (lipase) mL^{-1} solution in all cases.

Next, we investigated the far-UV CD spectrum in water, the ESR spectrum in isopropyl ether, and the initial rate for each enantiomer of **1**, in comparison with the Z- or the Z(NO_2)-lipase and the native one. The CD spectra in Figure 1 indicate that the covalent attachment of Z and Z(NO_2) leads to an increase of the relative intensity of the negative band in the range from 200 to 240 nm, thus corresponding to an increase of the secondary structure in these modified lipases. Then, to investigate the effect of the chemical modification on the conformational flexibility of the lipase, we measured the ESR spectra of the Z-lipase and the native one with a spin label (1-oxy-2,2,6,6-tetramethyl-4-piperidinyl ethoxyphosphorofluoridate) bound to the active site in isopropyl ether (Figure 2). As judged from the change in the ESR spectra, the peak (a discernible shoulder at the low-field component) of the isotropy of the lipase's spin label somewhat decreases upon the chemical modification with Z group. This spectral change suggests the lower flexibility of the Z-lipase. Taking into account these spectral results, one of the possible explanations for the lipase's structural change is as follows. The replacement of the

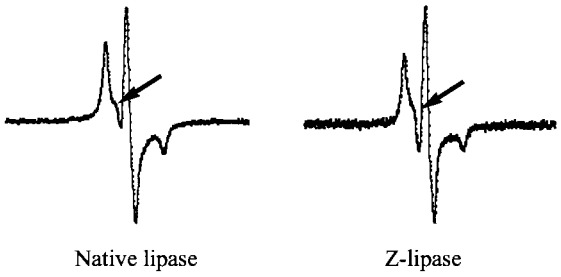

Figure 2. Effects of the chemical modification on the conformational flexibility of the native and Z-lipases with a spin label in isopropyl ether judged from the ESR spectra. The arrow (a discernible shoulder at the low-field component) shows the isotropy of the lipase's spin label.

charged amino acids on the lipase's surface with the hydrophobic group such as Z or Z(NO_2) may induce the conformational alteration in which the polar side chains of the amino acids tend to move away from its surface. In other words, this concentration of the amino acid side chains is assumed to produce a more compact conformation with a less flexibility around the active site of the lipase. In fact, as is seen from Table 2, the Z-modification causes a significant decrease in the initial rates for both enantiomers and the deceleration is more serious for the incorrectly binding *S* enantiomer than that for the correctly binding *R* enantiomer; the steric difficulty encountered by the *S* enantiomer would become more serious in fitting into the more compact binding pocket.[10] Thus, the large difference in their initial rates is attributed to the marked enhancement of the enantioselectivity in isopropyl ether. In addition, the use of isopropyl ether has an advantage of an increased solubility of a wide range of substrates. As to the modification-induced inversion of the solvent effects on the enantioselectivity, this is probably because the manner in the interaction between the lipase surface bearing the hydrophobic modifier such as Z or Z(NO_2) and the solvent molecules differs from that in the native one. In conclusion, the simple modification of lipase is found to provide a versatile tool to alter the features of lipase.

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10 On the other hand, as can be seen from the data of the initial rate (μmol h^{-1} mg^{-1}) in hexane (V_R = 70 and V_S = 0.35 for N-MY; V_R = 1.1 and V_S = 0.033 for Z(NO_2)-MY), the deceleration of the V_S value due to the Z(NO_2)-modification is somewhat relaxed as compared with that in isopropyl ether (Table 2), thus leading to the loss of the enantioselectivity of the modified lipase; in hexane, the Z(NO_2)-lipase seems to become less rigid by taking into account a more favorable association of a small amount of water in the reaction medium with the lipase in the hydrophobic solvents such as hexane than in isopropyl ether.