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Effects of Chiral Additives on Enantioselectivity for Lipase-Catalyzed Esterifications in an Organic Solvent. A Remarkable Enhancement of Its Enantioselectivity Due to Cooperative Effects of Two Kinds of Additives

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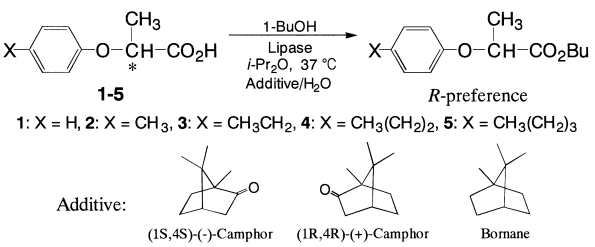
The enantioselectivity for the lipase-catalyzed esterifications in an organic solvent was found to be enhanced by addition of (+)- or (−)-camphor as a chiral additive. A more remarkable enhancement of its enantioselectivity was brought about by the cooperative effects of two kinds of additives: (−)-camphor and water.

Enhancing the enantioselectivity of enzymes remains a challenging problem in the field of the enzyme technology. Although various strategies have been introduced for this purpose, use of additives has been worthy of remark because it is a simple and a cheap way to alter enzyme functions.[1–8] Recently, a review concerning the additive effects on enzyme-catalyzed reactions has appeared.[9] Indeed, the satisfactory results of the enzyme reactions have been achieved by using certain additives in the reaction mixture.[9] In particular, the extensive investigations of the additive effects have been directed mainly towards lipases employed for organic synthesis, because they combine broad substrate specificity with high enantioselectivity.



Scheme 1. Lipase-catalyzed esterification of 2-(4-substituted phenoxy)propionic acids **1**–**5** with 1-butanol in isopropyl ether.

Here, we report the effect of camphor as a chiral additive on the enantioselectivity for the lipase-catalyzed esterification in an organic solvent. Furthermore, the use of the combined additives, camphor and a small amount of water, was found to display a more remarkable enhancement of enantioselectivity.

As a model reaction, we chose the esterification of 2-(4-substituted phenoxy)propionic acids with 1-butanol catalyzed by *Candida rugosa* lipase MY in isopropyl ether containing 0.15 vol% of water (Scheme 1). In this reaction, the lipase catalyzed preferentially the *R* enantiomer of all the substrates used. 2-Phenoxypropionic acids are well-known herbicides and also have other biological activities.[10]

Initially, we investigated the effects of (+)- or (−)-camphor as a chiral additive on the enantioselectivity in the model reaction. Table 1 summarizes the results of the variation of the enantioselectivity (E value[11]) at ca. 40% conversion in the esterification of the substrates **1**–**5** by addition of 5 mol% of camphor. Also, the enantioselectivity for **3** is strongly dependent on the content (mol%) of (+)- or (−)-camphor in the reaction medium (for (+)-camphor, $E = 24$ at 2.5 mol%, $E = 27$ at 5.0 mol%, $E = 30$ at 7.5 mol%, and $E = 39$ at 10 mol%; for (−)-camphor, $E = 36$ at 2.5 mol%, $E = 42$ at 5.0 mol%, $E = 44$ at 7.5 mol%, and $E = 58$ at 10 mol%). The data of Table 1 shows that the addition of (−)-camphor produces a more remarkable enhancement of the enantioselectivity than that of (+)-camphor for all the substrates **1**–**5**. Furthermore, the different behavior in the enantioselectivity between (+)- and (−)-camphors suggests that each enantiomer of camphor may interact with the lipase molecule in a different fashion.

To ascertain whether the carbonyl group of camphor plays an important role in the interaction with the lipase, bornane (obtained from reduction of camphor) was tested as an additive under the same conditions as the addition of camphor in the model reaction, thus resulting in the disappearance of the enantioselectivity enhancement ($E = 20$). Taking into account this observation, one could speculate that the camphor effect on the enantioselectivity may be mainly ascribed to a change in a flexibility and/or in a local conformation around the binding site of the lipase through an electrostatic interactions involving a hydrogen bonding between the carbonyl group of camphor and the lipase molecule.

To elucidate a mechanism of the enantioselectivity enhancement, we investigated the initial rate for each enantiomer of **3** in isopropyl ether by addition of 5 mol% of (−)-camphor (Table 2). As is seen in Table 2, although (−)-camphor brought about the decrease in the initial rates for both enantiomers as compared with that for no additive conditions, the larger deceleration was observed for the incorrectly binding *S*-enantiomer. Such a large difference in their initial rates is attributed to the enantioselectivity enhancement.

Our next step was to investigate the cooperative effects of two kinds of additives, 5 mol% of (+)- or (−)-camphor and 0.125 vol% of water, upon the enantioselectivity of **3** in the model reaction. Simultaneous addition of these additives produced a more remarkable enhancement of the enantioselectivity than expected from the sum of each effect (Table 3). To our

Table 1. Effects of (+)- or (−)-Camphor as a Chiral Additive on the Enantioselectivity at ca. 40% Conversion in the Lipase-Catalyzed Esterification of 2-(4-Substituted Phenoxy)propionic Acids **1**–**5** in Isopropyl Ether

Substrate	X	No additive				(+)-Camphor (5 mol%)				(−)-Camphor (5 mol%)			
		Time/h	Convn.%	ee/%	*E*	Time/h	Convn.%	ee/%	*E*	Time/h	Convn.%	ee/%	*E*
1	H	97	45	34	2.6	100	41	37	2.7	166	40	45	3.5
2	CH_3	40	41	82	18	37	41	83	19	50	41	86	24
3	CH_3CH_2	30	43	85	24	33	42	87	27	42	45	90	42
4	$CH_3(CH_2)_2$	50	44	81	18	53	43	81	18	67	40	88	28
5	$CH_3(CH_2)_3$	76	43	65	7.6	80	44	67	8.5	92	41	82	18

Table 2. Effects of (−)-Camphor as Additive on the Initial Rate for Each Enantiomer of **3** in the Lipase-Catalyzed Esterification in Isopropyl Ether

Additive	Initial rate/μM h^{-1}		V_R/V_S
	V_R	V_S	
None	0.70	0.080	8.8
(−)-Camphor	0.57	0.053	11

Table 3. Cooperative Effects of Two Kinds of the Additives, 5 mol% of (+)- or (−)-Camphor and 0.125 vol% of Water upon the Enantioselectivity at ca. 40% Conversion in the Lipase-Catalyzed Esterification of **3** in Isopropyl Ether

Additive	Time/h	ee/%	*E*
None	27	85	24
H_2O	10	91	26
(+)-Camphor + H_2O	11	93	46
(−)-Camphor + H_2O	14	96	84

knowledge, this is the first example of the enantioselectivity enhancement brought about by the cooperative effects of two kinds of additives. This observation can be explained by assuming that the flexible conformation of the lipase caused by water addition is liable to associate with the carbonyl group of camphor. As to the effect of added water on enzymes in organic solvents, it is known that water acts as a lubricant to increase the enzyme's flexibility, due to its ability to form multiple hydrogen bonds.[12,13] In fact, in anhydrous isopropyl ether, the enantioselectivity enhancement due to camphor becomes much smaller (data not shown).

For a brief test of the validity of the combined use of these additives, *Candida rugosa* lipase AY also displayed a marked increase in the enantioselectivity in the model reaction ($E = 45$ for no additive, $E = 54$ for (+)-camphor additive (5 mol%), $E = 57$ for (−)-camphor additive (5 mol%), $E = 57$ for water additive (0.125 vol%), $E = 66$ for (+)-camphor (5 mol%) and water additive (0.125 vol%), and $E = 73$ for (−)-camphor (5 mol%) and water additive (0.125 vol%)).

In conclusion, our approach based on the combined use of the additives demonstrates the feasibility of a more effective way to improve the outcome of enzyme reactions, as compared with the use of a single additive. In this approach, we recommend the combination of a hydrophobic additive and a hydrophilic one.

Experimental

Materials. Lipase MY and lipase AY were supplied from Meito Sangyo Co., Ltd., and Amano Pharmaceutical Co., Ltd., Japan, respectively, and were used without further purification. Isopropyl ether and (+)- and (−)-camphors were purchased from Wako Pure Chemical Industries, Ltd., Japan. Racemic 2-(4-substituted phenoxy)propionic acids **1**–**5** were prepared by the reaction of the corresponding 4-substituted phenol and ethyl 2-bromopropionate, according to the known method.[14]

Lipase-Catalyzed Esterification. The substrates **1**–**5** (0.36 mmol) and 1-butanol (1.08 mmol, 3 equiv) were dissolved in isopropyl ether (2 mL). To the solution, a small amount of the additives ((+)- or (−)-camphor, or water) was added, followed by ultrasonic dispersion, and then *Candida rugosa* lipase (30 mg) was added. The suspension was shaken (170 strokes/min) at 37 °C. The *E* value was calculated from the enantiomeric excess (ee) for the butyl ester produced, according to the literature.[11] The ee was measured by HPLC on a chiral column (Chiralcel OK, from Daicel Chemical Industries Co. Ltd., Japan).

Initial Rate for Lipase-Catalysed Esterification. According to our method, (*R*)- or (*S*)-ethyl 2-(4-ethylphenoxy)propionate **3** was prepared.[4] Each enantiomer was submitted to the model reaction under the same additive conditions. At an appropriate time interval, aliquots were withdrawn, and the supernatant was analyzed by HPLC on a chiral column to determine the conversion. Five data points (less than 10% conversion) were collected to determine the initial rate coefficient > 0.96.

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