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Drastic enhancement of the enantioselectivity of lipase-catalysed esterification in organic solvents by the addition of metal ions

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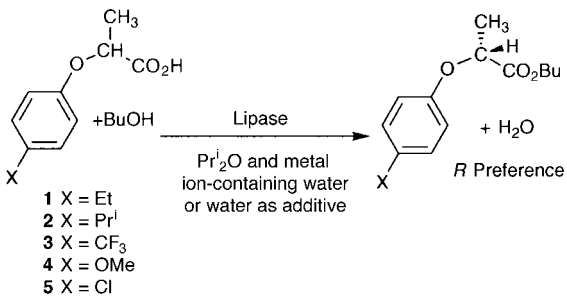
By the addition of metal ion-containing water, a marked enhancement of the enantioselectivity for the lipase-catalysed esterification of 2-(4-substituted-phenoxy)propionic acids in Pr^i_2O was observed, the mechanism of which will be discussed briefly on the basis of the initial rate obtained for each enantiomer of the substrate.

Recently, much attention has been paid to lipase-catalysed enantioselective transformations in organic solvents, because lipases display broad substrate specificities with high enantioselectivities.[1] For this reason, organic chemists often use lipases for the synthesis of useful optically active compounds. One of the most important factors for lipase-catalysed reactions is the control of their enantioselectivites as a function of reaction conditions.[1]

Here, we report a new and a simple method for improving markedly the enantioselectivity of lipase-catalysed esterification of 2-(4-substituted-phenoxy)propionic acids **1**–**5** in Pr^i_2O as a model reaction by the addition of alkaline metal ion (*e.g.* LiCl)-containing water (Scheme 1). This is the first example of an alkaline metal ion enhancing the enantioselectivity of a lipase in organic solvents. Furthermore, the mechanism of the enhanced enantiorecognition will be discussed briefly utilising data on the initial rate obtained for each enantiomer of substrate **1** in the model reaction.

All the substrates used in the model reactions, racemic 2-(4-substituted-phenoxy)propionic acids, were prepared from ethyl 2-bromopropionate and the corresponding 4-substituted-phenols according to the known method.[2]

In a typical enzymatic reaction, the substrates **1**–**5** (0.36 mmol) and a three-fold molar excess of BuOH (1.08 mmol, 80 mg) were dissolved in 2 ml of Pr^i_2O. To the solution, a small amount of 2.4 M metal ion-containing water or water (0–0.75 vol%) was added followed by ultrasonic dispersion, and then powdered lipase MY or lipase AY from *Candida rugosa* (30 mg) was added. The suspension was shaken (170–200 strokes min^{-1}) at 37 °C until *ca.* 40% of the substrate was converted to the corresponding butyl ester. The enantiomeric ratio (*E* value) was calculated from the enantiomeric excess (ee) for the butyl ester produced, according to the literature.[3] The ee was measured by HPLC on a chiral column [Daicel Chiral Cel OK].



Scheme 1

For the model reaction (Scheme 1), the *R* enantiomer of the butyl ester were preferentially produced in all the substrates **1**–**5**. We investigated the behaviour of the lipase's enantioselectivity caused by the addition of a small amount of alkaline metal ion (LiCl, NaCl or KCl)-containing water or a small amount of water to the Pr^i_2O of the reaction medium. Fig. 1 shows the variation of the *E* value for the lipase MY-catalysed esterification of **1** at *ca.* 40% conversion as a function of the amount of each additive. As is seen in Fig. 1, when a small amount of the alkaline metal ion-containing water was added to the reaction medium, the enantioselectivity was found to be dramatically enhanced, as compared with addition of water alone or no additive. In particular, upon addition of 0.5 vol% of LiCl-containing water, lipase MY displayed the highest *E* value (*ca.* 200), which was 5 and 50 times larger than that for water alone or no additive, respectively. A drop in the *E* value, however, was produced by the addition of a large amount of the metal ion-containing water (see the bell-shaped plots in Fig. 1). This can be explained by assuming that an excess of water molecules hydrated to the ion in the reaction medium causes the hydrolysis (the reverse reaction) of the corresponding ester product of **1**, thus leading to the loss of enantioselectivity, probably because the sites of the lipase molecule associated with the water molecule are restricted. The same trend was also observed for water alone (Fig. 1). In fact, the ester product of **1** was subjct to hydrolysis in Pr^i_2O with water or LiCl-containing water of 0.75 vol% or above.†

In order to investigate the scope of this enhancement effect, the other substrate **2**–**5** with a wide variety substituents and the other enzyme lipase AY were submitted to the model reaction. From the data summarized in Table 1, the optimum additive conditions (0.5 vol% of LiCl-containing water) were found to produce dramatically increased enantioselectivity for all the substrates **1**–**5** and both lipases, although lipase AY differs from lipase MY in its catalytic features.[4]

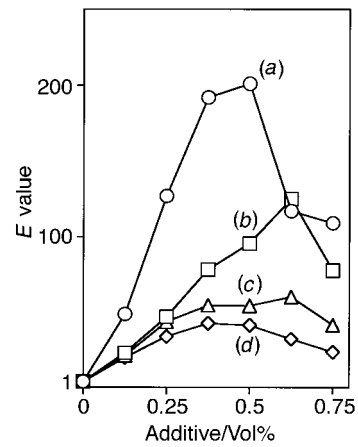


Fig. 1 The variation of the *E* value of lipase MY-catalysed esterification of **1** at *ca.* 40% conversion as a function of the amount of each additive: (*a*) LiCl, (*b*) NaCl, (*c*) KCl and (*d*) water.

Table 1 Enantioselectivity of lipase MY or AY-catalysed butyl esterifications of 2-(4-substituted-phenoxy)propionic acids under various additive conditions

	X	Lipase	Additive[a]	t/h	Conv. (%)	Ee (%)	*E*
1	Et	MY	A	120	25.0	52.3	3.8
			B	6	41.7	90.9	41
			C	16.5	39.5	98.1	201
2	Pr^i	MY	A	240	67.4	30.7	3.4
			B	12	43.5	84.1	22
			C	48	30.9	97.9	145
3	CF_3	MY	A	168	12.6	10.6	1.3
			B	24	40.8	74.6	11.4
			C	168	19.7	90.9	26.0
4	OMe	MY	A	168	6.65	17.8	1.5
			B	16	43.6	82.1	20
			C	168	33.9	92.8	43
5	Cl	MY	A	168	8.82	15.1	1.4
			B	24	38.1	61.4	6.0
			C	168	9.75	87.7	17
1	Et	AY	A	120	19.1	84.9	15
			B	24	43.9	84.7	24
			C	48	34.8	96.4	90
2	Pr^i	AY	A	200	5.5	53.8	3.4
			B	12	39.7	94.8	71
			C	120	37.1	97.9	170
3	CF_3	AY	A	168	1.93	11.6	1.3
			B	62	39.0	90.1	34
			C	168	26.3	95.1	56
4	OMe	AY	A	168	2.56	35.5	2.1
			B	62	48.8	82.7	25
			C	122	40.4	92.6	49
5	Cl	AY	A	168	2.89	17.9	1.4
			B	62	38.0	80.3	15
			C	168	33.9	87.1	23

[a] A: None; B: 0.5 vol% water; C: 0.5 vol% aq. LiCl (2.4 M).

It is known that a small amount of water improves the lipase's enantioselectivity in organic solvents, the origin of which is due to the enzyme's conformational flexibility arising from multiple hydrogen bond formation with water molecules.[5] This fact was also supported by solid-state NMR measurements as judged by tyrosyl ring motion in α-lytic protease.[6]

In order to gain an insight into the mechanism of the enhancement of the enantioselectivity by the metal ion and water, we investigated the initial rate for each enantiomer of **1** in the presence of the additives (Table 2). For addition of water alone, the initial rates for the *R* enantiomer (correctly reacting substrate) and the *S* enantiomer (incorrectly reacting substrate) were accelerated 37-fold and 12-fold, respectively, as compared with no additive conditions (see the relative ratio of the initial rate listed in Table 2). From the value of (relative ratio of V_R)/(relative ratio of V_S) = *ca.* 3 calculated from Table 2, the enhancement of the enantioselectivity was found to be mainly ascribed to the greater acceleration in the initial rate for the *R* enantiomer compared to that for the *S* enantiomer.

Table 2 The initial rate of the esterification for each enantiomer under the three conditions

Additive	V_R[b]	Relative ratio of V_R	V_S[b]	Relative ratio of V_S
None	9.0	1	0.56	1
Water (0.5 vol%)	340	37	6.9	12
LiCl (0.5 vol%)[a]	140	15	0.51	0.91

[a] The additive concentration was 2.4 M. [b] The V_R and V_S denote the initial rates of the *R* and *S* enantiomers, respectively, in nM s^{-1} (mg of protein)$^{-1}$.

In contrast, the addition of LiCl-containing water was found to bring about a slight inhibition (0.91-fold) of the initial rate for the *S* enantiomer, whereas that for the *R* enantiomer was accelerated (12-fold), as compared with the water alone additive (Table 2). Therefore, the larger value of (relative ratio of V_R)/(relative ratio of V_S) = *ca.* 18, arising from the reverse trend of each initial rate, is responsible for the significant enhancement of the lipase MY's enantioselectivity.

In conclusion, there is a difference in the mechanism of the enhancement of the lipase's enantioselectivity between the alkaline metal ion-containing water and water alone as the additive. Our method forms a useful tool to improve the enantioselectivity in lipase-catalysed reactions in organic solvents.

Notes and references

† For 0.75 vol% water of LiCl-containing water in Pr^i_2O *ca.* 20% of the butyl ester of **1** was hydrolyzed.

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