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Effects of Water and Water-Mimicking Solvents on the Lipase-Catalyzed Esterification in an Apolar Solvent

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The enantioselectivity, as well as the catalytic activity of Candida Cylindracea lipase was found to be affected by water and water-mimicking solvents in n-hexane. There existed a parallel relation between the reaction rate and the selectivity; the faster the former, the higher the latter.

An asymmetric hydrolase-catalyzed reaction in non-aqueous media has emerged as a powerful method to synthesize chiral synthons.[1] One of the most distinct feature of this process, as demonstrated by Klibanov and co-workers,[2,3] is that the enzyme's enantioselectivity can be altered dramatically by the nature of solvents, which is not feasible in water.

It is well documented that a small amount of water in organic solvents plays an essential role to determine the activity of biocatalysts.[4] The effect of water on the enzyme's enantioselectivity, however, has rarely been studied.[5] In this communication, we wish to report that the optimized amount of water and water-mimicking solvents can enhance lipase's capability to discriminate both enantiomers, with high catalytic activity.

The enantioselective esterification of α-bromopropionic acid 1 catalyzed by Candida Cylindracea lipase (Eq. 1), reported by Kirchner et al.,[6] was chosen for the present study. We found that the lipase's

$$\underset{\underline{1}}{CH_3\overset{Br}{\overset{|}{C}}HCO_2H} + C_4H_9OH \xrightarrow[\text{hexane}]{\text{lipase}} \underset{\underline{2a}}{(R)\text{-}CH_3\overset{Br}{\overset{|}{C}}HCO_2C_4H_9} + \underset{\underline{2b}}{(S)\text{-}CH_3\overset{Br}{\overset{|}{C}}HCO_2H} \quad (1)$$

activity is a strong function of the amount of water included. As seen in Fig. 1, the reaction was accelerated by 9-fold, when 0.125% of water was added.

The enhanced conformational flexibility, resulted by multiple hydrogen bonds with water molecules, has been considered responsible for water's effect to activate enzymes in organic solvents.[4] If added water

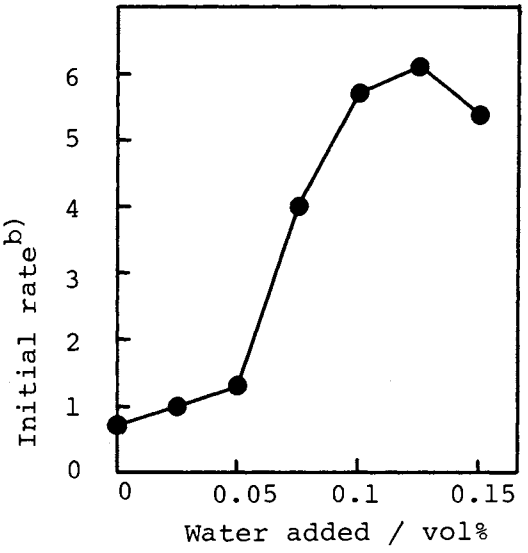

Fig. 1. Effects of Water on Lipase Reactivity[a)]

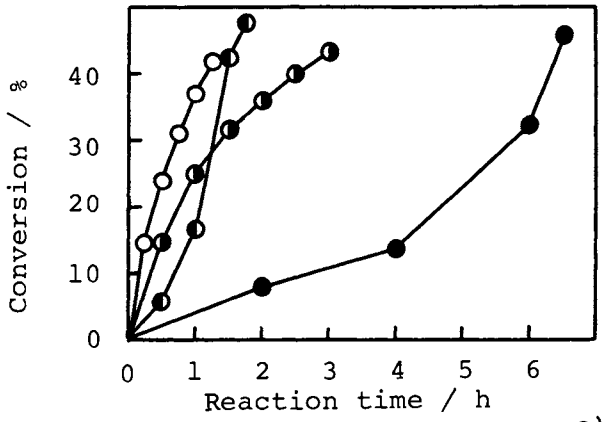

Fig. 2. Kinetic Resolutions[a)] with Graded Amounts of Water

● : 0%, ◐ : 0.05%, ◑ : 0.075%
○ : 0.125% of water was added.

a) 17.5 mg/mL lipase suspensions in n-hexane containing 0.2 $mol \cdot dm^{-3}$ of 1 and 1 $mol \cdot dm^{-3}$ of n-butanol were shaken at 30°C and 170 rpm. The reactions were followed by gas chromatography.

b) $mol \cdot h^{-1} \cdot (mg\ enzyme)^{-1}$

could change the flexibility of its active site's shape, it occured to us that the enantioselectivity of an enzyme might also be altered. On this assumption, we next examined preparative kinetic resolutions with graded amounts of water; 0, 0.05, 0.075, and 0.125%.

Figure 2 depicts timecourses of preparative resolutions. With 0 or 0.05% of water, which is not sufficient to activate the lipase judged by Fig. 1, the reaction became accelerated when the conversion reached 20-30%. This unusual phenomenon should be due to the effect of water formed during the dehydration reaction. In contrast, with sufficient amount of water, the reaction became decelerated while the conversion approached to 50%, as expected by the principle of kinetic resolution.[1a)]

Table 1 reveals that the lipase's enantioselectivity was indeed affected by water. With 0.125% of water, the enantioselectivity factor E[7)] became 5 times higher than that without it. Indicatively, there existed a parallel relation between the reaction rate and the selectivity; the faster the former, the higher the latter.[8)]

Recently, we reported that water mimicking solvents such as formamide and ethylene glycol, which can form multiple hydrogen bonds, can, at least partially, substitute for water as enzyme activators.[9,3)] Presuming that they activate the enzyme according to the same mechanism as that of water, water mimics can be expected to enhance the enantioselectivity besides the

Table 1. Effects of Water on the Enantioselectivity and the Reactivity of the Lipase-Catalyzed Reaction[a)]

Added water/%	Initial rate[b)]	Conversion/%	$[\alpha]_D$/deg.[c)]	e.e./%	E
0	0.69	44.5	+15.4	80	17
0.05	1.3	47.5	+16.4	85	29
0.075	4.0	43	+17.3	90	39
0.125	6.1	42	+18.2	95	81

a) Reaction conditions are the same as the footnotes of Fig. 1. The product 2a was isolated as described in Ref. 6, with yields of 36, 39, 32, and 32%.
b) $\mu mol \cdot h^{-1} \cdot (mg\ enzyme)^{-1}$.

Table 2. Effects of Water Mimics on the Lipase's Reactivity[a)]

Cosolvents	Initial Rate[b)]
none	0.69
0.05% water	1.3
0.05% water + 0.05% formamide	2.0
0.05% water + 0.05% ethylene glycol	2.4
0.05% water + 0.05% dimethylformamide (DMF)	1.4
0.05% water + 0.05% ethylene glycol dimethyl ether	1.5

a) Reaction conditions are the same as the footnotes of Fig. 1.
b) $\mu mol \cdot h^{-1} \cdot (mg\ enzyme)^{-1}$.

rate of the lipase-catalyzed reaction presently investigated.

The reaction rates in the presence of various cosolvents are shown in Table 2, where one can see that formamide and ethylene glycol could activate the lipase by 1.5-1.9 fold. Moreover, the fact that either DMF or ethylene glycol dimethyl ether had much less activating effect than their counterparts strongly suggests that hydrogen atoms which can form multiple hydrogen bonds are essential.[9)] The preparative resolution under the best condition in Table 2, with 0.05% of water and 0.05% of ethylene glycol, gave the ester 2a of 92% e.e.[10)] at 44% conversion.[11)] From these data, one can calculate E to 52,[7)] which is indeed 1.8 times higher than the selectivity without ethylene glycol.

The mechanistic detail of the effect of water and water mimics on the enantioselectivity is not clear. It seems, however, quite likely that the conformational flexibility of the lipase controls its enantioselectivity,

as well as the catalytic activity.

In summary, we demonstrated that the enantioselectivity of the lipase from Candida Cylindracea could be enhanced by adding a small amount of water or water mimicking solvents in n-hexane. These results suggest that, in general, the amount of water included in a reaction system, in addition to the nature of solvents[3] and substituents of a substrate,[5] should be closely examined in order to obtain an optimum and reproducible result for an asymmetric hydrolase-catalyzed reaction in non-aqueous media.

References

1) For a recent review, see: a) C.-S. Chen and C. J. Sih, Angew. Chem., Int. Ed. Eng., 28, 695 (1989); b) A. M. Klibanov, Acc. Chem. Res., 23, 114 (1990).
2) T. Sakurai, A. L. Margolin, A. J. Russell, and A. M. Klibanov, J. Am. Chem. Soc., 110, 7236 (1988).
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4) A. Zaks and A. M. Klibanov, J. Biol. Chem., 263, 8017 (1988).
5) It is reported that porcine pancreas lipase exhibits an improved enantioselectivity after dehydrated under vacuum, see; T. M. Stokes and A. C. Oehlchlager, Tetrahedron Lett., 28, 2091 (1988).
6) G. Kirchner, M. P. Scollar, and A. M. Klibanov, J. Am. Chem. Soc., 107, 7072 (1985). They achieved high enantioselectivity, when no water was added. The difference of the amount of water (ours 4.7%, theirs 6.1%(w/w)) included on crude enzyme powder (purchased from Sigma Chemical Co. and used without any pretreatment) seems responsible for this seeming discrepancy.
7) $E = \frac{\ln[1-c(1+ee(P)]}{\ln[1-c(1-ee(P)]}$, where c refers the degree of conversion and ee(P) does the enantiomeric purity of the product.
Strictly speaking, the equilibrium constant K should be considered to calculate E for a reversible reaction.[1a] We presume, however, that the back reaction (hydrolysis of the ester) is negligible compared to the forward one, based on the fact that this lipase-catalyzed esterification reached 100% conversion after 24 h.
8) Similar relations were observed in Refs. 3 and 5.
9) H. Kitaguchi and A. M. Klibanov, J. Am. Chem. Soc., 111, 9272 (1989).
10) $[\alpha]_D$ +17.6° (c 1.0, $CHCl_3$).
11) The timecourse of this reaction was quite similar to that with 0.075% of water in Fig. 2.

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