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Enhanced Enantioselectivity of the Lipase-Catalyzed Hydrolysis by the Addition of a Catalytic Amount of an Amino Alcohol

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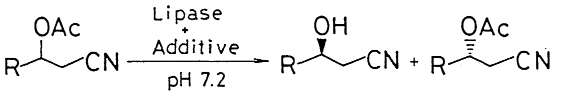
L-Methioninol (L-MetOH) was discovered to be an effective additive for improving the enantioselectivity of lipase-catalyzed hydrolysis. It was found that L-MetOH accelerated the enzyme-catalyzed hydrolysis of (*R*)-3-acetoxybutyronitrile (**1**) and inhibited that of (*S*)-**1**.

The synthetic value of enzymes is being increasingly recognized because enzymatic reactions are highly efficient and proceed under mild conditions.1) One such enzyme being recommended for use by organic chemists is lipase, which is especially useful,2) since lipases are uniquely characterized by their ability to catalyze the hydrolysis of many types of ester bonds, they can be used for the kinetic resolution of various racemic alcohols. However, there is only a limited number of commercially available lipases for practical use, and substrates which are applicable to effective kinetic resolution are limited at the present time. Therefore, a new method for improving the ability of the enzymes regarding both enantioselectivity and adaptability toward substrates, has been long awaited.

We have recently developed one such method using the modification of substrates: The introduction of a sulfur functional group to the acyl residue of a substrate increases the enantioselectivity of lipase-catalyzed hydrolysis.3) We now wish to report here another simple method which extends the synthetic utility of a lipase, an amino alcohol which acts as a very efficient additive to increase the enantioselectivity of a lipase catalyzed hydrolysis of 3-acetoxybutyronitrile.

Results and Discussions

Recently, Sih et al.4) reported that dextromethrophan (*d*-3-methoxy-*N*-methylmorphinan; DM) improved the enantioselectivity of lipase-catalyzed hydrolysis. Though this finding is very interesting when considering the nature of the enzyme, dextromethrophan, a derivative of morphinan, is not recommended for practical use since it lacks availability and is expensive. During our study of the lipase-catalyzed hydrolysis of 3-acetoxy nitriles, we found that the addition of a catalytic amount of diisopropylamine (33 mol% towards the substrate) considerably increased the rate of hydrolysis, although the enantioselectivity decreased.[#] This fact prompted us to survey other simple amines which have the ability to enhance the enantioselectivity of a lipase-catalyzed reaction. The effect of additives in lipase PS (*Pseudomonas* sp.)[##]-catalyzed hydrolysis of (±)-3- acetoxybutyronitrile (**1**) was examined (Eq. 1). The nitrile, **1**, should become a useful chiral building block for the synthesis of many biologically active compounds.5) Lipase PS (30 mg) and an additive were added to a suspension of (±)-**1** (60 mg, 0.47 mmol) in a 0.1 M phosphate buffer (pH 7.2, 5.0 mL); the resulting suspension was stirred at room temperature. The reaction was monitored by silica-gel TLC and was stopped when spots due to the ester and alcohol became the same size. The optical purity of the hydrolyzed alcohol, **2**, was determined by an ^{1}H NMR analysis of the corresponding (+)-α-methoxy-α-phenyl-α-trifluoromethylacetate (MTPA).7) The effect of various amines on the enantioselectivity of the lipase-catalyzed hydrolysis of (±)-**1** is shown in Table 1. Of the thirteen amines examined, four of them markedly enhanced the enantioselectivity of the reaction. Among them, DM (Entry 2) and (*S*)-2-amino-4-methylthio-1-butanol (L-MetOH) (Entry 8), which has been derived from L-methionine, showed a remarkable effect on the enantioselectivity. The other four amino alcohols derived from the corresponding amino acid, i.e. L-phenylalanine (Entry 12), L-leucine (Entry 13), glycine (Entry 11), and *dl*-serine (Entry 14), did not enhance the enantioselectivity of this enzyme. Since L-MetOH is easily prepared, we recommended L-MetOH be used as an additive for practical applications.



(±)-**1** **2** **3** (1)

[#] In the reaction conditions, the hydrolysis of **1** never occurred without the enzyme. Although the pH value of the reaction mixture was not affected by addition of this amount of *i*-PrNH$_2$ it seemed that this amine acted as an effective base in the enzymatic hydrolysis.

[##] Preliminary experiments involving the hydrolysis of **1** revealed that lipase PS (Amano, *Pseudomonas* sp.) was more promising than any other of the eight enzymes tested. However, it is fair to say that the result was not satisfactory because the ratio of specificity constants for the resolution was only 7. Lipases from *Aspergillus niger* (A and A6), *Candida* sp. (MY and M10), *Rhizopus* sp. (F-AP15 and Newlase F), Porcine Pancreatin (PPL), and Hog pancreatin (F) were not stereospecific.

Table 1. Effect of Various Amines on the Lipase-Catalyzed Enantioselective Hydrolysis of (±)-3-acetoxybutyronitrile (**1**)

Entry	Additive	mol%[b]	Time/h	%ee[a] of **2** (% Conv.)	E[c]
1	None	0	10	61(45)	7
2	DM	33	2.0	82(30)	15
3	Bn_2NH	33	3.0[d]	70(46)	10
4	DAPr	33	3.0	78(31)	11
5	Et_3N	33	1.0[d]	51(35)	4
6	i-Pr_2NH	33	0.17[d]	0(43)	1
7	i-$PrNH_2$	33	2.5[d]	63(31)	5
8	L-MetOH	33	16	69(56)	14
9	DL-MetOH	33	14	61(45)	7
10	D-MetOH	33	14	55(58)	8
11	$HOCH_2CH_2NH_2$	33	2.5	53(23)	4
12	L-PheOH	33	5.0	58(51)	7
13	L-LeuOH	33	3.0	58(38)	5
14	APD	33	6.0	53(23)	4

a) Determined by 200 MHz ^{1}H NMR analysis of (+)-MTPA ester of **2**. b) Based on the substrate. Where 33 mol% corresponds 28 mM. c) Calculated by the hydrolysis ratio (% Conv.) and ee of **2**.[6] d) Reactions were carried out at 35 °C. DM: Dextromethorphan. DAPr: 1,3-Diamino-2-propanol. L-MetOH: (2*S*)-2-Amino-4-methylthio-1-butanol. D-MetOH: (2*R*)-2-Amino-4-methylthio-1-butanol. L-PheOH: (2*S*)-3-Phenyl-2-amino-1-propanol. L-LeuOH: (2*S*)-4-Methyl-2-amino-1-pentanol. APD: 2-Amino-1,3-propanediol.

Table 2. Effect of Various Concentrations of Additives on the Enantioselective Hydrolysis of (±)-**1** Catalyzed by Lipase PS (*Pseudomonas* sp.)

Entry	Additive	mol%[a]	%ee of **2** (Conv. %)	E[6]
1	None	0	68(31)	7
2	L-MetOH	10	51(63)	8
3	L-MetOH	20	50(58)	8
4	L-MetOH	33	69(56)	14
5	L-MetOH	50	50(63)	8
6	L-MetOH	100	55(59)	8
7	L-MetOH	200	29(71)	4
8	DM	10	76(46)	14
9	DM	33	82(30)	15
10	DM	100	67(49)	10
11	DM	200	72(31)	8
12	DL-MetOH	10	42(63)	5
13	DL-MetOH	33	61(45)	7
14	DL-MetOH	100	37(64)	4
15	DL-MetOH	200	30(70)	4
16	D-MetOH	10	48(61)	6
17	D-MetOH	33	55(58)	8
18	D-MetOH	100	55(61)	9
19	D-MetOH	200	49(63)	7

a) Based on the substrate.

To gain insight into the mechanism of the action of L-MetOH, the hydrolysis was studied under various concentrations of the additive (DM or MetOH) (Table 2). The results can be summarized as follows: (1) L-MetOH is the best additive among isomers of MetOH, i.e. DL-, L- and D-MetOH. (2) The enantioselectivity of the reaction increases accordingly to the amount of L-MetOH until 33 mol% of **1**; the selectivity then decreases. (3) The effects of L-MetOH are similar to those of DM.

To study the mechanism regarding the action of L-MetOH, the enantiomer of **1** ((*R*)-**1**[3a] or (*S*)-**1**[3a]) was used as the substrate. The kinetic inhibition pattern for each enantiomer of the substrates could provide us with useful information relative to the inhibition mechanism.[4] We used an ^{1}H NMR analysis for determining the rate of hydrolysis. The enantiomer of **1** was subjected to hydrolysis and the reaction was stopped after 2.5 h. The relative rate of the reaction was calculated from the conversion rate at that time, since the hydrolysis of (*S*)-**1** is too small to

Table 3. Effect of Additives on the Rate of Hydrolysis of (±)-**1** Catlyzed by Lipase PS (*Pseudomonas* sp.)

Entry	Substrate	Additive	Concentration	Conv.	Rate[a]
1	(*R*)-**1**	None	0.0 mM	76%	4.0
2	(*R*)-**1**	L-MetOH	10.4 mM[b]	90%	4.7
3	(*R*)-**1**	D-MetOH	10.4 mM	79%	4.1
4	(*R*)-**1**	DM	10.4 mM	52%	2.7
5	(*S*)-**1**	None	0.0 mM	19%	1.0
6	(*S*)-**1**	L-MetOH	10.4 mM	0%	0.0
7	(*S*)-**1**	D-MetOH	10.4 mM	13%	0.7
8	(*S*)-**1**	DM	10.4 mM	11%	0.6

a) Results of comparing the % conversion with reference rate. The reference rate appears on Entry 5. b) 33 mol% based on the substrate.

determine detailed kinetic parameters (Table 3). As can be seen in Table 3, dextromethrophan (DM) inhibited the enzyme-catalyzed hydrolysis of both enantiomers of the acetate (Entries 4 and 8)[4]. It was found that D-MetOH was inferior compared to L-MetOH regarding the inhibitory action of hydrolysis of (*S*)-**1** (Entries 6 and 7). According to the results of hydrolysis under various concentrations of additives (Table 2) the effect of L-MetOH is similar to that of DM. However, the action of L-MetOH to the rate of hydrolysis of (*R*)-**1** was found to be different to that of DM. DM inhibits the hydrolysis of both enantiomers while an enzyme-catalyzed hydrolysis of (*R*)-**1** was not inhibited by L-MetOH. On the contrary, the reaction was accelerated by the addition of L-MetOH (Entry 2). On the other hand, the hydrolysis of (*S*)-**1** was strongly inhibited by L-MetOH and the hydrolyzed product in the presence of L-MetOH was not detected (Entry 6). Evidently, L-MetOH inhibited the hydrolysis of (*S*)-**1** and accelerated that of (*R*)-**1**. It seems therefore that there is a difference in the mechanism for the action to the enzyme between DM and L-MetOH.

According to the experimental results, it seems that L-MetOH and the substrate combine independently with the enzyme at different sites. A conformational change due to the binding of L-MetOH with a certain part of the enzyme may provide a change in the affinity towards the substrates. Therefore, a change of the conformation of the enzyme may provide an increase in the hydrolysis rate of (*R*)-**1** while decreasing the rate of the hydrolysis of (*S*)-**1**. This effect may provide an enhancement of the enantioselectivity of the resolution.

It is not clear at present what geometrical change in the conformation of the enzyme causes a change in the stereoselectivity during hydrolysis. In this study, however, it was established that L-MetOH is an efficient additive that can control the enantioselectivity of a lipase. Considering the broad substrate specificities of lipases, the present method is recommended for use in obtaining optically active alcohols under mild conditions using lipases.

Experimental

Instruments and Materials. NMR spectra were recorded on JEOL MH-100 and Varian VXR-200 spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Solvents and commercially available starting materials were generally used without additional purification, unless otherwise indicated. TLC analyses were carried out on Merck 60 F_{254} silica-gel plates and Wako gel B-5F. Pyridine and dichloromethane were refluxed over calcium hydride for 5 h, distilled and stored under argon in the presence of 4 Å molecular sieves.

Hydrolysis of 3-Acetoxybutyronitrile (1) by Lipase PS: A suspension of 3-acetoxybutyronitrile (**1**)[8] (60 mg, 0.47 mmol), Lipase PS (30 mg, 50 w%) and L-MetOH (21 mg, 0.16 mmol) in a mixed solvent of 0.1 M phosphate buffer (pH 7.2, 5.0 mL) and acetone(0.5 mL) was stirred by a magnetic stirrer at room temperature. After 14 h, the product was extracted from the reaction mixture with ethyl acetate, the extract was then filtered through a short silica-gel column (5 cm) for removing the L-MetOH, dried over anhydrous magnesium sulfate and evaporated in vacuo to give a crude oily product (160 mg). By ^1H NMR analysis of this crude product, the hydrolysis ratio was determine as a 56% conversion. Separation by silica-gel TLC gave **2** (22 mg, 0.26 mmol, 55%) and **3** (20 mg, 0.16 mmol, 33%). The optical purity of the produced alcohol, **2**, was determined by an ^1H NMR analysis of the corresponding (+)-MTPA ester. The enantiomeric excess (ee) of the remaining ester, **3**, was also determined by a conversion to the corresponding alcohol with LAH reduction (in THF at −50 °C) and esterification with 100% excess of (*S*)-(+)-MTPA chloride. **2**; 69 %ee; $[\alpha]_D^{23}$ +3.1°(*c* 1.0, EtOH, lit,[3a] +4.1° (*R*), 84 %ee). **3**; 80 %ee; $[\alpha]_D^{23}$ −19.0° (*c* 1.0, EtOH). The (*E*) value, which was calculated by ee of **2** and ee of **3**, agreed well with that calculated from the hydrolysis ratio and ee of **2**.[6]

Preparation of (*R*)- and (*S*)-3-Acetoxybutyronitrile (1): A solution of 3-(methylthioacetoxy)butyronitrile (**4**)[3c] (10 g, 57.7 mmol) in 0.1 M phosphate buffer (pH 7.2, 100 mL) and acetone (6.0 mL) was incubated with lipase P (2.96 g)at RT for 6 h. The mixture was filtered through a celite pad, and then extracted with ethyl acetate. Using silica-gel flash column chromatography, 8.24 g of the recovered ester and 1.25 g (14.7 mmol, 25% yield) of the product, (*R*)-**2**, were obtained. (*R*)-**2** ($[\alpha]_D^{23}$ +6.8°(*c* 1.33, EtOH); The ee of (*R*)-**2** (98%) was determined by 200MHz ^1H NMR analysis of the corresponding (+)-MTPA ester. The produced alcohol, (*R*)-**2**, was acetylated by acetyl chloride in the presence of pyridine in a usual manner to give (*R*)-**1**; $[\alpha]_D^{23}$ +19.0° (*c* 1.0, EtOH). To obtain (*S*)-**1** with a sufficient ee for practical use, the recovered acetate (8.24 g) was incubated once more with the lipase to give 2.68 g (36% yield) of (*S*)-(methylthio) acetate; $[\alpha]_D^{23}$ −17.6° (*c* 0.945, EtOH). LAH reduction of the ester in THF at −50 °C provided 98% ee of (*S*)-**2**; $[\alpha]_D^{23}$ −7.1° (*c* 0.98, EtOH), which was acetylated to give (*S*)-**1** ($[\alpha]_D^{23}$ −18.6°, *c* 1.0, EtOH).

Kinetics Experiment: Lipase PS (1.0 g) was dissolved in 20 mL of a 0.1 M phosphate buffer (pH 7.2) and centrifuged (2000 rpm, 2 min.). The resulting supernatant liquid was diluted with the buffer solution until the total volume was doubled and used as an enzyme solution. When the lipase was added as a powder to the reaction mixture, we could not obtain reproducible results concerning the determination of the % conversion. 3-Acetoxybutyronitrile (**1**) (20 mg, 0.157 mmol) and the additive (0.0145 mmol, 33 mol% based on the substrate) were weighed directly into a flask and dissolved into 1.0 mL of acetone. The enzyme solution (4.0 mL) was added and stirred for 2.5 h at 23 °C. The reaction was stopped by the addition of crashed ice; the reaction mixture was then extracted with ethyl acetate, dried, and evaporated. The ^1H NMR spectrum was then taken. The hydrolysis ratio was determined by a comparison of the peak intensity of 3-H signals, i. e. alcohols **2**; 3.9—4.1 (m), and the remaining ester **3**; 4.8—5.1(m).

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