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Enhanced Reaction Rate and Enantioselectivity in Lipase-Catalyzed Hydrolysis by Addition of a Crown Ether

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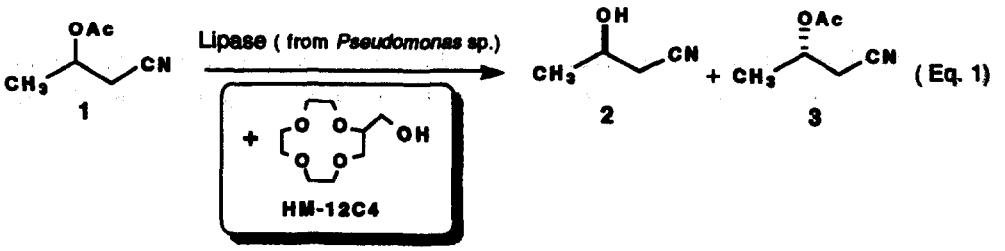
Abstract: Both reaction rate and enantioselectivity in a lipase-catalyzed hydrolysis of β-acetoxybutyronitrile were significantly enhanced by addition of hydroxymethyl-12-crown-4.

The synthetic value of lipases has been well recognized because the reaction is highly efficient and proceeds under mild conditions. [1] Since a limited number of lipases and substrates that are applicable to achieve kinetic resolution have been reported, a convenient method for improving the ability of the enzymes has been awaited. Two methods are recommended to enhance the enantioselectivity of the lipase-catalyzed hydrolysis. One is the modification of the substrate, [2] and the other is the addition of a compound that regulates the reactivity of the lipase. [3] Although the second approach is advantageous for its simplicity of use, only two effective compounds have been reported as such additives. [3] We now wish to report the finding of 2-hydroxymethyl-12-crown-4 (HM-12C4) as *a new type of additive* that enhances both reaction rate and enantioselectivity in the hydrolysis of β-acetoxybutyronitrile (**1**) by Lipase PS (Amano, *Pseudomonas* sp.) (Eq.1). *This is the first example* of a crown ether-type compound being used to enhance the enantioselectivity of an enzymatic reaction, though crown ethers have been reported as complexing agents for several types of proteins. [4]



We chose β-acetoxybutyronitrile (1) and Lipase PS as the control in this project, because the nitrile 1 has the potential to become a useful chiral building block and Lipase PS is recommended as one of the best enzymes applicable to a wide variety of substrates.[1c,d]

The hydrolysis of 1 was carried out in a non-buffered aqueous solution to exclude the effect of the metal cation complexation with a crown ether.[5] Typically, an additive and Lipase PS [6] (30 mg) were added to a suspension of (±)-1 (60 mg, 0.47 mmol) in a mixed solvent of 5.0 mL of water and 0.5 mL of acetone, and the resulting mixture was stirred at 35°C. The reaction was monitored using silica-gel TLC and was stopped when spots of the ester 1 and alcohol 2 became the same size. After GPC analysis of the crude product to determine the hydrolysis ratio, the resulting alcohol 2 and remaining ester 3 were extracted with ethyl acetate and isolated by silica-gel TLC (hexane / EtOAc = 2:1). The optical purity of the alcohol 2 was determined by an ^{19}F NMR analysis [7] of the corresponding (+)-α-methoxy-α-trifluoromethyl-α-phenylacetate (MTPA).[8]

Several crown ethers and acyclic analog were examined as additives. As can be seen in Table 1, both the reaction rate and enantioselectivity largely depended on the nature of the additive. The highest E value,[9] 18, was recorded when the hydrolysis was carried out in the presence of one equivalent of HM-12C4 (0.086 M) based on the substrate 1 (Entry 9). This concentration of HM-12C4 in the reaction mixture corresponds to 5,000 or more times the molarity based on the enzyme.[6] Addition of 12-crown-4 (12C4), 18-crown-6 (18C6), or tetraethylene glycol monomethyl ether (TEM) accelerated the hydrolysis, but the enantioselectivity was little enhanced (Entries 2, 3 and 4). Since 2-benzyloxymethyl-12-crown-4 (BnM-12C4) was also not effective in enhancing of the enantioselectivity (Entry 12), a combination of crown ring and hydroxymethyl side arm group is required to improve the reaction performance of this lipase. The chirality of the side arm of HM-12C4 may not affect the enzyme selectivity. The same E values were observed when the reactions were conducted in the presence of (±)-HM-12C4 or (S)-HM-12C4[10] (Entries 7 and 11).

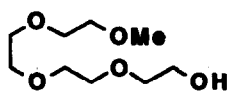
We previously reported that dextromethorphan (DM) and (S)-2-amino-4-methylthio-1-butanol (L-MetOH) were additives effectively enhancing the enantioselectivity of this lipase-catalyzed reaction.[3b] On addition of 33 mol% of DM or L-MetOH, the E values of the hydrolysis of 1 were recorded under the same conditions as 13 and 17, respectively (Entries 13 and 14). Results of the reaction rate are interesting. Addition of HM-12C4 or L-MetOH accelerated the hydrolysis with similar efficiency (Entries 7 and 14), while addition of DM slightly reduced the rate (Entry 13).

Although the origin of the effect of the additive on the reaction profile is not yet clear, it was established that the crown ether derivative has the potential ability

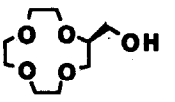
Table 1. Effect of Additive on the Lipase-Catalyzed Hydrolysis

Entry	Additive	mol% [a]	conditions	Time h	% Conv. (Yield of 2)	Rate [b]	%ee of 2	E [c]
1	none	0	H_2O	31	31(30)	1.0	76	10
2	12C4	33	H_2O	13	35 (34)	2.7	76	11
3	18C6	33	H_2O	16	35 (35)	2.1	76	11
4	TEM	33	H_2O	24	42 (41)	1.8	73	11
5	HM-12C4	5	H_2O	27	41 (39)	1.5	74	11
6	HM-12C4	10	H_2O	19	35 (34)	1.8	76	11
7	HM-12C4	33	H_2O	20	40 (40)	2.0	80	15
8	HM-12C4	50	H_2O	19	45 (44)	2.4	77	15
9	HM-12C4	100	H_2O	18	44 (42)	2.4	81	18
10	HM-12C4	200	H_2O	18	53 (51)	2.9	72	15
11	(S)-HM-12C4	33	H_2O	24	46 (45)	1.9	77	15
12	BnM-12C4	33	H_2O	24	42 (39)	1.8	74	11
13	DM	33	H_2O	48	47 (46)	0.98	74	13
14	L-MetOH	33	H_2O	31	59 (59)	1.9	66	17

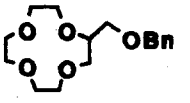
a) Based on the substrate. Where 100 mol% corresponds to 0.086M. b) Results of comparing the % conversion with the reference rate. The reference rate is shown in Entry 1. c) Calculated by the hydrolysis ratio (% Conv.) and ee of **2**.[9]



TEM



(S)-HM-12C4



BnM-12C4

to enhance the reaction rate and the enantioselectivity of the lipase-catalyzed hydrolysis. Our approach is therefore recommended as a new method to regulate the enzymatic reactions. Further research on the control of enzymatic activity by crown ether compounds is ongoing.

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2. For examples, see. (a) Itoh, T.; Takagi, Y.; Nishiyama, S. *J. Org. Chem.*, **1991**, *56*, 1521. (b) Tsukube, H.; Betchaku, A.; Hiyama, Y.; Itoh, T. *J. Chem. Soc., Chem. Commun.* **1992**, 1751. and references cited therein.
3. Dextromethorphan [3a] and (S)-2-amino-4-methylthio-1-butanol [3b] are reported as such effective additive compounds. (a) Guo, Z. -W.; Sih, C. J. *J. Am. Chem. Soc.*, **1989**, *111*, 6836. (b) Itoh, T.; Ohira, E.; Takagi, Y.; Nishiyama, S.; Nakamura, K. *Bull. Chem. Soc. Jpn*, **1991**, *64*, 624.
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5. FAB-Mass experiments indicated that the HM-12C4 bound Na^+ and K^+ cations more strongly than Li^+ cation. Complexation of the crown ether (0.0033M) with LiCl, NaCl, and KCl (0.0033M, each) in glycerol-H_2O (2:1) was studied by measuring the relative peak heights of [crown-M^+] ions: [crown-Li^+], 1.0; [crown-Na^+], 3.5; [crown-K^+], 2.9. ^{13}C NMR titration experiments also supported these behaviors.
6. The enzyme in Lipase PS(Amano) is a purified one which shows a single band in SDS disk gel electrophoresis experiments and has a molecular weight of 32,000. The enzyme content in Lipase PS employed here is less than 10% by weight and the remainder of Lipase PS is mostly amorphous inorganic compounds (celite). The content of the enzyme molecule is thus estimated as less than 3.1×10^{-3} mmol per gram of Lipase PS. We thank Mr. Yoshihiko Hirose of Amano Pharmaceutical Co. for giving us this information.
7. ^{19}F NMR experiments were performed at the SC-NMR Laboratory of Okayama University. The % ee of the remaining ester 3 was similarly determined after hydrolysis using Lipase MY (Meito, *Candida* sp.)
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10. (S)-HM-12C4 was derived from (S)-(+)-isopropyrideneglycerol by a modification of the published method: Miyazaki, T.; Yanagita, S.; Itoh, A.; Okahara, M. *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 2005.

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