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Enhancing the Enantioselectivity of Lipase in Transesterification by Substrate Matching: An Enzyme Memory Based Approach

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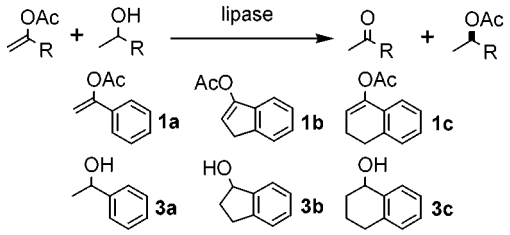
ABSTRACT



The substrate matching strategy is described as a new approach for effectively enhancing the lipase enantioselectivity in organic solvent. In the lipase-catalyzed transesterifications of 3a–c, higher enantioselectivities have been achieved using 1a–c, respectively, as the structurally matched acyl donors.

Transformations based on enzymatic catalysis in organic solvent provide useful methods for the synthesis of chiral molecules with high optical purity.[1] In many cases, however, enzymes show low catalytic activities and moderate selectivity in organic solvent. Among the strategies[2] that have been explored for overcoming these problems, one of the most practical is the molecular imprinting of enzyme.[3] When imprinted with a substrate or an inhibitor in water, proteases such as chymotrypsin and subtilisin exhibited markedly increased activity and more favorable substrate specificity in organic solvent.[4] The aqueous imprinting method, however, was rather ineffective in enhancing the activity and enantioselectivity of lipase to a satisfactory level.[5] We herein describe "substrate matching" based on enzyme memory as a new strategy for effectively enhancing the lipase enantioselectivity.

(1) Reviews: (a) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed.* **1989**, *28*, 695. (b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (c) Santaniello, E.; Fetrizia, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071. (d) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.

(2) The strategies other than molecular imprinting include enzyme modification (refs 2a,b), substrate modification (refs 2c−g), and additive addition (refs 2h−m). (a) Gu, Q.-M.; Sih, C. J. *Biocatalysis* **1992**, *6*, 115. (b) Tuomi, W. V.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 2638. (c) Scilimati, A.; Ngooi, T. K.; Sih, C. J. *Tetrahedron Lett.* **1988**, *29*, 4927. (d) Goergens, U.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1064 and 1066. (e) Kim, M.-J.; Choi, Y. K. *J. Org. Chem.* **1992**, *57*, 1065. (f) Kim, M.-J.; Lim, I. T.; Choi, G.-B.; Whang, S.-Y.; Ku, B.-C.; Choi, J.-Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 71. (g) Gupta, A. K.; Kazlauskas, R. J. *Tetrahedron: Asymmetry* **1993**, *4*, 879. (h) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6839. (i) Khmelnitsky, Y. L.; Welch, S. H.; Clark, D. S.; Dordick, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 2647. (j) Paradkar, V. M.; Dordick, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 5009. (k) Itoh, T.; Tagaki, Y.; Murakami, T.; Hiyama, Y.; Tsukuba, H. *J. Org. Chem.* **1996**, *61*, 2158. (l) Parker, M. C.; Brown, S. A.; Robertson, L.; Turner, N. J. *Chem. Commun.* **1998**, 2247. (m) Griebenow, K.; Laureano, Y. D.; Santos, A. M.; Clemente, I. M.; Rodríguez, L.; Vidal, M. W.; Barletta, G. *J. Am. Chem. Soc.* **1999**, *121*, 8157.

(3) Klibanov, A. M. *Nature* **1995**, *374*, 596.

(4) (a) Russell, A. J.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 11624. (b) Ståhl, M.; Jeppsson-Wistrand, U.; Mansson, M. O.; Mosbach, K. *J. Am. Chem. Soc.* **1991**, *113*, 9366. (c) Rich, J. O.; Dordick, J. S. *J. Am. Chm. Soc.* **1997**, *119*, 3245. (d) Ke, T.; Klibanov, A. M. *Biotechnol. Bioeng.* **1998**, *57*, 764.

(5) (a) Okahata, Y.; Hatano, A.; Ijiro, K. *Tetrahedron: Asymmetry* **1995**, *6*, 1311. (b) Kamiya, N.; Goto, M. *J. Ferment. Bioeng.* **1998**, *85*, 237.

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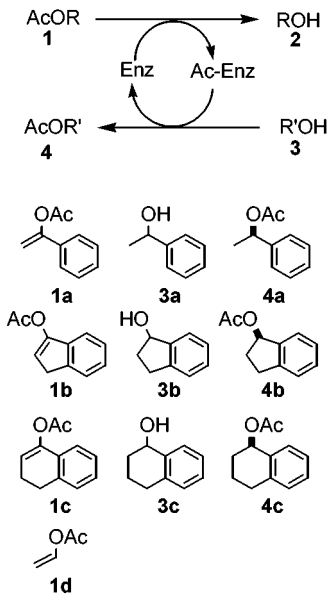
In the lipase-catalyzed transesterification following a ping-pong mechanism,[6] the enzyme reacts first with acyl donor **1** to give deacylated product **2** and acyl−enzyme intermediate and then the acyl−enzyme intermediate reacts with acyl acceptor **3** to give acylated product **4** and free enzyme (Scheme 1). On the basis of this mechanism, we imagined

Scheme 1. Lipase-Catalyzed Transesterification



that the enzyme would memorize **1** and **2** in the first half of the reaction and then the memory would be used for the recognition of **3** and the production of **4** in the second half of the reaction if the reaction is carried out in an anhydrous organic solvent where the enzyme has a high conformational rigidity. Accordingly, we hypothesized that the enantioselectivity of the lipase should be high in the reactions, where **1** and **2** are structurally close to **4** and **3**, respectively, and relatively lower in those where the former are significantly different from the latter. On other words, the two substrates **1** and **3** should be matched for high enantioselectivity.

To test this hypothesis, the transesterifications between enol acetates **1a**−**d** as acyl donors and secondary alcohols **3a**−**c** as acyl acceptors were studied with two lipases from *Candida antarctica* (CAL)[7] and *Pseudomonas cepacia* (PCL).[8] The reactions were carried out in anhydrous organic solvents at room temperature. The enantioselectivity in each reaction was determined by analyzing the optical purities of the unreacted substrate and acetylated product **4a** with chiral HPLC, which allowed us to measure the enantiomeric excess (ee) up to >99.5% ee. The enantioselectivity constant (E) was calculated using the equation $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$ or $\ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$, where $c = ee_s/(ee_s + ee_p)$.[9] It is noted that **1a**−**c** are structurally similar to **3a**−**c**, respectively, except for the acetyl moiety and chirality, and that popular acyl donor **1d** is very different from each of them.

The results from the lipase-catalyzed reactions of 1-phenethyl alcohol **3a** with **1a** (1-phenylvinyl acetate, PVA) and **1d** (vinyl acetate, VA) in several different organic solvents are described in Table 1. In the reactions with VA, CAL showed low enantioselectivity (E = 3−10) and PCL showed moderate to good enantioselectivity (E = 68−639). However, the enantioselectivities increased significantly in the reactions with PVA. The enantioselectivity of CAL increased to a moderate level (E = 18−86) and that of PCL increased to a high level (E = 445 to >2390). The largest enhancements in enantioselectivity realized were 33-fold and >17-fold for CAL and PCL, respectively, in THF.

Table 1. Enantioselectivity in Lipase-Catalyzed Reactions of **3a** with VA (**1d**) and PVA (**1a**) in Anhydrous Organic Solvents[a]

lipase	solvent	E(VA)[b]	E(PVA)[c]	E(PVA)/E(VA)[d]
CAL	toluene	4	42	11
	benzene	10	25	2.5
	tert-butyl methyl ether	2	18	9
	methylene chloride	6	21	3.5
	tetrahydrofuran	3	98	33
PCL	toluene	639	>2390[e]	>3.7
	tert-butyl methyl ether	216	445	2.1
	methylene chloride	175	>870[f]	>5.0
	tetrahydrofuran	68	>1160[f]	>17
	acetonitrile	161	>943[f]	>5.9

[a] In typical experiments, the reactions were carried out at room temperature with enol acetate (2−8 equiv), alcohol (30−50 mg), and enzyme (100 mg) in solvent (1−1.5 mL). The reactions were carried to approximately 40−50% completion, and then the optical purities of the remaining substrate and acetylated product were analyzed by HPLC using a chiral column (Chiralcel OD or Whelk-O-1). Analytical conditions: **3a**, Chiralcel OD, hexane/2-propanol = 98/2, flow rate 1.0 mL/min, UV 217 nm; **4a**, Whelk-O-1, hexane/2-propanol = 98/2, flow rate 0.5 mL/min, UV 217 nm. [b] The enantioselectivity in the reaction with VA (**1d**). [c] The enantioselectivity in the reaction with PVA (**1a**). [d] The ratio of the enantioselectivities between two reactions, indicating the enantioselectivity enhancement. [e] The optical purities (ee) of both remaining substrate and acetylated product were >99.5%. [f] In these cases, the optical purities (ee) of acetylated products were > 99.5%.

The results from additional lipase-catalyzed transesterifications between **1a**−**d** and **3a**−**c** in anhydrous THF are described in Table 2. In the CAL-catalyzed reactions of **3b**, the enantioselectivity was moderate (E = 21) with VA and significantly higher (E = >818) with **1b** (entries 1−2). In this case, at least a 40-fold enhancement in enantioselectivity was realized. Similar behaviors were observed in the reactions of **3b** catalyzed by PCL: E = 42 with VA and E = 1330 with **1b** (entries 3−4). Accordingly, at least a 50-

(6) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192.

(7) Three different types are commercially available: native and recombinant A and B. In this work, the native enzyme from Fluka was used as received.

(8) The enzyme from Amano was used after overnight vacuum-drying since it, when used as received, showed no significant difference in enantioselectivity in the reactions of **3a** with VA and **1a** in THF. In both cases, the enantioselectivity of nondried commercial enzyme was high (E = >800).

(9) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

Table 2. Enantioselectivity in the Lipase-Catalyzed Reactions of **1** with **3** in THF[a]

entry	lipase	donor	acceptor	*E*	*E*/*E*(VA)[b]
1	CAL	**VA**	**3b**	21	
2		**1b**	**3b**	>818[c]	>39
3	PCL	**VA**	**3b**	43	
4		**1b**	**3b**	>2390[d]	>56
5		**VA**	**3c**	48	
6		**1c**	**3c**	>2130[c]	>44
7		**1b**	**3a**	364	5.4
8		**1c**	**3a**	390	5.7
9		**1a**	**3b**	255	5.9
10		**1a**	**3c**	459	5.9

[a] The reactions were carried out as described in Table 1, and the optical purities of remaining substrates and acetylated products were determined by chiral HPLC using the following conditions: **3a** and **4a**, see Table 1; **3b** Chiralcel OD, hexane/2-propanol = 98/2, flow rate 0.5 mL/min, UV 217 nm; **3c**, Chiralcel OD, hexane/2-propanol = 99/1, flow rate 0.5 mL/min, UV 217 nm; **4b**,**c**, determined after hydrolyzed to **3b**,**c**, respectively. [b] The enantioselectivity ratio between the reactions of **1** except VA with **3** and the corresponding reaction of VA, indicating the enantioselectivity enhancement. [c] The optical purity (ee) of the acetylated product was > 99.5%. [d] The optical purities (ee) of both remaining substrate and acetylated product were >99.5%.

fold enantioselectivity enhancement was achieved in this case. In the reactions of **3c** catalyzed by the same enzyme, at least a 40-fold enhancement was observed when VA was replaced by **1c** (entries 5−6). Interestingly, the enantioselectivity enhancements were modest (5.4- to 9.6-fold) in the crossed reactions between **1a**−**c** and **3a**−**c** (entries 7−10).

All of the results from Tables 1 and 2 clearly indicate that the enantioselectivities of both lipases in the matched reactions between **1** and **3** are significantly higher compared to those in the poorly matched reactions in anhydrous organic solvents, supporting the theory that the enzyme memory induced by acyl donors in the first half of the reaction is effectively displayed toward acyl acceptors in the second half of the reaction. This memory process can be rationalized by assuming that acyl donors could bind to the active site in nearly the same mode as the fast reacting enantiomers of acyl acceptors do later in the second half of the reaction.[10] Here, the binding of **1a**−**c** into the hydrophobic active site should be energetically more favorable than that of **1d**. Accordingly **1a**−**c** must be more effective than **1d** in inducing the preorganized active site, thus resulting in better imprinting and significantly enhanced molecular recognition.

In conclusion, this work has demonstrated that the enantioselectivity of lipase in transesterification reactions can be maximized up to an almost perfect level by using two matched substrates. In our separate studies, we have shown that the lipase-catalyzed reactions between two matched substrates are synthetically useful.[11]

Acknowledgment. We acknowledge generous support of this work by the Postech/BSRI research fund and the BK21 program, the Ministry of Education, Korea.

Supporting Information Available: General procedure for determining the lipase enantioselectivity, some chromatograms to show the enantiomeric purities of acetylated products and remaining substrates from CAL-catalyzed reactions of **3a** and **3b** in THF, and tables describing the enantiomeric excesses (ee_s and ee_p) of the remaining substrates and acetylated products, conversion %, the enantioselectivity constants (*E*) for all reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) It was suggested previously for some lipases and proteases that enantiomers of acceptor substrate bind to the active site of enzyme in different productive modes. (a) Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166. (b) Tawaki, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1992**, *114*, 1882. (c) Uppenberg, J.; Öhner, N.; Norin, M.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838. (d) Rotticci, D.; Hæffner, F.; Orrenius, C.; Norin, T.; Hult, K. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 267. (e) Berglund, P.; Holmquist, M.; Hult, K. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 283.

(11) Jung, H. M.; Koh, J. H.; Kim, M.-J.; Park, J. *Org. Lett.* **2000**, *2*, 409.