Enzymatic Preparation of Alkanedicarboxylic Acid Monoesters

Eiji Ozaki,* Toshitaka Uragaki, Keiichi Sakashita,† and Akihiro Sakimae
Central Research Laboratories, Mitsubishi Rayon Co., Ltd., 20-1, Miyuki-cho, Ohtake, Hiroshima 739-06
†Head Office, Mitsubishi Rayon Co., Ltd., 2-3-19, Kyobashi, Chuo-ku, Tokyo 104

(Received March 28, 1995)

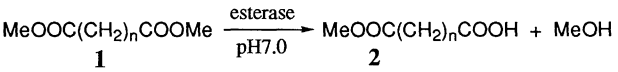
Hydrolysis of alkanedicarboxylic acid dimethyl esters using esterase from *Pseudomonas putida* MR-2068 gave exclusively pure monoesters. Hydrolytic activities were dependent on the carbon chain length of the substrates.

This esterase also catalysed enantio- and regio-selective hydrolysis of α-methylalkanedicarboxylic acid dimethyl esters.

The preparation of monoesters of alkanedicarboxylic acids are worth investigating when the corresponding cyclic anhydrides are not readily available. In particular, the preparation of monofunctionalized malonate and adipate were well studied because of their usefulness as starting materials for further transformations.[1-3] It is well known that porcine liver esterase (PLE) catalyses the hydrolysis of certain dicarboxylic acid dialkyl esters to give corresponding monoesters.[4] Furthermore, optically active α-substituted alkanedicarboxylic acid derivatives are useful chiral synthons for synthetic chemistry. In the preparation of chiral methylsuccinic acid derivatives, asymmetric reduction of itaconic acid[5-6] and enantioselective hydrolysis of the racemate by PPL were well studied.[7]

We have previously reported the novel esterase produced by a newly isolated strain, *Pseudomonas putida* MR-2068,[8] the cloning of its gene and the expression in *Escherichia coli*.[9]

Now we have found that this esterase can catalyse both the hydrolysis of alkanedicarboxylic acid dimethyl esters to give pure monoesters and enantio- and regio-selective hydrolysis of α-methylalkanedicarboxylic acid dimethyl esters to give optically active half esters and chiral diesters. The hydrolytic activities were dependent on the carbon chain length of the substrate.



1 **2**

Scheme 1. **a** : n=1 **b** : n=2 **c** : n=3 **d** : n=4 **e** : n=5

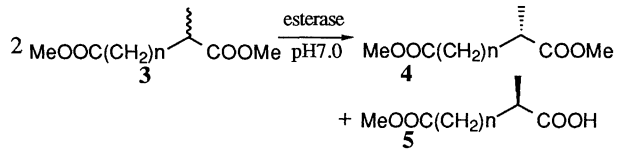
E.coli C600/pPE117 was used as an enzyme source. After cultivation at 37°C in an LB medium (10 g/l Bacto-tryptone, 5 g/l yeast extract, and 5 g/l NaCl) containing 50 μg/ml of ampicillin, bacterial cells were harvested and disrupted by sonication. The cell debris was removed by centrifugation and supernatant was concentrated with ammonium sulfate. The precipitate formed were collected by centrifugation and dissolved in a small amount of buffer A (50 mM Tris-HCl, 1 mM EDTA, pH7.5) and dialyzed against the same buffer. This enzyme solution was used for the hydrolysis reaction without further purification. The hydrolysis was carried out in 50 mM phosphate buffer (pH7.0) containing 30 mM of alkanedicarboxylic acid dimethyl esters at 30°C while adjusting the pH with 1 N NaOH. After the consumption of 1 equiv. of alkaline, unreacted diester was extracted with ethyl acetate. The aqueous layer was acidified to pH 2.0 or below with diluted sulfuric acid and extracted with ethyl acetate. After dried over Na_2SO_4, ethyl acetate extract was concentrated to give monoesters. Relative activities and selectivities of the reaction were summarized in **Table 1**.

Table 1. Esterase catalyzed hydrolysis of alkanedicarboxylic acid dimethyl esters

Substrate	Relative activity(%)[a]	Selectivity (%)[b]
1a	9	>99
1b	79	>99
1c	81	>99
1d	100	>99
1e	0	-

a Relative activities were expressed by initial rate of hydrolysis as a percentage that of dimethyl adipate taken as 100%.
b Selectivities were expressed as a percentage of monoester/(monoester+dicarboxylic acid) formed.

The hydrolytic activities were clearly dependent on the carbon chain length and drop sharply to zero when dimethyl pimelate(**1e**) was used as a substrate. The substrate binding site of the esterase has a fixed length and one methylene length of the substrate was precisely recognized. However, it is not clear why the hydrolysis activity for **1a** is less than 1/10 compared to that of **1d**. A possible explanation is that intramolecular closeness of ester groups cause steric hindrance to the active site of the esterase. In order to clarify this point, further study should be done including active site model study of the esterase. Once diester was hydrolyzed to monoester and released into the buffer, sodium salt was quickly formed and not accepted as substrate for the esterase. This simple process for preparation of monoesters, especially **2a** and **2d**, using inexpensive bacterial esterase is superior to the chemical hydrolysis or PLE catalysed hydrolysis.



3 **4** **5**

Scheme 2. **a**: n=1 **b**: n=2

When α-methylalkanedicarboxylic acid dimethyl esters were used as substrate under the same conditions, hydrolysis completed after 0.5 equiv. of NaOH consumption. Unreacted diesters and produced monoesters were separately collected as described above. ^1H and ^{13}C NMR studies for monoesters showed the esterase catalysed hydrolysis was regioselective.[10] After the esterification of monoesters with MeOH/HCl, the comparison of specific rotation of unreacted diesters and monoester-derived diesters showed that the hydrolysis were enantioselective. These results were summarized in **Table 2**. Enantioselectivity of this esterase is apparently superior to that of PPL.

Table 2. Optical resolution of α-methyl alkanedicarboxylic acid dimethyl esters

Substrate	Unreacted diester	Produced monoester	$[\alpha]_D^{25}$	C.P.(%)[a]	O.P.(%e.e.)[b]
3a	**4a**		-6.33	99.9	99.8
		5a	+6.33[c]	99.0	97.6
3b	**4b**		+25.9	99.2	>99.0[d]
		5b	-25.6[c]	99.0	>99.0[d]

a Chemical purity was determined by HPLC with ODS column.
b Optical purity was determined by HPLC with CHIRALCEL OD (DAICEL Co.,LTD., Japan) for **4a** and with CHIRALCEL OD-R (DAICEL Co.,LTD.) for **5a**.
c Measured after esterification by standard method.
d Determined by comparison of the optical rotations.

PPL catalysed hydrolysis of dimethyl methylsuccinate gave (S)-3-methoxycarbonylbutyric acid.[7] On the other hand, this bacterial esterase catalysed hydrolysis of the same substrate gave pure (R)-methyl 3-carboxybutyrate. Apparently, the regio- and enantio-selectivity of this esterase are different from those of PPL.

Thus we have found the bacterial esterase catalyses the selective hydrolysis of alkanedicarboxylic acid dimethyl esters($1 \leqq n \leqq 4$) and regio- and enantio-selective hydrolysis of α-methylalkanedicarboxylic acid dimethyl esters (n=1,2). Synthetic application of these useful monoesters will be reported elsewhere.

References and Notes

1 B. Rigo, D. Fasseur, P. Cauliez, and D. Couturier, *Tetrahedron Lett.*, **30**, 3073 (1989).
2 K. Matoba and T. Yamazaki, *Chem. Pharm. Bull.*, **31**, 2955 (1983).
3 H. Ogawa, T. Chihara, S. Teratani, and K. Taya, *J. Chem. Soc.,Chem.Commun.*, **17**, 1337 (1986).
4 M. Lobell and M.P. Schneider, *J.Chem.Soc.,Perkin Trans.1*, **15**, 1713 (1993).
5 K. Achiwa, *Chem. Lett.*, **1978**, 561.
6 I. Ojima, T. Kogure, and N. Yoda, *J. Org. Chem.*, **45**, 4728(1980).
7 E. G. Jampel, G. Roussean, and J. Salaun, *J. Chem. Soc. Chem. Commun.*, **1987**,1080.
8 A. Sakimae, R. Numazawa, and H. Ohnishi, *Biosci. Biotech. Biochem.*, **56**, 1341 (1992).
9 E. Ozaki, A. Sakimae, and R. Numazawa, *Biosci. Biotech. Biochem.*, **58**, 1745 (1994).
10 **5a** ; ^{1}H NMR($CDCl_3$) δ=1.25-1.28 (3H,d), 2.40-2.48 (1H,dd), 2.70-2.80 (1H,dd), 2.92-3.00 (1H,q), 3.69 (3H,s), 11.74 (1H,s); ^{13}C NMR($CDCl_3$) δ=16.57 (CH_3), 35.31 (CH), 36.66 (CH_2), 51.43 (CH_3), 172.11(C,ester), 181.20 (C,acid).
5b ; ^{1}H NMR($CDCl_3$) δ=1.20-1.23 (3H,d), 1.79-1.87 (1H,m), 1.96-2.04 (1H,m), 2.38-2.44 (2H,t), 2.49-2.58 (1H,q), 3.68 (3H,s), 11.58 (1H,s); ^{13}C NMR($CDCl_3$) δ=17.02 (CH_3), 28.59 (CH_2), 31.64 (CH_2), 38.65 (CH), 51.70 (CH_3), 173.69 (C,ester), 182.21 (C,acid).