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A Thioesterase for Chemoselective Hydrolysis of *S*-Acyl Sulfanylalkanoates

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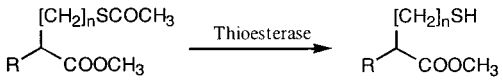
ABSTRACT



n = 0, 1
R = H, alkyl, phenyl

A thioesterase, isolated from a strain of *Alcaligenes* sp. ISH108, chemoselectively hydrolyzes thiol esters. The application of the enzyme has been demonstrated in the preparation of the antihypertensive agent captopril.

Although thiol esters are thermodynamically less stable than oxo esters, their rates of base-catalyzed hydrolysis in aqueous solutions are virtually identical.[1] The rates of ammonolysis of thiol esters are, however, several orders of magnitude higher than those of the corresponding oxo esters. Thus, neutral hydroxylamine has been used for the chemoselective hydrolysis of thiol esters, but it is not suitable for racemization-prone compounds in which a mercapto-bearing asymmetric carbon is adjacent to an electron-withdrawing ester, amide, or nitrile group.[2] Another disadvantage of using neutral hydroxylamine is that the acid component of thiol ester gets converted into an amide and cannot be recovered as such. Enzymatic hydrolysis of thiol esters has attracted very little attention compared to their oxygen or nitrogen analogues. Lipases and esterases have been used, but they are either not chemoselective or the chemoselectivity has been enforced by the substituents.[3,4]

We have recently purified a thioesterase from *Alcaligenes* sp. ISH108, which chemoselectively hydrolyzed thiol esters.[5] The enzyme is stable to a wide range of pH (6.0−10.5) and temperature (25−65 °C). Thioesterases are ubiquitous hydrolytic enzymes with a wide occurrence in plants, animals, and microbes, but they have not been utilized in organic synthesis.[6] We have evaluated the thioesterase for applications in organic synthesis. The results are presented in Table 1.

We started our investigations by studying the thioesterase-catalyzed hydrolysis[7,8] of methyl 3-acylsulfanyl-2-methylpropanoate (**1a**−**c**) in phosphate buffer at pH 6.8 and a

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(1) (a) Bruice, T. C.; Benkovic, S. J. *Bioorganic mechanisms*; W. A. Benjamin: New York, 1966; pp 268−294. (b) Jancks, W. P.; Gilchirst, M. *J. Am. Chem. Soc.* **1964**, *86*, 4651−4654. (c) Conners, K. A.; Bender, M. L. *J. Org. Chem.* **1961**, *26*, 2498−2504.

(2) Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664−3671 and references therein.

(3) Kumar, I.; Jolly, R. S. *Org. lett.* **1999**, *1*, 596−610.

(4) (a) Sproull, K. C.; Bowman, G. T.; Carta, G.; Gainer, I. L. *Biotechnol. Prog.* **1997**, *13*, 71−76. (b) Bianchi, D.; Cesti, P. J. *J. Org. Chem.* **1990**, *55*, 5657−5659.

(5) Isolation, purification, and characterization of thioesterase is being communicated separately.

(6) (a) Gokhale, R. S.; Hunziker, D.; Cane, D. E.; Khosla, C. *Chem. Biol.* **1999**, *6*, 117−125. (b) Lawson, D. M.; Derewenda, U.; Serre, L.; Ferri, S.; Szittner, R.; Wei, Y.; Meighen, E. A.; Derewanda, Z. S. *Biochemistry* **1994**, *33*, 9382−9388 and references therein.

(7) **In a representative experiment** a suspension of **1a** (0.440 g, 2.5 mM) in phosphate buffer (pH 7.00, 50 mL) was purged with a stream of nitrogen for 5 min, thioesterase (aliquot containing 1 mg pure protein) added, and the contents were stirred vigorously; the pH of the solution was kept at 7.00 by continuos addition of 0.1 N aqueous NaOH. After 10 min, when there was no further drop in pH, the reaction mixture was extracted with ether (3 × 10 mL). The organic extracts were washed with brine, dried (sodium sulfate), and then evaporated to give thiol **2**. The purification of thiol was effected by flash chromatography. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23 (3H, d, J = 6.85 Hz), 1.48 (1H, t, J = 8.2 Hz), 2.55−2.83 (3H, m), 2.87 (m, 1H), 3.69 (3H, s). On storage and partly during workup, thiol gets converted into disulfide. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28 (6H, d, J = 6.8 Hz), 2.70 (1H, dd, J = 6.6, 12.8 Hz), 2.72 (1H, dd, J = 6.6, 13.2 Hz), 2.88 (2H, m), 3.03 (1H, dd, J = 7.4, 12.8 Hz), 3.05 (1H, dd, J = 6.9, 13.2 Hz), 3.71 (6H, s).

(8) Compounds **1** and **3**−**12** are known compounds[2−4] and have been characterized on the basis of IR and NMR spectral data. The ee of optically active compounds was determined by methods described by Kellog.[2]

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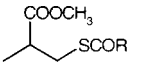
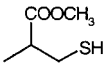
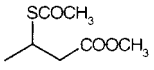
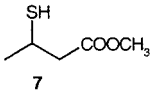
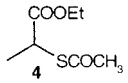
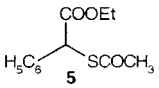
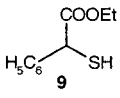
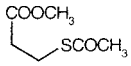
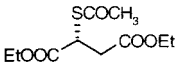
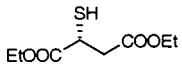
Table 1. Biocatalyzed Chemoselective Hydrolysis of *S*-Acyl Sulfanylalkanoates

entry	Substrate	Time (min.)	Hydrolyzed Product	Yield
1	 **1**, R = CH_3; **1a**, R = CH_2CH_3; **1b**, R = $CH_2CH_2CH_3$	10 - 15	 **2**	95 - 97
2	 **3**	20	 **7**	93
3	 **4**	45	 **8**	92
4	 **5**	40	 **9**	94
5	 **6**	30	 **10**	97
6	*R* - **4**[a]	45	*R* - **8**[b]	95
7	*S* - **5**[a]	40	*S* - **9**[b]	95
8	 *S* - **11**[a]	45	 *S* - **12**[b]	85

a) optically pure substrates were prepared as described[2]
b) ee was determined by ^{31}P method[2]

temperature of 37 °C. A rapid reaction occurred as evidenced by the drop in pH. The pH of the reaction mixture was maintained by the addition of 0.1 N NaOH. The reaction was stopped when there was no further drop in pH. The workup[7] followed by flash chromatography (silica gel; 1:10 ethyl acetate/hexane) gave the hydrolyzed product (**2**). The ^1H NMR spectral data of **2** revealed a peak at δ 3.69, indicating that the oxo ester group has remained intact, whereas the resonance peak due to thiol ester group was absent. In the absence of biocatalyst, **1a** was hydrolyzed to the extent of only 8% after 20 h.

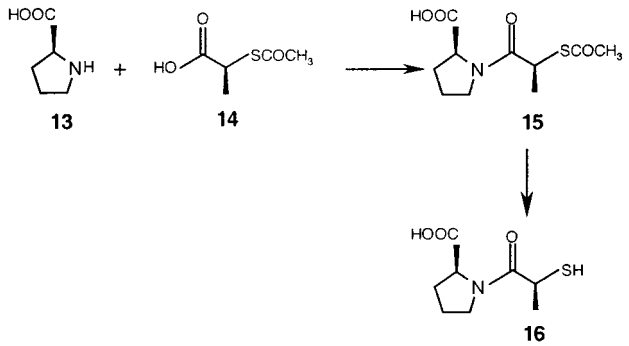
Similarly, the biocatalyzed reaction of methyl 3-acetylsulfanylbutyrate (**3**), ethyl 2-acetylsulfanylpropanoate (**4**), methyl 2-acetylsulfanyl-2-phenylacetate (**5**), and methyl 3-acetylsulfanylpropanoate (**6**) under the above reaction conditions gave the corresponding thiols, methyl 3-sulfanylbutyrate (**7**), ethyl 2-sulfanylpropanoate (**8**), methyl 2-phenyl-2-sulfanyl acetate (**9**), and methyl 3-sulfanylpropanoate (**10**), in near quantitative yield.

The oxo ester group remained intact in all the examples studied above, which demonstrates that the thioesterase is chemoselective for thiol esters. It may be argued that there is a kinetic bias of enzyme in favor of thiol ester and that once the thiol is formed, overall affinity of the substrate changes, making it a poor substrate for further reaction. Therefore, the ester group remains unaffected. However, the failure of the biocatalyst to hydrolyze the compounds containing only the oxo ester groups, viz., butyl acetate or dimethyl succinate, clearly demonstrate that the biocatalyst has no hydrolytic activity toward oxo esters.

Although the thioesterase was found to have absolute chemoselectivity, it failed to discriminate between enantiomers of any of the racemates studied above. However, the enzyme can still be used to advantage for the preparation of optically pure 2-sulfanylpropanoates such as **8**, **9**, and **12**. Such compounds are increasingly becoming important in pharmaceutical and other applications.[9] The ready availability of the corresponding thiol esters in optically pure form from the chiral pool of lactic acids and amino acids could provide an attractive route to these thiols in optically pure form. But the racemization-free deacylation of these thiol esters with a variety of reagents such as alcoholic HCl, ammonia, 4-chloroaniline, $Ti(OR)_4$, etc. has not been successful and leads to a varying degree of racemization.[2] We attempted the biocatalyzed hydrolysis of optically pure thiol esters *R*-(**4**), *S*-(**5**), and *S*-(**11**) under the reaction conditions described above. As expected, the reaction proceeded chemoselectively and without any racemization to give the corresponding thiols *R*-(**8**), *S*-(**9**), and *R*-(**12**) in optically pure form.

Finally, we report an application of the biocatalyst in the preparation of captopril (**16**), an ACE inhibitor having considerable pharmaceutical and commercial importance as an antihypertensive agent.[10] The steps involved in the commercial preparation of **16** are depicted in Scheme 1. We

Scheme 1



and others have already described a biocatalytic method for the preparation of crucial intermediate **14** in optically pure form.[3,11] Now, we report that intermediate **15** can be efficiently deacylated to captopril (**16**) in 97% yield by the

(9) For examples, see the following: (a) Analogue of Ala-82 in backbone of T4 lysozyme: Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197−200. (b) Ultra short acting ACE inhibitor: Baxter, A. J. G.; Carr, A. D.; Eyley, S. C.; Fraser-Reid, L.; Hallam, C.; Herper, S. T.; Hurved, P. A.; King S. J.; Megani, P. *J. Med. Chem.* **1992**, *35*, 3718−3720. (c) Platelet activating factor receptor antagonists: Tanabe, Y. Japan Kokai Tokkyo, Koho, JP 0495 092/1992; *Chem. Abstr.* **1993**, *118*, 234049e. Tanabe, Y.;

biocatalyzed method described above. At present, methanolic ammonia is used for this deacetylation step. Thus, the present biocatalytic method can be used as a viable alternative to the currently employed chemical method.

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Suzukamo, G.; Komuro, S.; Imaishi, S.; Morooka, S.; Enomoto, M.; Kojima, A.; Sanemitsu, Y.; Mizutani, M. *Tetrahedron Lett.* **1991**, *32*, 379−382. (d) In anti ulcer agents: Masai, N.; Enomoto, M.; Kojima, A.; Masumori, H.; Hara, N.; Hara, Y.; Morooka, S. EP Appl. 325496/1989; *Chem Abstr.* **1990**, *113*, 11529u.

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(11) (a) Sakimae, A.; Hosoi, A.; Kobayashi, E.; Ohsuga, N.; Numazawa, R.; Watanabe, I.; Ohnishi,H. *Biosci. Biotech. Biochem*. **1992**, *56*, 1252−1256. (b) Sakimae, A.; Numazawa, R.; Ohnishi, H. *Biosci. Biotech. Biochem.* **1992**, *56*, 1341−1341.