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Biocatalytic Deprotection of a Cetraxate Ester by Microbacterium sp. Strain 7-1W Cells

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Note

Biocatalytic Deprotection of a Cetraxate Ester by *Microbacterium* sp. Strain 7-1W Cells

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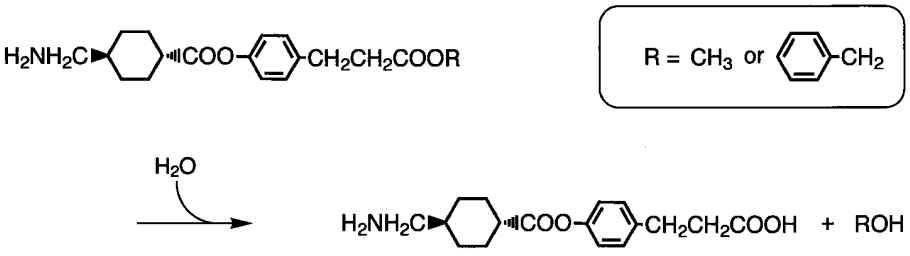
Enzymatic deprotection of the terminal ester bond of a cetraxate methyl ester was done with resting cells of *Microbacterium* sp. strain 7-1W, which produces an esterase catalyzing a regioselective hydrolysis reaction, as the catalyst. When 20 g of cetraxate methyl ester in 50 ml of a reaction mixture was incubated with 5 g of wet cells for 17 h, 96% of the substrate was converted to the desired product, cetraxate, quantitatively.

Key words: esterase; cetraxate; regioselective hydrolysis; *Microbacterium*

Cetraxate hydrochloride (*trans*-4-[[4-(aminomethyl)cyclohexyl]carbonyl]oxybenzenpropionate), which is widely used as an antiulcer medicine, has been industrially produced by chemical deprotection of the terminal ester of the esterified intermediate, benzyl cetraxate[1] (Scheme). But the regioselective deblocking of the terminal ester of benzyl cetraxate is a troublesome chemical reaction because of the coexisting labile phenyl ester. We previously reported the purification and characterization of a novel esterase that catalyzes the regioselective hydrolysis of cetraxate esters from the membrane fraction of *Microbacterium* sp. strain 7-1W.[2] Concerning the enzymatic deprotection of cetraxate esters, Kuroda *et al.* reported the regioselective hydrolysis of the terminal ester bond of benzyl cetraxate by an esterase, obtained from a commercial cellulase preparation of *Aspergillus niger*.[3,4] The enzymes of *Microbacterium* sp. strain 7-1W and *A. niger* both act on cetraxate esters, but these enzymes are quite different from each other. The *Aspergillus* enzyme catalyzes the hydrolysis of various benzyl esters, such as cetraxate benzyl ester and *p*-hydroxyphenyl propionate benzyl ester, but not the lower alkyl esters of these compounds. In contrast, the *Microbacterium* enzyme hydrolyzes the methyl and ethyl esters of cetraxate, and the activity toward benzyl esters relative to that of methyl esters is low. From a practical viewpoint, the hydrolysis of the lower alkyl esters of cetraxate, such as methyl ester and ethyl ester, is more advantageous than that of benzyl ester, because of the difficult synthesis of benzyl ester substrates and the considerable decrease in the molecular mass on deprotection. Here, we report the enzymatic preparation of cetraxate through the regioselective hydrolysis of a cetraxate methyl ester with *Microbacterium* sp. strain 7-1W cells, which were more easily prepared than the extracted enzymes, as the catalyst.

Microbacterium sp. strain 7-1W (AKU 163, Graduate School of Agriculture, Kyoto University) was cultured on a medium of 2% glycerol, 1% NH_4Cl, 1% yeast extract (Oriental Yeast Co., Osaka, Japan), 0.6% K_2HPO_4, 0.2% NaCl, and 0.02% $MgSO_4 \cdot 7H_2O$, pH 7.0, for 4 days at 28°C with shaking. After cultivation, cells were harvested by centrifugation and used in the reactions.



Scheme

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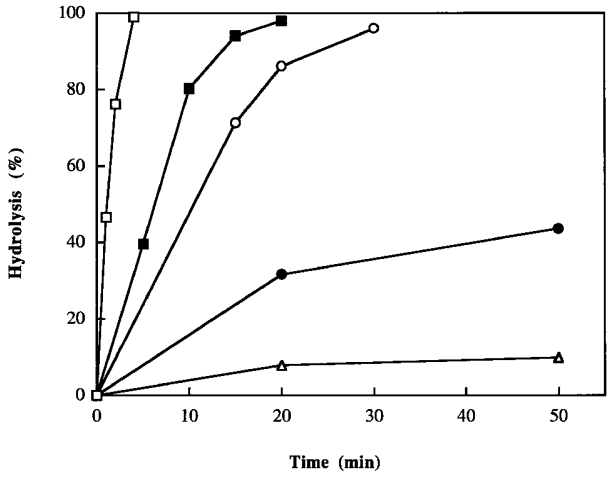

Fig. 1. Effects of the Substrate Concentration on the Hydrolysis Activity of *Microbacterium* sp. Strain 7-1W.

The reaction mixture contained 50 mM potassium phosphate buffer, pH 7.0, 80 mg/ml wet cells, and 1.0% (□), 2.5% (■), 5.0% (○), 7.5% (●), or 10% (△) cetraxate methyl ester, in a final volume of 10 ml. The reactions were done at 30°C with shaking (300 rpm), and the hydrolysis rate was measured periodically.

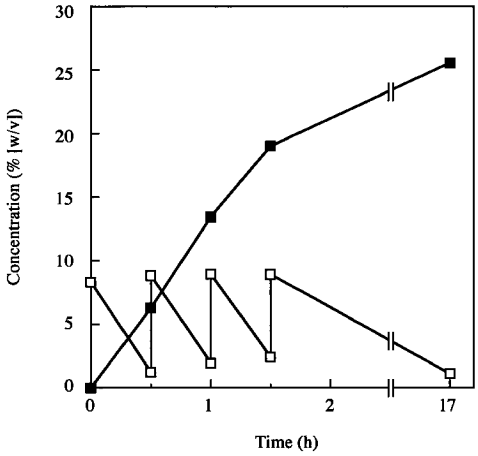

Fig. 2. Regioselective Hydrolysis of the Cetraxate Methyl Ester to Cetraxate with *Microbacterium* sp. Strain 7-1W Cells.

The reaction was done at 30°C with stirring, with the addition of 5.0 g of cetraxate methyl ester at 30-min intervals four times to the reaction mixture, 50 ml, with *Microbacterium* sp. strain 7-1W cells (5.0 g). The pH of the reaction mixture was controlled in the range of 6.5–7.5 with 6 M NaOH. □, cetraxate methyl ester; ■, cetraxate.

Both the cetraxate methyl ester and cetraxate were only slightly soluble in water. So, after reactions with high substrate concentrations, the reaction mixtures were diluted with water, and then the hydrolysis rates were measured by high-pressure liquid chromatography as described previously.[2]

The optimum temperature and pH for the regioselective hydrolysis of the cetraxate methyl ester by *Microbacterium* cells were about 45°C and pH 8.0, respectively. However, long-term reaction at high temperature and at pH 7.0 or above might cause not only inactivation of the enzyme but also spontaneous hydrolysis of the substrate to a by-product, 3-(4-hydroxyphenyl)propionic acid. Further investigation was done at 30°C and pH 7.0.

When 80 mg/ml of the cells (wet weight) was incubated with 1.0, 2.5, or 5.0% (w/v) of the substrate at 30°C, pH 7.0, the hydrolysis reaction proceeded efficiently and the substrate was almost stoichiometrically converted to the desired product, cetraxate (Fig. 1). When the substrate concentration was more than 5.0%, the enzyme activity was inhibited, and the reaction hardly proceeded with 10% cetraxate methyl ester. However, when the substrate was added to the reaction mixture little by little every few minutes, the hydrolysis reaction proceeded even with 10% cetraxate methyl ester, and after a reaction for 50 min, 90% of the initial substrate was converted to cetraxate. The sudden addition of large amount of the substrate probably caused a local drop of pH in the reaction mixture, because of the formation of cetraxate, and this change probably inactivated the enzyme.

On the basis of these observations, preparative-scale regioselective hydrolysis was done as follows: 5.0 g (wet weight) of *Microbacterium* cells was suspended in 50 ml of H_2O, and 5.0 g of cetraxate methyl ester was added slowly (within 2–3 min) to the reaction mixture. The reaction was done at 30°C with stirring. To avoid a pH drop in the reaction mixture because of the formation of cetraxate, the pH was controlled in the range of 6.5–7.5 with 6 M NaOH at appropriate intervals. Furthermore, 5.0 g of cetraxate methyl ester was added, at intervals of 30 min, three times to the reaction mixture. The total amount added was 20 g. After reaction for 17 h, 96% of the cetraxate methyl ester was converted to cetraxate, and little of the by-product 3-(4-hydroxyphenyl)propionic acid was detected (Fig. 2). Therefore, the inhibitory effect with the high concentration of cetraxate methyl ester did not arise from the concentrations of the products, cetraxate and methanol, but from the concentration of the substrate. The *Aspergillus* enzyme also is inhibited by a high (>5%) concentration of the substrate, the benzyl ester of cetraxate, and the inhibition seems to be caused by the accumulation of benzyl alcohol.[4] Probably, if the substrate was added in small portions several times, improvement in the hydrolysis rate could not be expected.

After the product had been collected by filtration and purified by recrystallization from 50% isopropanol, 17.7 g of cetraxate hydrochloride (molar yield, 92.2%) was obtained.

Microbacterium sp. strain 7-1W was useful as a catalyst for the production of cetraxate, and might be used for deprotection reactions in production processes.

Acknowledgments

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