A New Approach for Enzymatic Synthesis of d-3-Chlorolactic Acid from Racemic 2,3-Dichloropropionic Acid by Halo Acid Dehalogenase

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There is an increasing demand for the synthesis of optically active pharmaceutical compounds, because, in some compounds, only one chiral form is effective, and the other form works as a competitive inhibitor, for example, carnitine. Optically active 3-halolactic acid and its derivative, glycidic acid, are useful building blocks for the asymmetric synthesis of pharmaceuticals, such as \( \beta \)-adrenergic blocking agents, leukotrienes, and prostaglandins. 2-Halo acid dehalogenase (EC 3.8.1.2) catalyzes hydrolytic release of halogen from 2-halocarboxylic acids, and produces 2-hydroxy acids with inversion of the configuration. L-2-Halo acid dehalogenase acts on the L-isomer of 2-halocarboxylic acids and produces d-2-hydroxy acids.\(^1\) We thought that the enzyme could act on 2,3-dihalopropionic acids to yield optically active 3-halolactic acids.

The cells of *Pseudomonas putida*, strain 109 (FERM BP-2631) were cultivated by the method of Motosugi et al.\(^1\) L-2-Halo acid dehalogenase was prepared from the *P. putida* as follows; Cells (58.5 g) were suspended in 200 ml of 50 mM potassium phosphate buffer (pH 7.5), and were disrupted by sonication. After the cell debris was removed, the supernatant was brought to 30% saturation of ammonium sulfate. The precipitate was removed by centrifugation, and then ammonium sulfate was added again to the supernatant to 60% saturation of ammonium sulfate. The precipitate was removed by centrifugation, and then ammonium sulfate was added again to the supernatant to 60% saturation. The precipitate was dialyzed against 50 mM potassium phosphate buffer (pH 7.5). The enzyme activity was assayed by the previous method.\(^1\) 3-Chlorolactic acid, 2,3-dibromopropionic acid, and 2,3-dichloropropionic acid were measured using a Waters 600 Multi Solvent Delivery System with a HPX87 column (\( \phi \) 7.8 mm, 30 cm, BioRad, flow rate (0.01 M \( \text{H}_2\text{SO}_4 \)); 0.6 ml/min) and a differential refractometer (Waters R401).

Racemic 2,3-dichloropropionic acid was incubated with the crude preparation of L-2-halo acid dehalogenase from *P. putida*, Strain 109. As shown in Fig. 1, 2,3-dichloropropionic acid was converted to 3-chlorolactic acid. The reaction proceeded until the 2,3-dichloropropionic acid concentration decreased to exactly half of the starting one and 3-chlorolactic acid was formed correspondingly up to half of the initial 2,3-dichloropropionic acid concentration. This indicates that the reaction proceeds enantioselectively, that is, probably, only the L-2,3-dichloropropionic acid in the racemic 2,3-dichloropropionic acid is converted to D-3-chlorolactic acid due to the inherent specificity of the enzyme and only D-2,3-dichloropropionic acid remains unaltered after the reaction.

The initial dehalogenation rate of 2,3-dichloropropionic acid, as well as that of 2,3-dibromopropionic acid, by the enzyme is constant from pH 7 to 9. The enzyme reaction is retarded below pH 6 and 2,3-dihalopropionic acids are decomposed spontaneously over pH 9. The initial reaction rate of 2,3-dichloropropionic acid is about 100 times slower than that of 2,3-dibromopropionic acid, but in the case of 2,3-dibromopropionic acid dehalogenation, the L-2-halo acid dehalogenase was inactivated as the reaction proceeded.

To measure the enantiomeric purity of the product, we synthesized 3-chlorolactic acid on a preparative scale. The reaction mixture (161.5 ml) containing 40 mmol of potassium phosphate buffer (pH 8.0) and 400 units of L-2-dehalogenase was incubated at 30°C. The reaction was started by addition of 38.5 ml of 520 M 2,3-dichloropropionic acid solution (neutralized with NaOH). After 48 hr, the concentration of 2,3-dichloropropionic acid decreased to 50% of the initial concentration, while the product D-3-chlorolactic acid was increased to 47.9 mM. The solution was made acidic (pH 2) with conc. HCl, and the precipitate in it was removed by centrifugation. The D-3-chlorolactic acid and the remaining 2,3-dichloro-
propionic acid were extracted with ethyl acetate from the acidic solution saturated with NaCl. The ethyl acetate solution was evaporated, and the remaining solution was dissolved in 10 ml of water and put on a DEAE-Sephacel column (ø 4.5 cm, 30 cm) previously equilibrated with 0.01 N HCl. The D-3-chlorolactic acid and 2,3-dichloropropionic acid were separated on the column using 0.01 N HCl. The D-3-chlorolactic acid fractions were collected and extracted with ethyl acetate as described above. The ethyl acetate solution was dried with Na₂SO₄ and evaporated to dryness. We obtained 996 mg of D-3-chlorolactic acid (8.00 mmol, total yield 80% against the L-isomer of starting materials), mp 88-89°C, [α]D -3.55 (c=10, H₂O), 1H NMR (acetone-d₆) by Varian VXR 300 (300 MHz): 4.51 (1H, triplet, methylene-CH(O)carbonyl), 3.86 (2H, multi, Cl-CH₂-methine), IR (KBr) cm⁻¹: 3450 (OH), 1730 (O-C=O) Found: C, 28.77; H, 3.94; Cl, 28.49. Calcd. for C₃H₅ClO₃: C, 28.94; H, 4.05; Cl, 28.47 %

The measurement of the enantiomeric purity of the D-3-chlorolactic acid produced by the reaction was done by the method of Mosher et al., using 1H NMR. The D-3-chlorolactic acid was converted first into its methyl ester, and then to the (R)-(+) α-methoxy-α-(trifluoromethyl)phenylacetyl derivatives. The 1H NMR spectrum of the (R)-(+) α-methoxy-α-(trifluoromethyl)phenylacetyl derivative of the produced D-3-chlorolactic acid was compared with that of the racemic one. The product had no L-form contamination.

This experiment demonstrates that L-2-halo acid dehalogenase from P. putida produces optically active 3-chlorolactic acid. The initial velocity for 2,3-di-bromopropionic acid is faster than that of 2,3-di-chloropropionic acid, but 2,3-dichloropropionic acid is suitable as a substrate for practical synthesis, because the enzyme was inactivated when 2,3-dibromopropionic acid was used.

The optically active 3-chlorolactic acid can readily be converted into glycidic acid, which is also a useful building block as an epoxide for the synthesis of chiral compounds, because an epoxide can be cleaved regioselectively and stereoselectively.

The enzymatic reaction is very simple, and requires only dehalogenase as a catalyst, but no expensive cofactor, while the optically active 3-chlorolactic acid synthesis with lactate dehydrogenase as a catalyst needs an expensive cofactor, NADH, and organic synthesis of D-3-chlorolactic acid from d-serine methylester hydrochloride needs very intricate procedures. It is not necessary to purify the enzyme to homogeneity. Crude enzyme from an ammonium sulfate fractionation was sufficient for the reaction. The synthesis of optically active 3-chlorolactic acid using a microorganism has the same advantages as ours, but the yield of the reaction by dehalogenase (total yield containing both reaction and the purification steps, 40%) is higher than that of the reaction by microorganism (reaction 6-25%). The simplicity of the enzyme reaction indicates advantages in the practical production. Further, the high enantiomeric purity of the product is appropriate for pharmaceutical intermediates. Another attractive point of this enzymatic system in the practical application is that starting material, 2,3-dichloropropionic acid (Fig. 2(1)) can be synthesized from quite cheap material, acrylic acid (Fig. 2(2)).

Although we have here described the synthesis of D-3-chlorolactic acid, there is a possibility of the L-form synthesis by using DL-2-halo acid dehalogenase. The enzyme is known to act on both L- and D-2-halo acid with inversion of the configuration. As shown in Fig. 2, D-2,3-dichloropropionic acid (Fig. 2(4)) and D-3-chlorolactic acid (Fig. 2(3)) are synthesized by l-2-halo acid dehalogenase, because the enzyme reaction proceeds until l-2,3-dichloropropionic acid is consumed also completely, which is shown in Fig. 1. The D-2,3-dichloropropionic acid is converted into l-3-chlorolactic acid by the DL-2-halo acid dehalogenase in the next reaction.

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