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Novel and Selective Enzymatic Hydrolysis of γ-Ester of Dimethyl α-Dehydroglutamate with α-Chymotrypsin A

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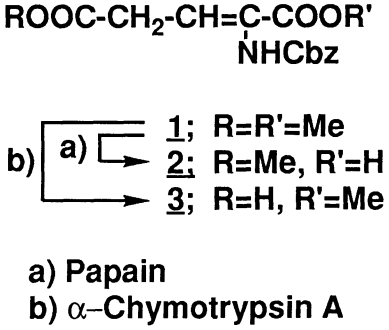
Selective enzymatic hydrolysis of γ-ester of Cbz-ΔGlu(OMe)-OMe with α-chymotrypsin at pH 9 was readily achieved to give the corresponding α-half ester (Cbz-ΔGlu-OMe) along with a small amount of Cbz-ΔGlu(OMe)-OH and Cbz-ΔGlu-OH.

Recently, we have reported on the interesting studies that the selective enzymatic hydrolysis of α-ester of N-benzyloxycarbonyl-α-dehydroglutamic acid dimethyl ester |Cbz-ΔGlu(OMe)-OMe; 1|[1] and facile coupling of 1 with leucine anilide by the catalytic action of thiol protease, such as papain, at pH 8 were accomplished to give Cbz-ΔGlu(OMe)-OH (2) and Cbz-ΔGlu(OMe)-LeuNHPh, respectively.[2,3] These two reactions of uncommon α-amino acid ester, in particular, such as α-dehydroamino acid, are thought to be first examples for the organic synthesis. In spite of the significance as the important starting materials to the syntheses of the wide variety of glutamate related to excitatory amino acids,[4] the selective synthesis of either 2 or Cbz-ΔGlu-OMe (3) alone by the chemical method has been found to be very difficult and so far unsuccessful.[1,2,5] However, surprisingly, by the use of an appropriate proteolytic enzyme, it was found that the regioselective hydrolysis of 1 took place smoothly to give the expected 2 and 3 in good yields, respectively. Here, we wish to report the selective synthesis of 3 by using α-chymotrypsin A |EC 3.4.22.2|, a kind of serine protease (Scheme 1).



Scheme 1.

According to the method reported previously concerning the treatment of 1 with papain,[2] a solution of 1 (1 mmol) and α-chymotrypsin A (46 units/mg, 150 mg) in McIlvaine buffer (55 ml) in the presence of $CaCl_2$ (110 mg) was stirred at pH from 4 to 9 and at 35 °C. To the every reaction mixture was added 10% citric acid (45 ml) and the resulting suspension was extracted

three times with ethyl acetate. The organic layer was washed with saturated NaCl aqueous solution and then dried. After concentrating in vacuo, the residual crystals were analyzed by HPLC (MeOH : H_2O = 50 : 50 v/v with 5% CF_3COOH), and the yields of products and their structures were determined by the HPLC peaks and by comparing with those of the authentic compounds,[1,2,5] respectively. As shown in Fig. 1(a), in the pH range of 4.0-9.0, the yield of 3 steeply increased at about pH 6 and reached 80% at pH 9 along with very small amount of Cbz-ΔGlu-OH (4)[1] and 2. Furthermore, Fig. 1(b) indicates that the hydrolysis is comparatively slow, being completed within 8 h under these conditions (pH 8). In order to obtain enough amount of pure 3, the large scale of 1 at pH 9 for 8 h was also worked up similarly to give 3 |mp 96-98 °C. IR (KBr): 3285 (NH), 1734 (COOMe), 1692 (COOH) cm^{-1}. ^1H NMR ($CDCl_3$):δ 6.71 (t, 1H, J=7.0Hz, -CH=), 3.31 (d, 2H, J=7.0Hz, $-CH_2-CH=$), 6.92 (br s, 1H, NH)|.

From the above result and the fact that the similar enzymatic hydrolysis of (L)-Cbz-Glu(OMe)-OMe (5) proceeded selectively to only (L)-Cbz-Glu(OMe)-OH almost quantitatively, the hydrolysis of α-ester of 1, depending on the peculiar structure of the substrate, may be related to the variation of the binding and active sites of α-chymotrypsin A.

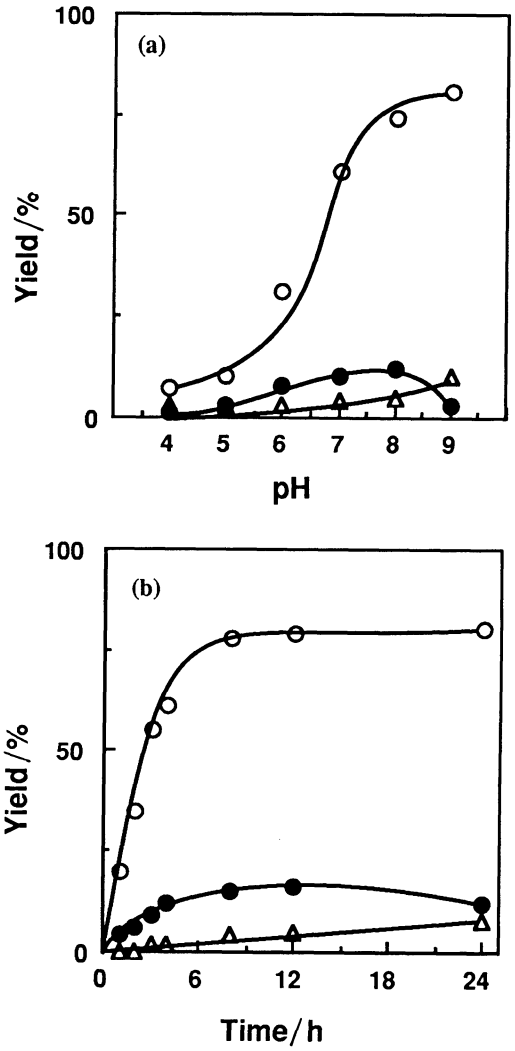


Fig. 1. Optimal conditions for hydrolysis of 1 with α-chymotrypsin A. (a) Effect of pH. The reaction mixture was incubated at 35°C for 24 h. (b) Effect of reaction time (pH 8). In (a) and (b): o, Cbz-ΔGlu-OMe (3); •, Cbz-ΔGlu(OMe)-OH (2); Δ, Cbz-ΔGlu-OH (4).

In conclusion, very interestingly, it can be seen that α-chymotrypsin catalyzed the ester hydrolysis of the quite different position of 1 and 5, whereas papain hydrolyzed the only α-esters of both 1 and 5.

References

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4) For example, H. Shinozaki, Gendai Kagaku, 199, 38 (1987).
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