CHEMISTRY LETTERS, pp. 2001-2002, 1988.

Very Selective Enzymatic Hydrolysis of α-Ester of Dimethyl α-Dehydroglutamate with Papain

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It was found that the selective ester hydrolysis of N-benzyloxycarbonyl-α-dehydroglutamic acid dimethyl ester [Cbz-ΔGlu(OMe)-OMe] with papain at pH 8.0 was achieved readily to give only a Cbz-ΔGlu(OMe)-OH almost quantitatively.

Recently, we have reported briefly on the synthesis of methyl γ-methyl-α-(N-benzyloxycarbonyl)-α-dehydroglutamate [Cbz-ΔGlu(OMe)-OMe] (1), derived by the condensation of methyl γ-methyl-α-oxoglutarate with benzyl carbamate, and the ester hydrolysis of 1 with LiOH under several reaction conditions.[1] However, at present, the direct and selective synthesis of Cbz-ΔGlu(OMe)-OH (2) from 1 is unsuccessful, consequently, the half ester (2) has to be derived by the hydrolysis of α,γ-dimethyl esters of 1 with LiOH, followed by the half-esterification with MeOH. Since the compound 2 is very important substrate for the synthesis of various kinds of N-carboxy-α-dehydroglutamic acid anhydrides and dehydroglutamyl-peptides, it is preferable to be accessible from 1 as direct as possible. Here, surprisingly, despite the uncommon α-amino acid, the enzymatic hydrolysis of 1 as an α-dehydroamino acid (DHA) ester with protease such as papain first took place to give 2 almost quantitatively, whereas the direct conversion of 1 to 2 with any base or acid was so far unsuccessful at all.

First of all, a solution of 1 (0.1 mmol) and papain (2.3 unit/mg) in McIlvaine buffer (5 ml) at pH 6.0 in the presence of 2-mercaptoethanol (0.1 ml) was incubated at 35 °C for 24 h. The reaction solution was washed with a mixture of saturated $NaHCO_3$ aqueous solution and ethyl acetate. The aqueous layer was made acidic to pH 2.0 with concentrated HCl and then extracted with ethyl acetate. The organic layer was washed with saturated NaCl aqueous solution and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residual crystals were recrystallized from benzene. Consequently, the expected hydrolysis was accomplished, according to Scheme 1, to give 2 as colorless needles,

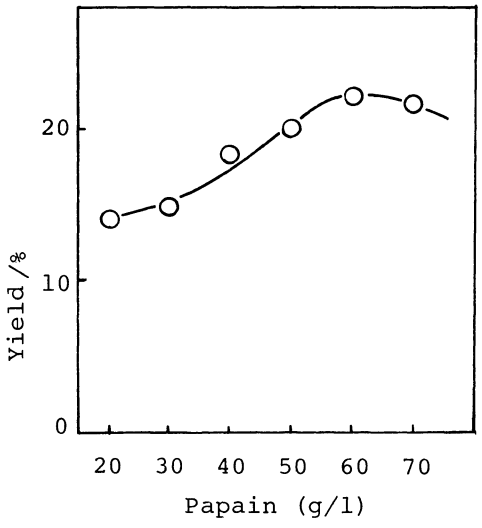
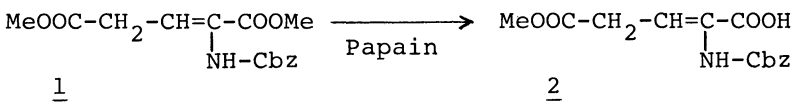


Fig. 1. Effect of enzyme concentration. Incubated in McIlvaine buffer (0.2 mol/l) at pH 6.0 and 35 °C for 24 h.



1 2

Scheme 1.

which were in complete accord with the authentic sample.[1] As Fig. 1 shows, although the yield of 2 increased gradually to reach ultimately 23% with increasing the enzyme concentration, the yield is still very low. From the result, if the amount of 1 is increased, it can be seen that the similar hydrolysis must be carried out in a fairly large amount of solution. Accordingly, this procedure was concluded to be undesirable for the mass production of 2.

On the other hand, generally, although the optimal pH of papain esterase action is well-known to be about 7.0, the relationship between the yield and the dependence of pH in the similar reaction was thoroughly examined. As a result, since it was found that the yield of 2 was extremely dependent upon the pH of McIlvaine buffer, the basic side of pH was variously altered. As shown in Fig. 2, in the pH range of 6.0-8.0, the yield of 2 increased steeply and reached almost 100% at pH around 8.0.

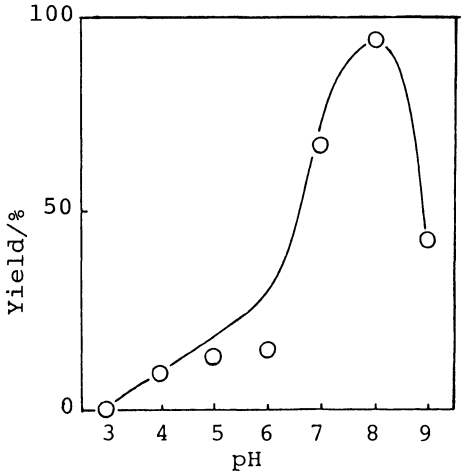


Fig. 2. Effect of pH. Incubated in McIlvaine buffer (0.2 mol/l) at 35 °C for 24 h.

Furthermore, in the case of Cbz-ΔGlu-OMe[1] too, the similar hydrolysis was worked up at pH 7.0 to give Cbz-ΔGlu-OH in a fairly good yield. Very interestingly, except for the cases of ΔGlu-OMe derivatives in high and those of ΔGln-, ΔOrn- and ΔPhe-OMe in low yields, the similar hydrolysis of the other DHA methyl esters,[2-4] for examples, ΔLeu-, ΔAsp-, ΔTrp-, ΔTyr-OMe, and so on, did not occur. In addition, it is interesting that only α-methyl ester of 1 is preferentially hydrolyzed with papain, whereas γ- or both α- and γ-methyl esters are chemically hydrolyzed by the action of LiOH.

In conclusion, it is worth noting that the substrate specific hydrolysis of ΔGlu-OMe derivatives as well as Cbz-Glu(OMe)-OMe with proteolytic enzyme became apparent. This fact indicates that the reverse reaction, i. e., peptide-bond formation can be also catalyzed with papain. In fact, the enzymatic syntheses of various dehydroglutamyldipeptides were successful. These results will be reported in detail elsewhere.

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(Received September 16, 1988)