CHEMISTRY LETTERS, pp. 747-748, 1989.

Enzymatic Peptide Synthesis by the Coupling of (Z)-α-Dehydroglutamate with L-α-Amino Acids Using Papain

Chung-gi SHIN* and Nobuyuki TAKAHASHI
Laboratory of Organic Chemistry, Faculty of Technology, Kanagawa University,
Kanagawa-ku, Yokohama 221

Enzymatic coupling of N-protected α,γ-dimethyl α-dehydroglutamate with L-α-leucine amide using papain at pH 8.0 was first achieved to give α-dehydroglutamylleucine derivatives.

In the preceding paper,[1)] we have reported the very selective and quantitative α-ester hydrolysis of α,γ-dimethyl N-benzyloxycarbonyl-(Z)-α-dehydroglutamate (Cbz-ΔGlu(OMe)-OMe) (1)[2)] using papain at pH 8.0 and 35 °C for 24 h. Moreover, in the report, it has been suggested that the reverse reaction of the enzymatic hydrolysis of 1, i. e., the peptide bond formation of 1 with L-α-amino acids (AA) can be also catalyzed with papain. In fact, here, very surprisingly, as a first example of the uncommon α-amino acid such as α-dehydroamino acid, the expected coupling of Cbz-ΔGlu(OMe)-OY (1; Y=H and Me) with L-leucine (Leu) amide or ester by the enzymatic peptide synthetic method was achieved. So far, since the syntheses of a number of peptides from various kinds of AA and small peptide as the carboxy (C)- and amino (N)-components by using an appropriate protease were reported[3,4)] and utilized already for the commercial production, similarly, for the C-component 1 three kinds of H-Leu-R (2: a; R=NHPh, b; R=NHNHPh, c; R=OCHPh$_2$) as the N-component and thiol protease papain were chosen (Scheme 1).

A solution of various mmoles of 2a, to 1 mmoles of 1 (Y=Me), and papain (150 mg, 2.3 unit/mg) in McIlvaine buffer (10 ml) was incubated with shaking at pH 8.0 and 35 °C for 24 h in the presence of 2-mercaptoethanol (0.1 ml). To the reaction mixture was added 10% citric acid (20 ml) and the resulting solution was then extracted with ethyl acetate. The organic layer was washed successively with $NaHCO_3$ and saturated NaCl aqueous solution and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residual syrup was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (20 : 1 v/v) as the eluent to give Cbz-ΔGlu(OMe)-Leu-NHPh (3a)[5)] as colorless powders. Although, in the case using an equimolar 1 (1.0 mmol) and N-component (2), the

$$\begin{array}{lclcl}
\textbf{Cbz-}\Delta\textbf{Glu(OMe)-OY} & + & \textbf{H-Leu-R} & \xrightarrow{\textbf{papain}} & \textbf{Cbz-}\Delta\textbf{Glu(OMe)-Leu-R} \\
\underline{\textbf{1}}\ \textbf{(Y=H and Me)} & & \underline{\textbf{2}} & & \underline{\textbf{3a}}\textbf{; 77\%} \\
 & & & & \underline{\textbf{3b}}\textbf{; 13\%} \\
 & & \underline{\textbf{a}}\textbf{; R=NHPh,}\ \ \underline{\textbf{b}}\textbf{; R=NHNHPh,}\ \ \underline{\textbf{c}}\textbf{; R=OCHPh}_2 & & \underline{\textbf{3c}}\textbf{; 10\%}
\end{array}$$

Scheme 1.

yield of 3a was low to reach only 35%, upon increasing the amount of the N-component from 1 to 6 mmoles under similar conditions, the yield was found to increase exceedingly to reach 77% at component ratio (N/C) 5.0, as shown in Fig. 1. On the other hand, regarding the yield of 3a versus the pH, the yield of 3a was also found to be the highest at pH 8.0, as shown in Fig. 2.

Similarly, the coupling of 1 with 2b gave Cbz-ΔGlu(OMe)-Leu-NHNHPh (3b)$^{5)}$ even in low yield, but the attempt to obtain Cbz-ΔGlu(OMe)-Leu-OCHPh$_2$ (3c) from 1 and 2c was unsuccessful at all. However, when the pH of the buffer was adjusted to 6.0, the enzymatic reaction took place ultimately to give the expected 3c.$^{5)}$

On the other hand, in the case of the coupling of an equimolar 1 (Y=H) with 2a at pH 8.0, dipeptide 3a was scarcely obtained, but the similar treatment with 5 mmole of 2a was carried out at pH 5.0 to give 3a in 23% yield.

In conclusion, since the other enzymatic syntheses of various dehydroglutamyldipeptides from 1 and AA amide, except Leu, have been also successful, it is highly expected that the excellent results obtained here will be able to contribute to the field of the chemistry of uncommon amino acid and its peptide.

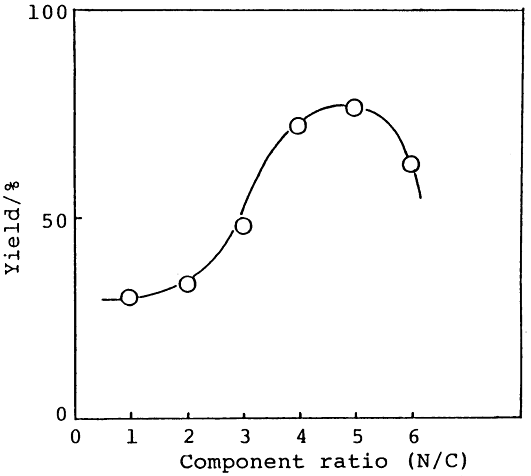

Fig. 1. The yield of 3a vs. the component ratio (pH 8.0, 35 °C, 24 h).

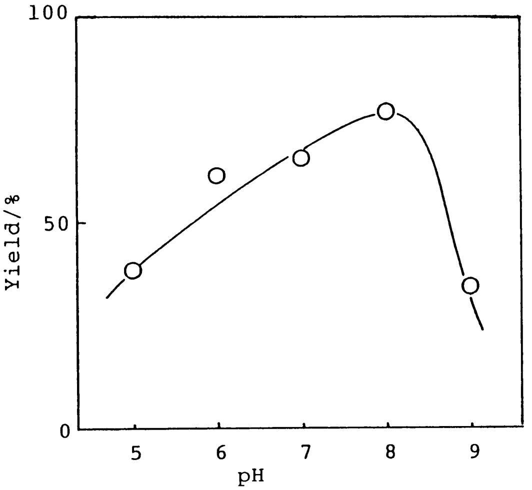

Fig. 2. The yield of 3a vs. pH (N/C=5.0, 35 °C, 24 h).

References

1) C. Shin, N. Takahashi, and Y. Yonezawa, Chem. Lett., 1988, 2001.

2) C. Shin, Y. Yonezawa, and E. Watanabe, Tetrahedron Lett., 26, 85 (1985).

3) Y. Isowa, M. Ohmori, M. Sato, and K. Mori, Bull. Chem. Soc. Jpn., 50, 2766 (1977).

4) K. Morihara and T. Oka, Biochem. J., 163, 531 (1977).

5) 3a: Mp 77.5-79.0 °C. IR (KBr): 1675, 1535 (CONH), 1640 (C=C) cm^{-1}. ^{1}H NMR (CDCl: δ 6.97 (1H, d, J=7.0 Hz, -CONH-), 6.32 (1H, t, J=7.0 Hz, -CH=), 4.66 (1H, m, -NH-CH-CO-). $|\alpha|^{20}_{D}$ -26.5° (c 1.0 in MeOH). 3b: Mp 63-64 °C. IR: 1680, 1525 (CONH), 1660 (C=C) cm^{-1}. ^{1}H NMR: δ 6.80 (1H, d, J=7.0 Hz, -CONH-), 6.34 (1H, t, J=7.0 Hz, -CH=), 4.58 (1H, m, -NH-CH-CO-). $|\alpha|^{20}_{D}$ -22.5° (c 1.0 in MeOH). 3c: Mp 92-93 °C. IR: 1680, 1515 (CONH), 1645 (C=C) cm^{-1}. ^{1}H NMR: δ 6.48 (1H, d, J=7.0 Hz, -NHCO-), 6.31 (1H, t, J=7.0 Hz, -CH=), 4.71 (1H, m, -NH-CH-CO-). $|\alpha|^{20}_{D}$ -7.2° (c 1.0 in MeOH).

(Received February 7, 1989)