HIGHLY SELECTIVE CLEAVAGE OF PYROGLUTAMYL-PEPTIDE BOND IN CONCENTRATED HYDROCHLORIC ACID

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Highly selective cleavage reaction of pGlu-peptide bond is described. Two model peptides, pGlu-X-Ala-Phe-OH (X = Ile and Ser), and dog neuromedin U-8 (d-NMU-8) (1-7)-OH (pGlu-Phe-Leu-Phe-Arg-Pro-Arg-OH) were hydrolyzed in concentrated HCl at 0 °C for from 6 days to 6 weeks to give the predominant cleavage products of pGlu-X linkage of pGlu-X-Ala-Phe-OH and the pGlu-Phe linkage of d-NMU-8 (1-7)-OH, respectively. The ring-opening reaction of the pyrrolidone moiety of the pGlu residue occurred to a considerably lesser extent.

KEY WORDS pyroglutamyl-peptide; pyroglutamyl-peptide bond; cleavage reaction; hydrolysis; hydrochloric acid

We revealed that pGlu-peptide is highly sensitive to dilute HCl under mild conditions,[1] generating not only the ring-opened product[2] of the pyrrolidone moiety of the pGlu residue, but also the cleavage product of the pGlu-peptide linkage. The rate of hydrolysis was affected by the reaction temperature, and the ring-opening reaction was greatly diminished at 4 °C compared with the cleavage reaction. This finding led to the highly selective cleavage of the pGlu-peptide linkage in conc. HCl. Here, we describe a new deblocking method of pGlu-peptide.

To develop a simple means of deblocking the pGlu-peptide prior to Edman degradation, we examined the susceptibility of the pGlu-peptide bond and the stability of the pyrrolidone moiety of the pGlu residue and internal peptide bonds to several concentrations of HCl at 0 °C. Concentrated HCl was the optimal concentration at which to selectively cleave the pGlu-peptide linkage. Two model peptides, pGlu-X-Ala-Phe-OH[3] (X = Ile and Ser), and dog neuromedin U-8[4] (d-NMU-8) (1-7)-OH (pGlu-Phe-Leu-Phe-Arg-Pro-Arg-OH[5]) were hydrolyzed in conc. HCl at 0 °C for 6 days-6 weeks. To analyze the acid hydrolysates, peptides were dissolved at a concentration of 10^{-3} mol/l in conc. HCl in polypropylene tubes under ice-cooling and divided into seven portions of 100 μl in polypropylene tubes with tight caps. These were maintained at 0 °C in a thermostatically controlled apparatus. Tubes were removed at 0 h or after various periods, and stored at -40 °C. An aliquot (15 μl) of this solution was examined by means of RP-HPLC to determine the amount of the starting material that remained and the amounts of the hydrolysates. The peak areas of the starting material and the hydrolysates were compared with those of standard samples. To identify the hydrolysates, each peak was collected, analyzed for amino acid composition, identified and confirmed by coelution with an authentic sample on the HPLC. RP-HPLC proceeded under the following conditions: columns, a

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YMC-ODS-5-AM (4.6 x 150 mm); elution, 8-19% MeCN in 0.1% TFA; flow rate, 1 ml/min; detection, 210 nm. The acid hydrolysates of each peptide were examined by means of HPLC in quadruplicate. The average values varied by ± 3.7%.

When a solution of pGlu-Ile-Ala-Phe-OH (10^{-3} mol/l in conc. HCl) was left at 0 °C for 6 weeks, decomposition resulted in the formation of major and minor hydrolysates (Table 1). The major product was H-Ile-Ala-Phe-OH (70.1%) by cleavage reaction, and the minor was H-Glu-Ile-Ala-Phe-OH (3.7%) by the ring-opened reaction of the pyrrolidone moiety of pGlu. The results indicated that the pGlu-Ile linkage was highly labile to conc. HCl hydrolysis, whereas the pyrrolidone moiety of pGlu residue was highly resistant under these conditions. It is well known that Ile containing peptide is resistant to acid hydrolysis due to the steric hindrance of the side chain. In fact, in 1 N HCl at 60 °C, the analog gave the ring-opened product, H-Glu-Ile-Ala-Phe-OH (43.8%), and the cleavage product, H-Ile-Ala-Phe-OH (18.1%), after a 6-h incubation, as reported previously.[3] The yield ratio of ring-opening reaction to cleavage of various pGlu-X-peptides was the highest when X was Ile. In contrast to the previous results, the cleavage reaction of pGlu-X bond proceeded almost exclusively in conc. HCl even when X was Ile without significant hydrolysis of pyrrolidone ring or internal peptide bonds.

The selectivity of the cleavage of pGlu-X bond was further emphasized when X was Ser. The Ser analog generated a higher yield ratio of the cleavage product, H-Ser-Ala-Phe-OH (91.6%) to the ring-opened product, H-Glu-Ser-Ala-Phe-OH (0.7%) (Table 1) at 6 d. The acid hydrolysis[3] of pGlu-Ser-Ala-Phe-OH in 1 N HCl at 60 °C for 6 h produced the major hydrolysates, H-Ser-Ala-Phe-OH (39.9%) and H-Glu-Ser-Ala-Phe-OH (24.4%). The cleavage predominated over the ring-opening reaction (molar ratio of about 1.6:1). On the acid hydrolysis in 1 N HCl at 6 °C for 12 weeks, the hydrolysates contained H-Ser-Ala-Phe-OH (37.5%) and H-Glu-Ser-Ala-Phe-OH (16.8%). The results indicated that the acid hydrolysis in conc. HCl at 0 °C favored the cleavage reaction. The acid hydrolysis of pGlu-X-Ala-Phe-OH (X = Ala, Glu, Arg and Tyr) under the same conditions gave a

Table 1. pGlu-X-Ala-Phe-OH and its Hydrolysates during an Incubation in 36% HCl at 0 °C

		Time *						
Peptide	X	0	1	2	3	4	5	6
pGlu-X-Ala-Phe-OH	Ile	100	79.0	59.5	47.4	42.9	32.2	25.7
	Ser	100	55.8	42.7	31.7	24.1	17.7	15.2
H-X-Ala-Phe-OH	Ile	0	21.0	32.6	42.4	56.7	64.2	70.1
	Ser	0	44.8	59.1	65.8	73.1	75.7	90.6
H-Glu-X-Ala-Phe-OH	Ile	0	1.2	1.8	2.2	3.2	3.3	3.7
	Ser	0	0.3	0.0	0.0	0.0	0.0	0.4

HPLC conditions: column, YMC ODS-5-AM (4.6 x 150 mm); elution, 8-18% MeCN in 0.1% TFA; flow rate, 1 ml/min; detection, 210 nm. The values for Ile and Ser analogs have an accuracy of about ±3.7 and 2.1%, respectively. * Ile analog, weeks; Ser analog, days.

highly selective cleavage product (data not shown).

Next, the degradation of a bioactive peptide fragment, d-NMU-8 (1-7)-OH, and the accumulation of its main hydrolysates in conc. HCl were investigated at 0 °C. Within 12 d, 63.4% of the starting material was hydrolyzed (Fig. 1). The main product was NMU-8 (2-7)-OH[1)] (H-Phe-Leu-Phe-Arg-Pro-Arg-OH) (52.3%), and the yield of the ring-opened product, [Glu[1]]-NMU-8 (1-7)-OH[1)] (H-Glu-Phe-Leu-Phe-Arg-Pro-Arg-OH), was only 1.7%. There was no significant cleavage at the internal peptide bonds in d-NMU-8 (1-7)-OH.

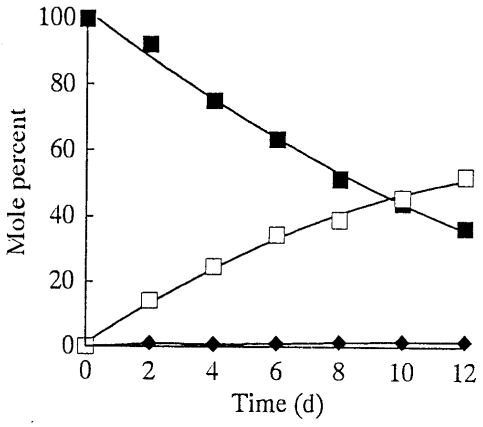

Fig. 1. Time Courses for d-NMU-8 (1-7)-OH and its Hydrolysates during an Incubation in conc. HCl at 0 °C for 12 d.
(■), d-NMU-8 (1-7)-OH; (□), NMU-8 (2-7)-OH; (◆), [Glu[1]]-NMU-8 (1-7)-OH.

Our study[1)] revealed that the hydrolysis of d-NMU-8 (1-7)-OH in 1 N HCl at 60 °C for 6 h and in 1 N HCl at 4 °C for 10 weeks gave [Glu[1]]-NMU-8 (1-7)-OH (42.0%) and NMU-8 (2-7)-OH (16.2%), and [Glu[1]]-NMU-8 (1-7)-OH (23.6%) and NMU-8 (2-7)-OH (19.2%) as main products, respectively. The ring-opened product, [Glu[1]]-NMU-8 (1-7)-OH, predominated slightly over the cleavage product, NMU-8 (2-7)-OH, on a mole percent basis even at 4 °C. The results indicated that the acid hydrolysis of the longer peptide fragment in conc. HCl at low temperature was superior to that in 1 N HCl at 4 °C for the highly selective cleavage of the pGlu-peptide bond.

This study revealed that the pGlu-peptide linkage is highly susceptible to conc. HCl at 0 °C compared with other peptide bonds and the pyrrolidone moiety of the pGlu residue. The results will contribute to the development of a simple means of deblocking pGlu-peptides prior to Edman degradation.

REFERENCES

1) Hashimoto T., Ohki K., Sakura N., *Chem. Pharm. Bull.*, **43**, 2068-2074 (1995).
2) Blombäck B., "*Methods in Enzymology*," ed. by Hirs C.H.W., Academic Press, New York, Vol 11, 1967, p. 398-411.
3) Saito S., Ohki K., Sakura N., Hashimoto T., *Biol. Pharm. Bull.*, in press.
4) O'Harte F., Bockman C.S., Abel P.W., Conlon J.M., *Peptides*, **12**, 11-15 (1991).
5) Sakura N., Kurosawa K., Hashimoto T., *Chem. Pharm. Bull.*, **43**, 1148-1153 (1995).

(Received February 9, 1996; accepted March 1, 1996)