

Hydrolytic Cleavage of Pyroglutamyl-peptide Bond. V. Selective Removal of Pyroglutamic Acid from Biologically Active Pyroglutamylpeptides in High Concentrations of Aqueous Methanesulfonic Acid

Junko KOBAYASHI, Kazuhiro OHKI, Keiko OKIMURA, Tadashi HASHIMOTO, and Naoki SAKURA*

Faculty of Pharmaceutical Sciences, Hokuriku University; Kanagawa-machi, Kanazawa 920-1181, Japan.

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Application of aqueous methanesulfonic acid (MSA) for selective chemical removal of pyroglutamic acid (pGlu) residue from five biologically active pyroglutamyl-peptides (pGlu-X-peptides, X=amino acid residue at position 2) was examined. Gonadotropin releasing hormone (Gn-RH), dog neuromedin U-8 (d-NMU-8), physalaemin (PH), a bradykinin potentiating peptide (BPP-5a) and neurotensin (NT) as pGlu-X-peptides were incubated in either 70% or 90% aqueous MSA at 25 °C. HPLC analysis of the incubation solutions showed that the main decomposition product was H-X-peptide derived from each pGlu-X-peptide by the removal of pGlu. The results revealed that the pGlu-X peptide bond had higher susceptibility than various internal amide bonds in the five peptides examined, including the Trp-Ser bond in Gn-RH, the C-terminal Asn-NH₂ in d-NMU-8, and the Asp-Pro bond in PH, whose acid susceptibility is well known. Thus, mild hydrolysis with high concentrations of aqueous MSA may be applicable to chemically selective removal of pGlu from pGlu-X-peptides for structural examinations.

Key words pyroglutamylpeptide; chemical cleavage; methanesulfonic acid; selectivity; hydrolysis

Biologically active peptides bearing pyroglutamic acid residue (pGlu) at the N-terminal are widely known. The removal of pGlu residue from a pGlu-X-peptide (X, amino acid residue at position 2) is required for the primary structure determination by Edman degradation. Our previous studies indicated that pGlu-X peptide bond is highly sensitive to mild acidic conditions, generating not only the ring opened product (H-Glu-X-peptide) at the pyrrolidone moiety of pGlu, but also the cleavage product (H-X-peptide) at pGlu-X linkage.^{1,2)} High selectivity to remove pGlu-OH from synthetic model pGlu-X-peptides was attained in concentrated hydrochloric acid³⁾ and in 70% aqueous methanesulfonic acid (MSA).^{4,5)} The aim of this study was to compare the selectivity of the cleavage reaction at pGlu-X bond in aqueous MSA with various internal peptide bonds in five biologically active pGlu-X-peptides, containing well-known acid sensitive amide bonds. The sequence of these peptides, namely, gonadotropin-releasing hormone (Gn-RH; **1**),⁶⁾ dog neuromedin U-8 (d-NMU-8; **2**),⁷⁾ physalaemin (PH; **3**),⁸⁾ a bradykinin potentiating peptide (BPP-5a; **4**),⁹⁾ and neurotensin (NT; **5**),¹⁰⁾ are shown in Fig. 1.

Experimental

General and Apparatus HPLC analysis was performed on a module consisted of a 7125 injector (Rheodyne Inc., U.S.A.), a 616 pump, a 600s controller, a 486 tunable absorbance detector, a 717 plus autosampler, and an SDM solvent degas module (all from Waters Corp., Milford, MA, U.S.A.).

Amino acid analysis was conducted on a 7300 Model amino acid analyzer system (Beckman Instruments Ltd., Fullerton, CA, U.S.A.). The hydrolysis of a synthetic peptide was performed by 6M HCl vapor at 130 °C for 3 h. Fast-atom bombardment mass spectra (FAB-MS) were obtained on a JMS-DX300 mass spectrometer (JEOL Ltd., Tokyo, Japan). Optical rotations of the peptides were measured with a DIP-370 digital polarimeter (Nippon Bunko Co., Ltd., Tokyo, Japan). HP-TLC analysis was carried out on pre-coated silica gel plates (Kieselgel 60; Merck, Darmstadt, Germany).

Peptides Syntheses of Gn-RH (**1**), PH (**3**), BPP-5a (**4**) and NT (**5**) (Fig. 1) and various fragment peptides related to these biologically active peptides (Figs. 2, 5, 6, 8, 9) were carried out according to a method previously reported for d-NMU-8 (**2**).¹¹⁾ Protected peptide resins were constructed by Boc strategy either on a benzhydrylamine resin or a chloromethylated polystyrene resin (Peptide Institute Inc., Osaka, Japan) using a peptide synthesizer (ABI 433A; Applied Biosystems, Foster City, CA, U.S.A.). Side chain protected Boc-amino acid derivatives (Watanabe Chemical Industries Ltd., Hiroshima, Japan) were Tyr(BrZ), Arg(Tos), Asp(OcHex), Lys(Cl-Z), Ser(Bzl), His(Bom) and Glu(OBzl). The protected peptide resins were treated with anhydrous liquid hydrogen fluoride (HF) containing 10% anisole under ice cooling for 45 min in a Teflon HF apparatus (Peptide Institute Inc., Osaka, Japan). After evaporation of HF *in vacuo*, the residual peptides were purified by HPLC, using a column of YMC-pack ODS-AM S-5 120 Å (20×150 mm) with 0.1% TFA-acetonitrile (MeCN) solvent system. Finally, the peptides were gel-filtered on a column of Toyopearl HW-40 (super fine) (1.5×47 cm, Tosoh Co., Tokyo, Japan) with 25% MeCN in 5×10⁻³ M hydrochloric acid as an eluent; and lyophilized. The amino acid analysis data of various synthetic peptides are shown in Table 1, and their FAB-MS analysis data and characteristics are in Table 2.

Cleavage Reaction of Peptides in Aqueous MSA Solutions of the biologically active peptides were prepared at a concentration of 10⁻³ M in either 70% or 90% methanesulfonic acid (MSA) under ice cooling. To examine the

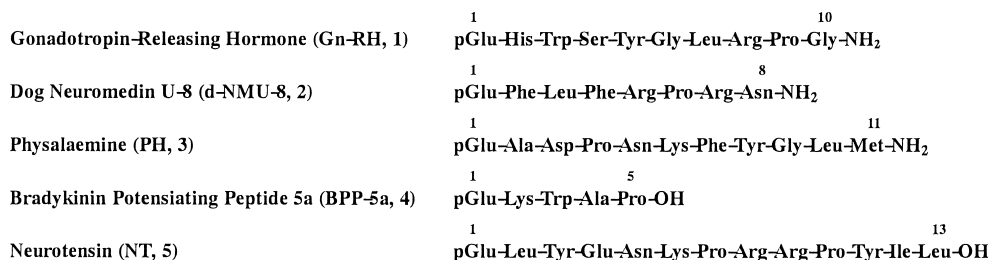


Fig. 1. Biologically Active Pyroglutamyl-peptides (pGlu-X-Peptides)

* To whom correspondence should be addressed. e-mail: n-sakura@hokuriku-u.ac.jp

Table 1. Amino Acid Analysis^{a)} of the Synthetic Peptides and Their Acid Hydrolysates

Peptides ^{b)}	Asp	Glu	Pro	Gly	Ala	Leu	Tyr	Phe	Arg	Other amino acids ^{c)}	NH ₃	Recovery (%)
Gn-RH (2—10)-NH ₂ (1a)	—	—	0.99 (1)	2.02 (2)	—	1.02 (1)	1.03 (1)	—	1.03 (1)	S: 0.93 (1)	1.24 (1)	86.0
[Glu ¹]-Gn-RH (1b)	—	1.09 (1)	0.98 (1)	2.01 (2)	—	1.01 (1)	1.02 (1)	—	0.99 (1)	S: 0.91 (1)	1.14 (1)	85.1
Gn-RH-OH (1c)	—	1.09 (1)	0.99 (1)	1.97 (2)	—	1.01 (1)	1.02 (1)	—	1.02 (1)	S: 0.92 (1)	0.58 (0)	86.6
Gn-RH (1—3)-OH (1d)	—	1.05 (1)	—	—	—	—	—	—	—	H: 0.95 (1)	0.19 (0)	94.4
Gn-RH (4—10)-NH ₂ (1e)	—	—	0.76 (1)	2.31 (2)	—	0.85 (1)	0.79 (1)	—	1.15 (1)	S: 1.15 (1)	0.85 (1)	92.3
d-NMU-8 (2—8)-OH (2e)	0.94 (1)	—	0.94 (1)	—	—	0.97 (1)	—	1.88 (2)	2.26 (2)	—	1.18 (1)	95.0
PH (2—11)-NH ₂ (3a)	1.99 (2)	—	1.03 (1)	1.01 (1)	1.00 (1)	1.03 (1)	1.02 (1)	0.94 (1)	—	M: 0.99 (1)	2.12 (2)	83.8
[Glu ¹]-PH (3b)	1.99 (2)	1.08 (1)	1.01 (1)	0.98 (1)	1.00 (1)	1.02 (1)	1.00 (1)	0.95 (1)	—	M: 0.98 (1)	2.07 (2)	81.8
PH-OH (3c)	1.93 (2)	1.03 (1)	0.94 (1)	0.97 (1)	0.97 (1)	0.99 (1)	0.97 (1)	1.23 (1)	—	M: 0.95 (1)	1.00 (1)	95.6
[Glu ¹]-PH-OH (3d)	1.94 (2)	1.05 (1)	0.96 (1)	0.98 (1)	0.98 (1)	0.99 (1)	0.96 (1)	1.23 (1)	—	M: 0.92 (1)	1.11 (1)	92.8
PH (2—11)-OH (3e)	1.92 (2)	—	0.97 (1)	0.98 (1)	0.95 (1)	0.98 (1)	0.96 (1)	1.22 (1)	—	M: 0.79 (1)	1.05 (1)	91.1
PH (4—11)-NH ₂ (3f)	1.03 (1)	—	0.99 (1)	1.03 (1)	—	1.01 (1)	1.00 (1)	0.99 (1)	—	M: 0.94 (1)	1.85 (2)	79.7
BPP-5a (2—5) (4a)	—	—	1.01 (1)	1.00 (1)	—	—	—	—	—	K: 0.99 (1)	0.38 (0)	84.7
[Glu ¹]-BPP-5a (4b)	—	1.07 (1)	0.97 (1)	—	0.99 (1)	—	—	—	—	K: 0.98 (1)	0.33 (0)	86.4
NT (2—13) (5a)	1.04 (1)	1.11 (1)	2.06 (2)	—	—	1.95 (2)	1.91 (2)	—	2.03 (2)	I: 0.87 (1)	1.21 (1)	75.3
[Glu ¹]-NT (5b)	1.01 (1)	2.19 (2)	2.00 (2)	—	—	1.99 (2)	1.96 (2)	—	1.97 (2)	I: 0.88 (1)	0.99 (1)	79.4
[Asp ⁵]-NT (5c)	1.01 (1)	2.18 (2)	2.01 (2)	—	—	1.96 (2)	1.94 (2)	—	1.97 (2)	I: 0.85 (1)	0.28 (0)	95.8
[Asp ⁵]-NT (2—13) (5d)	1.02 (1)	1.14 (1)	2.03 (2)	—	—	1.96 (2)	1.92 (2)	—	2.02 (2)	I: 0.88 (1)	0.31 (0)	77.2

^{a)} Hydrolysis at 130 °C for 3.0 h by 6 M HCl vapor containing phenol (3%). Number in parentheses are theoretical values. ^{b)} The structures of peptides are shown in the figure captions of Figs. 2, 5, 6, 8 and 9. ^{c)} S, Ser; H, His; M, Met; K, Lys; I, Ile.

cleavage reactions, these peptide solutions were divided into ten aliquots (100 μ l each) in polypropylene tubes (2 ml), which were tightly capped and kept at 25 °C in a thermostatically regulated apparatus. Samples were removed from the apparatus at an appropriate time (shown in Figs. 2—9), neutralized under cooling and kept in a freezer at -40 °C until analysis.

Identification of Peptide Fragments Produced Incubation solutions of biologically active peptides (3—5 mg) kept at 25 °C in aqueous MSA solutions (3—5 ml) were subjected to RP-HPLC employing a YMC-pack ODS-AM (1 $\times$ 25 cm) column. Materials corresponding to each peak were collected and lyophilized. Structures of the isolated peptide fragments were deduced by amino acid analysis and FAB-MS, and then confirmed by co-elution with authentic synthetic peptide on analytical HPLC using a PuresilTM C₁₈ (4.6 $\times$ 250 mm) column.

HPLC Analysis of Peptides To determine the amounts of the remaining starting materials and the cleavage products, aliquots (15 μ l) of each of the incubation solutions in aqueous MSA were subjected to RP-HPLC analysis. The peak areas of the starting materials and their hydrolysates were compared with those of synthetic peptide samples. HPLC analysis was performed using a PuresilTM C₁₈ (4.6 $\times$ 250 mm) column, a gradient elution with varying concentrations of MeCN [16 to 24% for Gn-RH (1), BPP-5a (4), and NT (5), 16 to 28% for d-NMU-8 (2), 20 to 25.6% for PH (3)] in 0.1% TFA for 40 min at a flow rate of 1 ml/min with detection at a wavelength of 210 nm. The HPLC analysis of the acid hydrolysates was repeated 4—6 times. The average values varied in the range of ± 2.5 to $\pm 5.0\%$.

Results and Discussion

Cleavage Reaction of Gn-RH (1) Gn-RH (1) degraded rapidly in 70% aqueous MSA at 25 °C giving various decomposed products. Thus, the decomposition reactions of Gn-RH (1) and its hydrolysates were examined in various concentration of MSA (10—90%) at 25 °C for 4 h. As shown in Fig. 2, the increase of MSA concentration promoted the degradation of Gn-RH (1). In 30% MSA, deamidation of the C-terminal Gly-NH₂ occurred predominantly, to produce Gn-RH-OH (1c), the amount of which decreased greatly in higher concentrations of MSA. In 70% MSA, Trp³-Ser⁴ peptide bond cleaved mainly, to produce Gn-RH (4—10)-NH₂ (1e) and its counterpart Gn-RH (1—3)-OH (1d). Decomposition reaction of Gn-RH (1) was promoted in higher concentration of MSA, but the cleavage at the N-terminal of Ser⁴ was suppressed in 90% MSA. The production of Gn-RH (2—10)-NH₂ (1a) markedly increased in 90% MSA. HPLC profiles of the incubation mixtures of Gn-RH (1) in 70% and 90% MSA are shown in Figs. 3A and B, respectively. As seen from the time courses of Gn-RH (1) and its hydrolysis products during incubation in 90% MSA at 25 °C (Fig. 4), pGlu-His peptide bond predominantly cleaved to yield Gn-RH (2—10)-NH₂ (1a) as a major product in 3 h, while Gn-RH (1—3)-OH (1d) and Gn-RH (4—10)-NH₂ (1e) were the minor products.

Reaction of d-NMU-8 (2) Decomposition of d-NMU-8 (2) in 90% MSA at 25 °C yielded H-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH₂ [NMU-8 (2—8)-NH₂ (2a)]¹²⁾ as the main product, and [Glu¹]-NMU-8 (2b),¹¹⁾ d-NMU-8-OH (2c),¹³⁾ [Asp⁸]-d-NMU-8-OH (2d),¹³⁾ and d-NMU-8 (2—8)-OH (2e) as minor products (Fig. 5B). However, in 70% MSA, the deamidation products (2c—e) at the C-terminal Asn-NH₂ of d-NMU-8 (2) increased greatly, and the main decomposition product was d-NMU-8-OH (2c) (Fig. 5A). The decomposition reaction of d-NMU-8 (2) proceeded much faster in 70% MSA compared to 90% MSA.

Reaction of PH (3) Incubation of PH (3) in 70% MSA at 25 °C for 12 h gave numerous peaks on HPLC chromatogram (Fig. 6), however, the main product was PH (2—11)-NH₂ (3a). Cleavage at Asp³-Pro⁴ peptide bond to yield PH (4—11)-NH₂ (3f) occurred to a less extent than at pGlu-

Table 2. FAB-MS Analysis and Characteristics of Synthetic Peptides

Peptides ^{a)}	Formula	FAB-MS Found [M+H] ⁺	[α] _D ²⁴ (c=0.5) (50% AcOH)	HP-TLC ^{b)}	
				R _f ¹	R _f ²
Gn-RH (2-10)-NH ₂ (1a)	C ₅₀ H ₇₀ N ₁₆ O ₁₁	1071	-25.2°	0.40	0.43
[Glu ¹]-Gn-RH (1b)	C ₅₅ H ₇₇ N ₁₇ O ₁₄	1200	-28.4°	0.32	0.29
Gn-RH-OH (1c)	C ₅₅ H ₇₄ N ₁₆ O ₁₄	1183	-40.8°	0.34	0.44
Gn-RH (1-3)-OH (1d)	C ₂₂ H ₂₄ N ₆ O ₅	453	-4.8° ^{c)}	0.38	0.51
Gn-RH (4-10)-NH ₂ (1e)	C ₃₃ H ₅₃ N ₁₁ O ₉	748	-40.8°	0.43	0.44
d-NMU-8 (2-8)-OH (2e)	C ₄₅ H ₆₈ N ₁₄ O ₉	949	-40.8°	0.33	0.46
PH (2-11)-NH ₂ (3a)	C ₅₃ H ₇₉ N ₁₃ O ₁₄ S	1154	-24.0° ^{c)}	0.36	0.49
[Glu ¹]-PH (3b)	C ₅₈ H ₈₆ N ₁₄ O ₁₇ S	1283	-23.2° ^{c)}	0.31	0.47
PH-OH (3c)	C ₅₈ H ₈₃ N ₁₃ O ₁₇ S	1266	-69.2°	0.36	0.48
[Glu ¹]-PH-OH (3d)	C ₅₈ H ₈₅ N ₁₃ O ₁₈ S	1284	-61.2°	0.26	0.44
PH (2-11)-OH (3e)	C ₅₃ H ₇₈ N ₁₂ O ₁₅ S	1155	-59.6°	0.32	0.46
PH (4-11)-NH ₂ (3f)	C ₄₆ H ₆₉ N ₁₁ O ₁₀ S	968	-14.8° ^{c)}	0.39	0.50
BPP-5a (2-5) (4a)	C ₂₅ H ₃₆ N ₆ O ₅	501	-27.6°	0.26	0.31
[Glu ¹]-BPP-5a (4b)	C ₃₀ H ₄₃ N ₇ O ₈	630	-44.8°	0.20	0.33
NT (2-13) (5a)	C ₇₃ H ₁₁₆ N ₂₀ O ₁₈	1561.9	-55.0° ^{c)}	0.37	0.16
[Glu ¹]-NT (5b)	C ₇₈ H ₁₂₃ N ₂₁ O ₂₁	1690.9	-69.5°	0.30	0.17
[Asp ⁵]-NT (5c)	C ₇₈ H ₁₂₀ N ₂₀ O ₂₁	1673.9	-79.3°	0.36	0.44
[Asp ⁵]-NT (2-13) (5d)	C ₇₃ H ₁₁₅ N ₁₉ O ₁₉	1562.9	-69.9°	0.33	0.22

a) The structures of peptides are shown in the figure captions of Figs. 2, 5, 6, 8 and 9. b) R_f¹, *n*-BuOH:pyridine:AcOH:H₂O (30:20:6:24); R_f², *n*-BuOH:AcOEt:AcOH:H₂O (1:1:1:1). c) *c*=0.2.

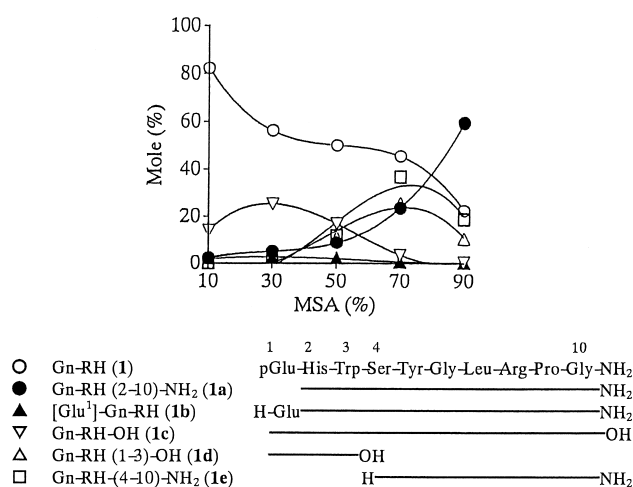


Fig. 2. Relationships between the Concentration of MSA and Gn-RH and Its Hydrolysates after Incubation at 25 °C for 4 h

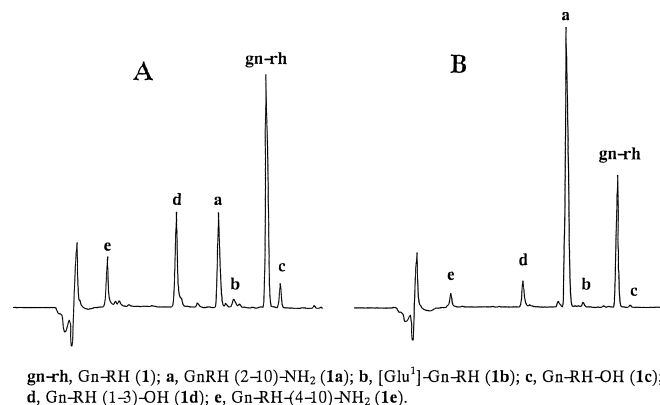


Fig. 3. HPLC Profiles for Gn-RH and Its Hydrolysates after Incubation in 70% (A) or 90% (B) MSA at 25 °C for 4 or 3 h

HPLC conditions: column, Puresil™ C₁₈ (4.6×250 mm); elution, linear 40 min gradient elution from 16 to 24% MeCN in 0.1% TFA; flow, 0.8 ml/min; detection, 210 nm. Retention times (*t_R*, min) were shown in parenthesis.

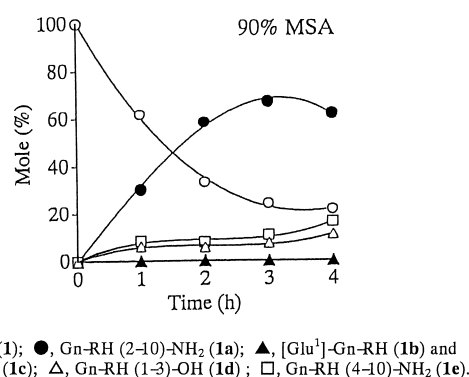


Fig. 4. Time Courses for Gn-RH and Its Hydrolysates during Incubation in 90% MSA at 25 °C

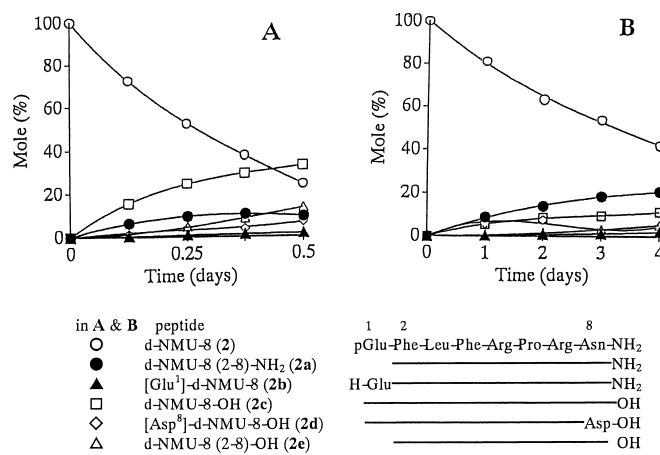


Fig. 5. Time Courses for d-NMU-8 and Its Hydrolysates during Incubation in 70% (A) and 90% (B) MSA at 25 °C

Ala bond, however, the production of PH (4—11)-NH₂ (**3f**) in 70% MSA was slightly higher than in 90% MSA (Figs. 7A, B). The deamidation at the side chain of Asn⁵ and at the C-terminal Met-NH₂ was insignificant.

Reaction of BPP-5a (4) HPLC profile of the incubation mixture of BPP-5a (**4**) in 70% MSA at 25 °C and the time courses of BPP-5a (**4**) and its hydrolysates are shown in Figs. 8A and B, respectively. After 2 d, 24% BPP-5a (**4**) remained in the incubate, and the yield of BPP-5a (2—5) (**4a**) was 56%, the ring-opened product [Glu¹]-BPP-5a (**4b**) was less than 1%, and the other minor products were not determined.

Reaction of NT (5) Incubation of NT (**5**) in 70% MSA at 25 °C for 24 h produced complex degradation mixture as seen from the HPLC chromatogram (Fig. 9A). Main peak in the chromatogram corresponded to NT (2—13) (**5a**). Four other peaks were also identified and the remaining small peaks were not determined. The time courses of the hydrolysis products are shown in Fig. 9B.

Selective cleavage of pGlu-X peptide bond was performed in either 70% or 90% MSA at 25 °C for all pGlu-X-peptides examined in this study. The reaction of Gn-RH (**1**) yielded more than 60% of the X-peptide [Gn-RH (2—10)-NH₂ (**1a**)] after 3 h in 90% MSA at 25 °C. The cleavage reaction at pGlu¹-His² peptide bond proceeded so rapidly that the other reactions such as the ring open reaction at pyrrolidone moiety of pGlu¹, the deamidation of C-terminal Gly¹⁰-NH₂ and

the hydrolysis of the other internal peptide bonds in Gn-RH (**1**) were suppressed. However, in 70% MSA, the cleavage of an internal peptide bond of Trp³-Ser⁴, was the predominant reaction. The cleavage of the peptide bond at the N-terminal of Ser was possibly *via* N-O rearrangement reaction followed by the hydrolysis of the ester bond as seen in concentrated hydrochloric acid.¹⁴ In 90% MSA, d-NMU-8 (**2**) yielded the X-peptide [d-NMU-8 (2—8)-NH₂ (**2a**)] as the main product. While, in 70% MSA, the greater decomposition of the C-terminal Asn⁸-NH₂ took place compared to the cleavage of pGlu¹-Phe² bond. Concerning the decomposition of peptides bearing C-terminal Asn-NH₂, we recently reported the stability of porcine neuromedin U-8 in detail, where it was shown that the Asn-NH₂ portion was hydrolyzed to produce peptides bearing Asn-OH, Asp-NH₂ and Asp-OH in dilute acidic solution.¹⁵ The hydrolysis experiments of Gn-RH (**1**) in 10—90% aqueous MSA (Fig. 3) showed that the deamidation reaction at C-terminal amide portion was enhanced in lower concentration of aqueous MSA. The reaction of PH (**3**) yielded the X-peptide [PH (2—11)-NH₂ (**3a**)] as a main product, demonstrating the cleavage of pGlu¹-Ala² peptide bond occurred to a greater extent than the internal peptide bond of Asp³-Pro⁴, which has previously been reported to be one of the most susceptible bonds to acidic hydrolysis.^{16—18} Treatment of BPP-5a (**4**) with 70% MSA yielded H-Lys²-Trp-Ala-Pro⁵-OH (**4a**), indicating that the cleavage occurred almost exclusive at pGlu¹-Lys² peptide bond in 70% MSA. NT (**5**) was decomposed by either a non-specific hydrolysis

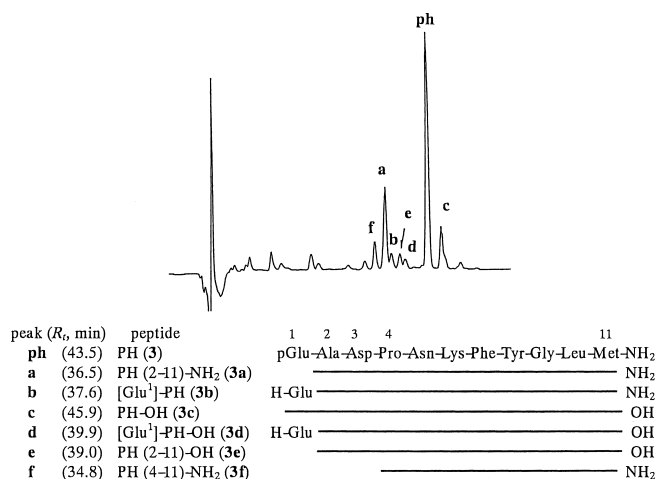


Fig. 6. HPLC Profiles for PH and Its Hydrolysates after Incubation in 70% MSA at 25 °C for 0.5 d

HPLC conditions: column, PuresilTM C₁₈ (4.6×250 mm); elution, linear 40 min gradient elution from 20 to 25.6% MeCN in 0.1% TFA; flow, 1.0 ml/min; detection, 210 nm.

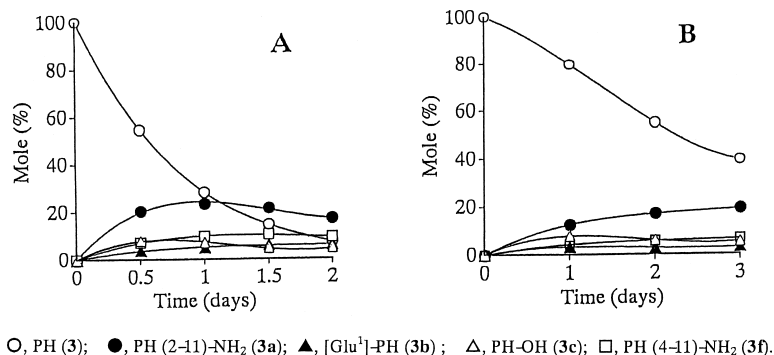


Fig. 7. Time Courses for PH and Its Hydrolysates during Incubation in 70% (A) and 90% (B) MSA at 25 °C

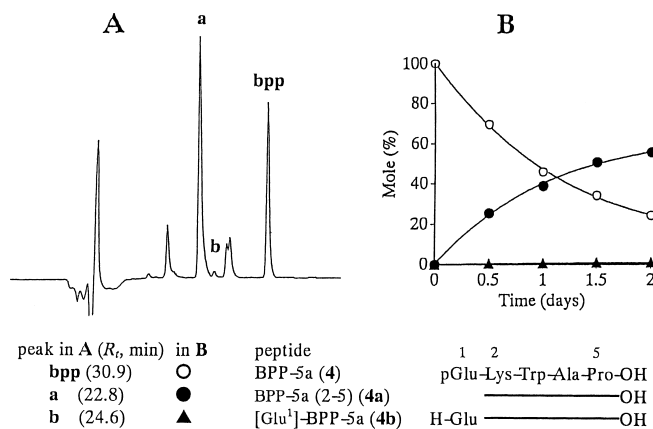


Fig. 8. HPLC Profiles (A) and Time Courses (B) for BPP-5a and Its Hydrolysates during Incubation in 70% MSA at 25 °C

HPLC conditions: column, PuresilTM C₁₈ (4.6×250 mm); elution, linear 40 min gradient elution from 16 to 24% MeCN in 0.1% TFA; flow, 1.0 ml/min; detection, 210 nm.

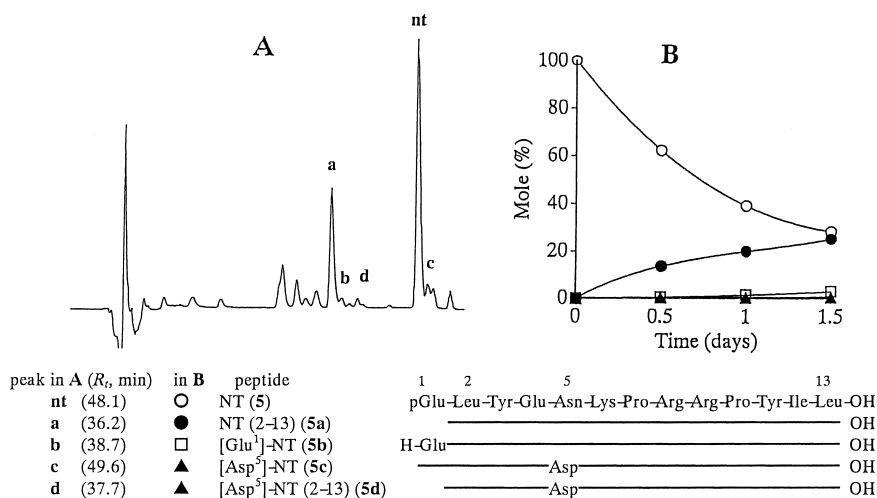


Fig. 9. HPLC Profiles (A) and Time Courses (B) for NT and Its Hydrolysates during Incubation in 70% MSA at 25 °C

HPLC conditions: column, Puresil™ C₁₈ (4.6×250 mm); elution, linear 40 min gradient elution from 16 to 24% MeCN in 0.1% TFA; flow, 1.0 ml/min; detection, 210 nm.

of the internal peptide bonds or the deamidation at the side chain of Asn at the position 5 in 70% MSA, resulting in the appearance of numerous peaks on HPLC chromatogram (Fig. 9A), with the peak of NT (2–13) (5a) decidedly the largest. When the hydrolysis was carried out in 90% MSA, the unknown peaks eluted earlier (t_R < 35 min) on HPLC chromatogram became larger than peak a (data not shown).

The half-life period ($t_{1/2}$) of Gn-RH (1) in 90% MSA at 25 °C was 1.4 h (Fig. 4). Previously we reported a highly selective cleavage of thyrotropin releasing hormone (TRH, pGlu-His-Pro-NH₂) under similar conditions, where more than 50% of TRH remained intact after 72 h.⁵⁾ Thus, there was a large difference in the susceptibility of pGlu-His peptide bonds in Gn-RH (1) and TRH under similar conditions. In each pGlu-X-peptide, the cleavage reaction of a pGlu-His peptide bond seemed to be greatly affected by the amino acid sequence that followed it. The $t_{1/2}$ values of the other biologically active peptides were quite varied at 72 h for d-NMU-8 (2) (in 90% MSA), 15 h for PH (3) (in 70% MSA), 20 h for BPP-5a (4) (in 70% MSA), and 15 h for NT (5) (in 90% MSA). Thus, the susceptibility of various peptide bonds including the pGlu-peptide bonds was dependent on the primary structures of these peptides.

Present study demonstrated that the selective removal of pGlu residue from various biologically active peptides could generally be attained by means of high concentration of MSA at low temperature, which may be applicable to structural examination of pGlu-peptides.

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