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Vespakinin-M, a Novel Bradykinin Analogue Containing Hydroxyproline, in the Venom of *Vespa mandarinia* Smith

A new hornet-kinin, "Vespakinin-M," was isolated from the venom of *Vespa mandarinia* which contained hydroxyprolyl residue in the sequence. The amount of the peptide in one venom sac was approximately 15 nmole which was much higher in concentration than other wasp venom.

In a previous paper,[1] we have identified alanyl-bradykinyl-isoleucyl-valine as hornet-kinin of *Vespa xanthoptera* and called the peptide vespakinin-X. In the venom of *Vespa mandarinia* (Japanese name Ohsuzumebachi), the biggest hornet in Japan, a novel bradykinin analogue which contained Hyp^3-bradykinin sequence in the peptide was isolated.

This communication deals with the separation of active principles in the venom and the sequential analysis of the peptide.

Separation of the Active Principles

Venom sacs of 27 hornets were homogenized with 7 ml of 0.6% trichloroacetic acid and the homogenate was centrifuged 3000 rpm for 15 min. The supernatant was chromatographed on a SE-Sephadex column with a linear concentration gradient from water to 1.0 N ammonium formate (pH 6.5) and the column was successively eluted with 1.0 N ammonium formate (pH 9.5). Five active fractions, H–I, –II, –III, –IV and –V, were obtained. These fractions, except H–II, contracted the isolated rat uterus, and the fraction H–II showed the haemolytic activity. The major active fraction, H–I, which was eluted from the column at the concentration of 0.25 N of the eluent, contracted the guinea pig ileum and showed hypotensive action on the rat blood pressure. This fraction was chromatographed further by Sephadex G-15 column. The similar chromatographic pattern to that in the case of *Vespa xanthoptera* venom was obtained and the fraction H–I was separated into two active fractions (H–I–1, H–I–2). The fraction H–I–1 was eluted in a column volume, while the fraction H–I–2 was adsorbed on the column and the latter was identified with serotonin by similar procedure reported in a previous paper.[1] The rat uterus contracting activity in the fraction H–I–1 was lost by chymotrypsin but not by trypsin digestion and was expected to be hornet-kinin. The active principle in this fraction was purified by successive SE-Sephadex chromatography with a flat concentration elution of 0.15 N ammonium formate (pH 6.5). The activity was eluted at 2.5 to 3.0 column volumes. The horne-tkinin of this fraction was observed as a single spot on a thin-layer chromatogram after dansylation and was considered to be homogeneous.

Sequential Analysis of the Peptide

After an aliquot of the peptide was hydrolysed with 6 N HCl at 110° for 24 hr, the amino acid composition of the peptide was determined as follows; Arg_2, Hyp_1, Asx_1, Ser_1, Pro_2, Gly_2, Ile_1, and Phe_2. The venom sac of one *Vespa mandarinia* contained approximately 15 nmoles of the peptide calculated from the data of the amino acid analysis. And this value showed 40 times higher comparing to the data of *Vespa xanthoptera*.

The N-terminal amino acid was identified with glycine by dansyl method. The dansyl derivative of the intact peptide was cleft into two fragments (DNS-T_1, T_2) by treatment with trypsin. The following amino acids were determined in the acid hydrolysate (6 N HCl, 90°, 16 hr) of DNS–T_1; DNS–Gly, Arg_2, Hyp_1, Ser_1, Pro_2, Gly_1, and Phe_2. The tryptic fragment, T_2, was separated by thin-layer chromatography after redansylation of the tryptic reaction mixture and purified as an usual manner.[2] The amino acid composition of the hydrolysate of

1) T. Yasuhara, H. Yoshida and T. Nakajima, sumitted to this bulettin.
2) Z. Tamura, T. Nakajima, T. Nakayama, J.J. Pisano, and S. Udenfriend, *Anal. Biochem.*, **52**, 595 (1973).

DNS–T_2 was determined as dansyl isoleucine and aspartic acid by thin-layer chromatography of polyamidelayer with the solvent system of benzene: acetic acid (9: 1) and by an amino acid analyser. The tryptic reaction products again, which contained DNS–T_1 and T_2, were directly treated with one cycle of Edman procedure and aspartic acid was detected from the reaction mixture by an amino acid analyser. The dansyl derivative of the intact peptide was cleft by chymotryptic treatment to give the dansyl fragment which contained the following amino acids; DNS–Gly, Arg_1, Hyp_1, Ser_1, Pro_2, Gly_1, and Phe_2. These results indicated that the C-terminal end of the peptide were –Phe–Arg–Ile–Asp. Dansyl–Edman procedure was carried out for the intact peptide and the sequence of the peptide from the N-terminal end was found to be Gly–Arg–Pro–Hyp–Gly–Phe–Ser–Pro–Phe–.

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The hornet-kinin in the venom of *Vespa mandarinia* was finally deduced to glycyl-(hydroxyproline^3-bradykinyl)-isoleucyl-aspartic acid By these experimental results.

Gly-Arg-Pro-Hyp-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Asp

(Vespakinin-M)

We have called the peptide "Vespakinin-M" being derived from the species name.

Hydroxyproline in vespakinin-M showed the same chromatographic behaviors on a thin-layer of Silica gel H and on an amino acid analyser (JEOL 5-AH) to 4-hydroxyproline of natural collagen and not to allo-4-hydroxyproline. This suggests that hydroxyproline in the peptide may be natural 4-hydroxyproline itself, although the amino acid was not able to examine further by physicochemical analysis for its minute amount.

It is little known on the presence of the hydroxyproline containing peptide of small molecular size in animal kingdom, except the cleft products of collagen, gelatin or such hydroxyproline containing proteins, which exist in serum or urine of mammals. The presence of this peptide in the insect venom also suggests the occurrence of proline hydroxylase in the invertebrates.

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