A new mast cell degranulating peptide, mastoparan-M, in the venom of the hornet *Vespa mandarinia*

YUKO HIRAI, TADASHI YASUHARA, HISANOBU YOSHIDA and TERUMI NAKAJIMA*

Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University, Kasumi, Hiroshima 734, and *Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Kanda Surugadai, Chiyodaku, Tokyo 101, Japan

ABSTRACT

A new mast cell degranulating peptide, *mastoparan-M*, was isolated from the venom of the Japanese hornet *Vespa mandarinia*. The peptide is a tetradecapeptide amide with the following sequence:

The hornet contained approximately 20 nmol of the peptide in one venom sac, and the peptide degranulated the rat peritoneal mast cells at the minimum concentration of 0.5 nmol/ml.

KEY WORDS mastoparan-M / mast cell degranulating peptide / hornet venom / *Vespa mandarinia*

Mastoparans, a series of tetradecapeptide amides in the vespid venoms, which have a potent action on the rat peritoneal mast cells, causing degranulation and the release of histamine from the cells, were recently characterized in our laboratories (1-3). These peptides were considered to be one of the components causing prolongation of the venomous action. Subsequently, we investigated such peptides from the other wasps and hornets and isolated a new peptide analogous to mastoparan from the venom of *Vespa mandarinia*, the largest hornet in Japan. We named the peptide mastoparan-M.

We report here on the isolation and chemical characterization of mastoparan-M.

The hornet venom sacs were homogenized in 0.5 ml of 5% trichloroacetic acid. The homogenate was diluted to 5 ml by adding distilled water and centrifuged at 1,500 g for 15 min. The lyophilized supernatant was used for further purification. The lyophilized material was dissolved in 0.5 ml of 0.05 M ammonium formate (pH 6.5) and chromatographed on a Sephadex G-25 column. The mast cell degranulating activity in each fraction was

assayed as described previously (5). Chromatographic details are shown in Fig. 1.

As shown in the chromatogram, two active fractions, fraction I (major) and fraction II (minor), were obtained. Fraction I was eluted at about half the column volume and fraction II at 1.5 column volume. Fraction I did not correspond to the chromatogram monitored at 280 nm but the activity in fraction II coincided with the UV absorbance at 280 nm. Fraction I lost its activity after treatment with trypsin and the active principle was considered to be a peptide. The active principle in fraction II was concluded to be serotonin based on the characteristics of the UV spectrum, biological action on the rat blood pressure and smooth muscle contraction of the rat uterus, and by high performance liquid chromatography.

An aliquot of the sample in each fraction tube of Sephadex G-25 chromatography was further chromatographed on a Silica gel plate (Merck HPTLC) with the solvent system of *n*-butanol : pyridine : acetic acid : water (15 : 10 : 3 : 12) and the plate was stained with 0.5% fluorescamine in acetone. A single spot was observed at the area of fraction I. When another

Fig. 1 The crude extract was chromatographed on a Sephadex G-25 column (0.9 cm $\times$ 78.5 cm). The column was eluted with 0.05 M ammonium formate (pH 6.5) and each 2 ml of the eluate was collected. The shaded areas showed the mast cell degranulating activity.

aliquot of the sample was labelled with dansyl chloride prior to thin layer chromatography, a similar result, i.e., a single fluorescent labelled spot, was observed with the solvent system of methylene chloride : methyl acetate : ethanol (5 : 5 : 1). This indicated that the active principle in fraction I was purified in one step of separation. The amino acid composition of this peptide was determined by an amino acid analyser (JEOL-5AH) after the usual hydrolysis with 6 N HCl at 110°C for 24 hr, and the following composition was obtained: Asp₁, Ala₄, Ile₂, Leu₄, and Lys₃. The yield of the peptide calculated from the datum of the amino acid analysis was approximately 0.2 μ mol from 10 venom sacs.

The aspartic acid residue was confirmed to be asparagine by the enzymatic digestion, in which 10 nmol of the peptide were digested by adding 10 μ g of aminopeptidase M (Protein Research Foundation, Osaka) and liberated amino acids were determined. The N-terminal amino acid was determined as isoleucine by the dansyl method.

The peptide of 20 nmol was dissolved in 90 μ l of 0.1 M triethylamine-bicarbonate (pH 8.5) and digested by adding 10 μ l of TPCK trypsin (Worthington Biochem. Co., 1 mg/ml in 0.001 N HCl) at 37°C overnight. The digest was separated on a thin layer of cellulose powder using the solvent system of *n*-butanol : pyridine : acetic acid : water (15 : 10 :

3 : 12). Fluorescamine staining at a part of the plate gave 4 fluorescent bands (T-1, T-2, T-3, and T-4). The corresponding areas were scraped, and each peptide fragment in the powder was extracted with 0.1 N HCl. The fragments were hydrolysed with 6 N HCl in the usual manner. The amino acid composition of these fragments was as follows: T-1 (Asp₁, Ile₁, Leu₁, Lys₁), T-2 (Ala₄, Ile₁, Leu₁, Lys₁), T-3 (Leu₂, Lys₁) and T-4 (Leu).

The chromatogram of T-4 did not coincide with leucine despite the fact that its composition was leucine, and this fragment was determined as leucyl-leucine amide by the dansyl-Edman procedure. Another aliquot of the intact peptide was cleft by the dansyl-Edman procedure to the last step. The results obtained from this degradation and the trypsin digestion experiments enabled us to deduce the sequence of the peptide as follows:

The venom of *Vespa mandarinia* contains histamine, serotonin, and a novel hornet kinin, vespakinin-M (Gly-[Hyp]³-bradikinin-Ile-Asp) (4). Mastoparan-M was the fourth active component of low molecular weight. It was assumed that more than 20 nmol of the peptide were stored in a single venom sac, which was a much higher concentration than in the venoms of other wasps.

Mastoparan-M consisted of the same amino acid residues as those of mastoparan, a similar peptide from the venom of *Vespula lewisii* (3). The difference between them was the replacement of isoleucine with leucine at position 6 and leucine with isoleucine at position 13 of the sequence. Mastoparan-M degranulated rat peritoneal mast cells at a minimum concentration of 0.5 nmol/ml, which was virtually the same as with mastoparan.

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