Studies on Wasp Venom, II

A New Mast Cell Degranulating Peptide from the Wasp *Paravespula vulgaris*

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Dedicated to Professor Dr. Ernst Bayer on the occasion of his 60th birthday

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Mast Cell Degranulating Peptide, *Paravespula vulgaris,* Edman Degradation, Fast Atom Bombardment Mass Spectra, HPLC

From the extract of 3991 venom glands of the most common European wasp species *Paravespula vulgaris* a novel basic pentadecapeptide was isolated and investigated by amino acid analysis, micro-Edman-degradation and fast atom bombardment mass spectrometry. The peptide has the sequence H–Ile–Asn–Trp–Lys–Lys–Ile–Lys–Ser–Ile–Ile–Lys–Ala–Ala–Met–Asn and exhibits strong mast cell degranulating activity similarly to the mastoparans isolated from various wasp species.

1. Introduction

After the identification of peptides as the major toxic principles of bee [1] and snake [2–4] venom various basic tetradecapeptide amides were isolated from wasps [5]. These mastoparans can be characterized by their mast cell degranulating properties, resulting in the release of toxic and allergenic mediators (histamine, serotonine) [6] causing hemolysis [7] or changing the ion conductivity of lipid membranes, due to their amphiphilic properties [8]. Mastoparans are of interest for studying immunological mechanisms of allergic reactions of the immediate anaphylactic type [9, 10]. As a consequence of the anaphylactic antigen-antibody interaction on the outer surface of mast cells these are degranulated in a way similar to the action of mastoparans. Eight mastoparans have so far been isolated from different *paravespula, vespa* and *polistes* species (Table I), but no investigations are known yet about similar peptides in the most common European wasp species *Paravespula vulgaris.* From the venom glands of 3991 wasps we have isolated a basic pentadecapeptide with mast cell degranulating properties. Its primary structure was derived by amino acid analysis, Edman degradation of the tryptic fragments and fast atom bombardment mass spectrometry.

2. Materials and Methods

Collection of wasp venom

Wasps were collected by deep-freezing complete nests with liquid nitrogen. After separating larvae and nest material the adult female animals were kept in liquid nitrogen. For isolation of the venom glands the animals were warmed up to room temperature and the venom apparatus removed under a stereomicroscope. The venom glands were transferred to bidistilled water. For precipitation of enzymes trichloroacetic acid was added until a concentration of 5% was reached. The solution was treated twice in an ultrasonic bath for 2 min and subsequently centrifuged for 20 min at 8000 cpm. The clear liquid was directly transferred to a column for gel filtration.

Gel filtration

The extract of 1000 venom glands was submitted to gel filtration on a column (80×1.6 cm) with Sephadex G 25 (fine) with ammonium formate (0.05 M) as eluent and UV detection (254 nm). The fractions were tested for mast cell degranulating properties and for their amino acid composition be-

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Table I. Amino acid sequences of mastoparans from wasps.

Mastoparan *(Paravespula lewisii)*
H-Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2

Mastoparan-M *(Vespa mandarinia)*
H-Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Ala-Lys-Lys-Leu-Leu-NH_2

Mastoparan-X *(Vespa xanthoptera)*
H-Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2

Mastoparan-A *(Vespa analis)*
H-Ile-Lys-Trp-Lys-Ala-Ile-Leu-Asp-Ala-Val-Lys-Lys-Val-Ile-NH_2

Mastoparan-T *(Vespa tropica)*
H-Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Phe-Ala-Lys-Lys-Leu-Leu-NH_2

Mastoparan-II *(Vespa orientalis)*
H-Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Val-Lys-Lys-Val-Leu-NH_2

Mastoparan-C *(Vespa crabro)*
H-Leu-Asn-Leu-Lys-Ala-Leu-Leu-Ala-Val-Ala-Lys-Lys-Ile-Leu-NH_2

Polistes mastoparan *(Polistes jadwigae)*
H-Val-Asp-Trp-Lys-Lys-Ile-Gly-Gln-His-Ile-Leu-Ser-Val-Leu-NH_2

fore and after total hydrolysis. Mast cell degranulating properties could be correlated to Sephadex G 25 fractions 4–9.

High pressure liquid chromatography

HPLC separations were performed on a Spectra-Physics Sp 8000 instrument with stainless steel columns (250×4 mm) with Lichrosorb RP-18 (5 μm, Knauer, Bad Homburg) together with a Lichrosorb RP-18 precolumn (70×2 mm) and UV detection at 215 nm. A linear gradient of bidistilled H_2O and acetonitrile (10 to 60% acetonitrile in 90 min) with 0.1% trifluoroacetic acid was used for elution at a flow rate of 1 ml/min at 323 K.

Amino acid analysis

Amino acid analyses were obtained on a Biotronic LC 6001 instrument with ninhydrin reagent for detection.

Acid hydrolysis

Small samples of the Sephadex G 25 or HPLC fractions were lyophilized in a glass ampoule. After addtion of 100 μl of 6 N HCl the ampoules were sealed and heated at 110 °C for 22 h. The solution was then lyophilized and the sample taken up in 100 μl of buffer for amino acid analysis.

Enzymatic hydrolysis

The lyophilized samples were dissolved in 50 μl of trisbuffer (0.01 M, pH 8.0). After adding dialyzed pronase E incubation proceeded for 1 h at 37 °C. The solution was lyophilized and taken up in 100 μl of buffer solution for amino acid analysis.

Sequence analysis

Thin-layer finger prints of the trypsin digestion products of 4 nmol of peptide were obtained on polygram plates (Cel 300, Macherey & Nagel, Düren). Electrophoresis at 450 V (1.5 h, 2% pyridine acetate, pH 4.5 with 5% acetone) was followed by chromatography in pyridine, *n*-butanol, acetic acid, H_2O (50:75:15:60, v/v; 4–5 h).

The plates were sprayed with 5% pyridine in acetone and, subsequently, with 1% fluram (Serva, Heidelberg) in acetone. The peptides were detected under UV-light and degraded according to the dansyl-Edman procedure [11–13]. Sequential degradation of the native peptide proceeded according to the dansyl- and dabsyl-Edman method using 5 nmol of sample.

Mass spectrometry

Fast atom bombardment (FAB) mass spectra were recorded on a Finnigan MAT 311 A instrument (negative ions) and on a Kratos MS 50 S instrument (positive ions) with xenon as collision gas and glycerol or 1-thioglycerol as matrix. Oxalic acid, acetic acid or HCl were added for better solution of the samples.

Biological tests

For detection of mast cell degranulation a phase contrast microscope with an amplification factor of 400 and 1200 was used. Mast cells were isolated from the peritoneum of rats according to the method of Bloom and Haegermark [14]. 5 μl of cell suspension (*ca.* 10^6 cells/ml in 0.9% NaCl solution), 2.5 μl of sample, dissolved in bidistilled H_2O and 2.5 μl of

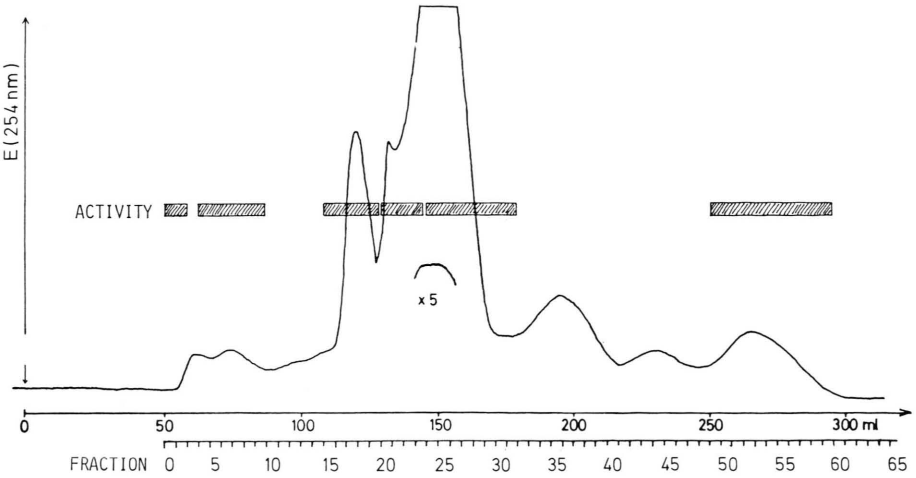

Fig. 1. Sephadex G 25 separation of *Paravespula vulgaris* venom.

1.8% NaCl solution were mixed on a glass plate. Immediate mast cell degranulation could be observed and continued for 10 min.

3. Results and Discussion

Concomitant biological tests revealed mast cell degranulating properties in Sephadex G 25 fractions 4–9 (Fig. 1). The biological activity could be correlated to a UV maximum at 254 nm and high yields of isoleucin and lysine in the amino acid analysis of these fractions, typical to mastoparans (Table I). The active fractions 4–9 were further separated by RP-18 HPLC. The biological activity could be located in HPLC fraction 17 (Fig. 2a) and isolated in almost pure form by micro-preparative HPLC (Fig. 2b).

Amino acid analysis after 6 N HCl hydrolysis indicated the presence of the following amino acids: Ala_2, Asx_2, Ile_4, Lys_4, Met_1, Ser_1. A peptide of this composition can not explain the UV absorption at 254 nm. We therefore performed an enzymatic hydrolysis with pronase E. In addition to the amino acids identified from acidic hydrolysis one tryptophane residue was detected. Also the asx residues were found to be asn residues. The complete amino acid composition of the mast cell degranulating peptide therefore must be as follows: Ala_2, Asn_2, Ile_4, Lys_4, Met_1, Ser_1, Trp_1.

For sequence analysis a tryptic digest of the purified peptide was investigated. Two-dimensional separation by electrophoresis and thin-layer

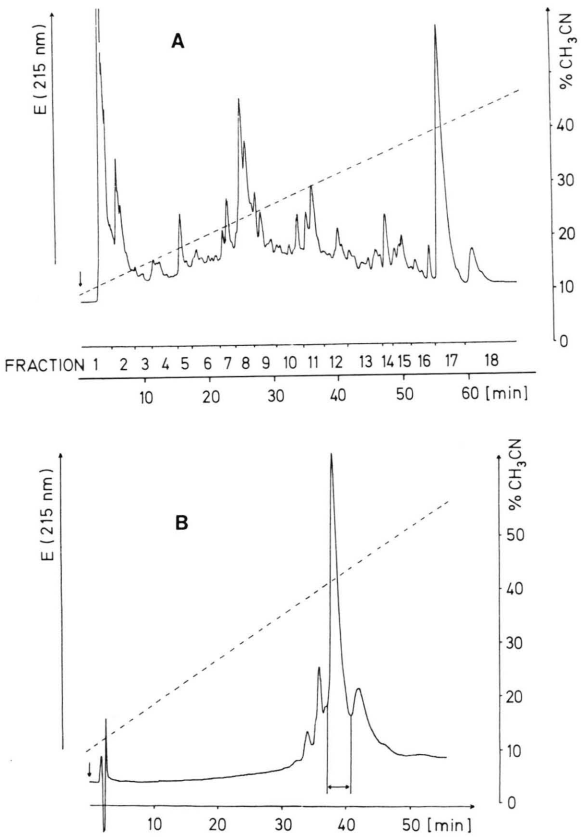

Fig. 2. a) HPLC chromatogram of Sephadex G 25 fractions of *Paravespula vulgaris* venom;
b) HPLC chromatogram of purified fraction 17 from a).

Table II. Tryptic fragments of pentadecapeptide from *Paravespula vulgaris*.

T 1	H–Ala–Ala–Met–Asn–OH
T 2	H–Ile–Asn–Trp–Lys–OH
T 3	H–Ser–Trp–Ile–Lys–OH
T 4	H–Ile–Lys–OH
T 5	Lys

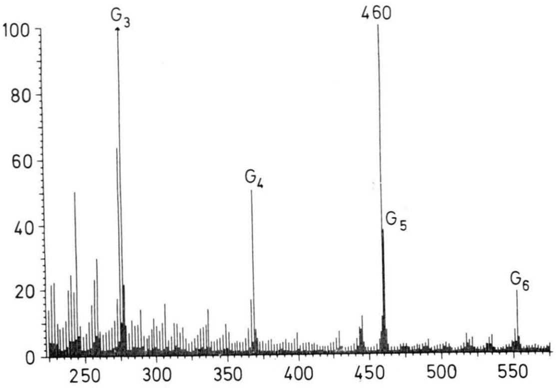

Fig. 3. Positive ion FAB mass spectrum of tryptic fragment T 3 (G3–G6 are cluster ions of the glycerol matrix).

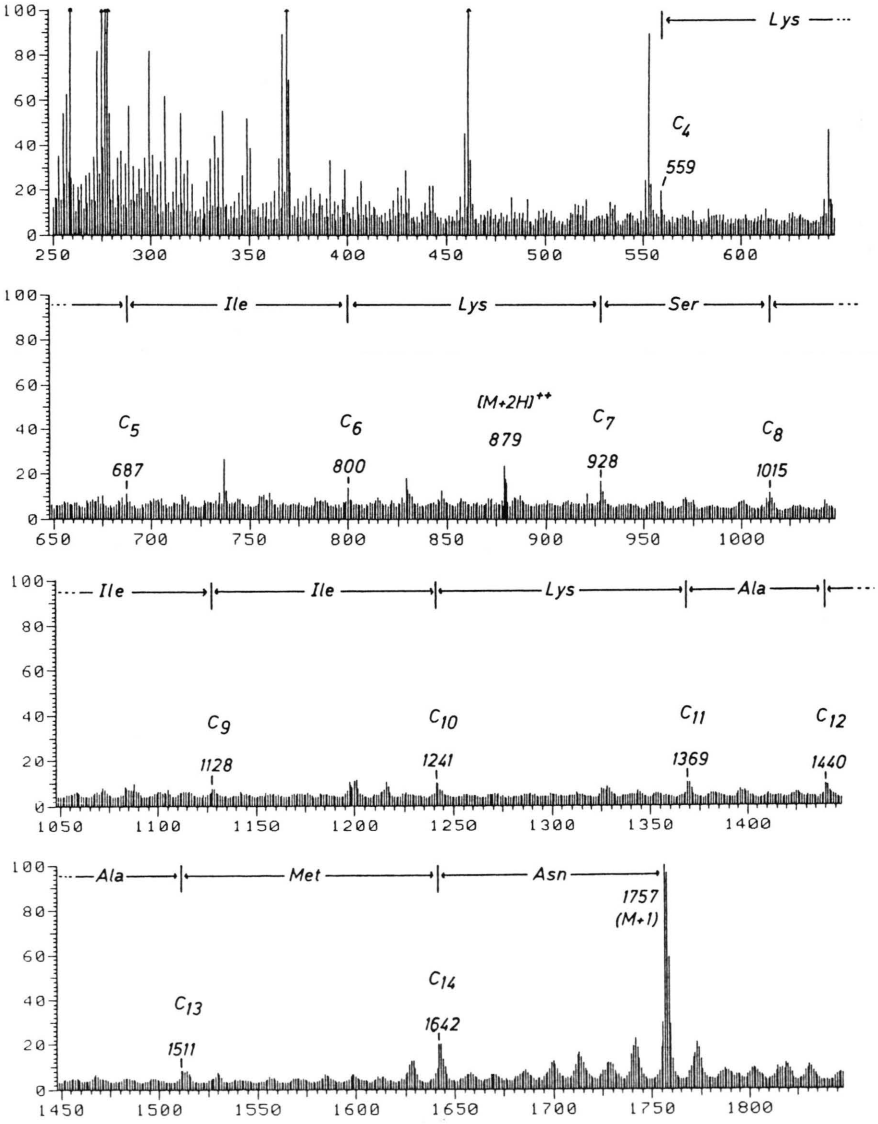

Fig. 4. Positive ion FAB mass spectrum of the mast cell degranulating pentadecapeptide from *Paravespula vulgaris* (C_4–C_{14} are sequence specific fragment ions).

chromatography yielded five spots. After isolation from the thin-layer plate the fractions were submitted to micro-dansyl-Edman degradation [11–13]. The fragments identified are shown in Table II. Stepwise degradation of the complete peptide sequence according to the micro-dansyl technique and degradation of the N-terminal residues 1–9 according to the dabsyl-Edman technique [15–17] in agreement with the fragments identified above yielded the following sequence:

H–Ile–Asn–Trp–Lys–Lys–Ile–Lys–Ser–
Trp–Ile–Lys–Ala–Ala–Met–Asn–OH

This sequence was not in agreement with the amino acid analyses, which consistently showed the presence of 4 Ile residues and only 1 Trp residue.

Mass spectrometric investigation by the FAB method (a technique that allows the recording of mass spectra of free peptides) [18, 19] of the tryptic fragment T3 (Fig. 3) clearly indicated a protonated molecular ion $[M+H]^+$ at 460 amu which agrees with the sequence H–Ser–Ile–Ile–Lys–OH. The composition of this fragment was also confirmed by amino acid analysis.

To prove this result FAB spectra of the complete pentadecapeptide were recorded (Fig. 4). A protonated molecular ion of high abundance appears at m/z = 1757. The molecular weight of 1756 amu is in accordance with the sequence:

H–Ile–Asn–Trp–Lys–Lys–Ile–Lys–Ser–
Ile–Ile–Lys–Ala–Ala–Met–Asn

In addition to molecular weight information in the mass spectrum sequence specific signals can be observed. Ions of the C-series are formed according to the fragmentation mechanism shown in Fig. 5.

An ambiguity which could not quite be ruled out concerns the C-terminal residue which was identified as Asn by amino acid analysis and Edman degradation, but which could also be an Asp–NH_2 residue. This isomer would hardly be discriminated by the analytical techniques applied. This question will possibly be answered after synthetic material will be available.

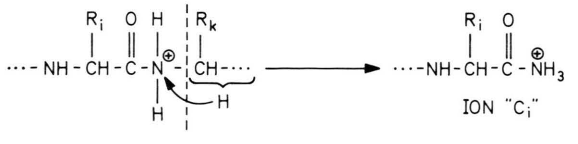

Fig. 5. Mode of fragmentation of a free peptide under formation of N-terminal sequence specific positively charged ions under FAB conditions.

From quantitative amino acid analyses it can be estimated that the mast cell degranulating pentadecapeptide amounts to about 0.2 mg/venom gland or 0.05% of the total venom of *Paravespula vulgaris*.

Although the pentadecapeptide resembles to the known mastoparans (Table I) only in the N-terminal region and inspite of the deviation in chain length the biological activity (strong mast cell degranulation at a concentration of 10 nmol/ml) justifies its classification as a mastoparan.

However, more detailed biological investigations should be performed after isolation of more material or with the synthetic peptide as soon as this will be available.

It was shown by Nakajima *et al.* [5] that histamine releasing properties are also observed with mastoparan analogues of shortened chain length.

Mast cell degranulation seems to depend on the ability of a peptide to form a hydrophobic α-helix in the cell membrane. Apparently the basic amino acid residues associate with negatively charged residues of membrane proteins. In this way the hydrophobic α-helix becomes fixed to the membrane [20].

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