[Chem. Pharm. Bull. 27(8)1945—1946(1979)]

UDC 547.466.1.02.04.09 : 615.275.2.011.5.015.44

A New Mast Cell Degranulating Peptide Homologous to Mastoparan in the Venom of Japanese Hornet (*Vespa xanthoptera*)

The haemolytic factor in the venom of Japanese hornet (*Vespa xanthoptera*) was identified as the new peptide consisting of the following sequence;

Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–Ala–Lys–Lys–Leu–Leu–NH_2

The structure is closely related to mastoparan, and the peptide revealed mast cell degranulating activity and released also serotonin from platelets. The peptide was named "Mastoparan-X."

Keywords——mast cell degranulating peptide; haemolytic peptide; serotonin release from platelet; hornet venom; *Vespa xanthoptera*; mastoparan-X

In the previous paper, we identified the new bradykinin analogous peptide, vespakinin-X, and active amines such as histamine, acetylcholine, and serotonin, in the venom of the Japanese hornet, *Vespa xanthoptera*.[1] We also reported in that paper the occurrence of some haemolytic principle in the venom. This haemolytic factor revealing also mast cell degranulating activity was found as the new peptide closely related to mastoparan.[2]

This communication deals with the isolation, chemical characterization and some pharmacological properties of this peptide.

Venom sacs of 900 pieces were used in this experiment. The trichloroacetic acid extract was chromatographed on a SP-Sephadex column in a similar manner to that described previously,[1] to remove active amines and vespakinin-X. The active fractions eluted 1.0 M of ammonium formate buffer (pH 6.5) were collected, and lyophilized and Sephadex G-10 column chromatography was employed for the next step of purification. The active principle was eluted near the void volume of the column. In the silica gel thin-layer chromatography of the active fraction, a single spot was observed after stained with fluorescamine, and the dansylated derivative also showed a single spot. This fraction lost its activity by trypsin or chymotrypsin digestion, and multiple spots were observed on a thin-layer chromatography after stained with fluorescamine of this digest. These results indicate that the principle in this fraction is the feature of polypeptide.

This peptide was constituted of the following amino acids which were determined by digestion of the peptide with aminopeptidase M and also by the usual acid hydrolysis with 6 N hydrochloric acid at 110° for 24 hr; Lys_3, Asn_1, Ala_3, Gly_1, Met_1, Leu_2, Ile_2, Trp_1. Tryptophyl residue was determined by ultraviolet (UV) absorption spectrum and by absorbancy at 280 nm. This composition is similar to mastoparan, with the difference of having an alanine and 2 leucine residues less, and a glycine, a methionine and a tryptophan residues more.

The N-terminal amino acid was identified with isoleucine by the dansyl method. The peptide was cleft with cyanogen bromide to give two fragments. One of which was considered to be the N-terminal portion of the peptide as it contained homoserine and isoleucine was at its N-terminus. The N-terminal amino acid of the other was alanine, and the dansyl Edman degradation led us to the sequence of Ala–Lys–Lys–Leu–Leu.

Trypsin split the C-terminal dipeptide amide from the intact peptide, which was developed at the highest *Rf* value when the digests was applied to a thin-layer chromatography of silica gel H using the solvent system of *n*-butanol: pyridine: acetic acid: water (90: 60: 18: 72). The sequence of this fragment was confirmed as Leu–Leu–NH_2 by dansyl Edman procedure.

1) T. Yasuhara, H. Yoshida, and T. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **25**, 936 (1977).
2) Y. Hirai, T. Yasuhara, H. Yoshida, T. Nakajima, M. Fujino, and C. Kitada, *Chem. Pharm. Bull.* (Tokyo), **27**, 1942 (1979).

The subtractive Edman degradation and dansyl Edman procedure were performed. The results of the both Edman procedures coincided with those obtained from cyanogen bromide cleavage and trypsin digestion. It is inconceivable to be no other amino acid but tryptophan at the third position from the N-terminus of the peptide. The structure of the peptide was deduced as follows, and named "mastoparan-X."

Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–Ala–Lys–Lys–Leu–Leu–NH_2

(mastoparan-X)

Ile–Asn–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–Lys–Ile–Leu–NH_2

(mastoparan)

Mastoparan-X has the very similar sequence to mastoparan. The positions of lysine and alanine and the N- and the C-terminal amino acids are common to those of mastoparan.

The mast cell degranulating activity of mastoparan-X was almost similar to mastoparan (0.5 μg/ml). Both mastoparans did not contract the rat uterus or the guinea pig ileum preparations, when 5.5 μg of the peptide was added to the 4 ml organ bath. The peptide of 20 μg injected intravenously to the anesthetized rat did not affect on the arterial blood pressure. These mastoparans did not potentiate the contracting activity of the bradykinin on the isolated rat uterus in the presence of 10 times excess of mastoparans. These features of mastoparans indicate that the peptides do not belong to the "kinin-like peptides" in the broadest sense.

Mastoparan-X revealed the haemolytic activity at the concentration of 10 μg/ml and also mastoparan-X (50 μg/ml) showed week activity of releasing serotonin from platelet, while mastoparan did react only for mast cells in such concentrations.

The biological activities of mastoparan-X were much more reduced, when the peptide solution was airated for 3 hr by bubbling which may be due to oxidation of the methionyl residue in the peptide.

Mastoparans are the sort of cytoactive peptide which may widely occurred in the vespid venom.

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Received May 21, 1979

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