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A New Mast Cell Degranulating Peptide, Granuliberin-R, in the Frog (*Rana rugosa*) Skin[1)]

A new mast cell degranulating peptide was isolated from the frog skin. The peptide possesses a notable sequence, in which hydrophobic amino acid residues are in the N-terminal, and hydrophilic and basic amino acid residues in the C-terminal region. Granuliberin-R belongs to a new family of the active peptides in the amphibian skin.

Keywords——*Rana rugosa*; mast cell degranulation; dansyl method; substractive Edman degradation; frog skin

The naturally occurring polypeptides in mammalian origin, such as histone, protamine and ACTH, have been known as the mast cell degranulating substance.[2)] In the invertebrates, especially in the bee venom components, the polypeptides of the mast cell degranulating activity have been characterized.[3)] The skin extract of *Rana rugosa* (Japanese name: Tsuchigaeru) also contains the another polypeptide of the mast cell degranulating, named granuliberin-R.[1)]

This report describes the isolation and the chemical characterization of granuliberin-R.

The brief detail of the isolation of the peptide was as follows: The frog skin (100 frogs) was extracted with 80% methanol containing 6% trichloroacetic acid, and the extract was evaporated to dryness by rotatory evaporator below 30°. The residue was dissolved in the lower layer of *n*-butanol: acetic acid: water (4: 1: 5) and was separated by droplet counter current chromatography (stationary phase; upper layer of *n*-butanol: acetic acid: water (4: 1: 5)). The material distributed in the stationary phase was collected by pushing out the stationary phase with nitrogen gas from the transfer tubes and the solution was evaporated under the reduced pressure. The droplet counter current chromatography was repeated with the same solvent system and the same fraction remained in the transfer tubes was collected. The dried residue was successively chromatographed on a SE-Sephadex column with the

1) A part of this work was presented at the 14th Symposium on Peptide Chemistry, Hiroshima, November, (1976).
2) H. Selye, "The Mast Cells" Washington Butterworths, 1965.
3) E. Habermann, "Venomous Animals and Their Venoms" Vol. III, ed. by W. Bücherl, and E.E. Buckley, Academic Press, New York, London, 1971.

linear concentration gradient elution from water to 1.0 N ammonium formate (pH 6.5). The fraction eluted at the concentration of about 0.5 N of ammonium formate was collected, lyophilized and then applied on a Sephadex G-15 column. The column was eluted with 0.05 N ammonium formate (pH 6.5). The fraction eluted at two third of the column volumes was collected. The gel permeation chromatography by Sephadex G-15 was repeated three times with the same condition, to remove the material eluted at the void volume and at the column volume. This fraction showed ultraviolet (UV) absorption at the region of 280 nm based on tyrosyl and tryptophyl residues. The fraction was further purified by SE-Sephadex chromatography by the flat elution with 0.2 N ammonium formate (pH 6.5) and the eluate was fractionated by monitoring of the UV absorption at 280 nm. The substance was separated into three peaks. The fractions in the former two peaks accompanied the oxytocic activity. The fraction in the last peak which had no oxytocic activity practically, showed UV absorption at 280 nm based on the tyrosyl residue and the substance in this fraction was considered to be the peptide. The peptide in this fraction was allowed to react with dansyl chloride by the procedure described previously,[4] revealed a single fluorescent spot on the thin-layer chromatography.

The amino acid composition after acid hydrolysis of the peptide was Arg_2, Ser_1, Pro_2, Gly_1, Ala_1, Ile_1, Leu_1, Tyr_1, and Phe_2, and a total of 230 nmol of the peptide was obtained. N-Terminal amino acid was phenylalanine. The peptide was digested by trypsin to give two fragments of the N-terminal peptide, Phe-(Pro, Gly, Ile, Leu, Tyr, Phe, Arg), and of the C-terminal peptide, Arg(Ser, Pro, Ala). Chymotrypsin also split the peptide into two fragments, Phe-(Pro, Gly, Ile, Leu, Tyr, Phe), and Arg-(Arg, Ser, Pro, Ala) respectively. The dansyl derivative of the chymotryptic N-terminal peptide was not cleft further by repeated treatment of chymotryptic digestion because of the poor solubility of this derivative. The substractive Edman degradation and dansyl-Edman procedure were performed using each 50 nmol of the peptide. The both Edman procedure gave the similar results for the sequence of the peptide except in the last step of degradation. In the last sequence of the peptide, dansyl serine amide and a trace of amount of dansyl serine were detected by dansyl-Edman procedure when the reaction product was detected directly by thin-layer chromatography.

As a result of these experiments the sequence of this peptide was deduced to

Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg–Arg–Pro–Ala–Ser–NH_2.

The peptide possesses the notable sequence, in which hydrophobic amino acid residues are located in the N-terminal region, and the c-terminal region is hydrophilic and basic. The peptide seems to be a property of the natural detergent. The peptide was tested for the rat peritoneal mast cells and bathophils (probably immature mast cells) on degranulating action and showed the degranulation in the concentration of 5×10^{-9} mol/ml.

The peptide was named granuliberin-R and has been synthesized chemically.

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