The amino acid sequence of a salivary peptide (F-A) with histamine-releasing property

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[Accepted for publication: September 18, 1985]

Key words: Human parotid saliva / histamine release / histidine-rich peptide / amino acid sequence

We have found that human parotid saliva contained the substances responsible for releasing histamine. They were basic peptides whose histidine contents were extremely high¹⁾. Human parotid saliva has previously been shown to contain a group of histidine-rich polypeptides (HRPs)^{2,3)}. However, their function is unclear and the sequence of the individual HRPs have not been determined. In this report, we further purified one of the HRPs, F-A with histamine-releasing property and estimated its amino acid sequence.

Materials and Methods

A histamine-releasing peptide, F-A from human parotid saliva, was prepared as described previously¹⁾. Responses in vascular permeability were estimated by the passive skin test in rats⁴⁾. Histamine releasing assay, using rat isolated mast cells was examined as reported previously⁵⁾. Amino acid analyses were performed on an automated amino acid analyzer (IRICA, model A-5500) after 24 h of hydrolysis *in vacuo* in 6 N HCl at 120°C. Sequence analysis was carried out by Edman degradation in an Applied Biosystems Gas-Phase model 470A automatic sequencer. Phenylthiohydantoin derivatives of amino acids were identified by high performance liquid chromatography (HPLC)⁶⁾.

Results and Discussion

Four fractions containing histamine-releasing peptides, F-A, F-B, F-C and F-D were

obtained from Bio-Gel fraction by subsequent chromatography with DEAE-Sepharcel and CM-cellulose. Polyacrylamide gel electrophoresis containing sodium dodecylsulfate and urea (SDS-PAGE) showed that F-A was homogenous and that its molecular weight was about 3000 as reported previously¹⁾. The F-A was further purified on a column (0.8×25 cm) of Cosmosil 5C₁₈ (Nakarai) by HPLC (Fig. 1). The result of the purification procedure was a single peak of peptide, the aliquote of which induced the histamine release from rat isolated mast cells. When the F-A was injected into rat skin intradermally, the vascular permeability was increased (Fig. 2). The response occurred with as little as 0.1 µg of F-A, and increased as the concentration of F-A was raised. The injection of histamine (1 µg) and a histamine releaser, compound 48/80 (0.5 µg), also induced the permeability response. These responses were completely abolished in all upon injection with 1 µg of antihistamine, diphenhydramine hydrochloride (Fig. 2).

The amino acid composition of the purified F-A is shown in Table 1. The peptide was sequenced from the NH₂-terminus in one continuous run. The sequence of F-A was estimated as Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr by Edman degradation.

Recently, the HRPs of human parotid saliva have been separated into 6 components by Baum *et al.*²⁾ and 14 by Mackay *et al.*³⁾ respectively, using cationic gel electrophore-

Fig. 1 High-performance liquid chromatogram of F-A. The mobile phase was the solvent of 7% n-propylalcohol in 0.1% trifluoroacetic acid. The flow rate was 1 ml per min. The column used was Cosmosil 5C₁₈ (0.8 × 25 cm).

Fig. 2 Vascular permeability responses of F-A and its prevention by antihistamine. The black areas show the leakage of Evans blue. Site 1: saline, Sites 2, 3 and 4: F-A (0.1, 0.5 and 1 µg), Site 5: compound 48/80 (0.5 µg), Site 6: histamine (1 µg). Sites 7, 8, 9, 10, 11 and 12: each of these sites tested with diphenhydramine (1 µg).

sis. The F-A is considered as a peptide of these HRP. The amino acid composition of the F-A was similar to HRP-5 reported by Baum *et al.*²⁾ who estimated the molecular weight as 5600. However, the molecular weight of F-A was 3000 as estimated by SDS-PAGE and its value was in good agreement with that (3015) calculated from the amino acid sequence. Therefore, the F-A is a new peptide of the HRP in human parotid saliva and it stimulates histamine release

Table 1 Amino acid composition of F-A

Amino acid	Residues/100 residues
Lys	16.8 (4)
His	28.6 (7)
Agr	13.3 (3)
Asp	4.6 (1)
Thr	—
Ser	7.2 (2)
Glu	4.5 (1)
Pro	—
Gly	8.3 (2)
Ala	4.1 (1)
Leu	0.3
Tyr	8.1 (2)
Phe	4.2 (1)
Total residues	24

Numbers in parentheses are estimated after sequence analysis.

from mast cells both *in vivo* and *in vitro*.

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