Purification of a novel pancreatic secretory factor (PSF) and a novel peptide with VIP- and secretin-like properties (helodermin) from Gila monster venom

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A combination of three HPLC procedures applied to the venom of Gila monster (*Heloderma suspectum*) has led to the purification to homogeneity of two bioactive components: (i) a 17.5 kDa protein, isolated on the basis of its potent secretory effect on dispersed rat pancreatic acini, was accordingly designated PSF (pancreatic secretory factor); (ii) a 5.9-kDa peptide, designated helodermin, was purified on the basis of its ability to stimulate adenylate cyclase in rat pancreatic membranes. PSF was unable to activate adenylate cyclase and, conversely, helodermin was devoid of secretory action.

Gila monster venom *Pancreatic secretory factor* *Helodermin* *Secretin* *Vasoactive intestinal peptide* *Adenylate cyclase*

1. INTRODUCTION

In 1982, it was observed [1] that the venom from Gila monster exerts a potent secretory effect on dispersed pancreatic acini from guinea pig. Moreover, the venom causes a 50-fold increase in cyclic AMP and inhibits dose-dependently and competitively the binding of ^{125}I-vasoactive intestinal peptide (VIP). The authors concluded that the venom contains a factor that interacts with VIP receptors and activates adenylate cyclase, thereby stimulating enzyme secretion from pancreatic acinar cells.

We discovered recently that this conclusion did not hold true for the rat pancreas as two distinct components (at least) were responsible for, respectively, the activation of adenylate cyclase in pancreatic plasma membranes and the stimulation of amylase secretion from dispersed acini. Indeed, when submitting the venom to anion exchange chromatography, at pH 7.0, a component stimulating adenylate cyclase eluted nearly immediately from the column while another factor stimulating amylase secretion eluted at higher ionic strength. We then decided to purify these two bioactive components, in order to circumstantiate their biological activities and chemical structure.

This first report describes the purification procedure of helodermin (a potent activator of adenylate cyclase) and PSF (the major pancreatic secretory factor) from Gila monster venom, by a combination of anion exchange HPLC, gel permeation HPLC, and reverse-phase HPLC.

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Abbreviations: VIP, vasoactive intestinal peptide; PSF, pancreatic secretory factor from Gila monster venom; SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2. EXPERIMENTAL

2.1. *Purification procedures*

The content of one flask (100 mg) of Gila

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monster venom (*Heloderma suspectum*, lot 42F-0747 from Sigma, St. Louis) was dissolved in 2 ml water and the resultant turbid solution was centrifuged for 30 min at 100000 × *g*. The clear supernatant was desalted by chromatography on a Sephadex G-15 column (1.2 × 15 cm) in 20 mM Tris–acetate (pH 7.0). The active fraction emerged with the void volume and represented 95 mg protein when assayed as in [2]. This fraction (4 ml) was diluted with 1-propanol (30% final concentration) and the resulting precipitate was discarded by centrifugation. The supernatant, containing the whole biological activity and 80 mg protein, was chromatographed by anion exchange HPLC on a TSK-545 DEAE column as indicated in fig.1. Fractions rich in PSF and helodermin were pooled separately and further purified by reverse-phase HPLC on a Lichrosorb RP8 column (as detailed in fig.2) and thereafter by gel permeation chromatography on two connected columns of TSK-G2000 SW and TSK-G3000 SW (see fig.3).

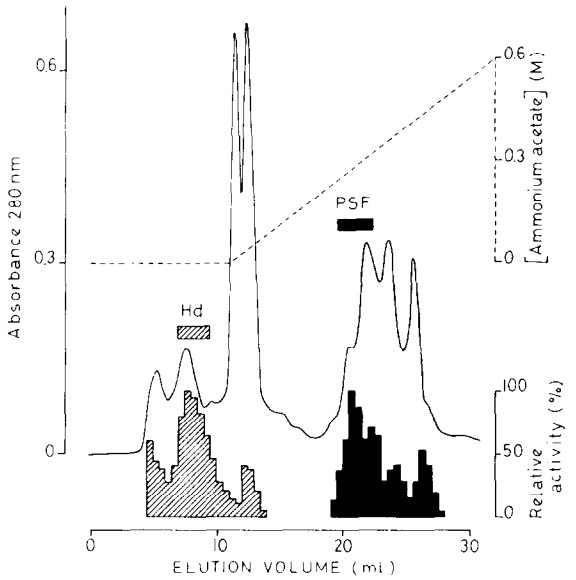

Fig.1. Anion exchange HPLC of Gila monster venom (80 mg protein) on TSK-545 DEAE (7.5 × 150 mm) equilibrated with 40 mM Tris–acetate buffer (pH 7.0) in 30% propanol and eluted at 0.5 ml/min with a linear gradient of ammonium acetate in the equilibrating buffer. Fractions of 0.5 ml were collected. Stimulations of adenylate cyclase activity (hatched columns) and amylase secretion (black columns) were measured on 1-μl aliquots of each fraction and expressed as % maximal activity. Helodermin (Hd) and PSF were collected as indicated.

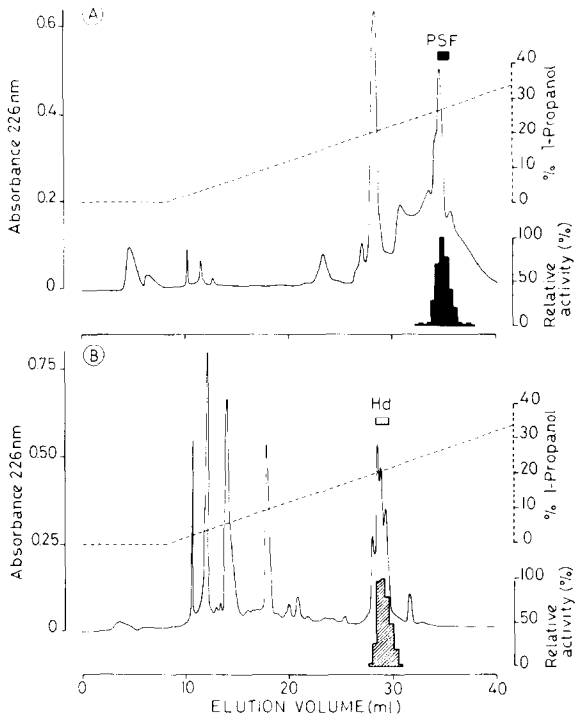

Fig.2. Reverse-phase HPLC on Lichrosorb RP-8 (4 × 250 mm) of PSF (A) and helodermin (B), collected after anion exchange HPLC (fig.1). The column was eluted at 0.4 ml/min (1 fraction/min) with a linear gradient of 1-propanol in 0.4 M ammonium acetate (pH 7.0). The active material was assayed as mentioned in the legend of fig.1 and collected as indicated.

Electrophoretic analysis was performed on SDS-polyacrylamide slab gels (18 × 20 × 0.7 cm) using the discontinuous buffer in [3] and a 10–20% acrylamide linear gradient gel with an acrylamide–bisacrylamide ratio of 20:1. The gels were fixed with formaldehyde and stained with Coomassie brilliant blue R 250 as in [4]. Protein standards were from Pharmacia Fine Chemicals (Uppsala). Synthetic porcine VIP was obtained from UCB Bioproducts (Brussels). Basic trypsin inhibitor was a gift from Bayer Pharma (Brussels). Synthetic porcine secretin was a generous gift from Dr W. König (Hoechst Aktiengesellschaft, Frankfurt/Main).

2.2. *Assays for biological activities*

The chromatographic tubes to be tested were

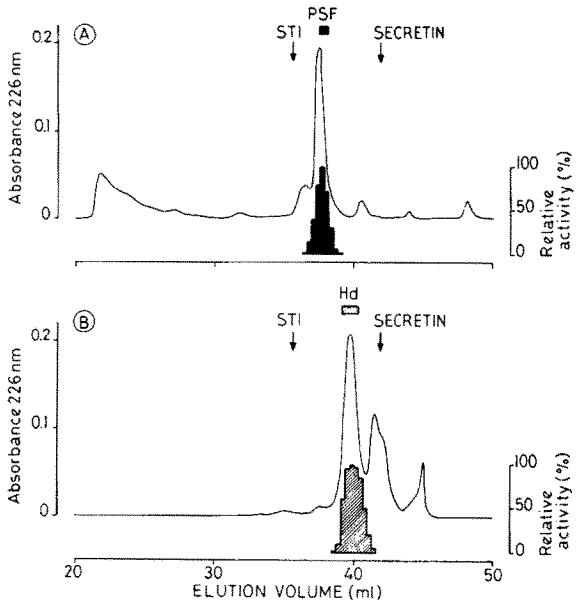

Fig.3. Gel permeation HPLC on two connected columns of TSK-G2000 SW (7.5 × 600 mm) and TSK-G3000 SW (7.5 × 600 mm) of PSF (A) and helodermin (B), collected after reverse-phase HPLC (fig.2). The columns were eluted at 0.2 ml/min (1 fraction/2 min) with 30% 1-propanol in 1 M ammonium acetate (pH 7.0). The active material was assayed as mentioned in the legend of fig.1 and collected as indicated, after checking the purity of each fraction by SDS–PAGE (see fig.4). The arrows indicate the elution position of soybean trypsin inhibitor (STI) and secretin.

lyophilized to dryness and the dry residue dissolved with the initial volume of distilled water. The equivalent of 1 μl of each fraction was used, per assay, for the measurement of either adenylate cyclase stimulation or amylase secretion.

Rat pancreatic membranes were prepared as in [5] but 2-mercaptoethanol was omitted from all buffers used. Adenylate cyclase activity was measured as in [6]; i.e., in the presence of 10 μM GTP and according to [7].

Rat pancreatic acini were prepared as in [8], and 0.2-ml aliquots of dispersed acini were incubated per assay. The released amylase activity was determined as in [9].

2.3. *Columns*

The columns used were: TSK-545 DEAE, TSK-G2000 SW and TSK-G3000 SW from LKB (Bromma) and Lichrosorb RP-8 from Merck (Darmstadt).

3. RESULTS AND DISCUSSION

Fig.1 shows the initial anion exchange chromatography separating PSF from helodermin. Only the major peaks of biological activities were pooled for further purification and there was no overlapping between the fractions stimulating adenylate cyclase activity of pancreatic membranes and those stimulating amylase secretion from isolated acini. PSF was, therefore, a potent secretagogue unable to activate adenylate cyclase. On the contrary, helodermin was a potent activator of adenylate cyclase activity (like VIP and secretin) but inactive on amylase secretion (unlike

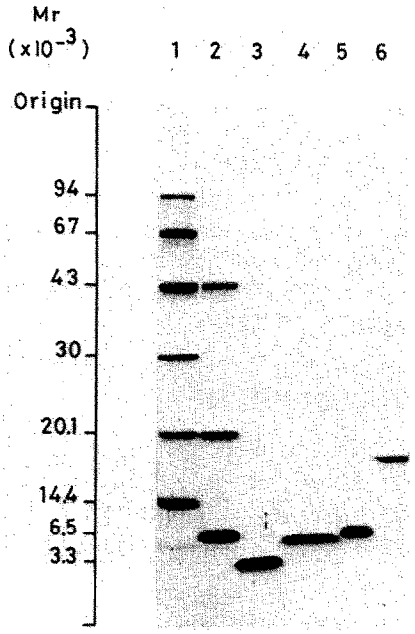

Fig.4. SDS–PAGE of chromatographically purified helodermin (lane 4) and PSF (lane 6). Electrophoresis was performed on a 10–20% acrylamide gradient as described in section 2. M_r standards were: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soybean trypsin inhibitor, 20100; and α-lactalbumin, 14400 (lane 1); ovalbumin, soybean trypsin inhibitor, basic trypsin inhibitor, 6500 (lane 2); VIP, 3300 (lane 3); basic trypsin inhibitor (lane 5).

secretin, and even unlike VIP which is still a poor secretagogue on the rat pancreas).

PSF and helodermin were further purified by reverse-phase HPLC (fig.2) and by gel permeation HPLC (fig.3). The purity of the fractions collected after gel permeation HPLC was checked by SDS–PAGE (see below) and the uncontaminated fractions, subsequently pooled as indicated in fig.3, yielded, respectively, 130 μg PSF and 380 μg helodermin when starting from 100 mg crude venom. The recovery of PSF could be improved by increasing the resolution power of both reverse-phase HPLC and gel permeation HPLC or by simply recycling the contaminated fractions.

The M_r value, as determined by SDS–PAGE, was 17500 for PSF and 5900 for helodermin (fig.4). Similar values could be deduced by gel permeation, i.e., under non-denaturing conditions, by comparing elution volumes with those of soybean trypsin inhibitor and secretin (fig.3). PSF was more lipophilic and anionic in character than helodermin. Helodermin itself comigrated with VIP by reverse-phase chromatography in 0.4 M ammonium acetate (pH 7.0) with a propanol concentration of about 20%. However, in 0.01 M HCl and using an acetonitrile gradient, VIP eluted before helodermin (not shown).

The biological properties of PSF and helodermin will be further documented in the two accompanying papers [10,11].

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