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INVESTIGATION OF THE BIOCHEMICAL PROPERTIES OF FRACTIONS OF THE SECRETION OF GABE'S GLAND OF *Varanus griseus*

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The optimum conditions for the fractionation of the saliva of the desert monitor by gel filtration have been determined. Three protein fractions have been obtained and their protein compositions have been subjected to electrophoretic analysis. The distribution of the activities of a number of enzymes and of a nerve growth factor over the fractions has been studied.

The secretion of Gabe's gland may serve as a convenient model for the study of the evolutionary transformation of reptilian saliva into venom. In representatives of the family Helodermatidae, this gland produces a most powerful venom, comparable in toxicity with snake venom. However, there is no toxin in the monitor saliva secreted by this gland [1], although the bite of the monitor is very painful and is accompanied by a local inflammatory reaction [2].

On biochemical investigation it was found that the saliva of the desert monitor contains enzymes that are characteristic for snake venoms — a hyaluronidase, a phospholipase A, and an endopeptidase — and also a nerve growth factor [3, 4].

We have made a selection of the optimum variants of the fractionation of *Varanus griseus* saliva and have investigated the distribution of its components over the fractions. One of the widely used methods of fractionating a protein mixture is gel filtration. In the separation of the components of snake venoms, solutions of metal acetates and acetic acid are successfully used as eluents. However, these solutions have proved unsuitable for dissolving dried monitor saliva since they cause coagulation of part of the saliva proteins. We therefore used 1/15 M phosphate buffer, pH 7.2, containing 0.2 M sodium chloride for the dissolution and gel filtration of the saliva. In working on columns with the supports TSK gel HW-60, TSK gel HW-55, and Ultragels AcA 44 and AcA 54 with various ranges of resolution it was found that fractionation was more successful on supports designed for the separation of proteins in the MM range of 500-100,000 kDa (Fig. 1).

In each case, the monitor saliva was separated into three fractions, designated in order of their issuance from the column as I–III. On TSK gel HW-55, fraction II was separated into two subfractions; and on Ultragel AcA 54 a considerable separation of the third fraction from the first two was achieved.

To study the distribution of the activities of the enzymes and of the nerve growth factor, each fraction was collected portionwise in an automatic collector. Hyaluronidase activity was detected mainly in fraction I, it regressed in fraction II, and was completely absent from fraction III (Fig. 2). Calculation of the hyaluronidase activity per 1 mg of protein indicated a 6.5-fold purification of this enzyme. Endopeptidase activity, as also the activity of the nerve growth factor, was concentrated in fraction II and was associated with its lower-molecular-mass components. In the following series of analyzed samples, the distribution of endopeptidase activity corresponded completely to the distribution of the nerve growth factor in them. It is possible that these proteins form a complex, as is observed in mouse saliva and in *Crotalus adamanteus* venom [5, 6]. A more detailed study of the nerve growth factor and the endopeptidase requires considerable amounts of starting material, since the concentration of protein with nerve growth activity is not more than 4.4% of the total amount of fractionated proteins of desert monitor saliva.

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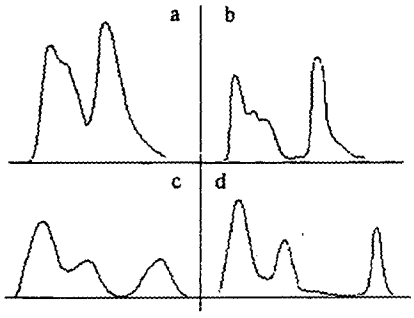

Fig. 1. Elution profiles in the gel filtration of desert monitor saliva (1/15 M phosphate buffer, pH 7.2, 0.2 M NaCl) on columns filled with: a) TSK gel HW-40 (1.4 × 40 cm); b) TSK gel HW-55 (1.4 × 105 cm); c) Ultragel AcA 44 (2 × 102 cm); d) Ultragel AcA 54 (1.6 × 84 cm).

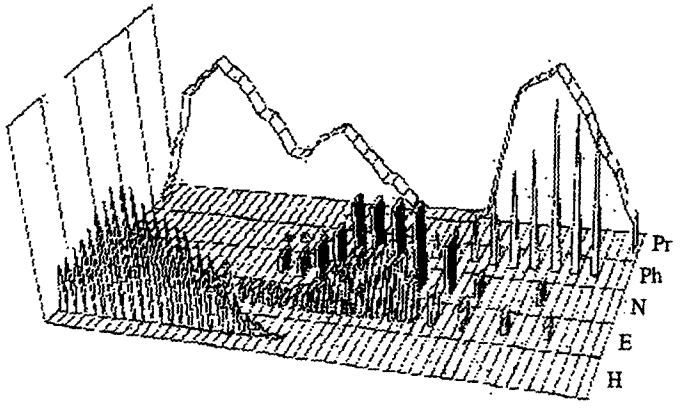

Fig. 2. Distribution of the activities of the enzymes (H — hyaluronidase; E — endopeptidase; Ph — phospholipase) and of nerve growth factor (N) in protein (Pr) samples in the gel filtration of the monitor venom on a column of AcA 44 (2 × 102 cm).

The phospholipase activity was localized in fraction III, as was established from the change in the optical density of a solution of egg yolk under the action of the enzyme in a specific test [7]. However, the components of this fraction had no effect on the coagulation of egg yolk on heating and did not cause hemolysis of erythrocytes: i.e., they did not possess the characteristic properties of the phospholipase A of snake venoms.

Subsequently, each fraction was desalted by dialysis and was freeze-dried. These procedures had an adverse effect on fraction I. It became sparingly soluble both in water and in phosphate buffer. An aqueous solution of redissolved fraction I contained 47% of the protein of the native fraction, and the hyaluronidase activity had decreased by a factor of 2.4. Dialysis and freeze-drying did not affect the solubilies of fractions II and III but led to a twofold decrease in the phospholipase activity of fraction III. The lability of the monitor saliva is therefore due above all to its high-molecular-mass components with hyaluronidase activity.

The compositions of the dried fractions I-III were analyzed by electrophoresis in alkaline and acid buffer systems in the presence of 0.1% Na-DDS and also by isoelectric focusing in PAAG. The proteins of fraction I were characterized by low electrophoretic mobility under both acid and alkaline conditions. In isoelectric focusing, eight protein bands were revealed at pH 5-8. In the presence of Na-DDS, this fraction was represented in the form of two major and two minor proteins with MMs greater than 50 kDa. Whether these proteins correspond to isoforms of hyaluronidase or represent individual proteins or a single complex will be determined by the further fractionation and analysis of fraction I.

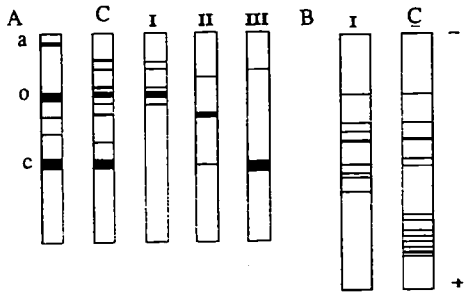

Fig. 3. SDS electrophoresis (A) and isoelectric focusing (B) in PAAG of fractions I–III and the whole saliva of the monitor (C). Markers: a) albumin; o) ovalbumin; c) cytochrome.

Fraction II contained a whole assortment of weakly acidic proteins of the monitor saliva, as was determined by electrophoresis under acid and alkaline conditions. In the presence of Na-DDS they all had practically the same molecular mass of 26-27 kDa.

Fraction III was characterized by a high electrophoretic mobility and was revealed in the form of a single protein band in an acid buffer system, while under alkaline conditions traces of protein were detected only in the starting zone of the gel. On Na-DDS electrophoresis, the protein of fraction III again appeared in the form of a single band, with MM 13 kDa.

Thus, gel filtration on supports designed for the separation of proteins in the MM range of 500-100,000 kDa and in 1/15 N phosphate buffer, pH 7.2 with 0.2 M NaCl permit not only the successful separation of monitor saliva proteins having various biological activities and differing both in molecular mass and in charge but also the isolation of an electrophoretically homogeneous protein (fraction III) in a single stage of purification.

EXPERIMENTAL

Fractionation on TSK gel Toyopearls HW-55 and HW-60 was conducted in accordance with the recommendations of the firm Toyo Soda (Japan), and on Ultragels AcA 44 and AcA 54 in accordance with the recommendations of the firm Pharmacia LKB (Sweden).

Proteins were determined by Lowry's method [8].

Meyer's method [9] was used for determining hyaluronidase activity, Joubert's [10] for phospholipase activity, Satake's for endopeptidase activity, and Fenton's [11] for nerve growth activity.

Disk electrophoresis was conducted in alkaline (7% PAAG, tris-glycine buffer, pH 8.3) and acid (15% PAAG, acetic acid–β-alanine buffer, pH 4.5) buffer systems, and also in 7.5% PAAG in phosphate buffer, pH 7.2 in the presence of 0.1% Na-DDS, using a Reanal (Hungary) instruments and Reanal reagents [12, 13]. The molecular masses of the proteins detected were calculated from a calibration graph of the dependence of relative electrophoretic mobilities on the molecular masses of standard proteins: BSA (67 kDa), ovalbumin (45 kDa) and cytochrome C (13 kDa).

Isoelectric focusing was conducted in a thin layer of 7% PAAG at pH 3.5-9.5 on a Multiphor instrument (Sweden). The gels were stained with a 0.1% solution of Brilliant Blue R

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