



PURIFICATION, SEQUENCING, CLONING AND EXPRESSION OF A HYALURONIDASE FROM THE CENTIPEDE *Scolopendra viridis* Say VENOM

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Introduction. Venoms from arthropods are a source of enormous diversity of high and low molecular weight biologically active components with potential novel pharmacological, therapeutic and research applications ⁽¹⁾. Centipedes have poison glands connected to a pair of forcipules, which they use to inject venom into their prey. Among the main components involved in local tissue damage and the spread of venom in the prey, there are metalloproteases and hyaluronidases ⁽²⁾.

The objective of this work is to clone and express a peptide of 8 kDa with hyaluronidase activity from the venom of *Scolopendra viridis* Say (SvS).

Methods. Centipedes are collected at the state of Morelos; and venom is obtained by electrical stimulation. Hyaluronidase activity was identified in the venom through zymograms, and the peptide with this activity was identified through a two-dimensional gel in the presence of hyaluronic acid (2 mg/ml of gel). Purification of the protein with hyaluronidase activity is performed by the electroelution method ⁽³⁾. The pure protein is passed through HPLC on a C18 column with a linear gradient; this is done to check the purity of the protein ⁽⁴⁾. With the purified protein is made of amino acid sequence by Edman degradation. It will analyze the sequence with other reported hyaluronidases. From the total RNA extracted from a pair of glands centipede SvS create a cDNA library to carry out the cloning and expression of the peptide in *E. coli*.

Results. A two-dimensional gel in the presence of hyaluronic acid shows several proteins with hyaluronidase activity (Fig. 1) in scolopendra venom.

Until now have not been reported small molecular weight proteins (<10 kDa) with hyaluronidase activity in other organisms.

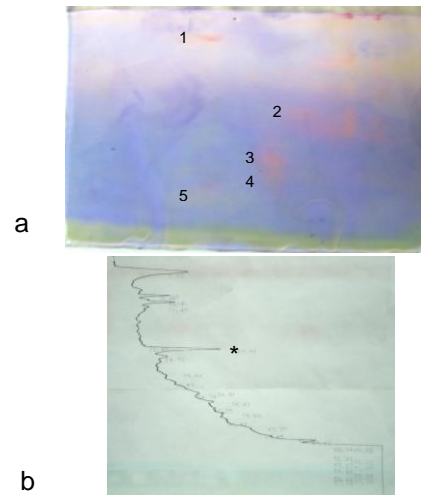


Fig.1 a) Two-dimensional gel the presence of hyaluronic acid. The presence of white spot indicates activity. b) Chromatogram from the 10 kDa protein, the asterisk indicates the peak of this.

Table 1. Molecular weight and pI of some proteins with hyaluronidase activity in the venom of the SvS

No.	PM (kDa)	pI
1	76	9.8
2	27	6.7
3	13	7.8
4	10	7.8
5	8	9.5

Conclusions. We identified several small molecular weight protein with hyaluronidase activity in the venom of the centipede SvS. Furthermore, the protein of 10 kDa showed a retention time of 22.31 min by HPLC. The amino acid sequence determination of the pure protein was performed by automatic Edman degradation.

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