ISOLATION OF TWO MAMMALIAN TOXINS FROM THE VENOM OF THE MEXICAN SCORPION *CENTRUROIDES ELEGANS* (THORELL)

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1. Introduction

The venom of several species of Mexican scorpions from the genus *Centruroides* contains polypeptides which display a high degree of toxicity toward vertebrates [1–3]. One of the challenging problems in the purification and characterization of such toxins is the relatively small amount of material that can be obtained from each animal. Often biochemical work is made possible only after collecting a few grams of venom from several thousand scorpions. We wish to report here the isolation of two pure protein components from four toxic fractions separated from the venom of *Centruroides elegans*. The experiments presented at this time were carried out with only 10 mg crude soluble venom.

2. Materials and methods

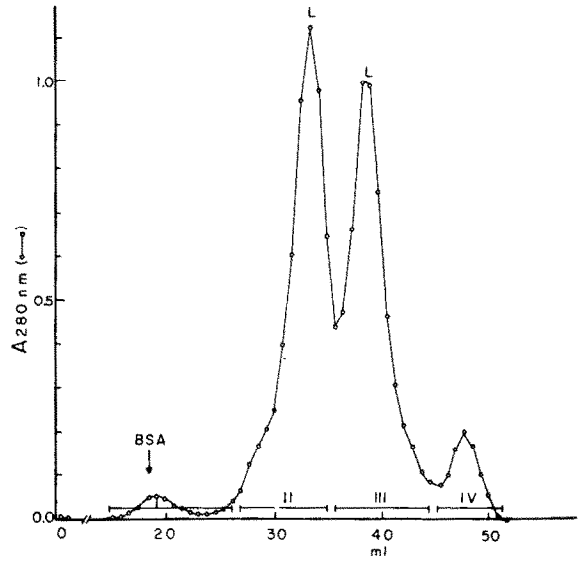
Only analytical grade reagents and solvents were used. The venom of 23 scorpions collected in Chamela, State of Jalisco, Mexico was obtained by electrical stimulation and immediately frozen. After 3 months storage at −20°C the venom was solubilized in cold distilled water and centrifuged at 27 000 × *g* for 30 min. The supernatant (A_{280} = 17.0 total) was used as starting material. Ten mg of this supernatant (assuming 1.0 A_{280} = 1 mg/ml) was chromatographed on a Bio-Gel P-10 (Bio-Rad Lab.) column, followed by rechromatography of the toxic fractions on a CM–Sepharose CL 6B (Pharmacia Fine Chemicals) column. The homogeneity of the chromatographed fractions was ascertained by polyacrylamide gel electrophoresis in β-alanine–acetate–urea system described in [4]. The homogeneous components were dialysed against 1 mM ammonium acetate buffer, pH 4.7, using Spectrapor Type 3 dialysis tubing (mol. wt ~3500 cutoff; Spectrum Medical Industries) and lyophilized. Duplicate samples of protein were prepared for acid hydrolysis in 6 N HCl with 0.5% phenol added for the protection of tyrosine in individually evacuated and sealed glass tubes. Hydrolysis was carried out on these samples at 110°C for 20 h, 40 h, 72 h and 120 h. Half-cystine content of this protein was determined as cysteic acid according to the method in [5]. Tryptophan was determined after hydrolysis of the protein in 4.2 N NaOH as in [6] modified for 100 μl vol. and substituting 4% thiodiglycol instead of starch. The amino acid analysis was carried out on the Durrum D-500 Amino Acid Analyzer modified for high sensitivity with the Mark II Data Analysis System.

Toxicity of samples removed during purification was determined by intraperitoneal injection into albino mice (local strain) weighing 20 g each.

3. Results and discussion

Injection of 100 μg crude soluble venom caused death of the mice with the following main symptoms: dyspnea, paralysis of hind limbs, convulsions and respiratory failure within 60 min after injection. Control animals injected with saline produced none of these effects.

Gel permeation chromatography was carried out on a Bio-Gel P-10 column (fig.1). Four main fractions were eluted on this column; fractions II and III were



lethal to mice on injection of 170 μg and 150 μg, respectively. Fractions I and IV were not toxic when the same amounts were injected.

Further purification of the toxic fractions isolated from the gel filtration chromatography was carried out by ion-exchange chromatography. Fraction II was rechromatographed on CM–Sepharose CL 6B cation exchange column as shown in fig.2a. None of the pooled peaks isolated from this chromatography were

Fig.1. Fractionation of soluble venom. Bio-Gel P-10 column (0.9 × 59 cm), equilibrated and eluted with 20 mM ammonium acetate buffer, pH 4.7, flower rate 3.2 ml/h, 0.8 ml fraction vol., loaded with 0.6 ml soluble venom. Horizontal bars with roman numerals indicate pooled fractions: L, lethal fractions; BSA, bovine serum albumin used as molecular weight marker for the exclusion volume of the column.

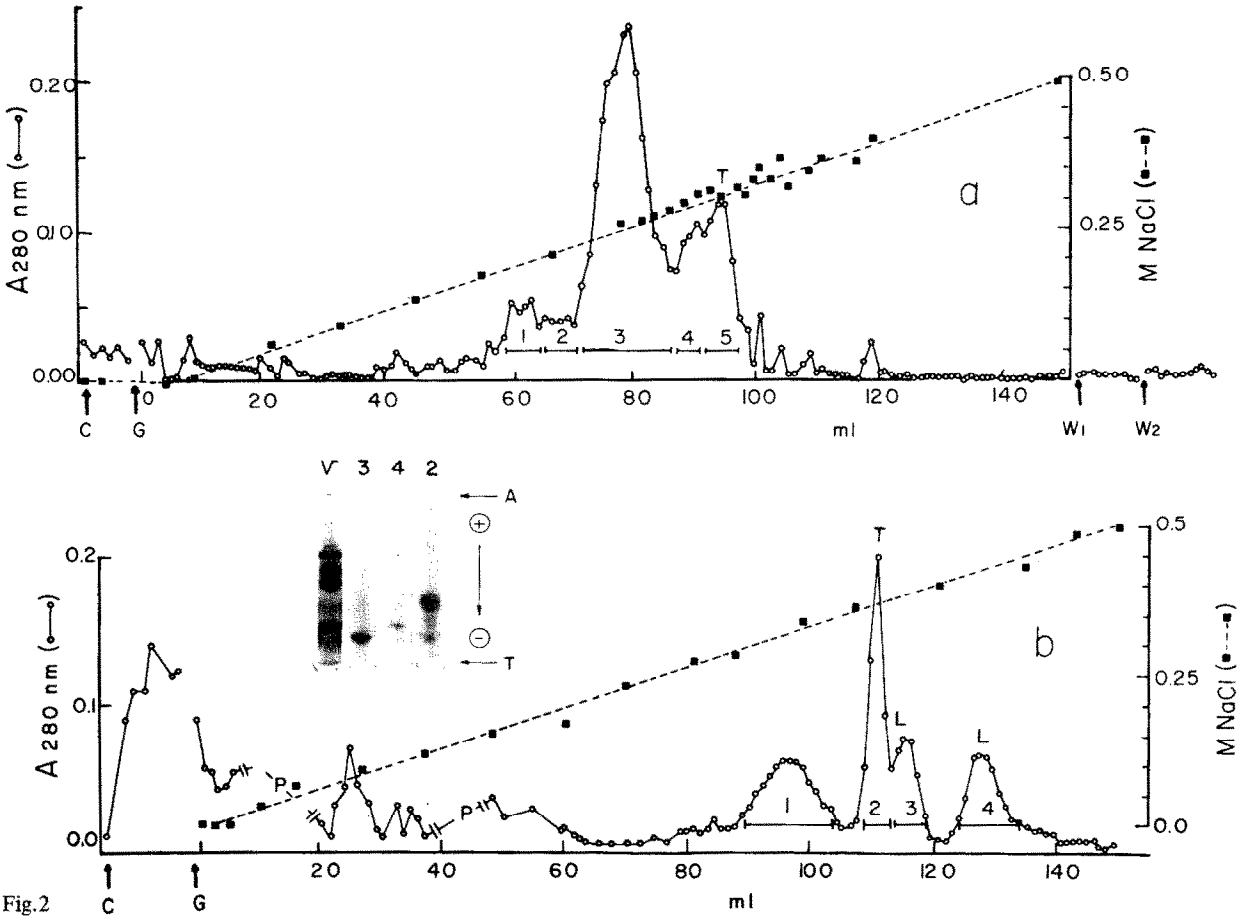


Fig.2

lethal in doses of 50–70 μg/mouse. Component II-5 demonstrated slight toxicity at 50 μg.

The three most basic components from the rechromatography of fraction III were toxic (fig.2b). Fractions III-3 and III-4 were lethal to mice injected with 39 μg and 34 μg, respectively. These fractions produced only a single Coomassie blue staining band on gel electrophoresis. Fraction III-2 produced 3 bands on gel electrophoresis and was toxic to mice at 80 μg. Components III-1 to III-4 accounted for 50% of the starting material. Overall recovery was 96% for both gel permeation and ion-exchange chromatography.

The component 3 of fig.2b named toxin III.3 was obtained in low amounts, allowing only the 20 h, 40 h, 72 h and 110 h acid hydrolysis of 300 pmol each for amino acid composition determination. No tryptophan or half-cystine was determined. The composition of toxin III.3 is as follows:

Asp 5, Thr 3, Ser 3, Glu 8, Pro 2, Gly 8, Ala 3,

Val 3, Met 0, Ile 0, Leu 4 or 5, Tyr 6, Phe 2, Lys 6,

His 2, Arg 2

with a total of 58–59 amino acid residues (plus Trp and Cys). Comparing this analysis with the composition of other toxins from the venom of scorpions of the genus *Centruroides* it has a clear similarity with toxin III, purified from *Centruroides suffusus suffusus* in [3]. In both cases no methionine or isoleucine were found and most of amino acids have the same value. The complete amino acid analysis of toxin III.4 (component 4 in fig.2b) using only 2.5 nmol material has revealed the following composition:

Asp 5, Thr 2, Ser 5, Glu 5, Pro 1, Gly 6, Ala 4,

Cys 4 or 5, Val 2, Met 0, Ile 1, Leu 3, Tyr 5, Phe 1,

Lys 4, His 2, Arg 2, Trp 1

with a total of 53–54 amino acid residues with only 4–5 cysteines that makes it a very different toxin from the others extracted from venom of the scorpions *Centruroides sculpturatus* [1,2] and *Centruroides suffusus suffusus* [3], as well as toxins extracted from scorpions belonging to other genera: *Buthus, Leirus, Androctonus* and *Tityus* [7–11]. Normally scorpion toxins have 8 half-cystines and are composed of 62–66 amino acids [7–11]. We are now determining the primary structure of this toxin. If the amino acid composition presently reported is confirmed by sequence analysis this toxin can be used to study the structural differences among other toxins from scorpion venom, that is an important contribution to the study of the relationship between structure and function of these toxic polypeptides.

In our opinion also one of the main contributions of the present communication is to show that it is possible to isolate and partially characterize toxins from the venom of small animals with small amounts of venom (10 mg) provided that techniques allowing good recoveries and high sensitivity instruments are available.

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Fig.2. Rechromatography of toxic fractions. CM–Sepharose CL 6B column (0.5 × 38 cm) equilibrated with 20 mM ammonium acetate buffer, pH 4.7, eluted with a linear gradient of NaCl from 0–0.5 M (75 ml each): flow rate 6.77 ml/h, 1.1 ml fraction vol. Horizontal bars with arabic numbers indicate pooled fractions. 'a' and 'b' refer to fractions II and III from Bio-Gel P-10 chromatography. T denotes toxic fraction, meaning increased excitability of the mouse accompanied by salivation. L denotes lethal fraction as described in section 3. Inset in 'b' indicates the electrophoretic pattern of V = whole soluble venom (400 μg), 3 = fraction III-3 (21 μg), 4 = fraction III-4 (24 μg) and 2 = fraction III-2 (36 μg), A = application point, T = tracking dye, C and G accompanied by arrow indicate loading of material and beginning of the gradient, respectively. W_1 and W_2 indicate successive washes of the column with 1 M and 2 M NaCl in the same buffer. P in 'b' indicates tubes with some precipitated material.

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