

The purification and amino acid sequence of the lethal neurotoxin Tx1 from the venom of the Brazilian 'armed' spider *Phoneutria nigriventer*

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A lethal neurotoxic polypeptide of M_r 8 kDa was purified from the venom of the South American 'armed' or wandering spider *Phoneutria nigriventer* by centrifugation, gel filtration on Superose 12, and reverse phase FPLC on columns of Pharmacia PepRPC and ProRPC. The purified neurotoxin Tx1 had an LD_{50} of 0.05 mg/kg in mice following intracerebroventricular injection. The complete amino acid sequence of the neurotoxin was determined by automated Edman degradation of the native and S-carboxymethylated protein in pulsed liquid and dual phase sequencers, and by the manual DABITC/PITC double coupling method applied to fragments obtained after digestions with the *S. aureus* V8 protease and trypsin. The neurotoxin Tx1 consists of a single chain of 77 amino acid residues, which contains a high proportion of cysteine. The primary structure showed no homology to other identified spider toxins.

Spider venom; Amino acid sequence; Polypeptide neurotoxin; *Phoneutria nigriventer*

1. INTRODUCTION

The venom of spiders contains several distinct classes of neurotoxins [1,2]. Numerous examples are known of low molecular weight acylpolyamine toxins which cause immediate, but reversible paralysis in insects associated with postsynaptic blockade of glutamate-sensitive receptor channels [3,4]. There has, however, been little study on the structures of the higher molecular weight neurotoxic polypeptides. So far the only complete amino acid sequences known are for robustotoxin and versutoxin from the Australian funnel web spiders *Atrax robustus* [5] and *A. versutus* [6], the insect toxin from the Russian cellar spider *Segestria florentina* [7] and the μ -agatoxins from *Agelenopsis aperta* [8].

The venom of the very aggressive South American solitary 'armed' or wandering spider *Phoneutria nigriventer* contains potent neurotoxins and is responsible for most human accidents of araneism, including the death of infants, in Central and Southern Brazil [9–12]. The major toxic effects of the crude venom appear to be caused by discharges of repetitive action potentials in nerve and/or muscle fibre membranes and have been ascribed to the activation of sodium channels [13,14]. Workers at the Fundação Ezequiel Dias in Brazil [15] have now purified 4 separate types of

neurotoxic polypeptides from the venom of this spider and here we report the purification and primary structure of the neurotoxin Tx1.

2. MATERIALS AND METHODS

2.1. Venom

The venom was collected from mature male and female spiders of *Phoneutria nigriventer* (Ctenidae, Labidognetha) maintained at the Fundação Ezequiel Dias in Belo Horizonte, Brazil. The venom ($\pm 10 \mu\text{l}$ per spider) was obtained at monthly intervals by electrical stimulation of live spider fangs and aspirated into capillary tubes held at 0°C. If not used immediately, the venom was lyophilized.

2.2. Purification of neurotoxin Tx1

Samples of venom (1–2 ml) were first centrifuged at $4000 \times g$ to remove an insoluble protein. Pooled samples of centrifuged venom (0.2–0.3 ml) or 20 mg of lyophilized venom dissolved in 0.2 ml of 0.15 M Na formate buffer pH 6.3 were subjected to FPLC gel filtration on a column (1 \times 30 cm) of Superose 12 (Pharmacia Ltd) in the same buffer (Fig. 1). The fractions eluted were assayed for toxicity in mice as described below. The pooled toxic fractions were lyophilized and then applied to a column (0.5 \times 5 cm) of PepRPC 5/5 (Pharmacia) in 0.1% aqueous trifluoroacetic acid. The column was eluted with a gradient (0–50% v/v) of acetonitrile in 0.1% TFA (Fig. 2).

The toxic material in the major peak which eluted at 22% concentration of acetonitrile was then rechromatographed on a reverse phase column (0.5 \times 10 cm) of ProRPC 5/10 (Pharmacia) using a linear gradient (0–50%, v/v) of acetonitrile in 0.1% TFA (Fig. 3).

2.3. Assessment of toxicity

Fractions were tested for toxicity by intracerebroventricular injection of 30 μl samples (in 0.15 M physiological saline) into 20–25 day old white mice (19 g body weight). Twelve animals per dose, at 6 dose

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Table I

The amino acid composition of *Phoneutria nigriventer* neurotoxin Tx1

Amino acid	Methods of hydrolysis			Sequence
	4 M MSA	5.6 M HCl	Average	
Asx	10	11.66	10.84	11
Thr	1	1.48	1.24	1
Ser	3	2.96	2.98	3
Glx	3	3.77	3.38	4
Pro	3	5.03	4.02	4
Gly	10	7.92	8.96	7
Ala	7	4.75	5.87	5
Cys	14	11.70	12.85	14
Val	5	4.66	4.85	5
Met	0	0.26	0.13	0
Ile	1	0.95	0.97	1
Leu	2	1.70	1.85	2
Tyr	3	2.96	2.98	3
Phe	1	1.23	1.11	1
His	2	1.64	1.82	2
Lys	6	5.90	5.95	7
Arg	3	3.82	3.41	4
Trp	+	n.d.	—	3

Values represent residues/mol

22% concentration of acetonitrile was finally purified by a further reverse phase FPLC step on a ProRPC column (Fig. 3).

Venom (2 ml) from 200 spiders thus yielded 1.35 mg of a product which was homogenous on SDS-PAGE and had an apparent M_r of 8 kDa. The concentration of Tx1 in the venom was 0.45% of the total protein. Purified Tx1 had an LD₅₀ of 0.05 mg/kg in mice when injected intracerebroventricularly. This value contrasts strongly with the figures of 0.355–0.424 mg/kg reported previously for the whole venom injected intravenously [13].

The amino acid sequence of neurotoxin Tx1 determined by both automated and manual sequencing methods is shown in Fig. 4. The manual DABITC method was particularly useful in confirming the identity of the TRP residues in positions 36 and 64, the LYS in position 71 and the four residues at the C-terminus. The sequence is in good agreement with the results of amino acid analyses shown in Table I, and the M_r of 8216 calculated from the sequence corresponds well with the estimates of 8 kDa made from SDS-PAGE.

In common with the toxins isolated from various snake, scorpion and other spider venoms, neurotoxin Tx1 from *Phoneutria nigriventer* has a high content of cysteine. However, comparison of the sequence of Tx1 with the sequences of the other known spider toxins [5–8] all of which are only half of the size of Tx1 revealed no significant similarities. Furthermore, no homologies were found when the sequence was com-

pared by computer with those of other proteins stored in the US National Biomedical Research Foundation 1988 Databank.

The results of further neurophysiological and pharmacological investigations on the *Phoneutria nigriventer* neurotoxin Tx1 will be presented elsewhere.

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