

## The Channel-Forming Component of the *Theridiidae* Spider Venom Neurotoxins

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**Abstract.** It is known that *Steatoda (Lityphantes) paykulliana* and *Latrodectus mactans tredecimguttatus* spider venoms are toxic to mammals and insects. These venoms act presynaptically eliciting massive release of transmitters. They also form channels in bilayer lipid membranes (BLM) that are selective for cations. Venoms of both spider species were fractionated by gel filtration on a Sephadex G-100 column. The fraction obtained were tested on neuromuscular preparations of frog and locust and on BLM. A fraction of low molecular weight components (about 5000 daltons and less) was disclosed. This fraction showed presynaptic and channel-forming effects similar to those of crude venoms and of high molecular weight toxin fractions, obtained simultaneously from these venoms. It was shown that channels formed in BLM by crude venoms and its different fractions are identical. Also, it was found that the low molecular weight channel-forming component is a construction element of high molecular weight toxins. On the basis of data obtained a toxin structure model of the *Theridiidae* family spider venoms was proposed.

**Key words:** Synapse — Bilayer membrane — Channels — Toxins

### Introduction

It has been shown previously that *Latrodectus* spider venoms contain components with a molecular weight of about 130,000 daltons (Frontali et al. 1976). Toxins selective for vertebrates and insects have been found among these components (Ornberg et al. 1976; Bettini and Maroli 1978). It has been established that all of them act presynaptically and increase the frequency of miniature end-plate potentials (MEPPs) (Smyth et al. 1978).

The present paper contains a report on spider venoms of *Latrodectus mactans tredecimguttatus* and *Steatoda (Lityphantes) paykulliana* their fractionation, separation of a low molecular weight component from neurotoxins, and on action of

crude venoms and some of their fractions on bilayer lipid membranes (BLM) and frog and locust neuromuscular synapses.

## Materials and Methods

The influence of venoms and their fractions on synaptic transmission was studied in neuromuscular preparations of frog (*Rana temporaria*) sartorius muscle and locust (*Locusta migratoria*) dorso-ventral wing muscle number 119 (according to the locust muscle nomenclature by Snodgrass 1929). Dissected preparations were mounted in an experimental bath with a volume of 2 ml, and perfused with saline. Solutions used had following compositions (in mmol/l): NaCl 108; KCl 2.5; CaCl<sub>2</sub> 1.8; MgCl<sub>2</sub> 2.0; NaH<sub>2</sub>PO<sub>4</sub> 0.8; Na<sub>2</sub>HPO<sub>4</sub> 2.1; pH 7.2 — for frog preparations; NaCl 134; KCl 6.0; CaCl<sub>2</sub> 2.0; MgCl<sub>2</sub> 2.0; NaH<sub>2</sub>PO<sub>4</sub> 6.0; KHCO<sub>3</sub> 4.0; pH 7.2 — for locust preparations. Intracellular synaptic potentials were recorded using conventional recording techniques. The microelectrodes were filled with 3 mol/l KCl and had a resistance of 5–20 MΩ. Before recordings were started the preparations were soaked in the solutions for about 30–60 min.

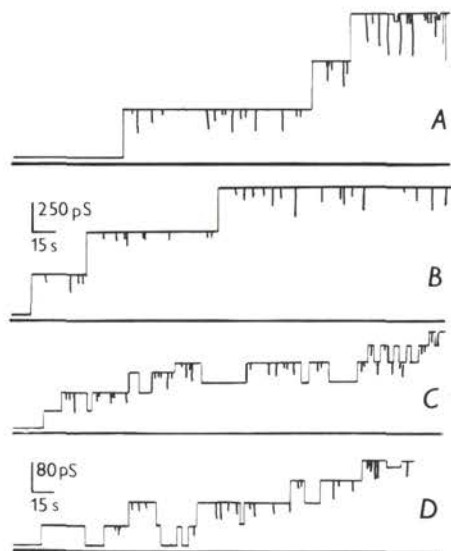
Planar bilayer lipid membranes were formed with lipids on a 0.3 mm hole of a Teflon cell. Total phospholipids from bovine brain were prepared by the method described by Folch et al. (1957). Small amounts of lipids in decane were aspirated into a micropipette and then applied to the hole in Teflon septum. During the application a fine cloud of material could be observed through the microscope which disappeared during the thinning process. The electric parameters of BLM were recorded in voltage clamp regime by an electrometric amplifier U 1–7. Ag–AgCl electrodes were used to apply fixed potentials from the voltage source and to measure membrane current. The membrane currents were recorded using a potentiometer recorder KSP 41. The experiments were performed at room temperature (20–24 °C).

Spider venoms were obtained by extraction of homogenized adult female venom glands. The obtained extracts were centrifuged at 10,000 g for 10 min and the supernatant was collected and lyophilized. All venom preparations steps and storage were carried out in cold at 0–4 °C.

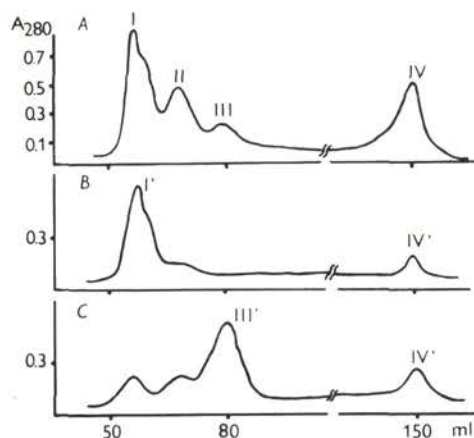
The spider venoms were fractionated by gel filtration on columns (1.5 × 90.0 cm) packed with Sephadex G-100 equilibrated with 0.01 mol/l phosphate buffer (pH 8.0), containing 0.01 mol/l NaCl. The elution was accomplished with the same buffer solution. The elution velocity was 4.5 ml/h. The fractions were combined according to their protein "peaks" and used in experiments, or concentrated, desalted on Sephadex G-25 column, lyophilized and assayed. Protein concentrations in venoms and their fractions were measured by the method of Lowry et al. (1951). Polyacrylamide disc gel electrophoresis was carried out by the method of Reisfeld et al. (1962). The molecular weight of protein fractions was estimated by the method of Andrews (1965). Sephadex G-100 columns were calibrated with Blue Dextran and the following series of protein standards: bovine serum albumin (mol wt 67,000); ovalbumin (mol. wt 45,000); cytochrome C (mol. wt 12,400); and cytoxin (mol. wt 7000) from *Naja naja oxiana* snake venom.

## Results

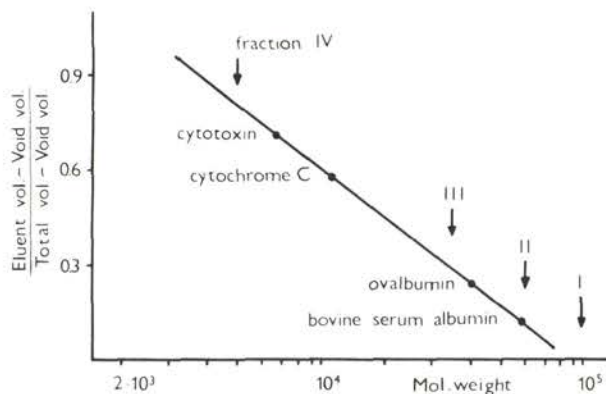
In preliminary experiments on BLM it was shown that crude venoms of *Steatoda paykulliana* spider as well as that of *Latrodectus m. tredecimguttatus* spider increase the integral membrane conductance and form a uniform population of cation selective channels (Usmanov et al. 1983). Under standard conditions with 100 mmol/l KCl or 100 mmol/l CaCl<sub>2</sub> in the medium, the channel conductance was



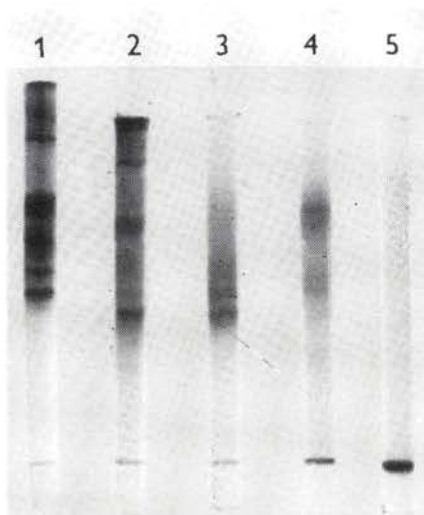
**Fig. 1.** Channel conductance data on bilayer membranes induced by *Latrodectus m. tredecimguttatus* (A, C) and *Steatoda paykulliana* (B, D) spider venoms. The membrane was formed in 100 mmol/l KCl; 5 mmol/l Tris (pH 7.5) (A, B) and 100 mmol/l  $\text{CaCl}_2$ ; 5 mmol/l Tris (pH 7.5) (C, D). Crude venoms were added to one chamber in a concentration of 0.10–0.12  $\mu\text{g/ml}$ . The membrane potential was clamped at 100 mV.



**Fig. 2.** The fractionation of venoms of *Latrodectus m. tredecimguttatus* (A) and *Steatoda paykulliana* (B) on Sephadex G-100. For details, see Materials and Methods.

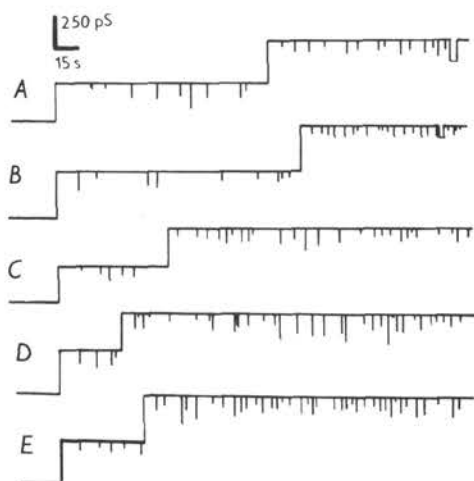


**Fig. 3.** Determination of molecular weights of fractions obtained by gel filtration on Sephadex G-100. Blue dextran was used as a marker for the void volume, and bovine serum albumin, ovalbumin, cytochrome C and cytotoxin were used as molecular weight standards. Arrows indicate the molecular weights of fractions.



**Fig. 4.** Gel electrophoresis of *Steatoda paykulliana* spider venom and its fractions obtained after fractionation. Standard 7 % polyacrylamide gels, pH 8.3, 7 ma per tube, run for 70 min at room temperature. The origin and cathode are at the top of the tubes. Staining with amido black 10B. 1-crude venom (200 µg protein); 2-fraction I (200 µg protein); 3-fraction II (200 µg protein); 4-fraction III (130 µg protein); 5-fraction IV (100 µg protein).



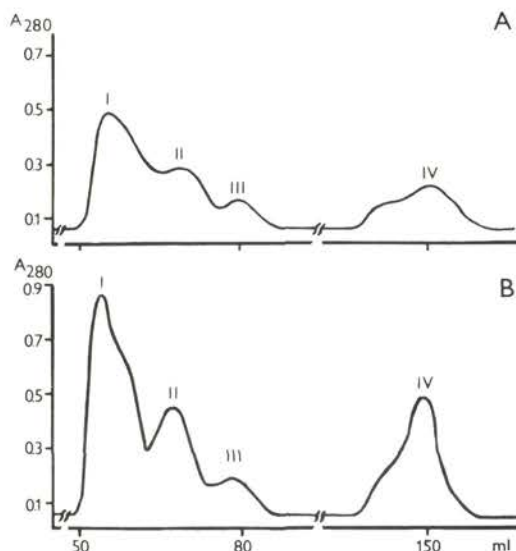


**Fig. 5.** Channel conductance on bilayer membranes induced by different fractions of the *Steatoda paykulliana* venom. The membrane was formed in 100 mmol/l KCl; 5 mmol/l Tris (pH 7.5). The membrane potential was clamped at 100 mV. A-fraction I (0.07  $\mu$ g protein/ml); B-fraction II (0.07  $\mu$ g protein/ml); C-fraction III (0.06  $\mu$ g protein/ml); D-fraction IV (0.04  $\mu$ g protein/ml); E-component obtained after rechromatography of fraction I (0.04  $\mu$ g protein/ml).

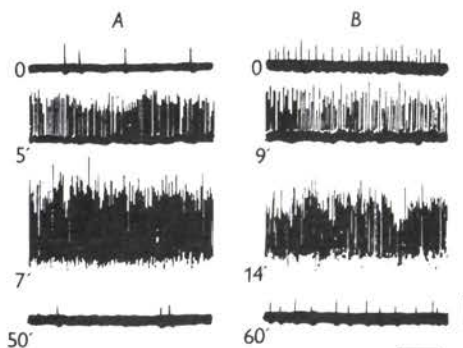
350 or 50 pS, respectively (Fig. 1). An analysis of the obtained data allowed to suggest that, in spite of their complex composition in all crude venoms, there was only one protein component able to form monotypic channels.

To identify this component, the spider venoms were fractionated on a Sephadex G-100 column (Fig. 2). Under our conditions, the crude venoms separated into 4 fractions containing protein components of following molecular masses: fraction I: 100,000 daltons and higher; fraction II: 60,000–70,000 daltons; fraction III: 30,000–40,000 daltons; and fraction IV: 5000 daltons and less (Fig. 3). Fractions I, II and III exhibited considerable heterogeneity on examination by disc gel electrophoresis (Fig. 4). Fraction IV always gave a single band and, as can be seen in Fig. 4, proteins with similarly high mobilities were seen in fractions I, II and III, respectively.

In experiments on BLM it was found that every venom contains at least four fractions capable of inducing an increase in integral membrane conductance. Under identical conditions, all of these fractions formed channels of similar selectivities and amplitudes (Fig. 5). It should be concluded that there are different polymerisation degrees of the same membrane active protein in different fractions. However, molecular weight of fraction IV was about 5000 daltons. These data were in disagreement with literature data showing that the molecular weight of neurotoxin separated from *Latrodectus* spider venoms is 130,000 daltons (Frontali et al. 1976)



**Fig. 6.** Rechromatography of fraction I (B) and fraction III (C) of the *Steatoda paykulliana* venom. Sephadex G-100 column (1.5 × 90 cm), eluted with 0.01 mol/l phosphate buffer, pH 8.0, containing 0.01 mol/l NaCl. A-crude venom.



**Fig. 7.** Increased frequency of MEPPs at frog (A) and frequency of MEPSPs at locust (B) neuromuscular junctions induced by fraction I (0.05 mg protein/ml) (A) and fraction III (0.02 mg protein/ml) (B) of the *Steatoda paykulliana* venom. The traces show records obtained at the times indicated following the application of the respective fraction. Calibration: 0.5 mV, 1 s.

or 70,000 daltons (Salikhov et al. 1982). We have suggested that the only channel former in these venoms is the small protein having a molecular weight of about 5000 daltons. This protein is a constituent of larger neurotoxins (Fig. 4). This suggestion has been strengthened by our finding that, at repeated rechromatography of fractions I and III, the component with a molecular weight of 5000 daltons was separated by several cycles of gel filtration (Fig. 6). This component also formed channels with characteristics similar to those of channels formed by the



**Fig. 8.** Volleys of MEPPs of frog (A) and MEPSPs of locust (B) neuromuscular junctions induced by fraction IV (0.01 mg protein/ml) of the *Steatoda paykulliana* venom. Calibration: 0.5 mV, 20 s.

crude venom and its high molecular weight fractions I, II and III (Fig. 5E).

To determine the biological action of the membrane active fractions obtained their influence on neuromuscular synapses of frog and locust was studied. It was found that fractions I of *Latrodectus m. tredecimguttatus* and *Steatoda paykulliana* spider venoms both contain a presynaptic toxin for vertebrates. This toxin increases the frequency of MEPPs in vertebrates, having no activity on insect neuromuscular synapses (Fig. 7A). Fractions III from these venoms contain a toxin that increases the frequency of miniature excitatory postsynaptic potentials (MEPSPs) in locust, while having no effect on vertebrate synapses (Fig. 7B).

An analysis of results obtained from experiments of this group showed that these fractions act on the respective synapse preparations in the same way as crude venoms. Initially, it was a progressive increase of MEPPs and MEPSPs frequencies after a latent period of 2–5 minutes. This event was followed by an exponential decrease of these frequencies to the control level. The low molecular weight fraction IV separated from these venoms induced bursts of miniature potentials in neuromuscular synapses of frog and locust (Fig. 8). These bursts were characterized by clear limits. They appeared 40–60 minutes after the addition of fraction IV. It may be that these bursts are a nerve terminal reaction to a discrete increase of presynaptic membrane permeability for calcium ions induced by channel-forming component of fraction IV.

## Discussion

The analysis of results obtained shows that the *Steatoda paykulliana* spider venom contains presynaptic toxins that are channel forming similarly as neurotoxins of the *Latrodectus* spider venom (Finkelstein et al. 1976). One of these high molecular