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INVESTIGATION OF THE TOXINS OF THE VENOM OF THE SPIDER *Linothele sp.*

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Two low-molecular-mass toxins — Ls1 and Ls2 — have ben isolated from the venom of a spider Linothele sp. *(fam. Dipluridae) with the aid of gel filtration and reversed-phase high-performance liquid chromatography (HPLC). It hs been shown that they are highly toxic on cerebroventricular injection into white mice: the LD-50 values of toxins Ls1 and Ls2 are 24 and 19 μg/kg, respectively, which are 25 and 31 time greater than the toxicity of the whole venon, the LD-50 of which is 0.6 mg/kg. According to mass spectrometry the molecular masses of toxins Ls1 and Ls2 are 337 and 505 Da, while their yields were, respectively, 0.21 and 0.43% of the weight of the whole venom.*

It is known that proteins and peptides predominate among the bioorganic compounds forming spider venoms; recently, low-molecular-mass polyamine derivatives have been found in the venoms of some spiders [1, 2]. It is just with these components of the venoms that the overwhelming majority of their biological effects are connected, including their influence on the generation, conduction, and transmission of a nervous impulse. The neurotoxic activity of spider venoms is characterized by a fairly high selectivity in relation to the animals of different systematic groups, and this established fact [3] is of particularly great scientific and practical interest. It is considered [4] that a detailed analysis of the neurotoxic activities of spider venoms with the aim of identifying the active principles and elucidating the nature of their toxicity and its selective manifestations will simultaneously promote an understanding of features of the organization and functioning of the nervous system in animals belonging to different taxons. If it is borne in mind that venoms of animal origin have been found to contain substances attacking various parts of the reflex arc, the inclusion of the corresponding investigations in a scientific program designed to solve the problem of the organization and mechanism of the functioning of the nervous system as a whole becomes obvious, which, of course stimulates scientific research in this direction.

The composition and properties of the venoms of spiders of the genus *Linothele,* fam. Dipluridae, have not been studied previously. Our results have confirmed the predominance (52.8%) in the venom of protein-peptide components having, according to the results of electrophoresis, molecular dimensions of from 100 to 5 kDa. No phospholipase A, protease, and direct hemolysins, which form the active principles of many zootoxins, were found in the protein-peptide fraction. In combination, these results witness a similarity of the material investigated by us to the venoms of spiders from other taxons [5]. Nevertheless, unlike them [6], the *Linothele* venom was ineffective on insects (cockroaches) even in a dose of 300 μg per animal. The evaluation of toxicity with the aid of intravenous and intraperitoneal injection also showed a substantially lower lethality of the venom for mammals; however, when the venom was injected intraventricularly — i.e., into ventricles of the brains of white mice — a fairly high toxicity was found: the LD-50 was 0.6 mg/kg body weight, which could indicate the presence in the venom of agents specifically attacking structures localized in the brain. To identify these factors, we fractionated the venom, with the parallel cerebroventricular testing of the fractions obtained.

Of the five fractions isolated when the venom was subjected to gel chromatography on a column of Sephacryl S-200 (Fig. 1), the last, containing the low-molecular-mass components of the fraction in a yield of 2.5% by weight, proved to be toxic. The results of electrophoresis, in agreement with those of gel chromatography, showed the presence in this fraction of

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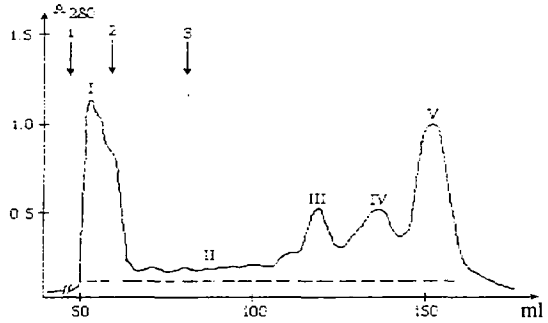

Fig. 1. Separation of the venom of the spider *Linothele* sp. on Sephacryl S-200: *I-V*) combined fractions; the figures *1-3* indicate the positions corresponding to the elution volumes of: *1*) blue dextran (2,000,000); *2*) BSA (66,000); and *3*) cytochrome C (12,500).

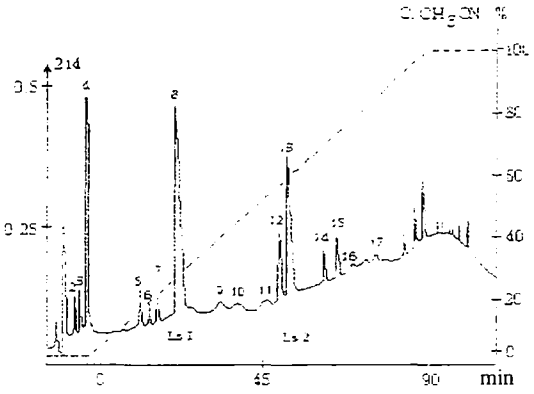

Fig. 2. Separation of fraction *V* of the *Linothele sp.* venom with the aid of HPLC on an Ultrasphere Octyl column: *1-17*) combined fractions, Ls1 (component *8*) and Ls2 (component *13*) being individual toxins.

compounds with molecular masses of 5 kDa and below. We achieved their further separation by reversed-phase high-performance liquid chromatography (HPLC) on an Ultrasphere Octyl column. By using a linear concentration gradient of acetonitrile it was possible to achieve the separate desorption from the column of 17 components (Fig. 2), of which only two — Ls1 and Ls2, obtained with yields of 0.21% and 0.43%, respectively — possessed toxic activity. The LD-50 values of toxins Ls1 and Ls2 for mice proved to be 24 and 19 μg/kg body weight, respectively. On comparing them with the toxicity of the whole venom (600 μg/kg) it can be seen that Ls1 and Ls2 were 25 and 31 times more active than the venom.

Analysis of the UV spectra of the isolated toxins (Fig. 3) revealed their total similarity to the spectra of toxic components of the venom of the spider *Argiope lobata*: Ls1 to pseudoargiopinine III, and Ls2 to argiopinine III. It is known [7] that the pseudoargiopinines and argiopinines are acylated low-molecular-mass polyamines with indolylacetic acid and 4-hydroxyindolyl acetic acid, respectively, as chromophores. The similarity observed in the UV spectra permits indolylacetic acid to be identified as the chromophore in Ls1 and 4-hydroxyindolylacetic acid in Ls2. Their similarity to the argiopines was supported by the results of a comparison of molecular dimensions.

Exclusion HPLC on UltraPac TSK G2000 SW columns in combination with SDS electrophoresis confirmed the individuality of toxins Ls1 and Ls2 and enabled their molecular masses to be determined in the range up to 600 Da for Ls1 and up to 800 Da for Ls2. Subsequently, by means of the method of fast-atom bombardment, the molecular masses were refined to 337 Da for Ls1 and 505 Da for Ls2 (Fig. 4). The molecular dimensions of other acylpolyamine toxins found in the

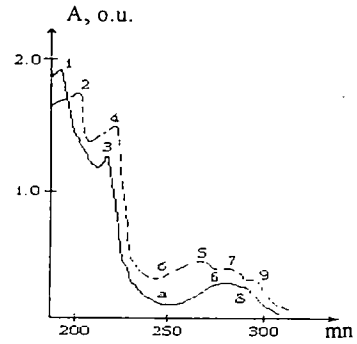

Fig. 3. UV spectra of toxins Ls1 (*a*) and Ls2 (*b*). The figures show the absorption maxima (nm): *1* (195), *2* (200), *3* (220), *4* (222), *5* (267.8), *6* (273), *7* (280), *8* (287), and *9* (291).

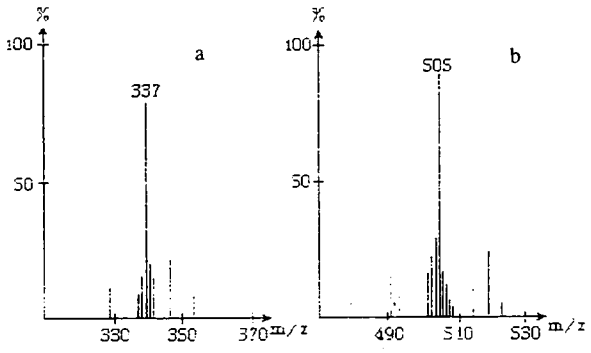

Fig. 4. Mass spectra of toxins Ls1 (*a*) and Ls2 (*b*).

venoms of the spiders *Argiope lobata, Agelenopsis aperta* and *Hololena curta*, belonging to different families of the Araneae order have been found to be in the same range of 400-700 Da [7-9]. Thus, the molecular mass of pseudoargiopinine III is 373 Da and that of argiopinine III, 659 Da [7]. The differences in the dimensions of the toxins being compared may be localized in the polyamine chain. At the same time, it must be mentioned that there are fairly substantial differences betwen the spider toxins being compared which consist in the fact that many of them (those of *A. aperta* and *H. curta*) contain not only polyamines but also polypeptide toxins that are composed of 36-38 amino acid residues and are structurally organized with the aid of disulfide bridges [8, 9], while we have detected no polypeptide toxins in *Linothele* venoms. These differences are fairly important for the action of the venoms, since polypeptide venoms paralyze slowly but irreversibly, while acylpolyamine toxins cause a rapid and reversible paralysis of insects [8]. This possibly is why the *Linothele sp.* venom, containing no polypeptide toxins, is characterized by a low efficacy of its lethal action on insects.

EXPERIMENTAL

The venom of the funnel spider *Linothele sp.* was obtained by aqueous extraction of the poison glands, followed by centrifugation to eliminate insoluble components. The supernatant was lyophilized and was stored at −15°C.

The amount of protein was determined by Lowry's method [10].

Toxicity was evaluated on white mice weighing 16-18 g by cerebroventricular injection [11], and LD-values were calculated by the Litchfield−Wilcoxon method [12].

Phospholipase activity was determined from the hydrolysis of lecithin [13], and proteolytic activity with casein [14]. In the determination of hemolytic activity we used the method of preparing a suspension of human erythrocytes and the calcu-

lations described previously [15], the degree of hydrolysis being monitored spectrophotometrically at 420 nm; as 100% we took the hemolysis of the same suspension in distilled water.

The chromatographic separation of the whole spider venom was conducted at 4°C on a column (1.5 × 90 cm) of Sephacryl S-200 in 0.05 M NH_4HCO_3, pH 8.2. Elution was carried out at a rate of flow of 5 ml/h. The protein in the fractions was determined spectrophotometrically at 280 nm.

Fraction V obtained after the gel filtration of the venom on Sephacryl S-200 was separated by reversed-phase HPLC on a column of Ultrasphere Octyl (4.6 × 250 mm, 5 μm) equilibrated with 0.1% trifluoroacetic acid. Elution was performed in a linear concentration gradient of acetonitrile of from 0 to 100% at a rate of flow of 1 ml/min.

Polyacrylamide electrophoresis was conducted in 15% gel in the presence and in the absence of 0.1% sodium dodecyl sulfate by the method of Weber and Osborn [16], using a slab or a cylindrical gel. The gel was stained with Coomassie Blue R-250. The molecular masses of the toxins were determined by two independent methods: HPLC on a column of UltraPac TSK G2000 SW (7.5 × 600 mm) equilibrated with 50 mM Tris-HCl, pH 7.5, containing 100 mM KCl and previously calibrated with marker proteins (elution with the same buffer at the rate of 0.5 ml/min), and by mass spectrometry. The substances were ionized in a glycerol matrix with bombardment by accelerated xenon atoms having an energy of 6-8 keV. The mass spectrum contained the peaks of the protonated molecular ions.

The UV spectra of the compounds isolated were taken on a spectrophotometer, 50 μg of the preparation in 0.4 ml of distilled water being taken for analysis. Measurements were made in the wavelength range from 200 to 340 nm.

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