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STRUCTURAL-FUNCTIONAL CHARACTERISTICS OF TOXINS FROM SPIDERS OF THE ARANEIDAE FAMILY

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With the aid of a series of chromatographic operations we have isolated 14 individual low-molecular-mass compounds (M_r — 624-744 Da) blocking postsynaptic glutamate and acetylcholine receptors from the venoms of species of spiders of the family Araneidae. It has been shown that from their structural characteristics and the nature of their action they can be divided into two families differing by their chromophoric groups and the degree of reversibility of their interaction with various receptors.

The venoms of some species of spiders possess a blocking action on the glutamate receptors of vertebrates and insects [1-3]. Earlier, from the venoms of the spiders *Argiope lobata* and *Araneus tartaricus*, fam. Araneidae, we isolated two neurotoxins irreversibly blocking insect glutamatergic synapses and reversibly blocking vertebrate cholinergic synapses [4]. In the present paper we consider the isolation and the physicochemical and structural analysis of blockers of glutamate and cholinergic synapses from the venoms of *Araneus cornutus*, *A. mongolicus*, and *A. angulatus*, which supplements information on the toxic principles of the venoms of the spiders of this family.

It is known that, with some differences in composition, the venoms of the spiders *A. lobata, A. tartaricus, A. cornutus, A. mongolicus*, and *A. angulatus* block synaptic transmission in vertebrates and insects, acting predominantly postsynaptically [5]. The active principles of the venoms, i.e., the postsynaptic toxins, are distributed among the low-molecular-mass components. By combining gel filtration with ion-exchange chromatography, which was replaced in individual cases by reversed-phase high-performance liquid chromatography (HPLC), we have isolated from these venoms 14 individual neurotoxins the homogeneity of which has been confirmed by HPLC and mass spectrometry. In all cases, isolation was begun by obtaining a low-molecular-mass fraction of the venom by gel filtration or extraction with ethanol; then the low-molecular-mass components were chromatographed on anion-exchange resins, and the toxins were finally purified on molecular sieves, using ordinary [4] or high-performance (Fig. 1) liquid chromatography. As a result we succeeded in isolating four toxins each from the venoms of *A. lobata* (Al-I, -II, -III, and IV) and *A. tartaricus* (At-I, -II, -II, and -IV) and two each from the venoms of *A. cornutus* (Ac-I and -II), *A. mongolicus* (Am-I and -II), and *A. angulatus* (Aa-I and -II), i.e., a total of 14. The yields of toxins were 0.88-3.90% of the whole mass of the venoms.

Electrophoretic analysis, conducted by D. Kalikulov, showed that all the toxins isolated interacted with a postsynaptic membrane, but in the light of specific features of their effects they can be divided into two groups: we assigned Al-I, At-I, Am-I, and Aa-I, reversibly blocking only the glutamatergic synapses of insects, to the first group, and toxins Al-II (argilobatin), Al-III, Al-IV, At-II, At-III, At-IV, Ac-II, Am-II, and Aa-II, irreversibly blocking insect glutamatergic synapses and reversibly blocking vertebrate cholinergic synapses, to the second.

It is interesting to compare the differences in the actions of these toxins with features of the organization of their structures. The physicochemical characteristics of the toxins that we had isolated, in comparison with the same parameters of other toxins from spiders of the Araneidae family are given in Table 1. Mass-spectrometric investigations showed that the toxins that we had isolated had molecular masses in the range of 624-744 Da. Similar values of the molecular masses have been obtained for the majority of the toxins of the venoms of spiders of the Araneidae family [6, 7, 9 12-15]. Compounds with

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TABLE 1. Characteristics of Toxins from Spiders of the *Araneidae* Family

Name of the toxin	Mol. m., Da*	Amino acid	Absorption maximum in the UV spectrum, nm	Chromophoric group
Al-I	637	Asx, Glu	215; 268; 280; 310	Not identified
At-I	637	Asx, Glu	215; 268; 280; 310	
Ac-I	637	Asx, Glu	215; 268; 280; 310	
Am-I	637	Asx, Glu	215; 268; 280; 310	
Aa-I	637	Asx, Glu	215; 268; 280; 310	
Al-II	657	Asx, Arg	200; 222; 267.8; 280; 291	4-Hydroxyindolyl-acetic acid
At-II	657	Asx, Arg	200; 222; 267.8; 280; 291	
Ac-II	658	Asx, Arg	200; 222; 267.8; 280; 291	
Aa-II	658	Asx, Arg	200; 222; 267.8; 280; 291	
Am-II	659	Asx, Arg	200; 222; 267.8; 280; 291	
Al-III	674	Asx, Arg	200; 222; 267.8; 280; 291	
At-III	744	Asx, Arg	200; 222; 267.8; 280; 291	
Al-IV	624	Asx, Arg	200; 222; 267.8; 280; 291	
At-iV	624	Asx, Arg	200; 222; 267.8; 280; 291	
Argiopine †	636	Asx, Arg	197.5; 280	2,4-Dihydroxy-phenylacetic acid
ArgTX636 ‡	636	Asx, Arg		
NSTX-3**	664	Asx, Arg		
JSTX-3**	565	Asx		
Argiopinine † I	759	Asx, Arg	200; 222; 267.8; 280; 291	4-Hydroxyindolyl-acetic acid
II	744	Asx, Arg	200; 222; 267.8; 280; 291	
III	659	Asx, Arg	200; 222; 267.8; 280; 291	
IV	630	@ Arg	200; 222; 267.8; 280; 291	
V	658	@@ Arg	200; 222; 267.8; 280; 291	
ArgTX659 ‡	659	Asx, Arg		
ArgTX673 ‡	673	Asx, Arg		
Pseudoargiopinine † I	743	Asx, Arg	220; 273; 280; 287	Indolylacetic acid
II	728	Asx, Arg	195; 220; 273; 280; 287	
III	373	Asx	220; 273; 280; 287	

*Molecular masses were determined by the method of fast-atom bombardment.

†Toxins from the venom of the spider *Argiope lobata* [Grishin et al., 1986, 1988].

‡Toxins from the venom of the spider *Argiope trifasciata* [Bateman et al., 1985; Budd et al., 1988].

**Toxins from the venoms of the spiders *Nephila maculata* [Kawai et al., 1987] and *Nephila clavata* [Kawai et al., 1983]; @) N-methyllysine; @@) N,N-dimethyllysine.

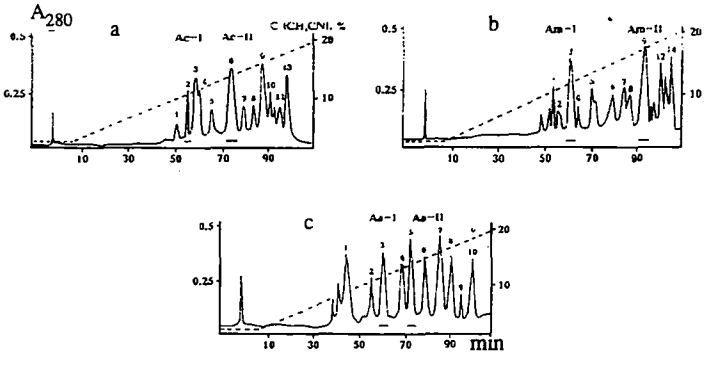

Fig. 1. Reversed-phase HPLC of the active fractions of the venoms of the spiders *A. cornutus* (*a*), *A. mongolicus* (*b*), and *A. angulatus* (*c*) on a column of TSK ODS 120T. The column (22.5 × 250 mm) was equilibrated with 0.1% TFA. Elution in a concentration gradient of acetonitrile (0-20%, indicated by a dotted line) at the rate of 10 ml/min. 1-13 (*a*), 1-14 (*b*), and 1-10 (*c*) are combined fractions, and Ac-I, Ac-II, Am-I, Am-II, Aa-I, and Aa-II are individual toxins.

molecular masses of 636 and 659 Da or close molecular masses are recognized as being the main toxic components of these venoms. In this connection, attention must be directed to the fact that all the toxins of the first group that we had isolated had a molecular mass of 647 Da, while the molecular masses of the second group of venoms were 657 Da (Al-II, At-II), 658

Da (Ac-II, Aa-II), 659 Da (Am-II), 674 Da (Al-III), 744 Da (At-III) and 624 Da (Al-IV, At-IV), i.e., their molecular masses ranged between 624 and 744 Da.

It is known [7, 9] that the molecular structures of the venom neurotoxins of Araneidae spiders are described as the amino acid Arg linked by a peptide bond with a spermine-like polyamine; the latter, in its turn is linked through a carboxy group with phenol (2,4-dihydroxyphenyl acetate) or indole (4-hydroxyindolyl acetate) radicals. Thus these compounds, containing different chromophores, may be differentiated as phenol or indole derivatives [7, 9].

Corresponding to their chemical structures, two families of venom toxins from orb-weaver spiders with phenol or indole chromphores are differentiated [7, 9, 13, 15]. From their structural features (nature of the chromophore, and the presence of Glu in place of Arg) the toxins of the first group that we isolated — Al-I, At-I, Ac-I, Am-I, and Aa-I – cannot be assigned to either of the structural families described above, i.e., they represent a new family of orb-weaver spider toxins. A capacity for reversibly blocking the glutamate synapses of insects corresponds to the characteristics of the structures of these toxins, containing Glu residues and an unidentified chromophore and differing in their spectral parameters. The toxins of the second group — Al-II — Al-IV, At-II — At-IV, Ac-II, Am-II, and Aa-II, with 4-hydoxyindolyl acetate as chromophore, may be assigned to the second family: slight differences in the molecular masses for some of them are most probably due to variations in the size of the polyamine moiety of the molecule.

The prime importance of the chromophoric part of the molecule for the biological action of the toxins [16] enables us provisionally to connect the functional differences of the toxins that we had isolated with this part. However, the differences in the chromophores do not change the direction of their main effect — the blockage of glutamate receptors — which shows the promising nature of their use in the study of membrane glutamate receptors.

EXPERIMENTAL

We used commercial preparations of freeze-dried extracts of homogenates of spider poison glands purchased from the Tashkent and Alma-Ata zoological combines.

The gel filtration of the venoms was performed on Sephadex G-75 in 0.05 ammonium bicarbonate buffer (pH 8.4) at a rate of flow of 10 ml/h, and the gel chromatography of the fractions was conducted on TSK Gel-Toyopearl HW40 in 0.15 ammonium bicarbonate buffer (pH 8.4) at a rate of flow of 15 ml/h, followed by gel chromatography of the active fractions on Ultragel AcA 202 in 0.05 M ammonium bicarbonate buffer (pH 8.4) at a rate of elution of 5 ml/h.

The ion-exchange chromatography of the fractions was conducted on DEAE- or CM-Toyopearl 650 M with the aid of a feed to the column of a stepwise or linear concentration gradient of ammonium acetate buffer (0.05-0.5 M) at a rate of elution of 30 ml/h.

Reversed-phase high-performance liquid chromatography was carried out on a TSK ODS 120T column in a concentration gradient (0-20%) of acetonitrile at the rate of 10 ml/min.

The separation procedure made use of LKB (Sweden) and Altex (USA) liquid chromatographs with a flow-through spectrometer at wavelengths of 280 and 214 nm.

The amino acid analysis of the components was conducted, after 24-hour hydrolysis in 6 N HCl at 110°C, in a D-500 analyzer (USA).

The UV spectra of the compounds isolated were taken on a Hitachi 150-20 spectrophotometer (Hitachi, Japan)

The mass spectra of the venom components were obtained on a MS-50 TC spectrometer (Kratos, United Kingdom). The substances were ionized in a glycerol matrix by bombardment with accelerated xenon atoms having an energy of 6-8 keV.

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