Molecular structures of spider toxins (JSTX-1, 2, 3 and 4) in the venom of *Nephila clavata* L. Koch

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Abstract: Spider toxins in the venom of *Nephila clavata* L. Koch were investigated and 4 kinds of closely related toxic components, JSTX-1, 2, 3 and 4, were chemically characterized as follows: JSTX-1, 2,4-dihydroxyphenylacetyl-asparaginyl spermine; JSTX-2, N-(2,4-dihydroxyphenylacetyl-ornithyl-asparaginyl)-N'-(putreanyl-putreanyl) cadaverine; JSTX-3, N-(2,4-dihydroxyphenylacetyl-asparaginyl)-N'-(aminopropyl-putreanyl) cadaverine; JSTX-4, N-(2,4-dihydroxyphenylacetyl-asparaginyl)-N'-(arginyl-ornithyl) cadaverine. The toxins inhibited glutaminergic neurotransmission in arthropods.

INTRODUCTION

The venom of *Nephila clavata* L. Koch (Japanese name: Jorogumo) is known as a potent blocker of glutaminergic neurotransmission (Kawai *et al.*, 1982a, b). It is by such action that the spider bites and paralyzes the insects trapped in their webs, since glutamate is the only neurotransmitter at the

neuromuscular junction of the insects. Our earlier communication (Aramaki *et al.*, 1986) reported the isolation of four kinds of toxic components (JSTX-1, 2, 3 and 4) from the venom of the spider. ¹H-NMR studies suggested that these 4 JSTXs were structurally similar to each other and all of them possessed 2,4-dihydroxyphenylacetyl moiety linked with polyamines and/or amino acids (Aramaki *et al.*, 1986). One major component, JSTX-3, was characterized chemically to be N-(2,4-dihydroxyphenylacetyl-asparaginyl)-N'-(aminopropyl-putreanyl) cadaverine (Aramaki *et al.*, 1987b). Presently we report the chemical characterization of the other JSTXs. We also discuss the toxicology of the venom of spiders belonging to the genus *Nephila*.

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MATERIALS AND METHODS

Spiders (*Nephila clavata*) were collected in the outskirts of Tokyo. Venom glands of the spiders were removed and kept in a deep freezer until use. A typical purification procedure of the toxin is given below. Twelve glands were homogenized in 1,000 μ l of 0.1% trifluoroacetic acid and centrifuged at 12,000 rpm for 5 min. The supernatant fluid was passed through a TOYOPAK-ODS cartridge (TOSOH Ltd.) pre-equilibrated with 0.1% trifluoroacetic acid, and the adsorbed material was eluted with 30% acetonitrile containing 0.1% trifluoroacetic acid. Both non-adsorbed and adsorbed fractions were evaporated under reduced pressure and the residues were chromatographed on a reversed phase ODS column as described previously (Toki *et al.*, 1988a). The HPLC fractions corresponding to four peaks monitored at 220 nm were collected separately. The biological activities of purified toxins were assayed for inhibitory effect on the excitatory postsynaptic potentials using a neuromuscular junction of lobster's walking leg (Kawai *et al.*, 1982a). Analytical methods and procedures employed in this experiment were as follows: Amino acids and polyamines obtained by complete hydrolysis of the toxins were determined by amino acid and polyamine analyzer with orthophthalaldehyde method as described by Nakajima *et al.* (1983). Sequential analysis of the polycationic part of the toxins were carried out by a combination of N-terminal analysis with dansyl method and fragmentation of toxins by partial acid hydrolysis (Toki *et al.*, 1988a, b). In essence, the intact toxin was first dansylated and secondarily hydrolyzed by the usual manner with 5.7 N HCl at 110°C for 20 hr. The dansylated amino acid or polyamines were identified by reversed phase HPLC using authentic samples as standards. Partial hydrolyzate with 5.7 N HCl at 100°C for 20 min was treated in a similar manner and the composition of the amino acids and polyamines of each fragment was determined. To distinguish between aspartic acid and asparagine in each sequence, the native toxin was first treated with bis-(1,1-trifluoroacetoxy) iodobenzene

which converted asparagine to α,β -diaminopropionic acid (Soby and Johnson, 1981). Confirmation of the presence of 2,4-dihydroxyphenylacetyl group was done by HPLC analysis of the hydrolyzed component. After hydrolysis of JSTXs with 0.025 M oxalic acid at 100°C for 16 hr, the hydrolyzate was injected on the reversed phase HPLC system (Aramaki *et al.*, 1987a). The substituent component was identified by the elution time and the UV spectrum monitored by a photodiode-array detector.

RESULTS

The chromatogram of the venom gland extracts is shown in Fig. 1a. To isolate toxic substances efficiently, the venom gland extracts were first passed through a TOYOPAK-ODS cartridge. Since the fraction eluted within 38 min in Fig. 1a, which contained JSTX-1, was not adsorbed on the cartridge, JSTX-1 was purified by rechromatography of the non-adsorbed fraction. This toxin was exactly the same as the one we synthesized in the course of our investigations on the structure-activity relationship of analogous compounds (Hashimoto *et al.*, 1987). The toxin was composed of 2,4-dihydroxyphenylacetic acid, asparagine and spermine. Spermine was linked at the terminus of the molecule, since N¹, N⁵, N¹⁰-tridansyl spermine was detected from the hydrolyzate of the dansylated JSTX-1. Other toxins (JSTX-2, 3 and 4) were isolated from the adsorbed fraction. The chromatogram of the adsorbed fraction is shown in Fig. 1b.

JSTX-3 was eluted at 10.6 min (Fig. 1b), which was confirmed by cochromatography with authentic JSTX-3. JSTX-2 and 4 eluted as adjacent peaks at 11.0 and 11.2 min, respectively. The large peaks eluted around 13 min (Fig. 1b) were a mixture of Nephilatoxins (NPTXs) (Toki *et al.*, 1988a, b) which not only inhibited the glutamate receptor, but also released histamine from the mast cells. The fractions containing JSTX-2 and 4 were rechromatographed with the flatter gradient system, and the components in each fraction were isolated. JSTX-2 contained 1 mol of asparagine, cadaverine ornithine and 2 mol of putrescine in addition to 2,4-dihydroxyphenylacetic acid. This

Fig. 1a Chromatogram of venom gland extracts of *Nephila clavata*. Column, TSK-gel ODS-120T, 0.46×250 mm (left).

Eluent, linear gradient from 0.1% trifluoroacetic acid to 30% acetonitrile containing 0.1% trifluoroacetic acid. Elution in 60 min at a flow rate of 0.7 ml/min. Column temperature, 25°C. Detection, UV 280 nm.

Fig. 1b Chromatogram of the fraction adsorbed on ODS-cartridge.

Column, TSK-gel ODS-80TM, 0.46×250 mm. Eluent, linear gradient from 0.1% trifluoroacetic acid to 30% acetonitrile containing 0.1% trifluoroacetic acid. Elution in 30 min at a flow rate of 1.0 ml/min. Column temperature, 50°C. Detection, UV 220 nm (right).

Fig. 2 Sequence analysis of JSTX-4.

amine component was the same as that of NPTX-3 (Toki *et al.*, 1988a). The amino acid and polyamine component of JSTX-4 was 1 mol of asparagine, cadaverine ornithine and arginine which is also the same as that of NPTX-9 (Toki *et al.*, 1988a). Sequence analysis of the polyamine part of these two

toxins gave the same results as those of NPTX-3 and 9, respectively. As an example, sequence analysis of JSTX-4 is summarized in Fig. 2.

Accordingly, the structure of JSTX-2 and 4 were deduced to be N-(2,4-dihydroxyphenylacetyl-ornithyl-asparaginyl)-N'-(putreanyl-putreanyl) cadaverine and N-(2,4-dihydroxyphenylacetyl-asparaginyl)-N'-(arginylornithyl) cadaverine, respectively. Structures of JSTXs and the related polyamine-like toxins isolated from spider venom are illustrated in Figs. 3a and 3b.

DISCUSSION

The venom of *Nephila clavata* was observed electrophysiologically to block irreversibly the neuromuscular junction of the

Fig. 3a Chemical structures of JSTXs.

Fig. 3b Structures of polyamine conjugated toxins.

walking leg of lobster (Kawai *et al.*, 1982a). They also noted that the venom was a potent blocker against quisqualate type glutamate receptor in mammalian brain (Kawai *et al.*,

1982b). They called the toxin in the venom, "Joro spider toxin (JSTX)." Chemical characterization of the venom leads us to the conclusion that JSTX was a mixture of

more than 16 kinds of closely related compounds. These toxins are named JSTX-1 to 4 and NPTX-1 to 12 (Aramaki *et al.*, 1986, 1987a, b; Toki *et al.*, 1988a, b). During the past few years, the toxins in the araneid spiders belonging to the genera, *Araneus*, *Argiope*, *Neoscone*, and *Nephila* have been characterized (Aramaki *et al.*, 1986; Grishin *et al.*, 1986, 1988; Adames *et al.*, 1987; Toki *et al.*, 1988b; *etc.*). Chemical structures of these toxins are similar to each other as shown in Fig. 3b, and their physiological action is practically the same with respect to the blocking action of the glutamate receptor. In this regard, the toxins in the araneid spiders may be classified as a new type of polyamine toxins. However there exists minor structural differences among them. Most of the toxins in *Nephila* possessing putreanine (carboxyethyl putrescine) which is linked directly with cadaveryl asparagine in the molecule. While putreanine has not been found as a component of toxins from the venom of the other genera, the toxins in the other genera contain longer chain polyamines instead of putreanine or N-methylated polyamines and/or N-methylated amino acids. However, this type of polyamine conjugated toxin may be characteristic in the venom of the spiders in the family, Araneidae, since in the chromatographic pattern analysis of the spider venom belonging to other families, such as *Loxoscelidae*, *Agelenidae*, *Clubionidae*, *etc.*, we could not find this type of polyamine conjugated toxins (Hagiwara *et al.*, unpublished). On the other hand, some kinds of solitary wasps belonging to the genera, *Pompilus*, *Eumenes*, *Sphex*, *etc.*, are known to contain similarly acting neurotoxins which cause paralysis in insects, and the wasps lay their eggs on preys anesthetized in this manner. δ -Phlanto toxin in the venom of a sphecoid wasp, *Phlanthus triangulum*, is the best investigated (Clark *et al.*, 1982; Eldeflawi *et al.*, 1988; Piek *et al.*, 1988) and, interestingly, the chemical structure of the toxic principle is also a polyamine conjugated toxin, (*n*-butyryl-tyrosyl)-1,12-diamino-5,9-diazadodecane. The above results suggest the use of a new class of polyamine-like toxins for the development of new pesticides and for the study of glutaminergic transmission.

REFERENCES

- Adames, M. E., R. L. Carney, F. E. Enderlin, E. T. Fu, M. A. Jamrema, J. P. Li, C. A. Miller, D. A. Schooley, M. J. Shapiro and V. J. Venema (1987): Structure and biological activities of three synaptic antagonists from orb weaver spider venom. *Biochem. Biophys. Res. Commun.*, **148**: 678-683.
- Aramaki, Y., T. Yasuhara, T. Higashijima, A. Miwa, N. Kawai and T. Nakajima (1987a): Chemical characterization of spider toxin, NSTX. *Biomed. Res.*, **8**: 167-173.
- Aramaki, Y., T. Yasuhara, T. Higashijima, T. Yoshioka, A. Miwa, N. Kawai and T. Nakajima (1986): Chemical characterization of spider toxin, JSTX and NSTX. *Proc. Jpn. Acad. Ser. B*, **62**: 359-362.
- Aramaki, Y., T. Yasuhara, K. Shimazaki, N. Kawai and T. Nakajima (1987b): Chemical characterization of Joro spider toxin (JSTX). *Biomed. Res.*, **8**: 241-245.
- Clark, R. B., P. L. Donaldson, K. A. F. Gration, J. J. Lambert, T. Piek, R. Ramsey, W. Spajer and P. N. R. Usherwood (1982): Block of locust muscle glutamate receptors by delta-Phlanto toxin occurs after receptor activations. *Brain Res.*, **241**: 105-114.
- Eldeflawi, A. T., M. E. Eldeflawi, K. Konno, N. A. Mansour, K. Nakanishi, E. Oltz and P. N. R. Usherwood (1988): Structure and synthesis of a potent glutaminergic receptor antagonist in the wasp venom. *Proc. Natl. Acad. Sci.*, **85**: 4910-4913.
- Grishin, E. M., T. M. Volkova and A. S. Arseniev (1988): Antagonists of glutamate receptors from the venom of *Argiope lobata* spider. *Bioorg. Khim.*, **7**: 883-892.
- Grishin, E. M., T. M. Volkova and A. S. Arseniev, O. S. Reshetova, V. V. Onoprienko, L. G. Magazanic, S. M. Antonov and I. M. Federova (1986): Structure-functional characterization of argiopine an ionchannel blocker from the venom of spider, *Argiope lobata*. *Bioorg. Khim.*, **12**: 1121-1124.
- Hashimoto, Y., Y. Endou, K. Shudo, Y. Aramaki, N. Kawai and T. Nakajima (1987): Synthesis of spider toxin (JSTX-3) and its analogs. *Tetrahedron Lett.*, **28**: 3511-3514.
- Kawai, N., A. Miwa and T. Abe (1982a): Spider venom contains specific receptor blocker of glutaminergic synapses. *Brain Res.*, **247**: 169-171.
- Kawai, N., A. Miwa and T. Abe (1982b): Effect of spider toxin on glutaminergic synapses in the mammalian brain. *Biomed. Res.*, **3**: 353-355.
- Nakajima, T., T. Yasuhara, N. Yoshida, Y. Take-

- moto, S. Shinonaga, R. Kano and H. Yoshida (1983): The pattern analysis of biologically active amines in some Hymenopteran venoms by high performance liquid chromatography. *Jpn. J. Sanit. Zool.*, **34**: 61-71.
- Piek, T., R. H. Fokkens, H. Karst, C. Kruk, A. Lind, J. van Marle, T. Nakajima, N. M. M. Nibbering, H. Shinozaki and W. Spanjar (1988): Polyamine-like toxins—A new class of pesticides? In *Neurotox. '88, Molecular Basis of Drugs and Pesticides Action* (ed. Lunt, G. G.), pp. 61-76, Excerptamedica, Amsterdam, New York, Oxford.
- Soby, L. M. and P. Johnson (1981): Determination of asparagine, glutamine in polypeptide using bis(1,1-trifluoroacetoxy)iodobenzene. *Anal. Biochem.*, **113**: 149-153.
- Toki, T., T. Yasuhara, Y. Aramaki, N. Kawai and T. Nakajima (1988a): A new type of spider toxin, Nephilatoxin, in the venom of Joro spider, *Nephila clavata*. *Biomed. Res.*, **9**: 75-79.
- Toki, T., T. Yasuhara, Y. Aramaki, K. Osawa, A. Miwa, N. Kawai and T. Nakajima (1988b): Isolation and chemical characterization of a series of spider toxin (Nephilatoxins) in the venom of Joro spider, *Nephila clavata*. *Biomed. Res.*, **9**: 421-428.

摘 要

日本産ジョロウグモ *Nephila clavata* の毒成分 (JSTX-1,2,3,4) について

日本産ジョロウグモ *Nephila clavata* の毒は、グルタミン酸レセプターの遮断剤として知られている。今回ジョロウグモ毒中に存在する4種類の毒成分、JSTX-1, 2, 3, および4について構造解析を行った。これらの毒成分はスベルミンまたはカダベリンの両端に塩基性アミノ酸あるいはポリアミンが結合し、置換基として2,4-ジヒドロキシフェニルアセチル基をもつ特異な構造を有していた。また単離した毒成分はいずれもグルタミン酸レセプター遮断活性を示した。