94. Chemical Characterization of Spider Toxin, JSTX and NSTX

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Spider toxin obtained from the venom of *Nephila clavata* (Joro spider) has been recognized as a new neurotoxin (JSTX) which suppresses irreversibly the excitatory postsynaptic potential (EPSP) and the glutamate potential in the lobster neuromuscular junction with high degree of specificity (Kawai *et al.*, 1982; Kawai *et al.*, 1984). JSTX is also pharmacologically active in the other systems where glutamate is a putative neurotransmitter (Kawai *et al.*, 1983).

We purified the active principles from the venom of *Nephila clavata* and *Nephila maculata*, a Papua New Guinean related species, and found that JSTX and that from *N. maculata* (NSTX) composed of many kinds of the structurally similar toxic principles. Some of these toxic principles (NSTX-3, JSTX-2, -3, -4) were isolated and chemically identified as their common structure composed of a 2, 4-dihydroxyphenylacetylasparginyl-cadaverinocarboxyethylamino-derivative. NSTX-4 contained additional arginylcadaverine, while JSTXs were composed of another type of polyamines and/or amino acids linked to the common structure.

This report deals with the purification of spider toxins and chemical characterization of toxic principles.

Materials and methods. Spiders (*N. clavata* and *N. maculata*) were collected on the outskirts of Tokyo, Japan and Wau, Papua New Guinea, respectively. After the venom glands of *N. clavata* were taken out, they were kept in a deep freezer (-80°C) to prevent possible degradation. In the case of *N. maculata* venom, the venom glands were immediately lyophilized after separation from the spider's body.

Both venom glands were homogenized with 60% acetonitrile and the homogenates were centrifuged at 3000 rpm for 5 min. The supernatant fluid was analysed by reversed phase high performance liquid chromatography with an octadecylsilane (ODS) column.

Biological activity was assayed by the neuromuscular preparation of the lobster's walking leg as described previously (Kawai *et al.*, 1983).

Results and discussion. Crude venom extracts from both spiders corre-

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sponding to a half of a venom gland were separated using an ODS column (Toyo Soda, TSK-120T, 4.6 mm i.d.×250 mm) with a linear concentration gradient from 0.02% HCl to 50% acetonitrile containing 0.02% HCl over 30 min at a flow rate of 0.5 ml/min. Chromatogram was monitored with a UV detector at 210 nm.

As shown in the chromatograms in Figs. 1a (*N. clavata*) and 1b (*N. maculata*), both venom extracts gave similar chromatograms. The peaks indicated by arrows showed the toxic activity for the lobster's leg preparations.

Fig. 1

Fig. 2

Figs. 1-2. 1: Chromatograms of spider venom extracts. a: *Nephila clavata* venom.

b: *N. maculata* venom. 2: ^1H -NMR spectra of spider toxin in D_2O .

By employing a wide bored column (21.5 mm i.d.×300 mm) of the same adsorbents, crude venom extracts corresponding to 200 spiders (*N. clavata*) and 100 spiders (*N. maculata*) were separated preparatively with similar elution system. The toxins eluted between 15 and 25 min were further purified and finally, 4 kinds (JSTX-1, -2, -3, -4) and 3 kinds (NSTX-1, -2, -3) of the toxins

from the respective venom extracts were isolated as a single peak by the repeated chromatography of similar elution system with a slower gradient.

All of these spider toxins possessed UV maxima at 210 and 280 nm, and exhibited natural fluorescence at 310 nm when excited at 280 nm. Acid hydrolysates of each toxin after usual acid hydrolysis with 6N HCl at 110°C for over-night gave aspartic acid and the other *o*-phthalaldehyde (OPA)-positive fragments except arginine from the hydrolysates of JSTX-4 and NSTX-3. One of these unidentified common fragments was identified as cadaverine by the polyamine analysis.

Fig. 3. ^1H -COSY-NMR spectrum of spider toxin (NSTX-3) in DMSO-d_6 . a: 2, 4-dihydroxyphenylacetic acid. b: asparagine. c: arginine. d: cadaverine. e: cadaverino- β -alanine.

It was shown by the measurement of ^1H -NMR spectra of some of these toxins (Fig. 2) that these toxins also showed similar pattern and the common signals around 6.5 and 7.0 ppm in D_2O . These signals were separated into three, 6.1 ppm(d), 6.3 ppm(s), 6.9 ppm(d), when the spectra were measured in DMSO-d_6 .

This part of component was readily hydrolysed by 0.025 M oxalic acid at 100°C for 16 hr., and was extracted with ethyl acetate. NMR analysis of this material was considered to be 2, 4-dihydroxyphenyl acetic acid.

¹H-COSY-NMR spectrum of NSTX-3 was shown in Fig. 3. Respective coupling and cross peaks were connected with lines. Lines *a* to *e* were the illustration of each component of NSTX-3, corresponding to 2, 4-dihydroxyphenyl acetic acid (*a*), asparagine (*b*), arginine (*c*), cadaverine (*d*) and cadaverino-β-alanine (*e*), respectively.

The facts that 2, 4-dihydroxyphenylacetic acid was readily hydrolysed and the NMR signal of the methylene group of this acid was split into quartet at 3.5 ppm (Fig. 2) indicated the existence of 2,4-dihydroxyphenylacetyl-asparagine moiety in the molecule. Time course study of the toxin for the partial acid hydrolysis and the analysis of liberated components suggested that -NH(CH₂)₅NHCO(CH₂)₂NH(CH₂)₅NH- took the next part of the toxin molecule; arginine was assumed to be connected by the peptide bond to this moiety, because we have isolated a similar toxin in which the arginine residue was absent.

Thus the structure of NSTX-3 was deduced to be 2, 4-dihydroxyphenylacetyl-asparaginyl-(arginyl-cadaverino-β-alanyl)-cadaverine. The FAB-MASS spectroscopic datum also supported this structure as the molecular ion peak was observed at *m/z*=665.

Similar NMR analysis was carried out to JSTX-2, -3 and -4. These structures have not been fully characterized. However, the occurrence of 2, 4-dihydroxyphenylacetyl-asparaginyl-cadaverino-β-carboxyethylamino moiety was commonly observed by ¹H-COSY-NMR spectroscopy. JSTX-3 was elongated -(CH₂)₃NH-(CH₂)₃NH₂ to this common moiety. While JSTX-2 linked -(CH₂)₃NH(CH₂)₄-NH-, and JSTX-4 attached another arginine to JSTX-2 with a peptide bond respectively.

Spider toxins (JSTX and NSTX) are pharmacologically and also structurally quite unique and new substances. The common part of the structure would be a minimum requirement for revealing their biological action on the glutamate receptor. Synthetic study to confirm or elucidate their structures has been started.

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