Communications to the Editor

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IODINATED JORO TOXIN (JSTX-3). ITS STRUCTURE AND BINDING TO THE LOBSTER NEUROMUSCULAR SYNAPSE

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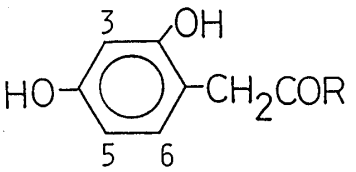
Synthetic Joro toxin (JSTX-3) was iodinated by the lactoperoxidase method and the reaction products were purified by high performance liquid chromatography. Proton-nuclear magnetic resonance spectroscopy revealed that iodination occurred mainly at position 3 of the benzene ring. The radio-iodinated JSTX-3 appeared autoradiographically to be accumulated in the synapse area of the lobster nerve-muscle preparation.

KEYWORDS——Joro toxin; JSTX; glutamatergic synapse; radio-iodination; nuclear magnetic resonance

Joro toxins (JSTXs) isolated from the venom of Joro spider (*Nephila clavata*) have been reported to be potent and irreversible inhibitors of the glutamatergic synapse.[1] For the further histological and biochemical research in this field, radiolabeled JSTXs are required. Since JSTXs are composed of the structurally related compounds in which the resorcinolic structure is included as the common component, radio-iodination[2] should be available for this purpose. We prepared iodinated JSTX-3 (one of the JSTXs) and iodinated 2,4-dihydroxyphenylacetic acid (DHPA) as the referential compounds (Fig. 1), by the lactoperoxidase method and defined their chemical structures. Also the distribution of the binding site of JSTX-3 on the lobster muscle was determined with radio-iodinated JSTX-3.

Synthetic JSTX-3[3] (2.6 μmol) was dissolved in 20 mM sodium acetate buffer (pH 5.6). Potassium iodide (15 μmol) and lactoperoxidase (Calzyme laboratories Inc., California, U.S.A., 3.8 nmol) were added to the solution, then hydrogen peroxide was added with mixing until the final concentration came to 0.1 mM.[4] After incubation at room temperature for 15 min, the reaction mixture was added to



JSTX-3; R = $NHCH(CH_2CONH_2)CONH(CH_2)_4NHCO(CH_2)_2NH(CH_2)_4NH(CH_2)_3NH_2$

DHPA ; R = OH

Fig.1. Structure of JSTX-3 and DHPA

sodium thiosulfate (20 μmol) and subjected to high performance liquid chromatography (HPLC)[5] on an octadecylsilane (ODS) column (Finepak Sil C_{18}, 4.6×250 mm). Besides the residual JSTX-3, three peak fractions appeared: the major product I, the minor products II and III (Fig. 2). The absorption spectrum of each product was measured with a MULTI-320 multi channel detector (Jasco, Tokyo, Japan) equipped with an HPLC system (Fig. 3a). Unlike native JSTX-3, the iodinated products did not show fluorescence (excitation 280 nm/ emission 320 nm, data not shown). Iodinated products of DHPA afforded chromatograms (i.e., product i, ii and iii) and absorption spectra similar to those of JSTX-3 (Fig. 3b). The absorption spectra show that the iodinated position in products I,II and III is identical to that in i, ii and iii, respectively.

Fig. 2. Purification of Iodinated Products of JSTX-3

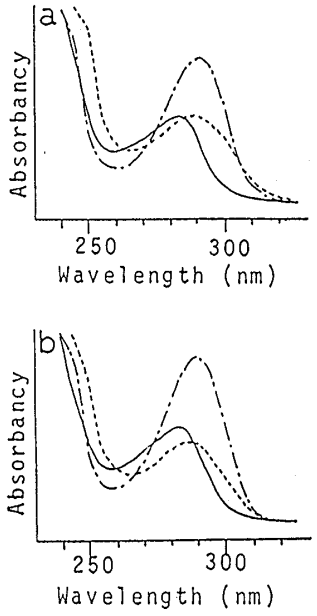


Fig. 3. Absorption Spectra of Iodinated JSTX-3(a) and Iodinated DHPA (b)

(a) ——— ,I; —·—·— ,II; ------ ,III

(b) ——— ,i; —·—·— ,ii; ------ ,iii

The proton-nuclear magnetic resonance (^{1}H-NMR)[6] spectra of native JSTX-3 and the major product I are shown in Fig. 4. The proton at position 3 of the benzene ring does not appear in the ^{1}H-NMR spectra of I, and the other signals, including amino and amide protons, correspond to those of native JSTX-3. This clearly indicates that iodination occurred at position 3 of the benzene ring. The minor product II appears to be a 5-iodinated compound since product ii lacks a proton signal at position 5 and the doublet proton at position 6 is converted to a singlet. A singlet signal (7.4 ppm) in the NMR spectrum of iii is derived from the proton at position 6, showing that III is 3,5-diiodinated.

The blocking activities against lobster neuromuscular synapse was examined in I and II. Both of them retained almost the same activity as native JSTX-3.[7] Therefore, using radio-iodinated JSTX-3, the distribution of the binding site of JSTX-3 on the muscle of lobster walking legs was autoradiographically investigated. Radio-iodination was peformed in the similar way as described above except that [^{125}I]-sodium iodide (New England Nuclear) was used. The radioactive spots were focused on limited points on the surface of muscle fibers (Fig. 5).

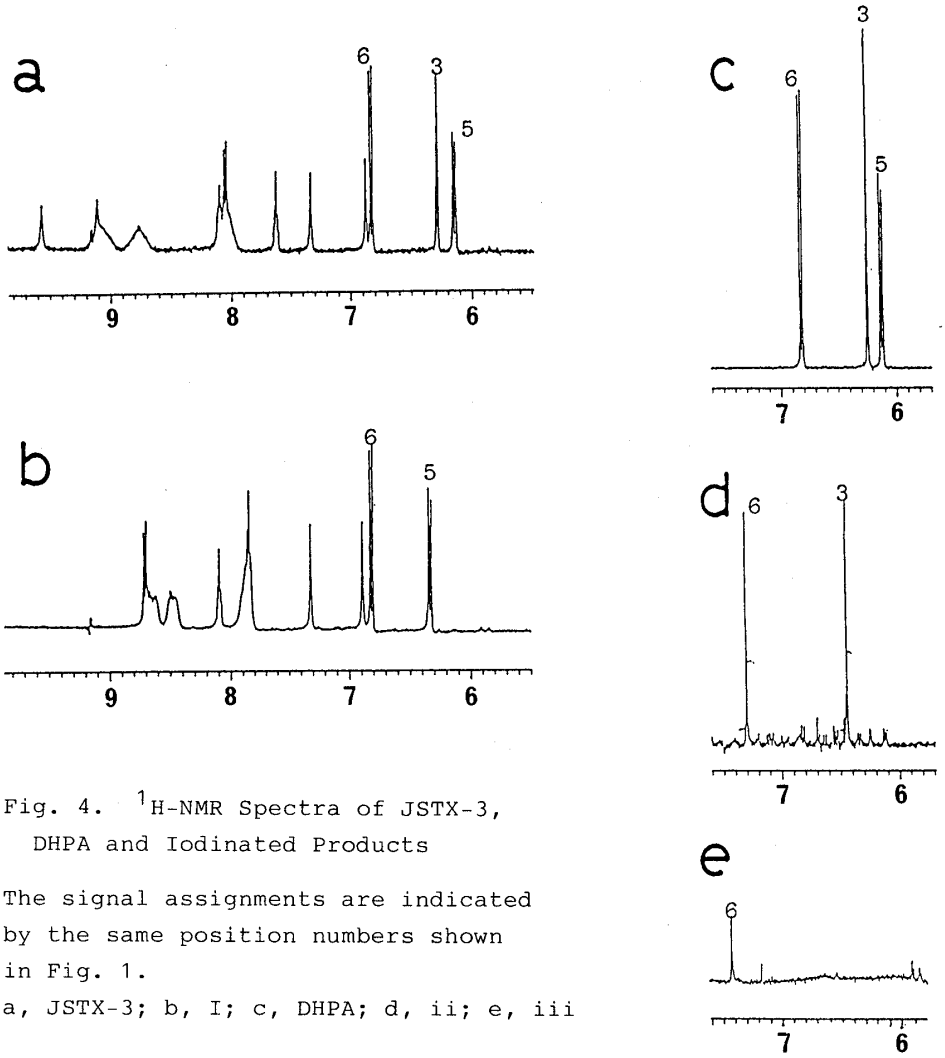


Fig. 4. ^{1}H-NMR Spectra of JSTX-3, DHPA and Iodinated Products

The signal assignments are indicated by the same position numbers shown in Fig. 1.
a, JSTX-3; b, I; c, DHPA; d, ii; e, iii

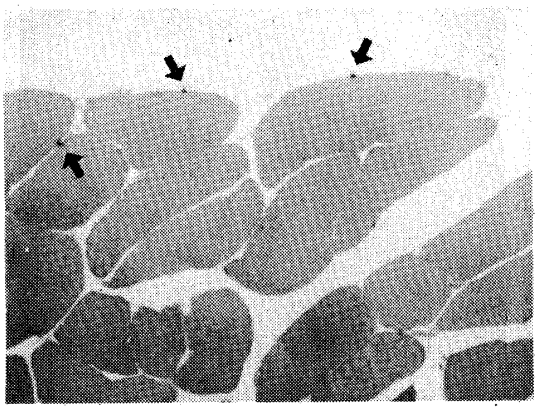


Fig. 5. Autoradiogram of Lobster Walking Leg Muscle Treated with ^{125}I-labeled JSTX-3

The radioactive spots (indicated by arrows) are accumulated in the synapse area on the surface of the muscle fibers.

These points were demonstrated to be the synaptic area by light microscopic observation with methylene blue staining and by electron microscopic observation with uranyl acetate-lead citrate staining.[7,8] This indicates the direct and selective binding of JSTX-3 to the crustacean neuromuscular synapse.

REFERENCES AND NOTES

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2) A.E.Bolton, *Methods Enzymol.*, 124, 18 (1986).
3) Y.Hashimoto, T.Yasuhara, Y.Endo, K.Shudo, Y.Aramaki, N.Kawai and T.Nakajima, *Tetrahedron Lett.*, 28, 3511 (1987).
4) The amount of JSTX-3 was calculated by quantification of aspartic acid and cadaverine after hydrolyzation in 6N hydrochloric acid (HCl) at 110 °C for 24 h. Lactoperoxidase and hydrogen peroxide were quantified by their molar absorptivity.
5) The HPLC system used was a TRI ROTAR-VI chromatograph (Jasco, Tokyo, Japan). The solvent system was 5.7 mM HCl/ acetonitrile (90/10, v/v) (A) and 5.7 mM HCl/ acetonitrile (50/50, v/v) (B). Elution was carried out with linear gradient from A to B over 30 min.
6) NMR spectra were measured in d_6-dimethylsulfoxide with a JNM-GX400 (400 MHz) spectrometer (JEOL, Tokyo, Japan).
7) K.Shimazaki, K.Hagiwara, Y.Hirata, T.Nakajima and N.Kawai, *Neurosci. Lett.*, 84, 173 (1988).
8) The nerve-muscle preparation from lobster walking leg was treated with ^{125}I-labeled JSTX-3, washed with saline and fixed in glutaraldehyde-formaldehyde solution. The tissue was postfixed in osmium tetroxide solution, dehydrated in ethanol, then embedded in epoxy resin (Quetol 651, Nisshin EM). Serial semi-thin (1.5 μm) sections were coated with nuclear emulsion (Sakura, NR-M2), exposed for 4 days at -80 °C and developed with Konidol X (Sakura).

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