Fast Atom Bombardment- and Secondary Ion-Mass Spectrometry of Paralytic Shellfish Poisons and Tetrodotoxin

Junichi Maruyama, Tamao Noguchi, Shigeki Matsunaga and Kanehisa Hashimoto

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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The application of fast atom bombardment- and secondary ion-mass spectrometry to the determination of molecular weights of non-volatile toxins was successfully examined with the use of authentic specimens of paralytic shellfish poisons (saxitoxin, gonyautoxin-1, gonyautoxin-2 and protogonyautoxin-1) and tetrodotoxin.

Two non-sulfated toxins, saxitoxin and tetrodotoxin, produced intense pseudomolecular ion peaks in both positive and/or negative ion detection. On the other hand, each of gonyautoxin-1 and -2 and protogonyautoxin-1, all sulfated, gave only small (M + H)⁺ ion peaks in the positive ion detection whereas exhibiting prominent (M - H)⁻ ion peaks in the negative ion spectrum.

The molecular weights thus determined were 299 for saxitoxin, 411 for gonyautoxin-1, 395 for gonyautoxin-2, 475 for protogonyautoxin-1 and 319 for tetrodotoxin, in good accordance with their molecular weights.

Paralytic shellfish poison (PSP), a most hazardous marine toxin, mainly originates in toxic marine dinoflagellates of the genus Protogonyaulax. PSP is accumulated by shellfish via the food chain and causes sporadic food poisoning in humans in many parts of the world including Japan. PSP is composed of more than ten components.¹ Their structures have so far been determined or assumed from physicochemical data obtained by various methods including X-ray analysis, and ¹H- and ¹³C-NMR spectrometry. Due to their non-volatile and thermolabile nature, however, electron impact (EI)-, chemical ionization (CI)- or field desorption (FD)-mass spectrometric techniques have not been successfully applied to PSP. So is the case with pufferfish toxin, tetrodotoxin (TTX).

Recently, Barber et al.² have demonstrated that fast atom bombardment mass spectrometry (FAB-MS) can provide much information on the molecular weights and structures of several peptides to which conventional mass spectrometric techniques are not applicable. On the other hand, secondary ion mass spectrometry (SIMS) has been applied to the molecular weight determination of various biorganic substances such as sugars and antibiotics.³ We have examined the availability of these techniques to determine the molecular weights of several members of PSP as well as TTX, and obtained some promising results.

MATERIALS AND METHODS

Paralytic shellfish poisons and tetrodotoxin. Of the preparations of PSPs used, saxitoxin (STX) was a gift from Dr. J. E. Campbell, Public Health Service, U. S. Department of Health, Education and Welfare. Gonyautoxin-1 (GTX₁)⁴ and gonyautoxin-2 (GTX₂)⁵ were isolated from toxic specimens of the scallop Patinopecten yessoensis, and protogonyautoxin-1 (PX₁)⁶ from toxic specimens of the oyster Crassostrea gigas, as reported previously. TTX was isolated and crystallized from the ovaries of the pufferfish Fugu vermicularis porphyreus essentially by the method of Goto et al.⁷

Mass spectrometry. In FAB-MS, a JEOL JMS DX-300 mass spectrometer equipped with a JEOL JMA-3100 data system was used. Xenon was used to provide the primary beam of atoms, the acceleration voltage of the primary ion being 3 KV. Scanning was repeated within a mass range of m/z 100 to 1000.
Each toxin preparation was dissolved in water or 0.05 M acetic acid at a concentration of approximately 10 μg/μl. One μl each of a given toxin solution with glycerol as the matrix was applied to the sample stage of the mass spectrometer, mixed well, and introduced into the ion chamber. Both positive and negative mass spectra were measured according to the brochure.

In SIMS, a Hitachi M-80B mass spectrometer equipped...
Mass Spectrometry of PSP and Tetrodotoxin

Fig. 1. Positive and Negative Fast Atom Bombardment (FAB) Mass Spectra of Saxitoxin (a, b), Gonyautoxin-1 (c, d), Gonyautoxin-2 (e, f), Protogonyautoxin-1 (g, h) and Tetrodotoxin (i, j).

FAB mass spectra were measured within a mass range of m/z 100 to 1000. The data above m/z 500 were omitted since there appeared no characteristic signals. G: glycerol.

Fig. 2. Positive Secondary Ion Mass Spectra of Gonyautoxin-2 (a), Protogonyautoxin-1 (b) and Tetrodotoxin (c).
with a Hitachi M-0101 data system was used. Acceleration voltages of the primary and secondary ions were 8 and 3 KV, respectively. Mass spectrometry was performed essentially in the same way as in FAB-MS, but only the positive mass spectra were measured.

**RESULTS**

Both the positive and negative ion FAB mass spectra of STX, GTX1, GTX2, PX1 and TTX are shown in Fig. 1, and the positive ion SIMS spectra of GTX2, PX1 and TTX in Fig. 2.

**Saxitoxin (1 in Fig. 3A)**

The absolute structure of STX has been elucidated by X-ray analysis of a crystalline derivative of this toxin.8>9) In the positive FAB-MS of STX (Fig. 1a), the ion peaks of (M+H)+, (M+H-H2O)+ and (M+H+glycerol-H2O)+ appeared at m/z 300 (base peak), 282 and 374, respectively. Both ion peaks at m/z 282 and 374 could result from dehydration of the hydrated ketone group of the molecule in the ionizing chamber. In the negative mode, an (M-H)~ ion peak was not recognized (Fig. 1b). Instead, two cluster ion peaks associated with one and two mol of hydrochloric acid per mol appeared at m/z 334 and 370, respectively, due to this toxin being isolated as a hydrochloride.8) The involvement of chloride in these peaks was verified by the relative intensities of the isotopic peaks at m/z 336 and 372. No other fragment peaks were assigned in either ionization mode.

**Gonyautoxin-1 (3 in Fig. 3A)**

The structure of GTX1 has been reported to be 11α-hydroxynoaxitoxin sulfate.10) Its positive FAB mass spectrum showed rather weak (M+H)+ and (M+H-SO3)+ ion peaks at m/z 412 and 332, respectively (Fig. 1c). On the other hand, the (M-H)~ ion peak was observed at m/z 410 as the base peak in the negative FAB mass spectrum (Fig. 1d).

**Gonyautoxin-2 (4 in Fig. 3A)**

The structure of GTX2 has been reported to be 11α-hydroxysaxitoxin sulfate.11) The positive FAB mass spectrum showed (M+K)+, (M+H)+ and (M+H-SO3)+ ion peaks at m/z 434, 396 and 316, respectively (Fig. 1e). In the negative spectrum, an intense ion peak of (M-H)~ was observed at m/z 394 (Fig. 1f). On the other hand, GTX2 exhibited (M+K)+, (M+H)+ and (M+H-SO3)+ ion peaks when analyzed by SIMS (Fig. 2a), in good agreement with the positive FAB mass spectrum.

**Protogonyautoxin-1 (5 in Fig. 3A)**

The structure of PX1 was carbamoyl-N-
sulf-11x-hydroxysaxitoxin sulfate.\textsuperscript{6)} The same structure had already been proposed for "Cl" of Wichmann et al.\textsuperscript{12)} by X-ray crystallography and for epi-GTX\textsubscript{8} by Kobayashi and Shimizu.\textsuperscript{13)}

In the positive FAB mass spectrum of PX\textsubscript{1}, a very weak ion peak of (M+H)\textsuperscript{+} appeared at m/z 476, along with rather intense peaks of (M+H−SO\textsubscript{3})\textsuperscript{+} and (M+H−2 SO\textsubscript{3})\textsuperscript{+} at m/z 396 and 316, respectively (Fig. 1g). In its negative spectrum, intense ion peaks of (M−H)\textsuperscript{−} and (M−H−SO\textsubscript{3})\textsuperscript{−} appeared at m/z 474 and 394, respectively (Fig. 1h).

On the other hand, PXX exhibited a very weak ion peak of (M+H)\textsuperscript{+} at m/z 476, and intense peaks of (M+H−SO\textsubscript{3})\textsuperscript{+} and (M+H−2 SO\textsubscript{3})\textsuperscript{+} at m/z 396 and 316, respectively, in the positive SIMS spectrum (Fig. 2b).

**Tetrodotoxin (Fig. 3B)**

The absolute structure of TTX was determined twenty years ago by three groups (Tsuda et al.\textsuperscript{14)}, Woodward\textsuperscript{15}) and Goto et al.\textsuperscript{13}). TTX disclosed (M+H)\textsuperscript{+} and (M+H−H\textsubscript{2}O)\textsuperscript{+} ion peaks at m/z 320 and 302, respectively, in the positive FAB mass spectrum (Fig. 1i), and an (M−H)\textsuperscript{−} peak at m/z 318 in the negative spectrum (Fig. 1j). SIMS presented essentially the same results as obtained by the positive FAB-MS (Fig. 2c).

**DISCUSSION**

The five toxins examined here have given rise to the pseudomolecular ions when applied to FAB-MS or SIMS.

STX and TTX, both non-sulfated, produced an intense (M+H)\textsuperscript{+} peak in the positive ionization mode, in contrast to the sulfated toxins which gave weak (M+H)\textsuperscript{+} peaks, along with rather intense fragment ion peaks resulting from the loss of SO\textsubscript{3}. On the other hand, the negative FAB mass spectra of all the sulfated toxins provided prominent pseudomolecular ion peaks. Poor pseudomolecular ions of the sulfate esters in the positive ion detection may be associated with their high acidity, which could contribute to rather rich production of (M−H)\textsuperscript{−} ions. Similar results have been obtained with some steroid sulfates.\textsuperscript{16)}

The molecular weights thus determined were 299 for STX, 411 for GTX\textsubscript{1}, 395 for GTX\textsubscript{2}, 475 for PX\textsubscript{1} and 319 for TTX, in good accordance with their reported molecular weights.\textsuperscript{1} Mass spectra of PSPs and TTX have not so far been measured successfully, since these toxins are non-volatile and, in addition, thermolabile to be applied to EI, CI or FD mass spectrometry. The structures of GTX\textsubscript{1} and GTX\textsubscript{2} have been proposed on the basis of their spectroscopic and chemical data, together with the absolute structure of STX. The present results obtained by FAB-MS and SIMS strongly support the proposed structures of both GTXs. It is worthwhile to note that the reliability of the proposed structure of GTX\textsubscript{1}, a derivative of neosaxitoxin (neoSTX, 2 in Fig. 3A), was obtained by determination of its molecular weight. The structure of neoSTX has been proposed as N (1)-hydroxysaxitoxin, from its \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra as compared with those of STX, from some conversion experiments of neoSTX to STX,\textsuperscript{17)} and also from comparison of the \textsuperscript{15}N-NMR spectra of neoSTX and GTX\textsubscript{2}.\textsuperscript{18)} Our results with GTX\textsubscript{1} are also in favor of the proposed structure of neoSTX.

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**REFERENCES**


