



HOKKAIDO UNIVERSITY

Title	STRUCTURE AND TOXICITY OF CLOSTRIDIUM BOTULINUM TYPE C TOXIN
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INFORMATION

Hokkaido University granted the degree of Doctor of Veterinary Medicine to the following two researchers on 25 December, 1980 under a new regulation (1962) authorizing the granting of the Doctor's degree to qualified researchers who were not graduates of the Graduate School of Veterinary Medicine.

The titles of their theses and other information are as follows :

STRUCTURE AND TOXICITY OF *CLOSTRIDIUM* *BOTULINUM* TYPE C TOXIN

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Clostridium botulinum type C toxin was purified and crystallized from the culture supernatant grown for 5-6 days at 33°C. The crystal system, lattice constants, and the molecular shape of the toxin crystal were analysed by the electron microscopy. The lattice constants of the crystal were $a=84.6 \text{ \AA}$, $b=146.7 \text{ \AA}$, $c=71.4 \text{ \AA}$, $\alpha=90^\circ$ and $\beta=94.3^\circ$. There were 4 molecules in a unit cell. The crystal system was monoclinic. The shape of the toxin molecule observed by the electron microscopy in the crystal was like the pressed ball with 73.3 Å of long diameter, 71.4 Å of short diameter and 42.3 Å of thickness.

Molecular weight of the purified toxin was determined as 141,000 daltons by the ultracentrifugal analysis, gel filtration and SDS-polyacrylamide gel electrophoresis. Reduction of the toxin molecule with 2-mercaptoethanol gave two components, heavy and light chains, with the molecular weights of 98,000 daltons and 53,000 daltons, respectively. These two components were separated and purified by ion-exchange chromatography on a column of QAE Sephadex A-50 in the presence of 5% of 2-mercaptoethanol at 0°C. No toxic activity was detected on both the chains. They were different from each other in antigenicity, amino acid composition and electric charge.

Reassociation of these two components gave a molecule with lower toxic activity (0.1-3% of the native toxin) in the delay of S-S linkage formation, whereas a molecule with high toxicity (70-90% of the native toxin) in the first formation of S-S linkage.

It was suggested by the results of the chemical modifications of the toxin molecule that at least one residue of tryptophane and 5-6 residues of tyrosine were contributed to constitute the toxic structure. The same suggestion was also obtained from the results of the solvent perturbation difference spectroscopy analyses of the accessible

fractions in the native and the low active reconstituted toxin. Toxic structure may be constituted by the specific cooperation of two components.

**PATHOLOGICAL STUDIES ON EQUINE SPINAL
ATAXIA IN JAPAN**

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Original report of this thesis appeared in "The Japanese Journal of Veterinary Science" Vol. 42, 681-694 (1980).

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The titles of their theses and other information are as follows:

**STUDIES ON THE CANINE TRANSMISSIBLE SARCOMA ORIGINATED
FROM THE NATURALLY OCCURRING VENEREAL SARCOMA:
SUCCESSIONAL TRANSPLANTATION AND GROWTH
AND REGRESSION OF THE SARCOMA**

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Original reports of this thesis appeared in "Japanese Journal of Veterinary Research" Vol. 22, 105-110 (1974), "Gann" Vol. 70, 115-118 (1979) and "Japanese Journal of Veterinary Science" (in press).