

Recent Progress in Jellyfish Toxin Study

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Recently, we have reported the chemical properties of some jellyfish proteinaceous toxins. These were the first chemical characterizations of jellyfish protein toxins to be reported. The isolation of the proteinaceous toxins in their active forms was the key step in the studies. We isolated the toxins from three box jellyfish (Cubozoa) species [*Carybdea rastoni* (*C. rastoni*), *Carybdea alata* (*C. alata*), and *Chiropsalmus quadrigatus* (*C. quadrigatus*)]. These toxins showed lethal toxicity to crustaceans and hemolytic activity. Furthermore, the full-length cDNAs and the deduced amino acid sequences of the toxins were clarified. All of these toxins have a molecular weight of around 45 kDa and their amino acid sequences showed homology with each other. The box jellyfish toxins represent a novel bioactive protein family.

Key words — jellyfish, protein, toxin, Cubozoa, sting

INTRODUCTION

Some jellyfish have venomous stings and are harmful to humans. For example, the deadly box jellyfish *Chironex fleckeri* (*C. fleckeri*) has caused many fatalities in Australia and the Portuguese man-of-war *Physalia physalis* also has a severe sting occasionally causing death.¹⁾ From the public health point of view, jellyfish toxins have been extensively examined and revealed to be mostly proteinaceous toxins.²⁾ However, the precise chemical nature of none of the jellyfish toxins has been successfully elucidated, because the lability of these toxins has hampered further studies.³⁾ Among the many jellyfish species, box jellyfish (Cubozoa) are especially recognized as hazardous and include *C. fleckeri*, *Chiropsalmus quadrigatus* (*C. quadrigatus*), *Carukia barnesi*, *Carybdea rastoni* (*C. rastoni*), and *Carybdea alata* (*C. alata*).⁴⁾ Thus our group has focused on the chemical characterization of box jellyfish toxins. This paper summarizes the recent progress in the study of jellyfish toxin, especially box jellyfish toxin.

Proteinaceous Toxins of *C. rastoni*

The box jellyfish (Sea Wasp) *C. rastoni* (andonkuraige in Japanese) is one of the most annoying marine organisms for swimmers and bathers at the Japanese seashore.⁵⁾ The stinging of *C. rastoni* causes cutaneous pain and inflammation in humans. Thus we choose *C. rastoni* as the first target of study. During that study, we observed that the toxicity of the proteinaceous toxins from *C. rastoni* was markedly decreased during purification, storage, and sample concentration. Determining the optimal conditions for the purification and storage of the toxins was the key step in characterizing them. It was found that ion-exchange chromatography, but not an ultrafiltration system, was suitable for concentrating toxins. We also found that the purified samples should be stored in a high salt-concentration solution, and should not be frozen or freeze-dried. Thus we stored the purified toxins in a solution with a high salt concentration such as 0.8 M NaCl and 5 mM phosphate buffer (pH 6.0) at 4°C. Under this condition, 90% of the hemolytic activity of *C. rastoni* toxins could be retained for more than 6 months of storage. Finally, two proteinaceous toxins, *C. rastoni* toxins A and B (CrTX-A, 43 kDa; CrTX-B, 46 kDa), were isolated in their active forms from the tentacle of the box jellyfish *C. rastoni*. Peptide mapping revealed some amino acid sequences of the peptide fragments originating from the toxins. Based on the

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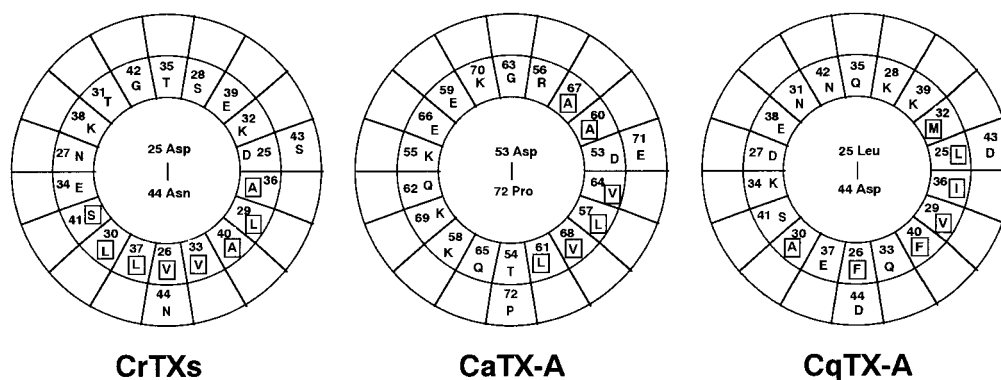


Fig. 2. Predicted Amphiphilic α -Helices in CrTXs, CaTX-A, and CqTX-A

The potential amphiphilic α -helices were predicted in the structures of CrTXs, CaTX-A, and CqTX-A. They are depicted by Edmundson helical wheels.¹⁰ Hydrophobic amino acids are boxed. CrTXs, from aspartic acid (Asp)-25 to asparagine (Asn)-44; CaTX-A, from Asp-53 to proline (Pro)-72; CqTX-A, from leucine (Leu)-25 to Asp-44.

Table 1. Comparison of Lethal Toxicity and Hemolytic Activity of the Box Jellyfish Toxins

| Toxin (Organism) | Lethal toxicity to crayfish (intraperitoneal, LD ₅₀ value) | Hemolytic activity (0.8% sheep red blood cells, EC ₅₀ value) |
|----------------------------------|--|--|
| CrTX-A (<i>C. rastoni</i>) | 5 μ g/kg | 2 ng/ml |
| CaTX-A (<i>C. alata</i>) | 5–25 μ g/kg | 70 ng/ml |
| CqTX-A (<i>C. quadrigatus</i>) | 80 μ g/kg | 160 ng/ml |

sequence using the BLAST algorithm revealed homology (43.7%) with that of CrTXs.⁶ The deduced CqTX-A amino acid sequence had 25.2% homology with that of CrTXs and 21.6% homology with that of CaTX-A.⁸ Comparison of the box jellyfish toxin amino acid sequences with those of other proteins using the BLAST (basic local alignment search tool) algorithm showed no significant similarity.⁸ Since a novel bioactive protein family has emerged from these studies of the box jellyfish toxins (CrTXs, CaTX-A, and CqTX-A), we propose here to designate these novel bioactive proteins as the box jellyfish toxin family. Secondary structural analysis of these toxins predicted the presence of α -helices that existed in the *N*-terminus region. Construction of an “Edmundson wheel”¹⁰ showed that the predicted α -helices were amphiphilic (Fig. 2). Previous studies suggested that the amphiphilic structures of some cytolytic protein toxins allow the formation of pores in cell membranes,^{11–13} and thus the amphiphilic structures of the box jellyfish toxins may explain their potent hemolytic activity (Table 1).

Lethal Toxicity and Hemolytic Activity

The LD₅₀ values (intraperitoneal injection) of CqTX-A, CrTX-A, and CaTX-A in crayfish

(*Procambarus clarkii*) were 80 μ g/kg, 5 μ g/kg, and 5–25 μ g/kg, respectively (Table 1). CqTX-A, CrTX-A, and CaTX-A caused 50% hemolysis in 0.8% sheep red blood cells at a concentration of 160 ng/ml, 2 ng/ml, and 70 ng/ml, respectively (Table 1).^{5,6,8} These data indicate that CqTX-A is less toxic than CrTX-A and CaTX-A. The number of tentacles of *C. quadrigatus* is several times greater than that of *C. alata* or *C. rastoni*. Furthermore, the length of the tentacles of *C. quadrigatus* is at least three times that of *C. alata* or *C. rastoni*. Therefore, in human stings, the amount of toxin injected into the victim by *C. quadrigatus* in one incident should be much greater than the amounts injected by *C. alata* or *C. rastoni*. This is one reason why *C. quadrigatus* stings are more dangerous than those of *C. rastoni* or *C. alata*. Massive regional stings were observed on the bodies at autopsy after fatal *C. quadrigatus* incidents. CqTX-A was the major toxin in the nematocysts, and thus CqTX-A is likely to be the causative toxin in fatal cases of *C. quadrigatus* stings. Further study on the mode of action of these toxins will lead to the development of effective and specific remedies for box jellyfish stings.

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