ELSEVIER

Toxicon 46 (2005) 768–774

TOXICON

www.elsevier.com/locate/toxicon

Novel peptide toxins from acrorhagi, aggressive organs of the sea anemone *Actinia equina*

Tomohiro Honma[a], Sonomi Minagawa[a], Hiroshi Nagai[b], Masami Ishida[b], Yuji Nagashima[a], Kazuo Shiomi[a,*]

[a]*Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan*

[b]*Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan*

Received 3 May 2005; accepted 2 August 2005
Available online 23 September 2005

Abstract

Two peptide toxins, acrorhagin I (50 residues) and II (44 residues), were isolated from special aggressive organs (acrorhagi) of the sea anemone *Actinia equina* by gel filtration on Sephadex G-50 and reverse-phase HPLC on TSKgel ODS-120T. The LD_{50} against crabs of acrorhagin I and II were estimated to be 520 and 80 μg/kg, respectively. 3′- and 5′-RACE established the amino acid sequences of the acrorhagin precursors. The precursor of acrorhagin I is composed of both signal and mature peptides and that of acrorhagin II has an additional sequence (propart) between signal and mature peptides. Acrorhagin I has no sequence homologies with any toxins, while acrorhagin II is somewhat similar to spider neurotoxins (hainantoxin-I from *Selenocosmia hainana* and Tx 3-2 from *Phoneutria nigriventer*) and cone snail neurotoxin (ω-conotoxin MVIIB from *Conus magus*). In addition, analogous peptides (acrorhagin Ia and IIa) were also cloned during RT-PCR experiments performed to confirm the nucleotide sequences of acrorhagins. This is the first to demonstrate the existence of novel peptide toxins in the sea anemone acrorhagi.

Keywords: Acrorhagi; *Actinia equina*; cDNA cloning; Peptide toxin; Sea anemone

1. Introduction

Sea anemones are a rich source of proteins and peptides that are cytolytic or neurotoxic. Three classes of toxins, 20 kDa pore-forming hemolysins (actinoporins) (Kem, 1988; Anderluh and Maček, 2002), 3–5 kDa sodium channel toxins (Kem, 1988; Norton, 1991) and 3.5–6.5 kDa potassium channel toxins (Castañeda et al., 1995; Schweitz et al., 1995; Cotton et al., 1997; Gendeh et al., 1997; Diochot et al., 1998; Minagawa et al., 1998), have been isolated from a number of sea anemones and well characterized. Besides these toxins, structurally novel toxins also exist in some sea anemones; for example, 60 kDa hemolysins (PsTX-60A and B) have been found in *Phyllodiscus semoni* (Nagai et al., 2002), an epidermal growth factor-like toxin (gigantoxin I) in *Stichodactyla gigantea* (Shiomi et al., 2003) and 6.5 kDa toxins (AETX II and III) with high crab lethality in *Anemonia erythraea* (Shiomi et al., 1997). Although, the sea anemone toxins described above have been isolated mostly from the whole body or tentacles, they are considered to be contained in tiny stinging organelles called nematocysts, which are distributed in various tissues, especially in tentacles. Upon chemical or physical stimulation the thread tubule folded in the nematocyst is discharged and penetrates the epithelium of the victim.

* Corresponding author. Tel.: +81 3 5463 0601; fax: +81 3 5463 0669.
E-mail address: shiomi@s.kaiyodai.ac.jp (K. Shiomi).

0041-0101/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2005.08.003

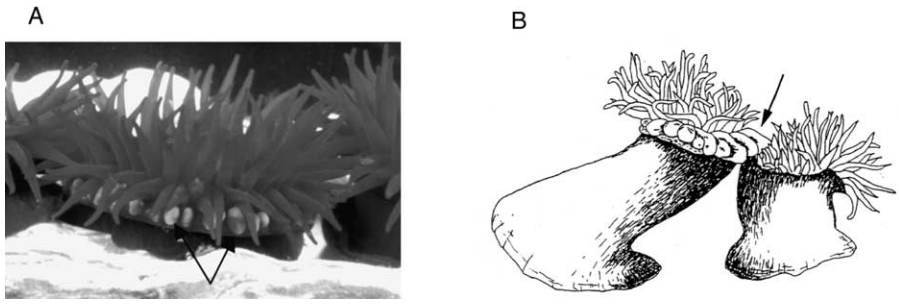

Fig. 1. Sea anemone acrorhagi. (A) *Actinia equina* and its acrorhagi (indicated by arrows). Note that the acrorhagi are somewhat dilated. (B) Aggression by acrorhagi. The aggressor (left) dilate acrorhagi (indicated by an arrow) fully and press them against the body of the victim (right).

Simultaneously, the toxins in the nematocyst are injected into the tissue of the victim through the thread tubule. It is thus assumed that the sea anemone toxins are primarily used in capture of prey animals.

It is known that certain species of sea anemones belonging to the family Actiniidae have evolved special aggressive organs (acrorhagi) in a ring around the base of the tentacles to fight with non-specific non-clonemates (Fig. 1; Williams, 1991). Acrorhagi are small sacs having a number of large holotrichous nematocysts that are morphologically distinct from those in other tissues. They can dilate and move to contact with an encroaching anemone. After contact, pieces of acrorhagial ectoderm of the aggressor adhere to the body of the victim, into which nematocysts are discharged. The victim then move away from the aggressor. Within few days, local necrosis or even death ensues in the victim, suggesting the occurrence of toxic principles in the acrorhagi. At present, however, no information is available as to what kinds of toxins are contained in the acrorhagi. This prompted us to isolate acrorhagial toxins using the colonial sea anemone *Actinic equina* with developed acrorhagi. *Actinia equina* is a relatively small species commonly found in the temperate, coastal waters of the world including Japan and is well known to possess hemolysins (equinatoxins; Ferlan and Lebez, 1974; Anderluh and Maček, 2002), a sodium channel peptide toxin (Ae I; Lin et al., 1996) and a potassium channel peptide toxin (Ae K; Minagawa et al., 1998). We report here the isolation and molecular cloning of two novel peptide toxins (named acrorhagin I and II) from the acrorhagi of *A. equina* that are structurally remote from Ae I and Ae K.

2. Materials and methods

2.1. Sea anemone

Live specimens of *A. equina* were collected along the coasts of Katsuura, Chiba Prefecture, in June 2003. They were kept in an aquarium until use.

2.2. Bioassay

Lethal activity was assayed using freshwater crabs (*Potamon dehaani*) weighing about 5 g purchased from the Tokyo Central Wholesale Market. Sample solutions were injected into crabs at the junction between the body and the leg. The injection volume was fixed at 10 μl/g of body weight of crabs. In order to calculate LD_{50} against crabs by the method of Litchfield and Wilcoxon (1949), groups of five crabs were challenged with various doses of toxin and observed for mortality up to 2 h.

2.3. Isolation method

Acrorhagi (2 g) were carefully collected from 33 live specimens (110 g) of *A. equina* and well macerated in a motor. The macerate was homogenized in 20 ml of distilled water and centrifuged at 18,800×*g* for 15 min. This extraction procedure was repeated once more. The combined supernatants were applied to gel filtration on a Sephadex G-50 column (2.0×45 cm; Amersham Biosciences, Piscataway, USA), which was eluted with 0.15 M NaCl in 0.01 M phosphate buffer (pH 7.0). Fractions of 3 ml were collected and measured for absorbance at 280 nm and crab toxicity. Toxin-containing fractions were pooled and subjected to reverse-phase HPLC on a TSKgel ODS-120T column (0.46×25 cm; Tosoh, Tokyo, Japan). The column was washed with 0.1% trifluoroacetic acid (TFA) and then eluted with two steps of linear gradients of acetonitrile (0–21% in 5 min and 21–38.5% in 60 min) in 0.1% TFA at a flow rate of 1 ml/min. Peptides were monitored at 220 nm with a UV detector. The eluate corresponding to each toxin peak was manually collected, lyophilized and dissolved in desired solvents for subsequent experiments.

2.4. Chemical analysis

Peptides were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Amino acid sequencing was performed with an automatic gas-phase protein sequencer (LF-3400D TriCart with high sensitivity chemistry; Beckman Coulter, Fullerton, USA). Molecular