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Putative γ-conotoxins in vermivorous cone snails: the case of *Conus delessertii*

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Abstract

Peptide de7a was purified from the venom of *Conus delessertii*, a vermivorous cone snail collected in the Yucatan Channel, Mexico. Its amino acid sequence was determined by automatic Edman degradation after reduction and alkylation. The sequence shows six Cys residues arranged in the pattern that defines the O-superfamily of conotoxins, and several post-translationally modified residues. The determination of its molecular mass by means of laser desorption ionization time-of-flight mass spectrometry (average mass, 3170.0 Da) confirmed the chemical data and suggested amidation of the C-terminus. The primary structure (ACKOKNNLCAITγMAγCCSGFCLIYRCS*; O, hydroxyproline; γ, γ-carboxyglutamate; *, amidated C-terminus; calculated average mass, 3169.66 Da) of de7a contains a motif (—γCCS—) that has previously only been found in two other toxins, both from molluscivorous cone snails: TxVIIA from *Conus textile* and γ-PnVIIA from *Conus pennaceus*. These toxins cause depolarization and increased firing of action potentials in molluscan neuronal systems, and toxin γ-PnVIIA has been shown to act as an agonist of neuronal pacemaker cation currents. The similarities to toxins TxVIIA and γ-PnVIIA suggest that peptide de7a might also affect voltage-gated nonspecific cation pacemaker channels.

Keywords: Conacea; Conidae; Cone snail; *Conus delessertii*; Gamma-conotoxin; Pacemaker channels

1. Introduction

From a structural point of view, peptide toxins found in the venoms of the marine predators belonging to the genus *Conus* (superfamily Conoidea, family Conidae), commonly known as cone snails or cones, have been divided into two main groups: those with zero or one disulfide bridge, called "conopeptides", and those with two to five disulfide bridges, named "conotoxins." The mature peptide toxins present in the venoms are generated from larger precursors by proteolytic cleavage and post-translational modifications. The precursors may be classified into gene superfamilies (A, T, O, M, P, I, S, for the "conotoxins"), accordingly to the sequences of the signal peptides, and a correlation has been found between the latter and the frameworks and connectivity of the Cys residues within the sequence of the mature toxins (for an excellent review, see reference [6]).

Conopeptides and conotoxins may also be classified into pharmacological families depending on the type of molecular target and the effect of the toxin upon it. Within the conotoxins, well-known pharmacological targets are voltage-gated ion channels (Na^+, K^+, Ca^{2+}), ligand-gated ion channels (nAChR, 5-HT_3R), G-protein-coupled receptors (α1 adrenergic) and neurotransmitter transporters (NET) [6].

The O-superfamily of conotoxins includes several types of peptides that have three disulfide bonds but different pharmacological targets, all of which are voltage-gated ion channels. Sodium channels are distinctly affected by the δ- and μO-conotoxins, whereas potassium channels are blocked by the κ-conotoxins, and calcium channels are the targets of ω-conotoxins. So far, the four pharmacological families of the O-conotoxins have been shown to have the same Cys arrange-

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ment (one pair of adjacent Cys residues flanked on both sides by two non-adjacent Cys residues) and disulfide connectivity (I–IV, II–V, III–VI). Thus, all of the O-conotoxins correspond to scaffold O-1 (formerly, frameworks VI/VII), according to the new nomenclature proposed by Olivera [6].

However, there are other conotoxins that also contain six Cys residues whose disulfide connectivity is unknown, and these could belong to the O-superfamily based on the arrangement of these amino acids along their sequences. The first class is defined by the "bromosleeper" peptide from *Conus radiatus*, a 33-residue molecule with three classes of post-translational modifications: bromination of the N-terminal Trp, γ-carboxylation of four Glu residues, and hydroxylation of two Pro residues [2]. Although this peptide clearly induces, in mice of all ages, a sleep-like state, its molecular target remains unknown [8]. The second class includes two excitatory peptides from molluscivorous species, TxVIIA from *C. textile neovicarius* [3,5], and γ-PnVIIA from *Conus pennaceus* [4]. Each of these acidic toxins contains two γ-carboxyglutamate (Gla) residues that are thought to have a functional role [3,5]. These peptides produce, with distinct specificity, depolarization and increased firing of action potentials in molluscan neuronal systems. Due to its activation of a slow inward cation current in the caudodorsal neurons of *Lymnaea*, toxin γ-PnVIIA has been proposed to act as an agonist of neuronal pacemaker cation currents and as the prototype of a new class of conotoxins, the γ-conotoxins [4]. These peptides not only posses an uncommon biological effect among conotoxins, but also a unique structural feature, the motif −SγCCS− (γ denotes a Gla residue).

Here, we describe the first purification and amino acid sequence determination of a peptide from the venom of *Conus delessertii*. This peptide contains several post-translational modifications and six Cys residues arranged in the pattern that defines the O-superfamily of conotoxins, and it was named de7a, accordingly to the nomenclature of conotoxins [7]. Moreover, peptide de7a contains the sequence −γCCS− that is contained within the motif −SγCCS− only found in the γ-conotoxins. Thus, *C. delessertii* is the first vermivorous species shown to contain this class of conotoxins, and this feature suggests that peptide de7a may act on voltage-gated, nonspecific cation pacemaker channels.

2. Materials and methods

2.1. Specimen collection and venom extraction

Specimens of the worm-hunting snail *C. delessertii* were collected by fishing vessels in the Mexican Caribbean Sea off the Yucatan peninsula and immediately frozen and stored at −70 °C until used. The venom ducts from four specimens were dissected out and homogenized in 5 mL of 0.5% (v/v) trifluoroacetic acid (TFA) in 50% (v/v) aqueous acetonitrile (MeCN). The homogenate was then centrifuged at 17,000 × *g* for 30 min at 4 °C. The supernatant was kept at −70 °C; it contains the peptides and is referred to as the venom extract.

2.2. Peptide purification

Venom extract was applied onto a reversed-phase C18 High-Performance Liquid Chromatography (HPLC) analytical column (Vydac, 218TP54; 4.6 mm × 250 mm, 5 μm particle size) provided with a guard column (Vydac, 218GK54; 4.6 mm × 10 mm, 5 μm particle size). The components of the extract were eluted at room temperature at a flow rate of 1 mL/min using a linear gradient from 5 to 95% solution B in 90 min. HPLC solutions were: (A) 0.1% (v/v) TFA in water, and (B) 0.085% (v/v) TFA in 90% (v/v) aqueous MeCN. The absorbance was monitored at 220 nm, and the chromatographic peaks were collected manually. To start the characterization of the toxins present in this venom, the same column was employed for further purification of a relatively abundant peak (arrow in Fig. 2A), using a linear gradient starting with 16% solution B, and increasing solution B by 0.33%/min, at 1 mL/min.

2.3. Molecular mass determination

Positive ion matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained in the linear and the reflector modes with a JEOL JMS HX110 double-focusing spectrometer.

2.4. Cysteine stabilization and amino acid sequence determination

An aliquot of peptide de7a was taken to dryness, and 150 μL of 17 mM Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) in 0.17 M citrate buffer, pH 3.0, were added. After mixing, the sample was incubated at 65 °C for 20 min. The reduced peptide was alkylated by the addition of 3 μL of 4-vinylpyridine/1 mL of peptide solution. After incubation at room temperature for 20 min in the dark, the solution was diluted to reduce the MeCN concentration, and the pyridylethylated peptide was repurified by reversed-phase HPLC. The same column as above was employed to purify the alkylated peptide, using a linear gradient starting with 16% solution B, and increasing solution B by 0.33%/min, at 1 mL/min.

The pyridylethylated peptide was adsorbed onto a Biobrene-treated glass fiber filter, and the amino acid sequence was analyzed by automated Edman degradation on an Applied Biosystems Model 477A Protein Sequencer.

2.5. Sequence alignment

In order to make sequence comparisons, one of the most robust multiple sequence alignment programs, CLUSTAL W (1.82), was employed in the pairwise mode, using the default settings (http://www.ebi.ac.uk/clustalw/, [9]).