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Contryphan Genes and Mature Peptides in the Venom of Nine Cone Snail Species by Transcriptomic and Mass Spectrometric Analysis

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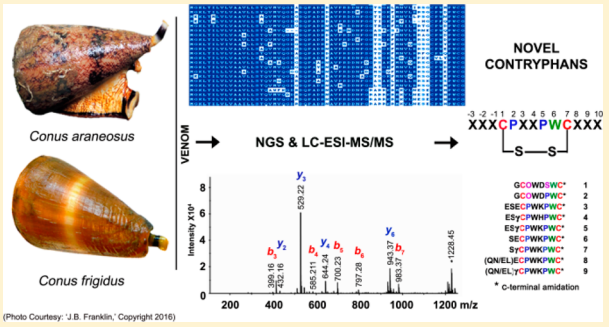
Supporting Information

ABSTRACT: The occurrence of contryphans, a class of single-disulfide-bond-containing peptides, is demonstrated by the analysis of the venom of nine species of cone snails. Ten full gene sequences and two partial gene sequences coding for contryphan precursor proteins have been identified by next-generation sequencing and compared with available sequences. The occurrence of mature peptides in isolated venom has been demonstrated by LC−ESI−MS/MS analysis. De novo sequencing of reduced, alkylated contryphans from *C. frigidus* and *C. araneosus* provides evidence of sequence variation and post-translational modification, notably gamma carboxylation of glutamic acid. The characterization of Fr965 (*C. frigidus*) provides a rare example of a sequence lacking Pro at position 5 in the disulfide loop. The widespread occurrence of contryphan genes and mature peptides in the venom of diverse cone snails is suggestive of their potential biological significance.



KEYWORDS: *conotoxins, contryphans, Conus venom peptides, peptide disulfides, mass spectrometry, next-generation sequencing, contryphan genes, cone snails*

INTRODUCTION

Marine cone snail venom contains a large number of peptides that exhibit high affinities for specific biological targets, most notably membrane ion channels and neurotransmitter receptors.[1−6] The conotoxins, a group of peptides ranging in length from 10 to 60 residues, have attracted a great deal of interest since the pioneering work of Olivera and coworkers revealed a dramatic spectrum of biological activities.[5−7] The majority of conotoxins that have been extensively investigated so far possess two or three disulfide bonds. *Conus* venom also contains peptides with a single disulfide bond and also sequences that lack cysteine (Cys) residues. The single disulfide bonded peptides include the conopressins[8,9] and contryphans.[10−15] Both linear and single-disulfide-bond-containing sequences have been reported for the conantokins, a class of *N*-methyl-D-aspartate (NMDA) antagonists.[16−18] The contryphans were first identified from the venom of the fish hunting snail, *Conus radiatus*, in 1996.[10] The contryphan sequences proved to be unique in containing a D-residue, in addition to other post-translational modifications commonly observed in *Conus* peptides like C-terminal amidation, proline hydroxylation, tryptophan bromination, and γ-carboxylation of glutamic acid.[19−22] The characteristic feature of contryphan sequences determined thus far is the presence of a tryptophan residue and one or more proline residues in the five-residue segment between the disulfide-bonded Cys residues (Figure 1). Contryphans exist in solution as a mixture of two conformers due to cis−trans isomerization around the N-terminal Cys−Pro peptide bond.[23]

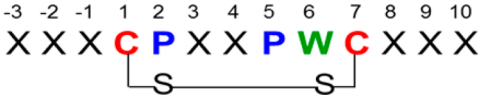


Figure 1. Numbering schemes for contryphans. Positions 2 and 5 are predominately Pro(P), while position 6 is Trp(W).

Following the discovery of contryphan, it was established that direct injection of this peptide into the central nervous system of mice resulted in responses that suggested that these peptides might contribute to the general excitotoxic shock response.[7,24] Subsequent studies have provided some evidence that the contryphan might act on Ca^{2+}-dependent K^+ channels.[13,25] The contryphans display interesting conformational properties because of the presence of multiple prolines within the disulfide loop,[26] raising the possibility that slow conformational isomerism may modulate observed biological activity. The presence of contryphans has been demonstrated in the venom of several *Conus* species. More recently, the

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