A conserved cysteine framework of toxins from *Viola tricolor* and *Conus brunneus* characterized in the *Drosophila melanogaster* Giant Fiber System

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ABSTRACT

Conotoxins are disulfide rich peptides present in the venom of cone snails, a genus of marine mollusks that prey upon fish, worms, and other mollusks. Conotoxins are promising drugs leads with great prospects in the treatment of diseases and disorders such as chronic pain, multiple sclerosis and Parkinson's and Alzheimer's diseases. Similar compounds can be found in plants; for example, cyclotides, which are cyclic peptides isolated from the Violaceae (violet), Rubiaceae (coffee), and Cucurbitaceae (cucurbit) families and they have a wide range of biological activities, such as anti-HIV, uterotonic, and antimicrobial. Cyclotides have a cyclic cysteine knot motif characterized by a cyclic backbone and six conserved cysteine residues that form the three disulfide bridges of the "knot". This motif provides cyclotides with superior stability against thermal, chemical, and enzymatic degradation; marking them as potential frameworks for peptide drug delivery. Cysteine framework IX conotoxins (C-C-C-CXC-C), isolated from the venom of *Conus brunneus*, contain the same cysteine framework, homologous sequences, and similar 3D structures to cyclotides. Presented are details on the isolation of these conotoxins and cyclotides, from *Viola tricolor,* and the characterization of their activity in the *Drosophila melanogaster* Giant Fiber System (GFS), which contains GAP, acetylcholine, and glutamate synapses.

BACKGROUND

Conotoxins of *Conus brunneus*

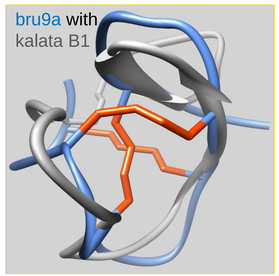
- Worm-hunting venomous marine mollusk
- P Superfamily Conotoxins
  - C-C-C-CXC-C
    - bru9a
    - bru9b
- 3 disulfide bonds (cysteine knot)
- Similar 3D structure to cyclotides

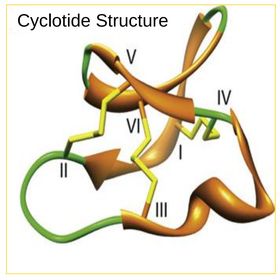
Cyclotides of *Viola tricolor*

- ~30 amino acids
- Head-to-tail cyclic backbone
- 3 disulfide bonds (cyclic cysteine knot)
- Activity
  - Uterotonic
  - Anti-bacterial & anti-fungal
  - Insecticidal – self defense

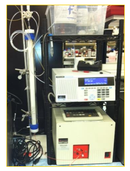
TOXIN FRAMEWORK AND PEPTIDE STRUCTURE

Peptide	MW	Sequence
bru9a	2523.8	-----SCGGSCFGG-CWOG---CSCYART--CFRD
bru9b	4133.1	SLDKGSNCGQDCSSDNCOSG---CFCYPRDNVCYVERRLN
varvA	2877.9	NGLPV--CGETCVGGTCNTPG--CSCSWPV--CTR
varvE	2892.2	NGLPI--CGETCVGGTCNTPG--CSCSWPV--CTR
vitriA	3152.8	NGIP---CGESCVWIPCITSAIGCSCKSKV--CYR





ISOLATION AND IDENTIFICAITON OF PEPTIDES



Peptide Extraction
↓
Size Exclusion HPLC
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Semi-Preparative RP-HPLC
↓
MALDI-TOF
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Analytical RP-HPLC
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MALDI-TOF
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Drosophila Assay

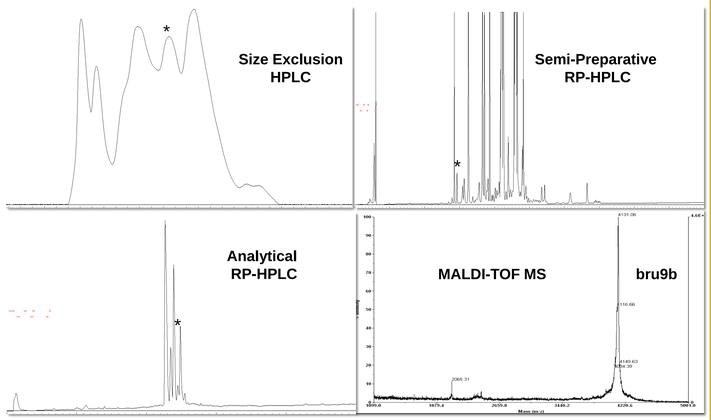


FIGURE 1: HPLC isolation and MALDI mass spectrometry conformation of conotoxin bru9b

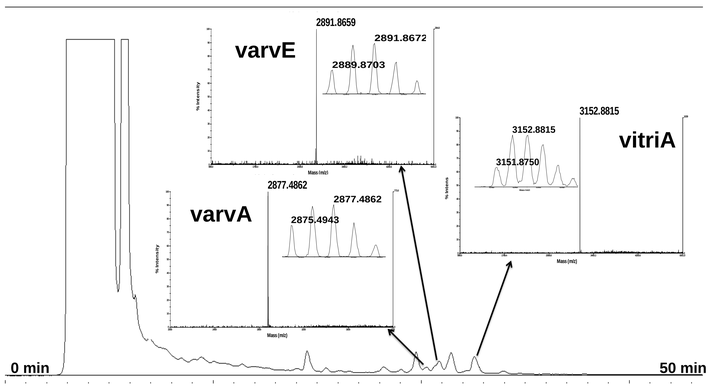
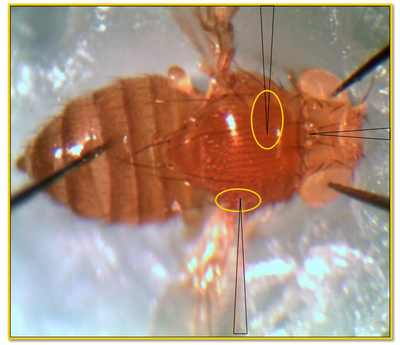
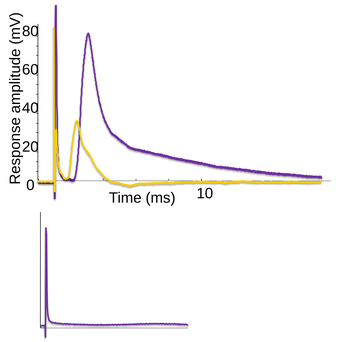
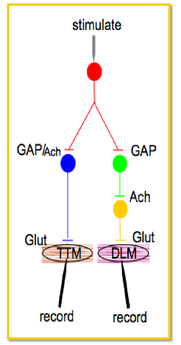


FIGURE 2: Semi-Preparative RP-HPLC and MALDI-TOF MS of cyclotides

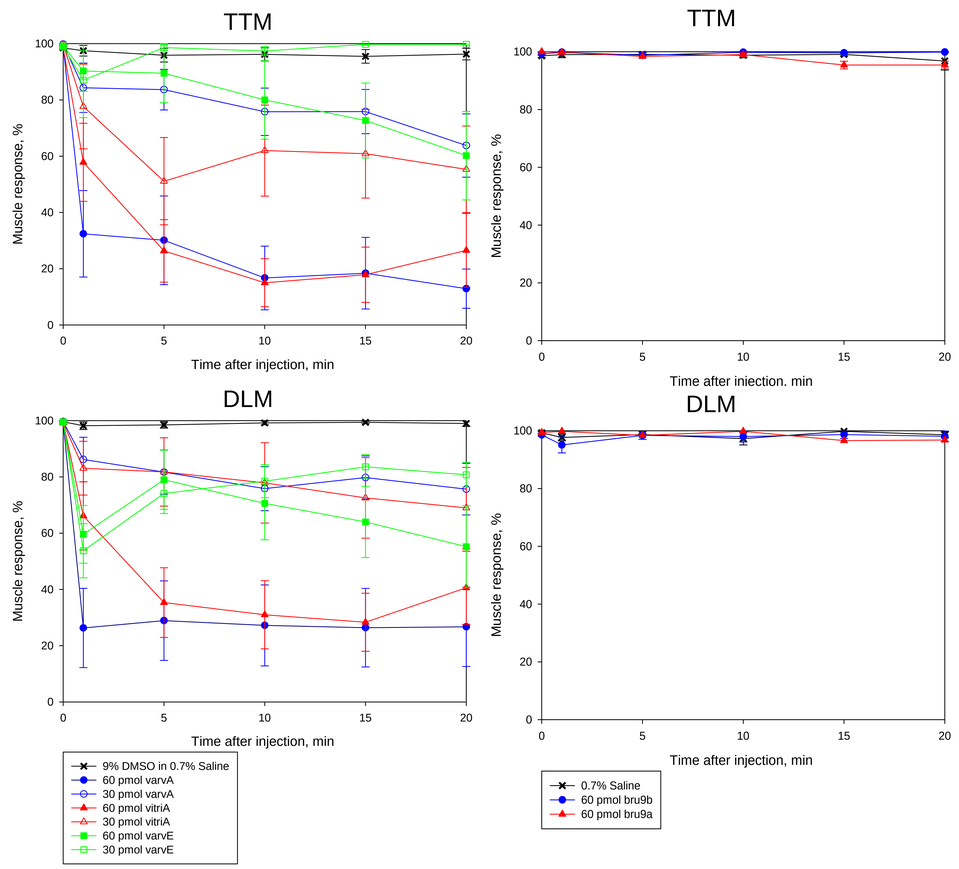
DROSOPHILA GIANT FIBER







RESULTS AND DISCUSSION



Cyclotides from *V. tricolor* affect both TTM and DLM muscle responses in the *D. melanogaster* assay; whereas, the conotoxins from *C. brunneus* have no affect. Future work consists of testing higher concentrations of peptide to identify the specific target of cyclotides within the GFS. The conotoxins will be tested in an *in vitro* immune cell flow cytometry assay to further assess their biological targets.

Refrences: Svangard, *J Nat. Prod,* **2004**. Mariamawit, *Phytochemistry Letters*, **2012.** Chen, *The Journal of Biological Chemistry,* **2005**. Mejia, *Toxicon*, **2010**. Tang, *Peptides,* **2010.** Claeson, *J. Nat. Prod.,* **1998**.

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