Title
Novel, Post-Translationally Modified Peptide Antibiotics from Solitary Tunicates

Authors
Vacquier, Victor
Taylor, Steven

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Novel, Post-Translationally Modified Peptide Antibiotics from Solitary Tunicates
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Co-Project Leaders: Victor Vacquier/Steven Taylor
University of California, San Diego
Scripps Institution of Oceanography
MC: 0202
La Jolla, CA 92093

Aims of the proposal:

Although the pharmaceutical industry has "mined" the world of soil bacteria, streptomycetes and fungi to find novel antimicrobial molecules, the emergence of resistant superbugs necessitates identifying new antibiotic sources. Recent research into the innate immune system has focused attention on antimicrobial peptides – molecules that equip animals to resist infection without assistance from antibodies and other accoutrements of adaptive immunity. In recent years, potent antimicrobial peptides have been identified in the blood cells (hemocytes) of horseshoe crabs, shrimp, mussels and tunicates ("sea squirts"). Our studies centered on three tunicates (*Styela clava*, *Styela plicata* and *Ciona intestinalis*) that are abundant in Southern California's shallow waters.

In our initial studies (Taylor, S.W., et al, J Biol Chem. 275:38417-38426, 2000), we observed that some tunicate antimicrobial peptides contained unusual post-translational modifications more commonly found in the classical "secondary metabolite" antibiotics produced by microbes. However, unlike most conventional antibiotics produced by prokaryotes, the endogenous antimicrobial peptides of animals are gene-encoded- a feature that offers exciting "down-the-road" possibilities for their recombinant production or transgenic expression in agricultural and aquacultural crops.

Research Findings:

Plicatamide is a modified octapeptide from the ascidian *Styela plicata* having the structure Phe-Phe-His-Leu-His-Phe-His-decarboxyΔDOPA (where decarboxyΔDOPA = decarboxy-(*E*)- α,β-dehydro-3,4-dihydroxyphenylalanine) (Tincu, Craig and Taylor Biochem Biophys Res Commun. 2000 Apr 13;270(2):421-4). During the course of the project we characterized this peptide's antimicrobial activity (Reference 1); identified a structurally related peptide (Reference 2) as well as caveats in the mass spectrometric characterization of this class of compounds (Reference 3). We also developed a new chemoenzymatic synthesis route to probe structure-activity relationships in plicatamide's antimicrobial activity (Reference 4).

We hypothesized that plicatamide was the product of post-translational cleavage from a polypeptide precursor as observed in many other antimicrobial peptides (References 5 and 6). Attempts to develop degenerate primers for use in polymerase chain reactions and library screens based upon the amino acid sequence of plicatamide were undertaken. The goal of the investigation was to address the biosynthetic origins of plicatamide utilizing the techniques of degenerate PCR, RNA ligase mediated rapid amplification of cDNA ends, and oligonucleotide screening of a cDNA library. Ultimately the eight residue sequence was insufficient for the identification of a polypeptide precursor and its existence remains unproven.

In efforts to gain insight to what blood cell types contain plicatamide in *S. plicata* we attempted immunolocalize the peptide. Native plicatamide and the synthethic derivative PL-101 (FFHLHFHY[$CONH_2$]) were coupled to keyhole limpet hemocyanin and injected in rabbits to produce ∝-plicatamide and ∝-PL-101 antibodies. ∝-Plicatamide antisera *did not* recognize plicatamide using western and dot blot analysis.

∝-PL-101 antisera *did* recognize PL-101 using western and dot blot analysis but *did not* recognize plicatamide using the same techniques. Additional synthetic derivatives of plicatamide were tested for reactivity with ∝-PL-101 antisera which partially determined the binding epitope of the ∝-PL-101 antibody which unfortunately has no utility for the immunolocalization of native plicatamide.

Two antimicrobial peptides from the ascidian *C. intestinalis* were isolated and partially characterized. These peptides, designated cionarin H and cionarin I, have many characteristics in common with larger molecular weight polypeptides and proteins isolated from ascidian blood cells including ferreascidin, the Ascidia and Mogula blood cell polypeptides and morulin Pm. These characteristics include resistance to Edman degradation sequencing and protease cleavage, possibly resulting from unknown post-translational modifications. Unlike these other peptides cionarins do not appear to contain DOPA or TOPA residues. While the cionarins eluded total characterization in the current study, preliminary results employing modern techniques of tandem mass spectrometry appear promising in elucidating the structures of this elusive class of biomolecules.

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2) Tincu JA, Taylor SW Tunichrome Sp-1: New pentapeptide tunichrome from the hemocytes of Styela plicata. J NAT PROD 65 (3): 377-378 MAR 2002.
3) Taylor SW, Kassel DB, Tincu JA, et al. Fragmentation of tunichrome Sp-1 is dominated by an unusual gas-phase intramolecular rearrangement. J MASS SPECTROM 38 (10): 1105-1109 OCT 2003.
4) Taylor SW. Chemoenzymatic synthesis of peptidyl 3,4-dihydroxyphenylalanine for structure-activity relationships in marine invertebrate polypeptides. ANAL BIOCHEM. 302(1): 70-4. MAR. 2002
5) Tincu JA, Taylor, SW Mini-Review. Antimicrobial peptides from marine invertebrates. ANTIMIC AGENTS AND CHEMO in press.
6) Lehrer RI, Tincu JA, Taylor SW, et al. Natural peptide antibiotics from tunicates: Structures, functions and potential uses. INTEGR COMP BIOL 43 (2): 313-322 APR 2003.