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Novel, Post-Translationally Modified Peptide Antibiotics from Solitary Tunicates

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Background

As is well recognized in the medical profession, the emergence of antibiotic-resistant bacteria creates serious public health problems. Most obviously, it makes it difficult to treat once easily cured infections, such as staph. It also increases the chances a patient will get an infection during a hospital stay. According to the Centers for Disease Control and Prevention, almost two million patients acquire an infection from a hospital each year. Of these, about 90,000 die from their infection, up from 13,300 patient deaths in 1992. Besides the consequences for human health, antibiotic resistance is yet another factor contributing to the rising cost of health care.

In the hopes of establishing a scientific base for developing new medicines, researchers have begun to look more closely at the innate immune systems of simpler life forms, such as marine invertebrates. An innate immune system is one in which an organism comes “hard-wired” with molecules that recognize, attack and kill pathogens such as bacteria and viruses. It contrasts with an adaptive (or acquired) immune system in which the body learns to recognize and defend against infection, through, for example, the production of antibodies in response to exposure to specific antigens. Humans have both innate and adaptive immune systems. Marine invertebrates have only an innate one.

Recent research has shown that certain peptides in the blood cells (hemocytes) of horseshoe crabs, shrimp, mussels and tunicates



Dr. Robert I. Lehrer. Images this story courtesy Lehrer Lab

are effective at killing bacteria that cause human disease. It is hoped that a greater understanding of the chemistry of these peptides, and how they fight infection, may one day lead to new antimicrobial medicines, ones that could treat currently resistant bacterial infections.

Project

California Sea Grant funded Robert Lehrer, M.D. of the David Geffen School of Medicine at UCLA, and Steve Taylor, currently a senior scientist at Amylin Pharmaceuticals, Inc., to elucidate the structures of antimicrobial peptides from the tunicates (“sea squirts”) *Styela clava*, *S. plicata* and *Ciona intestinalis*. These three species of primitive-looking organisms—tunicates look like fuzzy cucumbers but are actually simple protochordate ancestors of the vertebrates—thrive in microbe-laden shallow waters of Southern California. Because of this, researchers have long speculated these organisms must have effective and robust immune systems.

Previous research led by Lehrer has shown that antimicrobial pep-

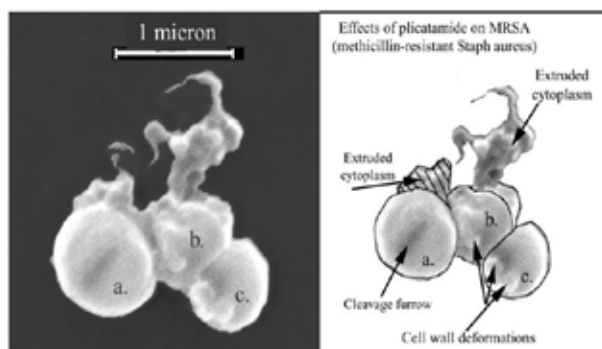
tides in tunicates have unusual post-translational modifications, meaning that the organisms are able to modify amino acids, creating compounds that resemble secondary metabolites produced by bacteria. Secondary metabolites from prokaryotes (bacteria and fungi) are the source of many conventional antibiotics (e.g., penicillin). Unlike conventional antibiotics, however, peptides from tunicates are gene-coded (i.e., the peptides are synthesized directly from genetic information), a feature that offers exciting “down-the-road” possibilities for their recombinant production or transgenic expression in agricultural and aquacultural crops. In other words, it may be possible to isolate the gene that codes for the production of a certain protein and to insert this gene into another organism.

Other properties of these peptides also favor their investigation. For one, the molecules tend to be small, making them easier to synthesize. Many also show remarkable specificity for prokaryotes with low toxicity for eukaryotic cells (the kinds of cells in humans). Yet another property encouraging investigators is that bacteria have difficulty developing immunity to these peptides.

In what follows, peptides isolated from each of the three tunicates are briefly described.

Plicatamide from *Styela plicata*

An important end-result of this project was the characterization of plicatamide, a small peptide isolated from the blood cells of *S. plicata*. Plicatamide is composed of only eight amino acids, making it one of the smallest antimicrobial peptides found to date.



Staphylococci after being treated with compound extracted from plicatamide.

Plicatamide was observed to have “excellent activity against methicillin-resistant *Staphylococcus aureus* and appeared to kill this important human pathogen in an unusual manner,” the researchers reported.

In addition to studying the native plicatamide peptide, researchers produced over 60 synthetic analogs of it to further assess its antimicrobial efficacy and to see if they could further reduce its size or broaden its antimicrobial spectrum.

Clavanins from *Styela clava*

Clavanins are peptides found in the blood cells of *S. clava*, a solitary tunicate. In previous research, the scientists described four alpha-helical peptides named clavanins A, B, C and D. These peptides contain 23 amino acid residues, are histidine-rich, and are C-terminally amidated. Clavanin A also displayed antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes*, and *Candida albicans* with a vigor related to pH.

In this research, they described an additional member of this family, which they named clavaspirin. Unlike clavanins A-D, clavaspirin had potent cytotoxic and also unfortunately hemolytic activity. A

compound’s cytotoxicity refers to its ability to kill bacterial cells. Its hemolytic activity is its propensity to “lyse” or burst red blood cells. The ideal drug is very cytotoxic but not hemolytic. Clavaspirin, as a non-specific killer in the immune system, is not a promising drug candidate.

Cionarins from *Ciona intestinalis*

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. (Pm is short for *Phallusia mammillata*, the sea squirt from which the compound was isolated.)

Summary

“In this project, we went searching for new antimicrobial agents from novel marine sources: tunicates,” Taylor explained. “We were able to find and characterize new peptides and to gain insights into the roles post-translational modifications play in their antimicrobial activity.”

“This is exciting because the features that make these peptides

unusual might be used as a template for a more stable peptide antibiotic,” he said. The fact these compounds are gene-coded opens the possibility of engineering drugs with desirable traits, using the genes in tunicates as a starting point. Modifications could, for example, reduce a compound’s hemolytic activity. “What we are looking at is potentially a whole new class of drugs,” Taylor said.

Collaborations

Stein-Oppeneheimer Program

Publications

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