## Di-Cynthaurin: a Novel Antimicrobial Peptide from the Hemocytes of the Solitary Tunicate, Halocynthia aurantium

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We have isolated and purified a novel antimicrobial peptide, di-cynthaurin, from hemocytes of a tunicate Halocynthia aurantium. The native peptide had a mass of approximately 6208, and was homodimeric, with two 30-residue cynthaurin monomers linked by a cystine disulfide bond. As confirmed in the primary structures of a variety of antimicrobial peptides, cynthaurin also has C-terminal amidation. Although we had obtained the peptide from the hemocytes of a marine invertebrate, its antimicrobial activity was salt-sensitive, with optimal activity displayed at NaCl concentrations below 100 mM. Under low salt conditions, the antimicrobial activity cynthaurin was effective against Grampositive and Gram-negative bacteria. However di-cynthaurin was inactive against the yeast-like fungus, Candida albicans. Native and synthetic di-cynthaurin tended to be more active than its monomeric counterpart when tested for antimicrobial or hemolytic activity. Cynthaurin lacked sequence homology to previously identified peptides, and is the first homodimeric  $\alpha$ -helical peptide implicated in antimicrobial defense.

## Purification and characterization of Antibacterial Peptide from Cell-free Plasma of Tunicate, *Halocynthia aurantium*

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Recently, we have found the cationic antimicrobial peptide in the cell-free plasma from immunized *H. aurantium* against *E. coli* through ultrasensitive antimicrobial assay. The peptide was isolated and purified by preparative acid-urea PAGE and reversed-phase HPLC. The molecular mass of the peptide seemed to be about 6.5 kDa on tricine SDS-PAGE gel. Our antimicrobial assay performed with the purified peptide revealed that it has potent antimicrobial activity against Gram positive and Gram negative bacteria. Although it has not yet been completely verified that the peptide is induced by bacterial infection, our finding of antimicrobial peptide in the cell-free plasma of tunicate may provide an important insight into the comparative immunological studies about invertebrates. This work presents the purification and physico-chemical characteristics of the peptide.