THE ROLE OF VANADIUM AS A CHEMICAL DEFENSE OF THE SOLITARY TUNICATE, *Phallusia nigra*

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TABLE OF CONTENTS

ABSTRACT

Ascidians may defend themselves from fish predators, fouling organisms, and bacterial infection by producing secondary metabolites or sequestering acid, but many species also accumulate heavy metals, most notably vanadium. The possible defensive functions of heavy metals in ascidians are unclear. Vanadium is a transition metal with oxidation states ranging from –1 to +5, but under physiological conditions occurs most often between +3 and +5. The black tunicate, *Phallusia nigra*, sequesters vanadium in blood cells and in the exterior tunic surface. Vanadium content of the whole tunic, the tunic bladder cell layer, whole soft body, and blood were determined by flame atomic absorption spectroscopy. The blood and soft body contain the highest vanadium concentrations; however, vanadium is also concentrated at the tunic surface. The concentration of vanadium within the soft body, blood and tunic exterior was significantly higher than that of the whole tunic. Vanadium accumulation, speciation, chelation environment, oxidation state and storage are associated with pH. The objective of this investigation was to attempt to decouple the defensive properties of vanadium from those of low pH in the solitary tunicate, *Phallusia nigra*. Results of feeding assays with the blue head wrasse, *Thalassoma bifasciatum* confirmed outcomes of past studies that demonstrated that vanadyl sulfate ($VOSO_4 \cdot 6H_2O$) and sodium vanadate (Na_3VO_4) were unpalatable to fish. However, the use of vanadium salts does not accurately reflect the chelation environment or oxidation state of vanadium *in situ* in *P. nigra*. The effects of vanadium chelated to naturally occurring compounds found within the blood of *P. nigra* and crude organic extracts of tunic and soft body tissues were evaluated in assays testing anti-predatory effects with the blue head wrasse, *T. bifasciatum*. The chelated vanadium compounds and crude organic extracts were also assessed for anti-microbial effects against a panel of 4 marine bacteria known to be pathogens of marine

invertebrates: *Vibrio parahaemolyticus*, *V. harveyi*, *Leucothrix mucor*, and *Deleya marina*. Crude organic extracts of whole tunic and soft body tissues are palatable to *T. bifasciatum* and do not inhibit the growth of any of the bacterial lines assayed. Non-acidic vanadium (+3) complexes do not deter predation or inhibit microbial growth, whereas acidic aqua vanadium (+3 and +4) complexes were unpalatable to *T. bifasciatum* and exhibited anti-microbial activity. Difficulties in decoupling acidity from oxidation state and chelation environment of vanadium prevent definitive conclusions regarding the relative importance of low pH and vanadium to the chemical defense of *Phallusia nigra*.

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LIST OF TABLES

LIST OF FIGURES