The Chemistry of Aloga Bay Ascidians

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ABSTRACT

This thesis investigates the chemistry of 25 ascidian species collected from Aloga Bay, South Africa with a concerted focus on metal accumulation by these ascidians and the possible interaction of these metals with ascidian metabolites. Chapter 2 details the screening techniques employed to establish the presence of nitrogenous metabolites (1H-15N HMBC), hyper-accumulated metal ions (ICP-MS) and potential metal ion/ascidian metabolite complexes (LC-ICP-MS/ESI-MS). Unfortunately, exhaustive attempts to detect intact metal ion/ascidian metabolite complexes through the use of liquid chromatography with parallel inductively coupled plasma mass spectrometry/electrospray mass spectrometry (LC-ICPMS/ESI-MS) were unsuccessful. However, the LC-ICP-MS/ESI-MS data obtained for the crude organic extracts of six of the Aloga Bay ascidian species, Distaplia skoogi, Aplidium monile, Aplidium sp., Didemnum sp., Leptoclindines sp. and Polycitor sp. enabled identification of a number of ten halogenated metabolites, namely the indoles 2.28-2.30, and the tyramine and tyrosine derivatives (2.31-2.33, 2.41, 2.43, 2.44 and 2.46), within the ascidian extracts. This study confirmed that LC-ICP-MS/ESI-MS is a powerful tool for the dereplication of halogenated metabolites in complex mixtures especially where these compounds are present in very small amounts. This study is also the first report of these compounds (eight of which are known) in African ascidians. Compounds 2.32 and 2.46 have not been reported before from a marine source. Compounds 2.28-2.30 and 2.33 were present in sufficient amounts in the respective ascidian extracts to allow their isolation and structure elucidation using standard spectroscopic techniques.

Chapter 3 explores the ability of ascidians to accumulate a wide range of metal ions at concentrations which are often orders of magnitude higher than those of the surrounding sea water. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the total ion concentrations of 24 metals in 25 Aloga Bay ascidian species. To the best of our knowledge this is the largest and most extensive investigation of metal concentrations in a group of different ascidians occurring in the same area. Hypothesizing that the metal ion concentrations for each ascidian specimen screened may represent a unique fingerprint for each specimen principal component analysis (PCA) was used in an attempt to establish whether there were spatial, temporal or phylogenetic relationships associated with the metal concentration fingerprints of the ascidians that formed part of this study. The PCA results showed that there were no statistically significant relationships between ascidian metal ion
concentrations and either the collection year or the collection site of the ascidians. However, species from the family Didemnidae provided the clearest statistical evidence supporting a phylogenetic relationship between these ascidians and their hyperaccumulated metal ion profiles. Furthermore, these results suggested that ascidian species are indeed actively concentrating metal ions from the surrounding sea water and are not simply sinks for passively accumulated metal ions. Interestingly, the concentration of vanadium in the set of iii ascidians studied did not appear to correlate with any of the other metals accumulated by these ascidians suggesting that there is possibly a unique method employed for the accumulation of vanadium by ascidians. Chapter 4 investigated this possibility further after the nucleosides 4.10, 4.11, 4.13, 4.15, 4.17 and 4.40 were isolated from the vanadium accumulating ascidian Aplidium monile. Studies into the interactions between nucleosides and vanadyl are unfortunately rare and usually qualitative in nature with limited information provided about the stability or structures of the complexes formed. The vanadyl accumulating aplousobranch ascidians e.g. Aplidium monile dominated our study of Algoa Bay ascidians therefore providing us with the rationale to investigate the relatively little studied binding ability and stability of vandyl-nucleoside complexes. Potentiometric studies were conducted to determine the stability constants of complexes formed between the oxovanadium ion vanadyl (VO2+) and the commercially available nucleosides 4.10-4.14. The data afforded by this analysis clearly confirmed the complexity of the vanadyl/nucleoside complexation and suggested that guanosine (4.12) formed the most stable complex with oxovanadium ions. We were also able to establish a third protonation constant for the hydroxyl moiety in 4.12 with a logK 8.87 which has not been previously reported. Finally, Chapter 5 revisited the cytotoxicity two Algoa Bay ascidians, Clavelina sp. and Atriolum marinense the extracts from which produced promising bioactivity results in previous studies against oesophageal cancer cells. The HP-20 fractionated extracts of Clavelina sp. and Atriolum marinense proved to be similarly cytotoxic to breast cancer cells. With the exception for the 100% acetone(aq)fractions the NMR data for both species suggested that most active non polar fractions were dominated by what appeared to be structurally unremarkable fatty acid glycerides and as such were not pursued further. Purification of the 100% acetone(aq)fraction of A. marinense resulted in the isolation of a styrene trimer, 5.1, common to both ascidian extracts. The NMR simulation software WIN-DAISY was employed to confirm the structure of 5.1. Attempts to establish if 5.1 was an isolation artefact or a product of marine pollution were inconclusive.