Enzymatic Detoxifying Systems for Diet-derived Chemicals in Herbivorous Marine Fish

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Introduction

Diverse chemical compounds play critical roles in the defenses of many organisms and can influence the community structure of entire ecosystems. Algae produce chemical classes similar to those found in terrestrial plants, including terpenes, acetogenins, alkaloids, and polyphenolics [1], and extensive research documenting the importance of these metabolites, most of which are lipid soluble, in mediating algal–herbivore interactions now exists [2-9]. Although the vast majority of vertebrates are fishes, little is currently known about how fish tolerate their preys' biochemical defenses – especially in tropical marine communities. It is important to understand how predators cope with poisons in their diet (from prey biochemical defenses) and how these systems parallel human's ability to remove foreign chemicals that are encountered in their every day lives (drugs, pollutants, toxins, etc.). In the absence of enzyme-driven degradation (metabolism), repeated consumption of seaweed chemical defenses would cause their accumulation within the body. The purpose of this project is to obtain NMR data to demonstrate and characterize how a marine herbivorous fish (*S. spinus*) uses its hepatic xenobiotic metabolizing enzymes to metabolize known chemical compounds (halimedatetraacetate and chlorodesmin) found within its natural seaweed diet (*Halimeda* and *Chlorodesmis* spp, respectively).

Experimental

A series of triplicate microsomal incubations (Table 1) were performed by sequentially adding 15 uL microsomal protein (0.25-0.5 mg/mL) and 6 uL of Halimeda compound(s) (ca. 600 uM in EtOH) to PBS buffer (25 mM NaPi, pH 7.8, plus 150 mM NaCl) and vortexing for 5 sec. The incubation is initiated through the addition of 1-2 mM NADPH (in buffer) at 29□C (pH 7.8) and allowed to continue for 45 min., before stopping and simultaneously extracting reaction products through the addition of 100 uL of EtOAc. Samples are prepared by the Biggs Lab and sent to the University of Florida for collection and analyses of NMR data by Luesch and Biggs.

Table 1. Constituent Matrix for microsomal incubations to demonstrate XME-driven metabolism.[a]

	Experimental *H_1: CYP-mediated*	Control (-) *Substrate*	Control (-) *All Protein*	Control (-) *NADPH*[b]	Control *Denatured Protein*
Buffer	564 uL	564 uL	564 uL	564 uL	564 uL
Substrate	6 uL	6 uL PBS	6 uL	6 uL	6 uL
Enzyme	15 uL	15 uL	15 uL PBS	15 uL	15 uL boiled
NADPH	15 uL	15 uL	15 uL	15 uL PBS	15 uL
EtOAc	100 uL	100 uL	100 uL	100 uL	100 uL

[a] Volumes can be scaled up as needed for analyses.
[b] Although CYPs require NADPH, other XMEs (e.g., GSTs) do not.

Results and Discussion

Unfortunately, the stability of *Halimeda* compounds and metabolites hindered our initial attempts to purify sufficient quantities of these compounds to conduct these incubations. We then switch to *Chlorodesmis* secondary metabolites within our incubations and were able to analyze the first set of reactions; however, unforeseen complications, mainly the presence of PEG analogs within the buffers used to create the microsomes, caused huge difficulties in downstream analyses (NMR). We are working to alleviate these issues

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy.

References

[1] Paul, V.J., Ritson-Williams, R. Natural Product Reports 25, 637–832. (2008)
[2] Hay, M.E., and Fenical, W. Annual Review of Ecology, Evolution, and Systematics 19, 111-146. (1988)
[3] Hay, M.E. The Ecology of Fishes on Coral Reefs. Acad. Press, San Diego, California, USA. 96-119. (1991)
[4] Paul, V.J. In: Paul, V.J. (ed) Ecological Roles of Marine Natural Products. Comstock, Ithaca. 24-50. (1992)
[5] Paul, V.J., *et al.* In: McClintock JB, Baker BJ (eds) Marine Chemical Ecology. 227-266 (2001)
[6] Van Alstyne, K.L., *et al.* Marine Chemical Ecology. CRC, Boca Raton, FL, 301-324. (2001)
[7] Hay, M.E. Journal of Experimental Marine Biology and Ecology 200, 103-134. (1996)
[8[Amsler, C.D., *et al*. Botanica Marina 52, 535–545. (2009)
[9] Jormalainen, V., and Honkanen, T. In Amsler, C.D. (ed) Algal Chemical Ecology. Springer. 291-313. (2008)