Mycosproine-like Amino Acid Levels in *Pisaster ochraceus* Related to Dietary Options

Ackley Lane

University of Oregon, OIMB, Charleston, OR 97420

Introduction

Zonation is a trend seen in intertidal areas worldwide. Zones are determined by both physical and biological factors. UV light as a physical constraint limits the upward bounds of many intertidal animals. The soft bodied animals of the intertidal are especially susceptible to tissue damage by UV light. Prolonged exposure to UV light is very dangerous, but it is often times unavoidable. One method of dealing with UV light is the ability to accumulate mycosporine-like amino acids (MAAs) in the skin (Shick and Dunlap, 2002). MAAs are proteins synthesized in plants and algae, which are consumed and utilized by animals of a higher trophic level. Algas that are exposed to UV light have higher concentrations of MAAs than those that don't (Korbee, 2006). Therefore, concentrations of MAAs are directly correlated to UV light absorbance (Shick and Dunlap, 2002). At Cape Arago where the animals were collected for the study, the sea star *Pisaster ochraceus* is considered a keystone species and is one of the top predators found in the intertidal. *P. ochraceus* is found in various habitats such as rocky boulder covered intertidal, to the severely wave beaten rocks where mussel beds are often found higher up. At these locations I have observed differences in food items that are available. Mussel beds are high in numbers of California mussels and gooseneck barnacles, while the boulder covered intertidal has numerous limpets, acorn barnacles and snails. Do these different prey items have varying amounts of MAAs? If so, can these variances be related to the levels of MAAs found in the skin of *P. ochraceus*? I hypothesize that any difference in UV

light absorbance in *P. ochraceus* taken from two different habitats can be positively correlated to levels of UV light absorbance found in their available food items.

Methods

Animals were collected from two locations, mussel beds at North Cove of Cape Arago, and from the boulder filled rocky intertidal of South Cove, Cape Arago. South Cove animals were collected at least twenty meters from the nearest mussels in order to examine *P. ochraceus* that did not eat mussels recently. These two locations represent the two different habitats to be compared. At each location two *P. ochraceus* were collected, an orange and purple morph from each. From the mussel beds at North Cove *one Mytilus californianus* and one *Pollicipes polymerus* were collected as representatives of available prey items of *P. ochraceus* in this habitat. At South Cove one *Lottia pelta,* one *Balanus glandula* and one *Chlorostoma funebralis* were collected to represent food items available. One *Leptasterias hexactis* was also taken from South Cove to serve as a control, based on the assumption that they prefer the underside of rocks due to low levels of MAAs and increased susceptibility to damage by UV light exposure. This control was used simply to check the reliability of the methods used and make sure organisms with lower amounts of MAAs show up as having lower levels of MAAs.

Two small tissue samples of approximately equal sizes were taken from each animal. These samples were then rinsed in filtered seawater and minced using a razor blade. The minced samples were approximately the same size, each covering about the area of a hole punch hole. Each sample was then soaked in 1.0 ml of 100% methanol pipetted into 1.5ml centrifuge tubes for twenty minutes. After soaking the samples were centrifuged and the supernatant was collected. The two methanol tissue solutions from each animal were then combined in cuvettes and examined using a spectrophotometer. Absorbance values at two wavelengths, 313nm and 340nm, were taken for each sample using pure methanol to zero the machine at each wavelength.

A BCA protein standard was made 250 ug/ml and absorbency of the standard and each sample was measured. 0.1ml of the standard was added to 2.0ml Working Reagent, the same was done with each sample, and allowed to stand for two hours. After two hours absorbency was measured at 562nm. This was done in order to standardize the measurements of absorbency at UV light wavelengths by comparing them to absorbency of a known concentration of protein solution.

Results

I found high levels of UV light absorbance in *Pisaster ochraceus,* (0.102 to 0.153 abs/ug/ml) and *Mytilus californianus* (0.125 to 0.157 abs/ug/ml). There was no consistant variation in absorbance between the *P. ochraceus* found in the two different locations (Fig 1, animals 1,2 vs animals 2,3). There were low levels of absorbance in all other animals sampled, *L. pelta, C. funebralis, P. polymerus, L. hexacti* and *B. glandula* ranging from 0.010 to 0.073 abs/ug/ml (Fig 1, animals 5-9). The lowest level was found in *L. pelta* and the control *Leptasterias hexactis* contained low levels of MAAs (Fig 1, animals 8 and 5 respectively).

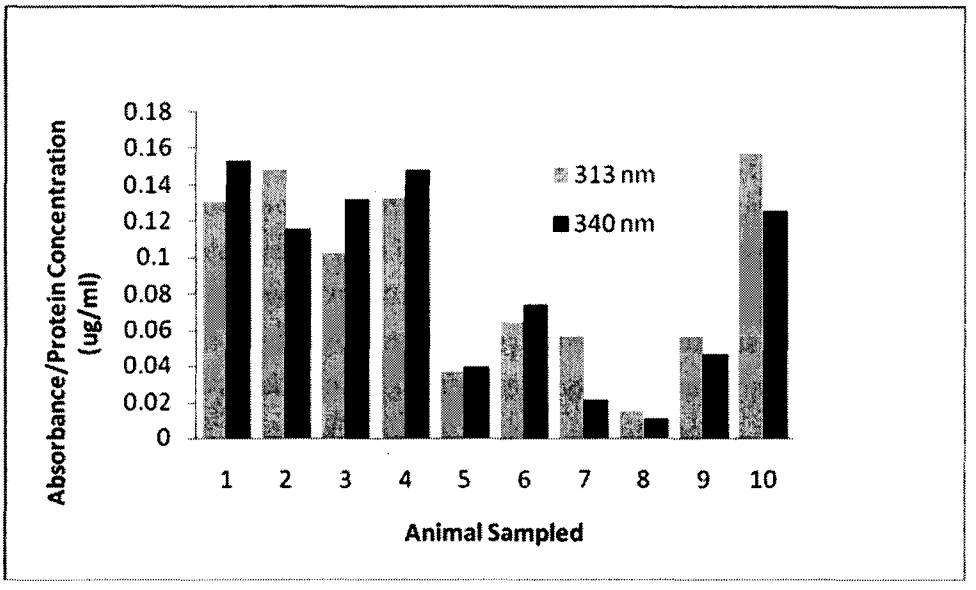

Fig 1. UV light absorbance per ug/ml protein at two UV light wavelengths, 313nm and 340nm. No correlation between levels of absorbance in available prey animals and levels found in *Pisaster ochraceus.* Animals Sampled (1) *Pisaster ochraceus* from North Cove mussel bed, orange morph, (2) *Pisaster ochraceus* from North Cove mussel bed, purple morph, (3) *Pisaster ochraceus* from South Cove, orange morph, (4) *Pisaster ochraceus* from South Cove, purple morph, (5) *Leptasterias hexactis,* from underside of rocks at South Cove, (6) *Balanus glandula* from South Cove, (7) *Pollicipes polymerus* from North Cove mussel beds, (8) *Lottia pelta* from South Cove, (9) *Chlorostoma funebralis* from South Cove, (10) *Mytilus californianus,* from North Cove mussel beds.

Discussion

My results did not support my hypothesis. *P. ochraceus* at the two locations did have variations in levels of MAAs, but they did not correlate with the levels of MAAs found in the food items. *M. californianus* had the highest levels of MAAs in their tissues, but the two *P. ochraceus* found in the mussel beds had slightly lower MAAs in their tissues. *L. hexactis* as a control worked very well, showing very low levels of MAAs compared to *P. ochraceus. L. pelta* also displayed very low levels of MAAs, this is presumably because its shell blocks UV light. *C.*

funebralis also has a shell, but the sample was taken from the foot which is often exposed to UV light and therefore would have need for MAAs. I did not test tissues from different parts of the animals so I do not know if they can specify where to accumulate MAAs or if it is just an even body wide dispersal.

One source of error in this exploratory came from the equipment used. The spectrophotometer was very susceptible to light pollution from lights and shadows in the room, especially because there was a gap into the chamber where the sample was placed. Measurements would fluctuate dramatically when somebody would walk in front of the machine. To lower this effect I was able to cover the opening to the machine with a piece of Styrofoam, but even then fluctuation was common and final measurements were usually the middle measurement from the range of fluctuation. A more reliable spectrophotometer would help in any repetitions of this experiment.

If the machine was working fine, then the next artifact would have to do with sample sizes. Two sea stars were taken from each location, and only one of each food item was sampled. This does not account for any variation that may exist within the population. Also, it is very difficult to know the amounts of each prey item any particular sea star eats. Sea stars in mussel beds may not eat many mussels and eat mainly gooseneck barnacles which have very low concentrations of MAAs in their tissue. Diets are most likely an important determining factor in amounts of MAAs consumed. In future work on this topic an experiment in the lab would probably be best, hold the *P. ochraceus* and feed different individuals different diets. Measure both the starting MAA levels, and the final MAA levels. This would show whether or not MAA concentrations are related to those available in the food items available. I do not know what the accumulation rates are for MAAs, but with MAA testing at different times that question could also be answered. I would also like to look at the effects of UV light on accumulation. If *P. ochraceus* does not encounter UV light, does it still accumulate MAAs?

Works Cited

Krobee N, Figueroa FL and Aguilera J. 2006. Accumulation of mycosporine-like amino acids (MAAs): biosynthesis, photocontrol and ecophysiological functions. Rev. chil. hist. nat. 79: 119-132.

Shick MJ and Dunlap WC. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-prtective functions in aquatic organisms. Annual Review of Physiology 64: 223-262.

Sept JD. 1999. The beachcomber's guide to seashore life in the Pacific Northwest. Harbour Publishing. Madeira Park, BC Canada.