

PRESENCE OF MAA'S IN THE TISSUES OF THREE CNIDARIANS: *POLYORCHIS
PENICILLATUS*, *CHRYSAORA FUSCESCENS*, AND *ANTHOPLEURA
ELEGANTISSMA*

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INTRODUCTION

Mycosporine-like amino acids (MAAs) are small molecules capable of absorbing ultraviolet radiation in the range 310 to 360 nm (Shick et al. 2005). The chemical structure of the 20 known MAAs shows that they are derivatives of aminocyclohexanone (Shick et al. 2002). They are present in almost all taxa of marine and freshwater cyanobacteria, algae, and metazoans. MAAs are synthesized by algae, cyanobacteria, fungi, and perhaps bacteria, but not by metazoans. This is because animals lack the biochemical pathway to synthesize them. Therefore, MAAs in symbiotic animals (corals and anemones) originate in the algal or cyanobacterial partner. Meanwhile, MAAs in aposymbiotic animals (tropical sea slugs, sea cucumbers, urchins, sea stars, polychaetes, etc) are acquired via the diet (Shick et al. 2005)

MAAs are ideal sun-screening agents because they are transparent to radiation in the photosynthetically active region but absorb strongly in the potentially damaging UV range. Therefore, they are capable of protecting an organism from high solar damage, and act as a sunscreen. This idea has been shown between locations; shallow-dwelling individuals typically have higher concentrations of MAAs in their tissues than their deeper-dwelling counterparts because of longer exposure in the sun (Wagner 2001). Their role as photo-protectants is further supported by the fact that a diverse range of marine benthic organisms provide their planktonic larvae with higher concentrations of MAAs than are found in adult tissues (Shick et al. 2005). Another indirect role for MAAs is that they perform a role similar to reproductive hormones. This was shown in the sponge *Dysidea herbacea* in which higher MAA concentrations were positively

correlated with reproductive cycle and there was no correlation between MAA concentrations and annual solar irradiance (Wagner 2001).

Many studies have been done on MAAs present in different cnidarians showing high concentration in both *Anthopleura* anemones, and different coral species (Shick et al. 2002). However, there is not much in the literature regarding the presence of MAAs in scyphozoans, however some have algal symbionts and most feed in the plankton at the surface of the water. This suggests that the mechanisms for accessing MAAs are present and that with sun exposure, MAAs could be a protective agent for larger jellies. *Chrysaora fuscescens*, a large scyphozoan found near-shore from the Gulf of Alaska to Mexico, could contain MAAs by using this logic. However, they are fairly mobile and move up and down in the water column suggesting that the amount of MAAs may not be as prevalent as in sessile organisms constantly exposed to the sun. Therefore, my null hypothesis is that *Chrysaora fuscescens* will have no MAAS in any of its tissues.

MATERIALS & METHODS

Three species of cnidarians were collected; five *Polyorchis penicillatus* and one *Chrysaora fuscescens* from the Charleston docks, and two *Anthopleura xanthogrammica* from the OIMB Beach. Both *P. penicillatus* and *A. xanthogrammica* were collected to use as comparisons in MAA values for *C. fuscescens*.

For each specimen, tissue samples of equal size were excised and minced using a razor blade and scissors. For *P. penicillatus* and *C. fuscescens*, 6-8 samples were taken from the exumbrella, subumbrella, tentacles, and oral lobes. For *A. xanthogrammica*, 4 samples were taken from both the tentacles and body wall. Each tissue sample was gently rinsed and blotted dry with a kimwipe to reduce variance associated with water

weight. Tissues were transferred to separate microfuge tubes into which 1ml 100% methanol was added. This solution was left for 20 minutes so that the methanol could extract the MAAs from the tissue. Each tube was centrifuged for one minute and the supernatant was transferred via pipette to cuvettes. A spectrophotometer was used to analyze samples for the presence of MAAs. The instrument was blanked with a cuvette filled with methanol. The UV absorbance of each tissue sample was measured at 313nm and 340nm and the presence of photosynthetic pigments was measured at 436nm.

To express the absorbance data from the spectrophotometer as absorbance units/mg protein, a protein assay was done. Protein concentrations in the tissue samples were compared to standards of bovine serum albumin (BSA), of which five standards were mixed (125, 250, 500, 750, and 1000 ug/ml). 0.1ml of each standard and tissue sample was mixed with 2.0ml of working reagent (50:1 Kit Reagent A to B) in individual test tubes. These mixtures were labeled and incubated at room temperature for two hours. The spectrophotometer was blanked with distilled water, and the absorbance value of each standard and unknown solution was measured at 562nm. Protein concentration for the experimental samples were calculated using a proportion between absorbance and known protein concentration based on the BSA standard solutions.

RESULTS

MAA values were calculated for four tissues in *P. penicillatus* and *C. fuscescens* : the exumbrella, tentacle, manubrium, and subumbrella. Data for *A. xanthogrammica* were inconsistent with the literature. Therefore, *A. elegantissima* absorbance values were used and were calculated using the concentration of *A. xanthogrammica* protein for two

different tissues: the column and tentacle. Data from three wavelengths, 313 nm, 340 nm, and 436 nm, are reported in Fig. 1 and Table 1.

The data show that there were very few MAAs and no algal symbionts in any of *P. penicillatus*' tissues, however both *C. fuscescens* and *A. elegantissima* show high values of MAAs. *C. fuscescens* has an overwhelming amount of MAAs that absorb 313nm, and algal symbionts were only present in the manubrium and subumbrella tissues. *A. elegantissima* shows relatively equal quantities of MAAs and algal symbionts.

DISCUSSION

I stated that *Chrysaora fuscescens* would have no MAAs in any of its tissues. The results show that there are MAAs present in all measured tissues and that there are particularly larger quantities of MAAs absorbing wavelengths at 313nm than 340nm (Fig 1). This trend is different from *A. elegantissima*, which has fairly equal quantities of MAAs at both 313nm and 340nm. This suggests that environmental factors may affect the types and quantities of MAAs found in an organism. *C. fuscescens*, a pelagic organism spends most of its time off the shore, and the particular species I found was in the docks with much pollution (Wrobel & Mills 1998). These factors may have affected the quantities and types of MAAs produced. Shick et al (2005) shows that different MAAs are associated with effects on photosynthesis, such as nitrogen levels, etc. This trend may also be because there are particular 340nm absorbing MAAs associated with zooxanthellae. This would explain why there is more MAAs that absorb 340 nm in the manubrium and subumbrella of *C. fuscescens*, where algal symbionts are present. Shick et al. (2002) showed that there are particular MAAs for *Anthopleura* species, thus showing that there is specificity in MAAs.

P. penicillatus had no algal symbionts or MAAs in any of its tissues. This shows that they are not getting any MAAs from symbionts or in the crustaceans and demersal zooplankton that they eat. This is a good indicator that MAAs are not a necessary adaptation as this organism spends much of its time on the bottom of bays (Wrobel & Mills 1998)

LITERATURE CITED

- Michalek-Wagner, K. 2001. Seasonal and sex-specific variations in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. *Marine Biology* 139: 651-660.
- Shick, J.M. Dunlap, W.C., Pearse, J.S., and V.B. Pearse 2002. Mycosporine-like Amino Acid Content in Four Species of Sea Anemones in the Genus *Anthopleura* Reflects Phylogenetic but Not Environmental or Symbiotic Relationships.
- Shick, J.M., Ferrier-Pages, C, Grover, R., and D. Allemand 2005. Effects of starvation, ammonium concentration, and photosynthesis on the UV-dependent accumulation of mycosporine-like amino acids (MAAs) in the coral *Stylophora pistillata*. *Mar. Ecol. Prog. Ser.* 295: 135-156.
- Wrobel, D. and C. Mills 1998. Pacific Coast Pelagic Invertebrates: A Guide to the Common Gelatinous Animals. Monterey Bay Aquarium, Monterey, CA.

Table 1. Absorbance units/mg protein of all three cnidarians (*P. penicillatus* [P], *C. fuscescens* [C], and *A. elegantissima* [A]) at three different wavelengths. Second letter refers to tissue type; E=exumbrella, T=tentacle, M=manubrium, S=subumbrella, and B=column. Codes are identical for x-axis categories in Fig. 1.

	313 nm	340 nm	436 nm
PE	3.3E-05	0	0
PT	1.7E-05	0	0
PM	4.1E-05	2.4E-05	0.0003
PS	1.3E-05	3.2E-06	0.0002
CE	1.2E-02	1.1E-03	0
CT	8.6E-03	4.7E-04	0
CM	3.6E-03	1.3E-03	7.2E-04
CS	5.7E-03	1.4E-03	8.3E-04
AB	0.0054	0.0052	0.0009
AT	0.0042	0.0045	0.0014

Units for all values are (absorbance units/mg protein)

Fig 1: Absorbance units/ mg protein for different tissues of three cnidarians

