Mycosporine-like amino acids in six scleractinian coral species

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Abstract

Mycosporine-like amino acids (MAAs) were studied in stony coral species (Fungiidae) along the Eastern coast of the Red Sea. Six species – *Fungia scutaria, F. danai*, *F. corona, F. repanda*, *Ctenactis echinata* and *Lithophyllor lobata* – were examined for MAAs at water depths of 5, 10, 15 and 20 m. Protein and chlorophyll were also determined and showed higher contents in winter than in summer. Generally, the total content of MAAs in summer was found to be approximately three times greater than in winter. Overall, concentrations of MAAs were greatest at a depth of 5 m. Porphyra-334 was the most abundant MAA in *F. scutaria* and *F. danai*, whereas asterina-330 was either not detectable (e.g. *L. lobata*) or present in low concentrations (e.g. *F. danai*, *F. repanda* and *C. echinata*). Shinorine was not detected in *F. danai* or *L. lobata*. Both *C. echinata* and *L. lobata* had the lowest concentrations of MAAs, presumably because of their large calcareous skeletons. The variation in MAA concentrations among seasons and water depths is probably due to a number of factors, including the intensity of solar radiation, turbidity and phylogenetic variation.

1. Introduction

Among the most ancient organisms of the marine world, coral reefs are commonly found in the tropical and sub-tropical regions of the marine environment. The responses of coral reefs to global changes confront us with a number of paradoxes. These organisms possess a powerful, adaptive and acclimatic mechanism associated with defence against the harmful effects of ultraviolet (UV) radiation. Environmental conditions allow the development of hermatypic corals, but the process by which corals defend themselves against elevated seawater temperature and solar radiation is not fully understood. However, a class of UV-absorbing compounds affording protection from the deleterious effects of high UV fluxes has been identified in many marine organisms. These effects include (i) damage to DNA and protein, (ii) oxidation of the membrane lipid, and (iii) inhibition of algal photosynthesis (Shick 1993).

The compounds that absorb UV radiation – mycosporine-like amino acids (MAAs) – play a role in UV-induced sporulation (Leach 1965). A family of water-soluble, low molecular weight compounds with absorption maxima between 309 and 360 nm, MAAs have been detected in both marine and terrestrial organisms. The frequently observed correlation between MAAs and solar radiation suggests that these compounds play an important part in protection against UV light (Drollet et al. 1997). Scleractinians have adapted to shallow waters, where the level of UV radiation is intense. Investigating seasonal variations on soft coral, Wagner (2001) found that MAAs are high during summer compared to winter and attributed this to the high incident radiation.

MAAs are known to be present in coral tissue, to absorb UV light, and to release it harmlessly as heat (Kuffner 2002). Each genotype seems to have a specific complement of MAAs. MAAs are products of a branch of the shikimate pathway and probably originate in the zooxanthellae. However, non-symbiotic metazoans also contain MAAs that they obtain from their food (Shick & Dunlap 2002). The host (coral) modifies the primary MAAs provided by zooxanthellae into an array of secondary MAAs (Shick et al. 1999). Apart from the normal MAAs, some unusual or substituted MAAs have also been isolated from stony corals (Wu Won et al. 1995). The MAAs of *Acropora* sp. decline gradually with depth (Dunlap et al. 1986). Very little work has been done on these types of compounds in the Red Sea (Salih et al. 2001, Woesik 2001, Al-Utaibi et al. 2006, Zeevi Ben-Yosef et al. 2006). The present work was undertaken in order to quantify MAAs in hard corals and to study variation in MAA concentration with respect to seasonal variation and water depth.

2. Material and methods

2.1. Sampling site

Coral samples were collected at depths of 5, 10, 15, and 20 m along the coast of the Red Sea near Jeddah at 21°52′12″N and 38°52′46″E during July 2002 and January 2003 (Figure 1). The average sea surface temperature during these periods was 24.5°C and 30°C and the salinity 39 PSU and 41 PSU, respectively. Specimens were transported to the laboratory in seawater and frozen at −20°C. The organisms belonged to Fungiidae genera and were identified as *Fungia scutaria* Lamarck, 1801, *Fungia danai* Milne Edwards & Haime, 1851, *Fungia corona* Döderlein, 1901, *Fungia repanda*

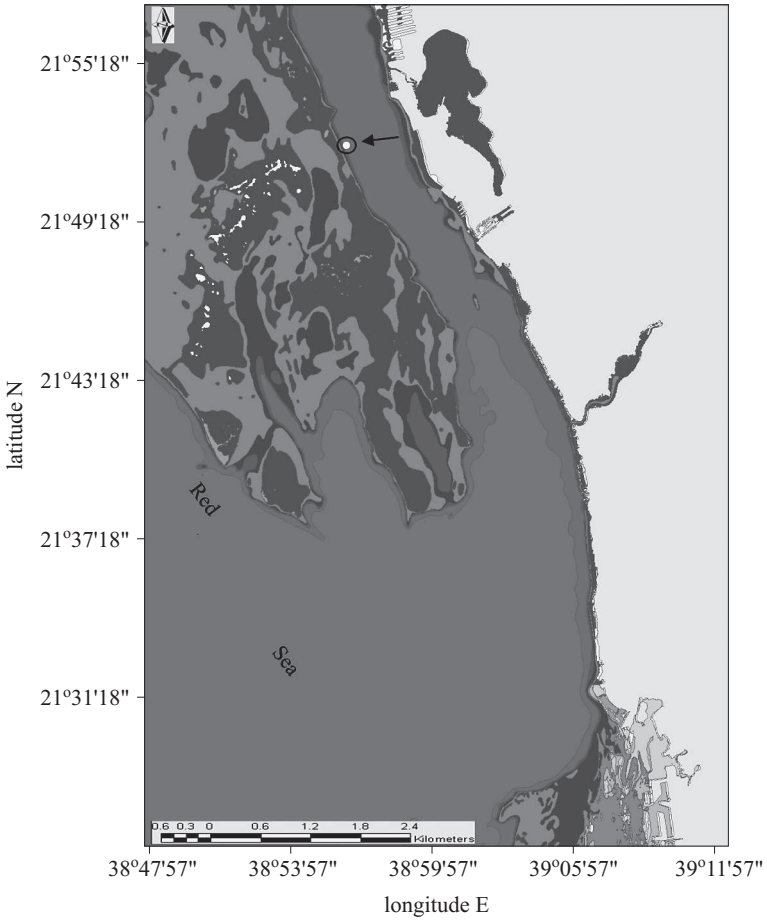

Figure 1. Eastern Red Sea coast showing the location of the sampling area. The site where the coral samples were collected is indicated (○)

Dana, 1848, *Ctenactis echinata* Pallas, 1766, and *Lithophyllor lobata* van der Horst, 1921.

2.2. Chlorophyll *a* and protein determination

Chlorophyll *a* was determined by incubating an aliquot of coral powder overnight at 4°C in 90% acetone. The chlorophyll concentration was estimated using a double beam spectrophotometer at 665 nm and calculated according to the equation of Lichtenthaler (1987):

$$\text{Chlorophyll } a = \frac{(11.2 \text{ Abs. } 665) - (2.04 \text{ Abs. } 645) \times \text{volume}}{1 \times 1000 \times \text{wt. (g)}}$$

A spectrophotometric method (Lowry et al. 1951) was used to quantify coral protein using egg albumin as protein standard. Briefly, an aliquot of coral powder was treated with hot alkaline solution (1 N NaOH) to digest the tissues, after which an aliquot of the supernatant was placed in a 1 cm quartz cuvette and its absorption determined at 660 nm on a double-beam spectrophotometer (Shimadzu UV-1601) against a blank.

2.3. Extraction and analysis of MAAs

Fresh samples of coral each weighing about 8 g each were powdered in a mortar, suspended in 90% aq. methanol and shaken at 25°C for 24 hours. Each sample was then centrifuged. The supernatant was isolated, after which the process was repeated twice. The bulk of the organic solvent was removed under pressure. The sample was freeze-dried and the extracts stored at −8°C. For MAA separation and quantification, the analysis was carried out using an HPLC system (Dionex UVD340S) equipped with a diode-array detector using isocratic reverse-phase HPLC. The dried extracts were resuspended in 50 to 100 μl of 25% methanol (v/v), and 20–70 μl aliquots were injected into a Phenosphere 5-μm pore size C8 column (250×4.5 mm) protected with an RP-8 (Brownlee) guard column. During the analysis, samples in the autosamplers were kept at 15°C while the column was maintained at 20°C. The mobile phase consisted of 0.1% acetic acid in 25% aq. methanol (v/v) at a flow rate of 0.79 ml min^{-1}. Peak absorbance at each of four pre-selected channels (310, 320, 334, and 360 nm) was measured (Sommaruga & Garcia-Pichel 1999). To quantify the MAAs, the peak areas of the unknowns were compared with those from a standard solution calibrated (mycosporine-glycine, shinorine, porphyra-334, palythine and asterina-330) using published molar extinction coefficients (ϵ). The concentration of MAAs in each sample was normalised to the protein content in the coral (expressed as μg mg^{-1} protein).

3. Results and discussion

The mean concentration of chlorophyll *a* was greater in winter (0.0014 mg g^{-1} dry wt. coral) than in summer (0.0009 mg g^{-1} dry wt. coral). The maximum amount was present at 5 m depth both in summer and winter (0.0014 mg g^{-1} and 0.0023 mg g^{-1} respectively). The minimum quantity in summer was recorded at 15 m depth in *Fungia danai* (0.0003 mg g^{-1}), whereas in winter the lowest quantity was found at 20 m depth in *Lithophyllor lobata* (0.0002 mg g^{-1}). Chlorophyll plays a vital role in the photosynthesis of biomolecules in the zooxanthellae. The chloroplasts diminish in size and chlorophyll concentrations are reduced as a consequence of sunlight intensity during the summer, whereas these two parameters increase during the winter. Photoinhibition is a function of light intensity and the duration of exposure to light (Dubinsky et al. 1984). There was inconsistency with respect to the protein content: in most cases it was found that a higher protein content meant fewer MAAs, whereas species with a low protein content contained more MAAs. The overall average of protein at four depths in summer was 0.356 mg g^{-1}, whereas this general average was approximately three times higher in winter (1.137 mg g^{-1}).

The average concentrations (n = 2) of MAAs were calculated in six Fungiidae species: *F. danai*, *F. scutaria*, *F. corona*, *F. repanda*, *Ctenactis echinata* and *L. lobata* (Table 1). Five MAAs were identified on the basis of retention times and wavelength maxima similar to those of MAA standards (Figure 2). *Fungia scutaria* exhibited the highest concentration of porphyra-334 during the summer at 5 m and 10 m depths, where the concentrations of MAAs were 38 and 15 μg mg^{-1}, respectively. The greatest concentration of palythine was found in *F. danai*, although its concentration was only 8 μg mg^{-1}. In *F. corona*, the most abundant (56% of the total) MAA component was mycosporine-glycine (6.8 μg mg^{-1}). The MAAs present in *F. danai* included shinorine and palythine but not asterina-330. During winter, mycosporine-glycine was the most abundant MAA in *F. danai* and *C. echinata*, whereas shinorine was the most abundant one in *F. repanda* (65% of total MAAs). The total concentration of MAAs was lower in *C. echinata* and *L. lobata* than in other species, probably because both of these two species have a calcareous skeleton. Palythine had the highest concentration (50% of total MAAs) in *L. lobata*. Palythine has also been found in soft corals like *Sinularia trocheliophorum* and *Sarcophyton polydactyla* (Al-Utaibi et al. 2006). In general, soft corals had a greater content of MAAs (both in summer and winter) compared to hard corals, indicating that they are probably more vulnerable to the deleterious effects of UV radiation and therefore produce more MAAs in self-defence.

Table 1. Concentration of chlorophyll, protein and MAAs in six species of Fungiidae from the Eastern Red Sea coast

Summer	Depth [m]	Chlorophyll mg g^{-1} dry wt.	Protein	MGL	SHI	POR	PIN	AST	MAAs
				μg mg^{-1} protein					
Fungia scutaria	5	0.0014	0.453	0.0	0.0	38.3	3.9	0.8	43.0
Fungia scutaria	10	0.0011	0.520	0.63	0.0	15.2	4.5	0.1	20.43
Fungia danai	15	0.0003	0.197	7.4	0.07	8.7	4.0	0.0	20.17
Fungia corona	20	0.0011	0.256	6.8	0.0	4.2	0.9	0.1	12.0
Winter									
Fungia danai	5	0.0023	0.296	20.3	0.0	0.0	0.0	0.3	20.6
Fungia rapanda	10	0.0021	1.967	1.3	4.9	0.6	0.4	0.1	7.3
Ctenactis echinata	15	0.0011	0.748	2.0	0.0	0.7	0.3	0.0	3.0
Lithophyllor lobata	20	0.0002	1.538	0.4	0.0	0.5	1.0	0.0	1.9

MGL = mycosporine-gly; SHI = shinorine; POR = porphyra-334; PIN = palythine; AST = asterina-330.

Variation in the concentration of MAAs among species could be explained by differences in anabolic pathways or differences in zooxanthellae strains.

Porphyra-334 was the most abundant MAA in the summer species, whereas mycosporine-glycine was the major MAA in the winter species. For example, in *F. scutaria* and *F. danai*, porphyra-334 was the most abundant MAA; concentrations of porphyra-334 during the summer were 38.3 and 8.7 μg mg^{-1} protein, respectively. During winter, the predominant MAA in *F. danai* was mycosporine-glycine (98% of total MAAs). The lower concentration of MAAs during the winter may have been due to the greater intensity and duration of solar radiation during the summer. This factor may also be responsible for the greater overall concentration of MAAs in summer.

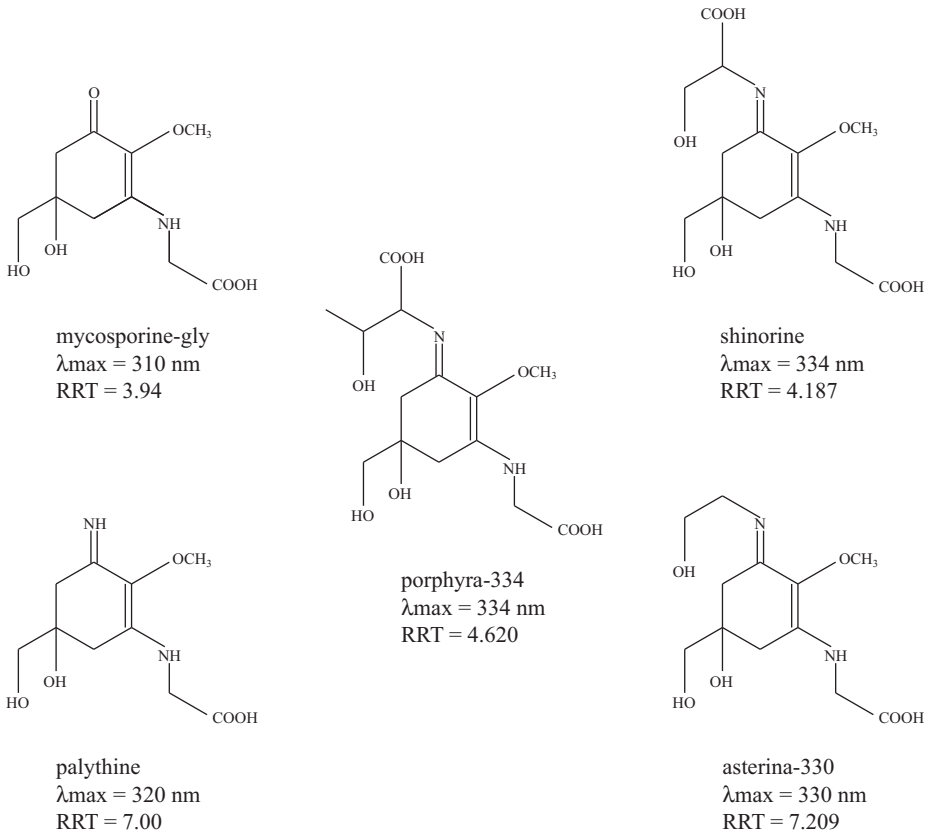

Figure 2. Molecular structure, wavelength maximum (λ_{max}) and relative retention time (RRT) of MAAs identified in the six scleractinian corals

Teai et al. (1997) analysed concentrations of MAAs in similar species (i.e. *F. scutaria* and *F. danai*) from Tahiti and Taumotu (French Polynesia). Their observations differed from ours, however: they found that mycosporine 2-glycine was the most abundant MAA in *F. scutaria* and *F. danai*, whereas mycosporine 2-glycine was not detected in the present study in either species. Also, shinorine was the most abundant MAA in *F. repanda* in this study and was the second most abundant MAA in *F. repanda* from Tahiti. This could be due to differences among phylogenetic clades. Some phylotypes have a greater predilection than others for the synthesis of MAAs. In 23 species of corals from French Polynesia, asterina-330 was the least common MAA (Teai et al. 1997). The present results also indicated that the content of asterina-330 was minimal in all specimens.

It was observed that when the solitary coral *Fungia repanda* was exposed to solar UV radiation for 18 months on Tahiti, the amount and

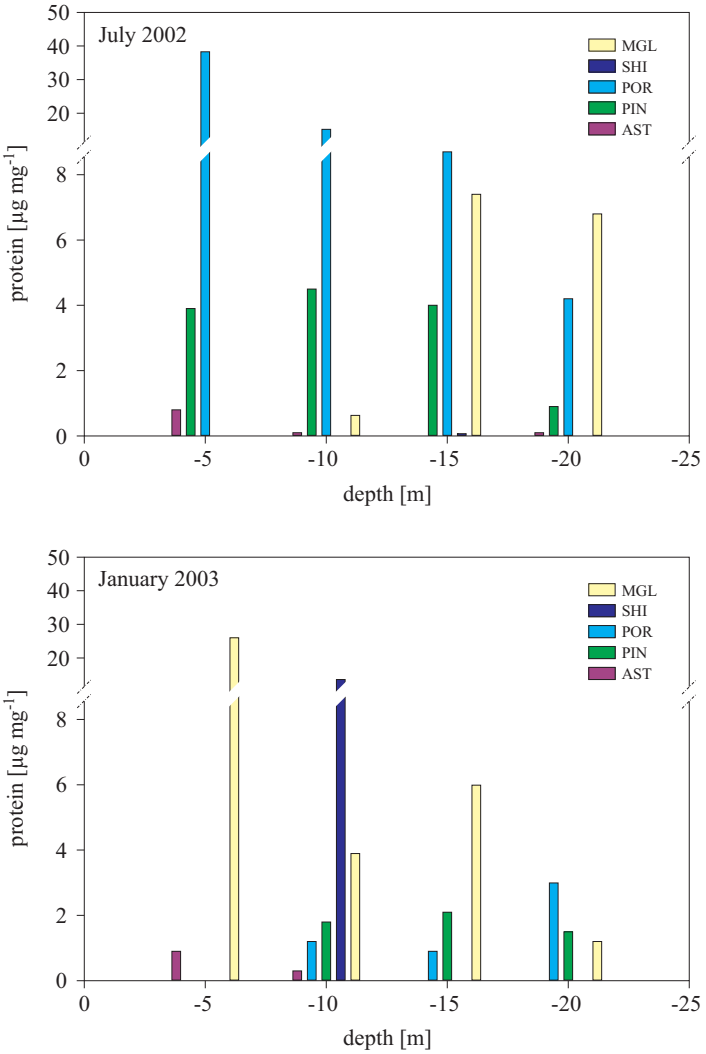

Figure 3. Concentration of MAAs (μg mg^{-1} protein) in different species of Fungiidae at different depths and seasons from the Eastern Red Sea coast

number of MAAs increased (Drollet et al. 1997). Photosynthetically active radiation and the daily light intensity were at a maximum in June (personal communication). Duration of daylight is also partially responsible for the high concentrations of MAAs in summer. In addition, in *F. danai*, shinorine, porphyra-334 and palythine were detected during the summer but not in winter. This indicates that the pattern of MAAs varies among species within an area, a situation that can be explained by the variation in the strains of the symbiotic algae during summer and winter (Banaszak et al. 2000).

Mycosporine-glycine, absent in *F. scutaria*, is the principal MAA synthesised in the zooxanthellae. Mycosporine-glycine gives rise to other mycosporine-like amino acids by forming imines with a number of amino acids such as glycine, serine and threonine; these reactions are metabolically reversible. Mycosporine-like amino acids may be synthesised rapidly, but they are also continuously utilised in the synthesis of other MAAs. A further explanation could be that the biosynthesis of mycosporine-glycine is inhibited when the concentration of MAAs reaches a steady state (Shick 2004). The concentration of mycosporine-glycine is greatest during summer and at a depth of 5 m. An inverse relationship between the presence or concentration of MAAs in corals and depth was noted previously (Gleason & Wellington 1993).

The overall concentration of MAAs was greater in summer than in winter, particularly at 5 m depth. Genotypes of zooxanthellae differ in summer and winter (Banaszak et al. 2000). In any given species of coral, genotypes of zooxanthellae can vary with environmental conditions, so the geographic variation in MAAs could be due to variation in the genotypes of zooxanthellae. Patterns of MAAs can vary within a given coral species growing at different locations but under similar environmental conditions owing to phylogenetic variation in zooxanthellae (Banaszak et al. 2006). In addition, the relationship between MAAs and protein is complex. The concentration of MAAs may not increase with protein content (Davidson et al. 1994).

The importance of solar radiation in the biology of coral reefs has been recognised for about three decades. In the coral *Porites compressa* Dana, growing in slowly flowing water, concentration of MAAs increased in colonies exposed to UV radiation. However, in colonies receiving no UV radiation, the concentration of MAAs decreased steadily, declining to 36% in six weeks (Kuffner 2001). In addition, the effect of UV radiation can vary among genotypes within a coral species (Gleason 1993). Shick et al. (1996) reported that an increase of UV radiation resulted in sublethal physiological manifestations in corals and other reef organisms. Torregiani & Lesser (2007) reported increased MAA concentrations when the Hawaiian coral *Montipora verrucosa* was exposed to UV radiation.

4. Conclusions

Five MAAs were identified and quantified in hard corals. Concentrations of each MAA varied from specimen to specimen. Overall concentrations of MAAs were approximately three times higher in summer compared to winter, which was probably due to the greater penetration of UV radiation during the summer. Concentrations of MAAs were also lower in

coral from deeper, slightly turbid water, as observed in *Ctenactis echinata* and *Lithophylor lobata*; both these species also exhibited a high mass of calcareous matter. Water turbidity and phylogenetic variation in the marine organisms play a vital role in this phenomena. Conversely, coral in clear, shallow water is vulnerable to damage caused by UV radiation; in turbid, deep waters this factor is reduced.

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