Comparative Biochemistry and Physiology, Part B 156 (2010) 216–221

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

Mycosporine-like amino acid content in the sea anemones *Aulactinia marplatensis*, *Oulactis muscosa* and *Anthothoe chilensis*

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ARTICLE INFO

Article history:
Received 22 December 2009
Received in revised form 26 March 2010
Accepted 27 March 2010
Available online 1 April 2010

Keywords:
Mycosporine-like amino acids
Intertidal sea anemone
Argentina

ABSTRACT

The occurrence of mycosporine-like amino acids (MAAs) in the sea anemones *Aulactinia marplatensis* (Zamponi, 1977), *Oulactis muscosa* (Drayton in Dana, 1846) and *Anthothoe chilensis* (Lesson, 1830), from the rocky intertidal habitats on the coast of Mar del Plata, Argentina, was assessed by HPLC. The pattern of MAAs in the mussel *Brachidontes rodriguezi*, main component of the diet for *A. marplatensis* and *O. muscosa*, was as well determined. The results were comparatively analyzed together and with previously reported MAA content in species mainly of the genus *Anthopleura*. The correlation between the MAA concentration and light availability of their habitats is in line with the photoprotective role assigned to the compounds. The high proportion of mycosporine-taurine in the three species and the results for the evaluation of MAAs in the mussels point to a non-dietary origin or a regulated biotransformation metabolism of dietary MAAs and/or their precursors that is common to sea anemones.

1. Introduction

Mycosporine-like amino acids (MAAs) are small, ultraviolet-absorbing (λ_{max} = 309–360 nm) molecules (Shick and Dunlap, 2002) which are widely distributed in marine and freshwater organisms (Bandaranayake, 1998), including sea anemones (Stochaj et al., 1994; Banaszak and Trench, 1995; Shick et al., 2002). Although more than 20 different chemical structures have been identified within this family of compounds, several additional MAAs have been recently detected as a consequence of the development of more efficient high-performance liquid chromatography (HPLC) separation techniques (Carreto et al., 2005; Carignan et al., 2009). MAAs have been implicated in many biochemical processes (Volkman, 1999; Shick and Dunlap, 2002) but the major emphasis has been directed to the presumed role in UV-protection (Dunlap et al., 1986; Carreto et al., 1990; Dunlap and Shick, 1998; Shick and Dunlap, 2002) sustained by some experimental evidence (Neale et al., 1998; Adams and Shick, 2001). *In vitro* studies of various MAAs have also given support to this function by confirming the high photostability and the release of heat to the medium as the main relaxation pathway of the photoexcited molecules (Conde et al., 2004, 2007). In addition, oxocarbonyl-MAAs such as mycosporine-glycine (Dunlap and Yamamoto, 1995; Suh et al., 2003; Yakovleva and Hidaka, 2004) and mycosporine-taurine (Zhang et al., 2007) have antioxidant activity capable of protecting against the cellular damage that high levels of reactive oxygen species (ROS) induce in organisms under different stresses.

Some actiniarians, commonly found in shallow waters or intertidally, are exposed to high levels of UV radiation and therefore require some means of protection. Stochaj et al. (1994) first reported that the temperate anemone *Anthopleura elegantissima* (collected in California coast, USA) contains four major MAAs: shinorine, porphyra-334 and two new compounds, mycosporine-taurine and mycosporine-2 glycine. As products of the shikimic acid pathway (Shick et al., 1999; Portwich and Garcia-Pichel, 2003) MAAs are expected to be synthesized by algae, bacteria and fungi and some protozoans; other marine organisms probably acquire and metabolize these compounds by trophic transference or by symbiotic or bacterial association (Shick and Dunlap, 2002). Thus, it has been thought that the MAAs found in symbiotic invertebrates have presumably their origin in the endosymbiotic algae, *via* the shikimate pathway (Shick et al., 1999). However the host tissue, and conceivably their associated bacteria, can bio-convert the primary MAAs produced by the zooxanthellae (mycosporine-glycine, shinorine, porphyra-334 and mycosporine-2 glycine) to yield an array of secondary MAAs (Shick and Dunlap, 2002; Shick, 2004; Banaszak et al., 2006). On the other hand, MAAs are not necessarily provided by zooxanthellae since azooxanthellate sea

Abbreviations: MAA, mycosporine-like amino acid; TFA, trifluoroacetic acid; SPE, solid phase extraction; R_t, retention time.

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doi:10.1016/j.cbpb.2010.03.011

anemones species such as *Actinia bermudensis* (McMurrich, 1889) (Stochaj et al., 1994) and *Anthopleura artemisia* (Shick et al., 2002), as well as other taxa of non-symbiotic invertebrates (Shick and Dunlap, 2002), have been found to contain these compounds. Moreover, Banaszak and Trench (1995) have found that MAA concentrations in symbiotic and aposymbiotic *A. elegantissima* anemones were not significantly different. Besides, MAAs were not detected in the symbiont, neither in culture nor in hospite, indicating that MAAs may be obtained from the diet, although probably modified by bacteria during their assimilation and/or by the invertebrate tissue during their accumulation. For instance, the MAAs in the diet consumed by holothuroid echinoderms (sea cucumbers) differ markedly from the complement of MAAs found in the consumer's tissues (Shick et al., 1992). The discovery of the capacity of *Vibrio harveyi*, a bacterium isolated from enteric fluids of the holothuroid *Thelenota ananas*, to mediate the transformation of shinorine and porphyra-334 in mycosporine-glycine supports the postulation of a bioconversion scheme that may explain the complementary changes observed in the enteric fluid of the tropical holothuroid (Dunlap and Shick 1998, Shick and Dunlap 2002).

In a broader study, Shick et al. (2002) have compared the patterns of MAAs distribution in four sympatric species of sea anemones in the genus *Anthopleura.* They have examined species having predominantly zooxanthellae or zoochlorellae as symbionts, those containing both kinds of algal endosymbionts, and naturally occurring aposymbiotic species collected from intertidal habitats on the Pacific Coast of temperate North America. Their results indicate that the complement of four major MAAs in these species of *Anthopleura* (mycosporine-taurine, shinorine, porphyra-334 and mycosporine-2-glycine) broadly reflect phylogenetic differences among the anemones rather than the taxon of endosymbionts, the presence or absence of symbionts or environmental factors.

A noteworthy feature is that the apparently unique MAA to this genus, mycosporine-taurine, has been found in the highest concentration in all the studied species, a situation that is not altered by controlled diets (Stochaj et al., 1994; Banaszak and Trench, 1995; Shick and Dunlap, 2002; Shick et al., 2002). These results and the observation that some non-symbiotic scleractinian corals can synthesize aromatic amino acids *via* the shikimate pathway (Fitzgerald and Szmant, 1997), support the hypothesis that some MAAs (i.e. mycosporine-taurine) might be *de novo* synthesized by the sea anemones (Shick et al., 2002).

In this paper we report for the first time on the occurrence of several MAAs in the sea anemones *Aulactinia marplatensis* (Zamponi, 1977), *Oulactis muscosa* (Drayton in Dana, 1846) and *Anthothoe chilensis* (Lesson, 1830), from the rocky intertidal habitats of Mar del Plata coast, Argentina. A comparative approach involving previous results on different species will allow us to establish the existence of recurrent patterns on the content of these metabolites in sea anemones.

2. Materials and methods

2.1. Sampling of sea anemones

Individuals of the species *A. marplatensis* (Zamponi, 1977), *O. muscosa* (Drayton in Dana, 1849) and *A. chilensis* (Lesson, 1830) were sampled from the rocky intertidal of Punta Cantera, Mar del Plata, Argentina (38°05′S, 57°32′W), during low tides in July 2008. The species *A. marplatensis* and *O. muscosa* (family Actiniidae) undergo sexual reproduction while those of *A. chilensis* (family Sagartiidae) belong to a single clone produced by longitudinal fission (Acuña and Zamponi, 1995, 1996, 1998, 1999; Acuña et al., 2001, 2007; Excoffon and Acuña, 1998). Individuals of the mussel *Brachidontes rodriguezi* were also collected from the same rocky intertidal area. After collection, all the specimens were immediately moved to the laboratory and frozen at −20 °C.

2.2. Analysis of MAAs

Samples were prepared from a batch of around 7–9 specimens of each species. Based on the procedure reported by Stochaj et al. (1994), 2 to 5 g of defrosted individuals (tentacles and column from anemones, and soft tissues from the mussels) were homogenized in 20 mL of 80% methanol by sonication on ice. The extracts were centrifuged at 5000 *g* during 10 min and filtered under vacuum. The extraction cycle was repeated twice and the supernatants combined to treat by Solid Phase Extraction (SPE) with ODS-C18 (AccuBOND II — Agilent Technologies) cartridges to eliminate lipids and lipophilic pigments. After removing the solvent under reduced pressure, the residue was redissolved in 20 mL of bidistilled water and passed through another ODS-C18 cartridge. Finally the solvent was evaporated and the sample taken in 50% methanol for further analysis. Preliminary analysis of the extracts was carried out by UV–vis absorbance spectrophotometry in a UV-2101PC Shimadzu scanning spectrophotometer. The variability of the extraction efficiencies was independently estimated as *ca.* 10%.

Qualitative and quantitative analyses of MAAs were achieved by HPLC in a Shimadzu-LC20 system consisting of a LC20-AT quaternary pump, a SIL20-ACHT automatic injector, a CTO20-AC oven and a DAD-SPD M20A detector. The methodology was that reported by Carreto et al. (2005), with a slight modification in the pH value of the mobile phase. Thus, aliquots (25–50 μL) of the extract were diluted in 100% HPLC-grade methanol to a final volume of 0.5 mL and dried in a Labconco-Centrivap centrifugal evaporator. The residues were taken in 0.2% TFA at pH = 3.05 (A eluent) and ultra-filtered in 100 kDa Whatman tubes under 11,600 *g* during 20 min. Twenty μL-samples of the resultant solution were injected in the HPLC system using a 1 mL min^{-1} flow rate. The signals were processed with the Class-VP software. Identification of MAAs was accomplished by their absorption maxima and retention time calibrated with authenticated standards (Carreto et al., 2005). Quantification was accomplished by comparing the area of the peaks with those from standard solutions calibrated by using the molar absorption coefficients (ε) at the wavelength of maximum absorption reported by Bandaranayake (1998). Since the absorption coefficients of mycosporine-2 glycine and mycosporine-taurine have not yet been determined, the values of the structurally closer compounds shinorine and mycosporine-glycine were used respectively, in order to quantify these MAAs. The relative standard deviation of the integrated peak area for repeated injections of calibrated MAA solutions made over several days was within the order of 1–2%. Standard deviation for retention time was around ± 0.1–0.2 min (Carreto et al., 2005).

3. Results

Fig. 1 shows the absorbance spectra of the 80% methanol extracts, partially purified after the ODS-C18 treatment, for the three anemone species. All spectra show an absorption maximum around 270 nm. In addition, the methanolic extracts of *A. marplatensis* and *O. muscosa* reveal less intense absorption bands with peak maxima between 310 and 340 nm respectively, which suggest the presence of MAAs.

The HPLC chromatograms of MAA extracts (Fig. 2) indicate that, collectively, the studied sea anemones contain ten known MAAs in quantifiable amounts: palythine-serine sulphate (1) stylophora-sulphate (2), shinorine (3), mycosporine-2-glycine (4), palythine (5), asterina-330 (6) porphyra-334 (7), mycosporine-glycine (8), palythinol (9), and mycosporine-taurine (10). It is interesting to remark that minor amounts of two highly polar MAAs (1 and 2), not previously detected in other species of sea anemones (Shick et al., 2002), occur in the extracts of the species, notably of *O. muscosa*. In addition, the chromatogram of the *A. chilensis* extract accounts for the presence of four non-identified peaks (U) with relatively high intensities at the following retention times: $R_t = 5.77$ min, $\lambda_{max} = 329$ nm; $R_t = 6.49$ min, $\lambda_{max} = 273$ nm;

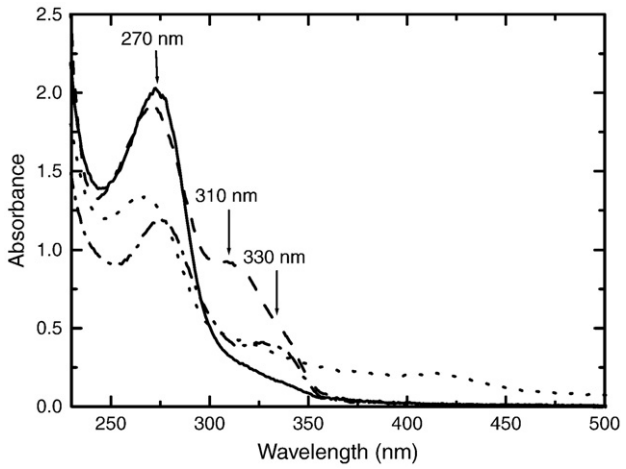

Fig. 1. UV–visible light absorption spectrum of 80% methanolic extract of *Aulactinia marplatensis* (– –), *Oulactis muscosa* (– · –), *Anthothoe chilensis* (——) and *B. rodriguezi* (· · · · ·).

$R_t = 14.08$ min, $\lambda_{max} = 252$ nm; $R_t = 19.08$ min, $\lambda_{max} = 340$ nm (Fig. 2). Whereas the absorption spectra of peaks U1 and U4 pointed to unknown MAAs, peak U2 showed a retention time and an absorption spectrum in agreement with substances such as gadusol and 4-deoxygadusol, postulated precursors of mycosporines (Chioccara et al., 1980; Bandaranayake et al., 1997). Co-chromatography of the extract with authenticated gadusol confirmed the occurrence of this compound in peak (U2) but also demonstrated the presence of other unknown substances in relatively higher amount which elute together with gadusol (results not shown).

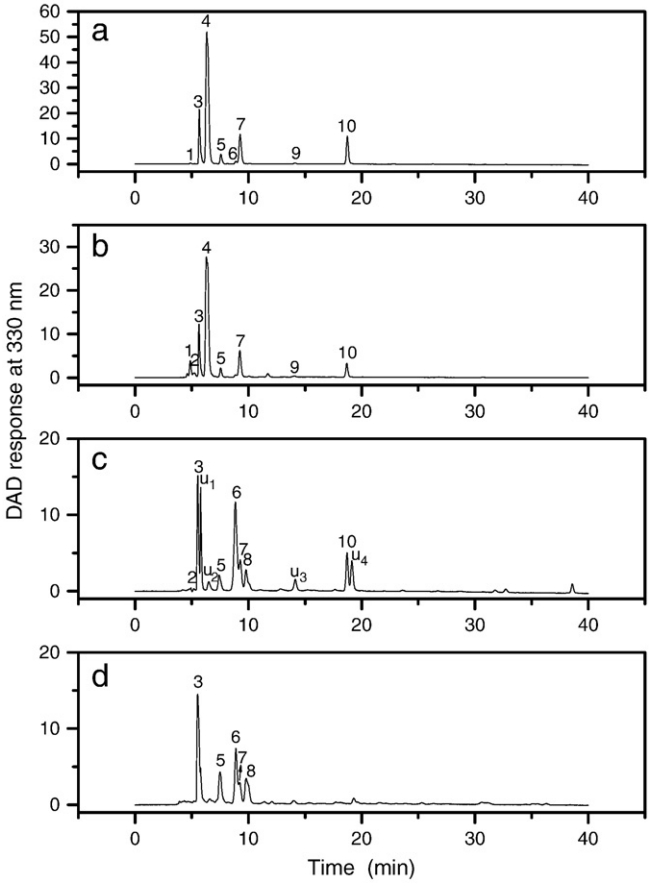

Fig. 2. HPLC-DAD (330 nm) chromatograms of the extracts from anemones a) *A. marplatensis*, b) *O. muscosa*, c) *A. chilensis* and d) the mussel *B. rodriguezi*.

In comparison with *A. marplatensis* (2.27 μmol g^{-1} wet tissue) and *O. muscosa* (1.13 μmol g^{-1} wet tissue), the total MAA content in *A chilensis* (0.31 μmol g^{-1} wet tissue) is significantly lower (Table 1; Fig. 3) and the MAA composition is also rather distinctive (Table 1; Fig. 4). *A. marplatensis* and *O. muscosa* contain the same four major MAAs previously found in several *Anthopleura* species (Shick et al., 2002): mycosporine-taurine, mycosporine-2-glycine, shinorine and porphyra-334, with a predominance of mycosporine-2 glycine (42.1% and 47.4%, of total MAAs, respectively) and mycosporine-taurine (36.3% and 20.7% of total MAAs, respectively) (Figs. 3 and 4).

A. chilensis differed from both of the foregoing species in that mycosporine-2-glycine, if present, occurs in undetectable amounts. In addition, mycosporine-glycine was found in *A. chilensis* as a major MAA with a molar proportion (26.1%) similar to that of the mycosporine-taurine (32.5%) (Fig. 4). Another distinctive characteristic of *A. chilensis* is the relatively high content of asterina-330 (19.1%), a MAAs scarcely represented in *A. marplatensis* and *O. muscosa* (Fig. 4).

The HPLC chromatograms of MAA extracts from the mussel *B. rodriguezi* (Fig. 2d) revealed that five MAAs were present in quantifiable amounts: mycosporine-glycine, shinorine, asterina-330, palythine and porphyra-334, being mycosporine-glycine the most concentrated (46.6%). Shinorine (24.5%), asterina-330 (12.8%), palythine (11.6%) and porphyra-334 (4.5%) were the next most abundant MAAs (Table 1). In comparison with the case of sea anemones, the total MAA content in *B. rodriguezi* (0.08 μmol g^{-1} wet tissue) was significantly lower (Table 1).

4. Discussion

As demonstrated for other non-endosymbiotic sea anemones species, such as *A. bermudensis* (McMurrich, 1889) (Stochaj et al., 1994) and *A. artemisia* (Shick et al., 2002) and for aposymbiotic specimens of *A. elegantissima* (Shick et al., 2002), the non-endosymbiotic species studied from the rocky intertidal of Mar del Plata (Argentina) contain significant amounts of MAAs. Our results also show that, in comparison with *A. marplatensis* (2.27 μmol g^{-1} wet tissue) and *O. muscosa* (1.13 μmol g^{-1} wet tissue), the total MAA content in *A. chilensis* (0.31 μmol g^{-1} wet tissue) is significantly lower (Fig. 3). It is difficult to compare the total MAA concentrations observed in the studied species with those reported for several species of sea anemones in the genus *Anthopleura*, since total MAA concentrations from these northern hemisphere species have been reported in terms of μmol g^{-1} dry tissue (Shick et al., 2002). Nevertheless, assuming that the averaged water content for sea anemones is about 85% (Brafield and Chapman, 1983), our recalculated values fall in the same range of concentrations (2.0 μmol g^{-1} dry tissue for. *A. chilensis* to 15.1 μmol g^{-1} dry tissue for *A. marplatensis*) reported for several *Anthopleura* species: asymbiotic (*A. artemisa*: ~1.5 μmol g^{-1} dry tissue), aposymbiotic (*A. elegantissima*: ~19.5 μmol g^{-1} dry tissue) and zooxanthellated anemones (*A. xanthogramica* (small): ~17.5 μmol g^{-1} dry tissue) (Shick et al., 2002).

The lowest MAA content in *A. chilensis* may relate to the typical sheltered habitat of this species, even during low tides. *O. muscosa*, which exhibits the next-lowest concentration of MAAs, is a low intertidal species that extends well into the subtidal; it occurs in tidepools or inhabits rocks buried in sand, so that only its oral disc is exposed. Thus, it experiences lower solar irradiances than the characteristically intertidal *A. marplatensis.* This last actiniarian, with the highest MAA concentration, is often found in areas directly exposed to sunlight in the intertidal zone during regular low tides. Its column is covered with attached gravel, providing a barrier against desiccation (Hart and Crowe, 1977). The same correlation between MAA concentration and light environment has been reported for several *Anthopleura* species: concentrations of MAAs are the lowest in *A. artemisia* (Pickering in Dana, 1846), which typically inhabits sheltered microhabitats whereas the highest content has been detected in the

Table 1
Mycosporine-like amino acid concentrations in the anemones *A. marplatensis*, *O. muscosa*, *A. chilensis* and in the mussel *B. rodriguezi*.

Peak	MAA	MAA concentration (μmol g^{-1} wet tissue)			
		A. marplatensis	*O. muscosa*	*A. chilensis*	*B. rodriguezi*
1	Palythine-serine sulphate	0.0046 ± 0.0001	0.0747 ± 0.0015	–	–
2	Stylophora sulphate	–	0.0249 ± 0.0005	0.0012±0.0001	–
3	Shinorine	0.2180 ± 0.0044	0.1200 ± 0.0024	0.0368 ± 0.0007	0.0196 ± 0.0004
4	Mycosporine-2-glycine	0.9545 ± 0.0191	0.5343 ± 0.0107	–	–
5	Palythine	0.0711±0.0014	0.0385 ± 0.0008	0.0135 ± 0.0003	0.0093 ± 0.0002
6	Asterina-330	0.0115 ± 0.0002	–	0.0591±0.0012	0.0103 ± 0.0002
7	Porphyra-334	0.1790 ± 0.0036	0.0996 ± 0.0020	0.0175 ± 0.0004	0.0036 ± 0.0001
8	Mycosporine-glycine	–	–	0.0807 ± 0.0016	0.0373 ± 0.0007
9	Palythinol	0.0046 ± 0.0001	0.0023 ± 0.0001	–	–
10	Mycosporine-taurine	0.8237 ± 0.0165	0.2332±0.0047	0.1006 ± 0.0020	–

characteristic intertidal species *A. elegantissima* (Shick et al., 2002). Therefore our results are consistent with the sunscreening role assigned to MAAs. However, some experimental evidence (Stochaj et al., 1994; Banaszak and Trench, 1995; Scelfo, 1988; Shick et al., 2002) indicates that light environment seems to be less important as a determinant of MAA concentrations in *Anthopleura* spp. than phylogenetic differences among the anemones (Shick et al., 2002).

The asymbiotic nature of the studied species suggests a dietary origin of MAAs or MAA precursors, such as gadusol and related compounds (Shick et al., 2002). Thus, the observed differences in the MAA pattern and content in these anemones may be originated in the MAA composition and distribution of the ingested food, but also in the transformations of dietary MAAs by action of specific anemone enzymes or bacteria in the gut flora of the cnidarians.

The feeding ecology of *A. marplatensis*, *O. muscosa* and *A. chilensis* has been analyzed by Acuña and Zamponi (1995, 1999) and Acuña et al. (2001). Their results have shown that these sea anemones are polyphagous opportunistic species. However, whereas the major component of the diet for the rocky intertidal sea anemones *A. marplatensis* and *O. muscosa* is the mussel *B. rodriguezi*, crustaceans (Gamaridae) are the main constituent of the diet in *A. chilensis* (Acuña and Zamponi, 1995, 1996, 1999; Acuña et al., 2001). The presence of MAAs in *B. rodriguezi* could be related with their photoprotective role on the siphonal mantle, since this part of the bivalves is usually exposed to sun radiation (Ishikura et al., 1977). On the other hand, the seasonal accumulation of MAAs in ovaries of marine invertebrates indicates their involvement with reproduction (Bandaranayake et al., 1997).

It is difficult to establish the relative importance of the natural diet in determining the MAA content of these anemones, since crustaceans were not analyzed for MAAs. Nevertheless, according to our results, MAA content was significantly higher in those intertidal species (*A. marplatensis*: 2.27 μmol g^{-1} wet tissue and *O. muscosa*: 1.13 μmol g^{-1} wet tissue) feeding on mussels.

In comparison, the MAA content of the mussel *B. rodriguezi* (0.08 μmol g^{-1} wet tissue) was several times lower than those exibited by the intertidal anemones, indicating that the studied species may differentially retain and accumulate MAAs from their food.

Other organisms have also been found to selectively accumulate MAAs from their diet. It has been proposed that UV-induced MAAs in *Phaeocystis antarctica* can be trophically transferred to the Antarctic krill so that MAA concentrations in krill are dependent on the MAA content of phytoplankton (Newman et al., 2000). Analogously, Carefoot et al. (1998, 2000) observed that the amounts of MAAs incorporated into the spawn in the sea hare *Aplysia dactylomela* are strongly influenced by the diet but specific MAAs were differentially sequestered. Adams et al. (2001) demonstrated the prevalent accumulation of dietary shinorine from algae in sea urchins. Similarly, Mason et al. (1998) proved that marine consumers, like Japanese rice fish (*Oryzias latipes*) fed with red algae, can acquire selectively the MAAs palythine and asterina-330, but do not seem to retain shinorine.

In contrast, recent bioinformatic analyses of the genome of the sea anemone *Nematostella vectensis* have revealed that genes encoding enzymes of the shikimic acid pathway have been transferred to the genome of the sea anemone from both bacterial and eukaryotic (dinoflagellate) donors. Additional genes encoding some enzymes of

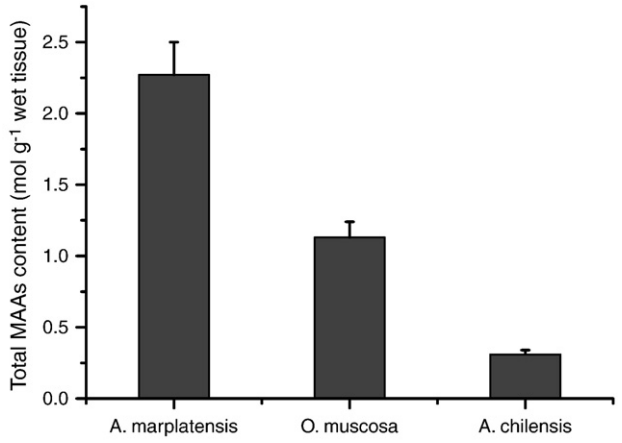

Fig. 3. Total concentration of mycosporine-like amino acids in the anemones: *A. marplatensis*, *O. muscosa* and *A. chilensis*. Vertical bars denote the estimated uncertainty of the determinations.

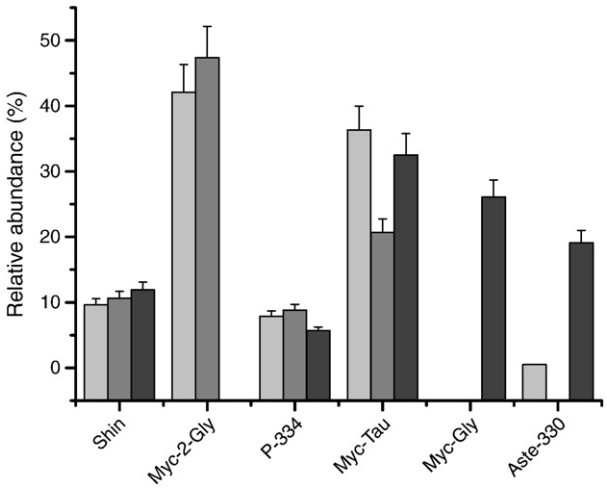

Fig. 4. Relative concentration (molar fractions) of the principal mycosporine-like amino acids (Shin: shinorine; Myc-2-Gly: mycosporine-2-glycine; P-334: porphyra-334; Myc-Tau: mycosporine-taurine; Myc-Gly: mycosporine-glycine; Aste-330: asterina-330) in the anemones: *A. marplatensis*, *O. muscosa* and *A. chilensis*. Vertical bars denote the estimated uncertainty of the determinations.

the pathway are present in a putative bacterial (*Flavobacteriaceae*) symbiont of this sea anemone, closely related to those of *Tenacibaculum* sp. MED152 (Starcevic et al., 2008). Moreover, a novel bacterial symbiotic species in the genus *Tenacibaculum* (*Tenacibaculum aiptasiae* sp. nov.) has recently been isolated from the tropical sea anemone *Aiptasia pulchella* (Wang et al., 2008). Therefore, MAAs may be synthesized *de novo* by the sea anemones and/or by their bacterial symbiont (Starcevic et al., 2008). On the other hand, the functionality of this metabolic pathway implies an additional energy expense to the anemones that lead us to suppose the existence of a complex regulation mechanism that needs to be determined but that might involve the light environment and the provision of dietary MAAs.

Nevertheless, diet does not explain the MAA complement observed in the studied anemones. Our results show that in the mussel *B. rodriguezi*—the principal dietary MAA source of *A. marplatensis* and *O. muscosa* (Acuña and Zamponi, 1995, 1996, 1999; Acuña et al., 2001) mycosporine-glycine, was the main MAA (46.6%) followed by shinorine (24.5%), asterina-330 (12.8%), palythine (11.6%) and porphyra-334 (4.5%). However, sea anemones are polyphagous opportunistic species and it cannot be excluded that a food source other than the mussel provided additional MAAs. Notably, in coincidence with the data for sea anemones species from the northern hemisphere (Shick and Dunlap, 2002; Shick et al., 2002), the non-diet MAA mycosporine-taurine was found in high proportion in the three non-endosymbiotic studied species from the southern hemisphere. These findings indicate a non-dietary origin of mycosporine-taurine (Starcevic et al., 2008) or perhaps a regulated biotransformation metabolism of dietary MAAs or their precursors that is common to sea anemones (Shick et al., 2002).

In addition to mycosporine-taurine, *A. marplatensis* and *O. muscosa* contain the same common major MAAs previously found in several *Anthopleura* species (Shick and Dunlap, 2002; Shick et al., 2002): mycosporine-2-glycine, shinorine and porphyra-334, with mycosporine-2-glycine and mycosporine-taurine being the most concentrated. Our results showed that the absolute and relative mycosporine-2-glycine content in these non-symbiotic species was similar to that reported for *A. elegantissima* having zooxanthellae. Therefore, as it has previously been discussed, the observed correspondence between the density of zooxanthellae and high levels of mycosporine-2-glycine is not absolute and remains to be clarified (Shick et al., 2002).

That such geographically, taxonomically and biologically distant sea anemone species would produce exactly the same suit of major MAAs is not surprising given that relationships among the Actiniidae are sometimes based on the absence of features rather than on synapomorphies, and that this group is known to be broadly paraphyletic. Furthermore, the genus *Anthopleura* (family Actiniidae) is polyphyletic with some members closely allied to members of other actiinid genera, and others associated with representatives of other families (Daly, 2003; Daly et al., 2008). For instance, among *Anthopleura* species, the MAA complement of the less closely related species, *A. artemisia* (Geller and Walton, 2001; Geller et al., 2005), differs from the other species in that it contains only traces of mycosporine-2-glycine and a major MAA, probably palythenic acid, (Whitehead et al., 2001) which has never been detected in any of the other studied species (Shick et al., 2002). Similarly, among the species studied here, *A. chilensis* had undetectable amounts of mycosporine-2-glycine, which clearly distinguishes this species from *A. marplatensis* and *O. muscosa*. Additionally, it contains high proportions of asterina-330 (12.8%) and mycosporine-glycine (46.6%), two compounds that have not been previously observed as major MAAs in sea anemones species (Shick et al., 2002).

This finding of a unique MAA composition together with the absence of mycosporine-2-glycine in *A. chilensis* is consistent with the phylogenetic conclusions of Daly et al. (2008) in the sense that family Actiniidae (*A. marplatensis* and *O. muscosa*) belong to the Endomiyaria clade, whereas the family Sagartiidae (*A. chilensis*) belong to the Acontiaria–Boloceroidaria–Mesomyaria clade (Daly et al., 2008). In addition, as already mentioned, *A. marplatensis* and *O. muscosa* (family Actiniidae) show sexual reproduction while *A. chilensis* (family Sagartiidae) reproduce by longitudinal fision (Zamponi and Excoffon, 1986; Excoffon and Zamponi, 1996; Excoffon and Acuña, 1998). Although we are not able to establish the relationship between MAA complement and reproduction mechanisms, certainly the mode of reproduction has profound implications in their ecology because colonies and solitary individuals interact with their environment very differently (Geller et al., 2005).

As Shick et al. (2002) have discussed elsewhere, some minor MAAs may arise from dietary origin, and therefore, the presence of asterine and palythinol in the non-symbiotic studied anemones is expected. However, the observed presence of palythine-serine-sulphate and mycosporine-sulphate ester from *Stylophora pistillata* is noteworthy, as these compounds have only been previously found in scleractinian corals (Wu Wong et al., 1997; Shick et al., 1999; Carreto et al., 2005; Carignan et al., 2009) but they were undetectable in the mussel *B. rodriguezi*, the principal item in the diet of *A. marplatensis* and *O. muscosa*. Thus, the origin of these minor compounds or their probable precursors (Shick, 2004; Carreto et al., 2005) are largely unknown. However it can be speculated that they might be synthesized by the anemones themselves, or more probably they may come from bacteria harbored in the anemones coelenteron or ectodermal tissue (Stochaj et al., 1994).

In conclusion, the MAA content in the three studied sea anemones from Mar del Plata coasts reflects phylogenetic relationships, similar to those observed by Shick et al. (2002) for sea anemones belonging to genus *Anthopleura* from the Pacific Coast of temperate North America.

Acknowledgements

This study has been supported by Universidad Nacional de Mar del Plata (15E-367). CONICET PIP 5009 and PIP 5909 provided partial financial support to this research. E.M.A. thanks CONICET for a studentship. This is a contribution of INIDEP No 1585.

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