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Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions

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Abstract The formation of UV-absorbing mycosporine-like amino acids (MAAs) as a photoprotective strategy against biologically harmful ultraviolet radiation was studied in Antarctic red macroalgae. After exposure to three different radiation treatments (PAR: 400–700 nm, PAR + UVA: 320–700 nm, PAR + UVA + UVB: 295–700 nm), using artificial irradiance sources under controlled conditions, the physiological capability to stimulate MAA synthesis was investigated. While 8 out of 18 species showed an induction of MAA formation and accumulation, the remaining ten, mainly deep water species, did not exhibit any traces of MAAs. The MAA-containing samples were divided into three physiological response types based on their MAA accumulation versus different radiation treatments. The first response type included *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens* and *Porphyra plocamiestris*, and exhibited additionally increasing MAA concentrations under the different radiation treatments, i.e. highest total MAA values were measured under the full radiation spectrum. The second response type included *Porphyra endiviifolium* and *Gymnogongrus turquetii*, showing highest MAA concentrations already under PAR + UVA. In contrast, *Neuroglossum ligulatum* and *Plocamium cartilagineum* exhibited a strong MAA decrease under PAR + UVR and were grouped in the third response type. No consistent MAA induction patterns could be found, even for individual MAAs, indicating that induction, formation and accumulation of individual MAAs is a very flexible and species-specific mechanism.

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Introduction

Benthic macroalgae are sometimes exposed to detrimental ultraviolet-B radiation (UVBR; 280–315 nm), affecting biomolecules, such as proteins and nucleic acids, by so-called direct mechanisms (Vincent and Neale 2000). These molecules can be photochemically degraded or transformed, resulting in impairment or even complete loss of biological function. In this way, UVBR may also influence the genomic stability of plant populations (Ries et al. 2000). In addition, after ultraviolet radiation (UVR; 280–400 nm), indirect exposure mechanisms, such as the production of reactive oxygen species, may negatively affect cells. Macroalgae have developed a number of mechanisms to cope with the deleterious effects of UVBR, such as avoidance, i.e. growth in deep waters, DNA repair by photoreactivation processes and protection, i.e. quenching of reactive oxygen species through antioxidants (Bischof et al. 1998; Aguilera et al. 1999; Cockell and Knowland 1999; Poll et al. 2001). Another protection mechanism against enhanced UVBR is the synthesis and accumulation of sunscreen compounds, such as mycosporine-like amino acids (MAAs), that have been found in many marine primary producers (Bandaranayake 1998; Dunlap and Shick 1998; Jeffrey et al. 1999; Karsten et al. 1998a,b). Due to high extinction coefficients these compounds are very effective UVR absorbers in the wavelength range between 309 and 360 nm (Dunlap and Shick 1998; Cockell and Knowland 1999). To date, 19 distinct molecular structures of MAAs have been identified. It is assumed that these structures are synthesized via the shikimate pathway, which only occurs in plants and microorganisms (Herrmann and Weaver 1999; Shick et al. 1999).

The Southern Ocean, as a habitat for Antarctic macroalgae, features some peculiarities in comparison to other marine regions. Besides high nutrient concentra-

tions (Drew and Hastings 1992; Prézelin et al. 1994) and low water temperatures (Antarctic Surface Water, ranging from −1.7°C to 2.0°C) (Schodlok et al. 2002), ice cover and high amplitudes of irradiance fluctuations typically result in total darkness in winter and 24 h daylight in summer, hence producing large seasonal variability. Furthermore, the terrestrial impact caused by riverine influx is limited, and therefore the waters are generally very clear.

High water transparencies are evidenced by low diffuse vertical attenuation coefficients (K_d, Kirk 1994), resulting in a 1% depth of the photosynthetic active radiation (PAR; 400–700 nm) of up to 40 m in coastal waters near King George Island (Gómez et al. 1997). UVR also penetrates deeply into the water column, in some Antarctic marine areas even to depths of about 60–70 m (Smith et al. 1992). This becomes ecologically important in a scenario with increasing UVBR at the earth's surface caused by ozone depletion in the stratosphere, particularly over the Antarctic. In September 2000, the "ozone hole" extended to the southern tip of South America, with ozone values 50% below normal conditions at 75–80°S, which is a new record low (WMO 2001).

While the occurrence of MAAs in a few selected Antarctic macroalgae was documented for the first time by Karentz et al. (1991) and McClintock and Karentz (1997), a comprehensive inventory of the MAA contents of Antarctic species has recently been undertaken by Hoyer et al. (2001), investigating brown, green and red algae and showing that mostly red algae contain MAAs. The dependence of MAA accumulation on depth distribution, as well as differences in MAA concentrations within algal thalli, was demonstrated. Additionally, Post and Larkum (1993) investigated seasonal effects on UV-absorbing compounds in three Antarctic algae without identifying the chemical structure of the compounds. In Arctic macroalgae such as *Palmaria palmata* and *Devaleraea ramentacea* (Karsten and Wiencke 1999; Karsten et al. 1999), as well as in cold-temperate species such as *Chondrus crispus* (Karsten et al. 1998a; Franklin et al. 1999), MAA synthesis was investigated in field studies, resulting in species-specific MAA inductions under different radiation conditions, i.e. mainly under PAR, UVA and/or UVB. Therefore, in the present study, the three physiological algal groups based on MAA contents in the field (group I – no MAAs at all, group II – MAAs inducible in variable concentrations, and group III – always high MAA values) as described by Hoyer et al. (2001) were photobiologically investigated under controlled laboratory conditions by applying well-defined spectral ranges. The main aim was to better understand the species-specific physiological capability of Antarctic red macroalgae to form and accumulate MAAs as a photoprotective strategy. Emphasis was placed on deep-water species, which seem to produce no or lower MAA concentrations when collected in the field, probably due to the lack of UVBR in that particular environment (Dunlap et al. 1986).

Materials and methods

The 18 red macroalgal species studied (Table 1) were isolated on King George Island (Antarctica) in 1994, according to the method of Clayton and Wiencke (1986), and established as unialgal cultures in the laboratory of the Alfred Wegener Institute (Bremerhaven, Germany). Plants were grown under the following conditions: Provasoli-enriched North Sea water (Starr and Zeikus 1987) at a salinity of 30–32 PSU, aerated with membrane-filtered air (pore size 0.2 µm), at a temperature of 0°C, and an illumination of 2.2–4.4 W m^{-1} irradiance provided by daylight fluorescent lamps (Lumilux Deluxe, OsramL 36 W/12/950, Germany). The irradiance

Table 1. Investigated red macroalgal species with details on respective habitats in Antarctica, according to Wiencke and Clayton (2002), and on the physiological capability to synthesize and accumulate UV-absorbing mycosporine-like amino acids (*MAA*) after treatment with various radiation conditions (see "Materials and methods")

Species	Habitat	MAA induction
Antarcticothamnion polysporum Moe & Silva (Ceramiales)	Sublittoral (15–25 m), endemic	No
Audouinella purpurea (Lightfoot) Woelkerling (Acrochaetiales)	Eulittoral, sublittoral, cosmopolitan	No
Ballia callitricha Kützing (Ceramiales)	Tide pools, sublittoral (0–45 m), cold-temperate	No
Delesseria lancifolia (J.D. Hooker) J. Agardh (Ceramiales)	Sublittoral (2–30 m), cold-temperate	No
Gymnogongrus antarcticus Skottsberg (Gigartinales)	Tide pools, sublittoral (0–15 m), endemic	Yes
Gymnogongrus turquetii Hariot (Gigartinales)	Tide pools, sublittoral (0–30 m), sub-Antarctic/Antarctic endemic	Yes
Hymenocladiopsis crustigena Moe (Rhodymeniales)	Sublittoral (2–30 m), endemic	No
Kallymenia antarctica Hariot (Cryptonemiales)	Sublittoral (5–35 m), endemic	Yes
Myriogramme smithii (J.D. Hooker & Harvey) Kylin (Ceramiales)	Sublittoral (8–43 m), endemic	No
Neuroglossum ligulatum (Reinsch) Skottsberg (Ceramiales)	Sublittoral (1–10 m), endemic	Yes
Palmaria decipiens (Reinsch) Ricker (Palmariales)	Lower eulittoral, sublittoral (0–30 m), endemic	Yes
Pantoneura plocamioides Kylin (Ceramiales)	Sublittoral (2–45 m), cold-temperate	No
Phycodrys austrogeorgica Skottsberg (Ceramiales)	Sublittoral (2–45 m), endemic	No
Phycodrys quercifolia (Bory) Skottsberg (Ceramiales)	Sublittoral (0–25 m), cold-temperate	No
Phyllophora ahnfeltioides Skottsberg (Gigartinales)	Sublittoral (0–30 m), endemic	No
Plocamium cartilagineum (Linnaeus) Dixon (Plocamiales)	Sublittoral (2–40 m), cosmopolitan	Yes
Porphyra endiviifolium Chamberlain (Bangiales)	Upper eulittoral, endemic	Yes
Porphyra plocamiestris Ricker (Bangiales)	Sublittoral (1–20 m), endemic	Yes

intensity was related to the species-specific light requirements of photosynthesis (Weykam 1996). The daylength in the culture room was varied between 5 h (winter) and 20 h light (summer), thereby, simulating fluctuating Antarctic daylengths (Wiencke 1990). All samples were taken under spring conditions, when the daylength reached 18 h.

For the induction experiments, the low-light-acclimated cultures were transferred to the following radiation conditions: (1) only white light (PAR, photosynthetic active radiation: 400–700 nm), (2) PAR plus UVA radiation (PAR + UVA: 320–700 nm) and (3) PAR plus UVR (PAR + UVA + UVB: 295–700 nm). Daylight fluorescent lamps (Lumilux Deluxe, OsramL 36 W/12-950, Germany) in combination with Q-Panel UVA-340 fluorescent tubes (Cleveland, USA) emitting a spectrum similar to solar radiation in the UV range were used. The glass vessels were covered with specific filters to cut off UVBR (320 nm cut-off: Ultraphan URUV, Digefra, München, Germany) and UVB + UVA radiation (400 nm cut-off: Folex PR, Folex, Dreieich, Germany). The containers under the full spectrum were also covered with a filter with no transmission below 295 nm (Ultraphan UBT, Digefra, München, Germany).

Irradiance was measured with a Spectro 320D spectroradiometer (Instrument Systems, Germany) (see Fig. 1). PAR was 5.4 W m^{-2}, UVA and UVB 4.1 W m^{-2} and 0.5 W m^{-2}, respectively, at the vessel's surface. The UVA radiation corresponded to values measured in 7–8 m water depth during a sunny summer day on King George Island (Hoyer et al. 2001). In all cases a 16 h PAR + UVR:8 h dark photocycle was applied.

During the experiments algae were kept in glass beakers filled with filtered Provasoli-enriched seawater plus 2.1 mM sodium hydrogen carbonate as an inorganic carbon source. All experiments were performed in a constant temperature room at 0°C, except for those with *Porphyra plocamiestris*, which was kept at 5°C. After 12 days of exposure to the various radiation conditions the algae were harvested, oven-dried at 50°C overnight, and stored in Eppendorf tubes under dry and dark conditions prior to MAA analysis.

MAA extraction and analysis

Samples of about 10–20 mg dry weight (DW) were extracted for 2 h in Eppendorf tubes filled with 1 ml 25% aqueous methanol (v/v) and incubated in a waterbath at 45°C. This procedure was sufficient to obtain > 99.5% of MAAs into solution. After centrifugation at 5000 *g* for 5 min, 800 µl of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 800 µl 100% methanol and vortexed for 30 s. Samples were analyzed with a Waters high-performance liquid chromatography (HPLC) system according to the method of Karsten and Garcia-Pichel (1996), with the following modifications. The MAAs were separated on a Phenomenex SphereClone RP-C8 column (5 µm, 250×4 mm i.d.) protected with a RP-C8 guard cartridge (4×3 mm i.d.). The mobile phase was 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min^{-1}. The MAAs were detected with a photodiode detector at 330 nm, and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time, and by co-chromatography with standards extracted from the marine red macroalgae *Chondrus crispus* and *Porphyra umbilicalis* from Helgoland, Germany, as well as from ocular lenses of the coral trout *Plectropomus leopardus* (kindly provided by Dr D. Bellwood, James Cook University, Townsville, Australia). Quantification was made using the molar extinction coefficients listed in Karsten et al. (1998c). All amounts are given as means (± SD) and expressed as concentration on a dry weight basis (mg g^{-1} DW).

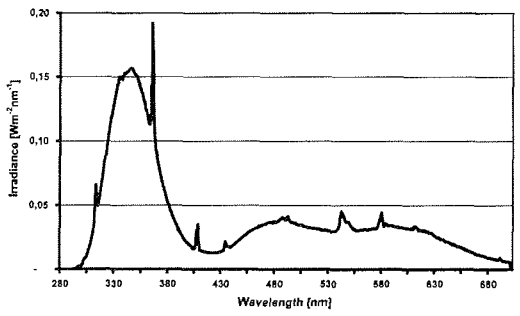

Fig. 1. Radiation spectrum in the range from 295 to 700 nm emitted by daylight fluorescent lamps in combination with Q-Panel UVA-340 fluorescent tubes

Statistical analyses

The influence of filter treatment on MAA content and significant differences within the different treatments were assessed by using one-way ANOVA followed by a multiple comparison test (Tukey–Kramer HSD-test). Differences were considered significant when probability was $P < 0.05$.

Results

Ten of 18 investigated red algae from Antarctica did not contain any MAAs, either in pre-culture or after exposure to various radiation conditions (Table 1). However, the other eight species exhibited seven different MAAs: shinorine, porphyra-334, palythine and asterina-330 as major components; palythinol in *Porphyra endiviifolium* in trace amounts as well as usujirene in *Palmaria decipiens*; and a minor content of an unknown substance (retention time: 4.6 min; absorption maxima: 332/3 nm) in *Neuroglossum ligulatum*. In this study, we refer mainly to the quantitatively dominant MAAs. Incubation under various radiation conditions resulted in species-specific differences in the quantity and quality of MAAs. Therefore, the macroalgae were grouped based on their response to the different irradiance treatments, resulting in distinct MAA induction/accumulation profiles (Table 2).

The first response type (a), including *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens* and *Porphyra plocamiestris*, exhibited an additional increase in total MAA concentration after incubation under PAR, PAR + UVA and PAR + UVA + UVB, with the highest concentrations under the latter treatment (Fig. 2A–D). The initial samples (before treatment) had a significantly lower MAA concentration compared to the exposed groups, except for *K. antarctica* and *P. plocamiestris*, in which no significant differences were found between the initial samples and the PAR treatment. The quantitative differences of MAAs between the PAR samples and those under the full radiation spectrum were statistically very significant ($P < 0.001$), except for *P. decipiens*. Additionally, significant differences were found in *G. antarcticus* between PAR and

Table 2. Classification of field-collected red algae in three physiological groups (*I–III*) based on MAA concentrations (Hoyer et al. 2001) and of cultured red algae belonging to the groups II and III, which were divided into three subgroups showing different responses (*response types a–c*) to MAA synthesizing and accumulation after treatment with distinct radiation conditions (PAR: 400–700 nm; PAR + UVA: 320–700 nm; PAR + UVA + UVB: 295–700 nm)

Field-collected algae	Group I	Group II		Group III
	Lacking MAAs	Inducibly variable MAA concentrations		Always high MAA concentrations
		Type a	Type b	Type c
Cultured algae after exposure to different radiation conditions	Lacking MAAs	Highest total MAA concentrations under PAR + UVA + UVB	Highest total MAA concentrations under PAR + UVA	MAA decrease under PAR + UVR

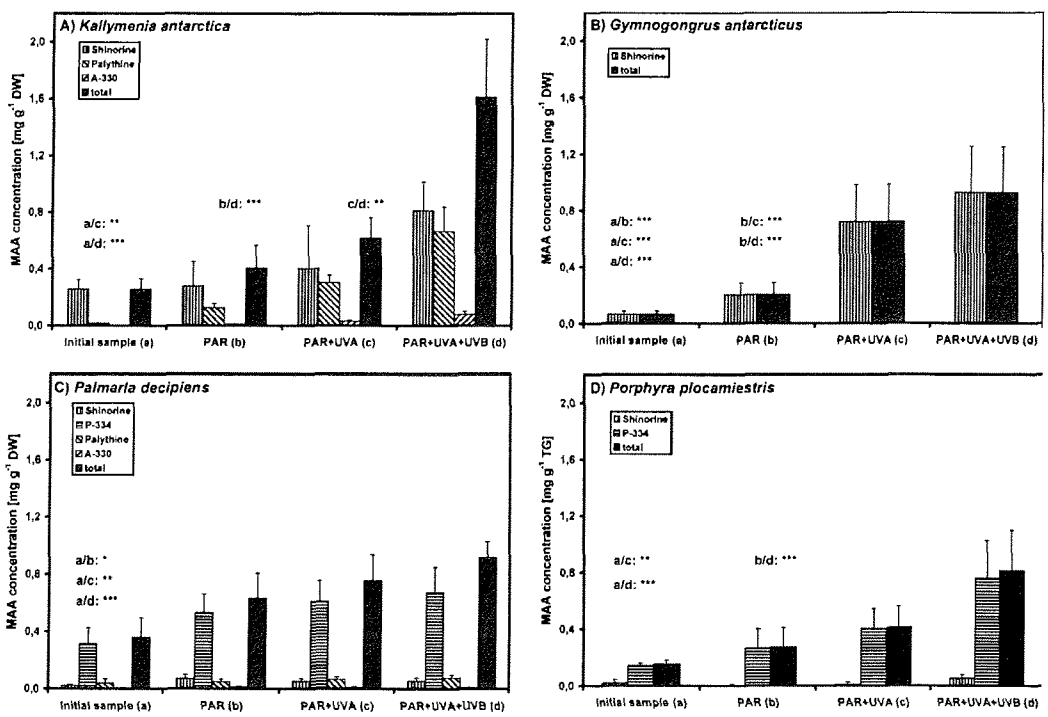

Fig. 2A–D. Shinorine, palythine, porphyra-334, asterina-330 and total concentration of MAAs in the first response type (a) of algal species showing a continuous increase of MAA concentration after 12 days of exposure to different radiation conditions. **A** *Kallymenia antarctica* ($n = 4–5$), **B** *Gymnogongrus antarcticus* ($n = 5–6$), **C** *Palmaria decipiens* ($n = 5–6$), **D** *Porphyra plocamiestris* ($n = 4–5$). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

PAR + UVA conditions ($P < 0.001$). The increase in total MAAs from the initial samples to those with the full spectrum treatment was species dependent: 2.6-fold in *P. decipiens*, 5.3-fold in *P. plocamiestris*, 6.3-fold in *K. antarctica* and 14-fold in *G. antarcticus*. The relative changes in MAA concentration under the different radiation treatments are shown in Fig. 5A.

Within the first response type, the MAA compositions were different. *G. antarcticus* contained only shinorine as the sole MAA, while in *P. plocamiestris*, in addition to this compound, porphyra-334 also occurred in large quantities. This species also exhibited traces of palythine, asterina-330 and palythinol (data not shown) under PAR + UVA + UVB treatment. *K. antarctica* and *P. decipiens* exhibited three (shinorine, palythine, asterina-330) and four different UV-absorbing compounds (shinorine, porphyra-334, palythine, asterina-330), re-

spectively (Fig. 2A–D). In *K. antarctica*, shinorine and palythine were the quantitatively dominant MAAs, and in *P. decipiens* porphyra-334 occurred in highest concentrations.

A second response type (b), with *Porphyra endiviifolium* and *Gymnogongrus turquetii*, showed the highest concentration of total MAAs already under UVA treatment (Fig. 3A, B). In *P. endiviifolium*, statistically significant increases of MAAs from the high-value initial sample to plants kept under PAR + UVR conditions ($P < 0.0003$, $P < 0.03$) were detected, as well as between the PAR- and PAR + UVA-treated samples ($P < 0.02$, Fig. 3A). After additional exposure to UVB, a slightly lower concentration of MAAs compared to the PAR + UVA treatment was observed. In *G. turquetii*, the MAA concentration significantly increased from the low initial value in all radiation treatments ($P < 0.001$). Significant differences in MAA concentrations were also found between samples under PAR and both UVR treatments ($P < 0.005$, Fig. 3B). Although MAA levels were slightly lower under PAR + UVA + UVB compared to PAR + UVA, this difference was not statistically significant. The rise of total MAA values from the initial samples to the PAR + UVA treatment was 1.9-fold in *P. endiviifolium* and 25.2-fold in *G. turquetii*. While both species exhibited shinorine and porphyra-334 as the main MAAs, in *G. turquetii* these compounds occurred in almost equimolar concentrations under all experimental radiation treatments, except in the initial sample where porphyra-334 was absent. In contrast, in all samples of *P. endiviifolium* porphyra-334 was the quantitatively dominant MAA, whereas shinorine occurred in low amounts.

Neuroglossum ligulatum and *Plocamium cartilagineum* represent a third response type (c), as both species contained lower or alternatively no MAAs after exposure to PAR + UVA + UVB (Fig. 4A, B). In *N. ligulatum*, exposure to PAR and PAR + UVA was accompanied by a 3.6- and 8.7-fold increase in total

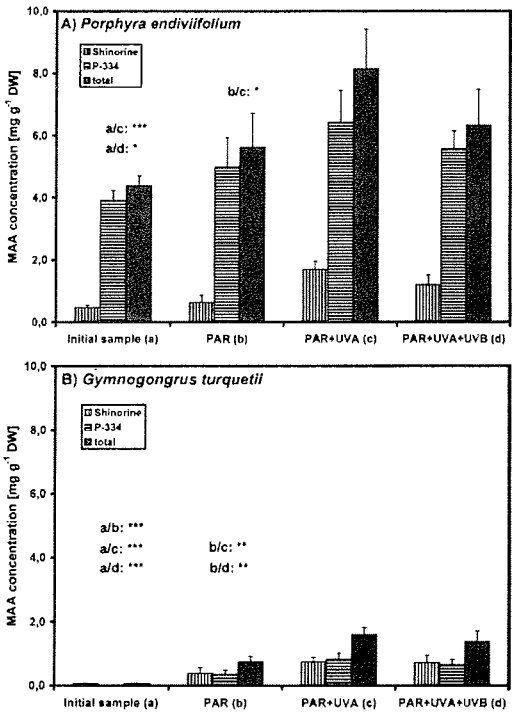

Fig. 3A, B. Shinorine, porphyra-334 and total concentration of MAAs in the second response type (b) of algal species showing the highest MAA concentration under PAR + UVA treatment after 12 days of exposure to different radiation conditions. **A** *Porphyra endiviifolium* ($n = 5$–7), **B** *Gymnogongrus turquetii* ($n = 4$–6). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

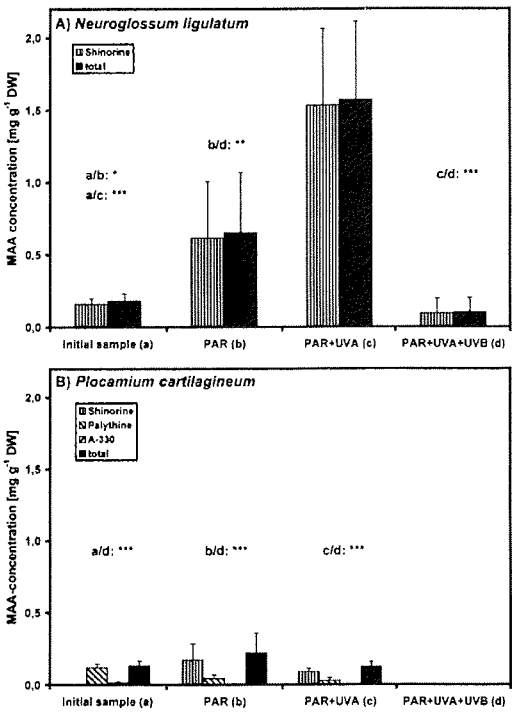

Fig. 4A, B. Shinorine, palythine, porphyra-334, asterina-330 and total concentration of MAAs in the third response type (c) of algal species showing a strong decrease of MAA concentration under PAR + UVR treatment after 12 days of exposure to different radiation conditions. **A** *Neuroglossum ligulatum* ($n = 3$–5), **B** *Plocamium cartilagineum* ($n = 3$–5). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

MAAs, respectively, followed by a decrease similar to the initial value under PAR + UVA + UVB. In contrast, only a 1.7-fold increase from the initial concentration to the MAA content after the PAR treatment was evident in *P. cartilagineum*. Although exposure to PAR + UVA already led to a strong decrease in the MAA concentration, resulting in values similar to the initial ones after exposure to the full spectrum, only trace amounts of palythine (0.002 mg g^{-1} DW) were present. The relative changes in MAA concentration for the second and third response types are summarized in Fig. 5B.

Based on MAA composition, shinorine was the most abundant sunscreen compound in *N. ligulatum*, in addition to traces of porphyra-334 and an unknown UV-absorbing substance (data not shown). Whilst the initial sample of *P. cartilagineum* contained palythine as a major compound along with small amounts of asterina-330; shinorine was the quantitatively dominant MAA after exposure to higher PAR intensities.

Discussion

The most important result of the present study is that the algal response to the application of different radiation conditions can be clustered into three subgroups (a, b, c; Table 2), here called response types, based on MAA accumulation versus different radiation treatments.

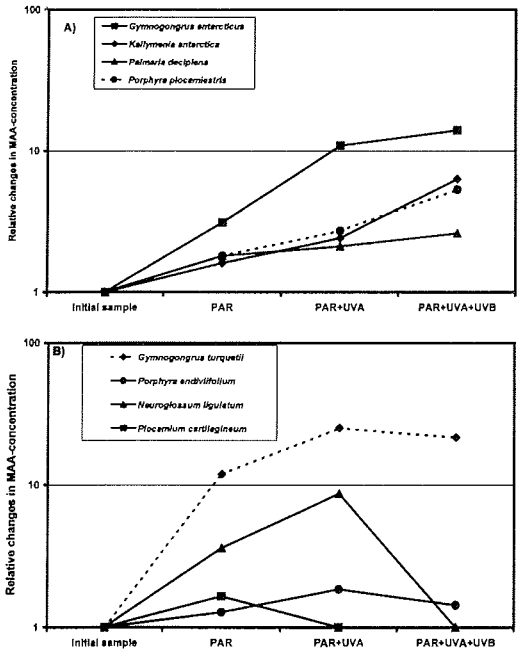

Fig. 5A, B. Relative changes in MAA concentrations under the different radiation conditions related to the normalized initial samples which were all set to 1. **A** First response type, **B** second and third response types of Antarctic algae after 12 days of exposure to different radiation conditions

The first response type (a) includes species that accumulated MAAs increasing under each treatment, i.e. strongest biosynthesis of total MAAs under the full artificial spectrum. The species of this response type (a: *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens*, *Porphyra plocamiestris*) are endemic to Antarctica and therefore generally well adapted to the harsh Southern Ocean environmental conditions, e.g. low temperatures (Wiencke and tom Dieck 1989; Bischoff-Bäsmann and Wiencke 1996; Eggert and Wiencke 2000). Furthermore, taxa of this subgroup are found over a wide depth range within the sublittoral, and *P. decipiens* occurs also in the eulittoral (Wiencke and Clayton 2002). This broad distribution pattern may result in adaptation to clear water conditions and consequently to UV radiation penetrating deeply into the water column. MAAs may thus be induced to assume the role of a sunscreen, as seen in *K. antarctica*, exhibiting high MAA concentrations even at a water depth of 20 m (Hoyer et al. 2001). The evolutionary development of endemism is coupled with environmental adaptation advantages with respect to temperature and irradiance. Such species flexibly allows the adjustment of their MAA concentrations to the prevailing radiation climate, as demonstrated for several field-collected Antarctic species from different depths (Hoyer et al. 2002).

Macroalgae of the second response type [b: *Gymnogongrus turquetii* (sub-Antarctic and Antarctic endemic), *Porphyra endiviifolium* (Antarctic endemic)] contain the highest MAA concentration under PAR + UVA, and additional UVB did not lead to any further MAA accumulation. The habitat of *P. endiviifolium* is the upper eulittoral, which is regularly exposed to full solar radiation. The perpetual high MAA concentrations found in this species have also been found in the closely related cold temperate and eulittorally growing *Porphyra umbilicalis* (Gröniger et al. 2000). High MAA concentrations are typically present in eulittoral species and, hence, may reflect a steady protective mechanism, thereby highlighting the photoprotective role of these UV-absorbing compounds (Karsten and West 2000). The generally lower MAA concentrations in *G. turquetii* can be explained by the broader depth range for growth (eulittoral to sublittoral down to 30 m, Wiencke and Clayton 2002), indicating that each algal species probably has an upper MAA concentration threshold that depends on the vertical distribution on the shore and the respective radiation conditions. However, one has to consider that the effect of dose, regardless of irradiance, was not explicitly tested. Therefore, we cannot ultimately conclude that the increase of MAAs is only due to the change in spectral composition.

Algae of the third response type (c) also exhibited higher MAA content after treatment with PAR or PAR + UVA. However, in contrast to type b, exposure to the full spectrum led to a strong decline in total

MAAs. Species of this type are the endemic *Neuroglossum ligulatum* and the cosmopolitan *Plocamium cartilagineum*. Under treatment with the full radiation spectrum, only traces of MAAs were found, which may either be explained by an almost complete degradation or decomposition of MAAs, or their possible leakage from the cells into the medium. However, the observation of thalli bleaching at the end of the UVR exposure experiment in these two species, indicates photodamage of the thallus. Additionally, this results in a decrease of MAA content per dry weight under the UVB treatment, whereas these plants show higher MAA concentrations under PAR/PAR+UVA conditions. Compared to *P. cartilagineum* from cold-temperate waters, the Antarctic isolate is extremely stenothermal, growing only at temperatures below 5°C and dying above 7°C (Bischoff-Bäsmann and Wiencke 1996), indicating the development of temperature ecotypes. The Antarctic *P. cartilagineum* seems to exhibit a high degree of physiological sensitivity to changing abiotic parameters, against which even the presence of MAAs does not provide full UV protection. Therefore, the presence of UV-absorbing compounds in *P. cartilagineum* and *N. ligulatum* is not sufficient to prevent bleaching of the tissue, suggesting that a MAA suite alone does not guarantee complete protection against UVR. However, even incomplete protection from UVR might reduce damage, so that any residual biological effects may be completely counteracted by other defenses, as suggested for phytoplankton species (Neale et al. 1998).

The MAA-containing species of this study belong to the two physiological groups II and III, classified by Hoyer et al. (2001). These algae also exhibited MAAs in the field (Karentz et al. 1991; McClintock and Karentz 1997). The generally lower MAA concentrations in this study may be due to the fact that cultured algae always show less MAAs due to the artificial and less extensive radiation climate (Carreto et al. 1990). The ten species which do not exhibit MAAs even after exposure to UVR belong to the first physiological group mentioned in Hoyer et al. (2001), including species such as *Hymenocladiopsis crustigena* and *Phycodrys austrogeorgica*, typically lacking MAAs in the field. These deep-water taxa are strongly shade adapted (Kirst and Wiencke 1995), and exhibit low photosynthetic light compensation and initial light-saturation points closely corresponding to the conditions of their habitat (Weykam et al. 1996). They are susceptible both to higher PAR and UVR and must live in a low radiation climate with little or no UVBR. This has also been demonstrated in the Arctic deep-water red algal species *Phycodrys rubens*. After transplantation from deeper to shallow water, strong photoinhibition and photobleaching occurred, suggesting that this plant is genetically adapted to a low-light environment and, hence, completely incapable of coping with higher ambient radiation (Karsten et al. 2001). In agreement with our data, these authors assumed that the lack of MAAs in *P. rubens* may be one reason for the observed response. Similarly, Bischof et al. (1998) found very strong sensitivity of photosynthesis to UVR, particularly in Antarctic deep-water species such as *Delesseria lancifolia* and *P. austrogeorgica*, both of which also lack MAAs (Table 1) shown by the present study. It may be possible that the presence or absence of MAA biosynthesis in Antarctic species may also be determined on a genetic level, and can be explained as adaptation to an almost UVB-free environment and to low light levels in general.

Six known MAA compounds (shinorine, porphyra-334, palythine, asterina-330, palythinol, usujirene) were detected in the Antarctic species studied here. The induction of individual MAAs is dependent on spectral radiation composition and intensity, resulting in species-specific induction patterns. The quantitatively dominant MAAs synthesized during the experiments were shinorine, porphyra-334 and palythine. The MAA composition differs across species, depending on the physiological and genetic characteristics of the individual algae. Cultured *P. endiviifolium* and *N. ligulatum* exhibited porphyra-334 and shinorine, whereas in field-collected samples palythine was also detected. The absence of some MAAs in cultured samples is a common observation, demonstrating that the artificial irradiance might not be efficient or natural enough to induce the whole MAA inventory. These data also indicate that red macroalgae must have highly specific trigger systems, which are capable of sensing solar radiation (dose, spectral composition, etc.) and which lead to a particular set of MAAs.

All data presented suggest that the induction, formation and accumulation of individual MAAs is a physiologically very flexible as well as species-specific process. The underlying mechanism seems to include several enzymatic steps for biosynthesis and interconversion depending on various environmental (radiation climate, water turbidity), ecological (growth habitat), physiological (enzymatic activity/regulation) and genetic factors (missing and/or silenced genes). Although experimental evidence for a particular trigger mechanism, as well as details concerning the biosynthetic pathway of individual MAAs, is still missing, we agree with the proposal of Franklin et al. (1999) that a signal transduction pathway or even interactions among various photoreceptors must be involved in the overall process leading to high MAA concentrations. At least two different photoreceptors should be taken into consideration, due to the two types of MAA induction patterns: one for the PAR range and another for UVR sensing.

As MAA induction occurs in *Chondrus crispus* under blue light and UVA, a cryptochrome photoreceptor should be involved in the triggering of MAA biosynthesis (Franklin et al. 2001). Furthermore, Portwich and Garcia-Pichel (2000) suggested reduced pterin as a UVB photoreceptor chromophore in the MAA metabolism of the cyanobacterium *Chlorogloeopsis* sp. PCC. Other mechanisms such as a redox reaction might be involved in blue-light-induced gene expression as well (Lin 2000).

As known from higher plant photobiology it seems that individual photoreceptors play unique roles in physiological regulation processes and that certain gene products are shared by light activation pathways initiated by different photoreceptors (Petridou et al. 1997 and references therein).

Although the trigger mechanism and the photoreceptor still have to be elucidated, it has been demonstrated that different radiation conditions lead to three different response types based on MAA formation. The MAA biosynthesis in mainly eu- and sublittoral red algae is a very flexible and species-specific biochemical pathway depending on irradiance, which may result in protection against harmful UVR as well as the production of other sunscreen substances important for life in the respective habitats.

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