Mycosporine-Like Amino Acids in Antarctic Sea Ice Algae, and Their Response to UVB Radiation

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Mycosporine-like amino acids (MAAs) were detected in low concentration in sea ice algae growing in situ at Cape Evans, Antarctica. Four areas of sea ice were covered with plastics of different UV absorption exposing the bottom-ice algal community to a range of UV doses for a period of 15 days. Algae were exposed to visible radiation only; visible + UV radiation; and visible + enhanced UV radiation. MAA content per cell at the start of the experiment was low in snow-covered plots but higher in samples from ice with no snow cover. During the study period, the MAA content per cell reduced in all treatments, but the rate of this decline was less under both ambient UV and visible radiation than under snow covered plots. While low doses of UVB radiation may have stimulated some MAA production (or at least slowed its loss), relatively high doses of UVB radiation resulted in almost complete loss of MAAs from ice algal cells. Despite this reduction in MAA content per cell, the diatoms in all samples grew well, and there was no discernible effect on viability. This suggests that MAAs may play a minor role as photoprotectants in sea ice algae. The unique structure of the bottom ice algal community may provide a self-shading effect such that algal cells closest to the surface of the ice contain more MAAs than those below them and confer a degree of protection on the community as a whole.

Introduction

The global increase in UVB radiation resulting from ozone depletion in the stratosphere is now well documented (Madronich et al., 1998). UVB radiation (280–320 nm) readily damages many of the organic molecules of living organisms, and is especially harmful to the components of the photosynthetic system within plant cells (Jordan 1996). In the Antarctic, ozone induced increases in UVB may be 50–100% higher than maximal values otherwise expected (Frederick and Lubin 1994). Some parts of the Antarctic ecosystem therefore may be particularly vulnerable to these changes, perhaps none more so than the sea ice algae, which may contribute between 10–50% of the primary production in some regions of the Antarctic during spring (Skerratt et al., 1998). The algae growing on the bottom of annual sea ice in Antarctica are primarily diatoms. The community is restricted to 10–15 species, and in the McMurdo Sound area, is dominated by Entomoneis kijellmannii, Berkeleya adeliense, and Nitzschia stellata (McMinn, 1998). Between late October to mid November, this algal community presents an ideal experimental system to examine the effects of UVB on microalgal productivity because several important environmental factors coincide. Firstly, during this period the ice is usually relatively transparent to UVB (Trodahl and Buckley, 1990; Ryan, 1992, 1994); secondly, ambient levels of UVB are unusually high due to springtime ozone depletion; finally, these organisms are trapped in the ice and unlike the phytoplankton of the open ocean, are unable to avoid the incident radiation dose.

The role of MAAs in UV photoprotection in algae is not as clearly defined as with the flavonoids of higher plants. While there have been a number of studies that indicate that production of MAAs is induced by increased levels of UV radiation in marine organisms (see Hannach and Sigleo 1998, for review), not all marine phytoplankton re-

Abbreviations: MAA, Mycosporine-like amino acids; PAR, Photosynthetically active radiation; UVA, Ultraviolet-A radiation; UVB, Ultraviolet-B radiation.
respond to UVR in this way. In particular, Jeffrey et al. (1999) noted that whereas most algal classes have representatives with both low and high UV absorptive capacity, most diatoms are on the low end of this scale. This indicates generally low levels of MAAs in these algae. Riegger and Robinson (1997) observed that the wavelengths most effective in inducing MAA production in diatoms are between 350–470 nm rather than in the UVB (280–320 nm). They therefore suggest that it is unlikely that sufficient MAAs would be induced during an increase in UVB resulting from a reduction in atmospheric ozone. Inducibility of MAAs in diatoms may be related to cell size (Riegger and Robinson, 1997), so that smaller diatoms gain no sun screening benefit (García-Pichel, 1994) from accumulation of MAAs and tend not to accumulate them.

Most studies examining the effect of UV radiation have been from cultures of phytoplankton samples in vitro. With ice algae, experiments have involved removal of bottom ice algal samples from the ice and subjecting them to melting, exposure to ambient air at temperatures much lower than that of sea water, culture at elevated temperatures etc. All of these treatments are likely to modify the response of the organism to the applied stress, perhaps in unexpected ways. For example, Roos and Vincent (1998) showed that cultures of sub-Arctic cyanobacteria grown at close to their natural temperature conditions (0–5°C) were more susceptible to UVB than those grown at 20°C. It should also be recognised that it is very difficult to provide an artificial source of radiation that matches solar UV and visible radiation. Queseda et al. (1995) have suggested that the balance between PAR/UVA/UVB may play a crucial role in the efficiency of damage/repair mechanisms and therefore laboratory experiments using artificial sources may not accurately reflect physiological effects of natural UVB exposure.

Methods

The study area was on sea ice at Cape Evans, Ross Island, Antarctica (77°38′S, 166°24′E). The weather during the study period (28 Oct. and 12 Nov. 1999) was generally overcast and the ice had a thin 1 cm cover of snow. Four adjacent areas of sea ice 4.2 × 8.4 m were covered with plastics of different UV transmissions to provide experimental plots of different UV treatments as described in Ryan et al. (submitted). The treatment regions were: photosynthetically active radiation (PAR) only, PAR + UVA; PAR + UVA + UVB; and an enhanced UVB region. The plastics used for the first three regions were polycarbonate, mylar, and cellulose triacetate respectively, and all reduced the transmission of PAR by approximately 10%. For this reason cellulose triacetate was also used to cover the UVB enhanced region. The lamps in the latter region provided enhanced UVB radiation immediately beneath the lamps, and a progressive reduction in intensity displaced horizontally. An additional area of ice approximately 200 m from this site was also used for some samples. This area similarly had no platelet ice but was covered with 5–10 cm of snow.

A Biospherical Instruments Inc (San Diego, USA) PUV500 submersible multichannel radiometer was deployed under the ice under each treatment region. The instrument recorded 4 bands in the UV centred on 305 nm, 320 nm, 340 nm, and 380 nm, as well as PAR, depth and temperature. The surface irradiance was recorded both before and after the under-ice irradiance measurements. Since ambient solar radiation changes constantly through the day, levels of under-ice irradiance are recorded here as percentages of that at the surface. The enhanced UVB treatment was about 7–10× the level of UVB in adjacent areas, but was still only 2% of that on the surface. This level is considerably less than ambient radiation we have previously observed in this area at this time of the year (Ryan and Beaglehole 1994), and is therefore ecologically relevant.

Five samples for chlorophyll-α and MAA analysis were taken from the edge of the treatment block before laying the plastics. Three replicate samples were taken from the PAR, PAR+UVA, and PAR+UVA+UVB plots approximately every five to seven days as weather permitted. However, samples were not taken during the experiment in the enhanced UVB plot because removal of samples would have interfered with the radiation field. Final samples were taken from all 4 plots as the plastics were removed at the end of the experiment. Samples were obtained by drilling with a powered auger to within 0.5 m of the bottom of the ice, and then a 150 mm core sample was re-
moved using a SIPRE corer. Cores were taken progressively down the central long axis of each experimental plot after removing part of the plastic cover. Three cores were taken at each sampling time, and after sampling, ice shavings were replaced into each hole to minimise disruption to the radiation field at the underside of the ice, and the plastic filter replaced. At each sampling time the three duplicate samples holes were drilled adjacent to each other, while subsequent sets of holes were taken 1.5–2 m further away. The cores were immediately enclosed in a black PVC bag for transport to the laboratory. Sub sampling occurred as soon as possible after the cores were taken.

High performance liquid chromatography (HPLC)

The lower 10 cm of the ice cores were melted and a 10 ml sample was extracted in methanol (MeOH) and stored at −20 °C and returned to New Zealand for subsequent analysis. A 5.0 ml aliquot from each sample was evaporated to dryness at room temperature under a stream of nitrogen and re-dissolved in 250 µl of methanol. The resultant solutions were analysed by reverse-phase HPLC using a modified method based on that described by Dunlap and Chalker (1986). Chromatography was carried out on a Philips Lichrosorb RP-8 column (250 × 5 mm ID; 5 µm) using a Phenomenex RP8 security guard cartridge (4 × 2 mm ID), a Waters (Milford, MA, USA) 600E solvent controller, a Waters 996 photodiode array detector, a Jasco (Tokyo, Japan) 851-AS intelligent sampler, and Millennium32 software. Injection volumes were 5 µl and elution was performed using a flow rate of 0.6 ml min⁻¹ at 30 °C. An isocratic mobile phase was used consisting of 0.1% acetic acid and 20% methanol in water. Spectral data for all peaks were accumulated in the range 250–400 nm, and chromatograms were plotted at 310, 330 and 360 nm. Due to the lack of observed MAA peaks and the potential for the injection of 5 µl of methanolic extract to obscure small peaks, the remaining volume (ca. 210 µl) was again evaporated to dryness. The samples were re-suspended in a solvent similar to the mobile phase by the addition of 40 µl methanol followed by 160 µl water containing 0.13% acetic acid. The resultant solutions were filtered through glass wool and injected onto the HPLC under the same conditions as above except an injection volume of 20 µl was employed. MAA peaks observed were identified based on comparison of their online absorption spectra and retention times with those of authentic standards. Approximate quantification was based on comparison of HPLC peak areas for identified MAA peaks at 330 nm with those of the authentic secondary standards. It was also confirmed that evaporation and resuspension of the authentic standards in either MeOH or the mobile phase had no significant impact on observed peak areas at 330 nm. The standards used were porphyra-334, shinorine, mycosporine-glycine, mycosporine-2-glycine, mycosporine-taurine, asterina-330, palythene, palythine, and palythinol. S. Newman kindly supplied these standards.

Statistical analysis

It was noted that, for each of the variates, the variance was not constant across the treatments. The slope of the plot of the log of the standard deviation against the log of the mean was used to determine the best transformation that could be applied to each variate to stabilise the variance, choosing from logarithm, and powers of 1, 1/2, −1/2 and −1. For each variate the logarithmic transformation was indicated and the transformed variates were then submitted to one-way analysis of variance. In each case, a quantile-quantile plot of the residuals indicated that the distribution deviated only mildly from the normal distribution. Hence the main assumptions of the analysis of variance were found to hold to a good approximation. The mean of each logarithmic variate and the standard error of the mean are given in Table I for each treatment. Statistical comparisons were made between each treatment using the Students t distribution.

Results

This study was conducted in a remote field camp 200 m off the coast at Cape Evans. The site was chosen firstly because the ice had been snow free for a period of over 2 weeks prior to our study, and secondly, because platelet ice was absent from the lower surface of the sea ice. Platelet ice is difficult to recover using coring methods and its presence would compromise biomass measurements. The lack of snow cover over a wide area around
our site (both prior to and during the study) ensured uniform PAR and UV radiation penetrating to the algae growing on the lower surface. This allowed two assumptions to be made. We assumed that the algae under study had already acclimated to the current radiation environment. We also assumed that the algal distribution under the ice was uniform both in density and in species composition. Over 120 m² of sea ice was covered with plastics in these experiments, and practical and environmental considerations precluded the use of replicate blocks in this remote and extreme field site.

During the period of the study (late October / early November 1999), the field site was exposed to continuous sunlight, with a maximum solar irradiance at solar noon and a minimum at midnight. Cloud cover considerably affected irradiances for much of the study period, although some clear days were recorded. Under ice irradiance measurements verified that the filters were effective in supplying the appropriate radiation regime (Ryan et al., 2001, submitted). For example, the highest level of UVB recorded under the cellulose acetate filter (PAR+UVB+UVA treatment) was 0.3% of the surface irradiance, while no UV was recorded under the polycarbonate filter (PAR treatment).

Methanolic extracts of sea ice algal samples had relatively low levels of absorbance in the UV (Fig. 1), although a small peak at 340 nm was observed, which may be due to MAAs. All samples were subsequently analysed by HPLC. Considerable sample processing was required to reveal very low levels of MAAs in the samples and most of these were consistent with porphyra-334. There was quite high variability between samples with the same treatment. In particular there was no significant difference between the PAR+UVA, and the PAR+UVA+UVB samples, and therefore these results were pooled. Riegger and Robinson (1997) noted that UV radiation in the UVA part of the spectrum was the most effective in inducing MAA production and therefore pooling data from PAR + UVA and PAR + UVA + UVB treatments is appropriate.

Changes in the concentration of MAA.m⁻² for the various treatment blocks are given in Table I. At the beginning of the study, the concentration of MAAs was lower below the snow-covered regions than below the clear ice although this difference was not statistically significant. The amount of snow cover had not changed for several weeks.

Table I. Results from ANOVA for MAA.m⁻², MAA.Chl-a⁻¹, and MAA.cell⁻¹. The data under the anova heading is log₁₀ of the mean with the standard error of the mean in brackets. The last row gives data for enhanced UVB treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>n</th>
<th>mean</th>
<th>anova</th>
<th>n</th>
<th>mean</th>
<th>anova</th>
<th>n</th>
<th>mean</th>
<th>anova</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snow</td>
<td>1</td>
<td>5</td>
<td>0.44</td>
<td>–0.36 (0.15)</td>
<td>2</td>
<td>0.0037</td>
<td>–2.43 (0.21)</td>
<td>5</td>
<td>115.9</td>
<td>2.06 (0.16)</td>
</tr>
<tr>
<td>Snow</td>
<td>7</td>
<td>5</td>
<td>0.02</td>
<td>–1.76 (0.15)</td>
<td>2</td>
<td>0.0001</td>
<td>–4.33 (0.21)</td>
<td>5</td>
<td>4.2</td>
<td>0.62 (0.16)</td>
</tr>
<tr>
<td>Snow</td>
<td>12</td>
<td>3</td>
<td>0.02</td>
<td>–1.66 (0.19)</td>
<td>3</td>
<td>0.0001</td>
<td>–3.89 (0.17)</td>
<td>3</td>
<td>3.6</td>
<td>0.56 (0.20)</td>
</tr>
<tr>
<td>Initial ambient</td>
<td>1</td>
<td>5</td>
<td>0.61</td>
<td>–0.21 (0.15)</td>
<td>4</td>
<td>0.0212</td>
<td>–1.67 (0.15)</td>
<td>4</td>
<td>511.7</td>
<td>2.71 (0.18)</td>
</tr>
<tr>
<td>PAR</td>
<td>7</td>
<td>3</td>
<td>0.27</td>
<td>–0.57 (0.19)</td>
<td>3</td>
<td>0.0026</td>
<td>–2.59 (0.17)</td>
<td>3</td>
<td>55.7</td>
<td>1.75 (0.20)</td>
</tr>
<tr>
<td>PAR</td>
<td>16</td>
<td>5</td>
<td>0.64</td>
<td>–0.19 (0.15)</td>
<td>4</td>
<td>0.0045</td>
<td>–2.35 (0.15)</td>
<td>5</td>
<td>66.1</td>
<td>1.82 (0.16)</td>
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<tr>
<td>PAR+UV</td>
<td>7</td>
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<td>0.76</td>
<td>–0.12 (0.14)</td>
<td>6</td>
<td>0.0067</td>
<td>–2.18 (0.12)</td>
<td>5</td>
<td>157.0</td>
<td>2.20 (0.16)</td>
</tr>
<tr>
<td>PAR+UV</td>
<td>16</td>
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<td>0.83</td>
<td>–0.08 (0.10)</td>
<td>9</td>
<td>0.0058</td>
<td>–2.23 (0.10)</td>
<td>9</td>
<td>167.1</td>
<td>2.22 (0.12)</td>
</tr>
<tr>
<td>PAR+ enh UV</td>
<td>12</td>
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<td>0.17</td>
<td>–0.76 (0.15)</td>
<td>5</td>
<td>0.0019</td>
<td>–2.72 (0.12)</td>
<td>5</td>
<td>20.1</td>
<td>1.30 (0.168)</td>
</tr>
</tbody>
</table>

Fig. 1. Absorbance of a raw methanol extract of an ice algal sample. The small peak at 340 nm is possibly due to MAAs. The wavelengths of three other peaks are also indicated.
prior to our experiments, and our initial measurements therefore reflect algae that had acclimated to these light levels. Over the period of the experiment the MAAs concentration dropped to almost zero in the snow-covered region, with some samples having no detectable MAAs. By day 7 the MAA concentration in this region was significantly different (p<0.01) from the other two treatments (Table I). In contrast, in those samples receiving higher levels of PAR and UV, the concentration of MAA.m⁻² remained at the same level throughout the study period. The MAA.m⁻² concentration in samples subjected to the highest UVB dose (ie the enhanced UVB block) dropped unexpectedly to low levels, even though the UVB enhancement was well within ambient levels previously recorded.

During the period of this study, both the chl-a concentration and the number of cells per m⁻² increased (Ryan et al., submitted). When these influences are included in the data, MAA content per unit of Chl-a or per cell declined for all samples (Table I). The trends and statistical significances were very similar for both parameters. The initial points at day 1 for the snow-covered and the clear blocks were significantly different (p<0.01) for both parameters (Table I), and both parameters declined to very low levels after 7 days in the snow-covered samples. The initial level of MAA per cell in algae acclimated to higher levels of PAR and UV was significantly higher than in the snow-covered samples. In samples subsequently exposed to treatments lacking any UV radiation, there was a rapid decline in the concentration of MAAs per cell to 10% of the initial levels within 7 days. While samples exposed to ambient levels of UV also experienced a reduction in MAA content per cell, the loss after 7 days was much less at only 50%. In both of these cases, the concentrations reached after 7 days were maintained for the remainder of the experiment.

Samples treated with enhanced levels of UVB had very low levels of MAA per cell after about 2 weeks of irradiation. The time course for this decline could not be determined, as sampling the region being irradiated would have modified the radiation field.

Discussion

The assumption that MAAs function as a sun screen in phytoplankton cells relies on their relatively high absorption of UVB wavelengths, their apparent photoinducibility (Carreto et al., 1990; Garcia-Pichel et al., 1993; Neale et al., 1998), and their photophysical properties (Conde et al., 2000). MAAs were found in very low concentration in our sea ice algal samples with the most prevalent compound detected being porphyra-334. This compound was also the most abundant MAA in ice algal communities near Palmer Station (Karountz 1994), and in phytoplankton assemblages (Villafane et al., 1995). The levels of MAAs observed in our diatom dominated bottom ice community were more than an order of magnitude lower than those observed in phytoplankton (Villafane et al., 1995). Our diatom dominated samples consequently had much lower absorbance in the UV (Fig. 1) than that reported by Villafane et al. (1995), and this supports an observation by Jeffrey et al. (1999) that diatoms tend to have low UV absorptive capacity. If MAAs play a role in the protection of phytoplankton cells from UVB, then low levels of MAAs may reflect a low level of protection from UVB induced damage in these sea ice algal samples. However, despite the low MAA content found in this study, all algae grew well and there was no discernible effect on viability. This suggests that MAAs may play a minor role as photoprotectants in sea ice algae.

Queseda et al. (1998) found that the concentration of MAAs in cyanobacterial mats exposed to partitioned natural radiation regimes similar to those employed here did not change over the 11 days of their treatment. Longer term observations of Antarctic macro algae also showed little change in MAA over time (Queseda et al., 1998). Similarly, the concentration of UVB absorbing pigments did not change significantly in five diatom species exposed to increasing UVB irradiances by Davidson et al. (1994), despite the fact that each species appeared to be capable of surviving high UVB doses. Similarly, the concentration of MAA per cell reported here was little affected by ambient levels of UVB, while enhanced UVB actually caused a greater reduction in MAA loading per cell. These results therefore support the conclusion of Davidson et al. (1994) that diatom survival
under elevated UVB irradiance results from processes other than screening mechanisms.

The algae from the snow-covered plots had very low initial levels of MAAs per cell, which declined to almost zero over the course of the study. Furthermore, these concentrations were significantly lower than in those from the clear ice regions. This low MAA concentration in the snow-covered samples may have resulted from these samples having a greater proportion of UVB sensitive species (Ryan et al., 2001, submitted). The low level of MAAs in the samples from under snow-covered regions may be due to an inability of the UV sensitive species to produce MAAs in response to UV radiation (Neale et al., 1998; Hannach and Sigleo, 1998). Alternatively, the lower MAA concentration in these samples may reflect a low transmission of visible and UV radiation due to the highly scattering nature of the snow cover. Under these low light conditions, reduced MAA photoinduction is likely.

It is possible that MAAs function as a sunscreen by acting at the community level rather than at the cellular level. The ice algal community presents a different structure to most marine phytoplankton because they are fixed in the ice, rather than free in the open ocean. In the sea ice algal community, those algae closest to the upper surface of the ice bear the highest burden of UV protection for all the algae in the ice. Initial levels of MAAs per cell were relatively high in our samples, but as the community grew, the net concentration of MAAs per cell reduced (Table I). However, the concentration per unit area did not alter in samples exposed to ambient levels of UV (Table I) despite the observation that the bottom ice algal layer grew in thickness from 2–5 mm to 20–30 mm over the period of the study. This may be because as new algal cells grew, those above already provided them with protection from UVB, and there was no need for further production of screening compounds. As a result, some photoprotection may ensue despite a reduction over time in the cellular concentration of MAAs within the whole ice algal community. However, we did not examine our samples for evidence of such stratification. Similar self-shading from UV has been suggested for Antarctic cyanobacterial mats (Vincent and Queseda, 1994).

Acknowledgements

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