ABSTRACT

Marine phytoplankton are sensitive to inhibition of photosynthesis by solar ultraviolet (UV) radiation, although sensitivity varies, depending on the growth environment. A mechanism suggested to increase resistance to UV inhibition is the accumulation of UV-absorbing compounds, such as the mycosporine-like amino acids (MAAs) found in many marine organisms. However, the effectiveness of these compounds as direct optical screens in microorganisms has remained unclear. The red-tide dinoflagellate Gymnodinium sanguineum Hirasaki accumulates about 14-fold more MAAs (per unit of chlorophyll) in high (76 W m\(^{-2}\)) than in low (15 W m\(^{-2}\)) growth irradiance. Biological weighting functions were estimated for UV inhibition of photosynthesis and showed that the high-light-grown cultures have lower sensitivity to UV radiation at wavelengths strongly absorbed by the MAAs. The time course of photosynthesis during exposure to UV radiation was measured using pulsed amplitude modulated (PAM) fluorometry and displayed a steady-state level after 15 min of exposure, indicating active repair of damage to the photosynthetic apparatus. Repair was blocked in the presence of the antibiotic streptomycin, yet high-light G. sanguineum remained less sensitive to UV radiation than did low-light cultures. These experiments show that MAAs act as spectrally specific UV screens in phytoplankton.

Key index words: biological weighting function; dinoflagellate; fluorescence; photoinhibition; photoprotection; photosystem II; quantum yield

Abbreviations: BWF, biological weighting function; chl, chlorophyll a; P-I, photosynthesis vs. irradiance; PSII, photosystem II

Solar ultraviolet (UV, 290–400 nm) radiation affects phytoplankton growth and survival in near-surface waters by the inhibition of photosynthesis (Smith et al. 1980, Helbling et al. 1992, Smith et al. 1992), damage to DNA (Karentz et al. 1991a, Buma et al. 1995), and effects on other processes (Holm-Hansen et al. 1993). Concern about stratospheric ozone depletion and the associated enhancement of middle ultraviolet (UVB, 290–320 nm) has motivated spectral assessments of UV inhibition of photosynthesis to distinguish effects by UVB compared to the near ultraviolet (UVA, 320–400 nm), which is unaffected by ozone depletion (Cullen et al. 1992, Lubin et al. 1992, Neale et al. 1994, Boucher and Prézelin 1996a, Neale et al. 1998a). Equally important, although less well understood, are protective mechanisms by which phytoplankton offset the negative effects of UV exposure (Vincent and Roy 1993). These mechanisms include protective processes that decrease the biological effectiveness of UV exposure and counteract processes, such as repair, reactivation, and protein turnover, that restore functions lost because of UV damage. Understanding such mechanisms is critical to assessing the long-term effect of changes in incident solar UV radiation on ecosystem processes (Bothwell et al. 1993).

One possible mechanism of increased protection from UV radiation is the accumulation of UV-absorbing compounds, or putative “screens” (Vincent and Roy 1993). The mycosporine-like amino acids (MAAs) are a class of about a dozen related UV-absorbing compounds that are widespread in marine organisms (Carreto et al. 1990a, Karentz et al. 1991b, Shick et al. 1992, Banaszak and Trench 1995, Dunlap and Shick 1998). MAAs have sharp (ca. 20-nm-bandwidth) absorption peaks varying between 300 and 360 nm (Carreto et al. 1990a, Karentz et al. 1991b, Shick et al. 1992, Stochaj et al. 1994). Accumulation of MAAs is correlated with exposure to UV radiation in many marine organisms, so their protective function has been suggested for some time (Shibata 1969, Yentsch and Yentsch 1982, Dunlap et al. 1986, Vernet et al. 1989, Carreto et al. 1990a, Vernet et al. 1994, Banaszak and Trench 1995, Helbling et al. 1996). However, the efficiency and mode of protection of MAAs is not well understood. Some studies have suggested that the presence of MAAs does not necessarily result in photoprotection. The colonial form of an Antarctic prymnesiophyte, Phaeocystis antarctica, accumulates MAAs; however, growth of this alga has been reported to be sensitive to UVB (Karentz 1994, Karentz and Spero 1995; see contrasting results of Davidson et al. 1996). Moreover, some species of Antarctic diatoms do not accumulate MAAs yet survive UVB exposure better than P. antarctica (Davidson et al. 1994, Davidson and Marchant 1994). Optical models indicate that MAAs might not be an effective sunscreen over the short path lengths characteristic of phytoplankton cells (Garcia-Pichel 1994). Previous attempts to directly measure a “sunscreen factor” for MAAs have revealed only small (around 10%) increases in spectral filtering (Garcia-Pichel et al. 1993) and no significant decreases in UV biological weight (Lesser 1996a, b). MAAs do...
protect against UV-induced developmental delays in the eggs of laboratory-reared green sea urchins, supporting a direct photoprotective role (Adams and Shick 1996). However, these authors also recognized that other mechanisms of MAA action might have provided protection (i.e. through antioxidant activity) (Dunlap and Yamamoto 1995).

Our experimental objective was to determine whether MAAs act as a direct, spectral screen against solar UV exposure in marine microorganisms. We quantitated the protective function of MAAs in a marine dinoflagellate as the increased resistance to inhibition of photosynthesis by UV radiation in the wavelength band absorbed by the MAAs. The spectral dependence of inhibition was described by a biological weighting function, or BWF (Cullen and Neale 1997). A BWF is similar to an action spectrum, the difference being that it is inferred from polychromatic exposures, as is appropriate for processes, such as photosynthesis, that respond to multiple wavebands (Cooihill 1991). Previous work has shown that photosynthesis as a function of UV and photosynthetically available radiation (PAR, 400-700 nm) is well described by a model, the BWF/P-I model, which combines the BWF with a saturating function of PAR irradiance (Cullen et al. 1992, Neale et al. 1998a). We examined the responses of Gymnodinium sanguineum Hirasaka, which can form extensive surface blooms (“red tides”) in marine coastal and estuarine waters, such as the Chesapeake Bay.

MATERIALS AND METHODS

Culture growth conditions. We used a clone of G. sanguineum, isolated by D. Wayne Coats from the Rhode River, a subestuary of the Chesapeake Bay, and grown under low light (LL) and high light (HL). Cultures were grown under cool-white fluorescent lights on a 14:10 h LD cycle at 25° C. The growth medium was a standard “f/2” enrichment of filtered seawater from the Gulf of Maine, supplemented with vitamins and trace elements (Cullen and Weidemann 1993). Chlorophyll a content (chl) of the culture (1 mL) was measured fluorometrically on aliquots concentrated on glass fiber filters (335, 345, and 360) or a GG400 filter that excludes all UV, and was corrected for path-length amplification as described (Cleve-...

\[
P^w = P^b \tan\left(\left(\frac{E(\lambda)}{E_b}\right)^{1/2} + 1\right)
\]

where \(P^w\) is photosynthesis normalized to chl a content (\(\text{g} \cdot \text{chl} \cdot \text{g}^{-1} \cdot \text{h}^{-1}\)), \(P^b\) is the saturated rate of photosynthesis in the absence of inhibition, and \(E_b\) is a saturation parameter for PAR irradiance (\(\text{W} \cdot \text{m}^{-2}\)). A dimensionless inhibition index, \(I^w\), is defined as

\[
I^w = \frac{\int_{\lambda}^{\lambda_f} \sum_{\lambda} v(\lambda) \cdot E(\lambda) \cdot d\lambda}{\int_{\lambda}^{\lambda_f} \sum_{\lambda} v(\lambda) \cdot E(\lambda) \cdot d\lambda}
\]

where \(v(\lambda)\) is biological weight (reciprocal mW-m\(^{-2}\)) at wavelength \(\lambda\) and \(E(\lambda)\) is spectral irradiance at \(\lambda (\text{mW-m}^{-2}\cdot\text{nm}^{-1})\). A detailed protocol described by Lewis and Smith (1985). Aliquots of culture (1 mL) were incubated in 7-mL scintillation vials under 37 light levels that were obtained by filtering irradiance from a 250-W halogen lamp with neutral density screens. Irradiance was measured with a quartz scalar sensor (QSL-100) mounted inside a scintillation vial. Quantum scalar irradiance (\(\mu\text{mol} \cdot \text{m}^{-2}\cdot\text{sec}^{-1}\)) was converted to \(E_{PAR}(\text{W} \cdot \text{m}^{-2})\) by application of a conversion factor (4.9 \(\mu\text{mol} \cdot \text{m}^{-2}\cdot\text{sec}^{-1}\)) determined from measured spectral irradiance. Photosynthetic parameters \(P^w\) and \(E_b\) were estimated by fitting the hyperbolic tangent curve as in Equation 1 (i.e. with \(E_{b0} = 0\)).

Cellular absorption and chlorophyll. Cells concentrated on glass fiber filters were scanned in a Cary 4 dual beam spectrophotometer, using a blank filter wetted with filtrate as a control. Spectra were corrected for path-length amplification as described (Cleveland and Weidemann 1983). Chlorophyll a concentration (chl) was measured fluorometrically on aliquots concentrated on glass fiber filters (Whatman GF/F) and extracted in 90% acetone at least 24 h in the dark at 4° C.

Mycosporine-like amino acids. For all samples, the extraction and analysis of MAAs were performed as described (Dunlap and Chalker 1986) with minor modifications. Briefly, approximately 50 mL of sample water were filtered onto GF/F filters and frozen (−70° C) until analysis. Filters were extracted overnight in 1 mL of 100% high-performance liquid chromatography (HPLC)–grade methanol at 4° C; the extracts were centrifuged, and the supernatant was used for MAA analysis. Individual MAAs were separated by reverse-phase, isocratic HPLC on a Brownlee RP-8 column (Spheri-5, 4.6 mm ID X 250 mm), which was protected with an RP-8 guard column (Spheri-5, 4.6 mm ID X 50 mm). The mobile phase consisted of 25% methanol (v/v), 0.1% glacial acetic acid (v/v) in water with a flow rate of 0.7 mL min\(^{-1}\). Detection of the peaks was carried out using a diode array, UV absorbance detector (Beckman Gold System). Standards were available for seven MAAs (mycosporine-glycine, shinorine, porphyra-334, pal- ythene, asterina-330, palythene, and palythene). Standards were originally isolated by Walter Dunlap and were kindly provided by Deneb Karentz and Michael Lesser. Identities of peaks were con-

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firmed by co-chromatography with standards and by the maximal wavelength of absorbance by on-line diode array spectroscopy. All MAA concentrations are expressed in nmol (nmol chl)−1.

Photochemical efficiency of photosystem II. A pulse amplitude modulated (PAM) fluorometer (Walz, Effeltrich, Germany) was used with a high-sensitivity detector as described (Schreiber 1994). One milliliter of culture was placed in a 1-cm-square quartz cuvette. The cuvette was illuminated from below using irradiance from a 150-W xenon lamp (Schoeffel) filtered through selected Schott long-pass filters. The fluorometer measures the steady-state in vivo chl fluorescence (Fv) of phytoplankton during illumination with actinic irradiance. At 1-min intervals a saturating flash (400-ms pulse duration) was applied to obtain a maximum yield (Fm). The relative efficiency of excitation energy capture by photosystem II (ΦPSII) is calculated as Fv/Fm, active fluorescence measurements of ΦPSII have been shown to be highly correlated with the overall efficiency (ΦII) and, thus, rate of photosynthesis (Genty et al. 1989). For each sample, there was an initial 10-min period without actinic illumination to measure maximum ΦPSII, followed by illumination with PAR only (GG-400 filter). During the initial few minutes of steady PAR illumination, ΦPSII, displayed short-term variations consistent with the activation of photosynthesis, but after 10 min, ΦPSII was nearly constant (Schreiber et al. 1986). Once steady-state ΦPSII was reached, kinetics of UV effects were observed through the decrease in ΦPSII upon supplementing the PAR illumination with UV. In some cases, a streptomycin solution in distilled water (final concentration 250 μg·mL−1 [Stein 1973]) was added after 10 min of PAR exposure, followed by another 10 min of PAR exposure before UV exposure began.

RESULTS AND DISCUSSION

The initial density of the G. sanguineum cultures after biweekly transfer was 100 cells·mL−1. Experiments were performed 11 days after transfer for HL cultures and 12 days after transfer for LL cultures (Table 1). Gymnodinium sanguineum grew 57% faster in HL compared to LL, and cell density of samples used for photosynthesis measurements was three times higher in HL. In contrast, the chl concentration was slightly higher in the LL cultures because of the much higher cellular chl content (Table 1). Growth in LL was limited by light, and G. sanguineum acclimated through the accumulation of photosynthetic pigments (Richardson et al. 1983, Falkowski and LaRoche 1991).

Photosynthesis. Estimates for the parameters of PAR-dependent photosynthesis were obtained by fitting Equation 1 to observations of carbon assimilation in the photosynthetron (halogen lamp) and during exposure to the xenon lamp (an example data set is shown in Fig. 1). Analysis of the photosynthetron results showed that E was significantly (ca. 30%) lower in LL cultures. Also, Fm was slightly (ca. 6%) higher in LL cultures (Table 2), but the difference was not very significant (P > 0.05). Analy-
was measured to be 24.6° C ± 0.3° C in each case.

The values of $E_s$ were lower under halogen lamp exposure than in HL cultures grown in high light (HL, five trials) and low light (LL, four trials). Mean ± standard error of estimates for the saturated rate of photosynthesis in absence of photoinhibition ($P_{\text{max}}^h$, mg C [mg chl]^{-1} h^{-1}) and light saturation parameter ($E_s$, W m^{-2}). Estimates were obtained using the photosynthetron data fitted to a hyperbolic tangent curve ($n = 37$ per curve) and the photoinhibitron data fitted to the BWF/P-I model ($n = 72$ per curve). Further details on measurement conditions and parameter estimation are given in Materials and Methods.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>HL</th>
<th>LL</th>
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<tbody>
<tr>
<td>$P_{\text{max}}^h$ (mg C [mg chl]^{-1} h^{-1})</td>
<td>6.47 ± 0.16</td>
<td>6.83 ± 0.11</td>
</tr>
<tr>
<td>$E_s$ (W m^{-2})</td>
<td>113 ± 8</td>
<td>78 ± 4</td>
</tr>
</tbody>
</table>

### Fig. 2

Average spectral absorbance ($a_{380}$, m² mg chl^{-1}) by *G. sanguineum* cultures for high-growth irradiance (HL, solid line, $n = 5$) and low-growth irradiance (LL, dashed line, $n = 4$). The maximum $y$ for the UV portion of the spectrum (left scale) is 10 times greater scale than the visible (right scale). The prominent peak in the 320–360-nm region is due to MAA absorbance.

Analysis of cell extracts revealed that the increased absorbance was due to the presence of MAAs, which were found in about 14-fold higher concentration in the HL cultures than in the LL cultures (Table 3). High concentrations of UV-absorbing compounds have been observed in several species of bloom-forming dinoflagellates that, by accumulating in surface waters, are exposed to HL conditions (Yentsch and Yentsch 1982, Balch and Hazo 1984, Vernet et al. 1989, Carreto et al. 1990b). Carreto et al. (1989) showed that transferring cultures of the red-tide dinoflagellates *Alexandrium excavatum* and *Procentrum micans* from low (20 µmol quanta m^{-2} s^{-1}) to high (250 µmol quanta m^{-2} s^{-1}) light intensity resulted in a rapid increase in the content of UV-absorbing compounds as determined by the ratio of 365:672 nm absorbance. This process was reversible in both species. Sev-
en different MAAs were later identified (Carreto et al. 1990b) in A. excavatum, four of which are found in G. sanguineum under our culture conditions (Table 3). Prorocentrum micans in culture also contained a complex of seven MAAs (Carreto, unpubl., in Carreto et al. 1990), whereas Lesser (1996a) reported four MAAs in the same species. Amphidinium carterae differs from other species of dinoflagellates in not accumulating high amounts of MAAs even under UV exposure (Hannach and Sigleo 1998). The only other dinoflagellate in culture with identified MAAs to date is Symbiodinium microadriaticum, the symbiont of Cassiopeia xamachana (Banaszak and Trench 1995). In laboratory experiments, greater concentrations of three MAAs (mycosporine-glycine, shinorine, and porphyra-334) were produced by S. microadriaticum in the presence of UV radiation and PAR than in the presence of PAR only (80 μmol quanta·m⁻²·s⁻¹) treatments. Whether high levels of PAR would induce the production of MAAs in the presence of UV radiation (Vilafañe et al. 1995, Riegger and Robinson 1997) or HL (UV and PAR) conditions (Helbling et al. 1996). Helbling et al. (1996) showed that two species of centric diatoms, Thalassiosira sp. and Corethron cryophilum, which had been growing in culture at 250 μmol quanta·m⁻²·s⁻¹, increased MAA concentration in response to exposure to natural solar radiation (ranging from 340 to 1320 μmol quanta·m⁻²·s⁻¹, depending on species and day of experiment). In contrast, two species of pennate diatoms, Pseudonitzschia sp. and Fragilariopsis cylindrus, which had much lower MAA concentrations compared to the centric diatoms, increased MAA production only in the presence of UV radiation. The bloom-forming pyrnesiochrome Phaeocystis pouchetii produces UV-absorbing compounds in response to exposure to UVB radiation and might provide UV protection for organisms present in the water column during a P. pouchetii bloom (Marchant et al. 1991).

Biological weighting functions. Four estimates of the BWF for inhibition of photosynthesis by UV radiation were obtained for each culture trial using from one to four spectral components as previously described (Neale et al. 1994, Cullen and Neale 1997). Increasing the number of components in the estimation of the BWF allows greater complexity of the spectral shape. However, additional components are accepted in the estimate only to the extent that they significantly increase variance explained by the BWF/P-I model (sequential F-test). For the LL cultures, two spectral components were sufficient to obtain maximum variance explained (R²); that is, no significant increase in R² occurred when a third component was added to the fit (mean F₁,₂₀ = 0.99, P > 0.25). Consistent with this result, there were no differences in the shapes of the BWFs for LL cultures estimated with two or three components. In contrast, incorporating a third component did increase R² for the HL cultures (mean F₁,₂₀ = 9.99, P < 0.05), indicating an inherently more complex shape. Incorporating a fourth component did not significantly increase R² in either the LL or the HL culture trials. Estimated weights were similar for the four trials conducted with LL-grown cultures (Fig. 3). In all

Table 3. Average concentration of mycosporine-like amino acids (MAAs, nmol·μmol chl a⁻¹) in G. sanguineum grown under high light (HL) and low light (LL). Mean of five replicate cultures grown under each regime, ± standard error. The ratio of the concentrations HL to LL is also given. Four MAAs were detected, and wavelengths of peak absorbance in methanol are given in parentheses.

<table>
<thead>
<tr>
<th>MAA</th>
<th>HL (nm)</th>
<th>LL (nm)</th>
<th>Ratio HL:LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycosporine-glycine</td>
<td>7.12 ± 0.57</td>
<td>0.66 ± 0.25</td>
<td>13.5</td>
</tr>
<tr>
<td>Palythine</td>
<td>0.53 ± 0.09</td>
<td>0.05 ± 0.02</td>
<td>6.2</td>
</tr>
<tr>
<td>Porphyra-334</td>
<td>9.10 ± 0.28</td>
<td>0.85 ± 0.10</td>
<td>13.3</td>
</tr>
<tr>
<td>Palythene</td>
<td>27.47 ± 0.90</td>
<td>27.47 ± 0.90</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Fig. 3. Biological weight for the inhibition of photosynthesis by UV (ε(λ)), reciprocal mW·m⁻² estimated by statistical analysis of data from LL cultures. Independent estimates from four trials are shown. The solid line shows the weights estimated using the LL data in Figure 1. The vertical bar shows the average 95% confidence interval as a proportion of the estimate over all four curves. The solid line is at the midpoint of the interval, which is arithmetically symmetrical around the estimate.
cases, there was significant weight for inhibition of photosynthesis by UV radiation ($\varepsilon(\lambda)$, reciprocal mW·m$^{-2}$) across the spectrum. Damaging potential decreased with an approximately exponential slope as a function of increasing wavelength. There was some variation in biological weight at the longer wavelengths; however, the variation between cultures was not significant.

The BWFs for the HL-grown cultures had more complicated shapes and were more variable between trials (Fig. 4). Weights were especially variable in the spectral range of 320–360 nm, in which the UV response was consistent with much lower biological weights compared to LL-grown cultures. In some cases, estimated weights were slightly positive and in others slightly negative. Negative weights occur when the beneficial effects of irradiance at a wavelength (i.e. as an energy source for photosynthesis or an inducer of repair) outweigh the damaging effects. However, the 95% confidence interval for all estimates in the 320–360-nm region overlapped zero, except for the BWF with the most positive $\varepsilon(\lambda)$, which had a 95% confidence interval overlapping zero in the 330–350-nm region.

The average BWF for HL and LL cultures over all trials for each culture regime is shown in Fig. 5. A 95% confidence interval for the mean weight was calculated by propagation of errors from the individual estimates (Bevington 1969). The greatest difference between HL and LL $\varepsilon(\lambda)$ is over the 325–355-nm range. Exposure to these wavelengths significantly inhibited photosynthesis in LL cultures; however, the $\varepsilon(\lambda)$ of HL cultures could not be statistically distinguished from zero. Outside this wavelength band (i.e. at both lower and higher wavelengths), the UV sensitivity of the HL culture approached that of the LL culture. The shape of the HL spectrum strongly supports the conclusion that MAAs provide direct photoprotection to the $G.$ sanguineum cells.

The greatest difference between the HL and the LL BWFs is for the 320–360-nm region (Fig. 6). The shape of the LL-HL difference spectrum is very similar to the wavelength band of largest enhancement of intracellular UV absorbance in the HL culture compared to the LL culture (Fig. 2). The decrease in sensitivity of the HL culture is especially dramatic for the spectral region in which solar radiation most inhibits photosynthesis in the LL culture (maximum of the product $\varepsilon_{\text{LL}}(\lambda) \cdot E(\lambda)$) (Fig. 6). This indicates that MAAs are specifically accumulated to provide spectral protection against solar UV.

**Repair.** Our results, showing that MAAs produce a spectrally specific feature in the BWF, are persuasive support for the hypothesis that MAAs act as potent UV “screens” in phytoplankton. The spectral difference between HL and LL BWFs strongly suggests that accumulation of MAAs accounts for the increased resistance to UV radiation acquired by HL cultures. However, organisms have multiple defenses against UV. Biological weighting functions (un-
like action spectra) are composite functions in which the weights include both the direct effect of a specific wavelength and interactive effects with other wavelengths (Coohill 1991). The interactive effect could be, for example, the induction of repair processes by UVA illumination (Hirosawa and Miyachi 1983, Greenberg et al. 1989). Thus, to better define the protective role of MAAs, we examined the extent to which HL cultures exhibited a greater resistance through processes that actively counteract UV damage during exposure. The presence of these opposing processes can be diagnosed from the time course of photosynthesis during UV treatment. A rapid decrease in photosynthetic rate, followed by stabilization of photosynthesis at a depressed but steady-state level, implies that damage is partially counteracted by ongoing repair of the target site(s) within the photosynthetic apparatus. We measured time courses of relative PSII efficiency ($\psi_{\text{PSII}}$) using active fluorometry, as previous studies have indicated that $\psi_{\text{PSII}}$ is closely coupled to the quantum yield of photosynthesis (Genty et al. 1989). Thus, $\psi_{\text{PSII}}$ should reflect damage and repair occurring anywhere in the photosynthetic apparatus, not only in PSII.

In time-series experiments with *G. sanguineum*, cultures were first exposed to a moderate level of PAR only (160 W-m$^{-2}$ of xenon irradiance filtered by a 400-nm long-pass cutoff filter), after which UV radiation was added, while PAR was maintained constant, by exchanging the filter with a 305-nm long-pass cutoff filter. Upon illumination with this broadband UV+PAR, there was a small drop in PSII efficiency in HL cultures, whereas PSII efficiency decreased but reached a lower steady-state level after about 15 min in LL cultures (Fig. 7). The lower PSII efficiency implies a lowering of photosynthetic rate. Indeed, the difference between HL and LL $\psi_{\text{PSII}}$ is consistent with the difference in photosynthesis by HL and LL cultures, as predicted by the BWF/P-I model under the time-series experiment conditions (Figs. 1, 3, 4) (Lesser et al. 1994). Agreement between changes in $\psi_{\text{PSII}}$ and predicted photosynthesis was also evident over a series of trials with both HL and LL cultures using a range of intensities and spectral treatments (data not shown). Steady-state $\psi_{\text{PSII}}$ after UV treatment for these trials ($n = 13$) was on average 71% of initial yield; application of the corresponding BWFs predicted that photosynthesis under UV exposure in the PAM would be 66% of the PAR-only control.

The rapid attainment of a steady state suggests...
that repair processes are partially counteracting UV damage and thus are an important factor in determining the overall response of dinoflagellates to UV. To determine the contribution of repair to the increased resistance observed in the HL cultures, cultures were treated with an inhibitor of chloroplast protein synthesis, streptomycin. This inhibitor limits repair capacity to the extent that damaged proteins cannot be restored to function through turnover processes (Samuelsson et al. 1985). Streptomycin was previously shown to enhance inhibition by UV radiation in a marine diatom (Lesser et al. 1994). After the addition of streptomycin, both HL and LL cultures were significantly more sensitive to UV radiation as evidenced by a greater decrease in PSII efficiency over the 45-min exposure (Fig. 7). Rates still approached a steady state; apparently, there was some residual repair capacity despite using the maximum recommended dose for algal cultures (250 µg·mL⁻¹ [Stein 1973]). The important point is that streptomycin treatment did not narrow the difference in sensitivity between HL and LL cultures. On the contrary, the difference in PSII efficiency between HL and LL cultures after a 45-min exposure is actually larger in the presence of streptomycin. These time series indicate that repair processes are active in both cultures, but the capacity for repair is not markedly augmented in HL cultures. We conclude that the increased resistance of the HL cultures to UV radiation is primarily due to enhanced photoprotection by MAAs.

Comparison with previous results. The sunscreen potential of MAAs has been frequently suggested on the basis of the correlative evidence that organisms (algae and invertebrates) with higher concentrations of the compounds appeared to be more resistant to UV radiation (Yentsch and Yentsch 1982, Dunlap et al. 1986, Vernet et al. 1989, Carreto et al. 1990a, Karentz et al. 1991b, Shick et al. 1992, Stochaj et al. 1994, Vernet et al. 1994, Banaszak and Trench 1995, Helbling et al. 1996), but little optical evidence was available to define the specific function of MAAs (Garcia-Pichel et al. 1993, Garcia-Pichel 1994). Our results provide optical evidence that MAAs are direct protectants in G. sanguineum and possibly in other MAA-accumulating phytoplankton with a similar cell size as G. sanguineum (mean diameter 44 µm in the HL culture). In the absence of data showing that protection was targeted to the spectral region of greatest MAA absorbance, previous studies could not determine which portion of the increased resistance was due to the MAAs as opposed to other photoprotective or repair processes that counteract UV damage that might be induced concomitantly with MAA accumulation. There are several reasons that our approach might have better distinguished the specific effect of MAAs. First, the MAA content of G. sanguineum varied simply as a function of PAR intensity. Supplementation of growth irradiance with UVR was not necessary. Such supplementation might confound comparative studies by inducing other effects besides MAA accumulation. Second, the reported BWFs are the average of four (LL) to five (HL) independent determinations; this enhanced the statistical power of the comparison. Third, photosynthesis was measured at high irradiance under which the consequences of differing BWFs are the most pronounced (Fig. 1).

A simple optical model (Garcia-Pichel 1994) suggested that MAAs are marginally efficient protectants in the size range of dinoflagellates. In most cases, organisms are found to have less than 1% dry mass of protectant. For cells with a 44-µm diameter, the optical model predicts that a 0.5%–1% investment in MAAs would screen 50%–70% of UV radiation. To obtain a more precise estimate of MAA optical protection in G. sanguineum, a sunscreen factor (S) was calculated directly from measured absorbance (Fig. 2) using the equations of Garcia-Pichel (1994). We estimated an S of 0.8 for HL cultures, assuming that the background absorbance was equivalent to the LL cells. A similar S was obtained by estimating the increase in UV absorbance from LL to HL cultures using measured MAA concentrations (Table 3) and the maximum extinction coefficient for MAAs (5.7 × 10⁻¹ L·g⁻¹·µm⁻¹), as reported by Garcia-Pichel (1994). An S of 0.8 implies that the increased MAAs account for an 80% decrease in UV radiation reaching cellular constituents. However, the lowering of UV weight (ε(λ)) between LL and HL cultures was greater than 80% (Fig. 5), implying that screening performance exceeds both model expectations for ≤1% MAA content and direct estimates of screening potential. The calculation of screening factor assumes a homogeneous distribution of MAAs in the cell (Garcia-Pichel 1994). This does appear to be the case for cyanobacteria (Garcia-Pichel and Castenholz 1993), but little is known about intracellular distribution of MAAs in eukaryotes. Targeting of MAAs around cellular structures containing sensitive targets might increase the efficiency of protection. Another possibility is that MAAs are also providing protection through secondary mechanisms, for example, as an antioxidant (Dunlap and Yamamoto 1995). However, such mechanisms would not provide spectrally specific protection and thus would be unlikely to induce the observed changes in BWFs.

Although intercellular distribution of screening compounds might affect the scale of cell sizes over which effective protection is obtained, the general principles of the Garcia-Pichel analysis probably still hold: As cell size becomes smaller, nonoptical defense strategies, such as antioxidants, repair, and reactivation processes, will have better cost/benefit ratios (Raven 1991). Indeed, the contribution of repair needs to be taken into account
for any cell size (Lesser et al. 1994). If there are offsetting processes to damage (as suggested by the observation of negative weights), even incomplete protection from UV radiation might reduce damage enough so that residual biological effects can be completely counteracted by other defenses. Thus, phytoplankton should be expected to have a range of strategies for defending against UV effects, with varying importance of photoprotection and other mechanisms.

CONCLUSIONS

Much progress has been made recently in developing quantitative approaches for estimating UV inhibition of marine photosynthesis (Cullen et al. 1992, Lubin et al. 1992, Neale et al. 1994, Boucher and Prézelin 1996a, Neale et al. 1998a). In principle, these approaches can be used to assess the effect of stratospheric ozone depletion on marine primary productivity (Cullen et al. 1992, Arrigo 1994, Neale et al. 1994, Boucher and Prézelin 1996b, Neale et al. 1998a). However, phytoplankton response to environmental UV radiation is variable (Neale et al. 1998a). Thus, general estimates of the effect of ozone depletion depend on a better understanding of both phenotypic and genotypic variation in UV response. We have shown that the influence of one factor, MAA concentration, can be quantitated through changes in the BWF. This is a first step toward better prediction of how phytoplankton respond to UV radiation on the basis of cellular characteristics and environmental conditions. We suggest that this capability will be further expanded by spectral studies of MAA effects in other species and for other growth conditions and by similar comparative studies to relate changes in the BWF to other photoprotective and repair mechanisms.

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