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Evidence Regarding the UV Sunscreen Role of a Mycosporine-Like Compound in the Cyanobacterium *Gloeocapsa* sp.

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The UV sunscreen role commonly ascribed to mycosporine-like amino acids (MAAs) was investigated with an isolate of the terrestrial cyanobacterium *Gloeocapsa* sp. strain C-90-Cal-G.(2), which accumulates intracellularly an MAA with absorbance maximum at 326 nm but produces no extracellular sunscreen compound (i.e., scytonemin). The intracellular concentrations of MAA achieved were directly related to the intensity of the UV radiation (maximum at 320 nm) received by the cells. However, the presence of high concentrations of MAA was not necessary for the physiological acclimation of the cultures to UV radiation. The measured sunscreen factor due to MAA in single cells was 0.3 (the MAA prevented 3 out of 10 photons from hitting potential cytoplasmic targets). High contents of MAA in the cells correlated with increased resistance to UV radiation. However, when resistance was gauged under conditions of desiccation, with inoperative physiological photoprotective and repair mechanisms, cells with high MAA specific contents were only 20 to 25% more resistant. Although UV radiation centered around both 320 and 365 nm resulted in chlorophyll *a* photobleaching and photoinhibition of photosynthesis, the difference in sensitivity correlated with MAA accumulation occurred only at 320 nm (absorbed by MAA) and not at 365 nm (not absorbed by MAA). This difference represents the maximal protection ascribable to the presence of MAA for single cells, i.e., if one does not consider the enhancing effects of colony formation on protection by sunscreens.

The mycosporine-like amino acids (MAAs) have often been thought to serve a UV sunscreen role in the biology of the organisms that produce or contain them, mainly because MAAs present strong absorbance in the UV region of the spectrum (between 310 and 360 nm) and because no other physiological function has been assigned to them (20). In some instances, circumstantial evidence supporting the sunscreen role has been obtained. This evidence includes the finding that UV radiation can trigger the production and accumulation of mycosporines in fungi (23), corals (11, 18), a dinoflagellate (4), and cyanobacteria (8). Responsiveness to external UV cues should be an evolutionarily sound regulatory capability, if MAAs are indeed acting as UV sunscreens. Additional circumstantial evidence comes from the fact that in multicellular organisms these substances are especially prominent in tegumentary or other peripheral tissues (19). This can be regarded as a way of optimizing the effectiveness of the sunscreen in the fashion of other known sunscreens, such as animal melanins (14), plant phenylpropanoids (21, 22), and cyanobacterial scytonemin (7).

The mere presence of an absorbing compound is not a satisfactory criterion for invoking its role as a sunscreen, and because an effective sunscreen is a passive means of photoprotection, obtaining direct proof of function may be difficult. Nevertheless, a series of necessary conditions that should be met by a potential sunscreen compound can be put forward. These would provide more direct evidence for the sunscreen hypothesis, as with the cyanobacterial extracellular pigment scytonemin (9). Failure to meet such conditions would effectively falsify the sunscreen hypothesis.

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Possibly the most crucial condition is that the compound should absorb a fraction of the incident radiation high enough to provide a meaningful benefit to the organisms. This fraction is the sunscreen factor (8). A second condition is that the presence of the compound in the organisms should be correlated with enhanced fitness under UV radiation (i.e., growth rates enhanced or survival increased compared with that of the same organisms when they lack the compound). However, the possible correlation between enhanced fitness or survival under UV with elevated concentrations of a sunscreen compound would not necessarily imply a causal relationship, especially if the organisms have had to be exposed to UV radiation in order to obtain cells with high concentrations of sunscreen (see references 3 and 17). Other photoprotective mechanisms may also have been triggered in the process. This leads to the third condition: because of the passive nature of the sunscreen effect, the correlation between the concentration of compound and resistance to UV should still be present under conditions of physiological inactivity or quiescence, when other, active photoprotective mechanisms are not functional (9). Finally, other approaches that can be used to show the sunscreen role include determining the "spectral correctness" of the effect (it should be maximal at the wavelengths of maximal absorption of the alleged sunscreen compound and negligible where the compound does not significantly absorb) and, if possible, the loss of protection after artificial removal of the compound.

In this article, we present the results from experiments carried out with the cyanobacterium *Gloeocapsa* sp. to assess the possible sunscreen role of MAAs. *Gloeocapsa* sp. strain C-90-Cal-G.(2) produces abundant cytoplasmic MAAs (maximal absorption at 326 nm) under inductive conditions and lacks other extracellular sunscreen pigments (8), making it an appropriate subject for this research.

MATERIALS AND METHODS

The strain of *Gloeocapsa* used [strain C-90-Cal-G.(2)] was originally isolated from a limestone quarry wall in Calafell (Catalonia, Spain) and was maintained in liquid culture by previously described procedures (7). The strain is presently not axenic, but the numbers of heterotrophic bacteria are usually very low in growing cultures (ca. 1.2% of the biomass).

Experimental cultures were grown on polycarbonate membrane filters (Nuclepore; pore size, 0.6 μm) as described previously (9). Briefly, the cells were inoculated onto filters and incubated floating on D medium (9) inside a plastic petri dish. Illumination was received from either above or below, depending on the circumstances. This technique allows one to avoid the typical problems encountered when working with nondispersible cultures of cyanobacteria, namely, (i) uneven irradiation, (ii) the impossibility of traditional replicate sampling, and (iii) self-shading. These problems are avoided by (i) irradiating a single, uniformly loaded plane, (ii) inoculating from artificially homogenized liquid cultures so that aliquot subcultures can be inoculated on filters to serve as replicate samples, and (iii) limiting the maximum loads per filter (cell population density) to avoid self-shading. The maximum loads can be experimentally determined (for our present conditions, they lie between 1 and 1.5 mg [dry weight] per filter).

Biomass was determined gravimetrically as dry weight with pretared filters as described previously (9). MAA contents were determined spectrophotometrically from 250- to 500-nm absorption spectra, after being extracted in aqueous methanol (20%, vol/vol) for 1 h at 45°C, by using the following expression (8): $A_{326}^* = A_{326} - 0.2A_{260}$, where A_{326}^* is the corrected value of A_{326}. In the absence of an absorption coefficient for the compound, its amounts have been expressed as A units, where A stands for the value of the absorbance when that amount is dissolved in 1 ml of solvent and measured with a 1-cm-pathlength cuvette. Specific contents are expressed as $A \times$ milligrams (dry weight)$^{-1}$.

Visible light was obtained from cool-white Very High Output fluorescent-light tubes (Sylvania). UV radiation with a maximum output at 365 nm was obtained from black-light fluorescent tubes (Sylvania), and UV with a maximum output at 310 nm was obtained from Philips FS-40 (suntanning) fluorescent bulbs. The spectral irradiance of these UV sources after passage through one plastic petri dish cover (Falcon) is as shown in Fig. 1. They have been calculated from previously published spectra (7, 13) and corrected by the spectral transmittance of a petri dish cover. These will be referred to as UV-320 and UV-365 radiation. For some experiments, the output from very high output tubes was filtered through interference or colored filters to obtain narrow-band radiation centered at 450 (blue), 530 (green), or >640 (red) nm. The full spectra of these have been described previously (7). Visible radiation from fluorescent tubes or narrow-band radiation in the visible spectrum (red, green, and blue) was measured with a QSL100 (Biospherical Instruments) quantum scalar irradiance meter; narrow-band UV radiation was measured with an International Light 1700 radiometer connected to a UV-A or UV-B sensor. The readings from the FS40 lamps (UV-320) needed to be corrected for the (nonflat) spectral responsivity of the UV-B sensor; this was done by integrating the responsivity-corrected spectral output of the lamps between 280 and 350 nm to obtain a correction factor. The radiometer readings (in energy units) were then transformed to quantum equivalents by regarding the radiation as monochromatic and centered at 320 or 365 nm so that 1 W m^{-2} at 365 nm equals 3 μmol m^{-2} s^{-1} and 1 W m^{-2} at 320 nm equals 2.7 μmol m^{-2} s^{-1}.

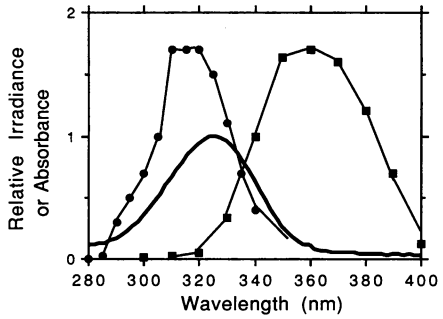

FIG. 1. Spectral irradiance of the UV sources used in the experiments after passage through a plastic petri dish cover (UV-320 [●] and UV-365 [■]) and absorption spectrum of the principal MAA from the *Gloeocapsa* sp. in 0.2% acetic acid in water (—).

UV excitation spectra for 461-nm fluorescence emission were measured with cells that had been stained with the DNA-binding dye DAPI (4',6-diamidino-2-phenylindole) to assess the magnitude of the sunscreen factor caused by MAAs. After culture homogenates were stained with a 60-μg liter^{-1} DAPI solution, the cells were rinsed with fresh medium by filtration and were resuspended. Excitation spectra were determined with a Perkin-Elmer MP66 spectrofluorimeter which was quantum corrected. Suspensions were sequentially diluted until the specific fluorescence remained constant upon dilution, indicating that the effect of self-shading was negligible. By comparison of cells with low and high contents of MAAs, the reduction in the ability of 326-nm UV to induce blue DAPI-DNA fluorescence relative to that at the maximum provides an indirect measure of the sunscreen factor.

Chlorophyll *a* (chl *a*) photobleaching rates were monitored by measuring the decay of A_{675} in filter cultures of the *Gloeocapsa* sp. by in vivo spectrophotometry as described previously (9). Dried filter cultures were treated with photobleaching radiation and rewetted at intervals to measure chl *a* absorption directly. After cultures were redried, treatments were resumed. A control which was kept in the dark but which had been submitted to the same drying-wetting cycles was used to obtain the zero rate of photobleaching. Cultures remained viable in a dry state.

Photosynthetic capacity was assessed by measuring $NaH^{14}CO_3$ photoincorporation into cell matter after periods of desiccation. In separate experiments (data not shown), it was demonstrated that ^{14}C incorporation proceeded immediately upon rewetting (the shortest incubation time was 0.25 h) and reached maximal, steady-state values at 0.5 h. Uptake was linear for up to 4 h. Experimental incubations were started 0.5 h after rewetting, and they lasted for 1.5 h. The values of dark incubations for each treatment were subtracted from the values of incubations in the light. The details of sample handling and radioactivity measurements were published previously (6).

RESULTS

Exposure of the *Gloeocapsa* sp. to UV-320 radiation resulted in an increase in the intracellular concentrations of a mycosporine-like substance (MAA). The kinetics of in-

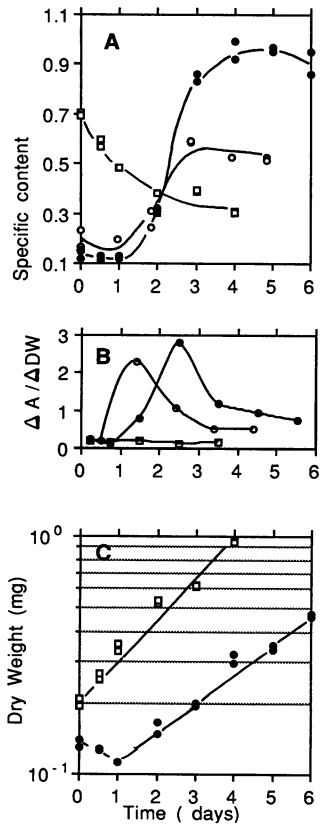

FIG. 2. Time course of MAA content, synthesis, and growth after shifts at time 0 in the UV-320 photon fluence rates. Shown are the shift-up from 0 to 6 μmol m^{-2} s^{-1} (●), the shift-up from 0 to 2 μmol m^{-2} s^{-1} (○), and the shift-down from 4 to 0 μmol m^{-2} s^{-1} (□). (A) Changes in specific content (A × milligrams [dry weight]$^{-1}$) of MAA; (B) rates of synthesis of MAA, measured as the difference in total MAA versus the difference in biomass; (C) kinetics of growth as dry weight.

crease in the specific content of MAA and growth after shift-up in the incident UV-320 radiation (from a virtual absence to 2 and 6 μmol m^{-2} s^{-1}) are depicted in Fig. 2A. The filter cultures were inoculated with cells grown at 50 μmol m^{-2} s^{-1} of white light and then incubated under the same levels of white light but supplemented with UV-320 radiation. During the shift-up experiments, three distinct phases in the kinetics of MAA synthesis were observed: a first phase, or lag period, lasting about 1.5 days, during which the specific contents remained low; a second phase during which the specific contents rose; and a third phase, or steady-state phase, during which the contents remained high. This steady state was reached at 3 to 4 days. Additionally, we followed the kinetics of MAA specific content and growth in a culture adapted to UV-320 radiation (4 μmol m^{-2} s^{-1}) that was submitted to a shift-down to no UV-320 (Fig. 2A). The rates of synthesis of MAAs with respect to the synthesis of new biomass are plotted versus time in Fig. 2B. These rates showed a clear peak at 1.5 and 2.5 days (in the 2 and 6 μmol m^{-2} s^{-1} shift-ups, respectively) with absolute maximal values between 2.3 and 2.8 ΔA × Δmilligrams (dry weight)$^{-1}$. During the steady-state phase there was noticeable production of MAA, but this was matched by increases in biomass, resulting in relatively constant specific contents.

TABLE 1. Instantaneous growth rates after shift-ups in UV-320 radiation in nonacclimated cells of *Gloeocapsa* sp.

Shift-up to: (μmol m^{-2} s^{-1})	Growth rate (day^{-1})[a]	
	Initial	After acclimation
No shift	0.36	0.36
2	0.36	0.40
6	−0.18	0.29
12	−0.20[b]	0.09[b,c]

[a] Values are means of two estimates.
[b] Only one estimate.
[c] Cells previously acclimated to 6 μmol of UV-320 m^{-2} s^{-1}.

In the shift-down experiment, specific contents of MAA declined continuously during the entire period in which they were measured, even though there were small but steady rates of synthesis (Fig. 2A and B). The kinetics of growth are plotted in Fig. 2C. In the shift-up experiments, there was an initial phase characterized by an absence of growth or even a decline in biomass, probably due to cell death and lysis, followed by a phase of exponential growth (see also Table 1), indicating that acclimation to the elevated UV fluxes had taken place. Of special interest here are the growth dynamics of the cultures submitted to shift-ups and their relationship with the changes in MAAs. The onset of exponential growth in shift-up experiments did not coincide with the time at which the maximal content of MAA was reached (Fig. 2A and C). Such a coincidence would be expected had MAA played a major role in the UV acclimation process. In the cultures submitted to a shift-down, as well as in control cultures in which there was no change in irradiation (Table 1), exponential growth started immediately. Acclimation to a UV-320 fluence rate as high as 12 μmol m^{-2} s^{-1} was possible, albeit at the cost of markedly reduced growth rates, and these only for cultures already adapted to 6 μmol m^{-2} s^{-1} (Table 1).

In shift-up experiments, the steady-state specific contents of MAAs attained were correlated with the magnitude of the UV-320 irradiance, so that the stronger the UV fluence rates, the higher the specific contents (Fig. 3). A maximal content of 1.55 A × milligrams (dry weight)$^{-1}$ was measured under 12 μmol of UV-320 m^{-2} s^{-1}.

A preliminary action spectrum for the effect of radiation on the levels of MAAs was carried out by measuring the steady states (at 4 days) in cultures after shift-ups of equal

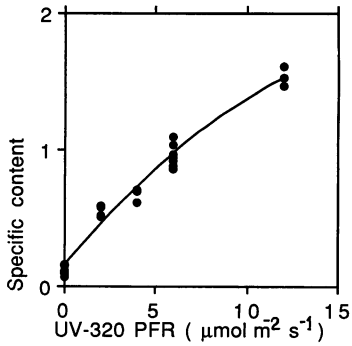

FIG. 3. Steady-state specific contents (A × milligrams [dry weight]$^{-1}$) of MAA in cultures exposed to UV-320. Data have been pooled from several experiments, but all measurements were taken 4 days after shift-ups. PFR, photon fluence rate.

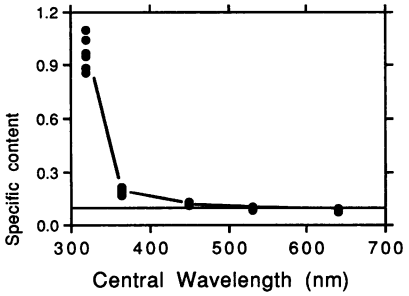

FIG. 4. Steady-state specific contents (A × milligrams [dry weight]$^{-1}$) of MAA in cultures exposed to 6 μmol m^{-2} s^{-1} of radiation of different central wavelengths. There was a background of 50 μmol m^{-2} s^{-1} of white light. Controls had nothing added, and their mean is the horizontal line.

photon fluence rate at different wavelengths. Figure 4 shows that UV radiation centered at 320 nm was by far the most effective in eliciting increases in MAA content; a small but significant increase was attained with UV-365 radiation, and neither blue, green, nor red light caused any significant deviations from control values (without additional radiation).

The excitation spectra for DAPI fluorescence in cells stained with the DNA-binding dye are shown in Fig. 5 (curves a and b). The spectra were normalized to equal fluorescence at the maxima, where there is no significant absorption by the MAA of the *Gloeocapsa* sp. In cells with high MAA contents (1.2 A × milligrams [dry weight]$^{-1}$) there was a clear depression in the abilities of shorter wavelengths to excite blue DAPI fluorescence compared with that in cells with low MAA contents (0.1A × milligrams [dry weight]$^{-1}$). The spectral characteristics of this effect are plotted as the fractional depression (Fig. 5, curve c). As expected when the fractional depression is due to differing MAA contents, it resembles the absorption spectrum of *Gloeocapsa*'s MAA. This fractional reduction is a measure

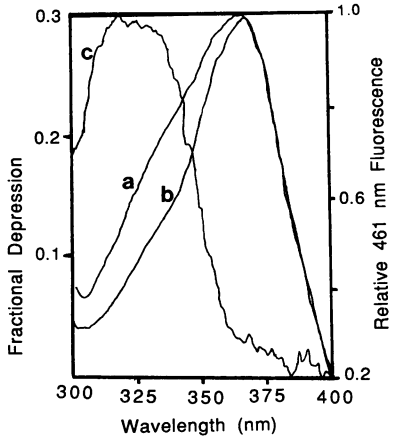

FIG. 5. Excitation spectra for blue fluorescence in DAPI-stained cells with low (a) and high (b) contents of MAA. Emission was measured at 461 ± 10 nm. The averages of 10 consecutive spectra are presented. The spectral fractional reduction (c) of the excitation spectrum calculated as (a − b)/a in cells with high contents of MAA resembles the absorption spectrum of MAA (Fig. 1) and has a maximum value of 0.3.

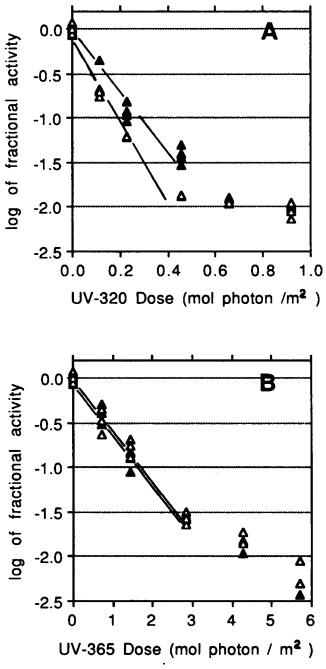

FIG. 6. Inhibition of ^{14}C uptake after exposure of *Gloeocapsa* cells to UV irradiation under desiccation. The data are expressed as the log of the fraction of ^{14}C-uptake activity remaining after a given dose, with respect to that of desiccated but nonirradiated cells. Irradiation with UV-320 (A) and UV-365 (B) is shown, as are cultures with high (▲) and low (△) MAA contents. See the text for details on curve fitting.

of the in vivo sunscreen effect due to 1.1A × milligrams (dry weight)$^{-1}$ of MAAs and has a value of ca. 0.3 at the maximum.

The decrease in photosynthetic capacity which was caused by UV irradiation under conditions of quiescence (desiccation) was measured by using $NaH^{14}CO_3$ photoincorporation. The experiment was designed to assess the extent of photodamage under conditions in which repair mechanisms should be inoperative. Aliquots of homogenized cultures growing under either 50 μmol m^{-2} s^{-1} of white light (nonacclimated cells, with low MAA contents) or 50 μmol m^{-2} s^{-1} of white light supplemented with 6 μmol m^{-2} s^{-1} of UV-320 radiation (acclimated cells, with high MAA contents) were deposited onto filters and desiccated. The desiccated samples were exposed to UV radiation in the dark (either UV-320 or UV-365) for different lengths of time (doses) or kept in the dark without exposure to UV. After the treatment, the filters were rewetted in the medium and ^{14}C photoincorporation rates were measured within 2 h to avoid posttreatment recovery effects. Log plots of the dose versus fraction of photosynthetic activity remaining are shown in Fig. 6. Regardless of the irradiation history, UV-320 was found to cause a loss of photosynthetic capacity much more effectively than UV-365 (note the differences in abcissa scales), which is in agreement with the previously published action spectra for UV photoinhibition in plant cells (2, 12). The D_{37} (dose at which only 37% of the control activity remains) for UV-365 corresponded to photon fluences of 0.70 (acclimated cells) and 0.71 (nonacclimated cells) mol m^{-2}, whereas for UV-320 only 0.12 (acclimated cells) and 0.08 (nonacclimated cells) mol m^{-2} were required.

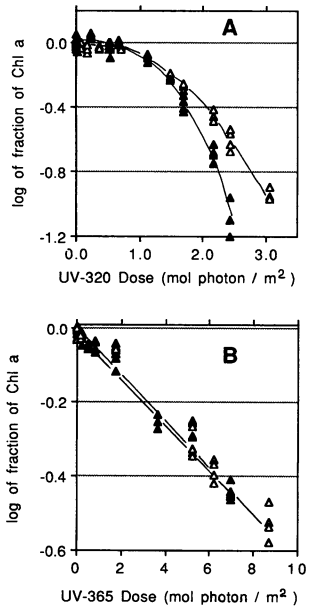

FIG. 7. Photobleaching of chl *a* by UV irradiation under desiccation. The data are expressed as the log of the fraction of chl *a* remaining after a given dose with respect to desiccated but nonirradiated cells. Irradiation with UV-320 (A) and UV-365 (B) is shown, as are cultures with high (△) and low (▲) MAA contents. See the text for details on curve fitting.

According to the single-target theory (5), linearity in the dose-versus-log fractional effect plots is to be expected; however, in some of our experiments, a departure from linearity clearly occurred at high doses (Fig. 6). Thus, the process could not be characterized rigorously by a mere exponential decay. Nevertheless, for our comparative purposes, we have fitted the data to log linear functions (exponential decay), excluding the data from the two highest doses in every case. This gave $R^2 \geq 0.95$ in all cases. Under UV-320 radiation, the rates of photoinhibition in acclimated cells were slightly but significantly lower than those for nonacclimated cells. The regressed values of the slopes were -3.115 and -4.126 m^2 mol^{-1}, respectively, and were found to be different at the 95% confidence level. With UV-365, slopes were -0.544 (acclimated cells) and -0.542 (nonacclimated cells) m^2 mol^{-1}, which were not significantly different even at the 50% confidence level. Thus, under desiccation, both types of cells were equally susceptible to UV-365 radiation. The significant difference found at the shorter wavelength (UV-320) provides a measure of the maximal possible sunscreen effect due to the different cellular contents of MAA. UV-320 inhibition of photosynthetic rates in cells with high MAA contents (1.1 A × milligrams [dry weight]$^{-1}$) was 25% lower than that in cultures with low MAA levels (0.1 A × milligrams [dry weight]$^{-1}$) (Fig. 6).

We also used photobleaching of chl *a* under desiccation as an alternative measure of photodamage (Fig. 7). The rates of chl *a* photobleaching by UV-365 fitted the log linear model, as has been found with other cyanobacteria (9). There were no significant differences between acclimated and nonacclimated cells (Fig. 7B). The dose-response rates for UV-320 photobleaching were nonlinear and showed the typical shapes of processes in which a certain carrying capacity for protection exists in the cells, such as those of some UV-A survival curves (24, 25). This indicates that the photobleaching of chl *a* was caused by different mechanisms under UV-365 and UV-320. A possible explanation is that with UV-365, the photobleaching was sensitized mostly by chl *a* absorption, whereas with UV-320 it was mostly mediated by other prooxidants or radicals, the effect becoming apparent only after the antioxidant capacity of the cells had been overcome. Regardless of the mechanisms, and similar to the effects on photoinhibition, a small difference between acclimated and nonacclimated cells exposed to UV-320 was found. To quantify the differences, we used the method of Novick and Szilard (15) as cited by Jagger (10). This involves the calculation of a dose modification factor by dividing the doses necessary for a given effect in nonacclimated cells by the dose needed for the same effect in acclimated cells, at doses in which the differences are measurable. Regression lines ($R^2 \geq 0.95$) of the data in Fig. 7 (at doses higher than 1 mol m^{-2}) gave significantly different slopes (95% confidence level). By using these lines, dose modification factors calculated for log fraction values of -0.4, -0.8, and -1 were 0.76, 0.72, and 0.82. The mean maximal sunscreen factor implied by these values (as 1 − dose modification factor) is 0.23.

DISCUSSION

The strain of the *Gloeocapsa* sp. used in these experiments was able to withstand very high fluence rates of UV-320 radiation and to thrive (Table 1). Fluence rates of 12 μmol m^{-2} s^{-1} are severalfold the values measured outdoors with the same instrument. For a reference, values in Eugene, Oreg., (at close to the same latitude as northeastern Spain) for a typically clear autumn day at noon in a horizontal plane are 2.2 μmol m^{-2} s^{-1}. This ability is to be expected in a strain that had been isolated from habitats exposed to full solar radiation. The results from the shift-up experiments indicate that the resistance to UV-mediated photodamage is not constitutive but develops after a process of acclimation, as evidenced by the growth lags or even death which occurs immediately after the shift-ups, but is followed by phases of exponential growth.

Two findings suggest that MAAs may be involved in the process of photoadaptation to UV radiation in the *Gloeocapsa* sp. The presence of increased levels of MAA in the cells was correlated with the incident levels of UV-320 (Fig. 3), and accumulation of MAA was shown to be induced most efficiently by UV-320 (Fig. 4). However, the onset of exponential growth during acclimation to UV radiation took place prior to the time at which cells attained the maximal content of MAA (Fig. 2). This suggests that the MAA was not a prerequisite for acclimation. In contrast, in a strain of *Chlorogloeopsis* sp., the onset of exponential growth after shift-ups in UV-365 occurred only after maximal levels of scytonemin had been attained (9). Moreover, fluorometric estimations of the sunscreen factor due to MAA (0.3 at 326 nm) for the *Gloeocapsa* sp. do not support the hypothesis that the acclimation to UV radiation could be due solely to the screening action of the MAA.

Both photobleaching of chl *a* and photoinhibition of photosynthesis were used as gauges of UV-induced photodamage to the cells. UV radiation is known to cause strong photoinhibition in photosynthetic microorganisms (1, 16), and the main target has been identified as the PSII D-1 protein in several organisms. Because a screening agent provides nonspecific protection regardless of the process affected (7), any measure of damage should be appropriate to reveal its action and does not imply that neither photoinhi-

bition of photosynthesis nor photobleaching of pigments are the primary targets of photodamage in the cells. These experiments were done under conditions of quiescence (desiccation) in order to circumvent the effect of repair mechanisms. The results of such experiments showed that the differences in the susceptibilities of cultures with high and low MAA contents to both photoinhibition and photobleaching under desiccation were significant, albeit not large, when they were induced by UV-320 (Fig. 6 and 7). Similarly acclimated or nonacclimated cells showed dramatically different behavior when exposed to UV-320 in growth medium; cells preacclimated to UV grew exponentially, whereas nonacclimated cells suffered population declines (Fig. 2C). That most of the difference in resistance to UV radiation between the two types of cultures was lost when the dosage was received under physiological inactivity clearly indicates that the differences in growth were mostly due to active photoprotective and/or repair mechanisms. The maximal possible effect of the MAA as sunscreens can only be the remaining 23 to 25% difference found under desiccation. Since the sunscreen factor due to MAA estimated by fluorometry is of the same magnitude (ca. 0.3 at 326 nm), it can be deduced that the differences between nonacclimated and preacclimated cells found under desiccation were in fact due to the sunscreen effect of the MAA. If the protective effect were due to MAAs entirely, it should not be detectable at the wavelengths at which *Gloeocapsa* MAA does not absorb. This is borne out by the results with UV-365 radiation that showed no significant differences in the rates of either photobleaching or photoinhibition between cells with high and low specific MAA contents. As a comparison, the measured maximal possible effect of MAAs in the *Gloeocapsa* sp. is smaller than that due to scytonemin in an isolate of a *Chlorogloeopsis* sp. acclimated to UV-365, which was 0.7 for UV-365 radiation (9). A comparison of the sunscreen factors due to MAAs and scytonemin and their complementation both at 320 and at 370 nm has been previously published (8).

The evidence gathered indicates that only a small sunscreen effect can be ascribed to the MAA in the *Gloeocapsa* sp. under these experimental conditions. Because the overall biological effects of UV radiation are not necessarily linearly related to the dose or the dosage rate, it is not possible to predict the benefit the cells derive from a ca. 23 to 30% reduction in the UV-320 reaching the cell matter. However, one can assert that the overall biological effect of such a reduction would be equal to that of lowering the doses (or dosage rates) by the same percentage. This may still be regarded as a good improvement for a small investment. The cost of maintaining the MAA levels at $1A \times$ milligrams (dry weight)$^{-1}$ for UV-320 fluence rates of 6 μmol m^{-2} s^{-1} corresponds to investments of about 0.7% of the cell dry biomass (8) and to sunscreen factors of 0.25 to 0.3, which cut down the effective UV-320 fluxes reaching the cells to 4.4 μmol m^{-2} s^{-1}. Such a difference in fluxes may be trivial (see shifts between 0 and 2 μmol m^{-2} s^{-1} in Table 1) or may be equated to a decrease in growth rate of 18% (interpolating between 6 and 12 μmol m^{-2} s^{-1} [Table 1]). The maximal depletion in the growth rates due to the burden investment on the sunscreen should be less than 1%.

These small effects were measured under conditions that prevented self-shading, namely, single cells or small groups of cells. When undisturbed, the *Gloeocapsa* sp. grows in a colonial form, with packages of many cells held together by an extracellular sheath. In such growth forms, the effective cellular pathlengths for radiation are greatly increased and so is the overall sunscreen factor for the colony. According to a previously published model (8), a single-cell-based sunscreen factor of 0.2 translates into values of 0.9 for a 0.1-mm-thick colony or biofilm. Possibly, it is in the colonial growth forms that the presence of MAAs and their screening action become major factors in resistance to UV radiation.

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