Occurrence of UV-Absorbing, Mycosporine-Like Compounds among Cyanobacterial Isolates and an Estimate of Their Screening Capacity

FERRAN GARCIA-PICHEL[†]* AND RICHARD W. CASTENHOLZ

Department of Biology, University of Oregon, Eugene, Oregon 97403

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A survey of 20 strains of cyanobacteria (belonging to 13 genera) isolated from habitats exposed to strong insolation revealed that 13 strains contained one or more water-soluble, UV-absorbing, mycosporine amino acid (MAA)-like compounds. Some of the compounds were identical in several strains. In all, 13 distinct compounds were found. The UV absorption spectra of MAAs complemented well that of the extracellular sunscreen pigment scytonemin, which many of the strains also produced. Even though the specific MAA contents were variable among strains, they were invariably higher when the cultures were grown with UV radiation than when it was absent. In five strains tested, the MAA complement accumulated as a solute in the cytoplasmic cell fraction. The sunscreen capacities of MAA and scytonemin and their combined capacity were estimated for each strain and condition on the basis of the specific contents, cell size, and cellular location of the compounds. The estimates suggested that significant, albeit not complete, protection from UV photodamage could be gained from the possession of either MAA or scytonemin but especially from simultaneous screening by both types of compounds.

Cyanobacteria are known to inhabit environments subject to intense solar radiation. They are often the primary colonizers of bare rock surfaces and soils (33). Under such conditions, they must possess efficient mechanisms to prevent or to counteract the harmful effects of strong solar radiation, including those caused by the particularly deleterious UV region of the spectrum. Mechanisms that counteract UV-mediated photodamage have been previously demonstrated in the cyanobacteria, and they include both dark DNA repair and photorepair (16, 31), the presence of detoxifying enzymes (17), and the actions of photoprotective carotenoids (2). Among the possible mechanisms to prevent UV photodamage are negative photomovements or avoidance responses (20) and the synthesis of UV sunscreen compounds (8). The presence of substantial amounts of UV-absorbing compounds in cyanobacteria has been documented in several instances (see reference 8 and references therein; 21, 23, 25, 30), and it has frequently been hypothesized that these substances may be playing a UV-photoprotective role in the organisms that contain them. One type for which a sunscreen role has been established (10) is the extracellular sheath pigment scytonemin (8). Scytonemin is a colored (i.e., it is a pigment), lipid-soluble compound with broad absorption in the UV and violet wavelengths (280 to 450 nm) and an *in vivo* maximum at ca. 370 nm. A second type or class includes a variety of colorless, water-soluble compounds with narrow absorption bands and maxima between 310 and 334 nm (see references 21, 23, and 25). These compounds are reminiscent in their physicochemical traits (i.e., absorption and polarity, etc.) of a family of compounds known as mycosporine-like amino acids (MAAs) that have been previously characterized in fungi (1, 6, 34), eukaryotic algae (26, 28, 29), corals (27), and starfish (19). They have been found in a wide variety of marine organisms (13).

Chemically, MAAs contain a substituted cyclohexenone linked with an amino acid (or its imino alcohol). They present a characteristic, single absorption band in the UV spectrum between 230 and 400 nm, with maxima ranging from 310 to 360 nm, and absorb only weakly below and above the main band. The role of any of the water-soluble compounds as sunscreens has not yet been determined rigorously, but some circumstantial evidence supporting the sunscreen hypothesis has been gathered in certain cases (see reference 11 for a detailed discussion).

To assess how common and diverse these substances are among cyanobacteria from habitats exposed to intense solar radiation, we analyzed a variety of isolates of cyanobacteria for MAA-like, UV-absorbing, water-soluble substances. The isolates were from such environments as desert rocks, mountain walls, pavement, and hot spring and saline cyanobacterial mats. We also investigated the cellular locations of the substances and the effect of UV radiation on their specific contents. Their possible role as sunscreen agents is discussed on the basis of their estimated specific absorption in the cells, the size of the cell, and the presence or absence of the extracellular sheath pigment scytonemin.

MATERIALS AND METHODS

Strains and culture. The strains used in this study are listed in Table 1. Isolation and culture methods were described previously (8). Briefly, cultures were grown on polycarbonate Nuclepore filters floating on medium-filled petri dishes receiving white light from cool-white fluorescent-light tubes, at photon fluence rates of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. UV radiation with maximal spectral irradiance at 320 nm was provided by Philips FS40 tubes as filtered through plastic petri dish covers (11). Radiation was measured with either a Biospheric Instruments QSL100 Quantum Scalar Irradiance Meter (visible) or an International Light 1700 radiometer with a UV-B sensor (ultraviolet). Characteristics of the light

* Corresponding author.

[†] Present address: Max-Planck-Institut für Marine Mikrobiologie, BITZ, Fahrenheitstrasse 1, D-2800 Bremen 33, Germany.

TABLE 1. Characteristics of cyanobacterial strains investigated

Strain	Taxonomic assignment ^a	Source	Source or reference	Presence (+) or absence (-) of the following UV-absorbing compound:	
				MAA	Scytonemin
O-89-H-Syn.	<i>Synechococcus</i> sp.	Pavement	A ^b	+	+
C-90-Cal-G.(2)	<i>Gloeocapsa</i> sp.	Quarry wall	11	+	-
M-88-VD-G.(1)	<i>Gloeocapsa</i> sp.	Desert rock	8	+	+
HW-91-Gth.(2)	<i>Gloeotheca</i> sp.	Tree bark	B ^c	+	+
WH-87-L.a.(1)	<i>Lyngbya aestuarii</i>	Intertidal mat	8	+	+
O-89-H-C.p.(1)	<i>Calothrix parietina</i>	Pavement	8	+	+
S-90-C.Rh.(1)	<i>Calothrix</i> sp.	Waterfall	8	+	+
Y-89-WT-C.(3)	<i>Calothrix</i> sp.	Hot-spring mat	8	+	+
UTEX 424	<i>Scytonema</i> sp. (<i>Tolypothrix</i> sp.) ^d	U. Texas.C.C. ^e		+	+
UTEX 1163	<i>Scytonema</i> sp.	U. Texas.C.C.		+	+
M-89-Dip.(2)	<i>Nostoc</i> (<i>Diplocolon</i> sp.) ^d	Desert rock	8	+	+
B-79-Scy.j.	<i>Scytonema javanicum</i>	Wall	8	+	+
O-89-Cgs.(1)	<i>Chlorogloeopsis</i> sp.	Pavement	8	+	+
AN-90-N.m.	<i>Nostoc microscopium</i>	Pond fringe	8	-	+
NZ-86-O.b.	<i>Oscillatoria boryana</i>	Hot-spring mat	4	-	-
OH-88-O.t.(1)	<i>O. terebriformis</i>	Hot-spring mat	C ^f	-	-
AN-90-O.p.	<i>O. priestleyi</i>	Salt pond	D ^g	-	-
NZ-19-O.a.-2	<i>O. amphigranulata</i>	Hot-spring mat	7	-	-
M-87-GN-Mic.	<i>Oscillatoria</i> sp. (<i>Microcoleus</i> sp.) ^d	Hypersaline mat	E ^h	-	-
M-87-GN-Cth.	<i>Cyanotheca</i> sp.	Hypersaline mat	E ^h	-	-
M-87-GN-Sp.s.	<i>Spirulina subsalsa</i>	Hypersaline mat	E ^h	-	-
ATCC 27179	<i>Synechococcus lividus</i>	Hot-spring mat		-	-

^a Supraspecific assignments are according to the microbiological usage. Specific assignments, when possible, are tentative and are according to the botanical usage.

^b From crust on concrete in Eugene, Oreg.

^c Hawaii.

^d Tentative genus.

^e University of Texas Culture Collection.

^f Hunter's Hot Springs, Oregon.

^g McMurdo Ice Shelf, Antarctica.

^h Exportadora de Sal's salt evaporation ponds, Guerrero Negro B.C.S., Mexico.

source and details on the measurement of fluence rates can be found in reference 11.

Determination of pigments and MAA-like substances. MAAs were extracted from cultures by submersion in 20% (vol/vol) aqueous methanol for ca. 1 to 2 h at 45°C. Further extractions yielded only insignificant ($\leq 2\%$ of the total) amounts of MAAs. This procedure did not extract photosynthetic pigments when the cells were not otherwise disturbed (i.e., ground or sonicated). Specific contents were determined spectrophotometrically from the aqueous methanol extracts by measuring the absorbance at the wavelength of MAA maximum absorbance, and corrections were made according to the following expression:

$$A_{\lambda}^* = A_{\lambda} - A_{260}(1.85 - 0.005\lambda) \quad (1)$$

where A_{λ}^* is the corrected value of the absorbance at the maximum, A_{λ} is the measured value of absorbance at the maximum, and λ is the wavelength (in nanometers) of maximal absorbance. This correction accounts for the absorbance due to water-soluble cell matter in the extracts that is not due to MAA, and it has been determined from the pooled spectra of extracts in three species that do not contain MAAs. In the absence of absorption coefficients for each compound, concentrations have been expressed as A (absorbance or optical density). One A equals the value of the absorbance when the amount of compound is dissolved in 1 ml of solvent and measured with a 1-cm-pathlength cuvette. Specific contents are expressed as $A \times \text{milligrams (dry weight)}^{-1}$. Subsequent extraction of extracellular scy-

tonemin and photosynthetic pigments was done when needed by grinding the remaining cell material in 100% acetone. The determination of scytonemin contents was done spectrophotometrically by using the trichromatic equations provided previously (8) and was also expressed in absorbance units for comparison.

Isocratic HPLC analysis. Aqueous methanol extracts (20%) were brought to dryness by rotary evaporation at 45°C, and the resulting dry matter was dissolved in 100% methanol. Methanol-insoluble residue was discarded by decantation and filtration through Whatman GFF filters. This residue had no absorbance due to MAA when it was redissolved in water. The methanol fraction was concentrated by rotary evaporation and stored at -20°C until it was analyzed. Aliquots of the methanol fraction were vacuum dried and dissolved in the same solvent mixture as that used in high-pressure liquid chromatography (HPLC). Normally, 25- to 100- μl aliquots of solutions containing 5 to 15 absorbance units per ml were injected for analyses. The isocratic reverse-phase HPLC system was similar to those used previously for MAA separation by other investigators (3, 5, 13, 18) and consisted of a Hypersil 5- μm -pore-size C_{18} (octyldesyl silane) column attached to a Waters HPLC pump (M6000A) system. The mobile phases were 0.2% acetic acid in water (solvent 1), 0.2% acetic acid-3% methanol in water (solvent 2), and 0.2% acetic acid-6% methanol in water (solvent 3). The chromatographies were run at 204 atm (1 atm = 101.29 kPa) with flow rates of 1 ml min^{-1} at room temperature. Detection of MAA in the eluate was by UV

absorption measured at 320 nm with a Waters Lambda-MAX M480 spectrophotometer. The HPLC peaks presenting UV absorbance were collected and analyzed by UV spectrophotometry. Fractions having absorption spectra typical of MAAs were stored at -20°C and further purified by HPLC in a second solvent. Only those putative MAA peaks having absorbances $\geq 1\%$ of that of the main MAA peak were analyzed and reported. Identities between MAAs which had equal absorption spectra and similar retention times but which had been obtained from different species were confirmed by cochromatography of the isolated compounds to a single peak in at least two different solvent systems.

Cell fractionation. Cell fractionation was carried out by differential centrifugation. Cells were harvested, suspended in 30 mM sodium citrate buffer (pH 5.5), and broken open by passage (twice) through a French press. The resulting suspensions were centrifuged at ca. $300 \times g$ for 2 min to discard the unbroken cells. The supernatants were then centrifuged for 50 min at $50,000 \times g$. This procedure pelleted a large fraction of the extracellular sheath material. The resulting supernatants were ultracentrifuged first at $75,000 \times g$ for 1 h and then at $200,000 \times g$ for 1 h to yield a cytoplasm-plus-membrane fraction and a cytoplasmic fraction, respectively. UV visible absorption spectra were taken of each supernatant to monitor the fate of the MAAs. Chlorophyll *a* absorption monitored the fate of cellular membranes, scytonemin absorption (where applicable) monitored the fate of the extracellular sheath, and phycobilin absorbance monitored the fate of the cytoplasmic fraction.

Estimation of the sunscreen factors. The effect of the alleged sunscreen compounds in shielding the cells was gauged by estimating a sunscreen factor, *S*, which is defined as the fraction of the incident radiation that is prevented from impinging upon cell matter because of the presence of the compound. The factor varies between 0 (no sunscreen effect) and 1 (all photons are prevented from hitting cell matter), and it is dependent on the wavelength (λ). For a sunscreen that is present in the extracellular layers, such as scytonemin, *S* simply equals the complementary value of the transmittance (fraction transmitted, *T*) of the compound in the layer, so that

$$S(\lambda) = 1 - T(\lambda) \quad (2)$$

If the sunscreen is located intracellularly and is homogeneously distributed, *T*(λ) and *S*(λ) also depend on the distance (*x*) from the point where the radiation hits the cell. The complementary value of *T*(λ , *x*) obtained by using the cell diameter (*d*) as the pathlength gives the maximal sunscreen factor at the distal end of the cell. The minimal value of *S*(λ , *x*), at the point in the cell surface at which the radiation impinges, is 0. An average sunscreen factor for the entire cell, *S*(λ), can be calculated by integrating the values of *T*(λ , *x*) between *x* = 0 and *x* = *d* and finding its complementary fraction; thus,

$$S(\lambda) = 1 - d^{-1} \int_0^d T(\lambda, x) \, dx \quad (3)$$

Assuming that *T*(λ , *x*) follows the Lambert-Beer Law, then

$$S(\lambda) = 1 - d^{-1} \int_0^d \exp[-\epsilon(\lambda)cx] \, dx \quad (4)$$

where $\epsilon(\lambda)$ is the extinction coefficient of the sunscreen and *c* is its concentration. Equation 4 has the following solution:

$$S(\lambda) = 1 - \frac{(T_d - 1)}{\ln T_d} \quad (5)$$

where *T_d* is the value of the transmittance at the distal end of a cell with diameter *d*. If both the value of transmittance or absorbance in the cells due to the sunscreen and the cell diameters are known, the sunscreen factors can be estimated from equation 2 or 5. We note that in these estimates, the effects due to cell morphology have been neglected.

We derive the values of absorbance within the cells from their specific contents of either MAA or scytonemin by using the following allometric constants: (i) live cells contain 75% of their weight as water, (ii) extracellular sheath material accounts for 25% of the dry weight (22, 32), and (iii) the density of the cells is $1.08 \text{ kg/liter}^{-1}$ (12). From these, we derive that 1 mg (dry weight) of biomass represents $2.8 \mu\text{l}$ of cell volume. The absorbance for 1 mg (dry weight) (specific content data) measured in extracts (*A_{ext}*; 1 ml of volume, 1 cm of pathlength) is then transformed into the absorbance within the cells (*A_d*; $2.8 \mu\text{l}$ of volume and the cell's diameter, *d*, in micrometers, as pathlength) with

$$A_d = 0.035 A_{\text{ext}} d \quad (6)$$

T_d values are obtained from *A_d* values as

$$T_d = \exp(-2.3A_d) \quad (7)$$

In the case of MAAs, sunscreen factors were obtained by using *T_d* values resulting from equations 6 and 7 in equation 5. The wavelengths of maximal absorbance were used, but for simplicity in comparisons, the results are assigned to a common wavelength of 320 nm. For scytonemin (extracellular), *A_d* values obtained from equation 6 were halved, since only half of the absorbance lies between the radiation and the cell and the other half at the distal end and is therefore useless in screening that cell. They were transformed into transmittance, and sunscreen factors were estimated from equation 2. This gave the sunscreen factor for scytonemin at its wavelength maximum (384 nm in extracts, ca. 370 nm in vivo). The sunscreen factor of scytonemin at 320 nm was also estimated in the same fashion, but the fact that *A_{ext}*(320) equals $0.4 A_{\text{ext}}(384)$, in accordance with the absorption spectrum of scytonemin, was taken into consideration (Fig. 1B). The combined sunscreen factor *S*^{*}(λ) for MAAs and scytonemin at 320 nm was calculated from the partial sunscreen factors as

$$S^* = 1 - \{[1 - S_{\text{(scyt)}}][1 - S_{\text{(MAA)}}]\} \quad (8)$$

RESULTS

From a total of 20 different strains of cyanobacteria (Table 1) belonging to 13 genera, 13 strains were found to contain water-soluble, UV-absorbing substances with spectra typical of those of MAAs, i.e., a band of absorption in the near UV and no absorption bands in the visible or between 230 and 280 nm. MAAs seem to be common but not universal among cyanobacteria from habitats with high insolation.

FIG. 1. Spectral characteristics of whole cells and their UV-absorbing substances. (A) In vivo absorption spectra of cell suspensions of *Gloeocapsa* C-90-Cal-G.(2) grown under white light ($\blacktriangle$) and under UV-supplemented white light ($\diamond$). Fluence rates were 50 (white light) and 6 (UV radiation; centered around 320 nm) $\mu\text{mol m}^{-2} \text{s}^{-1}$. The suspensions contained equal amounts of cells on a fresh-weight basis. Chl a, chlorophyll a; PC, phycocyanin; Car, carotenoids. (B) In vivo absorption spectrum of cells of *Diplocolon* M-88-Dip.(1) grown under UV-supplemented white light (+) and that of their cytoplasmic fraction (—) and absorption spectrum of extracted and purified scytonemin in chloroform (O). Spectra are not to scale.

Most of the isolates that contained MAA-like substances also possessed scytonemin (12 out of 13), and only one strain among those having scytonemin (13 in total) did not contain detectable amounts of MAA. Thus, our isolates tended to have either both types of compounds or none. In the

following section, we will discuss data obtained from the isolates that contained MAAs.

The contents of MAAs showed variations both within and among strains. When cultures were incubated under UV-supplemented radiation (6 μmol of UV $\text{m}^{-2} \text{s}^{-1}$ plus 50 μmol of visible light $\text{m}^{-2} \text{s}^{-1}$), the specific contents of MAA were invariably higher than those found for the same strain under visible radiation alone (Table 2). These differences in MAA content with UV treatment ranged from 2- to 30-fold. Under visible light alone, the specific contents in different species ranged between 0.02 and 0.32 $\text{A} \times \text{milligrams (dry weight)}^{-1}$, and under UV-supplemented radiation they ranged between 0.19 and 1.02 $\text{A} \times \text{milligrams (dry weight)}^{-1}$. The maximal MAA content found [for *Gloeocapsa* C-90-Cal-G.(2)] is estimated to be about 0.8% of the cell biomass (dry weight). The effect of those concentrations of MAA on the absorbance characteristics of the cells of the *Gloeocapsa* strain is depicted in the in vivo spectra of Fig. 1A. For this *Gloeocapsa* isolate, the value of absorbance due to MAA at the 326-nm maximum in cultures grown with UV amounts to ca. 2.7 times the absorbance due to the rest of the cell matter and is substantially larger than that attributable to any cellular pigment at any other wavelength. In the other isolates, which contained scytonemin, the in vivo absorption spectra did not reveal as clearly the magnitude of the MAA absorption band because of the overlapping of MAA and scytonemin in the 300- to 340-nm region, but the presence of MAA was evident both in the cytoplasmic fraction and in methanol-water extracts (Fig. 1B).

The aqueous methanol extracts revealed that the MAA maxima ranged between 309 and 332 nm and presented in some cases conspicuous shoulders at ca. 340 nm, suggesting that the complement of MAA varied with the species (Table 2). Subsequent HPLC-spectrophotometry analysis showed that a variety of compounds having typical MAA absorption were present in the isolates (Table 2). In some strains, only one such compound was detected, but in others, as many as four were detected. In most cases, however, there was clearly one compound that accounted for most of the absorbance, with other compounds, if they were present, being relatively minor (see the listing of relative contents in Table 2). Cochromatography also showed that some of the compounds were found in more than one species. In all, 13

TABLE 2. Distribution and total specific content of mycosporine-like, UV-absorbing compounds

Strain	Absorbance maximum in extracts (nm) ^a	Specific content		% of dry cell mass ^c under UV	Compound(s) detected ^d (relative contents in each strain)
		Under UV ^b	Without UV		
O-89-H-Syn.	310	0.19	0.10	0.16	II
C-90-Cal-G.(2)	326	1.02	0.13	0.84	XI (1)
M-88-VD-G.(1)	323	0.75	0.14	0.62	III (1), VIII (0.62)
HW-91-Gth.(2)	310	0.12	0.05	0.09	VII (1), IX (0.26)
WH-87-L.a.(1)	330	0.32	0.17	0.27	I
O-89-H-C.p.(1)	310 (340)	0.49	0.07	0.40	IV (0.09), VI (0.02), XI (0.01), XII (1)
S-90-C.Rh.(1)	310 (340)	0.68	0.32	0.56	IV (0.08), XIII (1)
Y-89-WT-C.(3)	332	0.63	0.02	0.53	V (0.06), VI (1), VII (0.09)
UTEX 424	326	0.50	0.09	0.42	X
UTEX 1163	309	0.39	0.01	0.32	II (1), VII (0.01)
M-89-Dip.(2)	310 (340)	0.38	0.04	0.31	IV (0.12), XIII (1)
B-79-Scy.j.	309	0.45	0.05	0.37	II (1)
O-89-Cgs.(1)	325	0.35	0.04	0.29	III (0.02), V (0.01), XI (1), XII (0.14)

^a Approximate wavelength of a conspicuous shoulder is shown in parentheses.

^b Expressed as $\text{A} \times \text{milligrams (dry weight)}^{-1}$.

^c Estimated by using an average extinction coefficient of 120 liters $\text{g}^{-1} \text{cm}^{-1}$ computed from values in the literature.

^d See Table 3 for a key to the compounds. Fluence rates of UV were ca. 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

TABLE 3. Key to the mycosporine-like compounds detected

Compound	Absorption maximum (nm)	HPLC elution time (min) for solvent:		
		1 ^a	2 ^b	3 ^c
I	330	4.4	3.2	2.9
II	310	5.4	3.4	
III	322	6.0	4.0	
IV	310 (340) ^d	7.1	4.1	3.8
V	332	8.0	5.2	3.6
VI	334	8.8	6.6	4.9
VII	310	10.8	8.3	5.8
VIII	324	11.1	6.2	
IX	310	16.0	9.1	
X	324	17.2	10.3	
XI	326	19.5	9.5	7.5
XII	332	30.4	12.9	8.8
XIII	310 (340) ^d	>60.0	45.0	16.2

^a 0.2% aqueous acetic acid.^b 0.2% aqueous acetic acid–3% methanol in water.^c 0.2% aqueous acetic acid–6% methanol in water.^d Approximate position of shoulder in parentheses.

different compounds were detected in the 13 isolates analyzed.

The designation, chromatographic behavior, and absorption maxima of the compounds isolated are summarized in Table 3; the full spectra of some of the compounds are presented in Fig. 2.

Preliminary data from chemical characterizations in progress showed that upon basic hydrolysis, compound VI afforded histidine, compound XI afforded lysine, and compound V afforded glycine. Even though the compounds were not characterized further, the fact that they yielded amino acids upon hydrolysis confirms their classification as mycosporine-like substances. Some may be identical to previ-

ously described MAAs, whereas some are clearly novel. Several MAAs with absorption maxima at 310 nm (like compounds II, VII, and IX) have been previously characterized (1, 6, 13). Compound VI, which could be identical to shinorine (from a red alga [28]) on the basis of retention times in the same solvent system and absorption maxima from the literature (18), is clearly novel in that it yields histidine upon being hydrolyzed. There are no MAA compounds with absorption maxima at 326 (compound XI) or 324 (compound X) nm reported in the literature; therefore, these are also novel.

A special mention should be devoted to compounds IV and XIII, since their absorption spectra were virtually identical but atypical for known MAAs, presenting a pronounced shoulder at ca. 340 nm (Fig. 2). Mild hydrolysis of compound XIII (40°C, 1 h, NH₄OH) afforded an MAA with maximal absorbance at 310 nm and lysine. Thus, compound XIII may contain two typical MAA chromophores. A spectrally similar, water-soluble compound, which may be identical to compound IV or XIII, has been previously isolated from a terrestrial cyanobacterium (*Nostoc commune* [21]) and found to be bound to polysaccharides. Further investigation will be needed to establish all of these identities.

In order to establish the cellular location of the MAAs, we monitored the fate of the cellular absorbance at the MAA maximum in several isolates during cell fractionation by differential centrifugation after cell breakage. We also compared these values to those obtained by extraction in aqueous methanol. With *Gloeocapsa* C-90-Cal-G.(2), the results were clear: 93% of the A_{326} remained after the suspension was cleared of extracellular sheaths, cell walls, and cellular membranes by ultracentrifugation, indicating that the MAA complement in this strain was cytoplasmic. In other isolates, MAA absorption bands were also detected in the cytoplasmic fraction (Fig. 1B), but, since these strains also contained substantial amounts of scytonemin, it could not be deduced with certainty that the amounts detected in the cytoplasmic fraction accounted for the bulk of the MAA content. In these cases, however, extraction of the washed centrifugal pellets with aqueous methanol gave no absorption bands due to MAA. Moreover, for all five strains tested, the absorbances of the cytoplasmic fraction agreed well with the contents measured by the extraction of whole cells. The values, expressed as the ratio of absorbances found in extract (uncorrected) versus those in the cytoplasmic fraction for strains, were 1.01 for C-90-Cal-G.(2), 0.96 for M-89-Dip.(2), 1.05 for O-89-Cgs.(1), 1.12 for Y-89-WT-C.(3), and 0.98 for O-89-H-C.p.(1) (taxonomic assignments appear in Table 1). These results clearly indicate that the bulk of MAA in the isolates was cytoplasmic, which is to be expected for small, water-soluble compounds.

The estimated sunscreen factors (*S*) due to either MAAs, scytonemin, or both types of compounds are presented in Table 4. The values for specific content and cell diameter used in the calculations are also included therein. All of the values used correspond to a single determination, but according to additional determinations (9) in several isolates, they are representative of both size and specific contents under the conditions used. *S* values obtained for MAAs range between 0.1 and 0.26, indicating that, at the single-cell level, MAA can only be expected to reduce the UV fluences reaching cell matter (at about 320 nm) by a small fraction. For scytonemin at 320 nm (not its maximum), *S* ranged between 0.02 and 0.55, indicating that the effective reduction was very much species dependent and could attain values higher than any assigned to MAAs. The combined *S* values

FIG. 2. UV absorption spectra of several MAAs isolated from cyanobacterial strains in 0.2% acetic acid in water. See Table 3 for key to the compounds.

TABLE 4. Estimated values of sunscreen factor due to MAA, scytonemin, and/or their combined action in single cells of isolates studied^a

Strain	Specific content ^b		Modal cell diam (μm)	Sunscreen factor ^c at:			
				Ca. 320 nm			Ca. 370 nm ^d
	MAA	Scyt		MAA	Scyt	Combined	
C-90-Cal-G.(2)	1.02		6.2	0.21		0.21	
M-88-VD-G.(1)	0.75	1.35	10.8	0.26	0.21	0.42	0.44
HW-91-Gth.(2)	0.12	0.45	23.1	0.10	0.15	0.24	0.34
WH-87-L.a.(1)	0.32	0.23	10.9	0.13	0.04	0.16	0.09
O-89-H-C.p.(1)	0.49	2.15	10.6	0.18	0.31	0.43	0.60
S-90-C.Rh.(1)	0.68	0.14	9.0	0.21	0.02	0.23	0.04
Y-89-WT-C.(3)	0.63	0.32	9.0	0.20	0.05	0.24	0.10
UTEX 424	0.50	1.10	7.7	0.14	0.12	0.25	0.29
UTEX 1163	0.39	0.20	13.8	0.19	0.04	0.22	0.10
M-89-Dip.(2)	0.38	3.79	10.8	0.15	0.48	0.55	0.81
B-79-Scy.j.	0.45	0.05	16.7	0.25	0.00	0.25	0.03
O-89-Cgs.(1)	0.38	6.53	7.7	0.11	0.55	0.60	0.86

^a Scyt, scytonemin.^b For cultures adapted to 6 μmol of UV (320 nm) $\text{m}^{-2} \text{s}^{-1}$ and 50 μmol of white light $\text{m}^{-2} \text{s}^{-1}$. Expressed as $A \times \text{milligrams (dry weight)}^{-1}$.^c Fractional reduction in the UV fluences caused by the sunscreen (see Materials and Methods for calculations and assumptions).^d Due only to scytonemin.

at 320 nm were less variable and ranged between 0.16 and 0.60, with most species having values of more than 0.2. At longer wavelengths, the S value due to scytonemin was very high for some species (up to 0.86) but low for others.

DISCUSSION

We have shown that MAAs are common but diverse among terrestrial cyanobacteria and that they most often occur in species with extracellular scytonemin. The fact that MAA concentrations in the cells invariably increased with increased UV fluence rates suggests that they may be involved in processes of photoacclimation. The evidence presented here does not provide proof that the MAAs have a sunscreen function in the cells; rather, it measures their ability to screen out UV radiation. The possibilities that MAAs have some other function or functions and that their screening ability is merely coincidental cannot be rejected.

The spectral complementation between the MAAs and scytonemin in the isolates (Fig. 1B) suggests that the combined action of scytonemin and MAA may be responsible for any sunscreen effect at shorter UV wavelengths (300 to 340 nm), whereas at UV wavelengths longer than 340 nm, the effect must be due solely to scytonemin. The relative contributions of MAA and scytonemin at shorter wavelengths varied with each isolate (Table 4). Interestingly, even though the complement of MAA compounds in our isolates was quite diverse, we found no MAAs such as palythene from zoanthids (wavelength maximum, 360 nm [27]) with absorption bands close to or coincident with the strong absorption band of scytonemin.

The specific contents of MAA found in our isolates were estimated to represent between 0.16 and 0.84% of the total biomass (dry weight). These values are in the range of those from natural samples of Antarctic red algae and much higher than those from brown and green algae, diatoms, and metazoans (13). Reported values of up to 10% of the dry weight for a UV absorbing-compound in *N. commune* (21) cannot be compared directly with our values, because the absorption coefficient used to calculate that percentage (4 liters $\text{g}^{-1} \text{cm}^{-1}$) was much lower than those typical of MAAs (average, 120 liters $\text{g}^{-1} \text{cm}^{-1}$), perhaps because the *Nostoc* compound

was found to be linked to polysaccharides. Values of 10% of dry weight with such an extinction coefficient amount to 0.4 $A \times \text{milligrams (dry weight)}^{-1}$, which is in agreement with the specific absorbances measured with our isolates. This implies that the single-cell sunscreen factors attained in *Nostoc commune* are of a magnitude similar to that of the ones reported here.

The sunscreen factors estimated with our isolates indicate that the presence of the measured concentrations of both intracellular MAA-like compounds and extracellular scytonemin have a significant effect in preventing UV radiation from reaching prospective targets in the cells. The magnitude of the effect, although different for different isolates, was never negligible. For MAAs in *Gloeocapsa* C-90-Cal-G.(2), sunscreen factors of 0.2 were estimated, which is in good agreement with the values of around 0.2 to 0.3 which were determined empirically (11). For scytonemin, high sunscreen factors were estimated for *Chlorogloeopsis* O-89-Cgs.(1), which is in agreement with empirical determinations of the sunscreen effect (10). In general, however, the sunscreen factors for single cells were not large enough to provide full protection against UV radiation. A question arises: if the function of MAAs and scytonemin is really that of sunscreen agents, why were specific contents not high enough in all cases to make the sunscreen factors more substantial? It is plausible that the intracellular accumulation of soluble substances to high concentrations may pose physiological burdens regarding osmotic characteristics of the cells (increased osmotic pressure as water tends to enter the cell) that offset the photophysiological benefits derived from the presence of the compound. Nevertheless, we note that the values were estimated considering that the cells interact with radiation as single units, separate from other cells. This allowed controlled experimentation and provided a conservative estimate of the sunscreen factors; however, it may not be realistic in natural conditions. Many, though not all, of the strains have colonial growth forms, and the effective pathlengths for radiation are then much longer. With longer pathlengths and similar concentrations of compounds, the magnitude of the sunscreen effect is increased substantially. For the same reasons, the sunscreen factors that one should expect from concentrations of MAAs com-

parable to those in our isolates should be much higher in larger organisms, such as the red algae, and they should be very small for bacteria of typical sizes (0.5 to 1 μm in diameter).

Interestingly, neither MAA nor scytonemin was found in some microbial mat isolates, such as *Oscillatoria terebriformis* (20), *O. boryana* (4), and *Spirulina subsalsa* (9), which are known to display behavioral migration away from the light in response to excessive radiation. We have also found typical MAA-like absorbance bands in field samples of planktonic cyanobacteria such as *Gloeotrichia*, *Aphanizomenon*, and *Microcystis* spp. (9), and such bands from field samples have also been previously reported in the literature for an oceanic oscillatorian (from the description given, it is possibly a *Trichodesmium* sp. [23]). This indicates that the presence of MAAs in cyanobacteria is not restricted to nonplanktonic species, as seems to be the case with the sheath pigments (8). Since there do not seem to be any taxonomic boundaries to the presence of MAA, it is likely that the ability to produce these compounds among cyanobacteria preceded their evolutionary radiation, presumably in the Precambrian era (15). At that time, the absence of an atmospheric ozone shield and, therefore, the higher fluxes of UV-B (280 to 315 nm) (14), may have added evolutionary pressure to select for efficient UV-protecting mechanisms.

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REFERENCES

- Arpin N., J. Favre-Bonvin, and S. Thivend. 1977. Structure de la mycosporine 2, nouvelle molécule, isolée de *Botrytis cynerea*. *Tetrahedron Lett.* 10:819–820.
- Buckley, C. E., and J. A. Houghton. 1976. A study of the effects of near UV radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. *Arch. Microbiol.* 107:93–97.
- Carreto, J. I., M. O. Carignan, G. Daleo, and S. G. De Marco. 1990. Occurrence of mycosporine-like amino acids in the red tide dinoflagellate *Alexandrium excavatum*: UV-protective compounds? *J. Plankton Res.* 12:909–921.
- Castenholz, R. W., B. B. Jørgensen, E. D'Amelio, and J. Bauld. 1991. Photosynthetic and behavioral versatility of the cyanobacterium *Oscillatoria boryana* in a sulfide-rich microbial mat. *FEMS Microbiol. Ecol.* 86:43–58.
- Dunlap, W. C., and B. E. Chalker. 1986. Identification and quantification of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. *Coral Reefs* 5:155–159.
- Favre-Bonvin, J., N. Arpin, and C. Brevard. 1976. Structure de la mycosporine (P 310). *Can. J. Chem.* 54:1105–1113.
- Garcia-Pichel, F., and R. W. Castenholz. 1990. Comparative anoxygenic photosynthetic capacity in 7 strains of a thermophilic cyanobacterium. *Arch. Microbiol.* 153:344–351.
- Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *J. Phycol.* 27:395–409.
- Garcia-Pichel, F., and R. W. Castenholz. Unpublished data.
- Garcia-Pichel, F., N. D. Sherry, and R. W. Castenholz. 1992. Evidence for a UV sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. *Photochem. Photobiol.* 56:17–23.
- Garcia-Pichel, F., C. E. Wingard, and R. W. Castenholz. 1992. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. *Appl. Environ. Microbiol.* 59:170–176.
- Guerrero, R., C. Pedrós-Alió, T. M. Schmidt, and J. Mas. 1985. A survey of buoyant density of microorganisms in pure cultures and natural samples. *Microbiología* 1:53–65.
- Karentz, D., F. S. McEuen, M. C. Land, and W. C. Dunlap. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar. Biol.* 108:157–166.
- Kasting, J. F. 1987. Theoretical constraints on oxygen and carbon dioxide concentrations in the precambrian atmosphere. *Precambrian Res.* 34:205–229.
- Knoll, A. H. 1982. Microfossils from the late Precambrian Draken conglomerate, Ny Friesland, Svalbard. *J. Paleontol.* 56:755–790.
- Levine, E., and T. Thiel. 1987. UV-inducible DNA repair in the cyanobacteria *Anabaena* spp. *J. Bacteriol.* 169:3988–3993.
- Mittler, R., and E. Tel-Or. 1991. Oxidative stress responses in the unicellular cyanobacterium *Synechococcus* PCC7942. *Free Rad. Res. Comm.* 12:845–850.
- Nakamura, H., and J. Kobayashi. 1982. Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 250:113–118.
- Nakamura, H., J. Kobayashi, and Y. Hirata. 1981. Isolation and structure of a 330 nm UV-absorbing substance, asterina-330, from the starfish *Asterina pectinifera*. *Chem. Lett.* 1981:1413–1414.
- Richardson, L. L., and R. W. Castenholz. 1987. Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. *Appl. Environ. Microbiol.* 53:2142–2150.
- Scherer, S., T. W. Chen, and P. Böger. 1988. A new UV-A/B protecting pigment in the terrestrial cyanobacterium. *Nostoc commune*. *Plant Physiol.* 88:1055–1057.
- Schrader, M., G. Drews, J. R. Golecki, and J. Weckesser. 1982. Isolation and characterization of the sheath from the cyanobacterium *Chlorogloeopsis* PCC6912. *J. Gen. Microbiol.* 128:267–272.
- Shibata, K. 1969. Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant Cell Physiol.* 10:325–335.
- Sivalingam, P. M., T. Ikawa, and K. Nisizawa. 1976. Physiological roles of a substance, 334, in algae. *Bot. Mar.* 19:9–29.
- Sivalingam, P. M., T. Ikawa, Y. Yokohama, and K. Nisizawa. 1974. Distribution of a 334 UV-absorbing substance in algae, with special regard of its possible physiological roles. *Bot. Mar.* 7:23–29.
- Takano, S., A. Nakanishi, D. Uemura, and Y. Hirata. 1979. Isolation and structure of a 334 nm UV-absorbing substance, porphyra-334, from the red alga *Porphyra tenera* Kjellman. *Chem. Lett.* 1979:419–420.
- Takano, S., D. Uemura, and Y. Hirata. 1978. Isolation and structure of two new amino acids, palythanol and palythene, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* 49:4909–4912.
- Tsujino, I., K. Yabe, and I. Sekikawa. 1980. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendo* Yamada et Mikami. *Bot. Mar.* 23:65–68.
- Tsujino, I., K. Yabe, I. Sekikawa, and N. Hamaoka. 1978. Isolation and structure of a mycosporine from the red alga *Chondrus yendo*. *Tetrahedron Lett.* 16:1401–1402.
- Turian, G. 1985. Colonisation primaire des murs de béton par une *Chrysocapsa* (Cyanobactérie) à pigment U.V.-protecteur. *Saurea* 16:43–48.
- Van Baalen, C. 1968. The effects of ultraviolet irradiation on a coccoid blue-green alga: survival, photosynthesis and photoreactivation. *Plant Physiol.* 43:1689–1695.
- Weckesser, J., K. Hofmann, U. J. Jürgens, B. A. Whitton, and B. Raffelsberger. 1988. Isolation and chemical analysis of the sheaths of the filamentous cyanobacteria *Calothrix parietina* and *Calothrix scopulorum*. *J. Gen. Microbiol.* 134:29–34.
- Whitton, B. A. 1987. Survival and dormancy of blue-green algae, p. 109–167. In Y. Henis (ed.), *Survival and dormancy of microorganisms*. John Wiley and Sons, New York.
- Young H., and V. J. Patterson. 1982. A UV-photoprotective compound from *Glomerella cingulata*—a mycosporine. *Phytochemistry* 21:1075–1077.