UV-B-Induced Synthesis of Photoprotective Pigments and Extracellular Polysaccharides in the Terrestrial Cyanobacterium Nostoc commune

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Liquid cultures of the terrestrial cyanobacterium Nostoc commune derived from field material were treated with artificial UV-B and UV-A irradiation. We studied the induction of various pigments which are thought to provide protection against damaging UV-B irradiation. First, UV-B irradiation induced an increase in carotenoids, especially echinenone and myxoxanthophyll, but did not influence production of chlorophyll a. Second, an increase of an extracellular, water-soluble UV-A/B-absorbing mycosporine occurred, which was associated with extracellular glycan synthesis. Finally, synthesis of scytonemin, a lipid-soluble, extracellular pigment known to function as a UV-A sunscreen, was observed. After long-time exposure, the UV-B effect on carotenoid and scytonemin synthesis ceased whereas the mycosporine content remained constantly high. The UV-B sunscreen mycosporine is exclusively induced by UV-B (<315 nm). The UV-A sunscreen scytonemin is induced only slightly by UV-B (<315 nm), very strongly by near UV-A (350 to 400 nm), and not at all by far UV-A (320 to 350 nm). These results may indicate that the syntheses of these UV screens are triggered by different UV photoreceptors.

The terrestrial nitrogen-fixing cyanobacterium Nostoc commune Vaucher flourishes in extremely cold and dry habitats which are characterized by intense solar radiation, extreme temperature differences, and regular periods of desiccation (34, 42; for a review, see reference 7). N. commune, in its natural habitat, forms macroscopic colonies with filaments embedded in gelatinous glycan. In the past, most studies concentrated on the extraordinary drought resistance of N. commune (33; for a review, see reference 29), but only few investigated its UV tolerance (35, 41).

Mechanisms counteracting UV-B damage have been demonstrated in plants and cyanobacteria. Besides repair of UV-induced damages of DNA by excision repair and photoreactivation (10, 26) and accumulation of detoxifying enzymes and carotenoids (24, 25), an important mechanism to prevent UV photodamage is the synthesis of UV-absorbing compounds. Several studies provided evidence that epidermally located phenylpropanoids, especially flavonoid derivatives, protect higher plants by absorbing harmful UV radiation (22, 37). Mycosporine amino acids (MAAs) are thought to fulfill a comparable purpose in lower organisms (14, 21). MAAs are water-soluble, substituted cyclohexenes which are linked to amino acids and iminoalcohols and have absorption maxima between 310 and 360 nm. Scytonemin, which has an in vivo absorption maximum at 370 nm and is located in the cyanobacterial sheath, has been proposed to serve as a UV-A sunscreen (13). It is a yellow-brown, lipid-soluble dimeric pigment of terrestrial cyanobacteria with a molecular mass of 544 Da and a structure based on indolic and phenolic subunits (30).

A UV-A/B-absorbing pigment with absorption maxima at 312 and 335 nm was found in N. commune colonies exposed to high solar radiation (35). Recently, its chemical structure has been shown to be an oligosaccharide MAA (OS-MAA) (1). It was the first mycosporine reported to be covalently linked to oligosaccharides and is located in the extracellular glycan, where it forms high-molecular-weight complexes which are attached to the cyanobacterial sheath by noncovalent interactions (1, 16). Because N. commune is subject to regular cycles of desiccation and rewetting and often must survive long periods in quiescence, during which repair mechanisms are ineffective, UV-absorbing compounds may play a key role in UV photoprotection of N. commune.

The aim of this work was to study, in a single organism, the sequence of UV-induced synthesis of carotenoids, scytonemin, and a mycosporine, which are suggested to provide protection against UV damage.

MATERIALS AND METHODS

Organism and growth conditions. The cyanobacterium N. commune Vaucher DRH1 (hereafter referred to as N. commune DRH1) was derived from field material of N. commune collected in Hunan province, People’s Republic of China (17). The strain grows in liquid media under laboratory conditions without producing a visible glycan sheath surrounding single filaments. For UV induction experiments, 50 ml of N. commune DRH1 liquid cultures was grown under nitrogen-fixing conditions at 30°C in a 200-ml flask in BG11 (32) medium with constant shaking (80 rpm) to avoid self-shading. The flasks (Duran; Schott, Mainz, Germany) function as UV-C filters. The cultures were illuminated from above. Visible light (ca. 2.4 W m⁻²) was obtained from a cool white fluorescent tube (L40W/25 S, Osram, Munich, Germany). Additional illumination was provided from a Philips TL40W/12 lamp with incident irradiances of 100 to 140 mW m⁻² nm⁻¹ at 310 nm and 50 to 70 mW m⁻² nm⁻¹ at 330 nm. UV-A control experiments were performed with a Philips TL36W/08 lamp with an emission maximum centered at 375 nm. Foils with a cutoff at 315 nm were used as UV-B-blocking filters. The spectral irradiance which was received by the cultures after passing the filter and filters is presented in Fig. 1. Since no measurements are available, the maximum values of incident solar UV-B radiation in the natural habitat of N. commune DRH1 were calculated by the method of Feister (11) to vary between 0.7 W m⁻² (winter) and 2 W m⁻² (summer), around noon and on cloudless days. The UV-B irradiation applied in our experimental setting was 1 W m⁻².

Photon flux density in the visible spectral region was measured with a photodiode (G1118; Hamamatsu Photonics K. K., Hamamatsu, Japan), which was...
FIG. 1. Comparison of the spectral characteristics of UV light received by cultures in flasks from different light sources and filters. Curves: 1, lamp TL-12 (100% acetone); 2, lamp TL-12 plus 315-nm cut-off filter (far UV-A only, \( \lambda_{max} = 330 \) nm, ca. 0.1 W of UV-A m\(^{-2}\)); 3, lamp TL-08 (near UV-A, \( \lambda_{max} = 375 \) nm, ca. 1.7 W m\(^{-2}\)).

TABLE 1. Growth response of \textit{N. commune} DRH1 cultures upon UV-B irradiation\(^a\)

<table>
<thead>
<tr>
<th>Exposure time (days)</th>
<th>Cell no. (10(^7) cells/ml)</th>
<th>Mean ± SD</th>
<th>Chl ( \mu(g/10(^7) cells))</th>
<th>Dry wt ((\mu g/10(^7) cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+UV-B</td>
<td>Control</td>
<td>+UV-B</td>
</tr>
<tr>
<td>1</td>
<td>2.2 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>6.5</td>
<td>9.3 ± 0.7</td>
<td>3.9 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

\(^a\) DRH1 was cultivated at 30°C with cold fluorescent light of 2.4 W m\(^{-2}\) and artificial UV-B irradiation of 1.0 W m\(^{-2}\) (emission spectrum 1 [Fig. 1]). The control culture received the same intensity of fluorescent light without UV-B.

\(^b\) No significant differences were detected (P < 0.05). Chl a, chlorophyll a.

\(^*\) ND, not determined.
exposure, the enhanced carotenoid-to-chlorophyll a ratio declined to about 115% compared to controls.

To analyze whether UV-B led to a general shift-up in all carotenoids or whether specific carotenoids were induced, carotenoid patterns were analyzed by RP-HPLC. Figure 3B shows the carotenoid pattern of *N. commune* DRH1 after 1 day of UV-B treatment and the corresponding pattern of the control culture. As reported for other cyanobacteria (15), the carotenoid composition of *N. commune* was dominated by β-carotene, echinenone, and myxoxanthophyll, while canthaxanthin and zeaxanthin were only minor components. Specific contents (milligrams of pigment/milligram of chlorophyll a) of echinenone, myxoxanthophyll, and canthaxanthin were significantly increased (*P*, 0.01), while β-carotene and zeaxanthin showed no significant differences in comparison to control cultures. No additional carotenoids were induced by UV-B irradiation. The carotenoids mainly affected by UV-B were echinenone and myxoxanthophyll. Their specific content increased about 40 to 50% in comparison to control cultures.

The induction of carotenoids was due to UV-B, since filters with a cutoff at 315 nm (Fig. 1) prevented an increase in carotenoids (Table 2). Cultures irradiated with near UV-A (λ_max = 375 nm) also showed an increase in carotenoid-to-chlorophyll a ratios, but the induction followed completely different kinetics because carotenoids increased continuously over five days (Table 2).

**Induction of mycosporines.** UV-B exposure led to the production of UV-A/B-absorbing OS-MAA with absorption maxima at 312 and 335 nm (Fig. 4). The total amount of OS-MAA per milliliter of culture increased during the entire UV-B exposure time, but the specific content rose to a maximum of about 4.5 mg per mg of chlorophyll a at 3.5 days and remained at this high value (Fig. 5A). Synthesis of the pigment was promoted neither by supplemented UV-A irradiation (Table 2) nor by other stresses such as increased temperature, desiccation, or salt (data not shown). Traces of OS-MAA could be detected in old control cultures not subjected to UV stress.

**Induction of scytonemin.** No scytonemin was detected in control cultures grown without UV. UV-B irradiation induced scytonemin production after an initial lag of about 1 day. The specific content rose to a maximum of about 0.38 mg/mg of chlorophyll a at 2.5 days and declined thereafter (Fig. 5B). The induction of scytonemin was mostly due to the UV-B part (λ_max = 315 nm) emitted by the UV-B light source, because cultures protected by filters with a cutoff at 315 nm (far-UV-A irradiation, λ_max = 330 nm) showed only a very low scytonemin production of about 6% compared to unfiltered cultures (Table 2). Even higher far-UV-A irradiations led to only very low scytonemin production, whereas near-UV-A irradiation (λ_max = 375 nm) induced scytonemin production two- to threefold in comparison to cultures treated with UV-B (Table 2).

**DISCUSSION**

**Synthesis of extracellular polysaccharides is induced by UV-B.** Long-time UV-B, but not UV-A, exposure of *N. commune* DRH1 led to a decreased cell number but increased dry weight in comparison to control cultures, while short-time UV-B exposure had negligible effects on the growth of *N. commune* (Table 1). We suggest that the decreased cell number observed after 6 days of UV-B exposure is due to a slower cell replication caused by the metabolic cost of increased glycopol production and is not a consequence of inhibition of cell replication by UV-B. To our knowledge, the influence of UV-B on extracellular glycopol production had not previously been studied. Since the UV-absorbing mycosporines induced simultaneously are located in the glycan sheath (1, 35), it is likely that an increased glycopol production serves to provide a matrix for the OS-MAA, which is closely attached to the glycan by noncovalent interactions (1, 16). A thicker sheath provides much longer effective path lengths for the absorption of radiation. Whether UV-B led to structural changes of exopolysaccharides is currently under investigation.

**FIG. 2.** *N. commune* DRH1 filaments grown in liquid culture; unstained light microscopic picture. (A) Typical appearance of *N. commune* DRH1 grown in liquid culture without UV-B; (B) DRH1 liquid culture after 72 h of UV-B irradiation (1.0 W m⁻²). Note that glycopol is absent around heterocysts. Bars indicate 10 μm.
Myxoxanthophyll and echinenone could be envelope membrane-bound UV photoprotectors. For photosynthetic organisms, the protective role of carotenoids against high visible radiation is well known (36; for a review, see reference 6), and a protective role of carotenoids in cyanobacteria against UV-A radiation was reported (3, 28). Only little is known about the role of carotenoids in photoprotection against UV-B radiation (24, 31). Cyanobacteria produce some unique types of xanthophylls, such as ketocarotenoids and glycosides (18). Interestingly, in N. commune DRH1, these unique types were induced by UV-B whereas β-carotene and zeaxanthin showed no response (Fig. 3B). Analysis of cyanobacterial envelope membranes demonstrated that xanthophylls are the predominant carotenoids, whereas β-carotene was found almost exclusively in the thylakoids (20, 27). Myxoxanthophyll, a pigment induced by UV-B in N. commune (Fig. 5B), has been shown to be the predominant pigment in the outer membrane of Synechocystis sp. strain PCC 6714 (19). Echinenone, the other carotenoid strongly induced by UV-B in N. commune DRH1 (Fig. 5B), has also been found in the outer membrane of Synechocystis, but only as a minor compound. The function of the carotenoids in outer membranes of cyanobacteria is still not clear. Since it has been shown that heterologous expression of carotenoid genes in Escherichia coli led to an increased resistance to UV radiation (38, 39), our results suggest that myxoxanthophyll and echinenone may, indeed, act as outer membrane-bound UV-B photoprotectors of N. commune. They may be induced as a fast, SOS-type response before extracellular UV sunscreens can be synthesized.

![FIG. 3. Influence of UV-B irradiation on carotenoid synthesis. (A) Changes in total carotenoid/chlorophyll a (Car/Chl a) ratios in response to UV-B irradiation of 1.0 W m⁻². Values for UV-exposed cells were calculated and compared with those for non-UV-exposed cells and presented as mean percentage increase (P < 0.05) ± standard error. (B) Changes in carotenoid patterns after 1 day of UV-B irradiation monitored by RP-HPLC. Stippled bars, UV-B irradiation; white bars, controls. Abbreviations: Myxo, myxoxanthophyll; Zea, zeaxanthin; Canth, canthaxanthin; Echin, echinenone; β-Car, β-carotene. ∆ denotes significantly different values compared to the control (P < 0.05).](http://jb.asm.org/)

### TABLE 2. Wavelength dependence of pigment induction

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Car/Chl a (%) increase</th>
<th>OS-MAA-Chl a (mg/mg)</th>
<th>Scyt/Chl a (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Far UV-A + UV-B¹²</td>
<td>Far UV-A only³</td>
<td>Near UV-A</td>
</tr>
<tr>
<td>1</td>
<td>37.3 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>26.2 ± 0.9</td>
</tr>
<tr>
<td>2.5</td>
<td>28.1 ± 0.7</td>
<td>5.2 ± 0.6</td>
<td>30.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(10.9 ± 0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>22.6 ± 0.4</td>
<td>0.2 ± 0.4</td>
<td>45.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2.0 ± 0.3</td>
<td>3.6 ± 0.5</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>0.05 ± 0.02</td>
<td>0.38 ± 0.03</td>
<td>0.23 ± 0.05</td>
</tr>
</tbody>
</table>

¹ Car, total carotenoids; Chl a, chlorophyll a; scyt, scytominin, ND, not determined, —, not detected. DRH1 cultures received visible light (about 2.4 W m⁻²) supplemented with following UV irradiations as indicated in footnotes b to d. For detailed spectrum characteristics of UV treatments, see Fig. 1.

² λmax = 315 nm, ca. 1.6 W m⁻².

³ λmax = 330 nm, ca. 0.5 W m⁻² (or 1.0 W m⁻²).

⁴ λmax = 375 nm, ca. 1.7 W m⁻².
served in OS-MAA-producing N. commune. UV-A- and UV-B-induced syntheses of scytonemin in N. commune, but not in response to UV-A, some correlation of value. As both OS-MAA and glycans syntheses increased due to using the same extinction coefficient, correlate well with this value. OS-MAA found in desiccated field material (35), estimated by about 2% OS-MAA by dry weight (this study). Amounts of bleached when it was treated with UV-B (9).

Potential UV photoreceptors. Our results suggest that OS-MAA synthesis and scytonemin synthesis may be regulated by different photoreceptors. The synthesis of OS-MAA is induced by a UV-B photoreceptor absorbing at wavelengths below 315 nm (Table 2). A separate UV-A photoreceptor probably regulates scytonemin because its synthesis is most pronounced at near-UV-A (350- to 400-nm) irradiation, whereas far-UV-A (320- to 350-nm) irradiation had little effect. In addition to the induction by UV-A, there is a slight induction of scytonemin by UV-B (Table 2). UV-B induction and UV-A induction of chalcone synthetase are regulated separately (4). Based on our data, however, it is not possible to assess the small effects of UV-B on scytonemin.

Conclusion. Photon fluence rates of UV-B which are within the magnitude of solar fluence rates induce a cascade of physiological reactions in N. commune. In its natural habitat, Nostoc has to cope with high solar radiation in its dry state, in which photodamage cannot be efficiently repaired. Therefore, passive photoprotective mechanisms are needed. The water-soluble OS-MAA provides passive protection against UV-B and far-UV-A irradiation (1), whereas the lipid-soluble scytonemin, besides some absorption in the UV-B range, absorbs mainly UV-A (12). Since carotenoid synthesis is induced very quickly upon UV-B irradiation, outer membrane-bound carotenoids may play a role in photoprotection immediately after rewetting of desiccated colonies when the OS-MAA content is low. We propose that carotenoids, in UV protection, provide a fast, active SOS response to counteract acute cell damage whereas the extracellular glycan with its UV-absorbing pigments is a passive UV screen against long-time exposure.

ACKNOWLEDGMENTS

The technical assistance of Manfred Bold in cell counting experiments and of Markus Woitke in HPLC analysis is gratefully acknowledged. We thank Uwe Fichter for calculating the incident UV-B irradiation in the natural habitat of N. commune DRH1. We also thank Stefan Schulz for help in preparing the manuscript. Wolfgang Bilger thanks the Deutsche Forschungsgemeinschaft for financial support (grant SFB 251).

REFERENCES

UV-A/B PHOTOPROTECTION IN *N. COMMUNE*