A New UV-A/B Protecting Pigment in the Terrestrial Cyanobacterium Nostoc commune

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S. Scherer, T.W. Chen, and P. Böger
Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany

ABSTRACT

A new ultraviolet (UV)-A/B absorbing pigment with maxima at 312 and 330 nanometers from the cosmopolitan terrestrial cyanobacterium Nostoc commune is described. The pigment is found in high amounts (up to 10% of dry weight) in colonies grown under solar UV radiation but only in low concentrations in laboratory cultures illuminated by artificial light without UV. Its experimental induction by UV as well as its capacity to efficiently protect Nostoc against UV radiation is reported.

Solar UV-B radiation (280–320 nm) has been shown to severely influence metabolism, growth, and competition of both higher plants (9, 20–22, 25) and microorganisms (6, 8, 10, 12, 27). In view of a seemingly decreasing stratospheric ozone shield (11, 15) it is particularly interesting to study the protective mechanisms against solar UV-B radiation which are poorly understood as yet (24). Terrestrial cyanobacteria such as Nostoc commune are well-adapted to extreme environments of arctic (5, 7) or arid (4) areas. Nostoc commune grows on soil as colonies, i.e., as mats with an area of several square centimeters even under full sunlight in semiarid habitats. Besides its capability to withstand extreme water stress (e.g., 13, 14, 18, 19), Nostoc should have an effective protection system against high solar radiation, similar to that postulated for higher plants (2). In the latter, flavonoids or anthocyanins have been suggested to be instrumental as UV-B protection pigments (1, 3).

MATERIALS AND METHODS

Nostoc commune was collected in semiarid and human areas of the People’s Republic of China. Details on the origin of the samples are given in the figure legends. The colonies were stored in dry state in plastic bags in the dark at room temperature. Growth in the laboratory was achieved by placing thoroughly washed field-grown colonies into Petri dishes containing 2% agar with BG-11 medium. After 1 to 3 weeks, the colonies formed little bulbs which were transferred to another Petri dish and used for the experiments. Colonies were incubated at 23°C and illuminated with 2.4 W m⁻² fluorescent light (Osram L 40W/25-1, Universal White). Additional artificial UV-B irradiation was supplied by a Philips TL 40W/12 lamp (Philips, Hamburg, FRG). Nostoc cultures were placed under the UV light source for 7 to 10 d at a distance of 45 cm, resulting in an UV intensity of approximately 100 to 140 mW m⁻² nm⁻¹ at 310 nm and 50 to 70 mW m⁻² nm⁻¹ at 330 nm, respectively. The plastic material of the Petri dishes (No. 1232102, Greiner, Nüttlingen, FRG) served as an efficient filter excluding UV-C (transmission below 285 nm was found to be zero). After rewetting the colonies for 30 min in water, photosynthetic pigments were extracted with methanol for 30 min at 60°C in the dark. Absorption spectra were recorded from the clear supernatant after centrifugation. The UV-absorbing pigment was extracted from wet colonies in 30% methanol (v/v) for 30 min at 50°C. From this extract, the UV pigment was isolated by Sevag precipitation with chloroform, DEAE-anion exchange chromatography, acetone precipitation, and finally gel-exclusion chromatography with Sephadex G-25. The extinction coefficient of the isolated pigment was found to be zero.

Fig. 1. Absorption spectra in methanol of Nostoc colonies grown under different conditions. A, Nostoc commune, collected in dry state in a semi-arid area of Ningshia province, Zhou-Ling county, northwestern part of People’s Republic of China, grown under full sunlight. B, Colony collected in the humid area of Wuhan, East Lake, central region of the People’s Republic of China, grown in humid cover with low light intensity (compare Fig. 2). C, Liquid laboratory culture of Nostoc spec SAG 70.79 (from the algae collection of Göttingen University, originally isolated from a terrestrial environment) cultivated with fluorescent light without UV-B as described (17).
Fig. 2. A, Absorption spectrum of the isolated UV-absorbing pigment of *Nostoc commune*, grown outdoors in China. B, Absorption spectrum of a single colony (approximately 2 mm in diameter) grown in the laboratory. C, Same as B but including UV irradiation. The same strain was as used in Figure 1, A to C, collected in Wuhan (see legend of Fig. 1B).

be 4 cm² mg⁻¹. A detailed procedure for isolation and a preliminary chemical characterization will be published elsewhere.

**RESULTS AND DISCUSSION**

**Pigment in field-grown colonies.** Absorption spectra of methanolic extracts of *Nostoc* are shown in Figure 1. A high carotenoid to Chl ratio can be inferred from the spectra of colonies grown in full sunlight (Fig. 1A), compared to laboratory cultures (Fig. 1C) or colonies grown under low sunlight (Fig. 1B). Whether this high ratio is due to photooxidation of Chl or additional synthesis of carotenoids cannot be decided yet. Interesting is the high absorption in the region of 310 to 315 nm and 330 to 340 nm, most prominent in *Nostoc* grown under high light intensity in the natural environment (see arrows in the figure). A survey of 10 different cyanobacterial species (e.g. *Aphanocapsa, Phormidium, Mastigocladus*, or *Anabaena*) grown in liquid laboratory culture showed no significant absorption in this spectral region, even in strains isolated originally from soil (such as *Nostoc* spec of Fig. 1C). *Nostoc* colonies grown under low intensity light in the natural environment were similar to *Nostoc* grown in the laboratory (Fig. 1B). *Nostoc commune* samples grown at different places were compared in Figure 1, A to C. We cannot exclude that genetically different ecotypes were used, the absorbance in the UV region thus being genetically determined independent of environmental factors. No morphological differences between the samples were observed and further laboratory experiments showed that the formation of the pigment is environmentally regulated (see below).

We have isolated the UV-absorbing pigment from material grown outdoors. It is water soluble and localized outside the cells in the polysaccharide matrix of the colony since it can readily be extracted with water at 50°C. The absorption spectrum of the purified pigment is shown in Figure 2A, having a maximum at 312 nm and a shoulder at 330 nm. Detailed analyses under way in our laboratory suggest the presence of a pigment having a polysaccharide structure with a high nitrogen content without amino acids present and a mol wt between 1,500 and 3,000. Our data suggest the presence of two different chromophores, with absorption maxima at 312 nm and approximately 330 nm, respectively. The structure of the chromophore(s) bound to the polysaccharide core is under investigation, but an aromatic or a flavonoid structure can be excluded (W. Fiedler, unpublished findings of this laboratory).

**Induction of the Pigment by UV-A/B.** *N. commune* collected in China was grown in the laboratory under fluorescent light yielding spherical colonies 2 to 4 mm in diameter containing the UV-absorbing pigment in low concentrations only (Fig. 2B). An artificial UV-B radiation led to an enormous increase of the pigment up to a factor of five and more (Fig. 2C). The pigment content of laboratory-grown, as well as field-grown and UV-treated *Nostoc*, is quantified in Table 1. Noteworthy is the increase of the pigment relative to fresh weight (column 1). In Figure 3 the dependence of pigment induction on the intensity of UV irradiation is shown. Intensities higher than 100 mW m⁻² nm⁻¹ at 312 nm and more than 40 mW m⁻² nm⁻¹ at 330 nm (using two UV-B light lamps) led to almost colorless colonies with high amounts of mucilage and UV-B pigment, but eventually killing *Nostoc*.

These data are interpreted in terms of the new pigment serving as an UV-protecting constituent in *Nostoc*, functionally comparable to flavonoids of higher plants (3). In plants, flavonoids can also be induced by UV-B (2, 22) and seem to be localized outside the photosynthetically active tissue in epidermal cells (16). To be effective as an UV protecting system, the *Nostoc* pigment should be present in concentrations high enough to absorb most of UV from natural radiation. By relating the pigment content of single colonies to the area covered by the colony and calculating the transmission of UV after passing through the colony

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**Table 1. Content of UV Absorbing Pigment in Field-Grown and Laboratory-Grown *Nostoc commune***

<table>
<thead>
<tr>
<th>Cultivation</th>
<th>UV-A/B Absorbing Pigment in mg per:</th>
<th>Transmission at 310–330 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g Fresh weight</td>
<td>mg Chl</td>
</tr>
<tr>
<td>Laboratory-grown <em>minus</em> UV-B light</td>
<td>0.5 ± 0.16</td>
<td>14.5 ± 3.3</td>
</tr>
<tr>
<td>Field-grown (collected in Hopei Province)</td>
<td>1.3 ± 0.4</td>
<td>29.0 ± 10.2</td>
</tr>
<tr>
<td>Laboratory-grown <em>plus</em> UV-B light</td>
<td>2.4 ± 0.5</td>
<td>91.4 ± 19</td>
</tr>
</tbody>
</table>

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**UV-A/B PROTECTING PIGMENT**

![Graph](image)

**Fig. 3.** Induction of the UV-absorbing pigment by increasing intensity of UV irradiation: 100% is equivalent to 100 - 140 mW m⁻² nm⁻¹ at 312 nm and 50 - 70 mW m⁻² nm⁻¹ 330 nm. Different intensities were achieved by plastic filters made of Petri dishes (see “Materials and Methods”). Colonies collected in Wuhan (see legend to Fig. 1B) were used in this experiment.

![Graph](image)

**Fig. 4.** Absorption spectra of intact cyanobacteria. Dashed line, Liquid culture of *Anabaena variabilis*, American Type Culture Collection No. 29413, whose density has been adjusted to give approximately the same transmission at 680 nm as a *N. commune* colony of about 0.5 mm thickness (solid line) grown in the field at Wuhan, East Lake, People’s Republic of China.

(Table I), it becomes evident that most of the incident UV radiation can be absorbed by the pigment. The pigment accounts for a significant fraction of biomass of field-grown colonies, the highest contents were 7 to 10% of dry weight. Apparently, the UV-pigment may additionally participate in water storage, since it is a polysaccharide and located outside the cell in the polysaccharide matrix, responsible for rapid uptake and storage of water (14, 26). The UV transmission of a terrestrial *Nostoc* colony grown outdoors is rather low at shorter wavelengths when compared with a cyanobacterium grown in the laboratory in liquid culture (Fig. 4). In the UV-A/B region, the transmission is practically zero, demonstrating the capacity of an effective UV absorption.

Our conclusion to deal with a pigment physiologically relevant for UV-B protection is strengthened by growth experiments with field-collected material using UV-containing light in the laboratory (data not shown). Field-grown *Nostoc* is contaminated with bacteria, green algae and fungi which will increase under normal experimental cultivation conditions. Interestingly, under light including UV (for intensities, see “Materials and Methods”), *Nostoc* survived and grew, seemingly undisturbed, while contaminants were killed.

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**LITERATURE CITED**


