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Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation

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Abstract

Three filamentous and heterocystous N_2 -fixing cyanobacteria, *Anabaena* sp., *Nostoc commune* and *Scytonema* sp. were tested for the presence of ultraviolet-absorbing mycosporine-like amino acids (MAAs) and their induction by solar ultraviolet-B (UV-B) radiation. High performance liquid chromatographic (HPLC) studies revealed the presence of only one type of MAAs in all three cyanobacteria, that was identified as shinorine, a bisubstituted MAA containing both glycine and serine groups having an absorption maximum at 334 nm and a retention time of around 2.8 min. There was a circadian induction in the synthesis of MAAs when the cultures were exposed to mid-latitude solar radiation (Playa Unión, Rawson, Chubut, Patagonia, Argentina) for 3 days, 4–6th February, 2000. Solar radiation was measured by an ELDONET (European Light Dosimeter Network) filter radiometer permanently installed on the roof of the Estación de Fotobiología Playa Unión (43°18' S; 65°03' W). The maximum irradiances were around 450–500, 45–50 and 1.0–1.2 $W m^{-2}$ for PAR (photosynthetic active radiation), UV-A (ultraviolet-A) and UV-B (ultraviolet-B), respectively. PAR and UV-A had no significant impact on MAA induction while UV-B induced the synthesis of shinorine in all three cyanobacteria. Shinorine was found to be induced mostly during the light period. During the dark period the concentration stayed almost constant. In addition to shinorine, another unidentified, water-soluble, brownish compound with an absorption maximum at 315 nm was found to be induced by UV-B only in *Scytonema* sp. and released into the medium. This substance was neither found in *Anabaena* sp. nor in *Nostoc commune*. Judging from the results, the studied cyanobacteria may protect themselves from deleterious short wavelength radiation by their ability to synthesize photoprotective compounds in response to UV-B radiation. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The stratospheric ozone layer shields the Earth from the biologically most hazardous short-wave solar radiation. Recent studies have confirmed a continuous depletion of the stratospheric ozone layer, as a result of anthropogenically released atmospheric pollutants such as chlorofluorocarbons, chlorocarbons and organo-bromides and the consequent increase in solar ultraviolet-B (UV-B; 280–315 nm) radiation reaching the Earth's surface [1–4]. This process is expected to increase and spread to a broader range of latitudes throughout the 21st century [5–8].

Cyanobacteria are the largest group of gram-negative photosynthetic prokaryotes, having a cosmopolitan dis-

tribution. Members of the cyanobacteria represent important components of both aquatic and terrestrial ecosystems and possess a central position in the nutrient cycling due to their unique capacity to fix atmospheric N_2 into NH_4^+ , a form through which nitrogen enters into the food chain [9]. N_2 -fixing cyanobacteria form a prominent component of microbial populations in wetland soils, especially in rice paddy fields, where they significantly contribute as a natural biofertilizer [9,10]. Considering the vital role of cyanobacteria in crop production, the fluence rates of UV-B radiation impinging on the natural habitats seem to be of major concern since the cyanobacteria depend on solar radiation as their primary source of energy [9,11]. Because of its high energy, UV-B easily alters proteins, DNA and other biologically relevant molecules [9,12–15]. A number of vital functions such as motility, growth, survival, pigmentation, nitrogen fixation, phycobilisome assembly, $^{14}CO_2$ uptake and membrane

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permeability have been shown to be affected by UV-B radiation in cyanobacteria [9,16–20].

Like all photosynthetic organisms, cyanobacteria must withstand the impact of UV-B since they are simultaneously exposed to visible and UV radiation in their natural habitat. During their evolutionary history, cyanobacteria have developed certain mechanisms to counteract the damaging effects of UV-B. Besides repair of UV-induced damage of DNA by photoreactivation and excision repair [21,22], and accumulation of carotenoids and detoxifying enzymes or radical quenchers and antioxidants that provide protection by scavenging harmful radicals or oxygen species [23,24], another important mechanism to prevent UV-induced photodamage is the synthesis of UV-absorbing/screening compounds [25–29]. The water soluble, mycosporine-like amino acids (MAAs) which are characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol, having absorption maxima ranging from 310 to 360 nm, are thought to protect cyanobacteria from harmful UV radiation [25–32].

This study was carried out to test the inducibility of MAAs in different species of cyanobacteria in response to natural solar radiation and to identify the most effective radiation waveband in eliciting the induction of MAAs. The circadian rhythmicity of MAA induction during light and dark periods was also monitored.

2. Materials and methods

2.1. Experimental site

Experiments on MAA induction in cyanobacteria by natural solar radiation were performed on the coast of Playa Unión, Chubut (Patagonia, Argentina), during February 2000. The solar radiation was measured in three channels (UV-B, 280–315 nm; UV-A, 315–400 nm and PAR, 400–700 nm) during the experimental period using an ELDONET (European Light Dosimeter Network) filter radiometer [33] permanently installed on the roof of the Estación de Fotobiología Playa Unión (43°18' S; 65°03' W).

2.2. Test organisms

Three filamentous and heterocystous cyanobacteria, *Anabaena* sp., *Nostoc commune* and *Scytonema* sp., were used for experimentation. Isolation and purification of the cyanobacteria are detailed elsewhere [17,34]. Briefly, cultures were routinely grown in an autoclaved liquid medium [35] in Erlenmeyer flasks filled to 40% of their nominal volume and placed in a culture room at a temperature of $20 \pm 2^\circ\text{C}$ and white fluorescent light at $12 \pm 2 \text{ W m}^{-2}$. Unless otherwise stated, all experiments

were performed with log phase cultures having an initial dry weight of approximately 0.1 mg ml^{-1} .

2.3. Exposure of cyanobacteria to solar radiation

Cultures of *Anabaena* sp., *Nostoc commune* and *Scytonema* sp. were transferred into shallow plastic trays ($3 \times 11 \times 12 \text{ cm}$) that were covered on top with 395-, 320- and 295-nm cut-off filter foils (Ultraphan, Digefra, Munich and Folex PR Montagefolie 320 nm Art. No. 10155 099, Germany). The trays containing the cultures were placed on a continuously running water bath to keep the temperature constant and exposed to solar radiation (13 h light and 11 h dark period) for 3 consecutive days. At regular intervals (1 h), the cultures were shaken to avoid self-shading. The experiments started at 9:00 h (local time). Samples (5 ml \times triplicate) were removed after alternating light (4, 8, 32 and 56 h) and dark (24, 48 and 72 h) periods. The samples were immediately centrifuged and the supernatant was kept at 4°C for further analysis. The pellet was resuspended in 2 ml of double distilled water and kept at 4°C until transported to Erlangen, Germany for extraction and analysis of MAAs.

2.4. Extraction of MAAs

MAAs were extracted following the method described earlier [28]. Briefly, cells were harvested by centrifugation and MAAs extracted in 2 ml of 20% (v/v) aqueous methanol (HPLC grade) by incubating in a water bath at 45°C for 2.5 h. After centrifugation (5000 g; GP centrifuge, Beckman, Palo Alto, USA) the supernatant was lyophilized (Lyovac GT 2, Leybold, Cologne, Germany) and redissolved in 0.2% acetic acid. The samples were filtered through $0.2\text{-}\mu\text{m}$ pore-sized microcentrifuge filters (Mikro-Spin Zentrifugenfilter, Roth, Karlsruhe, Germany) before being subjected to HPLC analysis.

2.5. High-performance liquid chromatography

Analyses and purification of MAAs was done using a HPLC system (Merck Hitachi; Interface D-7000, UV-Detector L-7400, Pump L-7100, Darmstadt, Germany) equipped with a LiCrospher RP 18 column and guard ($5 \mu\text{m}$ packing; $250 \times 4 \text{ mm}$ I.D.). The samples were injected with a Hamilton syringe (Switzerland) into the HPLC column through a Rheodyne (USA) injection valve equipped with a $20\text{-}\mu\text{l}$ sample loop. The wavelength for detection was 330 nm; the mobile phase was 0.2% acetic acid at a flow-rate of 1.0 ml min^{-1} . The MAAs were identified by comparing the absorption spectra and retention times with several standards available in our laboratory. Quantification was performed using the peak area. Shinorine prepared by semipreparative HPLC was used as an external standard. The molar extinction coeffi-

cient (44 700) was used to determine the shinorine concentration of the standard [36].

2.6. Absorption spectroscopy

Absorption spectra of all samples were measured in a single beam spectrophotometer (DU 70, Beckman, Palo Alto, USA). The raw spectra were transferred to a micro-computer and treated mathematically and statistically for the peak analyses of MAAs, using the software provided by the manufacturer.

3. Results

Fig. 1 reports the irradiance of solar radiation in three channels at the study site over the study period. The maximum irradiance at local noon was around 450–500, 45–50 and 1.0–1.2 W m^{-2} for PAR, UV-A and UV-B, respectively.

HPLC chromatograms of the samples revealed the existence of a single MAA (retention time 2.8 min) in all three cyanobacteria (Fig. 2). This compound was found to be considerably induced only in the samples which were covered by the 295 cut-off filters, having maximum induction only during the light periods (Fig. 2), indicating that UV-B radiation plays an important role in the induction process of MAAs in all three cyanobacteria studied so far. There was only little induction in MAAs in the samples which were covered by the 320 or 395 cut-off filters in all three cyanobacteria indicating that UV-A and

PAR have no significant role in MAA induction in cyanobacteria (data not shown).

Absorption spectroscopic analyses of the samples revealed that the MAAs in all three cyanobacteria had an absorption maximum at 334 nm. Together with the retention time this points to shinorine, a bisubstituted MAA containing both glycine and serine groups. The absorption spectra of all three species indicate that the maximum induction of MAAs occurs mainly during light periods and by UV-B (295 cut-off filters) radiation only (Figs. 3–5). On day one, a slight increase in the amount of MAAs was recorded only after 4 and 8 h of UV-B irradiation in *Anabaena* sp. (Fig. 3). There was a slight increase in the amount of MAAs during the dark period (24 h) as well, but this could be due to the fact that the second sampling was done at 17:00 (local time) h (after 8 h of solar radiation) and there was still enough light to continue the process. On the subsequent days the induction was seen only during light periods (32 and 56 h) and a slight decrease in the amount of MAAs was recorded during dark periods (48 and 72 h) showing the circadian behavior of MAA induction during light and dark periods (Fig. 3). More or less similar results were recorded with *Nostoc commune* (Fig. 4) and *Scytonema* sp. (Fig. 5). In addition to MAAs, a brownish colored unknown compound having an absorption maximum at 315 nm was found to be induced by UV-B radiation and released into the medium in *Scytonema* sp. (Fig. 6). The induction behavior during light and dark periods was similar as for the MAAs. There was neither induction nor release of this compound in the cultures receiving UV-A (320 cut-off filters) or PAR-only (395

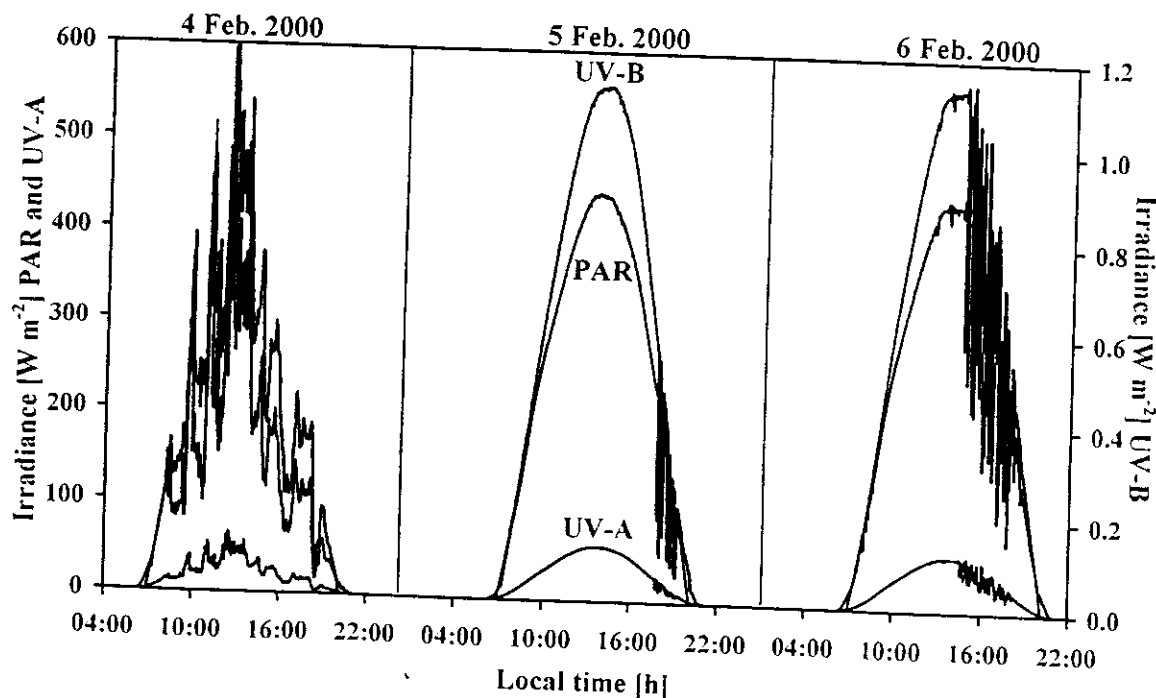


Fig. 1. Solar radiation (UV-A, UV-B and PAR) at the study site (coast of Playa Unión, Rawson, Chubut, Patagonia, Argentina) during the exposure period (4–6th February, 2000) as measured by the ELDONET (European Light Dosimeter Network).

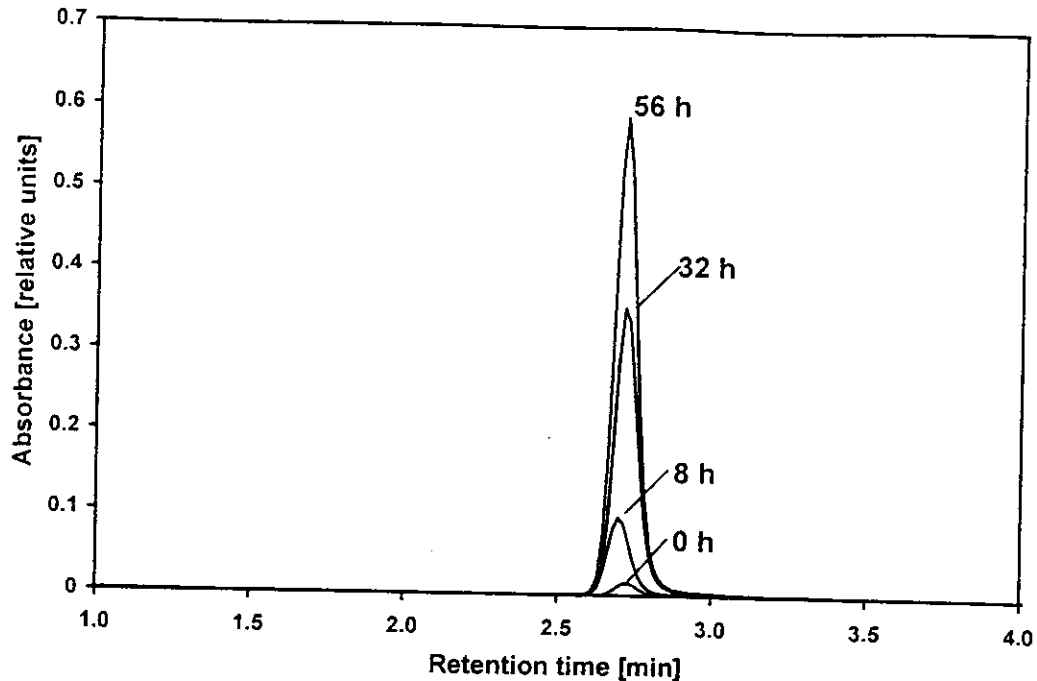


Fig. 2. Chromatograms showing the induction of MAAs (shinorine) in *Anabaena* sp. when the cells were exposed to solar radiation with 295 cut-off filters for indicated time periods. For clarity chromatograms for 4, 24, 48 and 72 h are not shown. Similar results were recorded with *Nostoc commune* and *Scytonema* sp. (data not shown).

cut-off filters) radiation. This compound was absent from the other two studied cyanobacteria (i.e. *Anabaena* sp. and *Nostoc commune*). Fig. 7 is constructed based on the results obtained on MAAs induction by solar radiation

under natural conditions in three studied cyanobacteria and summarizes the circadian induction of MAA in cyanobacteria mostly during the light period and by UV-B (295 cut-off filters) radiation. The results clearly show that

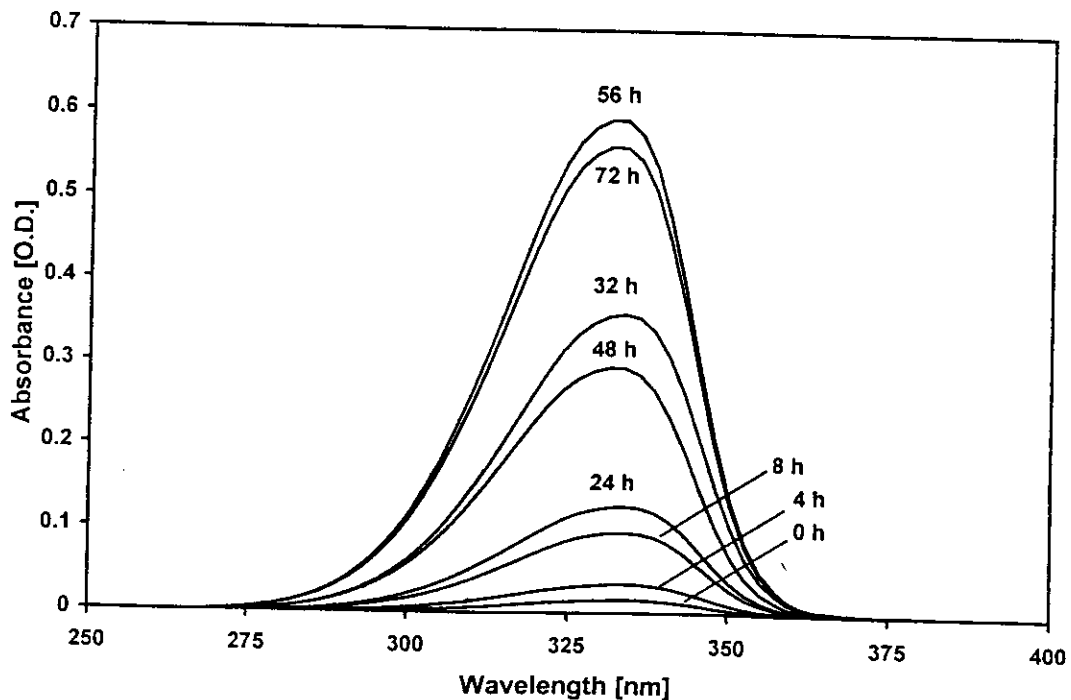


Fig. 3. Absorption spectra of the cells showing the induction of MAAs by UV-B (295 cut-off filter) radiation in *Anabaena* sp. after different durations of alternating light (4, 8, 32 and 56 h) and dark (24, 48 and 72 h) periods. There was only little induction in the amount of MAAs in the cultures covered with 320 and 395 cut-off filters (data not shown).

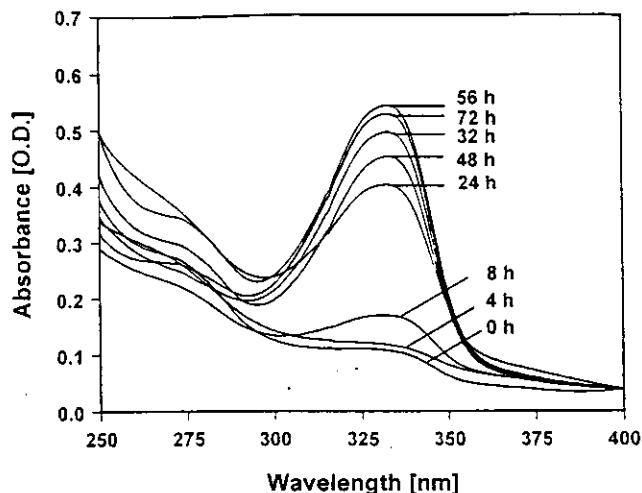


Fig. 4. Absorption spectra of the cells showing the induction of MAAs by UV-B (295 cut-off filter) radiation in *Nostoc commune*. Other conditions as in Fig. 3.

UV-A (320 cut-off filters) and PAR (395 cut-off filters) elicited only marginal or no induction of MAAs in all the studied cyanobacteria.

4. Discussion

The present investigation shows that the studied cyanobacteria are able to synthesize MAAs in response to UV-B radiation. *Scytonema* sp. seems to be more tolerant to UV-B radiation due to its ability to synthesize not only almost double the amount of MAAs but also to an additional unknown UV-B absorbing compound in comparison to *Anabaena* sp. and *Nostoc commune*. The unknown UV-B absorbing compound in *Scytonema* sp. seems to be a novel compound since its absorption and

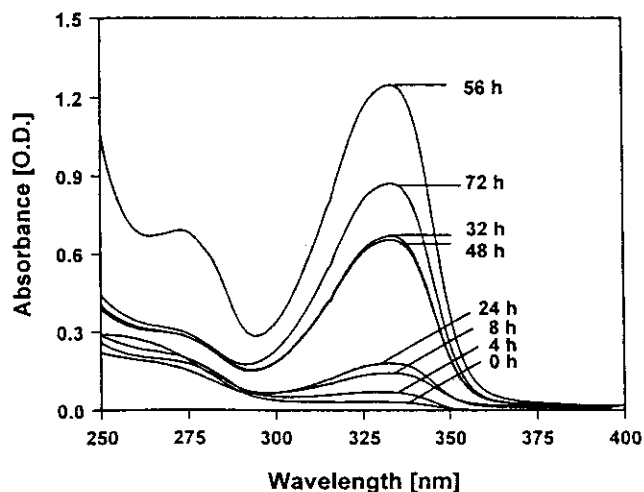


Fig. 5. Absorption spectra of the cells showing the induction of MAAs by UV-B (295 cut-off filter) radiation in *Scytonema* sp. Other conditions as in Fig. 3.

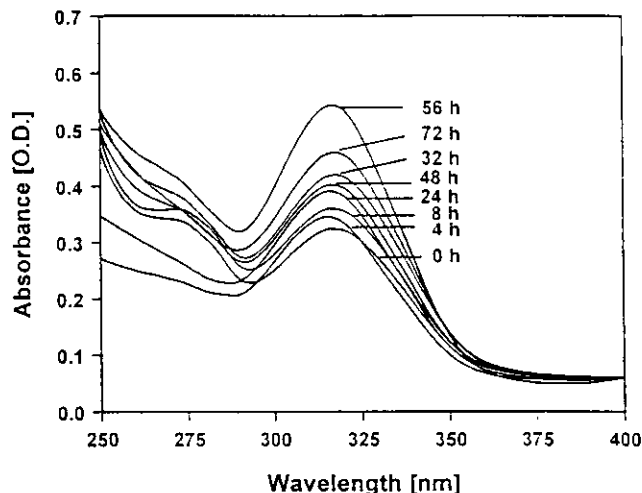


Fig. 6. Absorption spectra of the supernatant showing the induction of a brownish colored unknown compound (λ_{\max} 315 nm) in *Scytonema* sp. by UV-B radiation (samples covered with 295 cut-off filters). There was neither induction nor release of this compound by either UV-A or PAR radiation (cultures covered with 320 and 395 cut-off filters, respectively).

other properties neither matches with known MAAs nor with other lipid soluble UV protective compound such as scytonemin found in some cyanobacteria [27]. Earlier, Sinha et al. [17] have shown that *Scytonema* sp. was more tolerant to UV-B radiation in comparison to *Anabaena* sp. and *Nostoc* sp. The occurrence of high concentrations of MAAs in cells exposed to high levels of solar radiation has been described to provide protection as a UV-absorbing/screening compound [24,27,29] but conclusive evidence is lacking to a great extent for the exclusive role of MAAs as a sunscreen, and it is possible that they play more than one role in the cellular metabolism of all or some organisms [37]. It has been suggested that MAAs may act as antioxidants to prevent cellular damage resulting from UV-induced production of active oxygen species [38]. Synthesis and excretion of MAAs have been reported to be stimulated by UV radiation in a dinoflagellate, *Lingulodinium polyedra* [39]. MAAs were found to increase in response to PAR and UV radiation in a red alga, *Chondrus crispus* [40]. In many organisms MAA accumulation is strongly stimulated by UV-A [32] which has an about 50 times higher irradiance in solar radiation than UV-B. Laboratory experiments have shown the induction of MAAs by UV radiation in a rice-field cyanobacterium, *Anabaena* sp. [28]. The MAAs in *Nostoc commune* have been shown to be located extracellularly and linked to oligosaccharides in the sheath [41]. These glycosylated MAAs represent perhaps the only known example of MAAs that are actively excreted and accumulated extracellularly and therefore act as a true screen [30]. But intracellular MAAs are also effective protectants against UV-induced damage. MAAs have been reported to prevent 3 out of 10 photons from hitting cytoplasmic targets in cyanobacteria [26]. Cells with high concentrations of

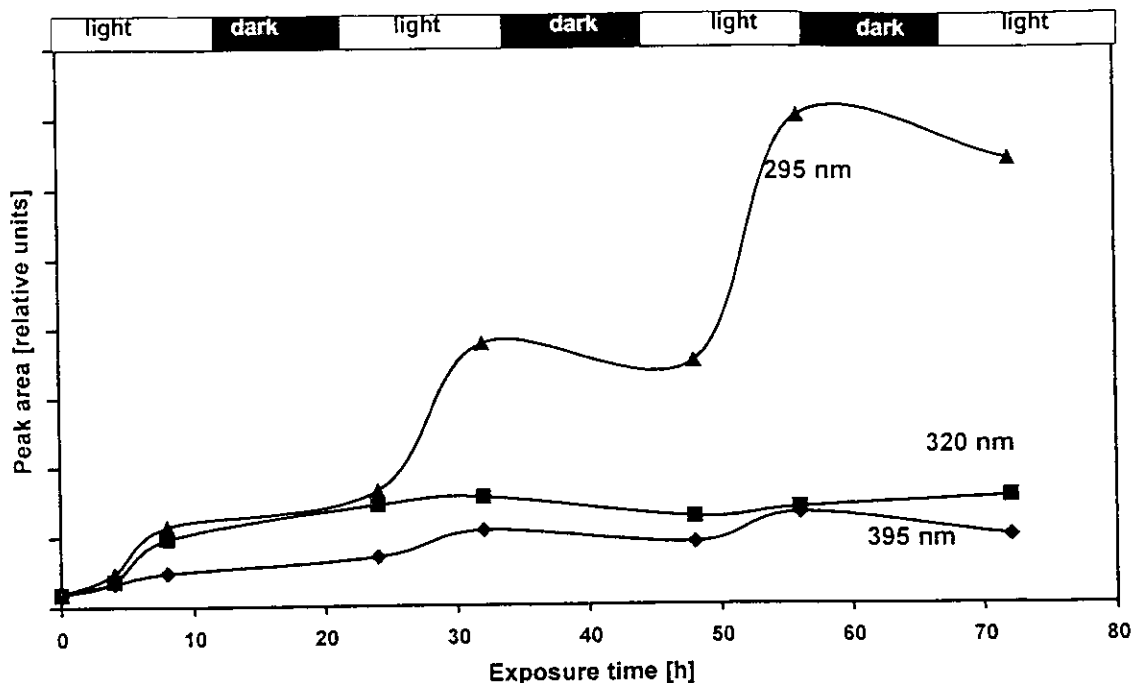


Fig. 7. Circadian induction behavior of MAAs in cyanobacteria in response to UV-B (295 cut-off filters) radiation. Note the pronounced induction of MAAs in the light phase. UV-A and PAR had very little impact on MAA induction in the studied cyanobacteria.

MAAs are approximately 25% more resistant to UV radiation centered at 320 nm than those with no or low concentrations [26]. However, there may be physiological limitations to the accumulation of osmotically active compounds such as MAAs within the cell, and it seems probable that the maximal specific concentration of MAAs in the cell is regulated by osmotic mechanisms which is reflected by the fact that field populations of halotolerant cyanobacteria contain an unusually high concentration of MAAs [42]. UV and osmotic stress have been reported to induce and regulate the synthesis of MAAs in the cyanobacterium *Chlorogloeopsis* sp. under laboratory conditions [43,44].

Under natural conditions, MAA induction in the studied cyanobacteria depends on the light quality and shows a circadian rhythm. The induction of MAA normally occurs during the light period and remains almost constant during the dark period. Out of the three wavebands, UV-B radiation plays an important role in MAA induction. Several other biological processes such as the synthesis of phycobilisomes and photosystem II reaction center proteins are regulated by light [45]. We conclude that the presence of MAAs in an organism and its circadian induction by short-wavelength radiation may provide protection to the internal structures and components from the impact of deleterious UV-B radiation. It is evident from the present investigation that the studied cyanobacteria are able to raise their MAA content in response to UV-B radiation and thus may be able to adapt to daily fluctuations in solar radiation impinging on their natural environment.

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