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Compositional changes in mycosporine-like amino acids induced by UV radiation: marine dinoflagellate *Scrippsiella sweeneyae*

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The compositional changes in mycosporine-like amino acids (MAAs) were investigated in the marine dinoflagellate *Scrippsiella sweeneyae* exposed to four different spectral compositions and five relative intensities of UV-B (280-320 nm) to UV-A (320-400 nm) + photosynthetically available radiation (PAR: 400-700 nm). Neither dose nor wavelengths of UVR significantly affected the growth rates. UVR caused a significantly increase in cell volume. Cell volume in the >280nm treatment was more than two times greater at 6.8 % of UVR intensity. Production of UVR induced MAAs was dependent on the dose of UVR. However, the induction of MAAs was related to the cell growth. Greater induction of MAAs was observed at shorter wavelengths. The composition of MAAs varied with increasing light intensity of UVR.

Key words: Absorption, Cell volume, Dinoflagellates, Mycosporine-like amino acids, PAR, Photoprotection, UV-B, UV radiation, UV-absorbing compounds

Introduction

Solar ultraviolet B (UV-B: 280-320 nm) irradiance has been one of the most significant variable in the aquatic environment throughout evolution [1]. UV-B radiation may impair the light-dependent responses of marine phytoplankton [2]. UV-B has a high energy level per photon and is effectively absorbed by nucleic acids, proteins, and pigments. Phytoplankton have developed defenses to protect their cells from its damaging effect. UV-absorbing compounds (absorbing in the range 310 to 360 nm) found in some microalgae act as a natural sunscreen [3]. One of the UV-absorbing compounds is known as mycosporine-like amino acids (MAAs). In a few species the effects of spectral composition and intensity of UV radiation (UVR) on the formation of MAAs have been investigated and are found to differ considerably among species [4-6]. In the present study we have investigated the influence of UV radiation on MAAs cellular content and compositional changes in MAAs of the marine dinoflagellate *Scrippsiella sweeneyae* exposed to four different spectral compositions and five relative intensities of UV-B to UV-A (320-400 nm) + photosynthetically available radiation (PAR: 400-700 nm).

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Materials and Methods

Incubation and Sampling

Scrippsiella sweeneyae Balech (NIES-684) were all grown in quarts bottle in GPM medium with a supplement of 10^{-8} M selenium [7] using filtered sea water from Sagami Bay, Japan.

Algal cells were exposed to PAR at 20.7W·cm^{-2} provided by a cool white fluorescence tube with 12 h light and 12 h dark cycle at 25°C. Cell density of the cultures was determined microscopically every day. Specific growth rate was calculated over the exponential growth phase. Once they reached the exponential growth phase, they were exposed further for three days to the same intensity of PAR with UVR provided by Toshiba fluorescence tube model FL202E [8]. A quartz bottle allowed penetration of all wavelengths (>280nm). The UVR screening filters were employed. UV-Guard filter (>400nm), mylar films (>313nm), and acetate films (>290nm). UVR was also controlled by neutral density filters to provide five intensities. Both PAR and UVR in μW·cm^{-2}·s^{-1} were

measured by Biospherical Instruments radiometer model GMR610 and PUV510, respectively. PAR, UV-A, and UV-B radiation was calculated using intensity of each wavelength gave from the instruments (Table 1) [9]. The spectral ratio was calculated as UV-B/(UV-A+PAR) using those integrated values for each wavelength region [10].

Samples were collected for cell volume and individual MAA analysis prior to additional UVR exposure (time zero) and also during the three days of UV exposure period.

Cell volume

The thickness, width, and height of 100 cells was measured randomly under the microscope and cell volume was estimated from the three cell dimensions assuming an ellipsoid shape for the cells [11].

Mycosporine-like amino acids.

Triplicate subsamples of cell suspension were filtered onto 25mm Whatman GF/A glass fiber filters, and extracted in 1ml of 20% methanol for at least 24 h at 37°C in the dark after sonication. This solvent mixture was then centrifuged at 10,000 rpm for 5 min. The supernatant (1ml) was taken to evaporate under reduced pressure, and redissolved in 1ml of 100% methanol, and centrifuged at 10,000 rpm for 5 min. A 900μl aliquot of the supernatant was evaporated for a complete dryness at 45°C. The residue was redissolved in 100μl of MilliQ water, and filtered through a 0.2μm membrane filter for HPLC analysis.

Extracts were eluted with 25:75:0.2 methanol:MilliQ water:acetic acid in Shiseido CAPCELL PAK C_{18} UG120 5μm (250mm×4.6mm I.D.) and Shiseido SG120 guard column 5μm (35mm×4.6mm I.D.) at a flow of $1 ml \cdot min^{-1}$. The column was re-equilibrated between samples with 10min of 80:20 MilliQ water:0.5 M ammonium acetate. Detection of peaks was by UV absorbance at 320 and 334nm. Identities of peaks were confirmed by co-chromatography using known standards. Detection of MAAs without standards were made using a photodiode array detector in the wavelength range between 200 and 400nm.

Results and Discussion

Cell growth and Cell volume

Neither dose nor wavelengths of UVR (Table 1) significantly affected the growth rates (χ^2=3.82, p>0.05, χ^2=3.13, p>0.05, respectively). However, the growth rate of cells at 6.8% of UVR intensity in the >280nm treatment showed a slight decrease of 22% as compare with PAR.

Average cell volume prior to additional UVR was 9850μm^3. However, increased UVR intensity caused a significantly increase in cell volume. Particularly, the shorter wavelengths significantly increased cell volume. Cell volume in the >280nm treatment was greater than two times at 6.8% of UVR intensity. Variations in cell size were considered significant in photoprotection since increased cell volume could protect sensitive sites, particularly centrally located DNA, by increasing the pathlength of light travelling through the cell [12]. In *Scrippsiella sweeneyae*, the increased cell volume without inhibition of growth rates suggests that increased cell volume might protect DNA by increasing the pathlength of the cell.

Table1. Integrated radiation of UV-B, UV-A, and PAR with a ratio of UV B to UV-A+PAR.

Experiment	UV-B (280-320 nm)	UV-A (320-400 nm)	PAR (400-700 nm)	UV-B/(UV-A+PAR)
		($W m^{-2}$)		(%)
1	0.061	0.299	20.7	0.29
2	0.585	0.565	26.1	2.2
3	1.040	0.784	28.1	3.6
4	1.820	1.170	36.9	4.8
5	2.620	1.540	36.9	6.8

Mycosporine-like amino acids

HPLC analysis of MAAs indicated the presence of a complex mixture of five MAAs in *Scrippsiella sweeneyae*. Of these five MAAs, shinorine, palythine, and porphyra-334 were identified in the present study. The other two were unknown MAAs, and the maximum absorption of these unknown MAAs was determined to be at 310 and 360nm, respectively. The cellular contents of shinorine, palythine, and porphyra-334 were changed by UVR exposure to 0.12-0.61 pmol/cell, 0.042-0.22 pmol/cell, and 0.020-0.19 pmol/cell, respectively. Shinorine was the most predominant MAA (50-70%) among these identified MAAs.

The cellular contents of shinorine, palythine, and porphyra-334 at time zero of additional UVR exposure were 0.20±0.03, 0.067±0.023, and 0.055±0.025 pmol/cell, respectively. MAAs were significantly increased by UVR exposure at 4.8% and 6.8% of UVR intensity. Especially, UV-B (>290nm and >280nm treatment) induced more effectively MAAs than UV-A (>313nm treatment). The shorter wavelengths of UV-B (280-290nm) induced more MAAs. In contrast, exposure to >280nm wavelengths resulted in decreased overall MAAs induction in dinoflagellate *Gyrodinium dorsum* [5]. The effects on MAAs induction by UVR exposure could vary among species. Induction of porphyra-334 was observed at 6.8% of UVR intensity, while it did not occur between 0.3% and 4.8%. At 6.8% of UVR intensity in the >280nm treatment of the second day of UVR exposure, the cellular contents of MAAs reached maximum contents of 0.61, 0.22, and 0.19 pmol/cell for shinorine, palythine, and porphyra-334, respectively.

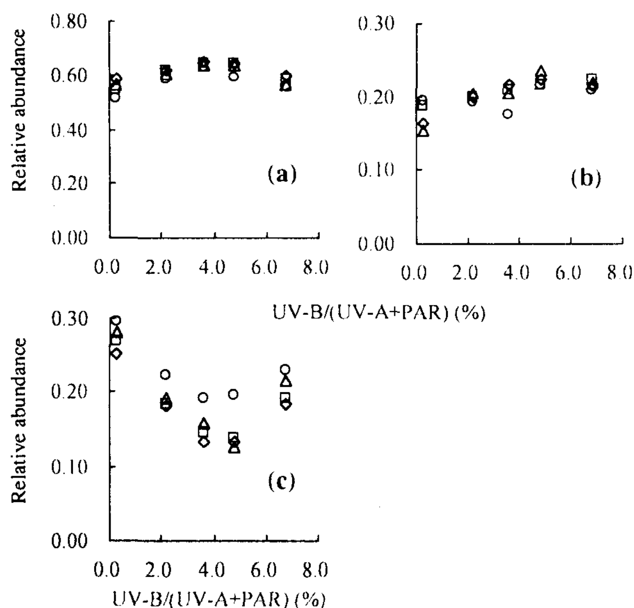

Figure 1. The relative abundance of induced shinorine (a), palythine (b), and porphyra-334 (c) at different intensities of UVR exposed to different spectral composition (○:>400nm, △:>313nm, □:> 290nm, and ◇:>280nm) of the second day of UVR exposure.

The induced MAAs was related to the cell growth. The induced MAAs of the third day of UVR exposure decreased at the end of experiment in the present study, except for palythine at 3.6% of UVR intensity. The induction of MAAs by UVR exposure was indicated to be influenced by the cell growth activity.

Compositional change in MAAs

UVR intensities induced changes in the composition of MAAs. (Fig. 1). The relative abundance in porphyra-334 was observed to vary with increasing UVR and also wavelengths. The relative abundance in shinorine and palythine also varied with UVR but their changes were not affected by wavelengths of UVR.

It might be concluded from the present study that in order to acclimate to the different UVR, *Scrippsiella sweeneyae* changed its composition of MAAs.

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