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Cellular Mycosporine-like amino acids protect photosystem II of the Dinoflagellate *Scrippsiella sweeneyae* from ultraviolet radiation damage

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ABSTRACT

Photo-damage to photosystem II (PSII) from ultraviolet radiation (UVR) was determined using chlorophyll fluorescence in relation to sunscreen factors on the dinoflagellate *Scrippsiella sweeneyae* based on the cellular mycosporine-like amino acid contents ($C_{MAAs,\ cell}$) and cell diameter (= light path, *d*). Three different $C_{MAAs,\ cell}$ were prepared by acclimating cells to three levels (30.8, 15.2, and 7.7 W m^{-2}) of photosynthetically active radiation (PAR). PAR-acclimated cells were exposed to PAR (0.64 W m^{-2}) + UVR (3.94 W m^{-2} = 2.51 W m^{-2} UVB + 1.43 W m^{-2} UVA) for 12 min. High PAR (HL) and medium PAR (NDF1) treatments acclimated cells to induce shinorine and porphyra-334 (longer λ_{max} at 333 and 334 nm); whereas, the low PAR (NDF2) treatment acclimated cells to induce mycosporine-glycine and palythine (shorter λ_{max} at 310 and 320 nm). Absorption spectra for the individual MAAs were reconstructed using the λ_{max} and $C_{MAAs,\ cell}$ and were summed to reconstruct the absorption of the total $C_{MAAs,\ cell}$ (m^2 cell^{-1}) to estimate the sunscreen factor ($S_{[\lambda]}$) at λ_{max}. The highest $S_{(\lambda max)}$ was obtained for cells that acclimated to the highest PAR (highest $C_{MAAs,\ cell}$ and longest *d*); whereas, the lowest $S_{(\lambda max)}$ was obtained for cells acclimated to the lowest PAR (the lowest $C_{MAAs,\ cell}$ and the shortest *d*). $C_{MAAs,\ cell}$ contributed approximately 94%, whereas *d* contributed < 6%, of the sunscreen factor ($S_{\lambda max}$). UVR-induced damage was indexed with a temporal decrease in the optimum quantum yield (F_v/F_m) in the Photosystem II. The highest damage was observed for cells acclimated to the lowest $S_{\lambda max}$ (lowest $C_{MAAs,\ cell}$ and shortest *d*); whereas, the lowest damage was observed for cells acclimated to the highest $S_{\lambda max}$ (highest $C_{MAAs,\ cell}$ and longest *d*). The $C_{MAAs,\ cell}$ mitigated most of the UVR-induced damage in photosystem II of the dinoflagellate *S. sweeneyae*.

1. Introduction

1.1. UV-absorbing compounds and cell volume

The effect of ultraviolet radiation (UVR) on the primary production or the photosynthesis of phytoplankton in the ocean has been studied extensively to accurately predict the effects of ozone depletion on the marine ecosystem [e.g., 1,2,3,4,5]. Phytoplankton has evolved to protect from DNA damage caused by UVR by synthesizing UV-absorbing compounds such as MAAs [6,7,8]. Mycosporine-like amino acids are effective UV-absorbing compounds present mostly in microalgae, particularly dinoflagellates [9]. The most common MAAs are palythene, palythine, mycosporine-glycine, palythenic acid, porphyra-334, and shinorine. The relative abundance and the occurrence of each MAA are variable among species of dinoflagellates. For example, palythenic acid is not observed in the dinoflagellate *Scrippsiella sweeneyae* [10]. Individual concentrations of all five MAAs are variable and exhibit a diel cycle [10]. The cellular contents of MAAs ($C_{MAA,\ cell}$) are also dependent upon cell volume (V_{cell}), which is influenced by UVR [11,12,13]. The capability to produce MAAs through photo induction is limited mostly to certain taxa, particularly dinoflagellates [14]. When the $C_{MAA,\ cell}$ are controlled by PAR [15,16], UVR research based on cells with known abundance of MAAs may provide fundamental cues based on the temporal photoprotection response of cells to UVR.

1.2. Exposure duration

In an earlier study [13], the dinoflagellate *Scrippsiella sweeneyae* could achieve optimal $C_{MAA,\ cell}$ and V_{cell} to mitigate possible UVR-mediated DNA damage in exponentially growing cells under the PAR + UVR condition. However, fresh exposure to the PAR + UVR condition from the PAR condition could provide an alternative evaluation on the role of MAAs based on a short-term experiment (~10 min) among different $C_{MAA,\ cell}$. The initial response of chlorophyll fluorescence to

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UVR is expected to have a time-dependent stage and assumed to be linear before transitioning to the asymptotic equilibrium stage [17,18]. The time resolution, which is commonly used in the conventional carbon fixation method (~30 min), was too broad to determine the suitability of the initial responses of chlorophyll fluorescence. To estimate particularly the initial slope of the photo responses of chlorophyll fluorescence, a shorter time resolution (< 10 min) is required. In various parameters obtained from chlorophyll fluorescence, the measurement of the optimum quantum yield (F_v/F_m) [19] can provide a sensitive indicator of photosynthetic performance [20]. Information on the protective capacity of a given species can be obtained by comparing the initial sensitivity of cells with different $C_{MAAs,\ cell}$, which are, for example, provided from bathymetrically variable habitats, to a given UVR dose in a field [21]. An alternative way to provide different $C_{MAAs,\ cell}$ to cells is the usage of PAR in a controlled laboratory [16]. Because the induction of MAAs was provided by a moderate PAR [22] but not too high of a PAR [23], PAR was applied to produce different levels of the $C_{MAAs,\ cell}$ in the present study.

2. Purpose

The aim of this study is to fit a linear relationship by developing shorter exposure experiments with UVR for *Scrippsiella sweeneyae* using fluorescent methods [20] with three different initial $C_{MAAs,\ cell}$, which are produced by exposing cells to different PARs as reported by Carreto et al. [16]. If a linear fit was in fact approved as a robust method for evaluating the photo damage to Photosystem II during UVR exposure, the evaluation would assist in understanding the universality and photo inducibility of MAAs in the species. All abbreviation was summarized in Table 1.

3. Materials and methods

3.1. Culture and experimental conditions

Scrippsiella sweeneyae Balech (NIES-684) was obtained from the algal collection at the National Institute of Environmental Science, Japan (http:/mcc.nies.go.jp). The algae were grown at 25 °C in a GPM medium [24], prepared with filtered sea water collected from Sagami Bay, Japan. The growth medium was supplemented with 10^{-8} M sodium selenite (Merck, USA) which was required to estimate the growth and cell yields of bloom-forming phytoplankton species [25].

3.2. Acclimation experiments

Semi-continuous cultures with cell densities of approximately1,600 ± 272 cells mL^{-1} were maintained in the exponential phase at a relatively high PAR (30.8 W m^{-2}) with cool white fluorescent tubes (FLR40S, Toshiba, Japan [13]) in a 2 L bottle.

To obtain the different cellular MAA contents, the cells were incubated in quartz bottles with acetate film and neutral density filters (NDF, 49.4% and 25%). Three levels of PAR were used: 30.8 W m^{-2} in the HL experiment, 15.2 W m^{-2} in the NDF1 experiment, and 7.7 W m^{-2} in the NDF2 experiment. The incubation was conducted from the beginning of the light period for 6 h because the species exhibited a diel cycle [10].

3.3. Exposure experiments

Exposure experiments on the acclimated cells were performed in six quartz bottles in front of two UVR lamps (FL202E, Toshiba, Japan) with a relatively low PAR (0.64 W m^{-2}) to enhance a proportion of UVR (3.94 W m^{-2} = 2.51 W m^{-2} UVB + 1.43 W m^{-2} UVA) in the total light (PAR + UVR). All cells in the quartz bottles were placed on a plankton wheel (Rotator RT-550, Taitec, Japan) so they would be exposed to equal amounts of light and to prevent the cells from sedimentation during the incubation. The same volume of subsamples (30 mL) were collected from each bottle every 2 min for 12 min, and pooled into an opaque bottle. Triplicate subsamples were collected to determine the cell density (D), the cell volume (V_{cell}), the $C_{Chl,\ cell}$, the $C_{MAAs,\ cell}$, the $a_{MAAs,\ cell}$, and the F_v/F_m. Each experimental run was conducted independently in triplicate.

3.4. Analysis

3.4.1. Cell density and volume

The cell density was determined under an inverted microscope (model Olympus, Tokyo, Japan). The cell volume (V_{cell}: μm^3) was estimated using the measurements of length (l), width (w), and height (h) for 100 cells from each exposure experiment by assuming an ellipsoid shape for the cell using the following equation described by Hillebrand et al. [26]:

$$V_{cell} = \pi lwh/6 \quad (1)$$

The equivalent spherical diameter (d) was estimated assuming the cell shape was spherical.

3.4.2. Chlorophyll a

Cell suspensions were filtered onto a 25-mm glass fiber filter (GF/A, Whatman, UK) and extracted in 1.5 mL of 100% methanol (High Performance Liquid Chromatography [HPLC] grade, Wako, Japan). Subsamples were then sonicated for 5 min in an ice bath and extracted for 24 h at − 4 °C in the dark. Extracts were filtered through 0.2-μm membrane filters (Mylex, Whatman, UK). The methanol extracts were analyzed on an HPLC (System Gold, Beckman, USA) with a C18 reversed-phase Ultrasphere 3-μm column using a solvent gradient system similar to those described by Head and Horne [27].

3.4.3. Mycosporine-like amino acids

Cell suspensions were filtered onto a 25-mm glass fiber filter (GF/A, Whatman, UK) and extracted in 1.5 mL of 20% methanol (HPLC grade, Wako, Japan) to remove nonpolar photosynthetic pigments [28] for at least 24 h at 37 °C in the dark. The solvent mixture was then centrifuged at 10,000 rpm for 5 min. The supernatant (1 mL) was evaporated under reduced pressure, and redissolved in 1 mL of 100% methanol, a process known to remove proteins and salts, which frequently pose a problem during a HPLC runs [28]. The extracts were vortexed and centrifuged again at 10,000 rpm for 5 min, and the supernatant (900 μL) was carefully separated and evaporated to complete dryness at 45 °C [28,29]. The residue was redissolved in 100 μL of a double-distilled water and filtered through a 0.2-μm membrane filter (Mylex, Whatman, UK) for HPLC analysis.

MAAs were separated using reverse phase HPLC in a 5-μm column (250 mm × 4.6 mm I.D., Capcell Pak C18 UG120 Shiseido, Japan) and a 5-μm guard column (35 mm × 4.6 mm I.D., SG 120, Shiseido, Japan). During each run, the column was equilibrated with a 100% solvent A (80% double distilled water and 20% [v/v] 0.5 M ammonium acetate [Wako, Japan]) run at a flow rate of 1 mL min^{-1}, followed by a 100% eluent B (75% MilliQ water, 25% [v/v] methanol, and 0.2% acetic acid [Wako, Japan]) run for 20 min. Elution of MAAs from the column was detected by measuring absorbance at both 310 nm and 334 nm [30]. MAA concentrations were calculated from peak areas (PA) detected at 334 nm using peak areas for an individual MAA standard during a calibration run. Common MAAs were identified by their retention time: absorption spectra from 290 nm to 400 nm: and co-chromatography with authentic standards for shinorine, *palythine*, porphyra-334, and palythene, which were obtained from the red algae *Tichocarpus crinitus, Chondrus yendoi, Porphyra yezoensis,* and *Palmaria palmata*, respectively [13]. Mycosporine-glycine was obtained from cultures of the dinoflagellate *Alexandrium tamarense* [10]. Quantification was made using the following molar extinction coefficients: shinorine: ε_{334} = 44,700 [31], palythine ε_{320} = 36,200 [32], porphyra-334: ε_{334} = 42,300 [33],