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Physiological response of marine centric diatoms to ultraviolet radiation, with special reference to cell size

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ABSTRACT

Three centric diatoms, *Thalassiosira pseudonana* (diameter ~4 μm), *Thalassiosira weissflogii* (~11 μm), and *Thalassiosira punctigera* (~47 μm), were exposed to low and high levels of UV radiation. UV-induced inhibition on photosystem II was correlated with cell size under high light levels, though it was insignificant under low light levels (PAR < 63 W m^{-2}). The highest inhibition (~15%) was observed for the smallest species. Several mechanisms may explain the observed relationship between cell size and response to UV. All three species counteracted UV-related photosystem damage via protein synthesis within the chloroplast. Non-photochemical quenching (NPQ) was induced when that process was blocked with an inhibitor in *T. pseudonana* and *T. weissflogii*, but not *T. punctigera*, as neither radiation nor the inhibitor had a significant effect on NPQ in this species. Moreover, UV-induced inhibition for cells treated with lincomycin was highest for *T. weissflogii*, which was in accordance with the highest UV exposure within the cell. The intracellular UV distribution was not associated with cell size, indicating that the package effect was not the only determinant of cell-size dependent UV sensitivity in phytoplankton.

1. Introduction

Phytoplankton in the euphotic zone utilize sunlight for photosynthesis, but may also be harmed by ultraviolet (UV) radiation at shallower depths [1]. The biological effects of UV radiation depend on wavelength [2]; the most detrimental radiation (UVC) is completely filtered out by diatomic oxygen and ozone, while the intensity of UVB is negatively correlated with the thickness of the ozone layer [3]. Though the use of CFC gases has been prohibited since the Montreal Protocol was established, some newly identified trace gases, such as halohydrocarbons, also destroy the ozone layer, resulting in increased UV radiation [4]. The precise effects of UV radiation in aquatic as well as terrestrial environments are still uncertain [5]. Phytoplankton that are exposed to high levels of UV radiation may be at risk for damage associated with climate change [6,7].

As the most productive phytoplankton group, diatoms account for 20% of the global primary production and play a fundamental role in the marine food web due to their abundance and size structure [8]. In particular, large diatoms contribute substantially to buried carbon owing to their high carbon content and fast sinking rate [9,10]. Therefore, the responses of differently sized diatoms to UV radiation may impact primary production and carbon export [9]. In theory, larger phytoplankton should be more resistant to UV radiation owing to package effects, i.e., more self-shading occurs in large than in small cells [11,12]. However, it is still unclear whether larger cells have more diluted pigments that would offset package effects, at least partially [13].

Field studies have been conducted to investigate the relationship between cell size and UV sensitivity, but the results of these studies vary. Some studies suggested that cell size is not a good indicator of UV sensitivity [14], but a recent study showed that photosynthetic carbon fixation of small phytoplankton was much more sensitive to UV than that of larger species in a coastal area [15]. At the molecular level, small cells are vulnerable to cyclobutane pyrimidine dimer accumulation, and photosynthesis is less inhibited by UV radiation [16], which may be attributed to faster photo-repair of small cells [17]. The light absorbed and scattered by cytoplasmic inclusion is an important determinant of the effects of UV on photosynthesis [11]. Additionally, phytoplankton species with differential pigments, geometry, and sub-cellular structures could differ significantly with respect to the intracellular light regime, and thus the UV exposure of photosynthetic organelles [11]. Figueroa et al. found that the bio-optical characteristics of a culture [18], rather than cell size or chlorophyll concentration, determine UV sensitivity. An intraspecific comparative analysis has shown that large species are less sensitive to UV than smaller species [19], indicated that the differences among species may outweigh the size effects; for example, *Phaeodactylum tricornutum* was more sensitive than *Thalassiosira pseudonana*, despite their similar sizes [20].

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The relationships between light absorption, cell size, and photosynthesis have been investigated [11,21,22], and recent studies have revealed a strong correlation between cell size and photo-inactivation in centric diatoms under high-PAR conditions [23]. Few laboratory studies have examined the UV responses of diatoms of various sizes, particularly using closely related species, which are expected to differ minimally with respect to traits such as cell geometry and pigmentation. In this study, we selected three species within the genus *Thalassiosira* that ranged in cell diameter from ~4 to ~47 μm to determine whether UV sensitivity is correlated with cell size and the underlying mechanisms that mediate this relationship.

2. Materials and Methods

2.1. Species and Culture Conditions

Thalassiosira weissflogii (CCMA102, ~11 μm) was isolated from the South China Sea in 2004 and obtained from the Center for Collection of Marine Bacteria and Phytoplankton (CCMA) of Xiamen University. *T. pseudonana* (CCMP1335, ~4 μm) was obtained from the National Center for Marine Algae and Microbiota (NCMA). *Thalassiosira punctigera* (CCAP 1085/19, ~47 μm) was obtained from the Culture Collection of Algae and Protozoa (CCAP).

Cells were inoculated in pre-aerated, sterilized filtered seawater and enriched with Aquil medium. Cultures were maintained semi-continuously with a maximal chlorophyll concentration of below 20 μg L^{-1} in polycarbonate bottles (500 mL). Cultures were illuminated with cool fluorescent tubes at a photon flux density of ~200 μmol m^{-2} s^{-1}, with a 12:12 light/dark cycle at 20 ± 1 °C, and culture bottles were manually shaken 3–4 times per day and then randomly distributed in the growth chamber.

2.2. Determination of Cell Absorbance, Chlorophyll Concentration, and Size

The 100-mL cultures (or media for blanks) were filtered on GF/F filters, and then gently attached to the filter holder in the spectrophotometer, which was equipped with an integrating sphere (Lambda950, PerkinElmer, Waltham, MA, USA). The absorbance was scanned between 280–750 nm and corrected using the blank filter. After measurements, filters were immediately extracted in absolute methanol for 2 h at room temperature and centrifuged. The supernatant was measured with a spectrophotometer (DU800, Beckman, Pasadena, CA, USA), and the chlorophyll concentration was calculated according to the methods of [24]. Cell size was measured under a microscope calibrated with a micrometer [25].

2.3. Experimental Set-up

The experiments were conducted under a solar simulator (Sol 1200, Hönle, Gräfelfing, Germany) with a 1000-W xenon arc lamp as a light source. Measurements of UV-B light (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm) were obtained using a broadband radiometer (PMA2100, Solar Light, USA). While the spectrum of UV within the quartz tube which covered with Ultraphan 295 filter, was measured with a spectrometer (HR4000, Ocean Optics, USA).

In the middle of the light period, cells in the exponential phase were harvested and transferred to quartz tubes (50 mL) directly at a density of less than 20 μg chl-a L^{-1}, dark-adapted for 15 min, and added with lincomycin (final concentration, 0.5 mg mL^{-1}) or milli-Q water (as a control). The tubes were then covered with Ultraphan 295 or 395 filters, which block radiation below 295 or 395 nm, respectively, to create PAR + UV-A + UV-B (PAB) and PAR treatments. Tubes were then incubated in a water bath under the solar simulator, and the temperature was controlled with a cooling system (CTP3000, Eyela, Tokyo, Japan). Two light levels were applied consecutively (for 60 min each) using a neutral-density mesh. The low-light conditions were PAR = 63.2 W m^{-2} and UVR = 13.1 W m^{-2}; high light conditions were PAR = 141.7 W m^{-2} and UVR = 35.1 W m^{-2}. Chlorophyll fluorescence was measured with an XE-PAM fluorometer (Walz, Eichenring, Germany) before and during light exposure at a time interval of 12 min.

2.4. Chlorophyll Fluorescence Measurements

A total of 12 tubes (four treatments) were dark-adapted for 15 min, and sub-samples were taken from each tube to measure the initial chlorophyll fluorescence with the XE-PAM. The quartz tubes containing the samples were placed in a water bath under low-light levels. After five rounds of measurements (60 min), samples were exposed to high-light levels by removing the neutral density screen, and measured following the same procedure.

2.5. Data Analysis

Photochemical yield and non-photochemical quenching (NPQ) were measured with the XE-PAM and calculated according to the following equations:

$$\text{Photochemical yield} = (F_m' - F_t)/F_m'$$

$$\text{NPQ} = (F_m - F_m')/F_m,$$

where F_m is the dark-adapted maximal fluorescence, F_m' is the effective maximal fluorescence, and F_t is the steady-state fluorescence under actinic light.

The relative inhibition of photochemical yield by UV was estimated according to the following equation:

$$\text{Relative inhibition}(\%) = (P_{PAR} - P_{PAB})/P_{PAR} \times 100,$$

where P_{PAR} and P_{PAB} represent the photochemical yield under PAR and PAB treatments, respectively. Relative inhibition was calculated when P_{PAR} and P_{PAB} were significantly different. Statistical differences among treatments were analyzed with a one-way analysis of variance (ANOVA) and Tukey's test, and the significance level was set at $p = 0.05$.

To estimate the UV distribution within the cell, cells were assumed to be spherical with an even distribution of pigments. Several parameters, i.e., the spectral absorbance of filters with intact cells, spectrum of UV within quartz tube, and cell density on the membrane, were used to derive the attenuation coefficient of UV radiation (280–400 nm). The relative UV intensity was plotted as a function of the optical length from the cell surface. The attenuation coefficient for UV was calculated as:

$$k_{uv} = -\text{Ln}\frac{\int_{\lambda=280}^{400nm} E_\lambda * T_\lambda}{\int_{\lambda=280}^{400nm} E_\lambda} \div L,$$

where E_λ is the relative UV intensity within the quartz tube, T_λ is the transmittance (in percentage) of the algal mat on the filter, while L (μm) represents the thickness of the algal mat.

The rate of UVR-induced damage to photosystem II (PSII) (k, min^{-1}) and the corresponding repair rate (r, min^{-1}) were calculated according to the following equation of [26]:

$$\frac{P_0}{P_t} = \frac{r}{k+r} + \frac{k}{k+r}e^{-(k+r)t},$$

where P_0 and P_t represent the initial photochemical yield or at a certain time point, respectively, and t is the exposure time in minutes.