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Effects of solar ultraviolet radiation on photosynthesis of the marine red tide alga *Heterosigma akashiwo* (Raphidophyceae)

Kunshan Gao [a,b,*], Wanchun Guan [a,b], E. Walter Helbling [a,c]

[a] *Marine Biology Institute, Shantou University, Shantou, Guangdong 515063, China*
[b] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China*
[c] *Estación de Fotobiología Playa Unión & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Casilla de Correos No. 15, 9103 Rawson, Chubut, Argentina*

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Abstract

In order to assess the short- and long-term impacts of UV radiation (UVR, 280–400 nm) on the red tide alga, *Heterosigma akashiwo*, we exposed the cells to three different solar radiation treatments (PAB: 280–700 nm, PA: 320–700 nm, P: 400–700 nm) under both solar and artificial radiation. A significant decrease in the effective quantum yield (Y) during high irradiance periods (i.e., local noon) was observed, but the cells partially recovered during the evening hours. Exposure to high irradiances for 15, 30, and 60 min under a solar simulator followed by the recovery (8 h) under dark, 9 and 100 μmol photons m^{-2} s^{-1} of PAR, highlighted the importance of the irradiance level during the recovery period. Regardless the radiation treatments, the highest recovery (both in rate and total Y) was found at a PAR irradiance of 9 μmol photons m^{-2} s^{-1}, while the lowest was observed at 100 μmol photons m^{-2} s^{-1}. In all experiments, PAR was responsible for most of the observed inhibition; nevertheless, the cells exposed only to PAR had the highest recovery in any condition, as compared to the other radiation treatments. In long-term experiments (10 days) using semi-continuous cultures, there was a significant increase of UV-absorbing compounds (UV_{abc}) per cell from 1.2 to $>4 \times 10^{-6}$ μg UV_{abc} $cell^{-1}$ during the first 3–5 days of exposure to solar radiation. The highest concentration of UV_{abc} was found in samples exposed in the PAB as compared to PA and P treatments. Growth rates (μ) mimic the behavior of UV-absorbing compounds, and during the first 5 days μ increased from <0.2 to ca. 0.8, and stayed relatively constant at this value during the rest of the experiment. The inhibition of the Y decreased with increasing acclimation of cells. All our data indicates that *H. akashiwo* is a sensitive species, but was able acclimate relatively fast (3–5 days) synthesizing UV-absorbing compounds and thus reducing any impact either on photosystem II or on growth.

Keywords: Growth; *Heterosigma akashiwo*; UV-absorbing compounds; Photosynthetic quantum yield; Toxic blooms; UVR

1. Introduction

Solar ultraviolet radiation (UVR, 280–400 nm), and the increase of UV-B radiation (280–315 nm) due to ozone depletion, has a range of effects on phytoplankton [1–3]. They include, among others, the impact on growth, metabolism, motility, photo-orientation, pigmentation and photosynthetic capability of phytoplankton [3–6]. One of the processes that received particular attention is photosynthesis, as solar energy is directly used by autotrophic organisms, and thus any change on UVR might affect primary production [7].

In short time scale (<day) dynamic photoinhibition or even irreversible photodamage of photosystem (PSII) have been reported in macroalgae and microalgae [8,9]. Photoinactivation, as the definitions proposed in a recent review of Franklin et al. [10], usually occurs when the D1 protein in PSII is damaged, causing a decrease in the electron transport [11,12]. It was found, however, that a fast synthesis of the D1 protein was enough to cope with photodamage,

* Corresponding author. Tel./fax: +86 754 2903977.
E-mail address: ksgao@stu.edu.cn (K. Gao).

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at least in *Synchocystis* sp. (PCC6803) and thus the organisms were not inhibited [13]. In addition, any impact of the photosynthetic quantum yield could be potentially recovered (dynamic inhibition) but the recovery time might vary from minutes to several hours after the stress of high irradiance is eliminated [14,15].

When considering relatively long-time scales (several days–weeks), autotrophic organisms might protect themselves through the synthesis UV-absorbing compounds, mainly mycosporine-like amino acids (MAAs) [16]. These compounds absorb UVR at a wavelength range between 310 nm and 360 nm and can be synthesized by many organisms [17–20]. Also many studies [21] have shown that they can be bioaccumulated through the diet by organisms at higher trophic levels that do not have the capacity of synthesis of them. The protective role of MAAs have been shown in previous studies (e.g. [22]); however, the size structure of the community is important as the useful concentration of these UV-absorbing compounds in small cells (e.g., <10–20 μm) would be too high and osmotically disadvantageous [23]. It is still uncertain, however, how different species can utilize them to cope with UVR, their rate of production or even the cell quota amount necessary to confer protection.

Harmful blooms of *Heterosigma akashiwo* have been reported in temperate waters [24,25], especially in Japan [26], New zealand [27], and China [28]. Some studies showed that *H. akashiwo* had anti-predatory activity and ichthyotoxicity [29]. At the same time, it had been shown allelopathic effects towards other algae, specifically diatoms [30]. The growth and toxicity of *H. akashiwo* can be mediated by hydrogen peroxide [24,31] or viruses [32,33]. In addition, and more relevant to the blooms *H. akashiwo* in a climate change environment, [34] showed that growth and toxicity of this alga was markedly influenced by changes in temperature and light intensity. Although many studies had focused on the ecophysiology of *H. akashiwo*, very little is known about the potential effects of solar UVR on this species. The aim of this study was to assess the short- and long-term impact of UVR on the effective quantum yield and growth of *H. akashiwo*. Our emphasis was on determining two different aspects, on one hand to establish the potential recovery after any damage occurred, and on the other hand the capability of the species to acclimate to an increase in solar irradiance to further prevent cellular damage.

2. Materials and methods

2.1. Species and culture conditions

The experiments to evaluate the effects of UVR on *H. akashiwo* (Hada) Hada were carried out at the Marine Biology Institute, Shantou University, during July–October, 2004 and during April–June, 2005. *H. akashiwo* is a bi-flagellated, unicellular golden-brown microalga (8–25 μm long and 6–15 μm wide) and was obtained from the Institute of Oceanology, Chinese Academy of Science (Qingdao) and maintained in f/2 medium [35], under cool-white fluorescent light at 60–70 μmol photons m^{-2} s^{-1} (12L:12D) and 25 °C in a illuminated growth chamber (White Westinghouse, model 515, USA). The cultures were shaken 2–3 times every day when indoor and aerated continuously when they were exposed outdoor.

2.2. Experimentation

2.2.1. Treatments

Samples were obtained when the culture was in exponential growth, diluted to 0.5–2.7 × 10^4 cells ml^{-1}, and used in short- (< day) and long-term (several days) experiments exposing the cells either to sunlight or using a solar simulator (Sol 1200, Dr. Hönle GmbH, Germany) provided with a 1000 W xenon arc lamp. After dilution the culture was placed in quartz tubes (59 mm in diameter and 350 mm high or 25 mm in diameter and 150 mm high), and maintained in a water bath with running water for temperature control (~21 °C). The culture was incubated under three radiation treatments: (1) PAB, tubes covered with a 295 nm cut-off foil (Ultraphan, Digefra, Munich, Germany), transmitting 295–700 nm; (2) PA, tubes covered with 320 nm cut-off foil (Montagefolie, Folex, Dreieich, Germany), transmitting 320–700 nm; and (3) P, tubes covered with a 395 nm cut-off foil (Ultraphan UV Opak, Digefra, Munich, Germany), transmitting 395–700 nm. Duplicate samples were used in all radiation treatments. The transmission spectra of these materials are published in Figueroa et al. [3].

2.2.2. Short-term experiments

These experiments were designed to evaluate the impact of UVR over a daily radiation cycle, the recovery of cells under different light conditions and the influence of cell concentration (shelf-shading) in the observed results. In these experiments three radiation treatments (as mentioned above) were used.

The impact of UVR on diurnal patterns of the effective quantum yield was obtained by exposing *H. akashiwo* cells to solar radiation from 8:00 to 18:00 (local time) and the effective quantum yield monitored every two hours (please see below). This set of experiments were conducted under high (July) and low (October) solar radiation.

The short-term impact of UVR on the photosynthetic quantum yield of *H. akashiwo* was obtained after 15, 30, and 60 min of exposure using a solar simulator. The recovery of the cell was followed for 8 h (measurements every 30–120 min) under three conditions: (a) darkness, (b) low PAR (9 μmol photons m^{-2} s^{-1}), and (c) high PAR (100 μmol photons m^{-2} s^{-1}). The irradiance received during the exposure in the solar simulator were: PAR, 245 W m^{-2} (1142 μmol photons m^{-2} s^{-1}), UV-A, 55 W m^{-2} and UV-B, 1.9 W m^{-2} with the culture placed at 120 cm from the light source. The light sources for recovery were cool-white fluorescent tubes.