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UV-B affects photosynthesis, ROS production and motility of the freshwater flagellate, *Euglena agilis* Carter

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ABSTRACT

The effects of ultraviolet B (UV-B; 295–320 nm) radiation on certain vital physiological (photosynthesis), biochemical (production of reactive oxygen species – ROS) and behavioral (motility and orientation) characteristics were investigated in the unicellular photoautotroph, *Euglena agilis* Carter. The photosynthetic performance of *E. agilis* was recorded after exposure of between 15 and 60 min followed by a period of recovery lasting 6–24 h under dim light (5–10 μmol photons m^{-2} s^{-1}). The maximum quantum yield of PS II (F_v/F_m) was reduced to 65% and 14% of initial values immediately following 15 and 30 min UV-B exposure, but recovered to 100 and 86% of the initials, respectively. Values of $rETR_{max}$ in *E. agilis* exposed to 15 min UV-B were similar to those of the initials, but a 30 min UV exposure resulted in 75% reduction of $rETR_{max}$ with only a 43% recovery as compared with the initial after 24 h recovery. After a 60 min UV-B exposure, there were no Chl *a* fluorescence signals, and hence no F_v/F_m or $rETR_{max}$. A UV dose-dependent increase in DCFH-DA fluorescence was found in *E. agilis* cells, reflecting an increase in ROS production.

After exposures to UV-B for between 15 and 60 min, the percentages of motile cells in the population decreased to 76, 39 and 15%, respectively. Following 24 h in dim light, the percentage of motile cells increased to between 66% and 95% of the initial value. The velocity of non-irradiated cells was 60 μm s^{-1}, which decreased to 16–35 μm s^{-1} immediately following exposure for 15–60 min. After periods of time in dim light (6, 12 and 24 h) velocities had recovered to between 44 and 81% of the initial value.

In untreated controls, the *r*-value was 0.23, indicating random movement of *E. agilis*, but it increased to 0.35 and 0.72 after exposure to UV-B for 30 and 60 min, respectively. There was a tendency towards vertical downward movement of cells proportional to the duration of exposure. The compactness of *E. agilis* decreased from 2.9 in controls to 1.8–2.3 in cells treated with UV-B although significant recovery followed. UV-B dose-dependent interaction between photosynthetic activity, ROS production and movement is discussed in terms of a UV-protective mechanism in *E. agilis*.

1. Introduction

Solar UV-B radiation is detrimental to all forms of life, but photosynthetic organisms, including microalgae, are particularly vulnerable due to their obligatory requirement for solar radiant energy for photosynthesis, growth and survival. It has been reported that UV-B radiation could alter the morphology, impair motility and photo-orientation, damage proteins and DNA, and inhibit growth, nitrogen metabolism, pigmentation and photosynthesis of various microalgae species (Häder and Häder, 1988; Ekelund, 1994; Beardall et al., 1997; Sinha et al., 1995; He and Häder, 2002; Laurion and Roy, 2009; Rastogi et al., 2010a). The ecological consequence of these effects on production rates and community structure of primary producers in aquatic ecosystems are of growing concern (Häder et al., 2011).

Of the various physiological processes, photosynthesis is one of the most prominent targets of solar UV-radiation causing a number of damaging effects such as the degradation of the D1 and/or D2 protein of PS II or RuBisCo, the destruction of photosynthetic pigments, perturbations in the electron flow between PS I and PS II, and the reduced expression of genes involved in photosynthesis (Holzinger and Lütz, 2006; Karsten et al., 2007).

UV-B is also known to impair the motility and swimming velocity of photosynthetic flagellates (Rai and Mallick, 1998). For example, motile phytoplankton face a dilemma in their orientation to light; to receive sufficient light for photosynthesis they need to

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be close to the water surface while exposure to intense solar radiation containing UV wavebands will result in bleaching and damage. Impairment of motility and/or swimming velocity caused by UV-B radiation would, therefore, limit the capacity of motile phytoplankton to adapt to the surrounding light environment, thus reducing photosynthesis and growth (Rai and Mallick, 1998).

UV stress imposed on microorganisms induces changes in oxygen metabolism, which can lead to oxidative stress. UV-B generates reactive oxygen species (ROS) through endogenous photosensitization reactions in photosynthetic microorganisms (Rastogi et al., 2010c). Severe oxidative stress inside cells can cause DNA damage, impair photosynthetic efficiency and alter the orientation of photosynthetic microalgae (Malanga et al., 1997; Vincent and Neale, 2000; Richter et al., 2003).

Conversely, microalgae have evolved certain protective strategies against UV-B radiation, which include avoidance, ROS scavenging by non-enzymatic and enzymatic antioxidant molecules, the synthesis of UV-absorbing/screening compounds such as the mycosporine-like amino acids (MAAs), scytonemin and sporopollenin, the repair of UV-induced DNA damage and the re-synthesis of damaged PS II proteins (Xiong et al., 1999; Rastogi et al., 2010b; Singh et al., 2010).

The vertical movement of motile microalgae is considered to be an avoidance strategy to protect these organisms from extreme UV irradiations. Vertical movement was previously regarded as a physical phenomenon based on the buoyancy effect due to an unequal mass distribution within the cells (Brinkmann, 1968). However, recent studies have shown that movement with respect to light (phototaxis) and gravity (gravitaxis) are active physiological mechanisms by which organisms are able to find the most safe and suitable position in the water column for optimal growth and photosynthesis (Richter et al., 2002, 2003, 2007). The ability of phytoplankton to avoid or repair damage caused by UV-B radiation may determine their potential to stand and survive high UV-B radiation stress.

The genus *Euglena* is composed of single celled organisms which are often considered to be members of either the protozoan order Euglenida or the algal division Euglenophyta. These organisms can grow photoautotrophically, heterotrophically or photoheterotrophically depending on environmental conditions (Ogbonna et al., 2002). Despite a number of reports on the effects of UV-B radiation on the motility and orientation of euglenophytes, simultaneous documentation of UV-induced responses in both the photosynthetic machinery and the parameters of motility has not been made.

In the present study, the effects of UV-B radiation on photosynthetic performance, intracellular ROS production and motility of *E. agilis* Carter have been investigated. Phytoplankton is the main biomass producers in aquatic ecosystems, contributing ca. 50% of the atmospheric carbon dioxide (Sebastian et al., 1994). Any negative effects of UV-B on the photosynthesis and motility or orientation of phytoplankton would be detrimental to entire aquatic ecosystems and food chains.

2. Materials and methods

2.1. Test organism and culture conditions

E. agilis Carter (obtained from Prof. Woonggi Shin, Chungnam University, Daejeon, South Korea) was grown in a mineral medium (pH 5; Checcucci et al., 1976) in 1 L Erlenmeyer flasks at 25 °C under white fluorescent light of 30 μmol photons m^{-2} s^{-1} (FL400, Kum-Ho, Korea) on a 16:8 h LD cycle. All experiments were performed using exponentially growing cells.

2.2. Acute UV-B exposure

Suspension of cells (10^5–10^6 cells/ml) were exposed to UV-B radiation (0.5 W m^{-2}; 295–320 nm), supplemented with PAR (photosynthetically active radiation, 400–700 nm; 30 μmol photons m^{-2} s^{-1}), for 15, 30 and 60 min in 6-well culture plates without lids (well diameter, 35 mm; SPL Life Sciences, Korea). Two UV-B tubes (TL20W/12, Philips, Germany) with a maximal output at 312 nm were used for UV irradiation and a 295 nm filter foil (Ultraphan, Digefra, Germany) was used to cut off UV-C wavelengths. PAR was provided by white fluorescent tubes (FL400, Kum-Ho, Korea). Radiation measurements were taken with a Li-Cor LI-1000 quantum meter (Li-Cor, Lincoln, USA) for PAR and a UV radiometer with UV-B sensor (DM series, Spectronics, USA) for UV-B radiation. All experiments were conducted in triplicate.

2.3. Measurement of Chl a fluorescence

The photosynthetic parameters were analyzed by measuring Chl *a* fluorescence with a pulse amplitude modulation (PAM) fluorometer (Maxi Imaging PAM, Walz GmbH, Germany). Immediately after the UV-B treatment and following a period of 24 h under dim light (5–10 μmol photons m^{-2} s^{-1}) *E. agilis* cell suspensions were dark-adapted for 15 min and the photochemical quantum yield of PS II was monitored from repeated measurements of selected fluorescence parameters: minimal fluorescence (F_0) that denotes the fluorescence yield when all PS II reaction centers are open with fully oxidized plastoquinone A (Q_A), maximal fluorescence yield (F_m) that is induced by a short saturating pulse (SP) of actinic light that reduces all *Q* and the derived maximal PS II quantum yield ($(F_m - F_0)/F_m$ or F_v/F_m). Rapid light curves (RLC) were measured using 10 s pulses of actinic light increased stepwise from 0 to 335 μmol photons m^{-2} s^{-1} (1, 11, 21, 36, 56, 81, 111, 146, 186, 231, 281 and 335 μmol photons m^{-2} s^{-1}) and provides an estimate of relative electron transport rate (rETR). The $rETR_{max}$ was calculated as described by Jassby and Platt (1976).

2.4. Measurement of reactive oxygen species

The generation of UV-B-induced ROS in *E. agilis* was estimated by adding 5 μM (final concentration) of DCFH-DA (Sigma Aldrich, USA; CAS No.: 4091-99-0), solubilized in ethanol, to cell suspension that was then incubated on a shaker at room temperature in the dark for 1 h. The fluorescent intensity was measured at an excitation wavelength of 485 nm and emission at 530 nm using a microplate fluorescence reader (Spectramax Gemini EM, Molecular Devices, USA).

2.5. Measurement of motility and orientation

Movements of the test organisms were measured using a manual ECOTOX biosystem, described by Tahedl and Häder (2001). The device comprises a horizontally positioned, custom made, microscope which is used to monitor swimming of cells within an observation cuvette. To prevent phototactic movements and stimulation of photosynthetic oxygen production from exposure to visible wavelengths of light during observations, cells were viewed under an infrared light source (IR diode; λ = 875 nm). The vectors of tracks were used to calculate the % moving cells ('Motility'), the mean direction of movement ('Upwards') and mean velocity and precision of orientation with the aid of software supplied with the ECOTOX biosystem (Tahedl and Häder, 2001). The precision of orientation was determined using the Rayleigh test, which yields a statistical value (*r*-value) of 0 (random orientation) to 1 (perfect orientation of all organisms in the same direction). The '*r* value' is essential for the characterization of the directional