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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

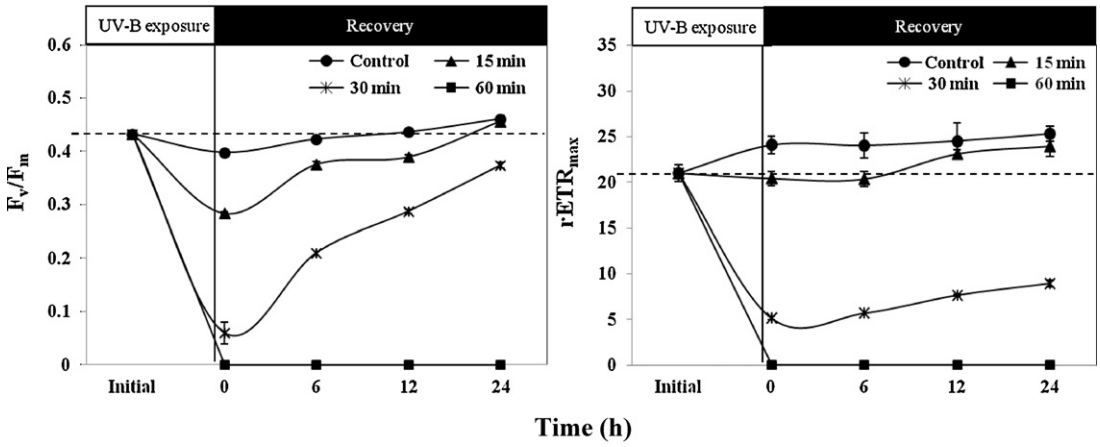

Fig. 1. The effects of different UV-B doses and subsequent recovery for 24 h in dim light on the maximal quantum yield of PS II (F_v/F_m) and relative electron transport rate ($rETR_{max}$) of *E. agilis*. The mean and 95% CI are shown ($n = 3$). Asterisks indicate no chlorophyll fluorescence.

movement behavior of the organism (Tahedl and Häder, 2001). Velocity of moving cells is expressed in $\mu m\ s^{-1}$. A minimum velocity of 15 $\mu m\ s^{-1}$ was set to avoid including sinking and non-motile objects.

2.6. Statistical analyses

The data presented in this study are mean values ±95% confidence intervals. All parameters were compared across treatments with a one-way ANOVA. A multiple comparison test using the least significance difference (LSD) was then performed to determine statistical significance at $P = 0.05$.

3. Results

3.1. Effect of UV-B on photosynthesis

Fig. 1 presents the photosynthetic performance of *E. agilis* after UV-B exposure for 15–60 min and then recovery for 6–24 h in 5–10 μmol photons $m^{-2}\ s^{-1}$. The F_v/F_m was reduced by 34% and 86% of the initial values after UV-B exposure for 15 and 30 min, respectively (Fig. 1). Values of $rETR_{max}$ in *E. agilis* exposed to UV for 15 min were similar to those of the initials, but a 30 min UV exposure resulted in a 75% reduction of $rETR_{max}$ as compared with the initial. After a 60 min UV-B exposure, no Chl *a* fluorescence signals were observed and, as a result, no F_v/F_m or $rETR_{max}$ (Fig. 1).

After UV exposures, *E. agilis* samples were transferred to low light (5–10 μmol photons $m^{-2}\ s^{-1}$) to allow for the recovery of photosynthesis. Under dim light, F_v/F_m values of *E. agilis* exposed to UV for 15 and 30 min showed a recovery to 100% and 86% of the initials, respectively (Fig. 1). In contrast, $rETR_{max}$ values of *E. agilis* irradiated with 30 min UV-B recovered to only 43% of the initial values. There was no recovery observed in either parameter in *E. agilis* exposed to UV-B for 60 min (Fig. 1).

3.2. Estimation of reactive oxygen species (ROS)

The increase in DCFH-DA fluorescence in UV-B irradiated cells indicated the production of ROS that was proportional to the duration of exposure (Fig. 2). The rise in DCF fluorescence was evident even within 15 min of UV-B exposure, and, in cells irradiated with UV-B for 30 and 60 min, the intracellular ROS levels were about four and five-fold higher than in the controls.

3.3. Effect of UV-B on motility parameters

Circular histograms showing the various motility parameters of *E. agilis* cells after each duration of UV-B exposure are shown in Fig. 3. UV-B radiation clearly caused a unidirectional and vertical downward movement of flagellate cells tested (Fig. 3). However, after a 24 h recovery period, the pattern of movement was randomized, and similar to that of controls.

In non-irradiated controls, 93% of the cells were motile, but this number decreased significantly with increasing UV exposure. Following exposure for 15, 30 and 60 min, the percentages of motile cells in the population were 76, 39 and 15%, respectively (Fig. 4). After 24 h in dim light, the motility of UV-irradiated cells showed levels of recovery ranging from 66% in cells exposed for 60 min to 95% in cells exposed for 15 min.

The average speed of non-irradiated cells was 63 $\mu m\ s^{-1}$, which decreased to 35, 23, and 16 $\mu m\ s^{-1}$ on exposure to UV-B radiation for 15, 30 and 60 min, respectively (Fig. 4). Under dim light, the speed of UV-irradiated cells showed a significant recovery ranging from 44% in 60 min UV-irradiated cells to 81% in 15 min UV-irradiated cells.

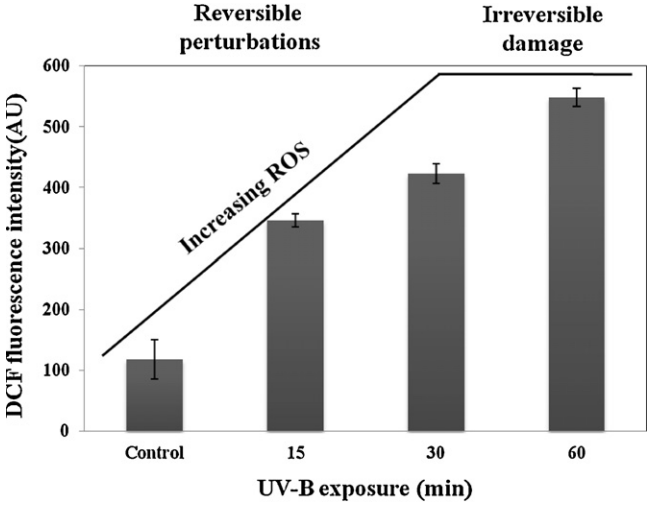

Fig. 2. The DCFH-DA fluorescence intensities from *E. agilis* cells after 15, 30 and 60 min of UV-B irradiation. Total UV doses were 0.45, 0.90 and 1.80 kJ m^{-2}. The excitation wavelength was 485 nm and the emission wavelength was 530 nm. The mean and 95% CI are shown ($n = 3$).

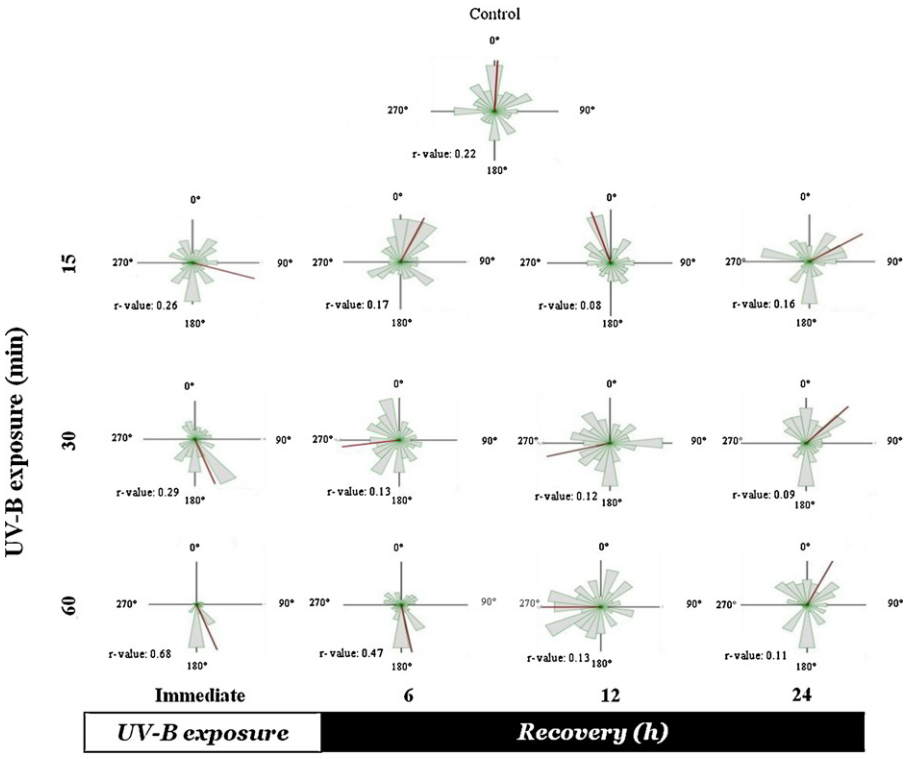

Fig. 3. Circular histograms showing the movement behavior of *E. agilis* immediately after various UV-B exposures and subsequent recovery.

The *r*-value was about 0.23 in controls, indicating a random movement of *E. agilis*. With exposure to UV the *r*-value increased to 0.35 in cells exposed to UV-B for 30 min, and 0.72 after a 60 min exposure, indicating a directional movement such as negative gravitaxis (Fig. 4). There was full recovery in the pattern of movement as indicated by lowered *r*-values. The compactness of *E. agilis* decreased from 2.9 in controls to 1.8–2.3 in cells treated with UV-B for 15–60 min. Following 24 h recovery, cells had compactness of between 2 and 2.8.

4. Discussion

UV-B radiation caused a significant decrease in the photosynthetic performance of *E. agilis*. A number of studies have reported severe effects of UV radiation on microalgae in terms of photosynthesis, pigmentation and respiration (Beardall et al., 1997; Villafañe et al., 2003; Holzinger and Lütz, 2006; Richter et al., 2007; Häder et al., 2011).

A decrease in the maximal quantum yield of PS II (F_v/F_m), referred to as photoinhibition, occurs when phytoplankton are exposed to photoinhibitory stress (Bracher and Wiencke, 2000). Photoinhibition can be either a dynamic or chronic phenomenon: dynamic photoinhibition involves the down-regulation of the photosynthetic apparatus and is associated with the dissipation of excess energy as heat, whereas chronic photoinhibition involves photodamage of the PS II reaction center D1 leading to a reduction in the number of functional PS II centers (Osmond, 1994; Hanelt, 1996). The values of F_v/F_m of *E. agilis* exposed to 15–30 min of UV-B were significantly decreased; however, a relatively fast recovery of F_v/F_m occurred within 6 h of recovery, followed by slower recovery until photosynthesis reached full capacity at 24 h (Fig. 1). This recovery is similar to that observed in the green macroalga, *Ulva rotundata*, by Franklin et al. (1992). The rapid recovery may occur as a result of the rapid reconversion of ineffective and dissipative PS II centers into active centers, whereas the slow recovery may be due to the slow removal and replacement of damaged D1 protein (Hanelt et al., 1997). Dynamic photoinhibition is considered to be a protective mechanism that enables the immediate recovery of photosynthetic activity once excess energy is removed.

In contrast, the significant decreases in the F_v/F_m of *E. agilis* exposed to 60 min of UV-B may indicate chronic photoinhibition or photodamage (Fig. 1). An extensive reduction in F_v/F_m followed by a slow and incomplete recovery may be ascribed to a degradation of the D1 protein or partial detachment of the antenna (Krause, 1988). UV-B radiation was reported to increase the net loss of D1 protein pools in natural phytoplankton (Bouchard et al., 2005).

Although F_v/F_m is widely used as a physiological stress parameter in algae, this parameter does not necessarily reflect the potential for photosynthesis as a whole. For example, limitations may occur elsewhere in the photosynthetic process such as in the dark reactions, without affecting PS II efficiency (Karsten et al., 2007). It should be noted that advances in instrumental methodology in the field of chlorophyll fluorescence have made it possible to measure PS II electron transport (Schreiber, 2004). The rate of PS II electron flow depends on the rate of photon absorption and the efficiency of PS II. Therefore, rETR provides an approximation of the rate of electrons pumped via PS II into the photosynthetic chain (Schreiber, 2004). rETR is also known to be linearly correlated with carbon fixation in microalgae (Barranguet and Kromkamp, 2000). In *E. agilis*, $rETR_{max}$ values were not significantly reduced after exposure to 15 min of UV-B in contrast to a significant decrease in F_v/F_m values after UV-B treatment (Fig. 1). This result confirms that a decline in F_v/F_m is not due to the permanent PS II damage, but instead to reversible perturbations, suggesting that the transfer of electrons generated by light absorption is normally operative. However, a 30 min exposure to UV-B resulted in a 75% decrease in the $rETR_{max}$ of *E. agilis* with only a 43% of subsequent recovery. This result is in marked contrast to the corresponding F_v/F_m showing an 86% of recovery.

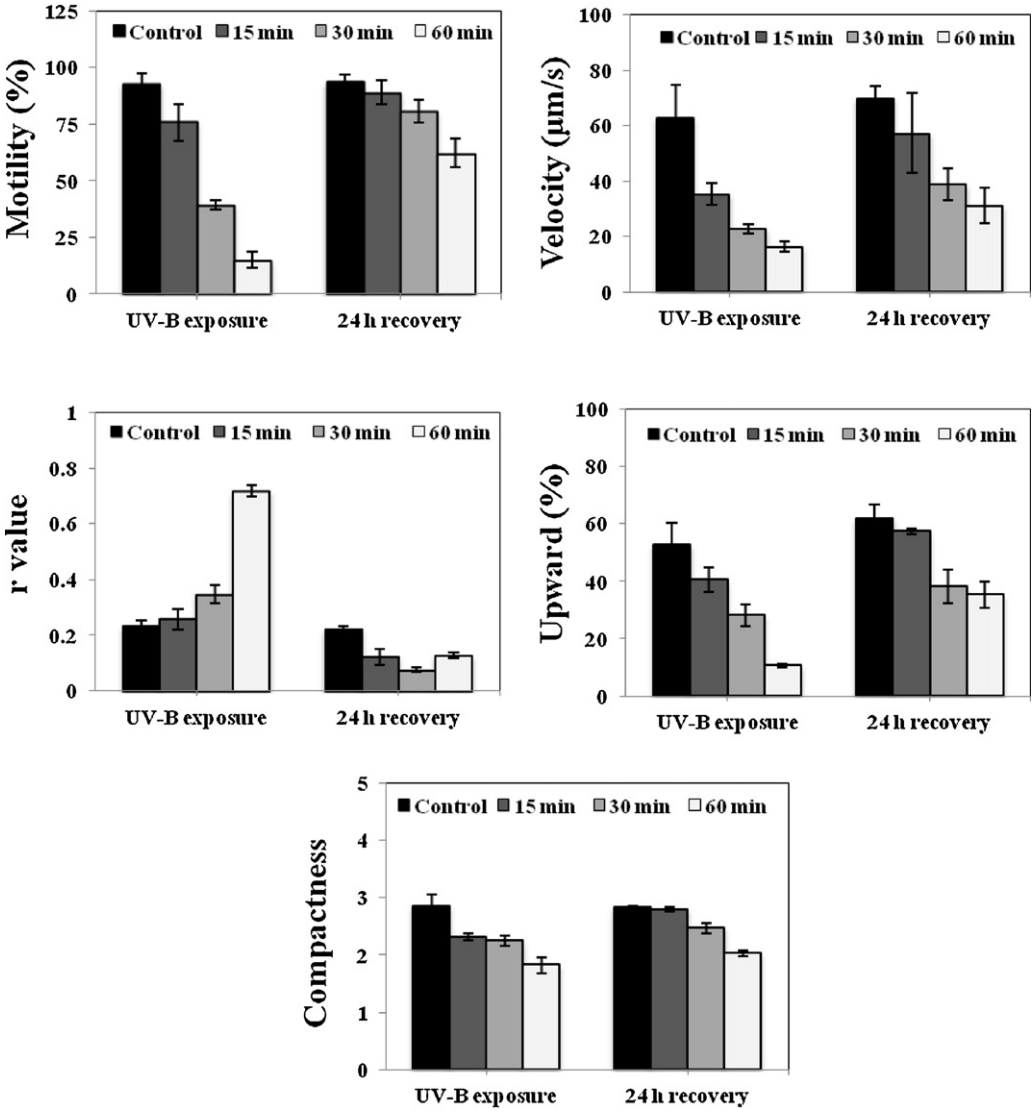

Fig. 4. The effects of UV-B doses and subsequent recovery for 24 h on motility parameters (motility, velocity, upward, *r* value and compactness) of *E. agilis*. The mean and 95% CI are shown ($n = 3$).

The disparity between F_v/F_m and $rETR_{max}$ simply indicates that the light energy absorption process is working normally in UV-B irradiated cells, but that the excited electrons are not properly transferred. The discrepancy in the dynamics of $rETR_{max}$ and F_v/F_m responses may be explained by the formation of alternative electron sinks such as ROS particularly, hydrogen peroxides, which withdraw electrons from the photosynthetic transport (Tartachnyk and Blanke, 2008).

It is noteworthy that UV-B exposure increased the cellular concentrations of ROS in *E. agilis* (Fig. 2). In chloroplasts, ROS is reported to cause inactivation and degradation of RuBisCo and other components of the Calvin cycle (Pätsikkä et al., 1998) as well as lipid peroxidation, which results in the disruption of photosynthetic pigments (Pätsikkä et al., 2002).

When a stress–strain response diagram is drawn on the UV-induced ROS production response, it shows that *Euglena* endures a certain range of ROS generated by 15–30 min of UV-B irradiation, which is evident from the significant recovery of photosynthetic capacity (Fig. 2). Although potentially dangerous, ROS production also induces defense mechanisms within the adaptive range of *Euglena* that protect the unicellular motile alga without causing oxidative stress (Mullineaux and Baker, 2010).

Within this context, increased ROS production in *E. agilis* following different doses of UV-B may be explained in different ways. First, ROS production of *E. agilis* exposed to 15–30 min of UV-B radiation may be considered as a powerful signal that causes a dynamic photoinhibition. Therefore, ROS may play a role in UV-B protection via the reversible photoinhibition of PS II activity. Few studies to date have considered photosynthetic ROS generation in the context of the light-driven production of powerful signaling molecules, whose abundance provides essential information to the cell regarding imbalances between energy-generating and energy-utilizing processes (Foyer and Shigeoka, 2011).

However, ROS produced after a 60 min UV-B exposure may cause oxidative stress, leading to the chronic photoinhibition or photodamage of *E. agilis* (Fig. 2). In fact, irreversible damage is followed by a loss of physiological competence and cell death when ROS were not rapidly scavenged, and when the rate of repair of damaged cell components fails to keep pace with the rate of damage (Nishiyama et al., 2001). This observation is in agreement with previous studies on the induction of UV stimulated oxidative stress in planktonic microalgae (Estevez et al., 2001; Kovacik et al., 2010). He and Häder (2002) demonstrated the highest generation of ROS in the cyanobacterium *Anabaena* sp. by UV-B radiation of

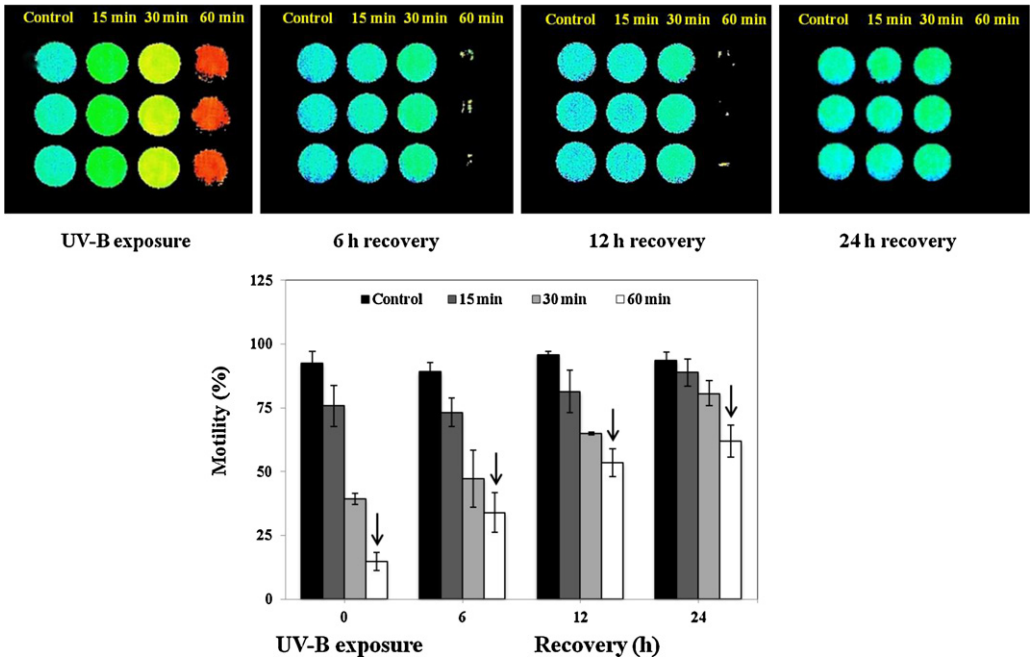

Fig. 5. The relationships among chlorophyll fluorescence image and motility immediately after different durations of UV-B exposure and subsequent recovery for 6–24 h. Chlorophyll fluorescence images were obtained by Maxi Imaging PAM which automatically provides false color imaging of the measured parameters. The blue and red colors represent the highest and lowest values of F_v/F_m, respectively.

0.18 W m^{-2} for 24 h, whereas Rastogi et al. (2010c) reported that after 12 h of UV-B (0.3 W m^{-2}) irradiation, the production of ROS in the cyanobacterium *Anabaena variabilis* PCC 7937 was enhanced, which indicated the generation of oxidative stress inside the cells.

Severe impairments in motility, orientation and moving velocity after UV-B exposure have been reported in a number of motile microalgae, including *Chlamydomonas*, *Cryptomonas*, *Euglena*, *Gyrodinium* and *Peridinium* spp. (Hessen et al., 1997). In the present study, the movement parameters of *E. agilis* decreased with increasing UV doses with the exception of *r* values which increased. A significant reduction ($P < 0.05$) in motility and swimming velocity in *E. agilis* (15% and 26% to the control, respectively) was observed within 15 min of UV-B exposure (Fig. 4). It appears that UV-irradiated *E. agilis* becomes slower rather than less motile.

Euglena cells became spherical in shape under high UV-B irradiation as indicated by a decrease in compactness from 2.9 in the control to 1.8–2.3 in UV-irradiated cells (Fig. 4). It has been reported that some species of the genus *Euglena* change their cell shape in response to increasing physical or chemical stressors (Häder et al., 1997; Takenaka et al., 1997). In fact, Gerber and Häder (1995) found that *E. gracilis* changed from a cylindrical to spherical shape after solar UV exposure. This change in cell shape impairs the cell's capacity to exert force on the cytoplasmic membrane which inhibits the random orientation of the swimming cells (Pettersson and Ekelund, 2006). It is interesting to note that the movement of *E. agilis* exhibited a UV dose-dependent decrease in upward movement, as shown by a decrease of upward values from 52 in the control to 10–40 in UV-irradiated samples (Fig. 4). Cell orientation represented by *r* value increased with increasing UV irradiances, which indicated a highly precise downward orientation (Fig. 4). Richter et al. (2007) reported a similar downward movement in *E. gracilis* in response to UV radiation and considered this to be a protective strategy that would endow the organisms with protection from harmful UV radiation. In the case of *E. agilis*, however, downward movement does not seem to be an active response to damaging UV-B radiation, but instead appears to be a settling movement related to decreased photosynthetic activity and/or reduced motility. Because the ability to move actively should entail a cost in terms of increased energy expenditure (Striebel et al., 2009), the UV-induced decrease in $rETR_{max}$ appears to be closely related to reduced movement within the UV exposure range of 15–30 min. However, this relationship does not apply to *E. agilis* exposed to a 60 min UV-B. Fig. 5 shows Chl *a* fluorescence together with the motility response to UV-B. Immediately after UV-B exposure, F_v/F_m decreased as indicated by a color change from blue (control) to red (60 min exposure) in parallel with a reduction in motility. During a 24 h recovery period, however, the F_v/F_m of *E. agilis* exposed to 60 min of UV did not show any sign of color or value, whereas motility recovered to 66% of the control. The disparity between these photosynthetic and motility parameters may indicate that, after 60 min of UV irradiation, *E. agilis* completely loses its photosynthetic function, but it is still capable of movement. This may be explained in two ways. The UV-irradiated *E. agilis* might use stored energy to sustain its movement for some time even when UV stress is removed. Alternatively, the alga loses its photosynthetic machinery due to UV-B radiation, which then triggers a transition in the *Euglena* from a plant-like to an animal-like form. *Euglena* is known to accumulate large quantities of paramylon as a storage carbohydrate when grown in the presence of utilizable carbons (Bäumer et al., 2001). It is also known that *Euglena* has two or three life forms, including photoautotrophic, heterotrophic and photoautoheterotrophic forms. The reduction in motility and orientation of *E. agilis* cells under UV-B stress is a rapid response and might not involve complex mechanisms, such as damage to DNA or other biomolecules (Häder and Häder, 1988). Richter et al. (2003) has proposed that tactic sign change of *E. gracilis* under certain stress conditions might involve ROS particularly hydrogen peroxide radicals. Thus, the production of ROS may be deeply involved in both the photosynthetic activity and motility response of *Euglena* to UV.

In summary, we propose a hypothesis for the UV-B protective/adaptive mechanism in *E. agilis*. UV-B radiation generates ROS which then down-regulates the photosynthetic activity and reduces motility. Therefore, after moderate levels of UV-B radiation,

ROS plays a signaling role to shut down photosynthetic system, preventing the absorption of impinging energy for protection against harmful UV radiation. This hypothesis is supported by a significant recovery in F_v/F_m and motility of *E. agilis* exposed to 15–30 min of UV.

On the other hand, excessive UV irradiation appears to damage the photosynthetic machinery of *E. agilis*, causing the organism to become animal-like. The advantages that *E. agilis* gains by taking an animal-like form is the subject of further investigation, but *E. agilis* exposed to excessive UV appears to invest all its stored energy into movement rather than into sustaining its photosynthetic machinery. This adaptation allows *E. agilis* to avoid harmful UV and seek a safe place where the organism may regain its photosynthetic capacity for survival. However, the possibility of maintaining an animal-like life form as long as there are plenty of carbon sources in the surrounding waters may not be completely ruled out, because the organism can save energy needed to construct biomolecules and produce enzymes required for the operation of its intricate photosynthetic system.

Experimental investigations using animal-like *Euglena* with no photosynthetic system are under progress to determine whether the presence and/or absence of carbon sources or light affect *E. agilis* behavior in cultures for prolonged periods.

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