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The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria

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ABSTRACT

In this study, we investigated the combined effects of UV-B irradiation and herbicides (glyphosate, GPS; 2-Methyl-4-chlorophenoxyacetic acid, MCPA-Na; 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU) and the antioxidant (ascorbic acid, ASC) on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria, *Anabaena* sp. and *Microcystis viridis*. UV-B irradiance increased reactive oxygen species (ROS) production, which decreased chlorophyll *a* fluorescence yield, pigment content and superoxide dismutase (SOD) activity, and increased malondialdehyde (MDA) content and caused serious DNA damage. The degree of these damages was aggravated by the addition of DCMU, GPS and MCPA, and was partially mitigated by the addition of ASC. During the recovery process, the degree and mechanism in restoring DNA damage and photosynthesis inhibition were different by the removal of UV-B and herbicides (DCMU, GPS and MCPA) in both cyanobacteria. These results suggest that the combination of UV-B and exogenous herbicides have detrimental effects on cyanobacterial metabolism through either a ROS-mediated process or by affecting the electron transport chain, and may cause the shifts in the phytoplankton community.

1. Introduction

The depletion of stratospheric ozone increases deleterious ultraviolet (UV) radiation at the Earth's surface. These biologically effective doses of UV-B radiation can penetrate deep into the water column, and cause leakage of reactive oxygen species (ROS) from the photosynthetic system in plant cells (Häder and Sinha, 2005). These increased ROS can easily destroy proteins, DNA and other biological molecules, and subsequently affect growth and reproduction, survival, photosynthetic energy harvesting enzymes, and the content of photosynthetic pigments (Wang and Zhang, 2001; He and Häder, 2002; Apel and Hirt, 2004; Holzinger and Lütz, 2006). UV radiation can also alter species composition, and the vertical distribution and metabolism of aquatic ecosystems, which causes shifts in phytoplankton community structure and the food web (Häder et al., 1995; Häder, 2000; Zeeshan and Prasad, 2009). However, cyanobacteria have also developed adaptation strategies to counteract the damaging effects of UV irradiance, which include avoidance, scavenging, screening, repair and programmed cell death (Quesada and Vincent, 1997; Sinha et al., 1998; Sinha and Häder, 2008; Singh et al., 2010).

The large-scale use of pesticides and herbicides in agriculture has caused concern among environmentalists. Herbicides used in agricultural practices are transported to water bodies through run-off, drift and leaching, which increase the risk of exposure in non-target organisms (Chen et al., 2007). Some herbicides cause metabolic alterations in algae due to oxidative stress (Romero et al., 2011), and others function via binding to the exchangeable quinone site in the photosystem II (PSII) reaction center, thus blocking electron transfer (Rutherford and Krieger-Liszkay, 2011), or by changing fluorescence emission and electron transport activities of the bio-samples (Ventrella et al., 2010). Since the region adjacent to Dianchi Lake in China is the main base for the supply of vegetables to Kunming city, a large number of herbicides are applied to control weeds, which cause serious environmental problems in Dianchi Lake (Xu et al., 2007; Yin et al., 2010).

Dianchi Lake is located in the south of Kunming city, Yunnan-Kweichow Plateau, China, where the UV radiation intensity was found to be over 7 W/m^2 at 312 nm from 12:00 to 14:00 (Guo et al., 2005). Recently, cyanobaceria blooms (CBs) dominated

Abbreviations: CBs, cyanobacteria blooms; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ET_o/ABS, the probability that an absorbed photon move an electron into the electron transport chain beyond Q_A^-; ET_o/TR_o, the efficiency that a trapped excitation can move an electron into the electron transport chain beyond Q_A^-; *Fv*/*Fm*, photochemical trapping efficiency of the dark adapted state; GPS, glyphosate; MDA, malondialdehyde; MCPA-Na, 2-methyl-4-chlorophenoxyacetic acid; (PSII), photosynthetic system II; RC/CS_o, the density of PSII reaction centers per excited cross-section; (ASC), ascorbic acid; SOD, superoxide dismutase

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by *Microcystis viridis* and *Anabaena* sp. have frequently been observed (Li et al., 2007; Wang et al., 2010). Due to the intense UV-B radiation and large-scale use of herbicides in the Dianchi Lake area, *Anabaena* and *M. viridis* may be suffered detrimental effects by the combination of UV radiation and herbicides. Based on the data on herbicide use in the Dianchi area, UV-B radiation and 3 herbicides (GPS, DCMU and MCPA) were selected to treat *Anabaena* and *M. viridis*, two cyanobacteria bloom species isolated from Dianchi Lake. Toxicity and survival mechanisms using the OJIP-test (Fluorescence transients) were investigated and analysis of pigment contents, antioxidant enzymes and DNA damage were performed in order to assess the damage of UV-B radiation and herbicides, and provide theoretical references for controlling CBs.

2. Materials and methods

2.1. Cells culture

Anabaena sp. and *Microcystis viridis*, two cyanobacteria isolated from Dianchi Lake, China, were stored in the FACHB collection (Freshwater Algae Culture Collection of Institute of Hydrobiology, The Chinese Academy of Sciences). Algal cells were cultured in BG-11 medium for 12 days (nitrogen-free BG-11 for *Anabaena* sp. and BG-11 for *M. viridis*, Rippka et al., 1979) at 25 ± 1 °C and illuminated with white light at 40 $\mu Em^{-2} s^{-1}$.

2.2. Treatment with UV-B radiation and exogenous chemicals

Exponentially growing cultures ($2–5 \times 10^4$ cells/ml) were placed in Petri dishes containing BG-11 or BG-11_0 medium, occupying a depth of 2.5 mm and supplemented with either 0.1 mM ASC or 10 μM DCMU, GPS or MCPA-Na, respectively. The cultures growing in Petri dishes with quartz lids were placed on a magnetic shaker to reduce cell aggregation and sedimentation, and then treated with UV-B radiation using a Spectronics Ultraviolet-BLE-1T158 tube (New York, USA) with the main output at 312 nm and a cellulose acetate filter to screen out UV-C, and measured with a spectroradiometer (Spectronics, XF-1000, BLE-6T312, USA). The irradiances used in the study was UV-B radiation (0–4 W/m^2) and 40 $\mu Em^{-2} s^{-1}$ PAR for different durations. The Non-irradiated cultures were used as controls.

During the recovery process, the cultures following 2 h exposure to 1 W/m^2 UV-B and herbicides were centrifuged (12,000g, 15 min) and washed 3 times to remove the herbicides, then resuspended in fresh medium and allowed to recover for 24 h under 40 $\mu Em^{-2} s^{-1}$ PAR.

2.3. Chlorophyll a fluorescence determination

The chlorophyll *a* fluorescence was determined by a portable plant efficiency analyzer (PEA, Hanstech, UK). Cultures treated with UV-B radiation and chemicals were dark-adapted for at least 15 min before measuring the fluorescence parameter *Fv*/*Fm* (PSII activity). The excitation light intensity was approximately 1500 $\mu Em^{-2} s^{-1}$ and the recording time was 5 s. The fluorescence intensities at 50 μs, 2 ms (J-step), 30 ms (I-step) and 1 s (P-step) were denoted as F_O, F_J, F_I and F_P of the JIP-test, respectively. The specific parameters ET_0/ABS, ET_0/TR_0 and RC/CS_0 were calculated according to the method of Wang et al. (2011).

2.4. Chlorophyll a (Chl a) and carotenoid analysis

Algal cultures were centrifuged at 12,000g for 15 min. The pellets were homogenized with a glass homogenizer for 5 min in an ice bath and then centrifuged at 12,000g for 10 min. Residues containing the homogenized algal cells were extracted in a 5 mL tube with 80% chilled acetone and kept in the dark for 2 h, then centrifuged at 12,000g for 10 min to obtain a clear supernatant. Absorbance of the extract was recorded at 660 nm for Chl *a* and at 470 nm for carotenoid in a spectrophotometer. The quantities of Chl *a* and carotenoid were calculated according to the method of Garcia-Pichel and Castenholz (1991).

2.5. Determination of SOD activity, MDA and protein contents

SOD activity was determined using the method previously described by Wang et al. (2007). Briefly, algal cultures treated with UV-B radiation and chemicals were centrifuged at 12,000g for 15 min, and the pellets were then ground to a powder in liquid nitrogen and homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 6.8). The homogenate were centrifuged at 12,000g for 20 min, and the supernatants were used for enzyme activity assays. One unit of enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro-blue tetrazolium reduction measured at 560 nm. Protein contents were determined according to the method of Bradford (1976), using BAS as a standard.

MDA content was prepared and assayed according to the method of Chen et al. (2009). Briefly, algal cultures were centrifuged at 12,000g for 15 min and homogenized with 10 mL 10% TCA. After centrifuged (6000g, 15 min), the supernatant (2 mL) was added to 2 mL 0.6% (w/v) thiobarbituric acid (TBA), and incubated in boiling water for 15 min. After centrifuged (10,000g, 10 min), the supernatant was measured and calculated as the method of Tang (1999).

2.6. Analysis of reactive oxygen species (ROS) generation and DNA strand breaks

ROS was analyzed by using the DCFH-DA method according to He and Häder (2002). DCFH-DA (final concentration 5 mM) was immediately added to the irradiated culture and incubated on a shaker at room temperature in the dark for 1 h. The fluorescence of the samples was measured with a fluorescence spectrophotometer (F-4500, Hitachi, Japan), with an excitation wavelength of 485 nm and an emission band between 500 nm and 600 nm. Fluorescence intensity at 535 nm normalized to the protein content was used to determine the relative ROS production.

DNA strand breaks were determined by fluorometric analysis of DNA unwinding (FADU) as described by He and Häder (2002), and modified by Chen et al. (2009). Briefly, cells were harvested by centrifugation (12,000g, 10 min). The pellet was washed with TE buffer and resuspended in solution A (50 mM Tris, pH 8.0; 50 mM Na_2EDTA; 1 M NaCl). Sarkosyl solution (10% N-lauroyl sarcosine, 10 mM Tris–HCl, pH 8.0; 20 mM EDTA) was added to the samples (final concentration 0.1%) and kept at 4 °C for 2 h. After centrifugation (7000g, 10 min), the pellet was resuspended in solution B (50 mM Tris, pH 8.0; 50 mM Na_2EDTA; 25% sucrose) up to a final volume of 184 μL. Then, 20 μL of 160 g L^{-1} lysozyme was added to the suspension to destroy the cell walls completely. The following steps were operated as the method of He and Häder (2002).

2.7. Data analysis

Data were analyzed using one-way analysis of variance (ANOVA), and values represent the mean of 3 replicates.

3. Results

3.1. Chlorophyll a fluorescence transients

To evaluate the effects of UV-B and chemicals on the electron transport of PSII, the Chl *a* fluorescence transients were recorded and analyzed according to the JIP-test, which quantifies the PSII performance. *Anabaena* and *M. viridis* controls exhibited a typical polyphasic rise in fluorescence induction (O–J–I–P) (Fig. 1). However, the fluorescence yield at phase J, I and P markedly declined with increasing UV-B doses and exposure time, and the transient almost reached a plateau after 4 h exposure to 1 W/m^2 or 2 h exposure to higher UV-B doses (Fig. 1). Following 24 h recovery, the UV exposed cells of *Anabaena* and *M. viridis* partially restored to the typical polyphasic rise in fluorescence induction, which showed a strong tolerance in response to UV-B (Fig. 1A and C).

The addition of exogenous ASC markedly increased the fluorescence yield at phase J, I and P in both cyanobacteria compared with exposure to UV-B alone (Fig. 2). However, the addition of GPS or MCPA-Na significantly decreased the fluorescence yield at phase J, I and P, while DCMU led to transformation of the O–J–I–P rise into an O–J rise compared with UV-B exposure alone.

3.2. Photosynthetic activity (Fv/Fm) and photosynthetic parameters

As shown in Fig. 3 and Table 1, exposure to 1 W/m^2 UV-B radiation for 2 h significantly ($p < 0.05$) inhibited *Fv*/*Fm*, ET_o/ABS, ET_o/TR_o and RC/CS_o in both cyanobacteria, which were significantly ($p < 0.05$) restored during the recovery process. The addition of ASC significantly increased *Fv*/*Fm* and the photosynthetic parameters of irradiated cells compared with exposure to UV-B alone, which were significantly ($p < 0.05$) restored during the