Environmental and Experimental Botany 68 (2010) 208–213

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Spiral breakage and photoinhibition of *Arthrospira platensis* (Cyanophyta) caused by accumulation of reactive oxygen species under solar radiation

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ARTICLE INFO

Article history:
Received 12 August 2009
Received in revised form
21 November 2009
Accepted 23 November 2009

Keywords:
Arthrospira platensis
Catalase (CAT)
Chlorophyll fluorescence
Morphology
Reactive oxygen species (ROS)
Superoxide dismutase (SOD)

ABSTRACT

Previous studies showed that exposure of *Arthrospira* spp. spirals to natural levels of solar radiation in the presence of UV radiation (UVR, 280–400 nm) led to the breakage of its spiral structure. However, the underlying mechanisms have not yet been explored. Here, we showed that associated accumulation of reactive oxygen species (ROS) resulted in the spiral breakage by oxidizing the lipids of sheath or cell membrane in *Arthospira platensis*, and presence of UVR brought about higher accumulation level of the ROS. Activities of superoxide dismutase (SOD) and catalase (CAT) were inhibited by high levels of solar PAR, addition of UVR led to further inhibition of CAT activity. High levels of ROS also decreased the content of photosynthetic pigments, damaged photosystem II (PSII) and inhibited the photosynthesis and growth. It is concluded that both UV and high PAR levels could generate higher amounts of ROS, which decreased the photosynthetic performances and led to spiral breakage of *A. platensis*.

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1. Introduction

Solar radiation drives photosynthesis, but also harms photosynthetic machinery (Aro et al., 1993). The UV radiation (UVR, 280–400 nm), especially UV-B (280–315 nm), is known to damage DNA and protein molecules (Niyogi, 1999) and therefore to affect physiology of cyanobacteria and algae (Häder et al., 2007). Light can also generate oxidative stress by producing reactive oxygen species (ROS) as inevitable byproducts of photosynthesis: reduction of oxygen on the acceptor side of photosystem I (PSI), as a result of the photosynthetic transport of electrons, leading to the formation of the superoxide peroxide (O_2^-), which can be further converted to hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) (Asada, 1999). Transfer of excitation energy from excited chlorophylls to oxygen in the light-harvesting complexes can lead to the formation of singlet-state oxygen ($^1O_2$) (Zolla and Rinalducci, 2002). Under less stressful conditions, these ROS can be reduced to tolerable levels by intracellular ROS-scavenging enzymes, such as superoxide dismutase (SOD) and ascorbate peroxidase, as well as antioxidants, such as β-carotene and α-tocopherol (Asada, 1999; Havaux et al., 2005). However, under stressful levels of photosynthetic active radiation (PAR), production of ROS is accelerated, giving rise to oxidative stress (Asada, 1999). Exposure to ROS, such as $^1O_2$, O_2^-, H_2O_2 and •OH, results in specific cleavage of the D1 protein, an essential component of PSII (Chen et al., 1992; Miyao et al., 1995; Okada et al., 1996). Recent studies showed that ROS preferentially inhibits the repair of PSII rather than damaging it (Nishiyama et al., 2005).

Arthrospira platensis, an economically important cyanobacterium, has been commercially produced for more than 30 years under solar radiation in artificial ponds (Belay, 1997). Its photosynthetic performance is low at noontime during such production, especially when the temperature deviates from the optimal range (Torzillo et al., 1998). The O_2 concentration in the commercial ponds may exceed five times the saturation level, leading to inhibited photosynthesis and growth (Vonshak, 1997). The accumulation of O_2 in the cultures of microalgae may enhance the production of ROS, which damages lipids, proteins and DNA by oxidation (Halliwell and Gutteridge, 1999; Nishiyama et al., 2005). Exposing indoor cultures of *Arthrospira* spp. to high oxygen concentrations leads to lower growth rate and bleached pigments (Marquez et al., 1995; Singh et al., 1995).

UVR is known to affect photosynthetic performance (Wu et al., 2005; Ma and Gao, 2009a), bleach photosynthetic pigments (Gao and Ma, 2008) and damage DNA (Gao et al., 2008) of *A. platensis*. Presence of UVR led to broken and compressed spirals of *A. platensis* (Wu et al., 2005). We showed previously that a 52.0 kDa periplasmic proteins was responsible for the compression of the spiral (Ma and Gao, 2009b). The trichomes of *Arthrospira* were surrounded by a thin, diffluent sheath similar to Gram-negative bacteria cell

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doi:10.1016/j.envexpbot.2009.11.010

wall, playing a role in maintaining the integrity of the filaments (Tomaselli, 1997). It is known that the main components of the sheath are peptidoglycans and lipids (Glauner et al., 1988; Sugai et al., 1990; Tavares and Sellstedt, 2000). On the other hand, ROS are harmful to many cellular components including lipids, proteins and nucleic acids (Oda et al., 1989; Miyao et al., 1995). We hypothesize that accumulation of ROS may lead to spiral breakage of the cyanobacterium. UV-B can induce formation of ROS in the cyanobacterium *Anabaena* sp. (He and Häder, 2002a). However, whether the accumulation of ROS in *Arthrospira* spp. contribute to the spiral disintegration remains to be investigated. Here, we report our results on the accumulation of ROS under UVR and PAR and its impacts on the morphology and physiology of *A. platensis*.

2. Materials and methods

2.1. Experimental organism

A. platensis (D-0083) was obtained from Hainan DIC microalgae CO. LTD., Hainan, China. A single healthy spiral was chosen and all the trichomes were propagated from it and cultured in Zarrouk's medium (Zarrouk, 1966). The cultures were aerated with filtered (0.22 μm) air at 30 °C and 60 μmol m^{-2} s^{-1} of cool-white light (12L:12D). Cells grown in the exponential growth phase were used in the following sub-cultures or experiments.

2.2. Measurement of solar radiation

Solar irradiances were measured using a broadband ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany) that has 3 channels for photosynthetically active radiation (PAR, 400–700 nm), ultraviolet-A radiation (UV-A, 315–400 nm) and ultraviolet-B radiation (UV-B, 280–315 nm), respectively (Häder et al., 1999). This instrument has been calibrated with the help of the maker regularly.

2.3. Radiation treatments and determination of antioxidant enzyme activity

To investigate the changes in activity of superoxide dismutase (SOD) or catalase (CAT) in the cells exposed to intensive PAR or full solar radiation, the trichomes were collected on GF/F filters and diluted with fresh Zarrouk medium to a biomass density of 0.33 g l^{-1} before transferred to quartz tubes (Ø 4.9 cm, 16 cm long). The re-suspended cells, while aerated with ambient air at 0.4 l min^{-1}, were then irradiated under PAR (P treatment, 260.86 W m^{-2}, about 1200 μmol m^{-2} s^{-1}) or PAR + UVR (PAB treatment, PAR of 260.86 W m^{-2}, UVA of 60.72 W m^{-2}, UV-B of 1.95 W m^{-2}) under a solar simulator (Sol 1200W, Dr. Hönle, Martinsried, Germany) for 4 h. For the P treatment, the tubes were wrapped with 395 cut-off foil (UV Opak, Digefra, Munich, Germany); for the PAB treatment, they were covered with 295 nm cut-off filter (Ultraphan, Digefra, Munich, Germany) to screen off UV-C. Transmission of the cut-off foils (about 90%) have been published elsewhere (Zheng and Gao, 2009); these foils reflected 4% of PAR under water (Gao et al., 2007). The tubes were placed a water bath in which the water temperature was controlled at 25 °C by a circulating cryostat (Eyela, CAP-3000, Tokyorikakikai Co. Ltd., Tokyo, Japan) set.

The harvested cells were sonicated in 10 ml ice-cold phosphate buffer, a small aliquot (1 ml) was used for total protein determination (Bradford, 1976) and the resulting supernatant was used for the assay of the antioxidant enzymes.

Total SOD activity was assayed by monitoring the inhibition of reduction of nitro blue tetrazolium (NBT) according to Giannopolitis and Ries (1977). 6 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT (nitroblue tetrazolium), 2 μM riboflavin, 0.1 mM EDTA and 1 ml of the enzyme extract. The reaction mixture was illuminated for 20 min with 80 μmol m^{-2} s^{-1} of PAR. Two blanks were prepared with the reaction mixture without the lysate, one was kept in light and the other in darkness. The former was taken as reference for calculating absorbance for one unit SOD and the latter was used as blank. One unit of the 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.

The activity of CAT was determined according to Cohen et al. (1996). Reagents and buffer for catalase assay were held at 0 °C in an ice-water bath, except for the 0.6N H_2SO_4 which is held at ambient temperature. The reaction kinetics was conducted at 0 °C with 6 mM H_2O_2. The reaction mixture (5 ml) contained 1 mM phosphate buffer (pH 7.0), 0.02N sulfuric acid 0.08 mM ferrous sulfate, 48 mM H_2O_2, 0.15 mM potassium thiocyanate (KSCN) and 0.5 ml enzyme extract. The samples were covered with aluminum foil to shield them from light and their absorbances at 492 nm (A_1) were measured after 20 min incubation. For the control, the enzyme extract was replaced with 0.5 ml phosphate buffer and its absorbance (A_0) was determined at same reaction time. The enzyme activity was determined as follows: U/mg protein = $(A_0 - A_1)$/protein.

2.4. Determination of ROS (H_2O_2)

To measure the diurnal variation of the ROS level under solar radiation, the cells at a concentration of 0.33 g l^{-1} got from linear of absorbance and dry weight were exposed to PAR or PAR + UVR (full solar spectrum) on a sunny day (21st June, 2007). The content of H_2O_2 was determined according to Rosenkranz et al. (1992) using a membrane-permeable non-fluorescent, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA can be deacetylated by esterase in the membrane and the hydrolyzed form, 2′,7′-dichlorodihydrofluorescein (DCFH), is very sensitive to oxidant species and can be oxidized to a highly fluorescent one 2′,7′-dichloroflurescein (DCF) *in vivo*. An aliquot of 20 μl DCFH-DA solution (5 mg ml^{-1}) was added in 4 ml algal suspension after the radiation treatments. The emission fluorescence at 538 nm was measured with a spectrofluorimeter (RF-5310PC, Shimadzu, Kyoto, Japan) with the excitation at 485 nm after 2 min dark incubation. The concentration of ROS was derived on the basis of the relationship of the fluorescence levels with the standard H_2O_2 concentrations determined in the same way.

2.5. Measurement of chlorophyll fluorescence

In order to investigate whether phycobilisome (PBS) and chlorophyll–protein complexes was damaged by ROS, we examined the changes in room-temperature Chl fluorescence in the cells treated with H_2O_2 at levels of 0.01, 0.1, 1 and 5 mmol g^{-1}-cells of dry mass. The Chl fluorescence emission spectra were measured with the spectrofluorimeter as mentioned above. The excitation wavelength was set at 436 for Chl *a* or 580 nm for PBS (Wen et al., 2005).

2.6. Determination of photosynthetic activity

To examine the effects of H_2O_2 on the photosynthetic capacity, the effective quantum yield (F_v'/F_m') and relative electron transport rate (rETR) were determined with a portable pulse amplitude modulated fluorometer (WATER-ED, Walz, Effeltrich, Germany). The actinic light was 80 with the measuring light of 0.3 and the saturating pulse of 5600 μmol m^{-2} s^{-1}.

Parameters of the rETR vs. *E* curves were analyzed according to Eilers and Peeters (1988) as follows: $rETR = E/(aE^2 + bE + C)$,

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