The involvement of the antioxidant system in protection of desert cyanobacterium *Nostoc* sp. against UV-B radiation and the effects of exogenous antioxidants

Gaohong Wang, Kun Chen, Lanzhou Chen, Chunxiang Hu, Delu Zhang, Yongding Liu*

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, People’s Republic of China

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Abstract

In this study, we found that UV-B radiation decreased photosynthetic activity and boosted lipid peroxidation of desert *Nostoc* sp., and exogenous chemicals (ascorbate acid (ASC), *N*-acetylcysteine (NAC), and sodium nitroprusside (SNP)) had obvious protective effects on photosynthesis and membranes under UV-B radiation. High-concentration SNP boosted the activities of antioxidant enzymes, but low-concentration SNP reduced the activities of antioxidant enzymes. Both NAC and ASC treatments of cells decreased activities of antioxidant enzymes. The results suggested that those chemicals possibly had different mechanisms of protection of algae cells against UV-B radiation. SNP might play double roles as a signal molecule in the formation of algae cell protection of Photosystem II under UV-B radiation and as a (reactive oxygen species) scavenger, while NAC and ASC might function as antioxidant reagents or precursors of other antioxidant molecules, which could protect cells directly against ROS initiated by UV-B radiation.

Keywords: Antioxidant system; Exogenous antioxidant; *Nostoc* sp.; Photosynthesis; UV-B radiation

1. Introduction

The UV-B radiation reaching the Earth’s surface has increased as a result of depletion of the ozone layer. UV radiation in solar light at low doses affects growth and metabolic activity in organisms, especially photoautotrophic organisms, which need light for synthesis of biomass and fixation of energy. Evidence indicates that increased UV-B radiation reduces photosynthesis by as much as 25% in the top 10–20 m of Antarctic waters and hampers CO₂ fixation (Malanga et al., 1999). On the other hand, high-intensity UV-B induces lipid peroxidation of biological membranes, which can be assayed by malondialdehyde (MDA) content, DNA damage, and hormone inactivation (He and Häder, 2002a). Photosynthetic apparatus is also impaired by UV radiation with pigment altered, composition, membrane damaged, and reaction center protein destruction (He and Häder, 2002b).

Cyanobacteria in desert regions are more easily subjected to UV-B radiation, which pressures them to be capable of adapting to the stress (Estevez et al., 2001; Dillon and Castenholz, 2003; Gorton and Vogelmann, 2003; Chen et al., 2003b). Some studies suggested that those algae might develop several strategies to survive under UV-B stress, including synthesis of UV-screening pigments, such as scytonemins, mycosporine-like amino acids, migration to escape from UV radiation, and induction of highly efficient antioxidant and DNA repair systems (Sinha et al., 2003). It has been found that the detrimental effects of UV-B radiation on cyanobacteria are always mediated by reactive oxygen species (ROS) (Wendehenne et al., 2004; Babu et al., 2003; He and Häder, 2002b; Agarwal et al., 2005). To quench ROS, organisms develop efficient antioxidant systems to scavenge them, including antioxidant molecules and antioxidant enzymes, which play obvious protective roles in organisms under oxidant stress.
(Gong et al., 2005; Costa et al., 2002; White and Jahnke, 2002; Fedina et al., 2003; Mallick, 2004; Sigaud-Kutner et al., 2005). Enzymatic components include superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), of which SOD is a major scavenger of O2 free radicals and converts them into O2 and H2O2. The H2O2 is then scavenged by CAT and variety of POD into H2O and O2. In addition to SOD, CAT, and POD, another major component of the enzymatic defense is the ascorbate (ASC) glutathione (GSH) cycle that involves four enzymes including ascorbate peroxidase (ASC-P), monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. It is good for *Nostoc* protection if those antioxidant elements increase when cells are under stress. Among them ASC-P is the major scavenger of H2O2, which requires reduced ASC provided by the ASC-GSH cycle for the reaction to occur (Ali et al., 2005).

With regard to the responses of antioxidant enzymes in desert organisms under UV radiation, there apparently are no reports. Of the small antioxidant molecules, ASC and tripeptide glutathione may be the most important antioxidants in plants and might play pivotal roles in quenching ROS (He and Hader, 2002). Research showed that *N*-acetylcysteine (NAC), a well-established thiol antioxidant, can be uptaken and converted to GSH in plants, which can function as effective exogenous antioxidant to scavenge ROS (Malanga et al., 1999). Recent evidence also indicated that nitric oxide (NO), a diffusible multifunctional molecule, was involved in various physiological processes, including salt tolerance, hypersensitive responses, radiation adaptation, growth, development, nutrition, and flowering in bacteria, fungi, animals, and plants (Zhang et al., 2003). Exogenous NO, supplied by sodium nitroprusside (SNP), always had the same effect on organisms when the endogenous NO synthesis pathway was inhibited. With regard to algae, it was found that both exogenous and endogenous nitric oxide can alleviate oxidative damage in the green alga *Chlorella pyrenoidosa* caused by UV-B radiation (Chen et al., 2003a). Research indicated that exogenous antioxidants including ASC, NAC, and SNP can protect algae under UV-B radiation (Chen et al., 2003a), but, with regard to the roles of exogenous antioxidants on the response to oxidant stress, there is also little information, especially about how they interact with endogenous antioxidant enzymes to protect organisms from oxidant stress (He and Hader, 2002a; Laspina et al., 2005; Malanga et al., 1999; Morley et al., 2003). In this study, these issues were investigated with aims to: (1) investigate the responses of photosynthesis and antioxidant enzymes of desert *Nostoc* sp. under UV radiation, (2) determine the roles of exogenous antioxidants (SNP, NAC, and ASC) on photosynthetic rates of desert cyanobacterium *Nostoc* sp. subjected to UV-B radiation, and (3) study the interaction of the exogenous antioxidant molecules and the endogenous antioxidant system of desert cyanobacterium under UV radiation and then discuss the mechanism of protection of exogenous antioxidants under oxidant stress.

2. Materials and methods

2.1. Cell culture

*Nostoc* sp. was obtained from Shapotou (37°27′N, 104°57′E) in the Tengger desert, Ningxia Hui Autonomous Region of China and collected by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, The Chinese Academy of Sciences. The organisms were found on the top of the soil and had thick polysaccharide sheaths (Hu et al., 2003), but unfortunately they were not identified because the species complete life history was not available. Prior to experiments, the species was cultured in BG110 (fixed nitrogen-free BG-11; Rippka et al., 1979) medium at 25 ± 1°C, 40 μmol photon m−2 s−1 light, and bubbled with air.

2.2. Treatment with UV-B radiation and exogenous antioxidant

Algae culture was selected for investigation and supplemented with BG11 medium (nitrogen free) containing a final concentration of 0.05–0.5 mM ASC, NAC, SNP (Chen et al., 2003a). Simulated solar radiation was obtained from a combination of fluorescent lamps (He and Hader, 2002a). UV-B from Philip Ultraviolet-B TL 40 W/12 (The Netherlands) tube with its main output at 312 nm, and cellulose acetate was used to filter out UV-C. The irradiances were 40 μmol photon m−2 s−1, photosynthetic active radiation (PAR) and UV-B radiation was measured with a double-monochromator spectroradiometer (OL 754; Optronic Laboratories, Orlando, FL, USA).

2.3. Measurement of photosynthetic activity

Photosystem II (PSII) activity was determined from samples using the Phyto-PAM Fluorometer (Walz, Germany) (Körner and Nicklisch, 2002). PSII activity was determined using quantum yield rate of photosynthesis. A more complete fluorometric characterization of photosynthetic activity was performed by recording electron transport rate versus PAR curves (Körner and Nicklisch, 2002; Wang et al., 2005).

2.4. Determination of activities of SOD, CAT, POD, ASC-P, and MDA and protein concentration

Extractions of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), and ASC-P (EC 1.11.1.11) were as described in Lin and Kao (2000) with few changes. Briefly, algae culture treated with UV-B and exogenous chemicals for 2 h was collected by centrifuging at 12,000 g for 10 min, ground to powder in liquid nitrogen, and homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 6.8). The homogenates were centrifuged at 12,000 g for 20 min, and the supernatants were used for enzyme activity assays.

POD activity was determined by measuring the rate of increase in absorbance at 470 nm of a mixture containing 1 ml 50 mM sodium phosphate buffer (pH 7.0), 0.95 ml 0.2% 2-methoxyphenol, 1 ml 0.2% hydrogen peroxide, and 0.05 ml enzyme extract or distilled water for control (total reaction volume 3 ml).

CAT activity was determined according to Cakmak and Marschner (1992) with slight modification. The reaction mixture in a total volume of 3 ml contained 1.9 ml 50 mM sodium phosphate buffer (pH 7.0) and 1 ml 0.2% H2O2. The reaction was initiated by the addition of 0.1 ml enzyme extract and activity was determined by measuring the initial rate of disappearance of H2O2 at 240 nm.

SOD activity assay was based on the method described by Giannopolitis and Ries (1977). 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.
ASC-POD activity was determined by following the decrease of absorbance at 290 nm using an extinction coefficient ($\varepsilon$) of 2.8 mM$^{-1}$ cm$^{-1}$ (Chen and Asada, 1989).

Lipid peroxidation (MDA) was assayed according to He and Häder (2002a).

Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

All experiments were carried out more than three times.

2.5. Statistics

All data were evaluated by one-way ANOVA (SPSS 6.0.1 for Windows; tests: least significant difference, Tukey’s honestly significant difference).

3. Results

3.1. UV-B inhibited the photosynthetic activity of cyanobacteria

It was found that UV-B decreased the photosynthetic activity of Nostoc sp (Fig. 1). The inhibition of photosynthetic quantum yield increased with increasing UV-B radiation intensity and exposure time. Low radiation intensity (0.025 mW) induced radiation resistance after 6 h exposure, whereas high radiation intensity (0.2 and 0.1 mW) had the strongest inhibition with increasing exposure time. After adaptation for 12 h under PAR radiation (without UV-B), the photosynthetic activity of cyanobacteria recovered to different degrees with respect to radiation intensity. With regard to the low-intensity-radiation group, the activity had returned to the normal state as did the control, but with regard to the high-intensity-radiation group, the photosynthetic activity returned only partly to the normal state, indicating that high-intensity UV-B radiation may produce more damage to the photosynthetic apparatus which cannot be recovered in a short time.

3.2. Exogenous chemicals had different mechanisms of protection of photosynthesis of algae under UV-B radiation

SNP is a NO donor, which can supply NO to the experimental system externally (Singh et al., 2004). NAC is the precursor of GSH synthesis and a ROS scavenger, which has been shown to be an effective protector under oxidant stress (Malanga et al., 1999; Morley et al., 2003; He and Häder, 2002a). ASC is an effective small antioxidant molecule, which plays very important roles in the scavenging of ROS in plants and animals, particularly hydrogen peroxide and the extremely reactive hydroxyl radical, and in the regeneration of $\alpha$-tocopherol (He and Häder, 2002a). It was found that ASC and NAC addition increased the activity of photosynthesis compared to that of the group under UV-B radiation without chemical addition during treatment, which indicated that ASC and NAC had obvious protection on the photosynthetic apparatus under UV-B radiation, but the effects of SNP are different from those of ASC and NAC.

Our results showed that SNP addition decreased photosynthetic activity after 2 h treatment but increased photosynthesis after 4–6 h treatment and 12 h adaptation (Fig. 2), which indicated that the effects of SNP addition on photosynthesis were changed during treatment and that SNP might induce some endogenous protective ability of algae under UV-B radiation. These effects are different from the effects of ASC and NAC addition which might merely quench ROS of algae under UV-B radiation and do not induce endogenous protective ability of algae. These results suggest that different chemicals might have different mechanisms of protection.
mechanisms of protection of photosynthesis under UV-B radiation.

3.3. Effects of exogenous chemicals on the antioxidant enzymes’ activity in algae under UV-B radiation

UV-B radiation increased the SOD activity of algae. Compared to the group under UV-B+PAR, high-concentration SNP addition increased SOD activity of algae (Fig. 3), but low-concentration SNP addition had an obvious effect of reduction on the SOD activity. For NAC and ASC addition, a different phenomenon is occurring because they reduced SOD activity of algae under UV-B radiation and the reductions were concentration dependent.

Fig. 4 shows that UV-B radiation also increased the CAT activity of algae. Compared to the group under UV-B+PAR, SNP addition increased CAT activity but NAC had the most efficient ability to reduce CAT activity of algae under UV-B radiation, ASC also had an obvious effect on CAT activity (Fig. 4). These results indicated that SNP had effects on CAT activity completely different from those of NAC and ASC.

UV-B radiation also improved POD activity of algae, and SNP, NAC, and ASC addition resulted in obvious reduction of POD activity under UV-B radiation (Fig. 5). SNP (0.05 mM) had the most efficient reduction of POD activity in our experiment, ASC addition also had efficient reduction of POD activity and NAC had the least reduction effect.

With regard to the ASC-POD activity, it was found that UV-B radiation increased the activity of ASC-POD of algae significantly (Fig. 6). With regard to the SNP addition, high concentration increased activity of ASC-POD in algae under UV-B radiation, but low concentration decreased enzyme activity of algae in our experiments. Fig. 6 also indicated that low-concentration NAC and ASC had no effect on ASC-POD activity, but high concentrations obviously indicated efficient reduction of the enzyme activity of algae under UV-B radiation.

3.4. Protection by exogenous chemicals to the membrane of algae under UV-B radiation

MDA content is used to assay the level of lipid peroxidation of organisms under stress. In this study, it was found that UV-B radiation obviously increased MDA
content compared to the control under PAR, which showed that lipid peroxidation had occurred in algae cells under UV-B radiation. SNP addition had the most efficient reduction of MDA content which indicated that SNP is the most efficient chemical to protect lipid membrane from peroxidation induced by UV-B radiation (Fig. 7). The effects of NAC and ASC were concentration dependent because low concentration had lesser effect and high concentration had greater effect.

4. Discussion

*Nostoc* is one of the common filamentous cyanobacterial genera in microbiological crusts that is always distributed at the surface of crust, which suggests that it must be capable of high resistance to the high intensity of solar light in desert regions (Hu et al., 2003). Photosynthesis is one of the most important metabolic activities of algae because it is the main source of biomass and energy for the support ecosystem in crust. So the effect of solar light on photosynthesis is critical for the survival of algae on the surface of a desert. Unfortunately, the relevant research has not been carried because it is not easy to obtain desert algae samples and pure desert algae strains.

It was found that UV-B radiation reduces photosynthesis by as much as 25% in Antarctic waters and hampers CO₂ fixation activity (Malanga et al., 1999). With regard to land plants, the photosystem is always the initial and major target for destruction by increased UV radiation. Pigments, proteins, and membranes of the photosystem are sensitive to UV radiation because chlorophylls, phycobiliproteins, and quinines of photoautophic organisms exhibit absorption in the UV range which can be photosensitive to the formation of ROS (He and Häder, 2002a). As a result, UV stress leads to the inhibition of photosynthesis, peroxidation of lipids, and damage of DNA (He and Häder, 2002a, b). Our results also showed that UV radiation hampered photosynthesis of desert *Nostoc* sp. and that the effect of the radiation was dependent on the radiation intensity and exposure time. SNP, ASC, and SNP also evidenced different levels of protection of photosynthesis activity under UV radiation. Evidence showed that the detrimental effects of UV-B radiation of solar light on cyanobacteria are always mediated by ROS, which cause cells to be under oxidant stress (Wendehenne et al., 2004; Babu et al., 2003; He and Häder, 2002b; Agarwal et al., 2005). With regard to the photosystem, the formation of ROS was suggested to be involved in the destruction of D1 protein. Photosynthetic membrane is another target of ROS in algae, which elevate lipid peroxidation and increase membrane leakage. Our results also showed that lipid peroxidation is boosted by UV-B radiation and that the level of lipid peroxidation is dependent on the intensity of radiation and exposure time.

To mitigate the oxidative stress caused by ROS, organisms develop efficient antioxidant systems to scavenge them, including antioxidant molecules such as carotenoids, tocopherols, ASC, and reduced GSH, and antioxidant enzymes such as SOD, CAT, and POD (Apel and Hirt, 2004; Gong et al., 2005; Costa et al., 2002; White and Jahneke, 2002; Mallick, 2004; Sigaud-Kutner et al., 2005). Exogenous antioxidants can also mitigate oxidant
stress in organisms, and SNP, NAC, and ASC are among the most efficient chemicals for protection of organisms under oxidant stress (Chen et al., 2003a; He and Häder, 2002a; Laspinas et al., 2005; Malanga et al., 1999; Morley et al., 2003). Our results showed that the lipid peroxidation of Nostoc under UV radiation is decreased significantly with exogenous antioxidant chemical addition, which implied that SNP, ASC, and NAC had obvious effects of protection on Nostoc under UV radiation.

NO is reported to be a regulatory molecule during plant development, cell differentiation, and response to abiotic stress (Huang et al., 2002; Hung and Kao, 2004; Urios et al., 2003; Zhao et al., 2004; Zhang et al., 2003). We have found that hormogonia differentiation of Nostoc sphaeroides is a NO-dependent process (Li et al., 2002) and that NO plays essential roles in the resistance against UV-B radiation (Chen et al., 2003a). SNP (NO generator) can also increase the resistance of plants to a variety of stresses including salt, heavy metals, aridity and UV-B radiation (Shi et al., 2005; Wendehenne et al., 2004; Singh et al., 2004; Chen et al., 2003a; Tyagi et al., 2003). NO is a special free radical which can play double roles as a scavenger for other free radicals and as a signal molecule to form resistance to oxidant stress. Our results indicated that SNP addition decreased photosynthetic quantum yield of Nostoc after 2–4 h treatment and then increased photosynthesis after 6 h treatment and 12 h adaptation under PAR compared to the control under UV radiation without chemical addition. This is also clearly different from the groups with ASC and NAC addition. Those results implied that SNP might induce some protection in cells of Nostoc step by step, which suggests a mechanism different from those of ASC and NAC. Some researchers think that NO protects cells from ROS-mediated cellular damage and cytotoxicity by changing the expression of cytoprotective proteins including CAT, SOD, and GST (Huang et al., 2002; Kopyra and Gwóźdź, 2003). Our data indicated that 0.5 mM SNP (NO generator) addition boosted significantly the activities of SOD, CAT, and ASC-POD of Nostoc under UV-B radiation, supporting this theory. At the same time, it was also found that the MDA content of all groups with SNP addition was significantly lower than that of the control under UV radiation without chemical addition. These results showed that SNP only induced the expression of antioxidant enzymes but did not increase the lipid peroxidation or damage to cyanobacterium, which suggested that NO generated by SNP might play a role as signal molecule for stress adaptation (Wendehenne et al., 2004). In our study, it was also found that 0.1 and 0.05 mM SNP addition decreased the activity of antioxidant enzymes of algae cells under UV radiation, but the effects of SNP are smaller than those of NAC and ASC, which suggested that the low concentration of NO might play the role of antioxidant and signal molecule simultaneously in organisms.

NAC is a well-established thiol antioxidant that after uptake, decacylation, and conversion to GSH, can function as a scavenger of reactive oxygen intermediates. Evidence indicates that NAC can maintain ascorbyl and lipid radical contents at the basal level in algae culture and soybean leaves exposed to UV-B (Malanga et al., 1999). NAC also can protect organisms from DNA damage under UV radiation (Morley et al., 2003). In Anabaena sp. under UV-B radiation, NAC addition reverses the oxidant stress and protects the organisms from chlorophyll bleaching and damage of the photosynthetic apparatus (He and Häder, 2002a). Our data showed that NAC addition can provide effective protection for photosynthetic activity of Nostoc under UV-B radiation and that the protective effect of NAC is greater than those of ASC and SNP addition after 2 h treatment. Also, the protective effect of NAC changed less than that of SNP addition with increasing exposure time of UV radiation. The level of lipid peroxidation was also reduced by NAC addition in our experiments, which indicated that NAC functioned as a scavenger of ROS and protected cells from oxidant stress induced by UV-B radiation. NAC addition also affected the activity of antioxidant enzymes: the activities of SOD, CAT, ASC-POD, and POD were reduced significantly in the cells treated with NAC. The results were also consistent with results of other research in Chlorella vulgaris (Malanga et al., 1999), which suggested that NAC may be as efficient scavenger of ROS, especially of H2O2, and might quench most ROS in the cells of algae under UV-B radiation. The results suggested that the role of NAC in cyanobacterium is to be an efficient scavenger of ROS, which is different from the role of NO which may be that of a signal molecule for stress adaptation.

With regard to the exogenous antioxidant ASC, evidence showed that it plays essential roles in the quenching of ROS in plants and animals, particularly H2O2 and the extremely reactive hydroxyl radical, and in the regeneration of α-tocopherol. In Anabaena, it was found that ASC exhibited significant protective effects on lipid peroxidation and DNA strand breaks, and the UV-B-induced damage to photosynthesis was also inhibited significantly by ASC addition (He and Häder, 2002a). ASC addition increased obviously the photosynthetic activity of Nostoc under UV radiation but its protective effects were smaller than those of NAC addition, which corroborated the research findings in Anabaena and suggested that it might share the same mechanisms in different species. ASC addition also affected the activity of antioxidant enzymes such as that of NAC addition, in which the activities of SOD, CAT, ASC-POD, and POD were reduced obviously in the cells treated with ASC. The major effect of ASC addition was the activity of POD, which suggested that ASC might quench most ROS caused by UV radiation, perform the same function as POD in vivo, and then inhibit excess expression of POD of cyanobacterium under UV radiation. The results suggested also that ASC may be an efficient scavenger of ROS but its protective effects are smaller than those of NAC.

On the other hand, the Nostoc species that we used is isolated from topsoil of desert crust which indicates that
it’s filaments in air have more polysaccharides than those in the aqueous solution in which our experiments were carried out. Furthermore, UV-B radiation can induce synthesis of polysaccharides and photoprotective pigments (Ehling-Schulz et al., 1997). Additional evidence indicates that polysaccharides are also effective antioxidants (Qi et al., 2005) under various environmental stresses including UV-B radiation (unpublished data), salt (Chen et al., 2003a), and dehydration. Thus the equivalent organism on land most likely has even more protection than the specimens in the aqueous solution. We have found that the type and ratio of polysaccharides of cyanobacterium changed under UV-B radiation and that exogenous polysaccharides addition can protect the photosynthetic apparatus under UV-B radiation (unpublished data). So it is necessary to consider the role of polysaccharides of algae under UV-B radiation.

In summary, it was found that UV-B radiation decreased photosynthetic activity and increased lipid peroxidation of desert cyanobacterium Nostoc sp. and that exogenous chemicals (ASC, NAC, and SNP) had obvious protective effects on photosynthesis and membrane of algae under UV-B radiation. High-concentration SNP boosted the activities of antioxidant enzymes, but low-concentration SNP reduced the activities of antioxidant enzymes. Both NAC and ASC treatment of cells decreased activities of antioxidant enzymes. Those results suggested that different chemicals may have different mechanisms for protection. SNP might play dual role as signal molecule to induce UV radiation resistance and ROS scavenger in the formation of algae’s protection of PSII under UV-B radiation, while NAC and ASC function as antioxidant reagents or precursors of other antioxidant molecules, which could protect PSII directly from UV-B radiation.

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