UV-B-induced Oxidative Damage and Protective Role of Exopolysaccharides in Desert Cyanobacterium \textit{Microcoleus vaginatus}

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Abstract

UV-B-induced oxidative damage and the protective effect of exopolysaccharides (EPS) in \textit{Microcoleus vaginatus}, a cyanobacterium isolated from desert crust, were investigated. After being irradiated with UV-B radiation, photosynthetic activity ($F_{v}/F_{m}$), cellular total carbohydrates, EPS and sucrose production of irradiated cells decreased, while reducing sugars, reactive oxygen species (ROS) generation, malondialdehyde (MDA) production and DNA strand breaks increased significantly. However, when pretreated with 100 mg/L exogenous EPS, EPS production in the culture medium of UV-B stressed cells decreased significantly; $F_{v}/F_{m}$, cellular total carbohydrates, reducing sugars and sucrose synthase (SS) activity of irradiated cells decreased significantly, while ROS generation, MDA production and DNA strand breaks of irradiated cells decreased significantly. The results suggested that EPS exhibited a significant protective effect on DNA strand breaks and lipid peroxidation by effectively eliminating ROS induced by UV-B radiation in \textit{M. vaginatus}.

Key words: carbohydrate metabolism; desert algae; extracellular polymeric substances; reactive oxygen species; UV tolerance.


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UV-B radiation is one of the most detrimental environmental factors for photosynthetic organisms in arid regions (Painter 1993; Bowker et al. 2002). Because of its high energy, it leads to the production of reactive oxygen species (ROS) in the evolution of photosynthetic processes in plant cells (Apel and Hirt 2004). It easily destroys proteins, DNA and other biological molecules (Sinha et al. 1998). A number of physiological and biochemical processes, such as growth, survival, pigmentation, photosynthetic oxygen production and phycobiliprotein composition, have been reported to be susceptible to UV-B radiation (Wang and Zhang 2001). Cyanobacteria have also developed mechanisms to counteract the damage effects of UV irradiance (Sinha et al. 1998). There are three adaptation strategies for cyanobacteria to take measures to avoid UV radiation damage: avoidance, repair and protection. For example, they can synthesize UV-absorbing substances or quenching agents to protect cells, or escape UV-B damage by migration, or repair photosynthetic apparatus and DNA strand breaks by UV-B radiation (Garcia-Pichel and Belnap 1996; Quesada and Vincent 1997).

As one of the most dominant species in desert microbiotic crust, \textit{Microcoleus vaginatus} excretes large amounts of extracellular polymeric substances (EPS) in the culture medium (Chen et al. 2003). The excretion of EPS can serve as a boundary between cells and the surrounding environment and fulfills a protective role against desiccation (De Caiola et al. 1996; De Philippis et al. 2001). It may be used for heterotrophic metabolism in algae in surviving prolonged periods under detrimental conditions (Gross et al. 1998). Some works also have...
revealed that UV-B induced the synthesis of exopolysaccharides in cyanobacteria since it only serves to provide a matrix for mycosporine amino acids (MAAs) and scytonemin (Ehling-Schulz et al. 1997; Quesada et al. 1999). It is conceivable that these pigments are covalently linked to the glycan after they are secreted from the cell (Potts 1999). So the changes of carbohydrate metabolism in cyanobacteria are related to UV-B absorbing substances. However, few works have revealed that EPS excretion is involved in enhancing UV-B tolerance in cyanobacteria. Since *M. vaginatus* was the preferential choice for desert crust formation (Chen et al. 2006), the present work is aimed at explaining the excretion of EPS in surviving UV-B damage, so as to increase the viability of inoculums on sand dunes.

**Results**

**Chlorophyll a fluorescence**

As illustrated in Figure 1, photosystem II (PSII) activity (*Fv/Fm*) of *M. vaginatus* decreased significantly (*P < 0.01*) after irradiation with doses of different levels of UV-B. *Fv/Fm* was inhibited intensively under the higher levels of UV-B radiation. After recovering for 16 h under 40 μE/m² per s white light, *Fv/Fm* was recovered to the normal at 1 W/m² and was not detected at 4 W/m². *Fv/Fm* of cells pretreated with exogenous EPS was significantly higher than that of untreated cells at various levels of UV-B radiation (Figure 2).

**EPS production**

As shown in Figure 3, EPS production of *M. vaginatus* in the culture medium increased slowly with the increase of culture time; however, it decreased significantly after it was irradiated with 2 W/m² UV-B (*P < 0.05*). After pretreatment with 100 mg/L EPS, exogenous EPS in the culture medium also decreased...
Protective Role of EPS to UV-B in *M. vaginatus*

Significantly (*P* < 0.01) when exposed to UV-B radiation. But EPS production increased significantly (*P* < 0.01) after it recovered for 18 h under white light. The results suggested that EPS in the culture medium could be absorbed by cultures after irradiation with UV-B radiation.

**Cellular carbohydrates and sucrose metabolism**

Cellular total carbohydrates, reducing sugars and sucrose were measured to study the effects of UV-B radiation on carbohydrate metabolism in *M. vaginatus* (Table 1). Compared with un-irradiated cells, cellular total carbohydrate and sucrose of irradiated cells decreased, reducing sugars increased significantly (*P* < 0.05). After pretreatment with 100 mg/L EPS, cellular total carbohydrates, reducing sugars and sucrose production were significantly higher (*P* < 0.05) at different levels of UV-B dose than cells stressed with UV-B alone.

Sucrose phosphate synthase (SPS) and sucrose synthase (SS) activity of un-irradiated or irradiated cells decreased significantly (*P* < 0.05) as shown in Figure 4. After being pretreated with 100 mg/L EPS, SPS activity didn’t have a significant change, whereas SS activity increased significantly (*P* < 0.05) compared with cells irradiated with UV-B alone.

**ROS generation, MDA production and DNA strand breaks**

UV-B radiation usually induces the generation of ROS in photosynthetic organisms. As shown in Figure 5, after irradiation with 2 W/m² UV-B, ROS generation measured by DCF (2',7'-dichlorodihydrofluorescein) fluorescence increased significantly (*P* < 0.05), and decreased significantly (*P* < 0.05) after pretreatment with exogenous EPS; malondialdehyde (MDA) production also increased significantly (*P* < 0.05) after being irradiated with UV-B, and decreased significantly (*P* < 0.05) at different levels of UV-B dose after being pretreated with exogenous EPS.

As shown in Figure 5, dsDNA (%) of *M. vaginatus* decreased significantly (*P* < 0.05) after irradiation with 2 W/m² UV-B, which indicated significant DNA strand breaks, whereas the addition of exogenous EPS exhibited a significant inhibition in DNA strand breaks (*P* < 0.05). The results suggested that membrane system and dsDNA were protected effectively from the oxidative damage induced by UV-B radiation after pretreatment with exogenous EPS.

**Discussion**

As one of the most common filamentous cyanobacteria, *M. vaginatus* is usually used to form man-made algal crusts in desert regions in recent years, but its low viability has restricted the colonization on sand surface because of the intensive UV-B radiation (He and Häder 2002b; Hu et al. 2002; Chen et al. 2006). In this study, organisms exposed to UV-B radiation decreased photosynthetic activity and induced significant oxidative damage.

Table 1. Changes of cellular total carbohydrates, reducing sugars and sucrose under different levels of UV-B radiation or with 100 mg/L exopolysaccharides (EPS).

<table>
<thead>
<tr>
<th>Cellular carbohydrate (mg/g DW)</th>
<th>0 (W/m²)</th>
<th>0.5 (W/m²)</th>
<th>1 (W/m²)</th>
<th>2 (W/m²)</th>
<th>4 (W/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar</td>
<td>74.09 ± 6.23</td>
<td>87.03 ± 7.61</td>
<td>95.84 ± 6.13</td>
<td>97.86 ± 8.85</td>
<td>85.40 ± 7.16</td>
</tr>
<tr>
<td>Sucrose</td>
<td>11.40 ± 1.37</td>
<td>11.52 ± 1.25</td>
<td>9.46 ± 0.80</td>
<td>6.55 ± 0.95</td>
<td>5.08 ± 1.45</td>
</tr>
<tr>
<td>Water soluble sugar</td>
<td>35.08 ± 1.23</td>
<td>37.27 ± 2.19</td>
<td>35.30 ± 2.36</td>
<td>28.34 ± 2.18</td>
<td>29.25 ± 1.66</td>
</tr>
</tbody>
</table>

Cellular carbohydrate after pretreated with 100 mg/L EPS

<table>
<thead>
<tr>
<th>Cellular carbohydrate</th>
<th>0 (W/m²)</th>
<th>0.5 (W/m²)</th>
<th>1 (W/m²)</th>
<th>2 (W/m²)</th>
<th>4 (W/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar</td>
<td>91.23 ± 8.32</td>
<td>127.43 ± 11.12</td>
<td>145.32 ± 10.09</td>
<td>149.15 ± 10.24</td>
<td>95.34 ± 8.79</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12.57 ± 1.03</td>
<td>16.78 ± 1.32</td>
<td>12.32 ± 1.56</td>
<td>9.74 ± 0.89</td>
<td>8.33 ± 0.76</td>
</tr>
<tr>
<td>Water soluble sugar</td>
<td>45.21 ± 3.78</td>
<td>52.36 ± 4.05</td>
<td>49.61 ± 5.06</td>
<td>38.99 ± 2.90</td>
<td>33.65 ± 2.25</td>
</tr>
</tbody>
</table>

DW, dry weight; EPS, exopolysaccharides. *P* < 0.01. Results shown are means ± SD.
stress (ROS), lipid peroxidation (MDA) and DNA strand breaks. However, photosynthetic activity of irradiated cells increased significantly following the addition of exogenous sucrose or EPS (Figure 2). The results indicated that exogenous EPS had a significant protective effect on *M. vaginatus* and could enhance the viability of irradiated cells (He and Häder 2002a).

Carbohydrate metabolism was improved after exposure to UV-B irradiance. PSII activity (*Fv/Fm*), EPS production and cellular sucrose of irradiated cells decreased significantly after irradiation with UV-B (Figure 1; Table 1). As we know, chlorophyll fluorescence (*Fv/Fm*) reflected light dependency of the quantum yield of PSII (Bilger et al. 1995). The damage of *M. vaginatus* did not result in lethal effects after irradiation with 2 W/m² UV-B since photosynthetic activity still remained. Since photosynthesis could offer the source of carbon and energy for the synthesis of sucrose, the partial inhibition of photosynthetic activity suggested the reduction of cellular sucrose. After pretreatment with exogenous EPS, reducing sugars and cellular sucrose of irradiated cells increased significantly after irradiation with UV-B. The advantage of reducing sugars synthesis is that it can undergo Maillard reactions with the amino groups of proteins that are related to carbohydrate assimilation (Potts 1999). The increase of cellular reducing sugar indicated the increase of assimilative reaction in carbohydrate metabolism (Page-Sharp et al. 1999). EPS production in the culture medium increased significantly after it recovered under white light (Figure 3). This suggests that EPS synthesis was prior to other carbohydrates. Therefore, the applied exogenous EPS of *M. vaginatus* might be absorbed and used as a carbon source for heterotrophic metabolism in surviving prolonged periods under detrimental conditions (Gross et al. 1998; Chen et al. 2003). Cellular sucrose significantly increased and coincided with the inhibition of SS activity (the main enzyme in the sucrose cleavage) should be advantageous for maintaining sucrose metabolism and thus protecting the membrane system (Potts 1999).

The photosynthetic electron transport system in an oxygen atmosphere is the major source of ROS in plant tissues (Wang and Zhang 2001). The electron leakages of PSI and PSII resulted in the formation of ROS in the light (Foyer et al. 1994). ROS generated under UV-B stress, especially hydroxyl radical (HO⁻) and singlet oxygen (¹O₂), react with sugars and purines (Apel and Hirt 2004). PSII activity (*Fv/Fm*) of cells pretreated with exogenous EPS was significantly higher than cells untreated with EPS, which showed that the UV-B-induced damage to photosynthesis was inhibited significantly. The increased formation of ROS is harmful to the normal metabolism and results in oxidative damage to photosynthesis targeted at lipid, proteins and DNA molecules, including lipid peroxidation, protein degradation and DNA damage (Kumar et al. 2004). As an end product of lipid peroxidation, MDA production showed the stability of the membrane system. MDA production and DNA strand breaks of irradiated cells increased significantly under UV-B stress (Figure 5). However, the addition of exogenous EPS (100 mg/L) to the culture medium of *M. vaginatus* protected the test organisms from oxidative stress, including photosynthetic activity, lipid peroxidation and DNA breaks. This might be
attributed to the decrease of ROS molecules in M. vaginatus after the addition of exogenous of EPS (Figure 5). As we have known, EPS could effectively scavenge cellular oxygen hydroxyl (OH), and demonstrates protective effects against the inhibition of the fusion between the membrane vesicles in cyanobacteria (Hill et al. 1997; Zhou et al. 1997). Physico-chemical structure of EPS of M. vaginatus showed that the major sugars present in the carboxylic moiety of biopolymer contained 7.5% to 50.3% protein and 16.2% to 40.5% carbohydrate, and the main carbohydrate chain consisted mainly of equal proportions of Man, Gal and Glc (Hokputsa et al. 2003; Hu et al. 2003). The water-soluble UV-absorbing pigments also contain chromophores with carbohydrate side chains (Boehme et al. 1995). Therefore, exogenous EPS could effectively protect cells from UV-B damage through scavenging of ROS and providing matrix for UV-absorbing pigment.

In conclusion, organisms exposed to UV-B radiation induced significant oxidative stress (ROS), lipid peroxidation (MDA) and DNA strand breaks, and decreased photosynthetic activity. The addition of exogenous EPS demonstrated significant protection against the oxidative stress, lipid peroxidation, DNA breaks and photosynthetic damage and thus significantly reversed the negative effects of UV-B on survival. Our findings indicate the unlikelihood that exogenous EPS used as a carbon source could improve carbohydrate metabolism, scavenge ROS generation and decrease DNA strand breaks, and thus enhance the UV-B tolerance of M. vaginatus.

Materials and Methods

Organisms and culture conditions

Microcoleus vaginatus Gom. was isolated from desert algal crust of Shapotou, Zhongwei County, Ningxia Autonomous Region, China (37°27′N, 104°57′E). It was gently dispersed with a glass homogenizer and grown in BG-11 medium at 25 °C and under continuous white fluorescent tubes at 40 μE/m² per s. After 18 d, cells were harvested and resuspended in fresh medium or with 100 mg/L EPS for 30 min before the experiment.

UV-B radiation was obtained from a lamp: UV-B VIX3W tube with its main output at 312 nm (Cole-Parmer Instrument Company, Paris, France). Cultures (10 mL) were placed in Petri dishes (diameter, 8 cm) and stirred with a shaker to reduce the cell aggregation and sedimentation. All experiments were replicated three times.

Chlorophyll a fluorescence

Chlorophyll a fluorescence was measured with a Plant Efficiency Analyzer (PEA, Hansatech, London, UK). Cyanobacterial cells were dark-adapted for at least 10 min before measuring the fluorescence parameter Fv/Fm (PSII activity). The excitation light intensity was about 1 500 μE/m² per s and the recording time was 5 s.

Carbohydrate analysis

Cellular total carbohydrates and reducing sugar were prepared according to the method of Li et al. (2001); EPS was prepared following the methods of Huang et al. (1998). Total carbohydrates, reducing sugars and EPS were quantified according to the phenol-sulphuric acid method of Chen et al. (2003), using glucose as a standard. Sucrose was extracted and quantified according to the method of Tang (1999), using sucrose as standard. Sucrose synthase (SS) activity and sucrose phosphate synthase (SPS) activity were analyzed according to the method of Chen et al. (2003).

MDA analysis

Cells were collected and homogenized with 10 mL 10% trichloroacetic acid (TCA). After centrifugation (4 000 g, 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. The reaction was stopped by placing the tube in an ice bath. After centrifugation (10 000 g, 10 min), the cells were supplemented with the supernatant measured at 450 nm, 532 nm and 600 nm with a spectrophotometer, and calculated according to the method of Tang (1999).

Detection of ROS using DCFH-DA

Reactive oxygen species production was detected by using DCFH-DA (2′,7′- dichlorodihydrofluorescein diacetate) (He and Hader 2002b). DCFH-DA (final concentration 5 mmol/L) 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 spectrofluorometer (F-4500, Hitachi, Japan) at room temperature, with an excitation wavelength of 485 nm and an emission band between 500 and 600 nm. The fluorescence intensity at 535 nm normalized to the protein content was used to determine the relative ROS production.

Analysis of DNA strand breaks

DNA strand breaks were determined by fluorometric analysis of DNA unwinding (FADU) as described by He and Hader (2002b), and modified as follows: briefly, irradiated or unirradiated cyanobacteria were harvested by centrifugation (5 000 g, 8 min). The pellet was washed with TE (Tris-ethylenediaminetetraacetic acid [EDTA]) buffer and resuspended in solution A (50 mmol/L Tris, pH 8.0; 50 mmol/L Na2EDTA; 1 mol/L NaCl). Sarkosyl solution (10% N-lauroyl sarcosine, 10 mmol/L Tris-HCl, pH 8.0;
20 mmol/L EDTA) was added to the samples (final concentration 0.1%) and kept at 4 °C for 2 h. After centrifugation (7000 g, 8 min), the pellet was washed twice with TE buffer. Subsequently, the pellet was resuspended in solution B (50 mmol/L Tris, pH 8.0; 50 mmol/L Na2EDTA; 25% sucrose) up to a final volume of 184 μL. Then, 20 μL of 160 mg/mL lysozyme was added to the suspension, and the mixture was incubated for 40 min at 37 °C to digest the cell walls completely. A 30 μL sample of 10% sodium dodecyl sulfate, 10 μL of 4 mol/L NaCl and 47 μL of TE buffer were added to a volume of 291 μL and incubated for 60 min at 37 °C. Finally, 9 μL of 10 mg/mL proteinase K was added to a final volume of 300 μL and incubated for 60 min at 37 °C to lyse the cells. The following steps were operated as the method of He and Hämmerl (2002b).

Data analysis

Data were analyzed using ANOVA and values shown are the means of three replicates.

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