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Effects of cadmium on the activities of photosystems of *Chlorella pyrenoidosa* and the protective role of cyclic electron flow

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HIGHLIGHTS

- Effects of $CdCl_2$ on PSI, PSII activities and CEF in *Chlorella pyrenoidosa* was studied.
- CEF was stimulated by Cd and played an essential role for the protection of PSI.
- PSII was more sensitive to Cd treatment than PSI.

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ABSTRACT

Cadmium (Cd) shows high toxicity to aquatic microalgae. Many studies showed that Cd inhibited activities of photosystem II (PSII) but the effects of heavy metals on photosystem I (PSI) and cyclic electron flow (CEF) were still controversial and unclear. The effects of $CdCl_2$ on the activities of PSI, PSII and CEF in *Chlorella pyrenoidosa* was measured simultaneously in the present study. In presence of 200 μM of Cd, ultrastructure of some cells was strongly modified. Cd exposure led to decrease of the activities of photosynthetic oxygen evolution and respiration. PSII was more sensitive to Cd treatment than PSI. Cd treatment showed significant inhibition on the photochemical quantum yield and electron transport rate of PSII. Cd increased the quantum yield of non-light-induced non-photochemical fluorescence quenching, indicating the damage of PSII. The activity of PSI showed tolerance to Cd treatment with concentration less than 100 μM in the experiment. Linear electron flow (LEF) made significant contribution to the photochemical quantum yield of PSI of the untreated cells, but decreased with increasing Cd concentration. The contribution of CEF to the yield of PSI increased with increasing Cd concentration. The activation of CEF after exposure to Cd played an essential role for the protection of PSI.

1. Introduction

Heavy metal pollution is one of the most important environmental problems today and increasing continuously as a result of industrial activities and technological development (Wang and Chen, 2009). Heavy metal contamination poses serious threat to the environment and human health. Cadmium (Cd) is considered as one of the major metal pollutants because of its wide distribution in aquatic ecosystems and its high toxicity (Nawrot et al., 2006; Zhou et al., 2006; Monteiro et al., 2011). It is not essential for life and extremely toxic to humans, animals and plants (Chen et al., 1999; Khattar and Shailza, 2009; Qian et al., 2009). It shows more serious toxicity to plants or algae than some other heavy metals or pesticides.

Effects of heavy metals on phytoplankton species including cyanobacteria have been extensively studied (Silverberg, 1976; Fargašová et al., 1999; Kola and Wilkinson, 2005). Phytoplankton species like cyanobacteria or green alga are also widely used as experimental materials to estimate the risk of heavy metals or other contaminants in aquatic systems (Qian et al., 2009; Wang and Pan, 2012). Many studies showed that heavy metals inhibited activities of photosystem II (PSII), which was suggested to be one of the sensitive target sites for environmental stress (Dewez et al., 2005; Perales-Vela et al., 2007; Pan et al., 2009). Exposure to heavy metals could lead to inhibition of capture and transfer of energy, quantum yield of photochemistry of PSII, and the synthesis of chlorophyll (Dewez et al., 2005; Zhang et al., 2010). Cd is thought to have toxicity to PSII by acting on the donor side or the acceptor side or inhibiting activity of oxygen-evolving complex (Atal et al., 1991; Siedlecka and Krupa, 1996; Zhou et al., 2006). A few studies investigated the relationship between heavy metals and the activity of photosystem I (PSI), showing that the effects of heavy metals

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on PSI were still controversial and unclear (Neelam and Rai, 2003; Zhou et al., 2006). Neelam and Rai (2003) found that Cd inhibited PSI activity in *Microcystis* sp. However, Zhou et al. (2006) reported that the activity of PSI in *Microcystis aeruginosa* increased due to Cd treatment.

The noninvasive chlorophyll fluorescence measurements can provide valuable information about the response of PSII to environmental stress (Appenroth et al., 2001; Pan et al., 2009). Although many studies investigated the effects heavy metals on PSII, toxicity of heavy metals to PSI and PSII has been rarely analyzed simultaneously. The simultaneous measurement of the photosynthetic activities of PSI and PSII are necessary to detect the effects of heavy metals on photosynthetic apparatus and the relation between PSII and PSI under stress. Klughammer and Schreiber (1994) introduced an improved method for the determination of PSI quantum yield and a Dual-PAM-100 system, which can simultaneously detect the chlorophyll fluorescence and $P700^+$ absorbance changes. Many studies have shown that the Dual-PAM-100 system becomes a powerful tool for investigation of response of PSI and PSII to environmental stress (Coopman et al., 2010; Huang et al., 2010).

The cyclic electron flow (CEF) around PSI was important for photoprotection and photosynthesis (Munekage et al., 2004; Suzuki et al., 2011), and the increase of CEF around PSI was suggested be one of the adaptive mechanisms to heavy metal stress (Zhou et al., 2006; Qian et al., 2009). However, effects of heavy metals like cadmium on CEF in green algae were still unclear.

In the present study, the activities of PSI, PSII and CEF in *Chlorella pyrenoidosa* was measured simultaneously to detect the quantum yields, electron transport and energy dissipation in PSI and PSII, and the relation between PSI and PSII under Cd treatment. In addition, toxic effects of $CdCl_2$ on the cell structure, photosynthetic oxygen development and respiration were tested.

2. Materials and methods

2.1. Culture of C. pyrenoidosa

C. pyrenoidosa (FACHB-9) was purchased from Freshwater Algae Culture Collection of Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and cultured in BG-11 medium (Stanier et al., 1971) at 25 °C under fluorescent white light (30 μmol photons $m^{-2}\,s^{-1}$) with a 12:12 h light–dark cycle. The cells in exponential growth phase were harvested for the following experiment by testing the growth state of cultures every day by measuring cell optical density at 680 nm (OD_{680}) with a UV2800 spectrophotometer (Unico, Shanghai, China).

2.2. Treatments

Exponentially grown cells used for different treatments were harvested and cultured in 50 mL flasks with 25 mL of BG-11 medium or prepared Cd solutions to make the final Cd concentrations be 0, 1, 25, 100 and 200 μM. Cd solutions were prepared by dissolving analytical-grade $CdCl_2$ in sterilized BG-11 medium at the desired concentrations just before the experiment. The samples without Cd were used as the control. During the whole experiment, all the samples were cultured under fluorescent white light (30 μmol photons $m^{-2}\,s^{-1}$) with a 12:12 h light–dark cycle at 24 ± 2 °C. The measurements were carried out at 0, 6, 12, 24, 48, 72 and 96 h after onset of different treatments.

2.3. Measurement of O_2 evolution and respiratory O_2 consumption

After the cells were exposed to various concentrations of Cd for different time, 2 mL of cells were added into the reaction cuvette of a Clark-type oxygen electrode (Oxygraph, Hansatech Instruments Ltd., King's Lynn, Norfolk, England). A white light provided illumination on the surface of the cuvette at about 400 μmol photons $m^{-2}\,s^{-1}$ during the measurement of O_2 evolution rate in the light (nmol $mL^{-1}\,min^{-1}$), which reached its stable ratio within 5 min. Then the cuvette was kept in darkness to detect the respiratory O_2 consumption rate in the dark (nmol $mL^{-1}\,min^{-1}$). The sum of O_2 evolution rate in the light and the respiratory O_2 consumption rate in the dark was calculated as net O_2 evolution rate (nmol $mL^{-1}\,min^{-1}$).

2.4. Transmission electron microscopy (TEM)

For transmission electron microscopy (TEM), algal cells were harvested by centrifugation (4000*g*, 5 min) after the cells were exposed to various concentration of Cd for 96 h. Algal cells were rinsed with culture medium for a short time and subsequently washed in glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). Then the cells were fixed for 24 h in 0.1 M phosphate buffer containing 2% glutaraldehyde. The fixed cells were centrifuged, re-suspended and rinsed several times in 0.1 M sodium cacodylate buffer containing sucrose. The algal cells were post-fixed with 2% osmium tetroxide. The cells were dehydrated in a graded acetone series after centrifugation and embedded in Epon 812 (Nishikawa and Tominaga, 2001). Ultrathin sections were stained with 1% uranyl acetate and lead citrate. Ultrastructure of cells was observed with a transmission electron microscope (SU080, Hitach, Japan).

2.5. Measurement of activities of the photosystems

2.5.1. Application of the Dual-PAM-100 system

The activities of PSI and PSII were measured with a Dual-PAM-100 system (Heinz Walz GmbH, Effeltrich, Germany). The cells used for measurements were injected into the DUAL-K25 quartz glass cuvette. The quartz glass cuvette was then sandwiched between the emitter head and detector head of the system. All the samples were dark-adapted for 5 min before measurement.

2.5.2. Measurement of the slow induction curve

The measurements were performed using the automated induction program provided by the Dual-PAM software (Pfündel et al., 2008) with a slight modification. After the samples were dark-adapted for 5 min, the minimal fluorescence after dark-adaptation, F_0, was detected by a measuring light at low intensity. A saturating pulse with duration of 300 ms and a light intensity of 10000 μmol photons $m^{-2}\,s^{-1}$ was then applied to detect the maximum fluorescence after dark-adaptation, F_m. The maximal change in $P700^+$ signal, P_m, was determined through the application of a saturation pulse after far-red pre-illumination for 10 s according to the methods of Klughammer and Schreiber (1994, 2008b).

After the determination of F_0, F_m and P_m, the slow induction curve was recorded with the routine of the Dual-PAM software. The actinic light was applied at the intensity of 30 μmol $m^{-2}\,s^{-1}$, as same as the light intensity at which the algae cells were cultured. The slow induction curve was used in the analysis of photosynthesis under light. A saturating pulse with duration of 300 ms was applied every 20 s after the onset of the actinic light to determine the maximum fluorescence signal (F'_m) and maximum $P700^+$ signal (P'_m) under the actinic light. The slow induction curve was recorded for 120 s to achieve the steady state of the photosynthetic apparatus, and then the actinic light was turned off. The data derived after the final saturating pulse was used in the analysis of the activities of PSI, PSII and CEF based on previously determined F_0, F_m and P_m.