



Simultaneous analysis of photosystem responses of *Microcystis aeruginosa* under chromium stress

Shuzhi Wang^{a,c}, Fulong Chen^{a,c}, Shuyong Mu^a, Daoyong Zhang^{b,**},
Xiangliang Pan^{a,*}, Duu-Jung Lee^{a,d}

^a State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

^b State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China

^c University of Chinese Academy of Sciences, Beijing 100049, China

^d Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan

ARTICLE INFO

Article history:

Received 19 September 2012

Received in revised form

9 November 2012

Accepted 9 November 2012

Available online 8 December 2012

Keywords:

Heavy metals

Cyclic electron flow

Electron transport rate

Freshwater algae

Photosystem I

Photosystem II

ABSTRACT

Chromium (Cr) is a toxic metal that poses a great threat to aquatic ecosystems. Information is limited on coinstantaneous responses of photosystems I (PSI) and II (PSII) to Cr(VI) stress due to lack of instruments that can simultaneously measure PSI and PSII activities. In the present study, responses of quantum yields of energy conversion and electron transport rates of PSI and PSII in *Microcystis aeruginosa* cells to Cr(VI) stress were simultaneously analyzed by a DUAL-PAM-100 system. Quantum yield of cyclic electron flow (CEF) under Cr(VI) stress and its physiological role in alleviating toxicity of Cr(VI) were also analyzed. At 5 mg L⁻¹ Cr(VI), quantum yield and electron transport rate of PSII decreased significantly, and light-induced non-photochemical fluorescence quenching lost. Cr(VI) also inhibited efficiency of PSII to use energy under high light more than of PSI. PSII showed lower maximal electron transport rate and light adaptability than PSI. Electron transport rate of PSI was higher and decreased less than that of PSII, implying less sensitivity of PSI to high light and Cr(VI). Energy dissipation through non-light-induced non-photochemical fluorescence quenching increased with increasing Cr(VI) concentration. CEF was stimulated under Cr(VI) treatment and made a significant contribution to quantum yield and electron transport of PSI, which was essential for protection of PSI from stresses of Cr(VI) and high light.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Chromium (Cr) caused widespread environmental problem since its compounds are widely used in various industrial processes such as leather tanning and textile (Yu and Gu, 2008). Cr occurs mainly as trivalent Cr(III) and hexavalent Cr(VI) species, and the toxicity of Cr depends on its species (Pan et al., 2009; Vignati et al., 2010).

Microalgae and cyanobacteria as important primary producers live widely in water bodies. They are also frequently used in environmental risk assessment due to their sensitivity to contaminants (Ma et al., 2004; Pan et al., 2008; Qian et al., 2009). Because Cr is a major pollutant in water bodies, phytoplankton species such as cyanobacteria are frequently exposed to Cr (Appenroth et al., 2001; Mishra and Doble, 2008). Physiological responses of microalgae and cyanobacteria to Cr stress have been investigated

extensively. Photosynthetic apparatus is one of the sensitive target sites to Cr toxicity. Cr(VI) is known to inhibit the photosynthesis of various phytoplankton species (Ait Ali et al., 2008; Perreault et al., 2009; Prasad et al., 1991) primarily targeting photosystem II (PSII) (Pan et al., 2009). The inhibitory effects of Cr(VI) were suggested to be located on D1 and oxygen evolving complex, oxidizing and reducing sides of PSII (Ait Ali et al., 2006; Perreault et al., 2009), electron carriers for electron transport between Q_A and Q_B (Appenroth et al., 2001; Pan et al., 2009), and PSII reaction centers (Pan et al., 2009).

Although there are quite a lot of studies on effects of heavy metals on PSII (Pan et al., 2009; Wang and Pan, 2012; Zhang et al., 2010), limited studies reported responses of photosystem I (PSI) to heavy metal treatment. Some researchers found that Cd inhibited PSI activity in *Microcystis* sp. (Neelam and Rai, 2003). On the contrary, Zhou et al. (2006) reported that Cd increased the PSI activity of *M. aeruginosa*.

In most cases, effects of heavy metals on PSII or PSI were studied separately. This means that sometimes such results might not reliably reflect status of PSII or PSI due to close relationship between them. Simultaneous measurements of PSI and PSII activities under stress of heavy metals are needed in order to get more accurate information about effects of heavy metals on photosynthetic apparatus.

* Correspondence to: Laboratory of Environmental Pollution and Bioremediation, State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830 011, PR China.
Fax: +86 991 7885446.

** Corresponding author at: State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China.

E-mail addresses: xiangliangpan@163.com, panxl@ms.xjb.ac.cn (X. Pan).

Perreault et al. (2009) investigated the toxic effects of dichromate on energy dissipation of PSII and PSI in *Chlamydomonas reinhardtii*, and found that both quantum yield of PSII and PSI were highly decreased under Cr(VI) stress. Cr(VI) strongly increased non-photochemical energy dissipation processes of PSII and PSI in *C. reinhardtii*. However, to date effects of Cr(VI) on PSII and PSI in cyanobacteria and responses of photosynthetic activities such as electron transport in PSII and PSI to Cr(VI) and increasing illumination are unclear. In addition, recent studies showed that cyclic electron flow (CEF) around PSI was stimulated and suggested to be important for photoprotection and photosynthesis under drought and chilling stress (Huang et al., 2010, 2012). Effects of heavy metals on CEF are unknown.

Microcystis aeruginosa has often been used as a model microbial species for examining effects of contaminants on photosynthesis (Wang et al., 2012; Zhou et al., 2006). In the present study, *M. aeruginosa* was used to detect the effects of Cr(VI) on PSII and PSI activities with the aid of a Dual-PAM-100 system. The aim of this work was to detect responses of complementary quantum yields of energy conversion in PSI and PSII and CEF of PSI at various concentrations of Cr(VI) and increasing illumination.

2. Materials and methods

2.1. Culture of *Microcystis aeruginosa*

M. aeruginosa (FACHB-905) was purchased from Freshwater Algae Culture Collection of Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The stock cultures of *M. aeruginosa* cells were carried out in a BG-11 medium (Stanier et al., 1971) at $24 \pm 2^\circ\text{C}$ under fluorescent white light ($30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, full spectrum lamp, Jinantenghao Scientific Instrument Co., Ltd., China) with a 12:12 h light: dark cycle. Growth of cultures was monitored every day by measuring the absorbance of the cells suspension at 680 nm (A_{680}) with a UV2800 spectrophotometer (Unico, Shanghai, China).

2.2. Cr(VI) treatments

Cells at exponentially growing phase (initial absorbance $A_{680} \sim 0.8$) were harvested for Cr(VI) treatment experiments. The cells were cultured in BG-11 culture medium containing various concentrations of Cr(VI). $\text{K}_2\text{Cr}_2\text{O}_7$ (analytical grade, Tianjin Chemical Reagent Research Institute, China) was applied to achieve the required concentrations of Cr(VI) ($0\text{--}5 \text{ mg L}^{-1}$). The samples without addition of Cr(VI) were used as the control. All treatments and controls were run at the same time. The samples were prepared in a final volume of 25 mL and cultured in 50 mL flasks. All the experimental cultures were incubated under the same culture condition as the stock culture. Measurement of PSII and PSI activities was carried out at 12 h after onset of treatments with various concentrations of Cr(VI).

2.3. Measurement of PSI and PSII activities

2.3.1. Application of the Dual-PAM-100 system

A dual-wavelength pulse-amplitude-modulated fluorescence monitoring system (Dual-PAM-100, Heinz Walz GmbH, Germany) was used to simultaneously measure the responses of PSI and PSII activities in *M. aeruginosa* to Cr(VI) (Huang et al., 2010; Suzuki et al., 2011). Measurements were performed using the automated induction program provided by the Dual-PAM software (Pfündel et al., 2008) with a slight modification. PSII and PSI activities were quantified by chlorophyll fluorescence and P700^+ absorbance changes.

The cell suspension (2.5 mL), which was cultured in BG-11 medium containing various concentrations of Cr(VI) for 12 h as described above, was injected into the DUAL-K25 quartz glass cuvette. It was then placed between the emitter head and detector head of the system. After the sample was dark adapted for 5 min, saturation pulse method was used to detect the maximum fluorescence and maximal change in P700^+ signal. The minimal fluorescence after dark-adaptation (F_0) was measured at light at low intensity (measuring beam at $0.2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). A saturating pulse at an irradiance of $10,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was then applied for 300 ms to detect the maximum fluorescence (F_m). P700 redox state was measured with a dual wavelength (830/875 nm) unit and was quantified as the difference between the signals at 875 nm and 830 nm. The maximal change in P700^+ signal (P_m) was determined through application of a saturation pulse after far-red pre-illumination for 10 s according to the methods of Klughammer and Schreiber (1994, 2008b).

2.3.2. Measurement of the slow induction curve under steady actinic light

After determination of F_0 , F_m and P_m , slow induction curve was recorded with the routine of the Dual-PAM software. The actinic light was applied at $30 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was the same as the light intensity for culturing the cyanobacterial cells. A saturating pulse with duration of 300 ms was applied every 20 s after onset of the actinic light to determine the maximum fluorescence signal (F_m') and maximum P700^+ signal (P_m') under the actinic light. P_m' was also defined in analogy to the fluorescence parameter F_m' and determined similarly to P_m but without far-red illumination (Huang et al., 2012). 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 for analysis of activities of PSI, PSII and CEF based on determined F_0 , F_m and P_m .

2.3.3. Quantum yields of the photosystems and CEF

Quantum yields of PSII and PSI were recorded at steady state under the actinic light. The effective photochemical quantum yield of PSII, $[Y(\text{II})]$, the quantum yield of light-induced non-photochemical fluorescence quenching, $[Y(\text{NPQ})]$, and the quantum yield of non-light-induced non-photochemical fluorescence quenching, $[Y(\text{NO})]$, were calculated by the Dual-PAM software (Kramer et al. 2004) and were transformed into the following simpler equations (Klughammer and Schreiber, 2008a; Suzuki et al., 2011):

$$Y(\text{II}) = (F_m' - F) / F_m', \quad Y(\text{NPQ}) = F / F_m' - F / F_m', \quad \text{and} \quad Y(\text{NO}) = F / F_m.$$

where F was the steady-state fluorescence; F and F_m' were detected at the final saturating pulse during the process of slow induction curve.

The effective photochemical quantum yield of PSI, $[Y(\text{I})]$, the quantum yield of non-photochemical energy dissipation in PSI due to donor side limitation, $[Y(\text{ND})]$, and the quantum yield of non-photochemical energy dissipation in PSI due to acceptor side limitation, $[Y(\text{NA})]$, were calculated according to Klughammer and Schreiber (2008b) and Suzuki et al. (2011) as mentioned below:

$$Y(\text{I}) = (P_m' - P) / P_m', \quad Y(\text{ND}) = (P - P_0) / P_m', \quad Y(\text{NA}) = (P_m - P_m') / P_m$$

where P was the P700^+ signal recorded just before a saturation pulse. Then a saturation pulse was applied to determine maximum P700^+ signal (P_m'). Finally at the end of the 1 s dark interval following each saturation pulse, P_0 was detected. The signals P and P_m' were detected referencing against P_0 (Pfündel et al., 2008). The quantum yield of CEF was calculated from the difference between $Y(\text{I})$ and $Y(\text{II})$ (Huang et al., 2010):

$$Y(\text{CEF}) = Y(\text{I}) - Y(\text{II})$$

2.3.4. Electron transport rates in PSI and PSII

Electron transport rates (ETRs) in PSI [ETR(I)] and PSII [ETR(II)] were recorded during the measurement of the slow induction curve and calculated by the Dual-PAM software as follows (Maxwell and Johnson, 2000; Suzuki et al., 2011):

$$\text{ETR(I)} = Y(\text{I}) \times \text{PAR} \times 0.84 \times 0.5$$

$$\text{ETR(II)} = Y(\text{II}) \times \text{PAR} \times 0.84 \times 0.5$$

2.3.5. Relation between cyclic electron flow (CEF) and linear electron flow (LEF)

CEF and LEF were detected by calculating the changes of the ratios of $Y(\text{CEF})/Y(\text{I})$, $Y(\text{CEF})/Y(\text{II})$ and $Y(\text{II})/Y(\text{I})$ after onset of various treatments for 12 h. $Y(\text{CEF})/Y(\text{I})$, $Y(\text{II})/Y(\text{I})$ and $Y(\text{CEF})/Y(\text{II})$ indicated the contribution of CEF to $Y(\text{I})$, the contribution of LEF to $Y(\text{I})$, and the relation between the quantum yield of CEF and LEF, respectively. The ratio of $Y(\text{II})/Y(\text{I})$ also showed the distribution of quantum yield between two photosystems (Huang et al., 2010).

2.3.6. Measurement of rapid light response curves of ETR(I) and ETR(II)

After the record for slow induction curve, the rapid light curves (RLCs) of ETR(I) and ETR(II) were recorded in the rapid light curve mode (RLC mode) with the routine of the Dual-PAM software to detect the response of the activities of electron transport in PSI and PSII to increasing illumination under exposure to Cr(VI). After the RLC mode was turned on, the actinic light was applied for 30 s to each of a series of increasing intensity ($0\text{--}1311 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). A saturating pulse was applied after each period of actinic light to determine F_m' and P_m' . RLCs with saturation pulse analysis were based on determined F_0 , F_m and P_m .

ETR(I) and ETR(II) were defined and calculated using the Dual-PAM software as described above. Descriptive parameters of ETR(I) and ETR(II) during the light response reaction, the initial slope of RLC of ETR(I) or ETR(II) (α), the maximal electron transport rates in PSI or PSII (ETR_{max}) and the index of light adaptation of PSI or PSII (I_k , $\text{ETR}_{\text{max}}/\alpha$) were derived from the RLCs, which were automatically calculated by the Dual-PAM software according to the exponential function described by Platt et al. (1980) and Kühl et al. (2001).

2.4. Statistics

Each treatment was replicated four times. Means and standard deviation (S.D.) were calculated for each treatment from four replicate samples and have been

Download English Version:

<https://daneshyari.com/en/article/4420793>

Download Persian Version:

<https://daneshyari.com/article/4420793>

[Daneshyari.com](https://daneshyari.com)