



RESEARCH ARTICLE

EFFECTS OF HgCl₂ ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF *Synechocystis* sp. PCC 6803

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Received 04th September, 2012; Received in Revised form; 17th October, 2012; Accepted 19th November, 2012; Published online 17th December, 2012

ABSTRACT

In this study, the physiological and biochemical responses of the freshwater microalgae *Synechocystis* sp. PCC 6803 to HgCl₂ were compared and evaluated using 96 h growth tests in a batch-culture system. The results showed that HgCl₂ had a significant inhibition on the growth and Chl a concentration of *Synechocystis* sp. PCC 6803. Moreover, remarkable physiological and biochemical responses also occurred when the microalgae were exposed to HgCl₂. The general increase in MDA content suggests that the toxic effects of metals were probably exerted through free radical generation; and the increase in SOD activity may be viewed as an active defense against metal stress by stimulating the synthesis of proteins against stress and quenching of free radicals. These observations suggest that HgCl₂ is toxic to *Synechocystis* sp. PCC 6803, and its use should be moderately restricted.

Key words: *Synechocystis* sp.; Metal stress; Malondialdehyde (MDA); Superoxide dismutase (SOD)

INTRODUCTION

Because of the increasing industrial and agricultural wastewater, contamination of aquatic environments by metals has become a focus of researchers and environmentalists in recent years. Once metals are introduced into aquatic ecosystems, they can affect the growth, development, morphology, physiological and biochemical metabolism of aquatic organisms through various ways (Van Assche and Clijsters, 1990; Bidar *et al.*, 2007). Furthermore, the non-degradability of metals, their accumulation in biota, and biomagnification along aquatic food chains (Azevedo *et al.*, 2007), will destroy aquatic ecosystem equilibrium. Toxicity mechanisms of metals include the binding to sulphhydryl groups in proteins or disruption of protein structure or displacement of an essential element (Tripathi *et al.*, 2006). In addition, metal stress would induce the uncontrolled and excessive production of reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions (Stohs and Bagchi, 1995), which can cause peroxidation of lipids, inactivation of enzymes, and damaged DNA and other constituents of cells (Mallick, 2004; Sabatini *et al.*, 2009; Gill and Tuteja, 2010; Ahmad, *et al.*, 2010). To minimize the damaging effects of ROS, algae have evolved antioxidant defense mechanisms, including enzymes which can catalyze reactions of ROS scavenging. Among the antioxidant enzymes, superoxide dismutase (SOD) is the most important enzyme and can detoxify superoxide anions (Beyer *et al.*, 1991; Mellado *et al.*, 2012). Moreover, malondialdehyde (MDA) is a cytotoxic

product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Janero, 1990). The unicellular microalgae *Synechocystis* sp. PCC 6803, ubiquitous in freshwater, was used in this study. The aim of this study is to explore the physiological responses of *Synechocystis* sp. PCC 6803 to mercury (Hg). The objectives of the study are as follows: 1) to evaluate the toxic effects of HgCl₂ on *Synechocystis* sp. PCC 6803; 2) to investigate the growth characteristics of *Synechocystis* sp. PCC 6803 under the stress of HgCl₂; and 3) to evaluate the changes in cell contents and antioxidant activity in *Synechocystis* sp. PCC 6803 induced by HgCl₂.

MATERIALS AND METHODS

Test chemicals and solutions

HgCl₂ was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Test solutions were prepared by adding a certain amount of HgCl₂ in BG11 medium (Stanier *et al.*, 1971). And the following concentrations were used according to the preliminary experimental results: 0, 2, 4, 6 and 8 mg/L (Hg) for the cytotoxicity test.

Microorganism

Synechocystis sp. PCC 6803 was obtained from Prof. Xiaowen Zhang (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences). The stock culture and inoculum were grown in BG11 medium. The inoculum was precultured aseptically in 500 mL Erlenmeyer flasks with 200 mL of BG11 medium. The flasks were placed in a 28 °C

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illuminated incubator (Jiangnan Instrument Factory, Ningbo, China) for 7 days under a 12 h light/12 h dark photoperiod and a light density of 40 $\mu\text{E}/\text{m}^2/\text{s}$.

Experimental set up

After precultivation, the algal inocula reached the exponential growth phase. Twenty mL of the algal inoculum was collected using centrifugation (4,000 g, 4 °C, 15 min). The deposited algal cells were washed twice with ddH₂O, and then inoculated into the growth medium. In the growth inhibition experiments, the cultures were grown in 250 mL Erlenmeyer flasks containing 150 mL of modified BG11 medium with different concentrations of HgCl₂, each in triplicate. And another set of flasks containing BG11 medium without test chemicals was prepared as the control, also in triplicate. The other cultivation conditions were as described above.

Microalgal growth analysis

Daily microalgal cell density was determined turbidometrically at 680 nm using a spectrophotometer (UV1800, Mapada Instruments Company, Shanghai, China). The relationship between microalgal cell density (D, cells/mL) and the optical density of the microalgal culture at 680 nm (OD₆₈₀) was shown in Eq. (1):

$$D = (0.5478 \times \text{OD}_{680} - 0.0034) \times 10^8, (R = 0.9968) \quad (1)$$

Chlorophyll a (Chl a) determination

Triplicate 5 ml of well-blended cultures were centrifuged at 4000 g for 15 min to discard the supernatants. And the pellets were homogenized with 80% (V/V) acetone for Chl a extraction. The mixtures were vigorously shaken using a vibrator, and then placed in a refrigerator in the dark at 4 °C for 24 h. Then the extracted samples were centrifuged at 10,000 g for 5 min to remove the pellets. Supernatants were transferred into 1×1 cm glass cuvettes, and measured for Chl a at 663 nm and 645 nm using a spectrophotometer. All absorbance values were corrected using the 80% acetone as control. The concentration of Chl a was calculated by the following equation (Hao *et al.*, 2007) (Eq. 2):

$$\text{Chl a (mg/L)}: \text{Ca} = 12.71A_{663} - 2.59A_{645} \quad (2)$$

SOD and MDA determination

After 48 and 96 h of test chemical exposure, 30 mL of well-blended cultures were harvested by centrifugation at 4,000 g for 15 min at 4 °C. The harvested microalgae were placed in 1.5 mL of extraction buffer containing 0.1 mol/L sodium phosphate buffer (pH 7.0), and immediately lysed by sonication (Scientz Biotechnology Co. Ltd, Ningbo, China) for 10 min with a repeating duty cycle of 5 s in an ice bath. The cellular homogenate was centrifuged at 12,000 g for 10 min at 4 °C, and the supernatant liquid was stored at -70 °C for use in the enzyme assay. The SOD and MDA assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). According to the manufacturer's instructions, SOD and MDA were extracted and determined from the supernatant liquid above. The results of SOD and MDA assay are given as units of the enzyme activity per 10⁷ cells (U/10⁷ cell) and nmol per 10⁹ cells (nmol/10⁹ cell).

Statistical analysis

SPSS PASW Statistics 18 software was used in all statistical analyses. The mean values, confidence intervals, and standard deviation values of the triplicates for each treatment were calculated. The effects caused by metal stress on the growth characteristics and antioxidant enzymes were evaluated using one-way ANOVA at $p < 0.05$.

RESULTS

Microalgal growth characteristics

Hg is a non-essential metal for the microalgal growth. Its effects on cell density and growth characteristics of *Synechocystis* sp. PCC 6803 were analyzed in this study. As shown in Figure 1, Hg inhibited the growth of *Synechocystis* sp. PCC 6803 in different treatments. Hg had great inhibitory effect on the growth of *Synechocystis* sp. PCC 6803, even in 2 mg/L HgCl₂. Visual and microscopic observation showed that the microalgal cells almost completely died after 72 h exposure with Hg and could not revive even in fresh, normal BG-11 medium.

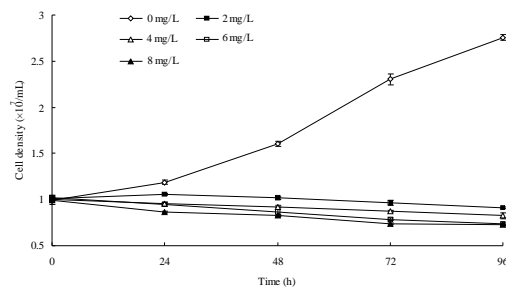


Fig. 1. Growth profiles of *Synechocystis* sp. PCC 6803 under different HgCl₂ concentrations (mg/L) during 96 h exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations

Chlorophyll a content

Figure 2 shows the changes of Chl a content in *Synechocystis* sp. PCC 6803 with HgCl₂ treatments. The Chl a greatly decreased, particularly after 48 h exposure. And then, the Chl a concentration was almost close to 0 at 72 and 96 h.

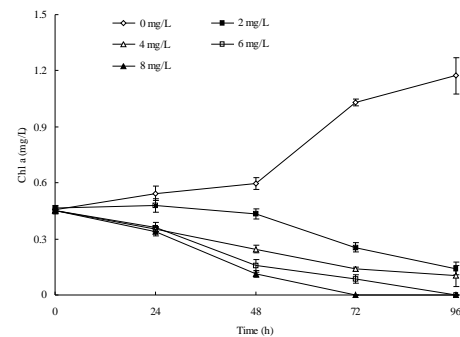


Fig. 2. Effects of HgCl₂ on chlorophyll a content of *Synechocystis* sp. PCC 6803 during 96 h of exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations

Lipid peroxidation

The level of lipid peroxidation was determined in terms of MDA content. As shown in Figure 3, an increase in the cellular MDA content was observed in all treatments. When the microalgae were exposed to HgCl_2 , the MDA content increased with exposure time and concentrations of HgCl_2 .

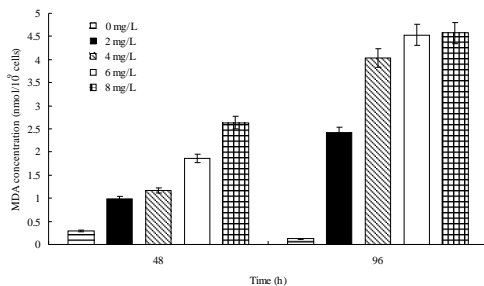


Fig. 3. Effects of HgCl_2 on intracellular malondialdehyde (MDA) content of *Synechocystis* sp. PCC 6803 after 48 and 96 h of exposure. Points represent means of three replicates ($n = 3$); error bars represent standard deviations

Antioxidase activity

Algae have various antioxidant enzymes to combat increased production of ROS caused by heavy metals. SOD is the most important enzyme among antioxidant enzymes, which can detoxify superoxide anions (Beyer *et al.*, 1991; Mellado *et al.*, 2012). In this study, the changes of SOD activity were determined and compared under different concentrations of HgCl_2 to investigate the tolerance of *Synechocystis* sp. PCC 6803 to HgCl_2 stress (Fig. 4). The SOD activity was generally improved with the increase of HgCl_2 concentrations. At 48 h HgCl_2 exposure, SOD activity significantly increased from 0.449 to 1.085 U/10⁷ cells when HgCl_2 were added from 0 to 8 mg/L. The SOD activity had also remarkable increase with the increase of HgCl_2 concentration at 96 h exposure, relative to controls. But higher concentrations (>6 mg/L) caused no remarkable difference in statistics ($p > 0.05$).

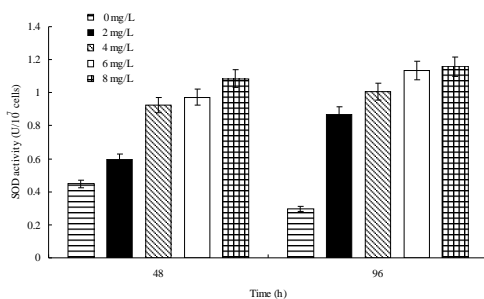


Fig. 4. Effects of HgCl_2 on superoxide dismutase (SOD) activity of *Synechocystis* sp. PCC 6803 after 48 and 96 h of exposure. Points represent means of three replicates ($n = 3$); error bars represent standard deviations

DISCUSSION

Information available regarding the toxicity of heavy metals on *Synechocystis* sp. PCC 6803 is scarce in peer-reviewed literature. This study examined the effects of HgCl_2 stress on the growth characteristics and physiological responses of unicellular microalgae *Synechocystis* sp. PCC 6803. Hg is a

non-essential metal for algal growth, which has become serious pollutant in water environment because of its widespread industrial, agricultural and anthropogenic use (Atli and Canli, 2010). Elevated levels of heavy metals elicit deleterious impact on algae and cyanobacteria. Toxicity of heavy metals to algae primarily results from their binding to sulphhydryl groups in proteins or disruption of protein structure or displacement of an essential element (Tripathi *et al.*, 2006). Hg can replace metals ion in photosynthetic pigments to cause a decrease in photosynthesis rates (Tabaldi *et al.*, 2007), and represents a major hazard to microorganisms at low concentrations. Aquatic plants can be affected by Hg in water at concentrations of 1 mg/L (Boening, 2000). In a study (De *et al.*, 1985) *Pistia stratiotes* was exposed for 2 days to mercuric chloride at concentrations between 0.05 and 20.0 mg/L. The highest dose of mercury decreased chlorophyll content, protein, RNA, dry weight, catalase and protease activity, and increased production of free amino acids.

In this present study, the growth of *Synechocystis* sp. PCC 6803 was inhibited by HgCl_2 , and chlorosis, a common symptom of metal toxicity, occurred before 48 h, which suggested that the Chl a formation was restrained by HgCl_2 . Some studies has also described the similar results in other metal treatments (Lee and Lustigman, 1996; Sabatini *et al.*, 2009; Wang *et al.*, 2011). MDA content is often considered as an indicator of lipid peroxidation status. In this work, an increase in cellular MDA content was observed in all treatments, which indicates free radical generation under HgCl_2 . It is similar to the effect of other contaminants on microalgal species (Choudhary *et al.*, 2007; Kumar *et al.*, 2008; Sabatini *et al.*, 2009; Hong *et al.*, 2009). When algae are exposed to various abiotic stresses, ROS production increased in the algal cells. ROS are highly reactive and toxic, and can causes damage to proteins, lipids, carbohydrates and DNA (Gill and Tuteja, 2010). Algal cells produce many antioxidant defense components, such as SOD and CAT, two important enzymes in ROS scavenging, to protect against ROS. In addition, the activity of these two enzymes may be enhanced following exposure to moderate environmental stresses (Malanga and Puntarulo, 1995; Kumar *et al.*, 2008; Qian *et al.*, 2008; Sabatini *et al.*, 2009). HgCl_2 greatly enhanced SOD activity compared with the untreated group. This was accounted as evidence of enhanced free radical production under metal stress.

Conclusion

The freshwater microalgae *Synechocystis* sp. PCC 6803 was used to compare and evaluate the toxicity of HgCl_2 in this study. The growth and Chl a concentration of *Synechocystis* sp. PCC 6803 were significantly inhibited by HgCl_2 . The observed growth inhibition of HgCl_2 provides sufficient energy for the induction of protective measurements such as the synthesis of antioxidants. In addition, rapid and remarkable physiological responses occurred when the microalgae was exposed to HgCl_2 . The general increase in MDA level suggests that the toxic effects of HgCl_2 were probably exerted through free radical generation. And the increase in SOD activity may be viewed as an active defense against metal stress by stimulating the synthesis of proteins against stress and quenching of free radicals.

Acknowledgment

This present study was supported by Jiangsu Provincial Natural Science Foundation of China (Grant No. BK2011493) and Visiting Fellowship of State Key Laboratory of Marine Environmental Science (Xiamen University) (No. MELRS1123).

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