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Effects of microcystin-LR on the metal bioaccumulation and toxicity in *Chlamydomonas reinhardtii*

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ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form
25 September 2011
Accepted 17 October 2011
Available online 25 October 2011

Keywords:
Bioaccumulation
Microcystin-LR
Toxicity
Trace metal

ABSTRACT

Microcystin-LR (MC-LR) is one of the most notorious toxins liberated from cyanobacteria in eutrophicated freshwater ecosystems. Its effects on the bioaccumulation and toxicity of Cd^{2+}, CrO_4^{2-}, Cu^{2+}, and Zn^{2+} in a green alga *Chlamydomonas reinhardtii* were investigated in the present study. The metal bioaccumulation in the alga was unaffected by MC-LR. The surface-adsorbed and intracellular metal concentrations in the treatments with and without the addition of MC-LR could be well simulated by a single Freundlich isotherm for each metal with their accumulation ability following the order of $Cu^{2+} > Cd^{2+} > Zn^{2+} > CrO_4^{2-}$. The bioavailable metal concentrations measured by diffusion gradients in thin-films remained unchanged when MC-LR was applied. Accordingly, the growth of *C. reinhardtii* was similarly inhibited at the same metal concentration regardless of the addition of MC-LR. The metal toxicity could also be well delineated with the classic free ion activity and biotic ligand models. However, the intracellular metal concentration was found to have the best predictability suggesting its more direct relationship with metal toxicity. Metal exposure induced the accumulation of MC-LR in the alga, which was leveled off at high metal levels. The underlying uptake mechanisms need to be further examined.

1. Introduction

Heavy metals with density higher than 5 g/cm^3 are widely used in various industries and a significant fraction would inevitably get to the environment (Fu and Wang, 2011). Substantially high concentrations of cadmium, chromium, copper, lead, mercury, and zinc, belonging to the thirteen priority metal/metalloid pollutants categorized by US EPA, have been found in lakes, rivers, estuaries and coastal areas, where harmful algal blooms frequently occur as a result of eutrophication (Diao et al., 2004). It has been reported that algae in bloom may take up abundance of metals, float with water current, and further strikingly influence the metal distribution in aquatic environments (Reynolds and Hamiltontaylor, 1992). On the other hand, various organic compounds (e.g., exopolymeric substances and toxins) could be released from the algae especially during the period of their decomposition, which may further play an important role in the metal bioavailability and toxicity to aquatic organisms (Haye et al., 2006).

As one of the most prevalent cyanobacterial toxins, microcystins are small monocyclic hepapeptides sharing a general structure of five identical but two variable L-amino acids in positions 2 and 4 (Babica et al., 2006). They are secondary metabolites produced by enzymes like microcystin, peptide or polyketide synthetases. Microcystins are specific

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0043-1354/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2011.10.035

inhibitors of eukaryotic protein phosphatases 1 and 2A, thus could increase the protein phosphorylation in the cells, destruct their cytoskeleton and further deregulate the cell division. They may also incur oxidative stresses, disrupt the osmotic and ion regulation in the organisms (Martins and Vasconcelos, 2009). Most of the current microcystin researches focus mainly on their behavior and fate in the environment as well as their accumulation, distribution, metabolisms, and toxicity in various organisms. However, their potential functions in cyanobacteria remain largely unknown.

Some putative roles of microcystins have been proposed such as the allelophathic effects on other algae/plants, cyanobacteria protection from their grazers, light absorption improvement (quorum sensing effects), toxic metal sequestration in the cells, and metal nutrient acquisition from the ambient environment (Hesse et al., 2001; Utkilen and Gjolme, 1995). The binding characteristics of microcystins with Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, and Zn^{2+} have been measured by differential pulse polarography, cyclic and anodic stripping voltammetry with intermediate affinity observed (Humble et al., 1997; Yan et al., 2000). Several microcystin–metal complexes (e.g., microcystin-Fe (II), -Zn, -Cu, -Mg etc.) were also detected using cryospray ionization-Fourier transform ion cyclotron resonance mass spectrometry (Saito et al., 2008). Zeng et al. (2009) compared the metal tolerance of a non-toxic and toxic strain of *Microcystis aeruginosa*, finding that the non-toxic strain was more sensitive to Cd^{2+} but both strains had similar tolerance to Zn^{2+}. It implies that microcystins may be involved in metal detoxification in a metal-specific manner. In contrast, the cellular microcystin concentration was found to be concomitant with the specific growth rate of *M. aeruginosa* rather than being triggered in response to Zn^{2+} and Cu^{2+} exposure, purporting a constitutive production of microcystins (Gouvea et al., 2008).

To further investigate how microcystins and trace metals may interact with each other, the most toxic and frequently observed microcystin, microcystin-LR (MC-LR), was chosen in the present study. The bioaccumulation and toxicity of the four metal ions, Cd^{2+}, CrO_4^{2-}, Cu^{2+}, and Zn^{2+}, in a classic freshwater green alga *Chlamydomonas reinhardtii* were compared with and without the addition of 1 μM MC-LR. Although mainly located inside the cells, a substaintial amount of MC-LR could be released into the ambient environment with the dissolved concentration varying from traces up to 1800 μg/l (approximately 1.8 μM) or higher, immediately after the collapse of a highly toxic bloom (Babica et al., 2006). According to Pearson's hard soft acid base principle (Pearson, 1997), Cd^{2+} is soft and would rather bind with sulfur containing functional groups than oxygen or nitrogen ones. Cu^{2+} and Zn^{2+} belong to the borderline group with intermediate affinity to all the three types of binding ligands above, whereas CrO_4^{2-} has negative charges. Different effects of MC-LR (if any) on the bioaccumulation and toxicity of these metals with different physicochemical properties would thus be expected. As the natural dissolved organic compounds (e.g, humic substances) are well documented to affect the metal speciation, accumulation and further toxicity in various organisms, it would be interesting to illuminate how MC-LR may be involved in the metal–organism interactions (Koukal et al., 2003; Lamelas and Slaveykova, 2007; Vigneault et al., 2000).

Both the free ion activity model (FIAM) and the biotic ligand model (BLM), in which the metal toxicity is predicted either by its free ion concentration in the media or by its adsorption on the biotic ligands (e.g., fish gills or cell membrane), have now been widely accepted (Campbell, 1995; De Schamphelaere et al., 2005; Slaveykova and Wilkinson, 2005). However, a number of exceptions were reported as both models are based on several assumptions and the metal toxicity was thus further related to their internal concentrations in the organisms or their subcellular distribution (Miao and Wang, 2007). In the present study, the metal bioaccumulation and their inhibition of algal growth were plotted against four types of metal concentrations (i.e., the total dissolved metal concentration $[M]_T$, the free metal ion concentration $[M]_F$, the surface-adsorbed ($[M]_{ads}$) and intracellular metal concentrations ($[M]_{intra}$)) to elicit whether the different models could still be applied in the presence of MC-LR. The overall objective is to examine whether the microcystins liberated from cyanobacteria could affect the metal bioavailability and toxicity to aquatic organisms.

2. Materials and methods

2.1. Phytoplankton culture conditions

An axenic culture of the Chlorophyta *C. reinhardtii* used in the present study was originally obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan. The algal cells were maintained in an artificial freshwater WC medium (Guillard, 1975) with its pH kept at 7.5 ± 0.1 by the addition of 5 mM 3-(N-morpholino) propanesulfonic acid (MOPS). The temperature was 25 °C with a light illumination of 50 μmol photons/m^2/s in a 12:12 Light–Dark cycle.

2.2. Effects of MC-LR on metal bioaccumulation and toxicity

MC-LR (formula: $C_{49}H_{74}N_{10}O_{12}$, molecular weight: 995.2, purity: >95%) was bought from the Express Bio-technology Co., Ltd., Beijing, China. There were two toxicity tests (i.e., one with and the other without the addition of 1 μM MC-LR) for each of the four metals with seven concentration treatments in 150 ml duplicates on average. MC-LR alone at the above-mentioned concentration had no notable effects on *C. reinhardtii*. A modified WC medium was used as the base of the toxicity media. Its components are shown in Table S1. Since a considerable amount of ethylenediaminetetraacetic acid (EDTA, 11.7 μM) was present in the original WC medium which could possibly hide the impacts of MC-LR, it was excluded from the toxicity media. The trace metal nutrient concentrations were thus reduced correspondingly to avoid their unnecessary precipitation (e.g., Fe^{3+}) or toxicity (e.g., Cu^{2+}) to the alga.

All the containers were soaked in 1 N HCl and then rinsed with Milli-Q water (18.2 MΩ) for at least six times. Trace metal clean technique was applied throughout the whole experiment. In our preliminary experiment, the dissolved metal concentrations were found to decrease remarkably as a result of their adsorption onto the container wall in the absence of