Journal of Hazardous Materials 252–253 (2013) 293–299

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Evaluation on the generative mechanism and biological toxicity of microcystin-LR disinfection by-products formed by chlorination

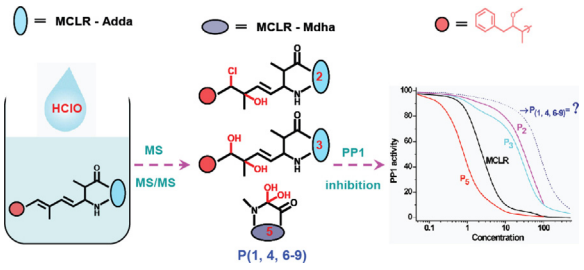
Wansong Zong[a,*], Feng Sun[b], Xiaojing Sun[a,b]

[a] College of Population, Resources and Environment, Shandong Normal University, 88# East Wenhua Road, Jinan 250014, PR China
[b] School of Environmental Science and Engineering, Shandong University, 27# Shanda South Road, Jinan 250100, PR China

HIGHLIGHTS

- Subject to chlorination, MCLR was transformed into 5 types of MCLR-DBPs.
- MS/MS analysis indicated that the conjugated diene in Adda was a major target site.
- Most MCLR-DBPs had lower toxicity on protein phosphatase 1 than MCLR.
- MCLR-DBPs still possessed certain biological toxicity and environmental risk.
- The secondary pollution of MCLR-DBPs in drinking water deserves further attention.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 November 2012
Received in revised form 9 February 2013
Accepted 6 March 2013
Available online 15 March 2013

Keywords:
Microcystin-LR
Disinfection by-products
Generative mechanism
Biological toxicity
Evaluation

ABSTRACT

To control the environmental risk of microcystin-LR disinfection by-products (MCLR-DBPs), we evaluated their generative mechanisms and biological toxicity by mass spectrometry technology and protein phosphatase inhibition assay. Subject to chlorination, MCLR was totally transformed within 45 min and generated 5 types of MCLR-DBPs with the chemical formulas of $C_{34}H_{54}N_{10}O_{12}$, $C_{49}H_{76}N_{10}O_{14}Cl_2$, $C_{49}H_{77}N_{10}O_{15}Cl$, $C_{49}H_{75}N_{10}O_{13}Cl$, and $C_{49}H_{76}N_{10}O_{14}$. Isomers for each MCLR-DBP type were identified and separated (products *1–9*), indicating that the conjugated diene in Adda residue was a major target site of disinfection. Though, subsequent toxicity test showed the toxicity of MCLR-DBPs on protein phosphatase 1 decreased with the extending of disinfection by and large, these DBPs still possessed certain biological toxicity (especially for product *5*). Combined with quantitative analysis, we thought the secondary pollution of MCLR-DBPs in drinking water also deserved further attention. This study offers valid technique support for MCLR-DBPs identifiation, contributes to a comprehensive cognition on their hazard, and thus has great significance to prevent and control the environmental risk induced by microcystins and their DBPs.

1. Introduction

The increasing frequency and intensity of cyanobacteria blooms by eutrophication posed serious threat to drinking water sources around the world [1,2]. A primary cause of cyanobacterial toxicity has been attributed to the typical metabolites, microcystins (MCs) [2–4]. MCs are cyclic heptapeptides that share a general structure. Based on the methylation pattern and the two variable amino acids at position 2 and 4 of the structure, more than 90 structural analogs have been identified [4]. Of all these variants, microcystin-LR (MCLR, Leu and Arg at positions 2 and 4 respectively, Fig. 1) is the most widespread and dangerous species.

Health-related incidents with humans, livestock and wildlife have been reported in association with MCs pollution [4–6]. The

* Corresponding author. Tel.: +86 531 88364868; fax: +86 531 88364868.
E-mail address: wansongzong@gmail.com (W. Zong).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.03.010

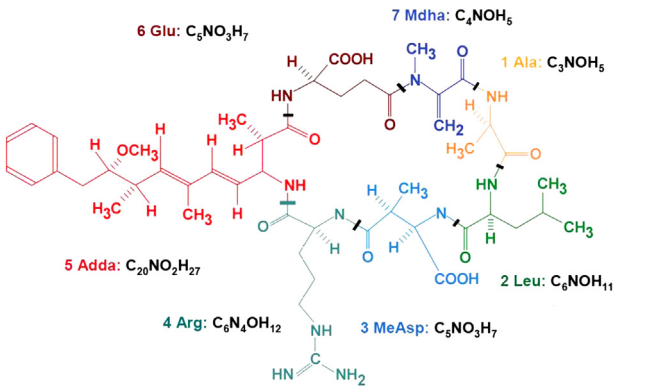


Fig. 1. Molecule structure of MCLR (Leu is at position 2, while Arg is at position 4).

primary target for MCs is the liver, as orally ingested MCs are actively absorbed to hepatic cells via a bile-acid transporter [7,8]. Within the cells, MCs irreversibly inhibit several protein phosphatases (PP1 and PP2A), subsequently leading to cell disruption, intrahepatic hemorrhage and death [9,10]. In order to protect consumer's health, guideline for these toxins has been proposed by the World Health Organization (e.g. 1 μg/L for MCLR).

Since MCs are potent hepatotoxins, controls on their levels in drinking water becomes of great importance [2,11]. Conventional water treatment processes like coagulation, flocculation, and filtration have been proven to be effective methods to reduce algal cells and cell-bound MCs [11,12]. However, they had less effect on dissolved MCs and were unreliable methods due to the fragile cyanobacteria cells (Inappropriate operations probably lead to secondary pollution). Activated carbon, in both granulated and powdered forms, could be used for the removal of dissolved MCs [13]. But it just worked as transfer media but could not eliminate the adverse effects of MCs. Disinfection methods including chlorine, ozone, permanganate and advanced oxidation processes also have been conducted for the remediation of MCs [12,14]. Disinfection involves the generation of oxidizing agents which effectively degrade MCs by destroying the crucial chemical structures (e.g. hydroxylation of Adda). In view of the above advantages, disinfection has become the key control strategy for MCs.

However, a major concern within disinfection was the formation of disinfection byproducts (DBPs) that could also bring secondary pollution [15–17]. Disinfection against MCs inevitably produced a variety of secondary MC-DBPs that might retain the original toxic groups and toxicity. Partial research gradually revealed that water samples subject to disinfection also showed certain biological toxicity [16,18]. For these reasons, there is a need for increased awareness and enhanced ability to detect, evaluate and control these DBPs. To date, this issue has not been investigated thoroughly yet, so no concrete conclusion was made. As Sylvain Merel concluded in a recent reference [3], chlorination by-products of MCLR have not been extensively studied and only two ones were mentioned in literature. Though they continued to find some new DBPs for MCLR, the biological toxicity for these DBPs has not been evaluated. We thought the lack of separation/identification methods for MCs related DBPs was one aspect of the problem; the other was the absence of evaluated methods for the toxicity of diverse DBPs from different disinfection techniques.

In the present work, a novel pipeline was established to evaluate the generative mechanism and biological toxicity of MC-DBPs involved in dringking water disinfection. MCLR (a typical microcystin) was selected as the target of chlorination (a widely used disinfection technique) and the primary disinfection byproducts, MCLR-DBPs, were separated and identified by MS, LC/MS and MS/MS. After chromatography preparation, the biological toxicity of MCLR-DBPs was evaluated by protein phosphatase inhibition assay. Our work not only offer valid technique support for microcystin DBPs identification, but also contribute to a comprehensive cognition on the hazard of these DBPs.

2. Materials and methods

2.1. Reactants

Ascorbic acid, bovine serum albumin (BSA), dithiothreitol, diethyl-p-phenylen-diamin, p-Nitrophenyl disodium orthophorphate (p-NPP), sodium thiosulfate, tris(hydroxymethyl)aminomethane (Tris), HCl, HClO, $MnCl_2$, and $MgCl_2$ were purchased from Sinopharm (Shanghai, China). MCLR, MCYR were purchased from Sigma (Saint-Quentin Fallavier, France). PP1 (1500 U/mL) from rabbit skeletal muscle was obtained from New England Biolabs Inc. and it consisted of a 37.5 kDa catalytic subunit. HPLC acetonitrile, formic acid and methanol were purchased from Merck (Darmstadt, Germany). All reagents were prepared with water produced by an alpha Q Millipore system (Molsheim, France).

2.2. Disinfection of MCLR with HClO

Reaction between MCLR and HClO was carried out in high purity water (to avoid the interferences of natural organic material) by mixing 50 mL MCLR and 200 mL HClO in 500 mL borosilicate glass bottles. The final concentrations of MCLR and HClO were 50 μg/L and 2 mg/L, respectively. Afterwards, the bottles were placed in darkness at 20 °C so as to avoid photo-degradation. Then, the disinfection process was stopped after 0–90 min through the addition of ascorbic acid stock solution (10 mg/L). For MCLR-DBPs identification, 5 mL reaction solution was pipetted and mixed with 1 mL ascorbic acid stock solution at each time. For MCLR-DBPs preparation, 50 mL ascorbic acid stock solution was added to the bottles. In each test series, a control sample was treated in the same way except for the addition of HClO.

2.3. Identification of MCLR-DBPs by LC and MS

2.3.1. Directed MS analysis of the disinfection samples

Candidate MCLR-DBPs were firstly analyzed by a maXis UHR-TOF mass spectrometer (Bruker Daltonios). Mass spectra were obtained in positive ion mode by direct injection of disinfection samples (mixed with isometric methanol containing 0.1% formic acid and 20 μg/L MCYR) into mass spectrometer using a syringe pump at 3 μL/min. Typical parameters were set as following: source voltage 4 kV, cone voltage 0.5 kV, desolvation gas N_2 0.4 bar, dry gas N_2 4 L/min, dry gas heater 180 °C, scan range 100–1200. Data acquisition was controlled with the Compass 1.3 software and MCLR-DBPs could be preliminarily identified according to the newly emerged ions.

2.3.2. LC/MS analysis of candidate MCLR-DBPs

Candidate MCLR-DBPs could be further identified by their specific retention times and chromatogram peaks in LC/MS analysis. MCLR-DBPs were separated using a Great Eur-Asia C_{18} column (4.6 × 150 mm, 5 μm, 120 Å) on a Dionex Ultimate 3000 HPLC system prior to MS analysis performed on a maXis a UHR-TOF mass spectrometer. The sample injection volume was 10 uL and the mobile phases used were (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. MCLR-DBPs were firstly eluted using 20% B for 5 min, then mobile phase B was gradually increased to 80% over 20 min. After a 5-min isocratic elution, mobile phase B was rapidly (within 0.1 min) switched to 20% to