

Transformation products of microcystin-RR with reactive species produced by radiolysis of water.

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Abstract Microcystins (MCs) are potent cyclic-peptide toxins produced by cyanobacteria during freshwater bloom episodes. They can severely impact drinking water supplies and recreational waters. More than 300 MCs are known today, which are toxic, mainly by inhibiting protein phosphatases. MC-RR is an important congener, as shown in several bloom episodes. There are still serious gaps of knowledge regarding the reaction pathways and transformation products of MCs with reactive species which have a role in advanced oxidation processes (AOPs) or in degradation processes in natural waters. In this study, we applied the principles of radiation chemistry of water to investigate the transformations of the less-studied MC-RR by a range of oxidizing (hydroxyl radical, superoxide ion, hydroperoxyl radical) and reducing (hydrogen atom, hydrated electron) species. We manipulated a steady-state radiation-chemical system using scavengers to investigate and quantify the effects of single species. We used high-resolution mass spectrometry combined with computational and visualization platforms to annotate MS features of transformation products and to compare the single-species reaction pathways. Our results contribute to risk assessment concerning the fate of MCs in water treatment processes and in the environment.

Keywords: Microcystins, water radiolysis, reactive species, transformation products, LC-HRMS

1. Introduction

Toxic cyanobacteria blooms in freshwater bodies can be a source of a large variety of noxious secondary metabolites, known as cyanotoxins. Microcystins (MCs) are among the most commonly occurring cyanotoxins into waterbodies, characterized by a cyclic heptapeptide structure and a strong hepatotoxic activity [1,2]. Moreover, their presence in drinking water has been linked to occurrences of liver cancer with the WHO suggesting a guideline value for MC-LR of 1 µg/L [3].

Conventional water treatment methods such as coagulation/flocculation/sedimentation, activated carbon adsorption and membrane filtration are not always effective in removing MCs. Advanced oxidation processes

(AOPs), on the other hand, have received increased attention as an attractive alternative for water detoxification. Generally, AOPs involve the formation of reactive oxygen species (ROS) such as hydroxyl radicals (OH·) which present high, non-selective reactivity with electron-rich organic compounds [4]. Several AOPs have been tested for the removal of cyanotoxins from drinking water, such as UV/H₂O, UV/O₃, UV/Vis/TiO₂, sonolysis, polyoxometalate photocatalysis and others [5]. However, the effects of individual reactive species (RS) generated during AOPs have not been well elucidated. In this study, radiation chemistry of water (water radiolysis) was applied as technique for the selective and quantitative production of RS, to study their reactions with MC-RR.

2. Experimental

2.1. Chemicals and reagents

Stock solutions of purified MC-RR (RGU, Aberdeen) were prepared by reconstitution in analytical grade water to a nominal concentration of 500 mg/L. The exact concentration of MC-RR was determined using a SPECORD S600 Spectrophotometer (Analytic Jena AG, Jena, Germany) at 238 nm ($\epsilon = 39.800 \text{ L mol}^{-1} \text{ cm}^{-1}$). Methanol (MeOH), acetonitrile (ACN), and water (LC-MS grade) were obtained from Fischer Scientific. Tertiary butyl alcohol (TBA) ($\geq 99.0\%$) and dichloromethane (DCM) of analytical grade were purchased from Sigma Aldrich, formic acid (HCOOH) ($>98\%$) and perchloric acid ($>98\%$) from Riedel-de Haen. High purity water (18.2 MΩ) was produced on-site, using a Temak TSDW10 system. High-purity oxygen, nitrous oxide and nitrogen were obtained from Linde Corporate Hellas.

2.2. Radiolysis experiments

Water radiolysis experiments were carried out in a ⁶⁰Co 6500 Ci Gamma Chamber (model 4000A, Isotope Group, Bhaba Atomic Research Centre, Trombay, India). The dose rate was 0.023 Gy s⁻¹, as measured using the Fricke chemical dosimeter [6]. Aqueous solutions of MC-RR (5ml, 10 mg L⁻¹) in borosilicate gas-sealed vials were

placed at specified positions in the irradiation chamber and were irradiated at specified times/doses. Samples (100 μ L) were collected for analysis. Experiments were carried out in duplicates. To study the effects of selected RS, radiolytic experiments were conducted by applying the appropriate conditions and scavengers, as illustrated in Table 1.

Table 1. Experimental conditions that were used for the selective production of selected RS. Radiation chemical yields (G-values) [7,8] are given in μ mol per J of absorbed energy.

Reactive Species	Experimental Conditions	G(RS) value (μ mol J ⁻¹)
OH \cdot	N_2O	0.56
e_{aq}^-	N_2O, TBA	0.28
H \cdot	Deaerated, TBA, pH=1	0.34
O_2^- / HO_2^-	$O_2, HCOOH$	0.62

2.3. Chemical analysis and data processing

Liquid chromatography coupled with high resolution tandem mass spectroscopy (LC-qTOF) were used to monitor the degradation of MC-RR and to identify transformation products. An Elute HPG1300 HPLC system (Bruker Daltonics) with an Atlantis T3 C18 column (100 \AA , 3 μ m, 2.1 mm X 100 mm, Waters) were used for separation. Mobile phases were water (A) and acetonitrile (B) both acidified with 0.1% formic acid. An Impact II qToF (Bruker Daltonics,) was used, equipped with an electrospray ionization source, operating in positive mode, in Auto MS (DDA) and MS1 modes (50-1300 m/z) for identification of products and quantification of MC-RR respectively. HyStar and Data Analysis software (Bruker Daltonics) was used for data acquisition and to convert HRMS data to mzXML files. HRMS data were pre-processed in MZmine3 (feature detection, feature alignment, deisotoping, gap filling) [9]. Feature-based molecular networks were developed in GNPS [10] and visualized in CytoScape [11]. Procedural and media blanks were utilized to remove “background features”.

3. Results

Degradation of MC-RR was observed under all experimental conditions in the presence of the selected RS (Table 1). Rates and kinetics of degradation depended on experimental conditions, showing the difference in reactivity among the various RS. An example of MC-RR degradation upon radiolysis of air-saturated solutions is shown in Figure 1.

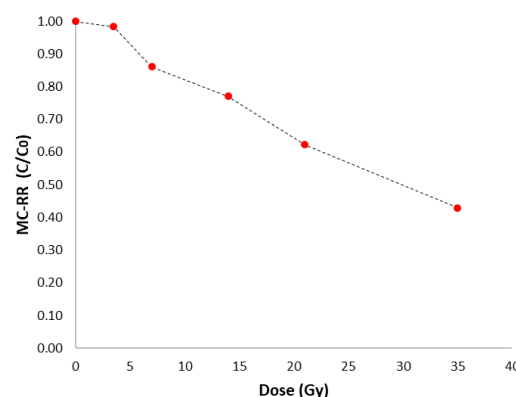


Figure 1. Degradation of MC-RR (10 mg L⁻¹) upon radiolysis of air-saturated aqueous solutions.

Under air-saturated conditions the majority of transformation products detected were generated via hydroxylation, oxidation, or cleavage of the aromatic ring. Figure 2 illustrates some of the byproducts that were detected as well as their proposed structures.

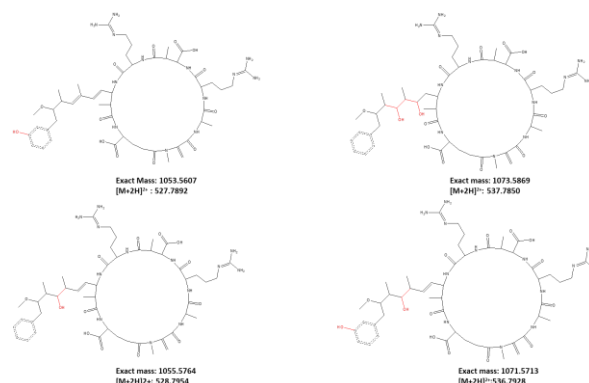


Figure 2. Proposed structures of transformation products formed upon radiolysis of air-saturated aqueous solutions of MC-RR.

Our work is now focusing on the complete elucidation of transformation pathways of MCs by RS. Of particular interest are reduction pathways, where reactions with the solvated electrons (e_{aq}^-) can shed light into underlying mechanisms in Advanced Reduction Processes (ARP) for water treatment.

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