Algal Research 18 (2016) 1–6

Contents lists available at ScienceDirect

Algal Research

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

Characterization of cylindrospermopsin decomposition products formed under irradiation conditions

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ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 25 May 2016
Accepted 27 May 2016
Available online xxxx

Keywords:
Cyanobacteria
Cylindrospermopsin
Decomposition products
Photosynthetically active radiation
UV-B

ABSTRACT

Cylindrospermopsin (CYN) is a cytotoxic alkaloid produced by several species of cyanobacteria. Research on its decomposition process and the products formed under abiotic factors is still limited. In this study, we examined the impact of UV-B or photosynthetically active radiation (PAR, 400–700 nm) on CYN stability, with particular emphasis on the structural characterization of decomposition products. CYN was stable under UV-B irradiation under acidic conditions, and its slow decomposition was observed in a neutral environment. Meaningful CYN decomposition under UV-B irradiation occurred under alkaline conditions. Within 24 h, the initial toxin concentration at pH 10 decreased to 2%, whereas at pH 12, the toxin degraded totally. Six CYN decomposition products were formed during UV-B irradiation under alkaline conditions. We elucidated their chemical structures and proposed fragmentation pathways. PAR, as well as irradiation in the presence of a photosensitizer such as phycocyanin, did not influence CYN stability in a wide range of pH values over a 24 h period. The obtained results contribute to a better understanding of CYN stability under abiotic factors.

1. Introduction

Cyanobacteria (blue-green algae) occur in many ecological niches all over the world [7,14]. However, human activity impairs proper functioning of water ecosystems by supplying industrial and municipal wastes, sewage and agricultural run-off, leading to massive development of cyanobacterial blooms. These microorganisms can provide food and be a source of biofuels or even various compounds used in medicine, pharmacy, cosmetology and biotechnology [1,10]. They have also the ability to produce many toxic secondary metabolites called cyanotoxins [8]. Depending on the species community, genetic differences and the conditions of water reservoirs, toxic blooms represent 25 to 90% of all cyanobacterial blooms [5,28]. The ageing and lysis of their cells cause the release of toxins into the water. Due to their harmful effects on vertebrates, cyanotoxins are divided into four groups: hepatotoxins (which cause liver damage), neurotoxins (which cause dysfunction of the nervous system), dermatotoxins (which cause irritation of the skin and mucous membranes) and cytotoxins (which exert negative effects on many organs). Moreover, these compounds can bioaccumulate in trophic chains, constituting a potential threat to the health of humans and animals [26,27]. Consumption of contaminated water, fish or seafood from regions where cyanobacterial blooms occur is not the only way to introduce cyanotoxins into the human body. The inhalation of toxin-contaminated aerosols, which can enter the respiratory system and impair the homeostasis of the organism, can also be dangerous [12]. In recent years, the number of studies concentrating on the harmful effects of cytotoxic cylindrospermopsin (CYN) on animals and humans has significantly increased [22]. CYN is an alkaloid (415 Da) possessing tricyclic guanidine moiety combined with hydroxymethyluracil that is produced by various species of cyanobacteria i.e., *Cylindrospermopsis raciborskii*, *Anabaena lapponica*, *Lyngbya wollei*, *Umezakia natans* and *Raphidiopsis curvata* ([25], for review: [9]). The biological activities of this toxin in animal and human cells include inhibiting protein synthesis, damaging nucleic acids, increasing the concentrations of reactive oxygen species, and causing aberrations during mitosis, as well as having cancerogenic properties and causing disorders of hormonal function (for review: [2]). Thus, the CYN degradation process and the methods used for its removal from water reservoirs should be investigated. There are only a few reports on its decomposition under the influence of factors present in the natural environment and its potential decomposition products [6,32]. In our previous study [3], we demonstrated that the stability of CYN was pH-dependent; in acidic and neutral pH, the CYN molecule was stable, whereas alkaline conditions accelerated its decomposition. A wide range of temperatures did not affect CYN stability as only boiling at high pH resulted in CYN decomposition. We described several decomposition products under alkaline conditions and alkaline conditions combined with high temperatures [3]. This study is a continuation of the research

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http://dx.doi.org/10.1016/j.algal.2016.05.027
2211-9264/

focusing on the stability of the CYN molecule under other abiotic factors. The aim of this study is to investigate the stability of the CYN molecule under the influence of UV-B or PAR (photosynthetically active radiation) irradiation, with particular emphasis on the characterization of its decomposition products.

2. Materials and methods

2.1. Growth conditions

CYN was extracted from *Cylindrospermopsis raciborskii* strain CS-505/7 cultured in BG11 medium [30]. Cyanobacteria were grown in a phytotron at 20 ± 1 °C under a 12 h light/12 h dark photoperiod and irradiated with 50 μmol m^{-2} s^{-1} of PAR (400–700 nm). After 1 month, the culture was filtered with GF/C glass fibre filters (Whatman, UK), and the cellular material was immediately frozen at −20 °C.

2.2. Extraction procedure

Extraction and purification of CYN from the lyophilized cells were performed as described by [3].

2.3. Sample preparation and irradiation conditions

A series of CYN solutions (20 μg mL^{-1}) was prepared in Britton-Robinson buffers of suitable pH: 3, 5, 7, 10 and 12. The pH was controlled with a glass pH microelectrode (InLab 423, Mettler Toledo, Switzerland). Afterwards, the CYN samples (2 mL) were placed in quartz glass containers and subjected to treatment with UV-B (36 μmol m^{-2} s^{-1}; two fluorescent tubes, Philips TL 40 W/12) or PAR (1500 μmol m^{-2} s^{-1}; two 1000 W Tungsram lamps) radiation. PAR was obtained by filtering the light through a heat cut-off filter (5 cm thick layer of a 4% aqueous solution of $CuSO_4$) and then concentrated with two independent optical systems; superimposed light spots were formed as a result [21]. Moreover, a photosynthetic pigment, phycocyanin C (Phy C, 250 μg mL^{-1}), was added to the buffers solutions of CYN and irradiated (100 μmol m^{-2} s^{-1}) with monochromatic red light at λ = 620 nm (maximum absorption of Phy C). The red light was obtained by passing the light through a 5 cm layer of distilled water (to eliminate the heat effects) and filtering it through a monochromatic filter (Zeiss, Jena, Germany) of λ = 620 nm with a half band of width of 7 nm. All experiments were conducted at 20 ± 1 °C during a 24 h period.

2.4. Analytical determination

Analysis of the changes in the CYN concentration and the determination of its degradation products were conducted by high-performance liquid chromatography (HPLC) according to a method described previously [3]. Briefly, chromatographic separation was achieved using a Waters Atlantis® dC18 column (3.9 × 100 mm, 3 μm) maintained at 35 °C and eluted under the following conditions: gradient elution from 99% to 88% of eluent A over 24 min at a flow rate of 1.0 mL min^{-1}. Eluent A was water/trifluoroacetic acid (0.05%, v/v), and eluent B was acetonitrile/trifluoroacetic acid (0.05%, v/v). CYN was identified by comparing its UV-spectrum with that determined for the standard, and then it was quantified by absorbance at λ = 262 nm. The post-reaction samples were analysed using an UPLC-MS/MS system coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were performed using an Acquity UPLC BEH (bridged ethyl hybrid) C18 column (2.1 × 100 mm, 1.7 μm) equipped with an Acquity UPLC BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 μm). The column was maintained at 40 °C and eluted under the following conditions: 100% of eluent A over 2 min and gradient elution from 100% to 30% of eluent A over 10 min at a flow rate of 0.3 mL min^{-1}. Eluent A was water/formic acid (0.1%, v/v), and eluent B was acetonitrile/formic acid (0.1%, v/v). The MS detection settings of the Waters TQD mass spectrometer were as follows: a source temperature of 150 °C, desolvation temperature of 350 °C, desolvation gas flow rate of 600 L h^{-1}, cone gas flow rate of 100 L h^{-1}, capillary potential of 3.00 kV, and cone potential of 20 V. Collision activated dissociation (CAD) analyses were conducted with an energy of 30 eV, and the all fragmentations were observed in the source. Consequently, the ion spectra were obtained by scanning from 30 to 500 *m/z*. The data acquisition software used was MassLynx V 4.1 (Waters).

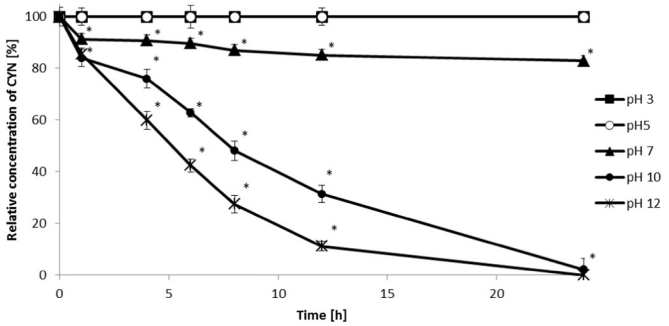

Fig. 1. Changes in concentration of aqueous CYN solution under UV-B irradiation at various pH during 24 h. Data are expressed as mean ± SD (n = 5). *significant difference from the control at $p < 0.05$.

2.5. Reagents

All reagents were analytical, HPLC or MS grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). The commercial standard of CYN was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure grade water (Milli-Q water) was obtained from Millipore (Bedford, MA, USA).

2.6. Statistical analysis

All data are expressed as the mean ± standard deviation (SD) of five independent replicates. All results were subjected to ANOVA.

3. Results

3.1. Characterization of the decomposition products of CYN after UV-B irradiation

CYN was stable under UV irradiation and at pH 3 and 5 for 24 h (Fig. 1). At neutral pH, UV irradiation reduced the CYN concentration to 83% (Fig. 1). Accelerated decomposition of the CYN molecule during irradiation was observed under alkaline conditions. In buffers of pH 10, its initial concentration decreased to 2%, whereas in buffers of pH 12, the toxin totally degraded (Fig. 1). The relationship ln (concentration) showed good correlation for linear regression model (coefficient of determination greater than 0.9), what allows for assumption, that the decomposition processes in the conducted experiments followed first-order reaction kinetics. The half-life, dissociation constant (k) and coefficient of determination (R^2) for those samples in which CYN decomposition took place are shown in Table 1.

The chromatographic (Fig. 2) and mass spectra (not shown) revealed the presence of six decomposition products under UV irradiation at pH 10.

Table 1
The kinetic parameters of the decomposition processes under UV irradiation.

pH	Dissociation constant [1/h]	Half-life [h]	Coefficient of determination (R^2)
7	0.01	69	0.98
10	0.14	7	0.97
12	0.17	5	0.99