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The photocatalytic destruction of the cyanotoxin, nodularin using TiO_2

Iain Liu [a], Linda A. Lawton [b], Detlef. W. Bahnemann [c], Peter K.J. Robertson [a,*]

[a] *Centre for Research in Energy and the Environment, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, UK*
[b] *School of Life Sciences, The Robert Gordon University, St. Andrew Street, Aberdeen AB25 1HG, UK*
[c] *Institut fuer Technische Chemie, Universitaet Hannover, Callinstrasse 3, D-30167 Hannover, Germany*

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Abstract

Titanium dioxide (TiO_2) photocatalysis has been used to initiate the destruction of nodularin, a natural hepatotoxin produced by cyanobacteria. The destruction process was monitored using liquid chromatography–mass spectrometry analysis which has also enabled the identification of a number of the photocatalytic decomposition products. The reduction in toxicity following photocatalytic treatment was evaluated using protein phosphatase inhibition assay, which demonstrated that the destruction of nodularin was paralleled by an elimination of toxicity.

Keywords: TiO_2; Photocatalysis; Nodularin; Cyanotoxin

1. Introduction

Cyanobacterial toxins produced and released by cyanobacteria around the world have been well-documented [1,2]. Nodularins (Fig. 1), produced by the cyanobacterium *Nodularia spumigena*, are structurally and biologically similar to microcystins (Fig. 2) and both groups of these toxins are among the cyanobacterial toxins usually detected in water [3]. It has been shown that the mode of action of these toxins at a molecular level is caused by the inhibition of serine/threonine protein phosphatases 1 and 2A. Chronic exposure due to the presence of hepatotoxic cyanotoxins in drinking water is thought to be a contributing factor in primary liver cancer (PLC) through the known tumour-promoting activities of these compounds [4–7].

Since cyanobacterial toxins pose a considerable threat to human health, various water treatment processes have been evaluated to degrade these toxins. It is believed, however, that conventional water treatment systems have proven unreliable for the removal of these toxins from water [8,9]. In recent years the use of titanium dioxide (TiO_2) as a photocatalyst for water treatment has been extensively reported. When TiO_2 is illuminated with light of an appropriate wavelength it generates highly active oxidising sites, which can potentially oxidise a large number of organic wastes such as dyes, pesticides, bacteria and herbicides [10–13]. TiO_2 is especially suitable as a photocatalyst for waste treatment, compared to other semiconductors, because it is highly photo-reactive, cheap, non-toxic, chemically and biologically inert, and photostable [14]. Previous work has demonstrated the effectiveness of TiO_2 photocatalysis for the destruction of microcystin-LR in aqueous solutions even at extremely high toxin concentrations, however, a variety of by-products were generated [15,16]. Further mechanistic studies of the process enabled the characterisation of some of the breakdown products and the assessment of their toxicity using protein phosphatase inhibition and brine shrimp bioassays [17,18]. The effectiveness of TiO_2 photocatalysis for destruction of other groups of cyanobacterial toxins such as nodularins has not yet been reported. In this study, we report the destruction of nodularin by TiO_2 photocatalysis. The toxicity of the photocatalytic reaction degradation products has been determined using a protein phosphatase inhibition bioassay.

* Corresponding author. Tel.: +44 1224 262301; fax: +44 1224 262222.
E-mail address: peter.robertson@rgu.ac.uk (Peter K.J. Robertson).

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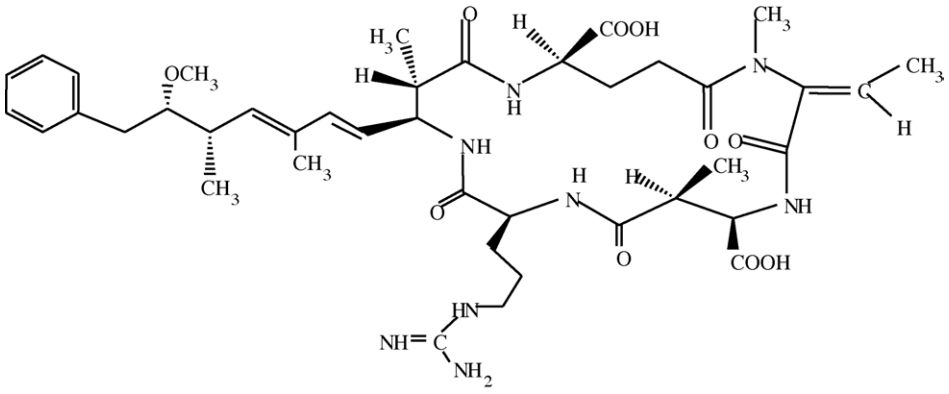

Fig. 1. Nodularin.

2. Experimental

2.1. Materials

Nodularin was obtained from a laboratory culture *N. spumigena* KAC 66 (University of Kalmar, Sweden). Harvested cells were frozen and then extracted in 50% aqueous methanol. The filtered extract was isolated by solid phase extraction followed by separation using preparative HPLC on a C18 column to obtain purified nodularin (18 mg). The purity of the nodularin was subsequently confirmed by comparison to previously purified standards by LC–MS data and by LC photodiode array analysis. Titanium dioxide (Degussa P-25) was used as received. Protein phosphatase 1 was obtained from Sigma, Pool, UK. All other reagents and solvents used were analytical grade or HPLC grade. Aqueous solutions were prepared in Milli-Q water.

2.2. Photocatalysis

Aqueous solutions (10 ml) of nodularin containing 0.1% (w/v) TiO_2 were illuminated in the presence of air with a 480 W Xenon lamp (Uvalight Technology Ltd.; spectral output 330–450 nm, with light filtered out below 350 nm). The reactions were carried out in glass universal bottles with constant stirring. The distance from the UV lamp to surface of the test solution was 30 cm and the light intensity at this distance was calculated to be 1.91×10^{-5} einstein s^{-1} using ferrioxalate actinometry. On irradiation temperature of the reaction solution stabilised at 30 °C. At timed intervals, samples were taken (0.5 ml) and centrifuged to remove TiO_2 prior to analysis by LC–MS and protein phosphatase inhibition assay. The initial concentration of nodularin was 1 mg ml^{-1} for the photocatalysis. Controls were performed, both in the dark and in the absence of TiO_2.

2.3. Analysis

The LC–MS system used in the study was a Waters Alliance 2690 HPLC Pump connected with Waters 996 PDA and Micromass ZQ Mass spectrometer with electrospray ionisation source (Manchester, UK). The HPLC column was a Waters Symmetry 300™ C18 column (5 μm, 2.1 mm × 150 mm, Waters, USA). Treated samples were diluted 10-fold with Milli-Q water before analysis and the injection volume was 10–50 μl. Mobile phases were water and acetonitrile, both containing 0.05% trifluoroacetic acids (TFA). Gradient elution was programmed as 5–20% of acetonitrile in 10 min increasing to 80% in 42 min. The flow rate was 0.3 ml min^{-1}. The eluent was directly introduced to the mass spectrometer ion source without a splitter. The mass data was obtained in the positive ion mode by full

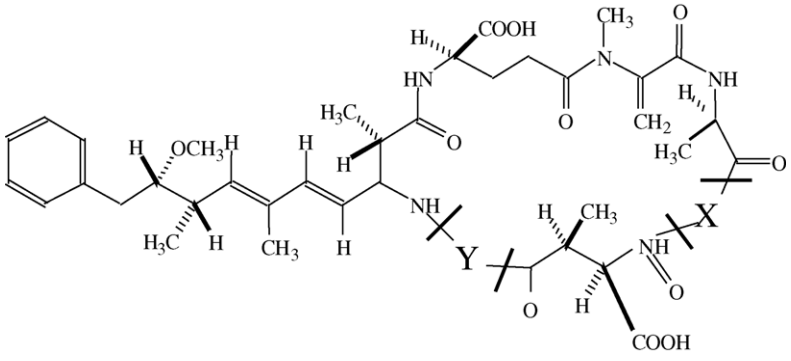

Fig. 2. Generic structure of microcystins where *X* and *Z* represent the variable amino acids and, D-Me-Asp is D-*erythro*-β-methylaspartic acid, Adda is (2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(*E*),6(*E*)-dienoic acid, D-Glu is D-glutamic acid and Mdha is *N*-Me-dehydroalanine.