Processes Influencing the Destruction of Microcystin-LR by TiO_2 Photocatalysis.

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Abstract.

We have previously reported the effectiveness of TiO_2 photocatalysis in the destruction of the cyanotoxin Microcystin-LR [1,2]. In this paper we report an investigation of factors which influence the rate of the toxin destruction at the catalyst surface. A primary kinetic isotope effect of approximately 3 was observed when the destruction was performed in a heavy water solvent. Hydroxylated compounds were observed as products of the destruction process. No destruction was observed when the process was investigated under a nitrogen atmosphere.

1. Introduction.

Microcystin-LR is a hepatoxin material released by the cyanobacteria microcystis [3]. This compound is a cyclic heptapeptide containing the amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (adda), with leucine (L) and arginine (A)

in the variable positions (figure 1). Microcystins have caused the deaths of both animals and humans as a result of ingestion of contaminated water [3,4,5]. It is also believed that longer-term exposure to sub-lethal levels of microcystins may promote primary liver cancer by disruption of protein phosphatases 1 and 2A. These enzymes are involved in regulating cell division particularly checking cell division. If this is inhibited tumour formation can occur [3].

Microcystins are chemically very stable [6] and conventional water treatment processes have so far failed to remove them, furthermore the use of more advanced methods such as granular carbon filtration and photochemical degradation have shown only limited efficacy [7,8]. We have previously reported the effectiveness of TiO_2 photocatalysis in the destruction of microcystin-LR [1,2]. In this paper we have extended the investigation to examine the mechanism of this destruction at the photocatalyst surface. The results of this work would indicate that the process occurs by attack of hydroxyl radical generated on the surface of the photocatalyst.

2. Experimental Details.

2.1. Materials.

Microcystin-LR was purified from a natural sample of Microcystis aeruginosa [9]. The cyanobacterial cells were extracted in methanol followed by C18 reverse-phase flash chromatography (Biotage UK). The final purification was performed by preparative C18 reverse-phase chromatograph.

Titanium dioxide (Degussa P-25) was used as received.

2.2. Photocatalysis.

Aqueous solutions of microcystin-LR (reaction pH 4 and temperature 306 K) were illuminated in the presence of air and a TiO_2 catalyst (1% m/v slurry of TiO_2 Degussa P-25) using a xenon UV lamp (280W UVASpot 400 Lamp, Uvalight Technology Ltd, Spectral Output 330-450 nm). The same procedure was adopted for the experiments performed in D_2O ( ).

2.3. Analysis.

The destruction of the samples was monitored by HPLC with a high resolution diode array detector (Waters 996 detector). Separations were performed on a symmetry C18 column (15x0.46 cm I.D.; 5mm particle size, Waters). Eluents were Milli Q water (Millipore, Watford, UK) and acetonitrile (Rathburn, Walkerburn, UK), both containing 0.1% trifluoroacetic acid (TFA). A linear gradient was employed starting at 30% (v/v) aqueous acetonitrile increasing to 35% over 5 minutes followed by an increase to 60% over the next 25 minutes. Detector resolution was set at 1.2 nm and the data acquired from 200 to 350 nm [10].

Mass spectrometry was performed using a Finnegan Masslab Navigator, with electrospray ionisation. This instrument utilises a quadrupole mass filter enabling measurement up to 1600 m/z.

3. Results

The destruction of microcystin-LR was investigated in both water (H_2O) and heavy water solvents (D_2O) (Figure 2). The rate of toxin destruction in the D_2O solvent was significantly slower than that observed in water. The primary isotope effect (k_H/k_D) for the process was calculated to be 3 (table 1). The intermediates of the destruction were investigated using mass spectrometry. Data from this analysis indicated that hydroxylated structures were generated (figure 3). A significant initial product of the photocatalytic process is a microcystin molecule with one of the unsaturated bonds on the ADDA group being hydroxylated. In the case of the D_2O reaction OD groups attached to the molecule (figure 3). The rapid loss of toxicity of the toxin during the course of the photocatalytic treatment would also be consistent with an attack on this bond [2].

When the destruction was investigated under a nitrogen solvent no significant disappearance of the toxin was observed. It was necessary to ensure that the solution was degassed in the presence of oxygen since even surface adsorbed oxygen was sufficient to allow the photocatalytic process to continue.

4. Discussion.

The role of the hydroxyl radical in as the main oxidant in the photocatalytic destruction of organic compounds has long been suspected. This concept has been validated for a

wide range of compounds with evidence such as the demonstration of kinetic isotope effects [11], the formation of hydroxylated intermediates [12] and the detection of $OH^{\bullet}$ in the reactor solutions [13]. A key paper by Turchi and Ollis has reviewed the evidence that supports this hypothesis [14]. This evidence included the observation of hydroxylated products of the process, the requirement for the catalyst to be hydroxylated for degradation to occur, the detection of $OH^{\bullet}$ by ESR and kinetic isotope effects.

The results for the destruction of microcystin-LR would initially appear to be consistent with a hydroxyl radical attack on the toxin molecule. This supposition is reinforced by the two factors:

1. the kinetic isotope effect observed in the D_2O solvent
2. the detection of hydroxylated products from the photocatalytic destruction process.

Cunningham and Srijaranai [11] observed a similar kinetic isotope effect for the destruction of isopropanol using TiO_2 to that reported here. The results of both their work and ours suggest that a rate limiting process in the photocatalytic system is the formation of the hydroxyl species. The reduced rate in D_2O is due to the lower quantum efficiency for the formation of $OD^{\bullet}$ radicals on the TiO_2 surface. Consequently there is a lower relative concentration of OD radical on the TiO_2 surface available for oxidation of the substrate. They proposed that this effect strengthened the supposition that the photogeneration of hydroxyl radicals was the rate determining process for the photocatalytic process. Few other workers have, however investigated the kinetic isotope effect with their particular systems.

Ollis and Turchi [14] established that using the same conditions the rate of destruction is same for a variety of different organic compounds. They proposed that the rate destruction would depend on a variety of parameters including catalyst structure and illumination intensity**.** In addition they noted that the method for determining the rate constant for the process form the intercept of a double reciprocal plot was sensitive to small variations in the data. It is interesting to note that the rates observed by various groups for a diverse range of compounds are of a similar order [15, 16, 17, 18].

Subsequent to the analysis by Ollis, Gerischer and Heller [19, 20] proposed that the rate-determining step for the destruction of organics by TiO_2 photocatalysis is the reduction of oxygen to superoxide radical anion. This observation confirmed that the rate of photocatalytic destruction would be independent of the substrate undergoing treatment since these compounds are not involved in the rate determining reaction.

In our investigation of microcystin we have also established that oxygen must be present for destruction of the toxin. This would indicate that neither microcystin nor any of the intermediates of destruction act as alternative electron acceptors. The level of oxygen required for this was in fact very small, with even pre-adsorbed gas allowing the destruction to proceed. Several other workers have also found that the necessity of the presence of oxygen for photocatalytic processes [21, 22, 23, 24].

Okamoto [25] and Anpo [26] have both proposed that the species formed as a result of the conductance band electron transfer to oxygen ($O_2^{\bullet -}$, $HO_2^{\bullet}$ and H_2O_2) are also involved in the photooxidation reactions. Linsebigler and Yates established such a mechanism for the destruction of chloromethane on TiO_2 [20]. Using $^{18}O_2$ they established that oxidation was initiated via species generated from valence band reduction.

The precise nature of the oxidising agent involved in the photocatalytic process is therefore, still a matter of debate. Product analysis may be inappropriate for assigning a particular mechanism. The presence of the hydroxylated products may not necessarily confirm attack by $OH^{\bullet}$ radicals. The substrates may undergo attack by direct hole oxidation and then subsequently may be hydrated by the solvent [27, 28, 29]. In order to determine the most likely primary oxidation process there is therefore a requirement for some other experimental evidence.

The fact that the kinetic isotope effect observed by us was of a similar magnitude to that observed by Cunningham may be significant. It is possible that the destruction of the toxin is mediated via hydroxyl radicals generated form the superoxide radical anion produced at the conduction band. This is subsequently hydrated or deuterated by the solvent. This may be rate determining since the O_2 has to be generated at the conduction band prior to interaction with the solvent and subsequent formation of $OH^{\bullet}$ or $OD^{\bullet}$ species. Therefore the kinetic isotope effect could be due to the interaction of the solvent

with the superoxide species rather than the attack on the toxin. If this is the case we should observe a similar kinetic isotope effect no matter what the substrate being treated. For the oxidation of microcystin to proceed there is therefore a requirement for hydroxyl radicals and oxygen. Whether the rate-determining step for the process is the degeneration of the hydroxyl radicals or the reduction of oxygen has yet to be determined.

Redox potential of OD radical.30

5. Conclusion.

The destruction of microcystin-LR appears to be initiated via hydroxyl radical attack on the ADDA group of the toxin. This premise is based on the fact that a kinetic isotope effect of 3 was observed when the destruction was investigated in D_2O. In addition hydroxylated compounds were observed as products of the decomposition process. Toxicity testing of solutions treated by this method have confirmed that the by-products of the photocatalytic process are non-toxic [2].

References

1) P.K.J. Robertson, L. A. Lawton, B. Münch and J. Rouzade, *Chem. Comm.*, *4*, (1997), 393
2) P.K.J. Robertson, L. A. Lawton, B. Münch and B. Cornish, J. Adv. Oxid. Technol., Accepted for publication.
3) W.W. Carmicheal, *Scientific American*, *2*, (1994), 64.

4) L.A. Lawton, C. Edwards, K.A. Beattie, S. Pleasance, G.J. Dear and G.A. Codd, *Nat. Toxins, 3,* (1995), 50.

5) J. Dunn, *Brit. Med. J., 313,* **(**1996), 1183.

6) K. Tsuji, T. Watanuki, F. Kondo, M. Wanatabe. S. Suzuki, H. Nakazawa, M. Suzuki, H. Uchida. K. Harada, *Toxicon, 33,* (1995), 1619.

7) C. S. Reynolds, in "Advances in Botanical Research", Ed J.A. Callow, Academic Press, London, 1987, vol. 13, 63.

8) K. Tsuji, S. Nalto, F. Kondo, N. Ishikawa, M.F. Watanabe, M. Suzuki and K.-I. Harada, *Environ. Sci. Technol. 28,* (1994), 173.

9) C. Edwards, L. A Lawton., S. M Coyle,. and P. Ross, *J. Chromatog. 734,* (1996), 163.

10) L.A. Lawton, C. Edwards and G.A. Codd, *Analyst, 119,* 1994, 1525.

11) J. Cunningham and S. Srijaranai, *J. Photochem., Photobio. A: Chem. 43,* (1988), 329

12) S. Turchi and D.F. Ollis, *J. Catal., 119,* (1989), 483.

13) C. D. Jaeger and A.J. Bard, *J. Phys. Chem., 83,* (1979), 3146.

14) C. S. Turchi and D.F. Ollis, *J. Catal., 122,* (1990), 178.

15) M. R. Hoffmann, S. T. Martin, W. Choi and D.W. Bahnemann, *Chem. Rev., 95,* (1995), 69.

16) R.W. Matthews, *J. Catal., 111,* (1988), 264.

17) R.W. Matthews and S.R. McEvoy, *J. Photochem. Photobiol. A: Chem., 66,* (1992), 355.

18) M. Trillas, M. Pujol and X. Domenech, *J. Chem. Technol. Biotechnol., 55,* (1992), 85.

19) H. Gerischer and A. Heller, *J. Phys. Chem., 95,* (1991), 5261.

20) C.M. Wang, H. Gerischer and A. Heller, *J. Am. Chem. Soc., 114*, (1992), 5230

21) G. Lu, A. Linsebigler and J.T. Yates, *J. Phys. Chem., 99*, (1995), 7626.

22) M. Barbeni, E. Pramauro, E. Pelizzetti, E. Borganello, M. Gratzel and N. Serpone, *Nouv. J. Chim. 8*, (1984), 547.

23) M.S. Diekmann and K. Gray, *Wat. Res., 30,* (1996) 1169.

24) B.R. Eggins, F.L. Palmer and J.A. Byrne, *Wat. Res., 31*, (1997), 1223.

25) K. Okamoto, Y. Yamamoto, H. Tanaka, M. Tanaka and A. Itaya, *Bull. Chem. Soc. Jpn., 58*, (1985), 2015.

26) M. Anpo, K. Chiba, M. Tominari, S. Coluccia, M. Che and M.A. Fox, *Bull. Chem. Soc. Jpn., 64*, (1991), 543.

27) R.B. Draper and M. A. Fox, *Langmuir, 6*, (1990), 1396.

28) Y. Mao, C. Schoneich and K.D. Asmus, *J. Phys. Chem., 95*, (1991), 1080.

29) E. R. Carraway, A.J. Hoffmann and M.R. Hoffmann, *Environ. Sci. Technol., 28*, (1994), 786.

Solvent	**Initial Rate/ μM min^{-1}**	**Apparent First Order Rate Constant / Min^{-1}**	**Relative Rate**
H_2O	5.62	0.088	1.0
D_2O	1.58	0.029	0.33

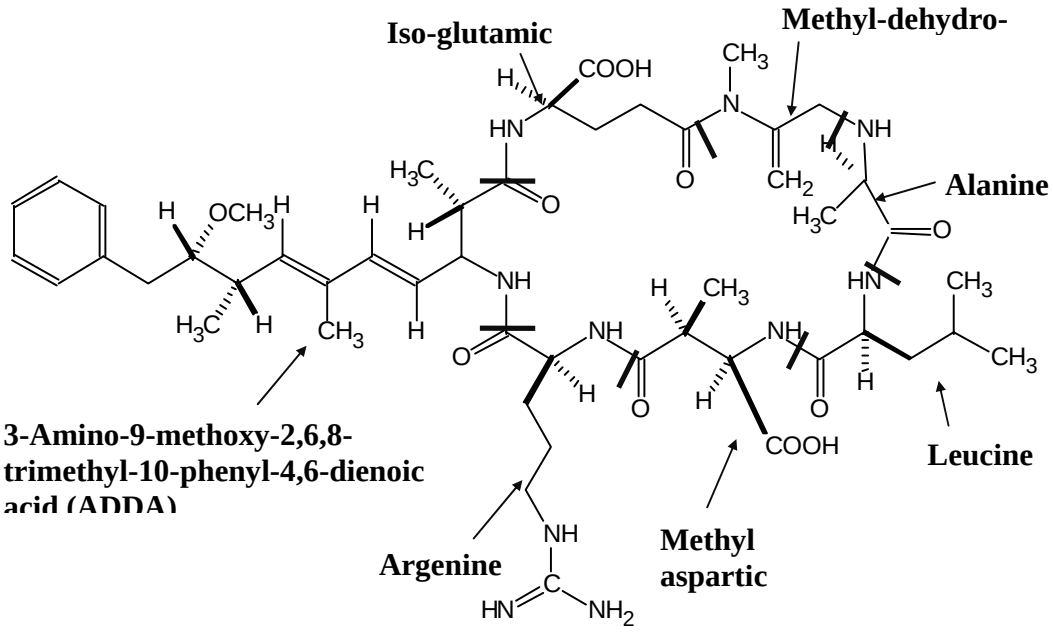
Iso-glutamic
Methyl-dehydro-
Alanine
Leucine
3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid (ADDA)
Argenine
Methyl aspartic

Figure 1.

R=H, D