Environ. Sci. Technol. **2005**, *39,* 6300−6305

Ultrasonically Induced Degradation and Detoxification of Microcystin-LR (Cyanobacterial Toxin)

WEIHUA SONG, TERRI TESHIBA, KATHLEEN REIN, AND KEVIN E. O'SHEA*

Department of Chemistry and Biochemistry, Florida International University, University Park, Miami, Florida 33199

Cyanobacterial toxins (CBTs), produced by glue-green algae, are one of the most common naturally occurring toxins found in potable waters. The microcystin family of CBTs present in drinking water sources poses a considerable threat to human health. In this study, we have demonstrated that ultrasonic irradiation at 640 kHz leads to rapid degradation of microcystin-LR (MC-LR). Degradation of MC-LR present in the crude cyanobacterial extracts containing cell constituents has been studied with ultrasound under a variety of conditions. The degradation of MC-LR was demonstrated over a concentration range from 0.03 to 3.0 μM. Hydroxyl radical scavenger experiments indicate that hydroxyl radical is responsible for a significant fraction of the observed degradation, but other processes (hydrolysis/pyrolysis) are also important. Analysis of the protein phosphatase inhibition activity of the reaction products indicates that the products from ultrasonic degradation of MC-LR do not exhibit any measurable biological activity. The results demonstrate that ultrasonic irradiation maybe an effective and practical method for the detoxification of microcystins from drinking water.

Introduction

The increased occurrence of cyanobacteria (blue-green algae) blooms and the production of associated cyanotoxins (algal toxins) have become major concerns for drinking water providers. Among the most common types of cyanotoxins found in potable water are microcystins (MCs), a family of cyclic heptapeptides containing substrates. MCs are strongly hepatotoxic and known to initiate tumor-promoting activity as a result of their potent inhibition of protein phosphatases (*1*). The LD_{50} values of ~65 different microcystin variants range from 50 to 300 μg/kg, with MC-LR being one of the most toxic variants, with an LD_{50} of 50 μg/kg (*2*). The presence of sublethal doses of MCs in drinking water was implicated as one of the key risk factors for an unusually high occurrence of primary liver cancer observed in China (*3*). Consumption of water contaminated with MCs has resulted in the fatalities of animals and humans (*4*, *5*). The acute and chronic toxicity of MCs has increased pressure to ensure their removal from potable water, and WHO published a guideline value for MC-LR of 1 nM in 1998 (*6*).

MCs contain two variable L-amino acids and two D-amino acids. The D-amino acids are *N*-methyldehydroalanine (MDHA), which hydrolyses to methylamine, and a unique nonpolar-linked amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, also known as ADDA (*7*). The key component for biological activity appears to be linked with the ADDA side chain, as cleavage or isomerization of the ADDA side chain from the cyclic peptide renders nontoxic products (*8*). Michael addition to MDHA also has been reported as a covalent binding process in the active site (*9*).

A variety of traditional water treatment methods, including chemical coagulation, flocculation, and sand filtration, have been attempted for the removal of cyanobacterial toxins, but with limited success (*10*, *11*). Removal of MCs by activated carbon can be an effective technology, depending on the contamination case, but may require relatively high doses and suffer breakthrough of toxin and reduced efficiency in natural water due to the presence of other organics (*12*). Treatment using chlorination requires high doses and long contact times and has the inherent disadvantage that chlorinated byproducts are generated (*13*). Ozonation can destroy MC-LR from both raw and clarified water (*14*, *15*), but unfortunately, the competition between the toxin and organic material in the raw water can lead to rapid depletion of ozone, resulting in incomplete oxidation of the toxin (*16*). The formation of bromate is also a general concern during ozone water treatment (*17*).

Advanced oxidation technologies (AOTs) are attractive alternatives to traditional water treatments and have received considerable attention recently. AOTs involve the generation of $OH^{\bullet}$ as the predominant species responsible for the degradation of pollutants/toxic materials. AOTs are effective for a variety of anthropogenic pollutants, but few reports have appeared on the treatment of natural toxins. A recent report established that TiO_2 photocatalysis, an AOT, leads to the degradation of MCs at high concentrations, and the resulting byproducts exhibit low toxicity (*18*). Iain Liu et. al. tentatively assigned structures to the oxidation byproducts using LC−MS (*19*). Fenton and photo-Fenton processes are also effective for the degradation of MC-LR (*20*). Ultrasonic irradiation, an AOT, is known to inhibit cyanobacterial growth (*21*) and has been suggested as a method to control algal growth (*22*), but the release of MCs into water during treatment is a concern.

Unlike AOTs involving photolysis and photocatalysis, ultrasound does not require addition of chemicals and can be used for treatment of turbid solutions. Ultrasonic irradiation promotes the growth and collapse of gas bubbles (cavitation), leading to extreme conditions (5000 K, 1000 atm) under which the pyrolysis of water produces $H^{\bullet}$ and $OH^{\bullet}$. In most cases, the reaction pathways observed for ultrasonic degradation are similar to those observed for other $OH^{\bullet}$-generating techniques. We report herein the ultrasonic-induced degradation and detoxification of pure MC-LR and microcystin-containing cellular extracts under a variety of conditions. Experiments conducted in the presence of hydroxyl radical scavengers suggest both hydroxyl radical and non-hydroxyl radical reactions are important for the degradation of microcystin during ultrasonic irradiation.

Experimental Section

Materials. MC-LR standard and protein phosphatase 1 were purchased from Sigma. CH_3OH (HPLC grade) and ammonium acetate were from Fisher. *p*-Nitrophenyl phosphate disodium salt was from Calbiochem. Terephthalic acid disodium, Tris-HCl, bovine serum albumin (BSA), $MnCl_2$, $MgCl_2$, and dithiothreitol were obtained from Aldrich. All solutions were prepared in Milli-Q water.

* Corresponding author phone: 305-348-3968; fax: 305-348-3772; e-mail: osheak@fiu.edu.

10.1021/es048350z CCC: $30.25
Published on Web 07/12/2005

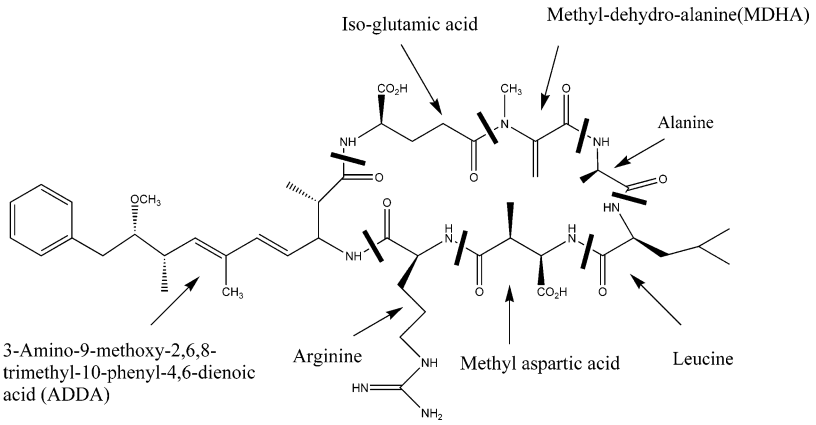

FIGURE 1. Structure of MC-LR containing three D-amino acids (glutamic acid, alanine, and methylaspartic acid), two unusual amino acids [*N*-methyldehydroalanine (MDHA) and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid (ADDA)], and two variable L-amino acids (leucine and arginine).

Isolation and Purification of MC-LR. A culture of *Microcystis aeruginosa* (UTCC299) was obtained from the University of Toronto culture collection. MC-LR was purified by employing a method that was developed from a literature protocol (*23*) as described below. Dried cyanobacteria obtained from the NIEHS Toxic Algae Culture Center at Florida International University, Miami, FL, was suspended in water in two centrifuge tubes packed with ice and sonicated with an ultrasonic cell disrupter four times for 30 s. After cell disruption, the two solutions were centrifuged at 11 000 rpm for 20 min at 4 °C, and the supernatant was removed and refrigerated. The above procedure was repeated after re-suspending the pellets remaining in the centrifuge tubes with additional water, and the two supernatants were combined. The extracts were filtered through 0.2 μm cellulose acetate syringes filters and stored at 4 °C. The extracts will be referred to as crude microcystin extracts.

One hundred milliliters of crude microcystin extracts was preconcentrated on 2-g C-18 Sep-pack cartridges. The cartridge was conditioned with 10 mL of CH_3OH, followed by 10 mL of water. The sample was then passed through by suction filtration at a rate of 10 mL/min. The cartridge was washed successively with 10 mL of 10% CH_3OH and 10 mL of 20% CH_3OH, the toxin was eluted using 10 mL 100% CH_3-OH, and the elutant was collected. The eluent fraction was rotary-evaporated to dryness at 40 °C. Final purification of crude MC-LR was performed by HPLC (*23*). Identity and purity were confirmed by mass spectrometry, ^{1}H NMR (*24*), and reverse phase HPLC comparison to an authentic sample (Sigma-Aldrich).

Ultrasound Equipment. An ultrasonic transducer (UES 1.5-660 Pulsar, Ultrasonic Energy Systems, Inc.) operated at 640 kHz equipped with a 40 mL reaction vessel was fitted with a Teflon window facing the transducer. The reaction vessel was centered at a distance of 4.50 cm from the face of the horn. Since modest heating is observed during ultrasonic irradiation, the entire assembly was submerged in an ice bath to maintain a constant temperature of 4 °C thoughout the reaction process.

Analytical Methods. The destruction of the MCs was monitored by HPLC with photodiode array detector (Beckman 166 detector). Separations were performed on a Spherisorb ODS column (25 cm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of 50% CH_3OH and 50% 20 mM ammonium acetate aqueous buffer solution. Chromatograms were analyzed and integrated at 238 nm. In some cases, the samples were preconcentrated prior to analysis. The concentration of MC-LR was determined by developing a calibration curve from commercially available MC-LR.

Analysis of Inhibition Activity. Protein phosphatase assays were performed using a modification of previously reported colorimetric procedures (*25*−*27*). Protein phosphatase 1 (PP1) was diluted to 0.05 units/μL in a buffer of 50 mM Tris-HCl, 1.0 g/L BSA, 1.0 mM $MnCl_2$, and 2.0 dithiothreitol, pH 7.4. A 5 mM *p*-nitrophenyl phosphate standard solution was prepared in buffer containing 50 mM Tri-HCl, 20 mM $MgCl_2$, 0.2 mM $MnCl_2$, and 0.5 g/L BSA with pH 8.1. All buffer solutions were freshly prepared. MC-LR and test samples were diluted with Milli-Q water.

Ten microliters of test solution was added to 10 μL of PP1 solution in a 96-well polystyrene microtiter plate. After a few seconds of gentle shaking, the microtiter plate was kept at room temperature for 5 min followed by addition of 180 μL of *p*-nitrophenyl phosphate solution (substrate). The plate was incubated at 30 °C and the production of *p*-nitrophenol was measured at 405 nm on a μQuant reader at 15 min intervals for 60 min. The dose-dependent kinetic activity of PP1 against substrate (*p*-nitrophenyl phosphate) was established to assess the baseline enzyme activity prior to sample testing. The inhibition of enzyme activity was measured at known MC-LR concentrations to establish the PP1 inhibition curve. All enzyme assays were performed in triplicate.

Results and Discussion

The structure of MC-LR is shown in Figure 1. Aqueous air saturated mixtures of MC-LR were subjected to ultrasonic irradiation at 640 kHz, and 1.0 mL samples were taken for analysis at various time intervals. The initial experiments were conducted using pure MC-LR at a starting concentration of 2.7 μM. MC-LR is rapidly degraded by ultrasonic irradiation, and within 3 min, less than 50% of the starting material remains, as illustrated in Figure 2. After 6 min, the concentration of MC-LR is reduced to ~0.4 μM.

Cyanobacteria are known to produce a number of toxins and other cell constituents that may interfere and inhibit degradation of MC-LR. With this in mind, we conducted ultrasound experiments on cellular extracts to evaluate the extent that MC-LR is destroyed in the presence of other dissolved organic matter. While the structures of the cyanobacterial cell constituents may vary significantly from those of humic materials found in natural waters, we expected both to react at similar rates with the radical species generated during ultrasonic irradiation. Hence, experiments conducted in the presence of the cellular extracts are more indicative of the types of samples that would be encountered in actual water treatment applications. Under our experimental conditions, MC-LR in cellular extracts is readily degraded

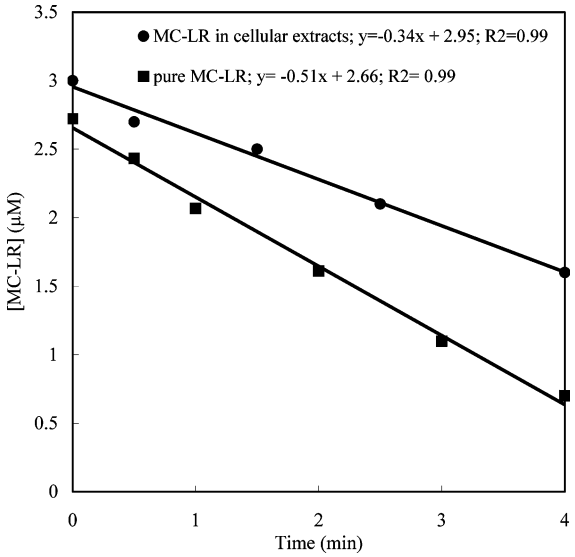

FIGURE 2. Concentrations of purified MC-LR and MC-LR in cellular extracts as a function of irradiation at 640 kHz. Initial concentration of MC-LR is ~2.7 μM in air-saturated aqueous solution. The experimental error in the analytical measurements is <2% on the basis of triplicate runs.

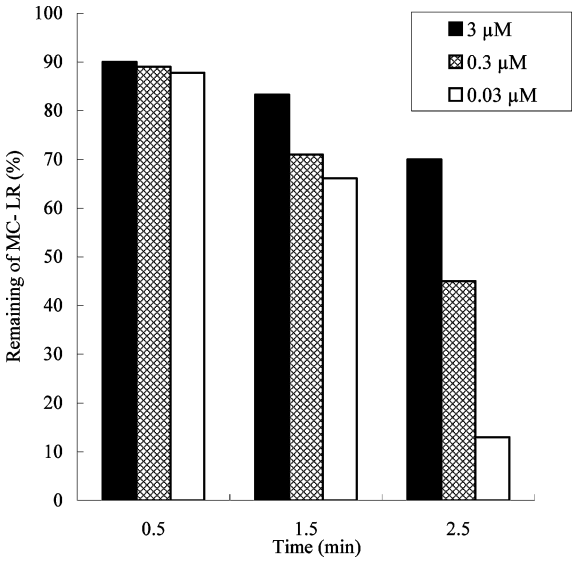

FIGURE 3. Loss of MC-LR from cellular extracts at different initial concentration under ultrasonic irradiation (640 kHz). The reproducibility is estimated to be ≥90% on the basis of duplicate runs.

under ultrasonic irradiation. Noteworthy is the observation that the MC-LR in the cellular extracts is destroyed at an only slightly reduced rate compared to the rate observed for pure MC-LR, illustrated in Figure 2. The degradation of pure MC-LR and crude MC-LR appears linear for the initial stages of the treatment. It is clear that the large amount of nonspecific cyanobacterial exudate present in the cellular extracts did not dramatically inhibit the destruction of MC-LR.

The degradation of MC-LR from the cellular extracts can be achieved with ultrasound over a MC-LR concentration range of 0.03−3.0 μM, as represented in Figure 3. The results clearly demonstrated that rapid ultrasonic-induced destruction of MC-LR is achieved over a range of concentration and in the presence of cellular constituents. If radical chain oxidation processes were predominant in the destruction of MC-LR under our experimental conditions, one would expect similar extents of degradation over a range of concentrations. However, we observed that the concentration of MC-LR is

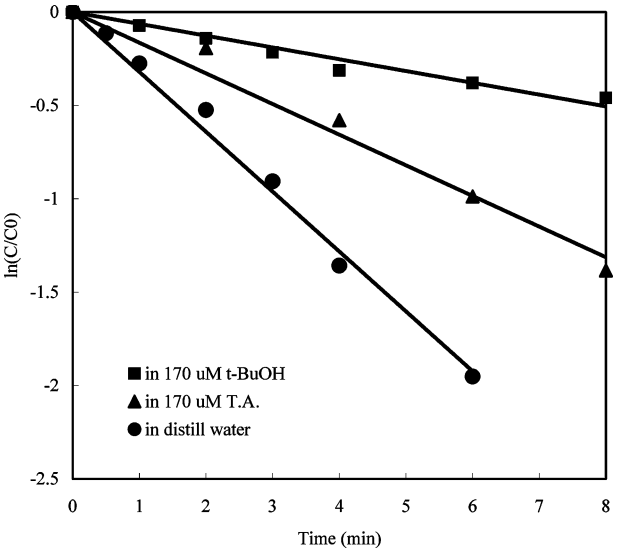

FIGURE 4. Degradation of pure MC-LR in aqueous solution and in the presence of hydroxyl radical scavengers TA and TBA. Experiments were performed under air-saturated conditions and an initial MC-LR concentration of 3.0 μM. The reproducibility of the analytical measurements is <2% on the basis of triplicate runs.

an important factor in the degradation. The order of the reaction appears to increase as the concentration of MC-LR decreases, as indicated by Figure 3. Such behavior would be expected in cases where the limiting factor becomes the concentration of the reactive species (hydroxyl radicals) or reaction sites (local concentration of MC-LR at or near the cavitation bubble interface). At higher MC-LR concentrations, more reaction byproducts are produced that will compete for the reactive species initiating the degradation process, consistent with the slower degradation observed at higher initial MC-LR concentrations.

Ultrasonic-induced degradation of organic compounds is a complex process that can involve hydroxyl radicals, supercritical water oxidation, and pyrolysis. Experiments were conducted to probe the mechanistic details of the degradation process, as described in the following sections. Lawton reported that hydroxyl radical attack destroys the conjugated diene structure in MC-LR to form the dihydroxylated products as a predominant degradation pathway during TiO_2 photocatalysis (*18*). Microcystin cellular extracts contain only ~3 wt % MC-LR (*28*). If hydroxyl radical degradation is the predominant pathway, the degradation rate of MC-LR in the cellular extracts is expected to be slower than for pure MC-LR, since the cellular extracts contain ~97% non-MC-LR organic matter that can compete for •OH in the bulk solution. The similar reaction rate observed for extracts and pure MC-LR imply that •OH is not solely responsible for the degradation and the reactions may be occurring in different regions: gas, interface, and the condensed phase (solution bulk) during cavitation bubble collapse. While ultrasonically induced degradation can occur in the vapor phase via pyrolysis, given the high molecular weight and nonvolatility of MC-LR, it is highly improbable that the observed degradation involves pyrolysis in the vapor region.

To further explore the role of •OH and its reactivity in different reaction zones produced during the ultrasonically induced degradation of MC-LR, •OH scavengers, terephthalate (TA) and *tert*-butyl alcohol (TBA), were added prior to irradiation. The solution pH in all cases was 7.0 ± 0.1. While the reactions of TBA and TA with hydroxyl radical are nearly diffusion controlled, these substrates are dramatically less reactive toward other reactive oxygen species, such as superoxide anion radicals and hydrogen peroxide, produced

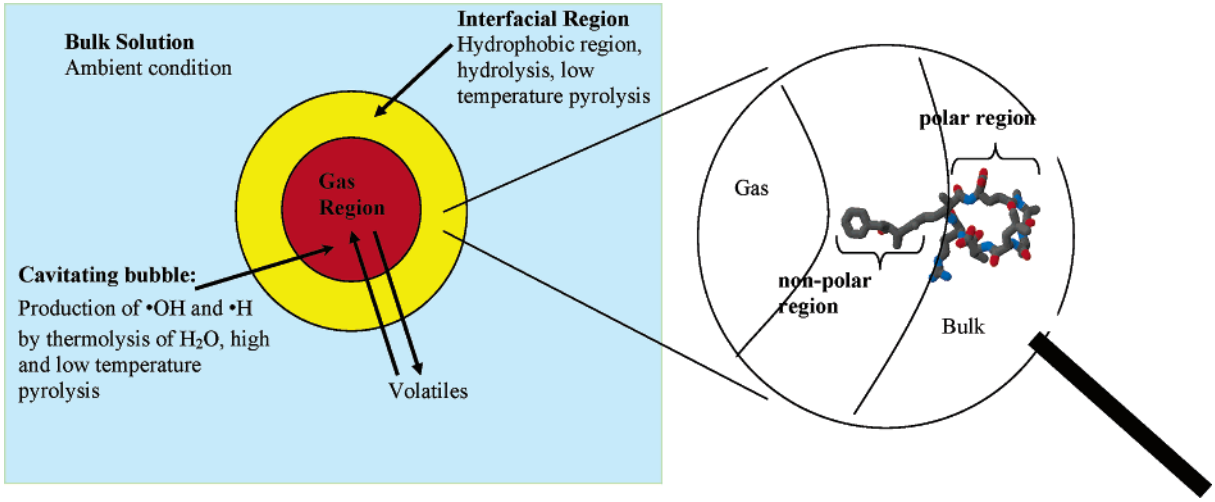

FIGURE 5. Proposed localization of MC-LR at the interfacial region during cavitation, with the hydrophobic ADDA portion localized near the (supercritical) interfacial region and the cyclic peptide localized toward the bulk solution.

during ultrasonic irradiation (*29*). Under our experimental conditions, TA is anionic and will reside in the bulk solution not at the interface or in the vapor phase. The reaction rate of •OH radical reaction with TA is nearly diffusion controlled ($k = 3.3 \times 10^9$ mol dm^{-3} s^{-1}) (*29*) and the rate constant for •OH reaction with MC-LR is expected to be on the same order of magnitude on the basis of the reactivity of hydroxyl radicals with the functional groups (*29*) present in MC-LR. Assuming homogeneous type reaction kinetics and more than 50-fold excess of TA (170 μM) relative to MC-LR (3.0 μM), we expect that >95% of the reactions of •OH in the bulk solution will involve TA. The overall degradation process of MC-LR is consistent with first-order kinetics, as illustrated in Figure 4. In the presence of TA, the initial degradation rate for a 50% reduction in the MC-LR concentration in Milli-Q water without scavenger is 0.54 μmol/L min, while in the presence of TA the observed rate is 0.33 μmol/L min, representing a decrease of 39% in the presence of a bulk solution •OH scavenger. In previous studies we determined that the rate of hydroxyl radicals produced under our experimental conditions in the bulk solution is 0.20 μmol/L min (*30*). This is consistent with the observed decrease of 0.21 μmol/L in the degradation of MC-LR with the addition of TA and leads us to the conclusion that •OH in the bulk solution is responsible for ~39% of the MC-LR degradation. Hence, the majority of MC-LR degradation must not be occurring in the bulk solution by hydroxyl radical pathways. Because of the low volatility of MC-LR, it is highly improbable that MC-LR can partition to the vapor phase and undergo subsequent reactions. With this in mind, it appears that the majority of the mechanisms responsible for the degradation occur at the cavitation bubble interface and likely include hydroxyl radical reactions, hydrolysis, and/or pyrolysis.

To probe the contribution of hydroxyl radicals at the interface, a volatile hydroxyl radical scavenger, TBA, was added to the solution. TBA is expected to partition into the vapor phase during the growth of the cavitation bubble, and upon bubble collapse, the TBA scavenges the hydroxyl radicals in the vapor and interfacial regions before they can diffuse to the bulk solution. The degradation rate of MC-LR in the presence of TBA is 0.14 μmol/L min, notably slower than the rates in distilled water (0.54 μmol/L min) and in the presence of TA (0.33 μmol/L·min). Assuming that the cavitation dynamics are not significantly changed by the TBA (*31*), our results indicate that hydroxyl radical reactions in the bulk solution occur at 39% (0.21/0.54) and at the interface at 35% (0.19/0.54), representing the major pathways for the degradation of MC-LR during ultrasonic irradiation. The remaining portion of the degradation, 26% (0.14/0.54), is likely due to the hydrolysis and pyrolysis processes at the interfacial region.

MC-LR is a large complex molecule containing a variety of polar and nonpolar functional groups. The cyclic region is comprised of polar hydrogen-bonding groups, which are expected to be solvated by water under ambient conditions. With this in mind, one might expect the MC-LR to reside primarily in the bulk solution (ambient water conditions) during cavitation and not at the gas−liquid interface that possesses hydrophobic properties, as the conditions exceed the critical point of water ($T_c = 647$ K and $P_c = 221$ bar) and are often compared to the properties of supercritical water. Since MC-LR also possesses a nonpolar aliphatic chain, it may be possible for this molecule to partition at the interface with the nonpolar ADDA side chain localized near the supercritical region (interface) and the polar cyclic peptide portion of MC-LR residing toward the bulk solution as illustrated in Figure 5. We propose the partitioning of MC-LR at the interface of these two regions would lead to the reactions of hydroxyl radicals from both the interface and bulk solution region, consistent with our hydroxyl radical scavenging experiments. In addition, hydrolysis and pyrolysis reactions are also expected to occur at the interface and can account for the observed non-hydroxyl radical reactions that are not inhibited by cellular constituents. While we discuss the interfacial region as a uniform layer, the portion closest to the vapor phase will have supercritical water properties (hydrophobic) with rapid changes toward ambient water conditions as you move away from the cavitation site, leading to localization of hydrophobic substrates at or near the interface (*32*). While ultrasonically induced mechanical shearing can lead to the degradation of polymers, such processes are unlikely for MC-LR because of its relatively small size (*33*). Hence, we conclude that the rapid degradation of MC-LR during ultrasonic irradiation occurs primarily at and near the cavitation bubble interface. Similar observations are expected under conditions where the substrates localized at or near the interface are injected into the "hot spots" upon collapse of the cavitation bubble.

While our results demonstrate that ultrasonic-induced degradation of MC-LR is rapid, it is critical to establish the biological activity of the resulting treated solution or the individual breakdown products. In general, ultrasonic irradiation leads to a complex mixture of products in low overall yields. A number of UV-detectable products have been reported during TiO_2 photocatalysis of MC-LR (*19*), but we did not observe the same byproducts using analogous MC-

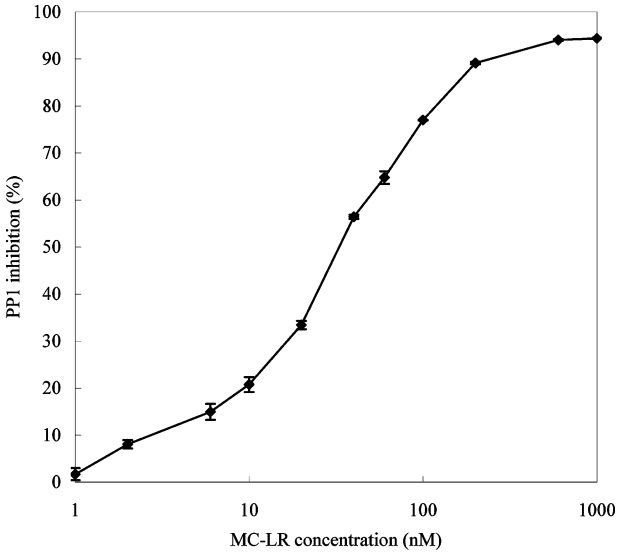

FIGURE 6. Standard inhibition curve of MC-LR against protein phosphatase 1 (PP1) $IC_{50} = 36 \pm 2$ nM. Analyses were preformed in triplicate, and the error bars indicate the standard deviation of the mean.

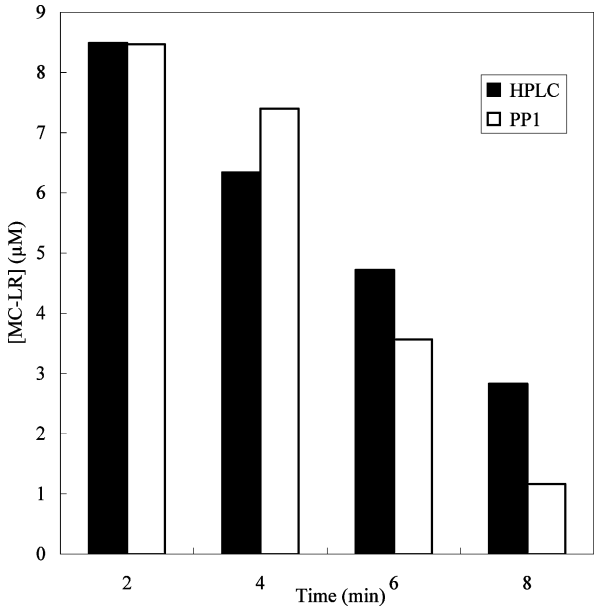

FIGURE 7. Degradation of purified MC-LR by ultrasound irradiation ($[MC\text{-}LR]_0 = 11.4\ \mu M$). HPLC and PP1 methods were used to monitor the concentration of MC-LR. Samples run in triplicate indicate the PP1 assay, and HPLC analyses are reproducible to within 10 and 2%, respectively.

LR concentrations and analytical methods to assess the products formed during ultrasonic irradiation. Given the high molecular weight and variety of functional groups present in MC-LR, it is a daunting task to identify the individual reaction byproducts and assess their individual biological activities. We chose to use a protein phosphatase assay to assess the biological activity of the ultrasound-treated solutions at various reaction times. Analysis of the PP1 inhibition activity may help to assess the tumor-promoting potential of the treated solutions.

A calibration curve for the PP1 inhibition as a function of the concentration of MC-LR was constructed using a MC-LR standard, as illustrated in Figure 6. Ninety percent inhibition of the PP1 enzyme occurs at MC-LR concentrations ≥200 nM. A lower detection limit of approximately 10 nM was established from the calibration curve. The linear region of the inhibition curve appears between 10 and 100 nM MC-LR with IC_{50} concentration of 36 ± 2 nM. The initial concentration of MC-LR in solutions subjected to ultrasonic irradiation was outside the linear region of the calibration curve and hence diluted accordingly before running the assays.

A 30 mL aqueous solution of pure MC-LR at a concentration of 11.4 μM was subjected to ultrasonic irradiation for 0-, 2-, 4-, 6-, and 8-min time intervals. The samples were diluted 100 times for assessing the activity using PP1. The results show that the biological activity of the treated samples decreases steadily with treatment, implying that toxic breakdown products are not formed to a significant extent and/or do not exhibit inhibition of PP1, as shown in Figure 7. HPLC determination of the concentration of MC-LR indicates that the observed biological activity of the treated samples parallels the concentration of MC-LR. The HPLC−diode array analyses of the treated solutions did not indicate any persistent or significant concentrations of specific breakdown products as observed during TiO_2 photocatalysis based on side-by-side experiments run in our laboratory. Ultrasound irradiation rapidly degrades MC-LR, and the byproducts do not exhibit any appreciable inhibition of PP1.

In summary, ultrasonic irradiation effectively and rapidly degrades pure MC-LR and MC-LR contained in cellular extracts. PP1 inhibition assays, a measure of the tumor-promoting potential, indicate rapid reduction in this activity of MC-LR-containing solutions upon ultrasonic-induced degradation. The efficient removal of MC-LR without the formation of toxic or present byproducts indicates that ultrasonic irradiation is an attractive treatment method for such toxins in potable water supplies. Ultrasonic irradiation appears to be an effective method for the control cyanobacterial blooms and the treatment of the associated toxins. Studies are underway to optimize the process and establish its value under a variety of water quality conditions and at environmentally relevant concentrations.

Acknowledgments

K.E.O. gratefully acknowledges support from the NIH/NIEHS (grant no. S11ES11181) and the Dreyfus Foundation. We thank the reviewers for valuable insight and suggestions. W.S. is supported by a Presidential Fellowship from the University Graduate School at FIU.

Literature Cited

(1) Honkanen, R. E.; Zwiller, J.; Moore, R. E.; Daily, S. L.; Khatra, B. S.; Dukelow, M.; Boynton, A. L. Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases. *J. Biol. Chem.* **1990**, *265* (32), 19401−4.

(2) Chorus, I.; Bartram, J. *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*; E& FN Spon: London, 1999.

(3) Ueno, Y.; Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Watanabe, M. F.; Park, H. D.; Chen, G. C.; Chen, G.; Yu, S. Z. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis* **1996**, *17* (6), 1317−21.

(4) Kuiper-Goodman, T.; Falconer, I.; Fitzgerald, J. *Toxic cyanobacteria in water*; E& FN Spon: London, 1999.

(5) Carmichael, W. W. The toxins of cyanobacteria. *Sci. Am.* **1994**, *270* (1), 78−86.

(6) WHO, *Guidelines for drinking-water quality*; World Health Organization: Geneva, 1998.

(7) Ressom, R.; Soong, F. S.; Fitzgerald, J.; Turczynowicz, L.; El Saadi, O.; Roder, D.; Maynard, T.; Falconer, I. *Health effects of toxic cyanobacteria (blue-green algae);* Australian Government Publishing Service: Canberra, 1994.

(8) MacKintosh, C.; Beattie, K. A.; Klumpp, S.; Cohen, P.; Codd, G. A. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *FEBS Lett.* **1990**, *264* (2), 187−92.

(9) Trogen, G. B.; Annila, A.; Eriksson, J.; Kontteli, M.; Meriluoto, J.; Sethson, I.; Zdunek, J.; Edlund, U. Conformational Studies of Microcystin-LR Using NMR Spectroscopy and Molecular Dynamics Calculations. *Biochemistry* **1996**, *35* (10), 3197−205.
(10) Keijola, A. M.; Himberg, K.; Esala, A. L.; Sivonen, K.; Hiisvirta, L. Removal of cyanobacterial toxins in water treatment processes: Laboratory and pilot-scale experiments. *Toxicol. Assess.* **1988**, *3* (5), 643−56.
(11) Lahti, K.; Hiisvirta, L. Removal of cyanobacterial toxins in water treatment processes: Review of studies conducted in Finland. *Water Supply* **1989**, *7* (4), 149−54.
(12) Falconer, I. R.; Runnegar, M. T. C.; Buckley, T.; Huyn, V. L.; Bradshaw, P. Using activated carbon to remove toxicity from drinking water containing cyanobacterial blooms. *J. Am. Water Works Assoc.* **1989**, *81* (2), 102−5.
(13) Tsuji, K.; Watanuki, T.; Kondo, F.; Watanabe, M. F.; Nakazawa, H.; Suzuki, M.; Uchida, H.; Harada, K.-I. Stability of Microcystins from cyanobacteria—iv. Effect of chlorination on decomposition. *Toxicon* **1997**, *35* (7), 1033−1041.
(14) Hart, J.; Fawell, J. K.; Croll, B. The fate of both intra- and extracellular toxins during drinking water treatment. *Water Supply* **1998**, *16* (1/2), 611−6.
(15) Rositano, J.; Nicholson, B. C.; Pieronne, P. Destruction of cyanobacterial toxins by ozone. *Ozone: Sci. Eng.* **1998**, *20* (3), 223−8.
(16) Shawwa, A. R.; Smith, D. W. Kinetics of microcystin-LR oxidation by ozone. *Ozone: Sci. Eng.* **2001**, *23* (2), 161−70.
(17) Gunten, U.; Bruchet, A.; Costentin, E. Bromate formation in advanced oxidation processes. *J. Am. Water Works Assoc.* **1996**, *88* (6), 53−65.
(18) Lawton, L. A.; Robertson, P. K. J.; Cornish, B. J. P. A.; Jaspars, M. Detoxification of microcystins (Cyanobacterial Hepatotoxins) Using TiO_2 Photocatalytic Oxidation. *Environ. Sci. Technol.* **1999**, *33* (5), 771−5.
(19) Liu, I.; Lawton, L. A.; Robertson, P. K. J. Mechanistic Studies of the Photocatalytic Oxidation of Microcystin-LR: An Investigation of Byproducts of the Decomposition Process. *Environ. Sci. Technol.* **2003**, *37* (14), 3214−9.
(20) Bandala, E. R.; Martinez, D.; Martinez, E.; Dionysiou, D. D. Degradation of microcystin-LR toxin by Fenton and Photo-Fenton processes. *Toxicon* **2004**, *43* (7), 829−832.
(21) Ahn, C. Y.; Park, M. H.; Joung, S. H.; Kim, H. S.; Jang, K. Y.; Oh, H. M. Growth Inhibition of Cyanobacteria by Ultrasonic Radiation: Laboratory and Enclosure Studies. *Environ. Sci. Technol.* **2003**, *37* (13), 3031−7.
(22) Nakano, K.; Lee, T. J.; Matsumura, M. In situ algal bloom control by the integration of ultrasonic radiation and jet circulation to flushing. *Environ. Sci. Technol.* **2001**, *35* (24), 4941−4946.
(23) Lawton, L. A.; Edwards, C. Purification of microcystins. *J. Chromatogr. A* **2001**, *912* (2), 191−209.
(24) Harada, K. I.; Ogawa, K.; Matasuura, K.; Murata, H.; Suzuki, M.; Watanabe, M. F.; Itezono, Y.; Nakayama, N. Structural determination of geometrical isomers of microcystins LR and RR from cyanobacteria by two-dimensional NMR spectroscopic techniques. *Chem. Res. Toxicol.* **1990**, *3* (5), 473−481.
(25) Carmichael, W. W.; An, J. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. *Nat. Toxins* **1999**, *7* (6), 377−85.
(26) An, J.; Carmichael, W. W. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon* **1994**, *32* (12), 1495−507.
(27) Ward, C. J.; Beattie, K. A.; Lee, E. Y.; Codd, G. A. Colorimetric protein phosphatase inhibition assay of laboratory strains and natural blooms of cyanobacteria: Comparisons with high performance liquid chromatographic analysis for microcystins. *FEMS Microbiol. Lett.* **1997**, *153* (2), 465−73.
(28) Feitz, A. J.; Waite, D.; Jones, G. J. Photocatalytic degradation of the Blue Green Algal Toxin Microcystin-LR in a natural Organic-Aqueous Maxtrix. *Environ. Sci. Technol.* **1999**, *33* (2), 243−249.
(29) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. P. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($\bullet OH/\bullet O^-$) in aqueous solution. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513−886.
(30) Kim, D. K.; O'Shea, K. E.; Cooper, W. J. Degradation of MTBE and Related Gasoline Oxygenates in Aqueous Media by Ultrasound Irradiation. *J. Environ. Eng.* **2002**, *128* (9), 806−12.
(31) Parusel, A. B. J.; Kohler, G.; Grimme, S. Density Functional Study of Excited Charge Transfer State Formation in 4-(N, N-Dimethylamino)benzonitrile. *J. Phys. Chem. A.* **1998**, *102* (31), 6297−6306.
(32) Henglein, A. Chemical effects of continuous and pulsed ultrasound in aqueous solutions. *Ultrason. Sonochem.* **1995**, *2* (2), S115−S121.
(33) Gronroos, A.; Pirkonen, P.; Ruppert, O. Ultrasonic depolymerization of aqueous carboxymethylcellulose. *Ultrason. Sonochem.* **2004**, *11* (1), 9−12.

Received for review October 21, 2004. Revised manuscript received May 24, 2005. Accepted June 8, 2005.

ES048350Z