

Photodegradation of Domoic Acid

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ABSTRACT

The effect of light exposure on domoic acid stability was tested by exposing three water types to different wavelengths of light in a commercially available irradiance chamber. Domoic acid (ca. 75 ng mL⁻¹) was added to deionized water, artificial seawater (amended with iron, or not) and to filtered seawater. The irradiance conditions were: full-spectrum light, and light blocked by filters that cut out wavelengths <320 nm, <375 nm, <385 nm and <400 nm. Control samples were kept in darkness. Domoic acid was not degraded when the samples were kept in darkness. Exposure to full-spectrum light for 22 h resulted in domoic acid degradation in all three water types in the absence of added iron: 36% for deionized water, 44% for artificial seawater and 41% for natural seawater, relative to the time zero value. No detectable domoic acid remained in the deionized water with iron added, after the light exposure. Photodegradation was most rapid within about the first 5 hours of exposure to the full-spectrum light. The greatest decrease in domoic acid occurred when wavelengths <370 nm were present, and in full-spectrum light. These early results show that exposure of domoic acid to light must be considered when carrying out culture experiments and when studying the fate of domoic acid in the ocean.

INTRODUCTION

Domoic acid is a water soluble neurotoxin produced and released by several species of the diatom *Pseudo-nitzschia* and some red macroalgal species (Bates *et al.*, 1998; Bates, 2000), yet there is no evidence that it accumulates in the ocean. Few bacteria are capable of biodegrading domoic acid (Windust, 1992). So far, only those bacteria isolated from within blue mussels (*Mytilus edulis*) and some soft-shell clams (*Mya arenaria*) have exhibited growth with and biodegradation of domoic acid (Stewart *et al.*, 1998). What, then, is the fate of domoic acid, once released into the water column?

In a previous study (Bates *et al.*, 2003), soluble domoic acid produced by *Pseudo-nitzschia multiseries* unexpectedly declined by ca. 60% during a 10 day period under the irradiance conditions used for the culture (i.e. fluorescent light; ca. 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ continuous irradiance). In a follow-up experiment during that same study, domoic acid added to sterile seawater declined by ca. 70% over a 12 day period under these same light conditions. There was less of a decline when bacteria were added, and even less in darkness. The above results prompted a further investigation into the possible photodegradation of domoic acid by light as a function of wavelength, type of water, and presence or absence of added iron.

MATERIALS AND METHODS

Domoic acid (ca. 75 ng mL^{-1}) was added to 10 mL of deionized water, artificial seawater, or filtered seawater (ca. 31 ppt, from the Damariscotta River, Maine) in duplicate or triplicate 15 mL acid-cleaned Teflon cups covered with saran wrap to minimize evaporation. The deionized and artificial seawater were made with added iron (225 nM ferric chloride) and in the absence of added iron, under trace metal clean conditions. The water samples were then exposed at 20°C for 22 h or 24 h to simulated solar irradiation in a Suntest ChamberTM (Model CPS+, Atlas Material Testing Technology, Chicago, IL) (Fig. 1). The irradiance conditions were: full-spectrum light, and light blocked by filters that cut out wavelengths $<320 \text{ nm}$, $<375 \text{ nm}$, $<385 \text{ nm}$ and $<400 \text{ nm}$. Control samples were kept in darkness. Duplicate aliquots of 4 mL were taken and stored frozen in 5 mL cryotubes until the domoic acid was analyzed by the FMOC-HPLC technique (Pocklington *et al.*, 1990).



Fig. 1. Suntest tabletop xenon chamber used to expose samples to UV light, showing chamber (left) and Teflon cups (right).

RESULTS

Domoic acid was not degraded when the samples were kept in darkness. However, exposure to full-spectrum light for 22 h resulted in a similar amount of photodegradation in all three water types (Fig. 2). In the absence of added iron, the decrease in domoic acid concentration (relative to the time zero value) was 36% for deionized water, 44% for artificial seawater and 41% for natural seawater. For the deionized water with iron added, the decrease was more dramatic; no detectable domoic acid remained ($< \text{ca. } 1 \text{ ng mL}^{-1}$) after the light exposure. Photodegradation also occurred when iron was added to the artificial seawater, but there was no substantial difference (48% decrease) relative to the condition with no added Fe (44% decrease).

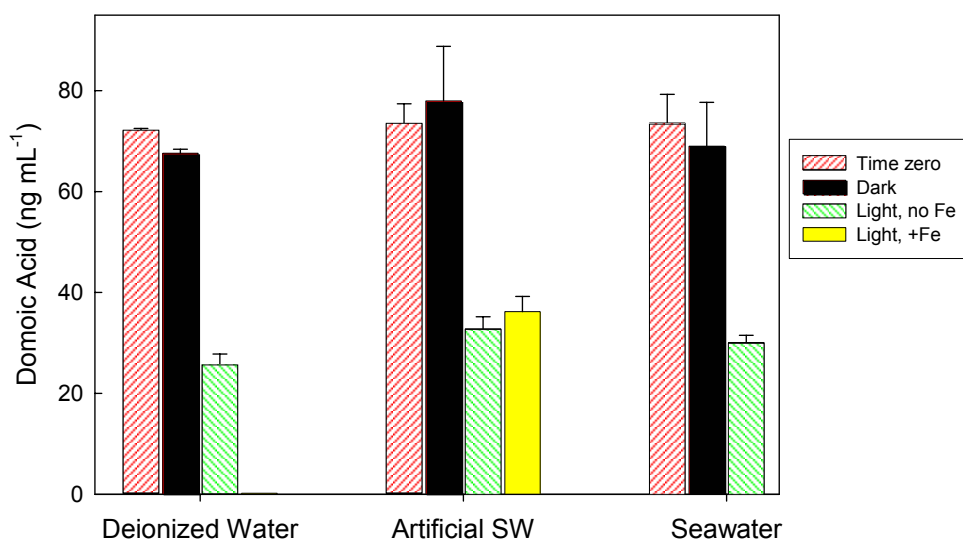


Fig. 2. Change in domoic acid concentration in three types of water and in the presence or absence of added iron, after exposure for 22 h to full-spectrum light; mean of triplicate beakers \pm SD. No iron was added to the natural seawater, which already contained natural concentrations of iron.

Domoic acid in seawater was photodegraded at all wavelengths tested relative to the time zero value and the dark control, but the greatest amount of photodegradation occurred when wavelengths <370 nm were present (Fig. 3A). This included the beakers exposed to full-spectrum light.

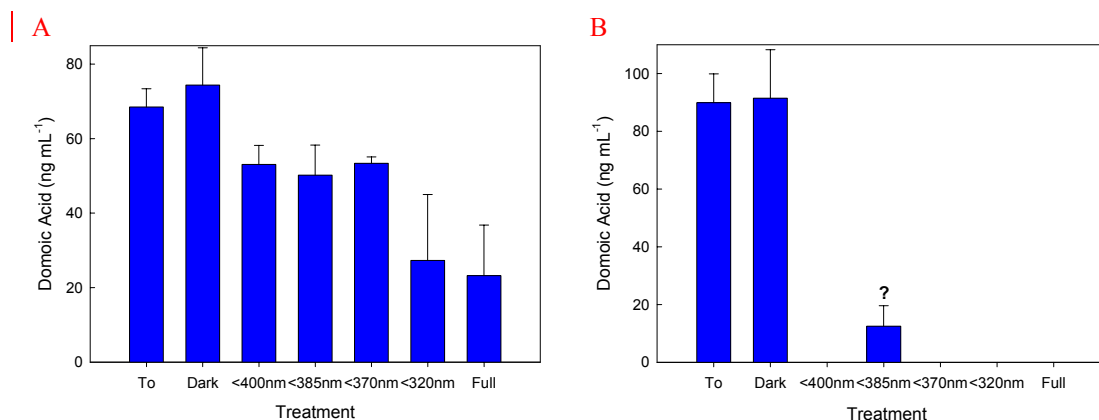


Fig. 3. Photodegradation of domoic acid in (A) seawater and (B) deionized water containing added iron. Samples were exposed for 22 h to full-spectrum light and to light that was filtered by cutting out wavelengths less than the number indicated. To = time zero. The control beakers were kept in darkness. Mean of duplicate beakers, with error bars showing the range.

With the exception of the <385 nm treatment, domoic acid in deionized water containing added iron was completely photodegraded (Fig. 3B); a similar result was shown in Fig. 2. We have no explanation for the ca. 16 ng DA mL⁻¹ that remained in the duplicate samples exposed to light filtered by the <385 nm cutoff filter.

The photodegradation was most rapid within about the first 5 hours of exposure to the light (Fig. 4). As in the above experiment (Fig. 3A), photodegradation was similar in the treatments that cut out wavelengths <400 nm, <385 nm and <370 nm. Also as in Fig. 3A, photodegradation was greatest in the <320 nm treatment and in the full-spectrum light exposure.

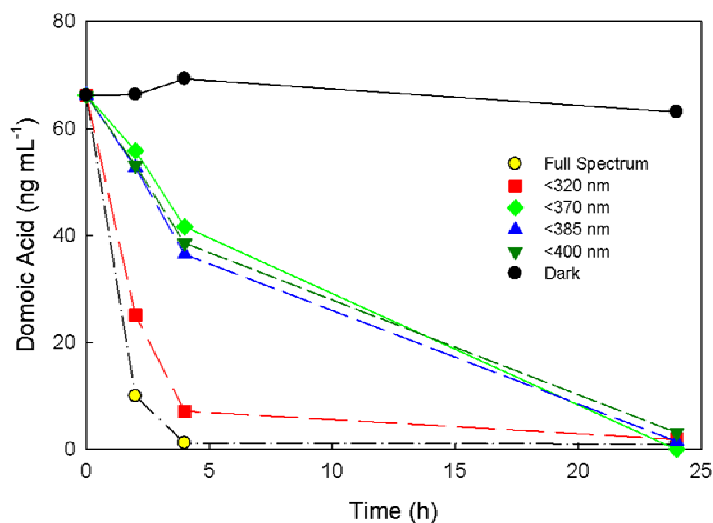


Fig. 4. Time course of domoic acid photodegradation in artificial seawater. Mean of duplicate samples normalized to the dark control.

DISCUSSION

Little is known about the fate of domoic acid in the ocean, once it is released by domoic-acid-producing *Pseudo-nitzschia* spp. and certain macroalgae. Aside from possible bacterial degradation (Windust, 1992; Stewart *et al.*, 1998), one earlier study did show that domoic acid can undergo photolysis (Wright *et al.*, 1990). After a short exposure (15 min) at 30°C to ultraviolet light (250 nm), domoic acid was converted to the geometrical isomers 2, 3 and 4; maximum yield of isomers was reached after 9-12 min (Wright *et al.*, 1990). Our study showed that the photolysis of domoic acid was most rapid within the first 5 h of exposure to the different wavelengths (Fig. 4). We have not yet investigated the photodegradation products.

With the exception of microcystins (Welker and Steinberg, 1999; Feitz and Waite, 2003), few other phycotoxins have been studied with respect to photodegradation. Our results confirm earlier initial findings showing that domoic acid may become photodegraded (Bates *et al.*, 2003).

In the present study, no degradation of domoic acid was seen in darkness, whether or not added iron was present. Therefore, iron, alone, was not responsible for the disappearance of domoic acid. Similarly, our stock solutions of domoic acid in distilled water have remained stable for up to 2 years in darkness at temperatures of 3-4°C in a refrigerator.

The absorption of sunlight (especially in the UV region of the spectrum) by dissolved organic and inorganic compounds in natural waters leads to the production of a variety of transient species, e.g. hydrogen peroxide, singlet oxygen, hydroxyl radicals and superoxide ions (Hoigné, 1990). These transient species are highly reactive and can promote the photodegradation of different organic molecules (Momzikoff *et al.*, 1983).

The affect of iron on enhancing the photodegradation of domoic acid in deionized water, but not necessarily in artificial seawater, must be studied further. For now, we know that domoic acid may chelate iron (Bates *et al.*, 2001; Rue and Bruland, 2001). One mechanism for domoic acid photodegradation may therefore be via an indirect photolysis, i.e. a photon hits the domoic-acid-iron complex, an electron moves from the domoic acid molecule to the Fe ion, causing it to become reduced and in the process causing the molecule to fragment during its oxidation. Alternatively, the degradation may occur via a direct photolysis, i.e. a photon may directly hit the domoic acid molecule, thereby resulting in its oxidation. Variable amounts of organic material and/or contaminating iron in the domoic acid stock solution, ~~and/or in the distilled water and artificial seawater solutions~~, may result in the slightly different amounts of photodegradation observed in the three experiments shown.

These early results show that exposure of domoic acid to light must be considered when carrying out culture experiments and when studying the fate of domoic acid in the ocean.

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