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Biomethylation and volatilization of arsenic by the marine microalgae *Ostreococcus tauri*

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HIGHLIGHTS

- *O. tauri* could tolerate 100 μM As(III) or As(V).
- *O. tauri* is able to biomethylate and biovolatilize As.
- As(V) reduction was speculated to be the rate-limiting factor for As methylation and volatilization.

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ABSTRACT

Ostreococcus tauri is a marine green microalga, recognized as a model organism of the marine phytoplankton assemblage and widely distributed from coastal to oligotrophic waters. This study showed it could tolerate both arsenite and arsenate concentrations of up to 100 μM, and cellular As concentration increased significantly ($P < 0.01$) with increasing concentration of As(V) in the medium (0–50 μM). It was revealed that As biotransformations were mediated by algal cells. Volatilized As was detected and the ability of As biovolatilization by *O. tauri* was demonstrated. The reduction of As(V) to As(III) might be the limiting step for As methylation and volatilization from seawater since the treatment with As(III) yielded five times more volatile As as compared to that with As(V). Arsenic biogeochemical cycle in the marine environment might play an important role based on the huge surface area of ocean (71%) and the massive number of marine phytoplankton.

1. Introduction

Arsenic (As) is highly toxic and broadly distributed in the lithosphere, hydrosphere, atmosphere and biosphere (Ng, 2005; Bhattacharya et al., 2007). The transport and transformation of As in the environment are governed by geochemical as well as biological processes, generating an As biogeochemical cycle (Wang and Mulligan, 2006).

The marine environment plays an important role in the biogeochemical cycle of metals and metalloids since it covers 71% of the earth surface. In seawater, the average concentration of As is about 0.035 μM (Mukhopadhyay et al., 2002) and occurs mainly as inorganic As, in both the trivalent (As(III)) and pentavalent (As(V)) state (Morita and Shibata, 1990). Small quantities of organic As species, such as monomethylarsonate (MMA) and dimethylarsenate (DMA), were also found in seawater, particularly under conditions of increased biological activity (Francesconi, 2005). Algae are widely distributed in oxygenated seawater, where As occurs as a toxin mainly in the pentavalent (As(V)) form (Francesconi, 2005). Although the average concentration of As in seawater is typically low (Mukhopadhyay et al., 2002), as an analog of phosphate, As(V) absorption by marine algae is enhanced along the uptake patterns of phosphate which occurs at low levels (0.065–0.113 μM) in marine environments (Sanders, 1980; Takahashi et al., 1990; Rahman et al., 2008). Marine algae have the ability of accumulate As 3–4 orders of magnitude higher than that in seawater, yielding less toxic or non-toxic organic arsenic compounds (Edmonds and Francesconi, 1981; Šlejkovec et al., 2006). Many studies on As speciation in marine organisms have established that arsenobetaine (AsB) is the major As species found in animal tissues, while in plants like algae, arsenosugars are the most frequently occurring As species (Murray et al., 2003).

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Marine microalgae have been the subject of many As metabolic studies because of their ecological and nutritional importance. Much of what we know about As detoxification in marine microalgae derived from studies focusing on As methylation and arsenosugar formation. For example, unicellular microalgae grown in radiolabeled (^{74}As) As(V) were reported to produce As(III), MMA and DMA (Andreae and Klumpp, 1979). Edmonds et al. (1997) reported that when the unicellular microalga, *Chaetoceros concavicornis* was grown axenically in As(V) enriched water, oxo-arsenosugar-sulfate (arsenosugar 4) was identified as the major As metabolite. Cullen et al. (1994) cultured a unicellular microalga, *Polyphysa peniculus*, in artificial seawater with As(V) and As(III), and identified DMA as the major metabolic product in both the cells and the medium. However, little is known about whether marine microalgae can volatilize As and their potential contribution to the biogeochemical cycle of As.

Arsenic biovolatilization has first been recognized in a number of fungi in the late 1800s. The product, a gas with garlic odor remained unidentified until 1933 when Challenger et al. (1933) established its identity as trimethylarsine (TMAs). It has since been reported that organisms can volatilize inorganic As to arsines (arsine AsH_3, mono-, di- and trimethylarsines, $MeAsH_2$, Me_2AsH, and TMAs, respectively), and contribute to the As biogeochemical cycle (Mestrot et al., 2011). Recently, some studies reported As volatilization from soil, geothermal environments and freshwater into the atmosphere by bacteria, thermoacidophilic eukaryotic microalga and fresh water microbes. Qin et al. (2006, 2009) showed that volatile trimethylarsine (TMAs) is the final product of the methylation pathway both in bacteria, as in *Rhodopseudomonas palustris*, and in the thermoacidophilic eukaryotic microalga, *Cyanidioschyzon* sp. isolate 5508. Similarly, arsenic methylation and volatilization were also reported in three freshwater cyanobacteria, significantly contributing to As biogeochemical cycling (Yin et al., 2011). Currently, studies on As biovolatilization in marine environment are very scarce. Although not well studied yet, biological production of volatile As compounds in marine environments is considered to be an important part in the global As biogeochemical cycling (Michalke and Hensel, 2004).

Ostreococcus tauri is ubiquitous and was isolated in 1994 as the smallest free-living eukaryotic organism. As a model organism of natural marine phytoplankton assemblage with cosmopolitan distribution from coastal to oligotrophic waters, its genome was sequenced in 2006 (Derelle et al., 2006). However, few studies of this marine microalga on metal metabolism have been carried out, and whether it is able to tolerate and accumulate high levels of arsenic as many marine microorganisms do remains unknown. Even less is known about the ability of these microalgae for As biotransformation and biovolatilization. In this manuscript, we report As transformation and volatilization by this marine unicellular microalgae, suggesting the potentially significant contribution made by marine phytoplankton to the global biogeochemical cycling of As.

2. Materials and methods

2.1. Growth conditions for O. tauri

O. tauri was obtained from the Roscoff Culture Collection, France and was grown in 100 mL of Keller medium in Artificial Seawater (KASW) (Keller et al., 1987) in 250 mL conical flasks. Experiments were carried out in a controlled-environment growth chamber under the following conditions: 16-h light period/day with a light intensity of approximately 280 μmol m^{-2} s^{-1}, 22/20 °C of day/night temperatures, and 60% relative humidity. The inoculum used in the experiments came from an exponential phase culture.

2.2. Growth of microalgae exposed to As

Arsenic was supplied as $NaAsO_2$ and Na_3AsO_4 at indicated concentrations (10, 30, 50, 100, 500 μM) to investigate As tolerance and accumulation. The As concentrations were selected for potentially contaminated environments, not to represent the ambient pristine coastal environment. *O. tauri* (10 mL at the exponential growth stage) was incubated in 90 mL KASW. The experiment was conducted in triplicate. Aliquots of 2 mL medium were taken at 0, 2, 4, 6, 8, 10, 12, 14, 16 d after addition of As, and growth was measured as optical density at 600 nm (OD_{600}). Growth rates determined by optical density measurements at day 16 *versus* the control were used as biological end points to estimate the effect of As species on *O. tauri*. Results were fit with a dose–response curve equation, incorporated in the OriginPro 8 program, and EC50, the concentration corresponding to 50% inhibition of the growth rate was evaluated.

2.3. Cellular As speciation after exposure to inorganic arsenic

After exposure to 30 μM As(III) or As(V) for 4, 8, 12, or 16 d, microalgae were collected by centrifugation (6000 rpm), and subsequently rinsed with deionized water and ice-cold phosphate buffer (1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$) for 10 min to remove apoplastic As. Microalgal cells were oven-dried at 70 °C and kept in 50 mL polypropylene tubes with 10 mL of 1% HNO_3 overnight. The samples were extracted with microwave assistance (CEM Microwave Technology Ltd., Matthews, NC, USA). The working program was as follows: 55 °C for 10 min, 75 °C for 10 min, and 95 °C for 30 min, with 5 min ramp time between each stage (Zhu et al., 2008; Yin et al., 2011). The supernatants were filtered through 0.45 μm filters and kept in −20 °C before analysis. The speciation of As in microalgal cells was determined by HPLC–ICP–MS (7500a; Agilent Technologies) as described in Section 2.7. In order to determine whether the As biotransformation are results of adsorption to dead cells or enzymatic activity of live microalgal cells, the experiments with dead cells (medium incubated with *O. tauri* was sterilized at 121 °C for 20 min) were carried out as the control treatment following the same procedure as described above.

For arsenosugar analysis, microalgae were exposed to 10 or 30 μM As(V) for 4 weeks, and collected in the same way as described above. Microalgal cells were freeze-dried and extracted in 2 mL deionized water following a previously developed microwave-assisted extraction method (García-Salgado et al., 2011). The extraction process was: 90 °C for 5 min, and repeated two times. The extracts were centrifuged at 4 °C (6000 rpm) for 10 min, the two supernatants were mixed and filtered through a 0.45 μm filter and kept in −20 °C before further analysis.

2.4. Arsenic transformation by cells and cell-free supernatant

O. tauri was cultivated for 2 weeks, after which the medium and microalgal cells were separated by centrifugation (6000 rpm) under sterile condition. The supernatant was filtered by a sterilized 0.22 μM nylon filter. Microalgal cells were transferred to the new sterile fresh medium. Arsenate (1.6 μM) was added to the supernatant and medium in triplicates each. Aliquots of 2 mL medium were taken at 0, 10, 20, 40, 60, 90 h and filtered through a 0.45 μM nylon filter and kept in the refrigerator at −20 °C until further analysis.

2.5. Arsenic biomethylation by O. tauri

O. tauri was exposed to 30 μM As(V) during a time-course experiment to investigate the process of As biomethylation by *O.*