Marine Chemistry 157 (2013) 78–85

Contents lists available at ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

The influence of arsenate and phosphate exposure on arsenic uptake, metabolism and species formation in the marine phytoplankton *Dunaliella tertiolecta*

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ARTICLE INFO

Article history:
Received 22 February 2013
Received in revised form 19 August 2013
Accepted 23 August 2013
Available online 3 September 2013

Keywords:
Arsenic cycling
Nutrient cycling
Phosphate
Arsenate
Dunaliella tertiolecta
Marine phytoplankton
Batch phytoplankton cultures

ABSTRACT

The influence of As(V) (50 μg L^{-1} & 2 μg L^{-1}) and PO_4^{3-} (3 mg L^{-1} & 0.12 mg L^{-1}) exposures on arsenic cycling by the marine phytoplankton *Dunaliella tertiolecta* was investigated both separately and in combination.

Total arsenic concentrations in *D. tertiolecta* tissue ranged from 8 to 10 μg g^{-1} when exposed to 50 μg L^{-1} As(V), and 2–4 μg g^{-1} when exposed to 2 μg L^{-1} As(V), with PO_4^{3-} exposure having little to no influence on total arsenic concentrations.

Arsenic was evenly distributed (≈33%) between the three major biochemical cell fractions (lipid, water, residue) in cultures exposed to high As(V):high PO_4^{3-} concentrations. Under low As(V):high PO_4^{3-} exposures arsenic was predominantly lipid-soluble (40–55%) and residue bound (30–40%). When exposed to high As(V):low PO_4^{3-} concentrations arsenic was predominantly residue bound (50–70%) and lipid-soluble (20–40%), whilst under low As(V):low PO_4^{3-} exposures arsenic was predominantly residue bound (75–80%).

As(V) was the dominant arsenic species present in the water-soluble cell fractions of all *D. tertiolecta* cultures. Under high PO_4^{3-} concentrations As(V) accounted for greater than 93% of the extractable water-soluble arsenic, with As(III) the only other species detected. Conversely, under low PO_4^{3-} concentrations As(V) accounted for between 66 and 71% of the extractable arsenic with As(III) (16–26%) and PO_4-riboside (8–13%) present.

Under high As(V):high PO_4^{3-} concentrations As(V) was the major arsenic species in hydrolysed lipid extracts accounting for approximately 56% of the extractable arsenic, with Gly-riboside (26%), PO_4-riboside (13%) and As(III) (5%) also present. Under low As(V):high PO_4^{3-} conditions As(V) only accounted for approximately 18% of the extractable arsenic with Gly-riboside (44%) and PO_4-riboside (38%) more prominent. Under low PO_4^{3-} conditions Gly-riboside was the major arsenic species (84–92%) irrespective of As(V) exposure with As(V) (8–10%) and PO_4-riboside (6%) also present.

This study demonstrates that *D. tertiolecta* alters its arsenic and nutrient cycling processes depending on the chemical composition of the culture media or surrounding environment. Future research should endeavour to culture laboratory phytoplankton at nutrient and arsenic concentrations that mimic the natural environment to ensure that observations generated in laboratory experiments can be used to describe arsenic cycling in marine systems.

1. Introduction

The uptake and accumulation of arsenic by marine primary producers have been linked to the availability of phosphate (PO_4^{3-}) (Hellweger et al., 2003). PO_4^{3-} (phosphate) and arsenate (As(V)) are structurally similar species (i.e. PO_4^{3-} & AsO_4^{3-}) (Button et al., 1973; Meharg and Macnair, 1990; Oremland and Stolz, 2003; Rothstein and Donovan, 1963; Wolfe-Simon et al., 2011). Consequently it has been hypothesised that PO_4^{3-} availability regulates As(V) uptake in marine primary producers as they cannot discriminate between As(V) and PO_4^{3-} (Hellweger et al., 2003).

The influence of PO_4^{3-} availability on As(V) uptake, metabolism and species formation by marine phytoplankton has been investigated (Foster et al., 2008b). Foster et al. (2008b) illustrated that little difference in arsenic uptake, accumulation and species formation was evident when *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* were exposed to PO_4^{3-} concentrations varying from 0.6 to 3 mg L^{-1} and As(V) concentrations of 2 μg L^{-1}. Although the variation between the PO_4^{3-} exposures used in the study by Foster et al. (2008b) was considerable, PO_4^{3-} concentrations of 0.6 mg L^{-1} typically exceed concentrations found in uncontaminated marine environments (Downing, 1997). The use of PO_4^{3-} concentrations that exceed concentrations found in uncontaminated marine environments is common in laboratory studies

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http://dx.doi.org/10.1016/j.marchem.2013.08.004

investigating arsenic cycling by marine phytoplankton (Cullen et al., 1994; Duncan et al., 2010, 2013; Foster et al., 2008b; Sanders et al., 1989; Sanders and Windom, 1980; Takamura et al., 1996; Yamaoka et al., 1999). Consequently, it is uncertain as to whether data generated from these laboratory-based experiments can be used to describe arsenic uptake, accumulation and species formation in natural systems.

The link between PO_4^{3-} exposure and arsenic cycling in marine primary producers may also result from metabolic similarities between As(V) and PO_4^{3-}. It has been proposed that arsenic is accumulated by marine primary producers to form various arsenolipid species, some of which have been shown to be phospholipid analogues (García-Salgado et al., 2012). This raises the possibility that arsenic may serve a functional role within the cells of primary producers as phospholipids are known to be a major component of cellular membranes (García-Salgado et al., 2012; Suzumura, 2005; Van Mooy et al., 2009).

As is the case with nutrient exposures, As(V) exposures used in laboratory-based marine phytoplankton arsenic cycling studies are also often in the mg L^{-1} concentration range (Cullen et al., 1994; Sanders et al., 1989; Sanders and Windom, 1980; Takamura et al., 1996; Yamaoka et al., 1990, 1999). As(V) concentrations in uncontaminated marine environments are typically between 1 and 2 μg L^{-1} (Andreae, 1978; Edmonds and Francesconi, 2003; Neff, 1997). Consequently, the use of As(V) exposures in the mg L^{-1} concentration range may result in laboratory experiments having limited environmental validity especially if arsenic is able to replace phosphorus in the synthesis of arsenolipids as has been recently suggested.

This study investigates three hypotheses; (1) whether As(V) uptake, accumulation and species formation in the marine phytoplankton *D. tertiolecta* are influenced by PO_4^{3-} exposure; (2) whether As(V) exposure influences arsenic uptake, accumulation and species formation in *D. tertiolecta*; and (3) whether As(V):PO_4^{3-} ratios or absolute concentrations of As(V) and PO_4^{3-} drive As(V) uptake, metabolism and species formation in *D. tertiolecta*.

2. Methods

2.1. Experimental design

This study consisted of a pilot study used to determine the lowest PO_4^{3-} exposure that could be utilised to culture sufficient *D. tertiolecta* tissue mass for analysis (approximately 0.1 g dry mass) and a major study, which cultured *D. tertiolecta* at varying As(V) and PO_4^{3-} concentrations.

To summarise, the pilot study cultured *D. tertiolecta* in a sterilised seawater f/2 medium (Guillard and Ryther, 1962) as per recent studies (Duncan et al., 2010, 2013; Foster et al., 2008b). PO_4^{3-} concentrations within these cultures were adjusted to either 3 mg L^{-1} (normal f/2 strength), 0.6 mg L^{-1} (f/10 strength — lowest PO_4^{3-} exposure used in Foster et al. (2008b)), 0.12 mg L^{-1} (f/50 strength) or 0.06 mg L^{-1} (f/100 strength).

The major arsenic cycling experiment cultured *D. tertiolecta* under the As(V) and PO_4^{3-} exposures listed in Table 1.

As(V) exposures were selected on the basis that 2 μg L^{-1} represented an environmentally realistic As(V) concentration (Andreae, 1978; Edmonds and Francesconi, 2003; Neff, 1997) that has been used by us previously in previous studies (Duncan et al., 2010, 2013; Foster et al., 2008b). An elevated As(V) concentration of 50 μg L^{-1} was also selected to investigate if arsenic cycling in *D. tertiolecta* changes with increased As(V) exposure. In addition, this As(V) concentration allowed for the investigation of whether As(V):PO_4^{3-} ratios or absolute As(V) and PO_4^{3-} concentrations drive arsenic cycling by *D. tertiolecta* as the same As(V):PO_4^{3-} ratio was investigated at different absolute concentrations (Table 1).

For clarity throughout the remainder of the manuscript "high PO_4^{3-} exposures" will represent 3 mg P L^{-1} exposures, whilst "low PO_4^{3-} exposures" will represent 0.12 mg P L^{-1} exposures. In a similar vein, "high As(V) exposures" will represent 50 μg As L^{-1} exposures, whilst "low As(V) exposures" will represent 2 μg As L^{-1} exposures.

Table 1
Approximate As(V) and PO_4^{3-} concentrations and ratios used in *Dunaliella tertiolecta* cultures.

Treatment	As(V) (μg L^{-1})	PO_4^{3-} (mg L^{-1})	As(V):PO_4^{3-} (μg L^{-1}:μg L^{-1})
High As(V):high PO_4^{3-}	50	3	1:60
Low As(V):high PO_4^{3-}	2	3	1:1500
High As(V):low PO_4^{3-}	50	0.12	1:2.4
Low As(V):low PO_4^{3-}	2	0.12	1:60

2.2. Phytoplankton culture maintenance

D. tertiolecta cultures were obtained from the CSIRO Centre for Analytical Chemistry (CSIRO, Lucas Heights Science and Technology Centre, NSW, Australia).

All *D. tertiolecta* stock cultures were operationally sterile (defined in this context as containing no culturable microbes on PYEA marine agar) and were prepared using autoclave sterilised 0.3 μM filtered seawater f/2 culture media (Guillard and Ryther, 1962) under aseptic conditions in a laminar flow hood (Gelaire, BSB-12, Sydney, Australia). Cultures were incubated in plant growth chambers (3504 process controller, Eurotherm, Australia) under a light intensity of approximately 110 μmol photons m^{-1} s^{-1}, over a 12 hour light exposure period. Temperature was maintained between 20 and 25 °C during light periods and was held at 20 °C during dark periods, with salinity maintained at 35‰.

Cultures were shaken daily to ensure cell motility. All stock cultures plus all prepared media were tested for the presence of culturable micro-organisms using marine PYEA marine agar plates (Foster et al., 2008b).

2.3. Batch culture preparation — pilot study

Operationally sterile *D. tertiolecta* cultures (30 mL) were transferred aseptically to sterile 5 L Erlenmeyer flasks, containing 3 L of autoclaved sterilised 0.3 μM filtered, f/2 seawater nutrient media with PO_4^{3-} concentrations amended at either 3 mg L^{-1}, 0.6 mg L^{-1}0.12 mg L^{-1} or 0.06 mg L^{-1}.

Two cultures were prepared for each of the PO_4^{3-} exposures used (see *Experimental design*) with one culture from each treatment incubated in separate plant growth cabinets (n = 2) (3504 process controller, Eurotherm, Australia) under the same conditions described previously (see *Phytoplankton culture maintenance*). Cultures were randomly moved throughout each chamber on a daily basis to negate any potential light and temperature gradients.

Assessment of viable cell numbers was performed daily via haemocytometer slide as described in Foster et al. (2008b). As well as measuring total cell numbers, *D. tertiolecta* cells were also analysed for viability, with motile and actively dividing cells present throughout incubation period. In addition, all cultures were tested for the presence of culturable microbes though the use of marine PYEA marine agar plates (Foster et al., 2008b) at the commencement and conclusion (day 7) of the incubation process.

2.4. Batch culture preparation — major study

All *D. tertiolecta* cultures were prepared and incubated as per those in the pilot study with the exception that PO_4^{3-} exposures were amended to either 3 mg L^{-1} or 0.12 mg L^{-1}.

After four days incubation, all cultures were exposed to As(V) under aseptic conditions with the As(V) concentration in the culture equating to either 50 μg L^{-1} or 2 μg L^{-1} (Table 1). All cultures were subsequently incubated for a further three days (7 days total). After seven days incubation cells were harvested via centrifugation at 4500 ×*g* for 10 min