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The role of phosphorus in the metabolism of arsenate by a freshwater green alga, *Chlorella vulgaris*

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ABSTRACT

A freshwater microalga, *Chlorella vulgaris*, was grown in the presence of varying phosphate concentrations (<10–500 μg/L P) and environmentally realistic concentrations of arsenate (As(V)) (5–50 μg/L As). Arsenic speciation in the culture medium and total cellular arsenic were measured using AEC-ICP-MS and ICP-DRC-MS, respectively, to determine arsenic biotransformation and uptake in the various phosphorus scenarios. At high phosphate concentration in the culture medium, >100 μg/L P, the uptake and biotransformation of As(V) was minimal and dimethylarsonate (DMAs(V)) was the dominant metabolite excreted by *C. vulgaris*, albeit at relatively low concentrations. At common environmental P concentrations, 0–50 μg/L P, the uptake and biotransformation of As(V) increased. At these higher As-uptake levels, arsenite (As(III)) was the predominant metabolite excreted from the cell. The concentrations of As(III) in these low P conditions were much higher than the concentrations of methylated arsenicals observed at the various P concentrations studied. The switchover threshold between the (small) methylation and (large) reduction of As(V) occurred around a cellular As concentration of 1 fg/cell. The observed nearly quantitative conversion of As(V) to As(III) under low phosphate conditions indicates the importance of As(V) bio-reduction at common freshwater P concentrations. These findings on the influence of phosphorus on arsenic uptake, accumulation and excretion are discussed in relation to previously published research. The impact that the two scenarios of As(V) metabolism, As(III) excretion at high As(V)-uptake and methylarsenical excretion at low As(V)-uptake, have on freshwater arsenic speciation is discussed.

Introduction

Elevated arsenic (As) concentrations, whether from natural or anthropogenic origins, are of major concern due to a variety of health effects connected with human exposure to arsenic (Abernathy et al., 1999). The toxic properties of arsenic are dependent on its chemical speciation (Aposhian et al., 2003), and therefore environmental transformations, which affect the speciation of this trace element, are important to study in order to assess this contaminants' risk to humans and the environment.

In fresh surface waters, arsenic is found predominantly as oxidized inorganic arsenate, As(V). The reduced inorganic arsenite, As(III), is routinely found in fresh water environments at higher concentrations than thermodynamic calculation would predict (Cullen and Reimer, 1989). Organic arsenic

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compounds are also found in freshwater environments, generally as minor constituents and are only present in significant concentrations in limited scenarios (Sohrin et al., 1997).

The increased presence of the reduced inorganic and methylated organic forms of As in freshwater environments suggests that factors other than the redox potential determine As speciation. Correlations between biological productivity and As transformations have been made (Chen et al., 2008; Hellweger and Lall, 2004; Howard et al., 1995; Maki et al., 2007; Sohrin et al., 1997; Wang et al., 2014; Wurl et al., 2013), identifying the conversion as a biologically driven process. Algal cells take in As(V) through phosphate transporters due to chemical mimicry (Rosen and Liu, 2009). Arsenic cellular metabolism proceeds with As(V) being reduced to As(III) followed by a, kinetically slower, stepwise methylation through monomethylarsenate, MMAs(V), to DMAs(V) (Challenger et al., 1951). Microorganisms have demonstrated the excretion of both the reduced As(III) form (Knauer and Hemond, 2000) and/or the methylated forms (Hasegawa et al., 2001).

Biotransformation of arsenic has been heavily studied in the marine environment, but comparatively less is known regarding what regulates transformations of arsenic in freshwater environments (Rahman et al., 2012), although some explanations have been suggested. Due to the competitive interaction between arsenate and phosphate, the influence of phosphorus on arsenic speciation under the presence of freshwater algae has been the topic of speculation by many researchers (Baker et al., 1983; Hellweger et al., 2003; Rahman et al., 2012). While researchers have begun to associate arsenic speciation with phosphorus condition, strong empirical data is lacking and divergent results have generated confusion. Studies which have explored arsenic-phosphorus interactions with freshwater algae in laboratory exposures, as well as freshwater observations of arsenic speciation, are summarized in Table 1.

Researchers have demonstrated a higher level of As(V) bio-reduction to As(III) at low phosphorus concentrations, including in work with freshwater green algae (Knauer and Hemond, 2000; Wang et al., 2013) and cyanobacteria (Guo et al., 2011; Markley and Herbert, 2010). Contrastingly, Hellweger et al. (2003) developed a model from arsenic speciation observed in laboratory cultures (Hasegawa et al., 2001) which implicated high phosphorus conditions, and the luxury uptake of phosphorus by algae, as the cause of increased bio-reduction rates of As(V). Meanwhile, methylarsenicals have been connected to highly productive waters (Hasegawa et al., 2009), yet, methylarsenicals can predominate at low phosphorus conditions (Baker et al., 1983; Hasegawa et al., 2001). These incongruities regarding the effect of low or high phosphorus conditions on the bio-production of arsenicals could lead to confusion in the assessment of arsenic speciation in freshwaters.

Phosphorus concentrations in freshwater lakes range from <10 μg/L in oligotrophic waters to >100 μg/L in eutrophic waters (Rast and Holland, 1988); previous research examining arsenic interactions with algae has often used extremely elevated P concentrations, tested only one or two concentrations of P (*e.g.*, a low and a high) and/or has indicated an initial P concentration without tracking the change in, or maintaining, the levels of this nutrient in the exposure. Overall, most research has lacked a quantitative approach to the phosphorus dynamics of the algal system. In addition to elevated P concentration, studies have employed arsenic at concentrations which are orders of magnitude above freshwater levels.

Table 1 – Overview of previous studies in which freshwater algae were exposed to arsenate at varying phosphorus conditions.

Algal species	Stage of algal growth	Phosphorus (μg/L)	As(V) (μg/L)	As speciation	Role of P	Reference
Chlorella sp.[a]	Logarithmic	6 or 6 × 10^3	0.075 to 75	Quick reduction to As(III). Methyl-arsenicals not analyzed.	Increase in bio-reduction under low P concentration	Knauer and Hemond, 2000
C. aciculare[a]	Logarithmic and Stationary	341 or 589	0.75 or 900	DMAs(V) dominant in stat growth while As(III) dominant in log growth.	DMAs(V) production increased with decrease in P:As ratio.	Hasegawa et al., 2001
C. reinhardtii[b] and *S. obliquus*[b]	Logarithmic	−P (limited) +P (enriched)	7.5 to 750	Quick reduction to As(III). Methyl-arsenicals not detected.	Intracellular As:P best determinant of toxicity. Increase in bio-reduction under P-limited condition.	Wang et al., 2013
Chlorella sp.[a] and *M. arcuatum*[a]	Logarithmic	150 or 1.5 × 10^3	100 to 40 × 10^3	As(III) excreted at high (*M. arcuatum*) and low (*Chlorella*) levels. No methylarsenicals excreted after 72 hr.	Increase in P concentration decreased toxicity of As(V).	Levy et al., 2005
Variety	Logarithmic and Stationary	<1–100 (Rast and Holland, 1988)	<1–10 (Terlecka, 2005)	DMAs(V) during stationary growth and As(III) spike associated with logarithmic growth (*i.e.*, algal blooms)	Limiting nutrient which, generally, stimulates growth of freshwater algae. Competes with As for uptake.	Freshwater observations (Hasegawa et al., 2010, Hasegawa et al., 2009; Hellweger and Lall, 2004; Hellweger, 2005; Howard et al., 1995)

[a] Isolated algae;
[b] Laboratory strain.