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European Journal of Protistology 48 (2012) 227–236

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Arsenate toxicity and stress responses in the freshwater ciliate *Tetrahymena pyriformis*

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Received 16 August 2011; received in revised form 12 January 2012; accepted 13 January 2012
Available online 16 February 2012

Abstract

The arsenic metabolism in different biological organisms has been studied extensively. However, little is known about protozoa. Herein, we investigated the cell stress responses of the freshwater ciliate *Tetrahymena pyriformis* to arsenate toxicity. An acute toxicity assay revealed an 18-h EC_{50} arsenate concentration of ca. 40 μM, which caused significant changes in the cell shape, growth and organism mobility. Whereas, under exposure to 30 μM arsenate, *T. pyriformis* could grow reasonably well, indicating a certain resistance of this organism. Arsenic speciation analysis revealed that 94–98% of the total arsenate in cells of *T. pyriformis* could be transformed to monomethylarsonic acid, dimethylarsinic acid and a small proportion of arsenite after 18 h of arsenate exposure, thus indicating the major detoxification pathway by arsenic oxidation/reduction and biomethylation. Finally, comparative proteomic analysis unveiled significant changes in the expression of multiple proteins involved in anti-oxidation, sugar and energy metabolism, proteolysis, and signal transduction. Our results revealed multiple pathways of arsenate detoxification in *T. pyriformis*, and indicated that protozoa may play important roles in the biogeochemical cycles of arsenic.

Keywords: Arsenate toxicity; *Tetrahymena pyriformis*; Biomethylation; Proteomics

Introduction

Arsenic (As) is a ubiquitous and potentially toxic element in the environment (Matschullat 2000). Weathering of rocks and anthropogenic point sources contribute mainly to As found in the environment, such as smelter slag, coal combustion, and runoff from mine tailings, etc. Chronic exposure to arsenic through contaminated drinking water or foods occurs worldwide, and is associated with a variety of diseases, including cancer, diabetes and developmental disorders (Kapaj et al. 2006; Tchounwou et al. 2004; Tseng et al. 2002). In natural waters, arsenic exists predominantly as the pentavalent form, As (V). Its mobility and bioavailability is influenced not only by abiotic factors, but also by the activities of aquatic plants, animals and microbes (Alvarado et al. 2008; Čerňanský et al. 2007; Liao et al. 2008).

Arsenic metabolisms in microbes, such as archaea, bacteria, and fungi, have been extensively studied in the past (Tsai et al. 2009). Microbes respond to arsenic in a variety of different ways, such as compartmentalization, exclusion, and immobilization. And many of them can methylate arsenic giving rise to monomethyl, dimethyl, and/or tri-methyl derivates, which are volatile and are rapidly released to the atmosphere

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doi:10.1016/j.ejop.2012.01.005

(Qin et al. 2006; Tsai et al. 2009). However, so far only little is known about arsenic metabolism in free living protozoa, an important and abundant component in aquatic ecosystems. Early in 1988, Amaral et al. (1988) investigated the protein responses of a protozoan *Tetrahymena pyriformis* to arsenite exposure and revealed an inducement of two main group of stress proteins with molecular masses in the ranges 70–75 kDa and 25–29 kDa, whereas due to the technical limitations at that time, not much information was obtained as to what these proteins are and what functions will they perform in the responses to arsenite stress. To the best of our knowledge, this represents the first and only report on arsenic toxicity in a free-living protozoan.

The free-living protozoa are ubiquitous and abundant component of microbial communities, playing a pivotal role in many major element cycles (i.e. carbon, nitrogen, phosphorus, and sulfur). They are represented in all aquatic ecosystems including lakes, reservoirs, streams, wetlands, seas and soils (Corliss 2002; Finlay and Esteban 1998; Hahn and Höfle 2001). Moreover, as unicellular organisms with a short regeneration time, protozoa respond rapidly with great sensitivity to the presence of pollutants in nature. This has resulted in them being used as test systems for assessing ecological risk (Sauvant et al. 1999). In fact, most organisms, from bacteria to mammals, have developed various strategies to counter-act arsenic toxicity or to utilize arsenic as an electron donor/receptor in energy production (Oremland and Stolz 2003; Tsai et al. 2009). Considering the high abundance and important ecological status of protozoa in natural aquatic ecosystems, we hypothesize that protozoa may have also developed certain approaches to tolerate arsenic toxicity. Most recently, Yin et al. (2011) reported the rapid biotransformation of arsenic by the common ciliated protozoa *Tetrahymena pyriformis*. Studies on arsenate toxicity and stress responses in *T. pyriformis* are urgently needed. On this basis, *T. pyriformis* was employed as one case to study the arsenate toxicity as well as the cell stress responses. The results will contribute to our knowledge on the poorly understood arsenic activities and metabolism in protozoa.

Material and Methods

Cell culture

Tetrahymena pyriformis Ehrenberg, 1830 (strain GL-C) was obtained from the National Tetrahymena Stock Center of Cornell University, USA. It was maintained in a 100 ml of modified Neff medium containing 0.25% proteose peptone, 0.25% Difco yeast extract, 0.5% glucose and 3.33 μM $FeCl_3$ at 30 °C without shaking.

Arsenate toxicity

To evaluate the arsenate toxicity, we analyzed growth curves for *T. pyriformis* under a set of different arsenate concentrations (0, 10, 50, 100, 150, 200 μM $Na_3AsO_4{\cdot}12H_2O$) using the same incubation conditions as described above. Arsenate was added into each culture at 21 h post-inoculation when *T. pyriformis* grew into the early exponential phase. Three replicates of each concentration were run. From the beginning of inoculation, subsamples were taken at 3 h intervals and fixed with glutaraldehyde (2.5%). Then the cell abundance of *T. pyriformis* was enumerated by the hemocytometer method using an inverted microscope. Based on growth curves, 18-h arsenate EC_{50} concentrations were determined, causing a 50% decrease in growth rate of *T. pyriformis*.

Cell morphology of *T. pyriformis* grown at different arsenate concentrations (0 and 40 μM) was evaluated with light and scanning electron microscopy methods. For scanning electron microscopy observations, 10 ml of subsamples from each culture were collected at 6, 12, 18 and 24 h after arsenate exposure, and fixed with glutaraldehyde as described above for 30 min at 4 °C. Via centrifugation (1800 × *g*, 4 °C, 10 min), the cell pellets were collected and washed by phosphate buffer (0.1 M, pH 7.2) three times. Finally, micrographs were taken with a field emission scanning electron microscope (Hitachi S-4800, Japan).

Arsenate resistance

To investigate which concentrations of arsenate *T. pyriformis* can bear, the cells were incubated in a set of media containing increasing arsenate concentrations from 10 to 40 μM at intervals of 5 μM. Finally, growth curves were drawn to observe whether *T. pyriformis* can grow well at different arsenate concentrations.

Determination of arsenic species in *T. pyriformis* and its culture medium

Arsenic species in the cells of *T. pyriformis* were determined using a modified method as previously described by Zhang et al. (2009a,b). Briefly, three groups (i.e., the Control, Env and Exp) of *T. pyriformis* culture (100 ml) at early exponential growth phase were exposed respectively to 0, 0.67 (50 ppb) and 40 μM of arsenate as described above. Three replicates were run for each concentration. At 18 h post exposure, all the remaining cells in flasks were finally collected by centrifugation and freeze dried in a Freeze Dryer (Labconco Corporation, USA). Freeze-dried cells were extracted with 5 ml of 1% nitric acid in a microwave-accelerated reaction system according to Zhu et al. (2008). The temperature was gently raised, first to 55 °C and then to 75 °C, with holding times of 10 min. Finally, the digest was heated at 95 °C for 30 min before cooling. The extract solutions were centrifuged and passed through nylon filters with a pore size of 0.45 μm. Filtrates were kept on ice and in the dark, and analyzed within a few hours after extraction.