Selenium accumulation in unicellular photosynthetic algae *Chlamydomonas reinhardtii* grown in Se(IV)-contaminated media

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Abstract

This work is a continuation of our studies on the accumulation and the effects of several elements on the unicellular photosynthetic alga *Chlamydomonas reinhardtii*. Cells were grown in media polluted with Selenite (Se(IV)), at several non-lethal concentration levels. Growth curves were constructed and a Se-dependent anomaly on the steady state part of the curves was observed. The Se content of individual *C. reinhardtii* cells was quantitated at certain time-points by conventional ICP-MS and single cell (SC) ICP-MS and the lipidome of the cells was studied by using easy ambient sonic-spray ionization mass spectrometry (EASI-MS). Comparison of the results with observations from a similar study with As(V) shows completely different impact of the two metalloids on the investigated organism.

Keywords: Selenium, Selenite, *Chlamydomonas reinhadtii*, Single Cell ICP-MS, EASI-MS

1. Introduction

To gain a better insight into the metallome of a biological organism it is important to quantitatively determine metals and/or metalloids in individual cells, the basic biological units of all living organisms. This is needed in order to overcome population/size heterogeneities, behavioural differentiations, etc. and to make analytical measurements biologically relevant. Single-Cell (SC) analysis by means of single-particle inductively coupled plasma mass spectrometry (SP ICP-MS) is in the position to reveal the metal distribution in a cell population. On the other hand, knowledge on how metal uptake affects cellular metabolism is also very important, and molecular mass spectrometric techniques are used in order to address these issues. In this work, Se(IV) accumulation and its impact on the lipid profile of cell membranes were studied by SC ICP-MS and EASI-MS, respectively.

2. Materials and Methods

Cell Cultivation: *Chlamydomonas reinhardtii* cells, (CC-1690) were cultivated mixotrophically, under continuous illumination at 25° C in TAP media with CH_3COOH as organic carbon source. Amounts of stock solutions of sodium selenite were added to the media, for the decided concentrations to be reached. The growth of the culture was monitored spectophotometrically every twelve hours at 750 nm. Cells were harvested 96 h, 132 h and 192 h after inoculation, washed by a washing buffer and kept in a high density sucrose solution at -80 ° C until use.

Single Cell ICP-MS Analysis: Cell counting was carried out with Flow Cytometer. Cell suspensions (10^5 cells/mL) in d.H_2O, were analyzed for their Se content, either after treatment with EDTA to remove cell wall-adsorbed Se or untreated. The instrument was operated in Time-Resolved Analysis mode with a dwell time of 10 ms. In order to achieve high sensitivity and low background intensities, Se was monitored at the m/z of 80 by introducing O_2 in the Dynamic Reaction Cell.

Bulk analysis of cells was carried out using conventional ICP-MS, following digestion of cells with nitric acid for 3-4 hrs with repeated cycles of sonication until the cell pellet was completely dissolved.

EASI-MS Analysis: Cell samples were washed three times with d.H_2O by centrifugation. The resulting cell pellets were resuspended in 10-20 μL of d.H_2O, and 1 μL of the cell suspension was placed on glass slide and left to dry under ambient conditions for approximately 25 min prior to analysis. The spraying solvent was a mixture of ACN/DMF 1:1 v/v delivered at a flow rate of 3-5 μL min-1. The nebulizing gas was nitrogen (N_2) at a backpressure of 8 bar. The instrument was operated in both positive and negative ion mode. Mass spectra were recorded in the m/z 500 – 1000 range in the full scan mode.

3. Results and Discussion

Growth curves of *C. reinhardtii* exposed to 20, 50, 100, and 150 μM Se(IV) show a relatively small effect on the growth rate in the log phase, and a Se-dependent anomaly on the steady state (Fig. 1). The Se(IV) content of individual *C. reinhardtii* cells, harvested after 96 h, 132 h, and 192 h of Se exposure was measured by SC ICP-MS and conventional ICP-MS with and without EDTA treatment (Fig. 2). It is shown that a significant amount of Se(IV) is attached on the cell walls and is removed by EDTA (blue bars, Fig. 2). Also shown is that the longest

exposure to Se causes a significant increase of accumulation in the cells (green bars). Interestingly, Se content of cells harvested after 132 hours of exposure (where the Se-dependent anomaly is close to maximum), is, in general, lower than the corresponding content observed for the other two exposure times. This observation is under investigation.

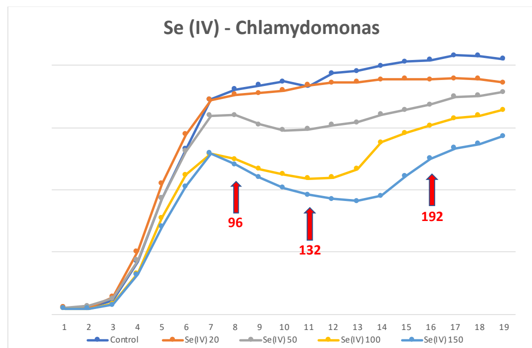

Figure 1. Growth curves of *C. reinhardtii* cells exposed to four levels of Se(IV). The arrows show the time-points of cell harvesting.

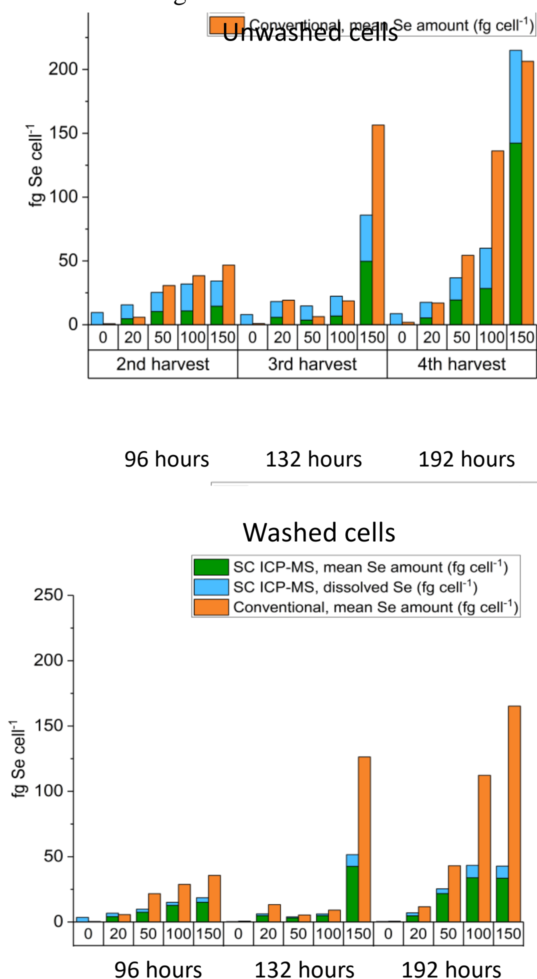

Figure 2. Mean Se amount (fg/cell) obtained by using SC-ICP-MS (green) compared to conventional ICP-MS (orange) for unwashed (upper plot) and washed cells (lower plot). Blue bars represent the dissolved Se determined by using SC-ICP-MS and normalized per cell.

Lipidomic analysis was performed on *C. reinhardii* cells by EASI-MS, as described in Materials and Methods and in Mavrakis *et. al.* (2019). Potential effects of Se(IV) on the saturation of the fatty acid chains of certain classes of lipids (diacylglyceryl-trimethyl-homoserine, DGTS, phosphatidylglycerol, PG, and sulfoquinovosyldiacyl-glycerol, SQDG), were investigated. The ratios of the more saturated to less saturated molecules are presented as box-whisker plots (Fig. 3). The results show no impact on the saturation levels of these membrane lipids, in contrast to our As(V) study (Mavrakis *et. al.* 2019).

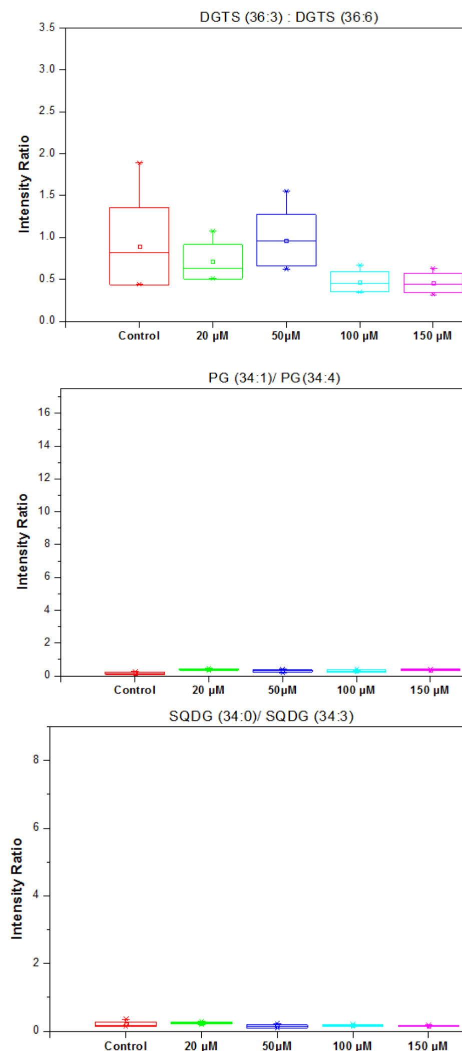

Figure 3. Box-whisker plots of lipid species intensity ratio for control and Se(IV)-incubated cells.

4. Conclusions

The Se(IV) levels tested in this study did not affect significantly the logarithmic growth of *C. reinhardtii* cells, however a Se-dependent anomaly at the steady state of the cultures was observed. The Se accumulation pattern, and the effect of Se(IV) on the fatty acid saturation status of certain membrane lipids were different from corresponding for As(V) exposures of *C. reinhardtii* cells (Mavrakis *et. al.* 2019).

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References

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