HEAVY METAL EFFECTS ON THE GREEN ALGA CHLAMYDOMONAS REINHARDTII : A GENE TRANSCRIPTION STUDY

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Introduction

Unicellular photosynthetic organisms are very important members of the marine and freshwater ecosystems. They are at the base of the food chain, the most important of the Earth's biomass producers, and it is estimated that 50%, or even more, of the oxygen in the atmosphere is produced by their photosynthetic activity. Detection of pollutants (quantitative analysis) or their effects (biochemical and molecular biological analysis) in photosynthetic algae are early and timely indicators of potential hazards in aquatic systems. Biochemical and molecular biological approaches for the detection of environmental stress in microalgae should be seriously considered in any environmental assessment program (Torres et al., 2008). Exposure of these organisms in heavy metal-polluted environments may cause both inhibition of photosynthesis and transport of these pollutants to higher organisms through the food chain(Perales-Vela et al., 2006). Hence, homeostasis of these organisms is very important. Unfortunately, homeostasis mechanisms, at the molecular level, regarding heavy metals, are largely unclear. Similar to higher organisms, phytoplanktonic cells have developed a defense system for their protection against exposure to heavy metals, which contains the synthesis of heavy metal-induced specific proteins (phytochelatins, PCs, metallothioneins, MTs, heat shock proteins, HSPs), or other metabolites with specific functions. This response of the cells exposed to heavy metals can be studied at the RNA transcription level, and important knowledge can be acquired towards the elucidation of the mechanisms involved in the defensive functions of the organisms, as well as for the utilization of these analyses as reliable probes for early detection of pollution events in the environment.

In this study, a unicellular freshwater and soil model organism, *Chlamydomonas reinhardtii*, was exposed to several sub-lethal levels of Cd, Ni, and Pb. The effects of the metal exposure on the cell growth rates, the transcript response, and the levels of certain peptides and metabolites were determined.

Methods

Cells were exposed to two levels of Cd, Ni, and Pb, under continuous illumination, and their growth curves for each condition were constructed. Samples were collected at the middle and at the end of the logarithmic phase of the growth, and the accumulation of the metals (adsorption on the cell walls and insertion in the cells) were determined by ICP spectroscopy. The levels of the mRNA for specific heavy metal stress-related enzymes was determined by reverse transcription and qPCR analyses for all the conditions studied. The –SH containing peptides were determined and quantified by SDS-PAGE electrophoresis and the Ellman method. Finally, the synthesis of certain metabolites (i.e. chlorophyll, carotenoids) was determined spectrophotometrically.

Results

Previous studies (Jamers et al., 2013) have shown that there is a time of exposure and level of pollution dependence of both gene transcription and certain metabolite profiles. In this work we expand our study to three heavy metals and to different genes and metabolites. We will present data showing that the bioaccumulation level and the percentage of metal insertion in the cellsare metal-dependent. The effects of the different metals on the gene expression levels of certain peptides, as well as on the synthesis of certain metabolites will be presented. Data on the heavy metal-induced synthesis of low molecular weight, sulfohydryl containing peptides, will also be shown. These results will be evaluated as potential probes for the assessment of pollution by heavy metals of their environment.

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