Regulatory Aspects of Sulfur and Selenium Assimilation in Chlorella sorokiniana


INTRODUCTION

Selenium is a trace element that acts either as an essential microelement or as a toxic element depending on its concentration in the medium. It is of fundamental importance to human health; selenium bioremediation is mainly involved in immune function, reproduction, metal toxicology and other biological functions in humans. Microalgae can accumulate selenium. In nature, selenium is present in its three oxidation states (selenite, selenate (VI)) and elemental selenium (0) over a range of natural water chemical conditions. Selenium is the dominant dissolved form, representing more than 67% of the total dissolved selenium concentration. Both selenium and selenite are taken up by microalgae and converted to protein-bound selenocysteine and selenomethionine, soluble inorganic forms, several free selenocysteine, and volatile organoselenium compounds.

The physical and chemical reactivity between selenite and selenate (S) helps to explain the intricate association between S and Se metabolism. The biological activities of Se are also similar to that of S. Selenium and selenite can be imported into cells via SO32- transporters and incorporated into organic molecules by enzymes of the SO4 2- assimilation pathway. It has been described in higher plants that the biosynthesis of most Se compounds may depend on the enzymes involved in the assimilation pathway but no many reports in microalgae have been described.

The present work, Chlorella sorokiniana was chosen as a representative green microalga to study some regulatory aspects of S and Se metabolism using OASTL activity under different sulfur and selenium nutritional conditions. Results show that OASTL activity was increased in S and Se-starved cells when compared to a sulfur-replete cell culture. These data are discussed in the light of the key role of OAS in cysteine synthesis.

MATERIALS AND METHODS

Experimental Conditions (enzymology):
- 16-well microplates, 96-well microtiter plate (fulldish illumination)
- Temperature: 30°C
- Modified kit medium (pH 2.5)
- Air with 5% of CO2 (argon-carbon source)

Selenium (indicated and sulfate) treatment:
- 100 ng/ml (final concentration)

Determination:
- Posthypothetic activity (to check viability)
- Biomass concentration: cell number and dry weight: Chlorophyll and total carotenoids
- Total selenium and selenium species: selenocystine (SeCys2), selenomethionine (SelMet)

Crude extract:
- Cells were collected (25 µg Chlorophyll), washed three times with standard medium, broken by freezing in liquid nitrogen for 2 min and thawed in enzyme specific buffer (100 mM potassium phosphate, pH 7.0)

OASTL determination:
- OASTL (O-acetyl-L-serine (thiolase, EC 2.4.2.8) activity was assayed as apoprotein to a 1:5 ratio, reaction mixture containing: 30 µl potassium phosphate buffer (pH 7.0), 20 µl O-acetyl-L-serine, 19 µl NAD and crude extract. The samples were incubated at 50°C for 10 min and the reaction was stopped by adding 25 µl of 20% trichloroacetic acid. Cysteine formed was determined as described by Galesse (1971)

Instrumentation:
- HPLC-MS method for the detection of selenium was used.
- HPLC columns were connected directly to the reductor of the indirectly coupled plasma mass spectrometer using three valves to build a column switching system and species were on-line detected by ICP-MS.