Regulatory Aspects of Sulfur and Selenium Assimilation in *Chlorella sorokiniana*

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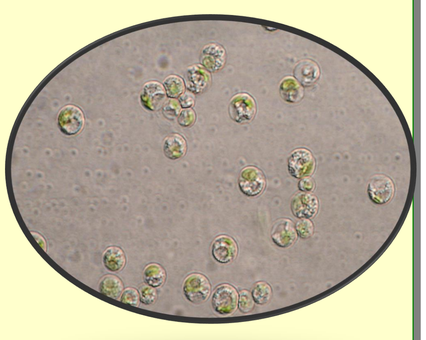
INTRODUCTION

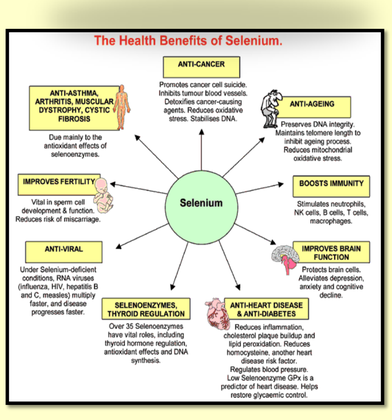
Selenium is a trace element that acts either as an essential micro-nutrient or as a toxic element depending on its concentration in the medium. It is also of fundamental importance to human health; selenium bioeffects are mainly involved in immune function, reproduction, metal toxicity resistance and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

The physical and chemical resemblance between selenium (Se) and sulphur (S) help to explain the intimate association between Se and S metabolism. The biological activities of Se are also similar to that of S.

Selenate and selenite can be imported into cells via SO_4^{2-} transporters and incorporated into organic molecules by enzymes of the SO_4^{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

Cysteine is the end product of the assimilatory sulfate reduction pathway in bacteria, fungi algae and plants. L-cysteine biosynthesis proceeds via two enzymic reactions. Serine acetyl transferase (SAT) first catalyzes the acetylation of L-serine by acetyl-CoA. The product, O-acetylserine, then reacts with sulphide to yield L-cysteine in a reaction catalyzed by O-acetylserine(thiol)lyase (OASTL). In the present work, *Chlorella sorokiniana* was chosen as a representative green microalga to study some regulatory aspects of S and Se metabolism testing OASTL activity under different sulphur and selenium nutritional conditions. Results show that OASTL activity was increased in S and Se -starved cells when compared to a sulfur repleted cell culture. These data are discussed in the light of the key role of OAS in cysteine synthesis.





MATERIALS AND METHODS

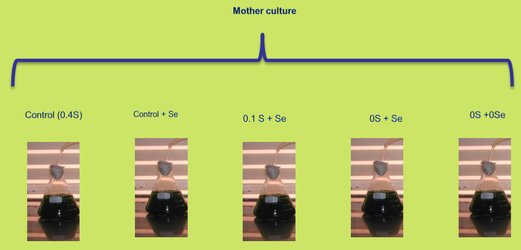
Experimental Conditions (erlenmeyers):

- 160 micromoles photons m^{-2} s^{-1} (continuous illumination)
- Temperature: 30ºC
- Modified K9 medium (pH 2.5)
- Air with 5%v/v of CO2 (unique carbon source)

Selenium (selenate) and Sulfur (sulfate) treatment:

25µg Chl ml^{-1} (cells in exponential phase)

Se and S concentrations added: Se (50 ppm in sodium selenate form) and S at the following concentrations (0.4 and 0.1 g/l in sodium sulphate form)



Determinations:

Photosynthetic activity (to check viability)

Biomass concentration: cell number and dry weight. Chlorophyll and total carotenoids,

Total selenium and selenium species: selenocystine ($SeCys_2$); selenomethionine (SeMet); selenomethylselenocysteine (SeMeSeCys)

Crude extract:

Cells were collected (25 µg Chl•mL^{-1}), washed three times with standard medium, broken by freezing in liquid nitrogen for 2 min and thawed in enzyme specific buffer100 mM potassium phosphate (pH 7.5)

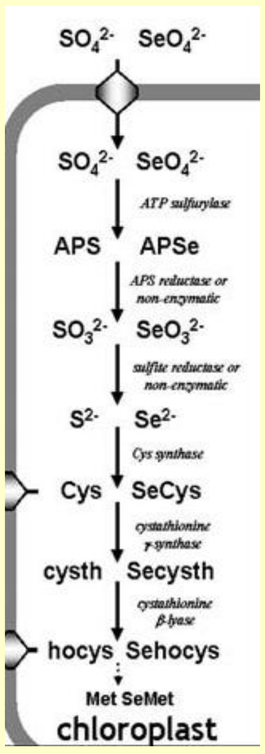
OASTL determination:

OASTL (O-acetyl-L-serine (thiol)lyase, EC 4.2.99.8) activity was assayed in a 1.5 mL reaction mixture containing: 50 µmol potassium phosphate buffer (pH 7.5), 20 µmol O-acetyl-L-serine, 10 µmol Na_2S and crude extract. The samples were incubated at 50 °C for 10 min and the reaction was stopped by adding 25 µL of 25% trichloroacetic acid. Cysteine formed was determined as described by Gaitonde (1967)

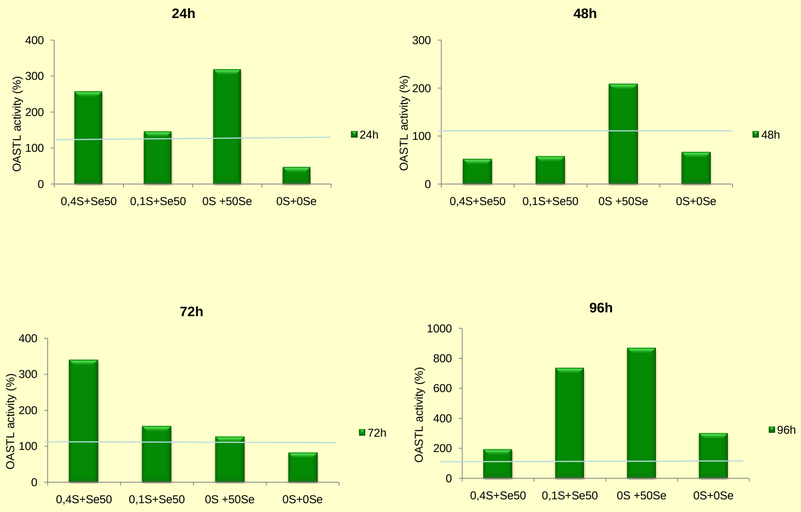
Instrumentation:

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

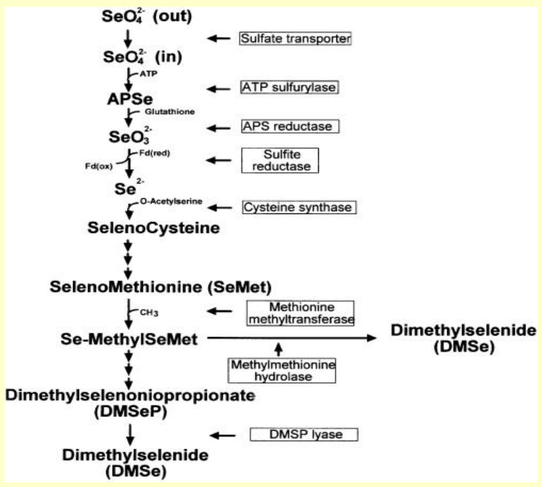
RESULTS and DISCUSSION



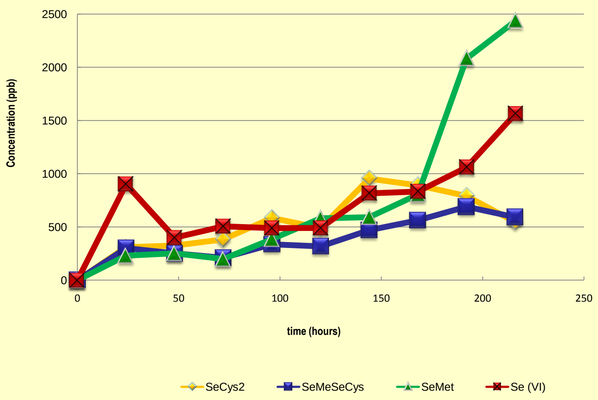
Pathway of sulfate and selenate assimilation in *C. sorokiniana*



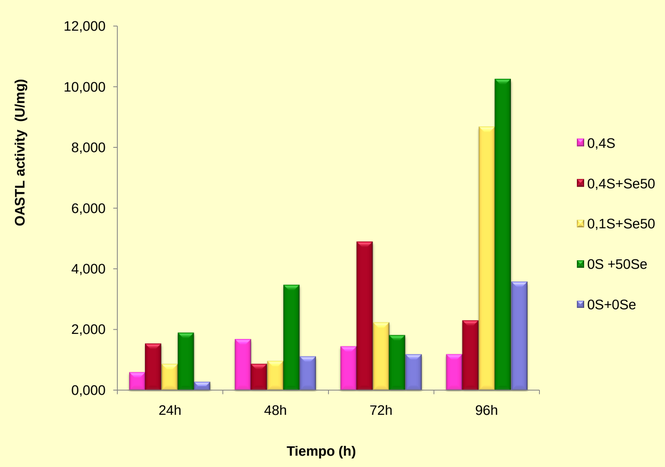
Effect of Se and S on the OASTL activity from *C. sorokiniana* crude extract. Standard autotrophic cultures, at the logarithmic phase of growth, were supplemented with the indicated concentrations of sulfate and selenate (see M &M) and the corresponding OASTL activity in crude extract was measured as describes in (M &M) at the indicated times (24, 48, 72 and 96 hours). Blue line represents OASTL 100% activity from control culture.



Selenium metabolism in plants, marine algae, bacteria and yeast



Biotransformation of inorganic selenate in *C. sorokiniana* cells. Standard autotrophic cultures, at the logarithmic phase of growth, were supplemented only with 50 ppm of Se (+VI). At the indicated times selenate (SeVI) and organic selenium species consisted of selenocysteine (SeCys2), selenomethylselenocysteine (SeMeSeCys) and selenomethionine (SeMet) biotransformed inside cells were determined by HPLC/ICP-MS.



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➢Se increases the enzyme activity of OASTL involved in the cysteine biosynthesis in presence or starvation of sulfur. Both elements share common metabolic pathway but *C. sorokiniana* preferentially uptake and transport selenate compared to sulfate suggesting thay S and Se compete for the enzyme.

➢Our results revealed that most of inorganic selenate was biotransformed into organic selenium species consisted of selenocysteine, selenomethylselenocysteine and selenomethionine during the culture, either as protein-bound or free Se-metabolites. In first 96 h main Se compunds were SeCys and SeMeSeCys. SeMet appeared at the end of the experiment (about 180h).

➢The complete understanding of Se and S metabolism in microalgae requires that more detailed enzymatic and biochemical studies to give us key information to drive algal Se-metabolism toward acumulation of the desired Se-metabolites, mainly selenomethionine

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