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Regulation of O-acetyl-L-serine Sulfhydrylase in Eukaryotic Algae

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K e y w o r d s : *Chlamydomonas reinhardtii*, *Monoraphidium braunii*, cysteine synthase, sulfur starvation.

S u m m a r y

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Two isoenzymes with O-acetyl-L-serine sulfhydrylase (OASS, EC 4.2.99.8) activity are present in *Chlamydomonas reinhardtii* and *Monoraphidium braunii*. Total OASS activity was strongly enhanced in S-deprived cells. The supply of O-acetyl-L-serine in the medium, was not needed for this enhancement. In *C. reinhardtii*, this increase in activity was mainly due to $OASS_1$. The increase was light-dependent and was negatively affected by L-cysteine or L-methionine in the medium. The purified OASS isoenzymes from these algae were inhibited by L-methionine, and only $OASS_1$ from *C. reinhardtii* was negatively affected by L-cysteine.

I n t r o d u c t i o n

Sulfate assimilation pathway may be controlled by sulfur availability, both in higher plants (SCHMIDT 1986) and microalgae (KRAUSS & SCHMIDT 1987), in the sense that sulfur starvation/limitation may result in an enhanced sulfate uptake capacity (LASS & ULLRICH-EBERIUS 1984), and in enhanced activities of ATP-sulfurylase (ZINK 1984) and O-acetyl-L-serine sulfhydrylase (LEON & VEGA 1991). Besides, in *Lemna minor* it has been observed that sulfate assimilation may be down-regulated by darkness, and it may be limited by the availability of O-acetyl-L-serine (NEUENSCHWANDER & al. 1991). Furthermore, plants growing with an excess of sulfur supply may accumulate thiol compounds, mainly glutathione and cysteine (BUWALDA & al. 1988). It has been proposed that reduced sulfur compounds may be involved in feedback inhibition of sulfate assimilation

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(BRUNOLD 1990). Previous data from *C. reinhardtii* show that L-cysteine and L-methionine inhibited OASS activity *in vitro* (LEON & VEGA 1991). The present paper is focussed on the effect of different nutritional conditions and S-amino acids on the OASS activity in eukaryotic algae, because of the key position of this enzyme between sulfur, nitrogen and carbon metabolisms.

Materials and Methods

Chlamydomonas reinhardtii, wild strain 21gr, and *Monoraphidium braunii*, wild strain 202-7d, were grown in standard liquid medium with 10 mM nitrate and 1 mM sulfate, as sole N and S source respectively. The cultures were bubbled with air containing 5 % CO_2 (v/v) and continuously illuminated with cool- and day-light fluorescent lamps (30 W m^{-2}) (LEON & VEGA 1991). L-Methionine was determined with the Cd-ninhydrin reagent (MATOH & al. 1980) and L-cysteine was done by the method of GAITONDE 1967. O-Acetyl-L-serine sulfhydrylase activity was assayed and purified as previously reported (LEON & VEGA 1991).

Results and Discussion

M. braunii can grow under autotrophic or heterotrophic conditions using different organic or inorganic nutrients as C-, N- or S-source. Therefore this alga was used to study the effect of different nutritional conditions on the intracellular OASS activity level in order to evaluate the regulatory properties of this enzyme. The level of OASS was not significantly influenced by the carbon source used by cells, or by light provided glucose was available. On the other hand, nitrate-supplied cells have similar OASS activity level as N-deficient ones, while ammonium or hydroxylamine, which can not be used by *M. braunii* as N-source for growth, have negative effect on the enzyme level, which may be 80 % decreased by 5 mM hydroxylamine in the culture medium (data not shown). However, leaves of N-deprived maize plants showed low OASS activity level (GHISI & al. 1986) while in tobacco cell cultures using nitrate as unique N-source the enzyme level was 2-fold higher than that shown by cells growing with nitrate plus ammonium, this observation being paralleled with a large release of glutathione to the medium (BERGMANN & al. 1980).

Particularly interesting is the effect of the S-source in the culture medium on the OASS activity level of *M. braunii*, while sulfite, thiosulfate, sulfide, L-cysteine, L-methionine, or glutathione-grown cells showed similar activity level as sulfate-supplied ones, the S-starvation almost doubled the initial activity, similar to tetrathionate-grown cells (Fig. 1,A). It has been observed that S-starvation not only increased the OASS activity level but also the sulfate uptake and other enzymes such as thiosulfate reductase in plants (SCHMIDT 1986), however, the activity of the sulfate activation system decreased in S-starved *Chlorella fusca* cells (KRAUSS & SCHMIDT 1987). The OASS intracellular activity level was 7-fold increased in *C. reinhardtii* S-limited cells (Fig. 1,B). The increase in OASS activity was light-dependent but the supplement of O-acetyl-L-serine in the medium was not required; the later was necessary for induction of OASS in prokaryotic organisms

(KREDICH 1971). In N-limited cells culture or under heterotrophic conditions the increase of OASS activity was reduced probably because of protein synthesis limitation under such conditions.

C. reinhardtii cells are not able to consume L-cysteine or L-methionine when present as unique S-compound in cultures of normally fed cells, however, these amino acids can be actively consumed by N-starved cells (Fig. 2, inset). The increase in OASS activity overexpression, in S-limited cells was negatively affected by L-cysteine or L-methionine. Even under N-limited conditions where these amino acids are actively consumed by the cells the OASS activity was negatively affected (Fig. 2). In *Salmonella typhimurium* the OASS activity synthesis could be repressed by L-cysteine (KREDICH 1971), while in *Lemna minor* the enzyme was not regulated by S-amino acids (SUTER & al. 1986).

L-Cysteine inhibited *in vitro* only the $OASS_1$ from *C. reinhardtii*, but it did not inhibit the isoenzymes from *M. braunii* (data not shown). Moreover, L-methionine acted as an uncompetitive inhibitor with respect to O-acetyl-L-serine of the *M. braunii* OASS (Fig. 3 A,B). However, L-methionine acted as the non-competitive inhibitor of the *C. reinhardtii* isoenzymes of OASS (Fig. 3 C,D). ASCAÑO & NICHOLAS 1977 observed that the OASS from wheat leaves was non-competitively inhibited by L-cysteine and competitively by L-methionine with respect to sulfide.

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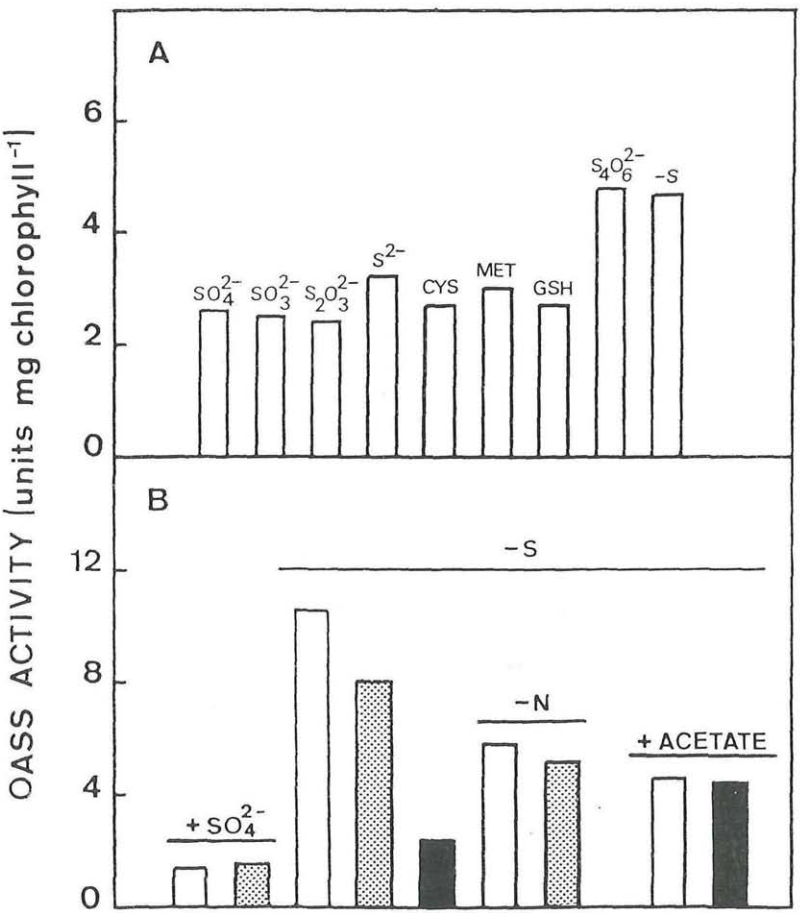

Fig. 1. Effect of different nutritional conditions on the OASS activity level in eukaryotic algae. Cells grown under standard conditions were transferred to fresh medium (20 µg Chl ml^{-1}) containing, where indicated, 24 mM acetate, 6 mM glucose, or 1 mM of the corresponding S-source. Black bars indicate cultures under darkness and grey bars those containing 1 mM O-acetyl-L-serine. The OASS activity was measured after 24 h growing under the stated nutritional conditions, and the experiments shown in A were done with *M. braunii* cells, while B corresponds to *C. reinhardtii* cells.

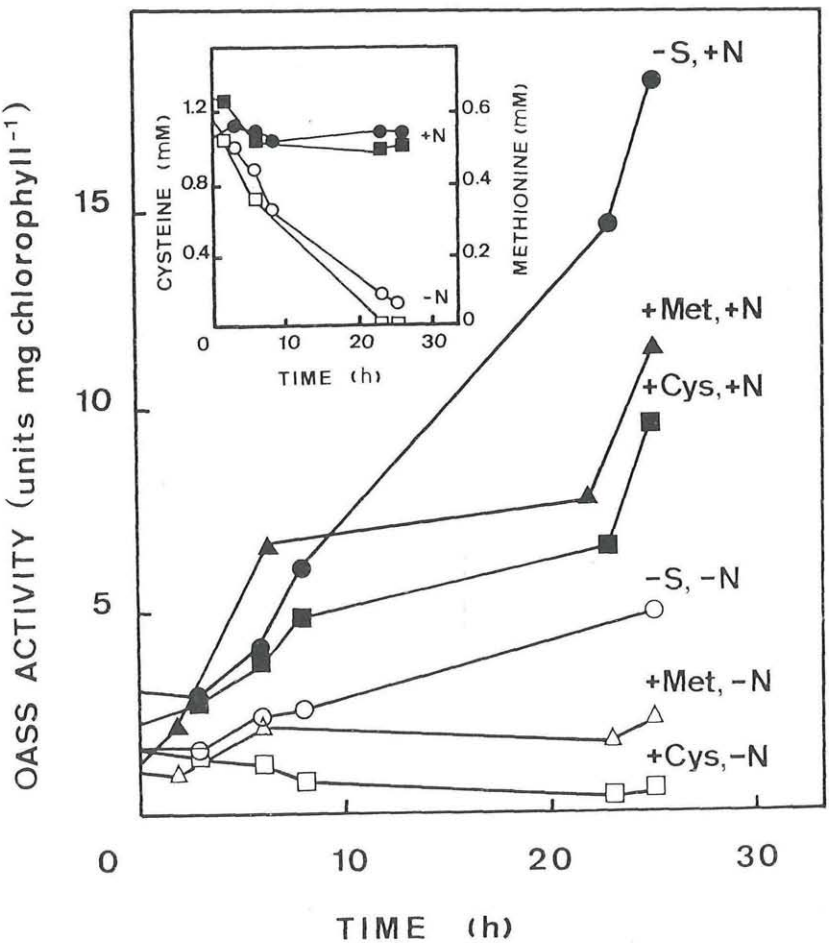

Fig. 2. Effect of L-cysteine or L-methionine on the OASS activity in *C. reinhardtii*. Cells were grown under standard conditions and transferred to S-free fresh medium (20 µg Chl ml^{-1}), with 10 mM ammonium (+N) or without an N-source (-N). Where indicated 1 mM L-cysteine or L-methionine was added (zero time). The figure inside shows the consumption of L-cysteine or L-methionine in ammonium-supplied (closed symbols) or N-starved (open symbols) cell cultures.

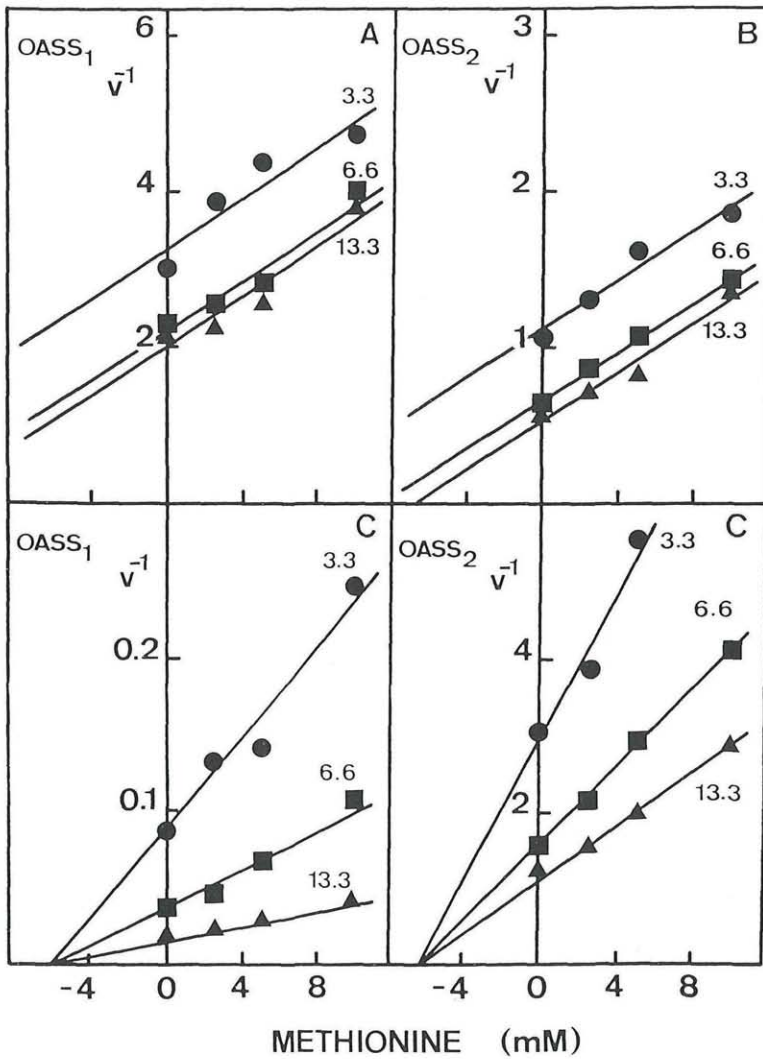

Fig. 3. Inhibition by L-methionine of OASS activity from eukaryotic algae. Partially purified preparations of $OASS_1$ and $OASS_2$ from *M. braunii* (A,B) or *C. reinhardtii* (C,D) cells were used, to show the Dixon plot of the data obtained using the indicated concentration of O-acetyl-L-serine and L-methionine.