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Regulation of Sulfate Assimilation in Tobacco Cells

EFFECT OF NITROGEN AND SULFUR NUTRITION ON SULFATE PERMEASE AND *O*-ACETYLSERINE SULFHYDRYLASE[1]

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IVAN K. SMITH
Department of Botany, Ohio University, Athens, Ohio 45701

ABSTRACT

The effect of nitrogen and sulfur nutrition on sulfate permease and *O*-acetylserine sulfhydrylase was studied in tobacco cells.

Sulfate transport rates increased 10-fold in cells transferred to sulfur-deficient B-5 medium. The addition of either sulfate or L-cysteine reduced transport 95 and 80%, respectively. The pools of sulfate, cysteine, glutathione, and methionine declined in sulfur-starved cells. The addition of either sulfate or L-cysteine increased the pools of sulfur-containing compounds, but major quantitative differences were measured. Nitrogen-starved cells had low transport rates which were not increased by addition of nitrate/ammonia. The pools of sulfate, cysteine, and methionine were high in nitrogen-starved cells and remained high upon addition of a nitrogen source. The results show that sulfate transport is regulated by the intracellular sulfate pool.

***O*-Acetylserine sulfhydrylase was not affected by sulfur nutrition. The extractable activity was high in B-5-grown cells, sulfur-deficient cells, and cells to which either sulfate or L-cysteine had been added. In contrast, the enzyme declined in cells transferred to nitrogen-deficient medium and the amount of enzyme/g fresh weight increased 10-fold when nitrate/ammonia was added. The addition of nitrate/ammonia had no effect on the cysteine or methionine pools but increased the total amino acid pool. The amount of *O*-acetylserine was positively correlated with extractable enzyme activity. This enzyme is positively regulated by an effector (possibly *O*-acetylserine) which is high under conditions of net nitrate assimilation.**

The rate of sulfate transport in suspension-cultured tobacco cells is controlled primarily by the size of the intracellular sulfate pool (18); transport rates are high in cells with small sulfate pools and low in cells with large sulfate pools. Frequently, modulation of the sulfate pool has no effect on the soluble cysteine and methionine content of plant cells (5, 18). ATP sulfurylase is regulated negatively by an end product of the sulfate assimilation pathway and positively by an end product of the nitrate assimilation pathway (15, 16). The result of this integrated regulation is that the ratio of nitrogen to sulfur assimilation is kept constant, whereas the absolute rates are allowed to rise and fall with the demand for net protein synthesis (6).

The regulation of *O*-acetylserine sulfhydrylase in suspension cultured tobacco cells was investigated because it catalyzes a reaction involving three potential regulatory molecules, namely, *O*-acetylserine, sulfide, and cysteine. Regulation of the enzyme has been studied in *Phaseolus* (1, 17) and *Lemna minor* (3). The enzyme is slightly elevated in sulfur-deficient *Phaseolus* (17) plants but is not affected by the growth of *Lemna* on L-cysteine (3).

MATERIALS AND METHODS

Tobacco XD-cell line (*Nicotiana tabacum* L. var. Xanthi) were cultured in modified B-5 medium (7). Standard B-5 medium contains 25 mM KNO_3, 1 mM $(NH_4)_2SO_4$, and 1 mM $MgSO_4$ as nitrogen and sulfur sources. Components other than these were not changed in the modified medium. Modified B-5 contained 25 mM KNO_3, 1 mM $(NH_4)_2SO_4$, and 1 mM $MgCl_2$. Nitrogen-deficient B-5 contained 25 mM KCl and 1 mM $MgSO_4$. Sulfur-deficient B-5 contained 25 mM KNO_3, 2 mM NH_4Cl, and 1 mM $MgCl_2$.

Sulfate Transport. Cells were harvested by vacuum filtration and washed with 30 ml transport medium minus sulfate. The washed cells (0.25–0.5 g fresh weight) were placed in a 125-ml Erlenmeyer flask containing 40 ml transport medium composed of 1% (w/v) sucrose, 0.5 mM $CaCl_2$, 5 mM bis-Tris-propane (pH 7) and 50 μM $Na_2{}^{35}SO_4$ (0.1 Ci/mol). The flasks were stoppered with cotton plugs and placed on a rotary shaker (80 rpm) at 25 C. At the end of the experiment, the cells were harvested by vacuum filtration, washed with 30 ml transport medium minus sulfate, and placed in 10 ml liquid scintillation fluid containing 1 ml H_2O. Radioactivity was determined with a Packard 3310 Tri-Carb scintillation spectrometer with external standardization.

Isolation and Assay of *O*-Acetylserine Sulfhydrylase. Cells (0.5 g) were harvested by vacuum filtration and manually ground in 5 ml 0.1 M bis-Tris-propane (pH 8.6), 0.1 mM pyridoxal phosphate, 10 mM DTT, and 1% (w/v) soluble PVP-10 (Sigma) using a conical Potter-Elvehjem homogenizer. The homogenate was centrifuged for 30 min at 10,000*g* and the supernatant was used in the enzyme assays. The reaction mixture (1 ml) contained 0.1 M bis-Tris-propane (pH 7.6), 0.1 mM pyridoxal phosphate, 20 mM *O*-acetylserine, 1 mM $Na_2{}^{35}S$ (0.5 Ci/mol), and protein. Reaction mixtures were incubated for 20 min at 30 C and the reaction was terminated by the addition of 0.2 ml 1.5 M trichloroacetic acid. L-Cysteine was isolated and quantified as reported previously (20). This assay measures total OAS[2] sulfhydrylase activity. The presence of two isozymes has been reported (1).

Measurement of Metabolite Pools in Tobacco Cells. Cells were grown in 80 ml modified B-5 medium containing 10 μCi 1 mM $Na_2{}^{35}SO_4$. Sulfate and sulfur-containing metabolites were isolated and quantified as previously described (18).

Carbon-containing metabolites were isolated as previously described from cells grown in 80 ml modified B-5 medium containing 30 μCi [U-^{14}C]sucrose (19).

Determination of Protein. Protein (1 ml) was precipitated with an equal volume of 1.5 M trichloroacetic acid, heated at 100 C for

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[2] Abbreviation: OAS sulfhydrylase (OASS in figures), *O*-acetylserine sulfhydrylase (EC 4.2.99.8).

5 min, sedimented by centrifugation, and washed 5 times with 95% ethanol (2.5 ml). The latter was necessary to remove PVP which interfered with the protein assay. The protein was dried and dissolved in 0.1 M NaOH. The protein content was determined using the method of Lowry *et al.* (10).

RESULTS AND DISCUSSION

Effect of Nitrogen and Sulfur Nutrition on Fresh Weight and Protein. Fresh weight increased from 1 to 14 g/80 ml medium over a 10-day culture period in modified B-5 (Fig. 1). Net protein synthesis ceased after 5 days and, as a result, the amount of protein/unit fresh weight declined. At least three phases were recognized in the cultures. During the first phase, lasting 2 days, the cells divided infrequently (Mitotic Index less than 0.5%), but net protein synthesis occurred. Second, from 2 to 5 days, net protein synthesis continued, the mitotic index increased to above 2%, and the fresh weight increased. Third, from 5 to 10 days, net protein synthesis ceased, the division rate declined, and the fresh weight increased due to cell expansion (K. Clark and I. K. Smith, unpublished results). Similar differences in cell populations during the culture cycle of bush beans were reported by Owens and Poole (12). The volume of cytoplasm as a percentage of cell volume varied from 45% at the start of the phase of rapid cell division to 10% during stationary phase. The amount of protein varied from 40 to 10 mg/g fresh weight. The average cell weight decreased 75% during the period of rapid cell division. The rate of increase in cytoplasmic volume was the only parameter which showed no detectable change during the prestationary culture cycle.

Cells inoculated into nitrogen-deficient medium did not grow or synthesize net protein (Fig. 1). Protein/unit fresh weight declined due to cell expansion.

Cells transferred to sulfur-deficient medium exhibited a pattern similar to cells inoculated into B-5, although there was less fresh weight gain and protein synthesis (Fig. 1). An explanation for these gains is the significant pool of sulfate and sulfur-containing amino acids present in the inoculated cells (Fig. 6).

Effect of Sulfur Nutrition on OAS Sulfhydrylase and Sulfate Transport. OAS sulfhydrylase specific activity was slightly higher in cells grown in sulfur-deficient medium (Fig. 2). The total amount of enzyme in the culture flask however was not affected by sulfur deficiency. The effect of adding either sulfate or cysteine (final concentration, 0.5 mM) to cells starved of sulfur for 9 days is shown in Figure 3. Addition of either sulfur source caused a resumption of protein synthesis and an increase in the amount of protein/unit fresh weight. Sulfate and cysteine slightly depressed OAS sulfhydrylase specific activity, but the total amount of enzyme in the culture flask was increased. It was concluded that OAS sulfhydrylase is not affected by the sulfur status of the cells, which agrees with previous studies of the enzyme. In *Phaseolus vulgaris*, OAS sulfhydrylase specific activity is slightly elevated by sulfur starvation, but there is a decline in the amount of protein/unit fresh weight, so that the amount of enzyme/unit fresh weight remains relatively constant (17). Similarly, growth of *L. minor* on L-cysteine has no effect on the amount of extractable enzyme (3).

Sulfate transport rates are low in cells grown on B-5 medium but increase 10-fold in cells grown on sulfur-deficient medium (Fig. 4). The decline in transport after 7 days is probably due to changes in the cell population. The decline in protein/unit fresh weight in stationary cells (Fig. 1) is indicative of increased vacu-

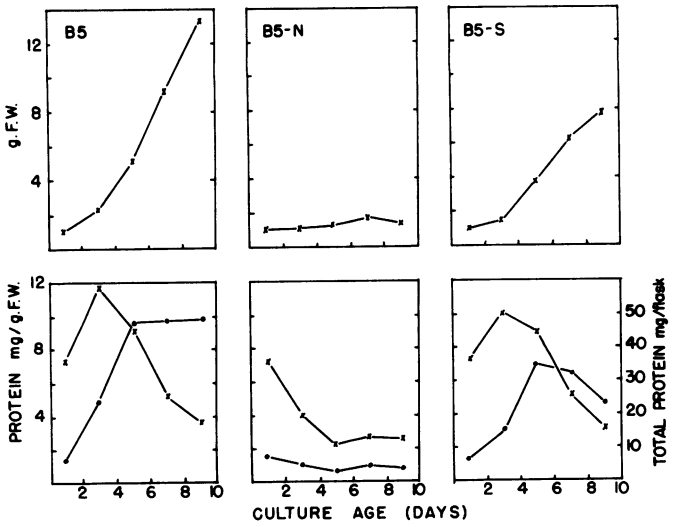

FIG. 1. Effect of nitrogen and sulfur nutrition on increase in fresh weight and protein. Cells were grown in 80 ml modified B-5, nitrogen-deficient B-5, or sulfur-deficient B-5 medium. The protein/g fresh weight (×——×) and total protein/flask (●——●) are shown in the lower three figures.

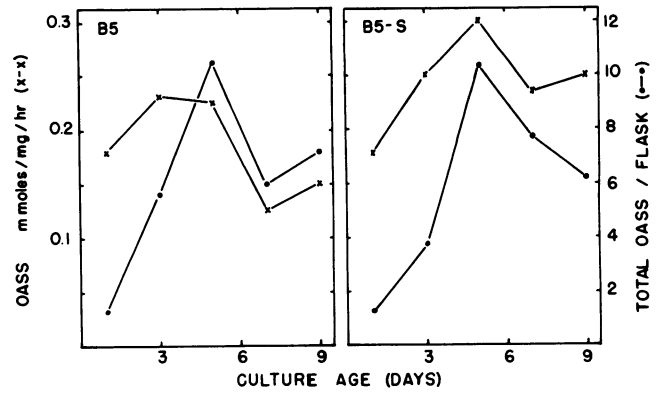

FIG. 2. The effect of sulfur nutrition on extractable OASS activity. Cells were inoculated into either modified B-5 or sulfur-deficient B-5. The extractable OASS specific activity (×——×) and the total OASS activity/flask (●——●) are recorded.

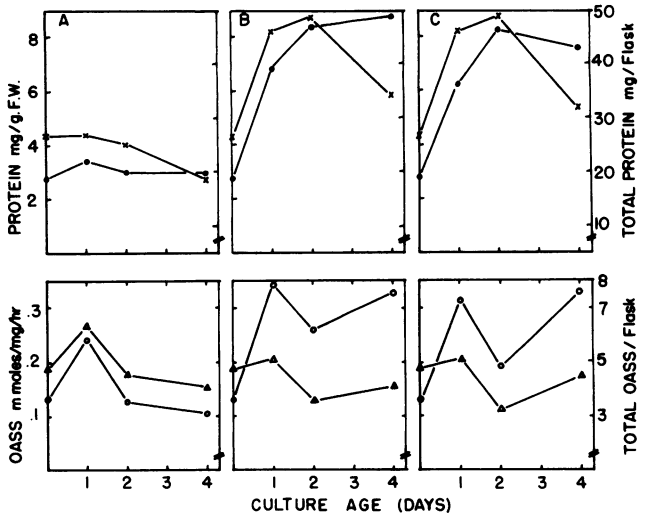

FIG. 3. The effect of sulfate and cysteine on the amount of extractable OASS activity. Cells were grown in sulfur-deficient B-5 medium for 8 days. The cells were maintained on this medium (A), 0.5 mM sulfate was added (B), or 0.5 mM L-cysteine was added. The upper three figures illustrate changes in protein/g fresh weight (×——×) and total protein/flask (●——●). The lower figures show changes in extractable OASS specific activity (△——△) and total OASS activity/flask (○——○). There was no significant change in the amount of fresh weight over the 4-day culture period.

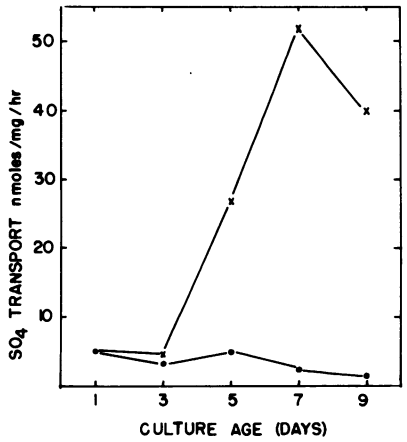

FIG. 4. The effect of sulfur nutrition on sulfate transport. Cells were grown in either modified B-5 (●——●) or sulfur-deficient B-5 medium (×——×).

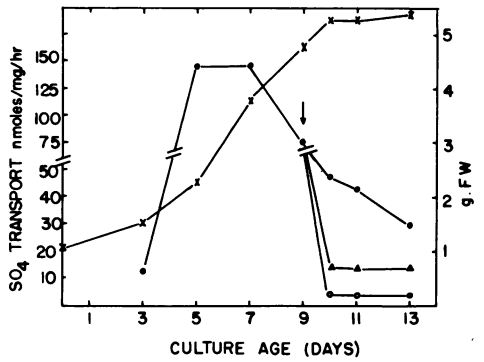

FIG. 5. The effect of adding either sulfate or cysteine on sulfate transport. Cells were grown in sulfur-deficient B-5 medium and the fresh weight (×——×) and sulfate transport rate (●——●) were measured. At 9 days, either 0.5 mM sulfate (○——○) or 0.5 mM cysteine (△——△) was added, using a syringe containing a Millipore filter.

olation, and it is suggested that these cells have lower transport rates than less vacuolated cells. The addition of either sulfate or cysteine (final concentration, 0.5 mM) reduces transport 95 and 80%, respectively (Fig. 5). Previously, it was shown that sulfate is more effective than cysteine in reducing sulfate transport rates (18).

Effect of Sulfur Nutrition on Sulfur-containing Metabolites. The intracellular sulfate and sulfur-containing amino acid pools decline when cells are transferred to sulfur-deficient medium (Fig. 6). Quantitatively, the greatest decline was in glutathione, but the cysteine and methionine pools also dropped 75% (Fig. 7). The presence of large amounts of sulfate and sulfur-containing amino acids initially indicated that the cells were not sulfur-deficient when they were inoculated and explains the growth of cells in sulfur-deficient medium (Fig. 1).

A large pool of sulfate (1 μmol/g fresh weight) is established within 24 h after the addition of sulfate to sulfur-deficient cells (Fig. 6). Sulfate is rapidly metabolized to rebuild the cysteine, glutathione, and methionine pools (Fig. 7). Fifteen μmol sulfate are transported in the first day after sulfate addition (Fig. 6); of this, 6 μmol are present as intracellular sulfate (1.2 μmol/g fresh weight × 5 g fresh weight; Fig. 5) and 4 μmol are present as sulfur-containing amino acids. Because these compounds account for 90% of the soluble sulfur-containing metabolites, the remaining 5 μmol must have been metabolized to protein and other insoluble compounds. Sulfate is transported from the medium at a rate of 5 μmol/day for the next 3 days without any major adjustments in

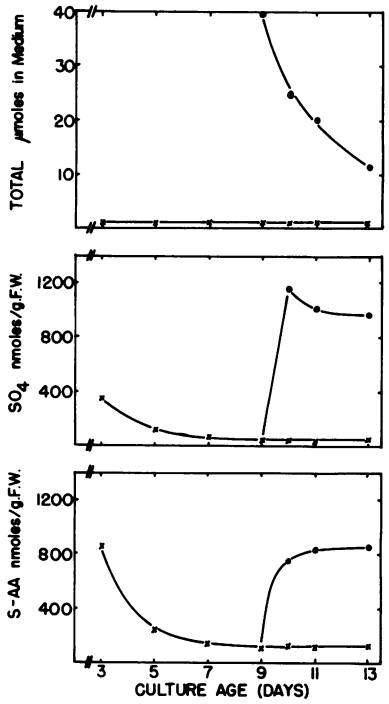

FIG. 6. The effect of sulfate on intracellular sulfate and sulfur-containing amino acids. Conditions were as described in Fig. 5. (×——×), cells grown on sulfate-deficient medium; (●——●), cells to which was added 0.5 mM sulfate.

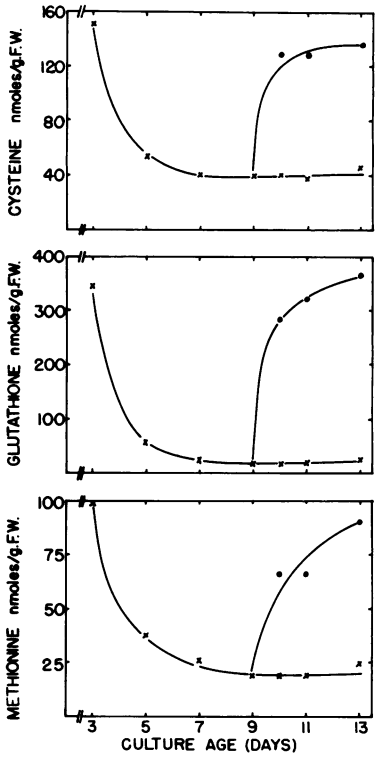

FIG. 7. The effect of sulfate on intracellular cysteine, glutathione, and methionine. Conditions were as described in Figure 6.

the soluble pools (Fig. 6). It was concluded that, upon addition of sulfate to sulfur-deficient cells, there is a rapid establishment of steady-state pools, followed by a regulated flux of sulfate through the sulfate assimilation pathway into protein and other insoluble compounds.

The sulfur-containing amino acid and sulfate pools are elevated upon addition of L-cysteine to sulfur-deficient cells (Fig. 8). The cysteine pool is 5-fold higher and the methionine pool is 2.5-fold higher (Fig. 9) than in cells to which sulfate is added (Fig. 7). The elevation of the methionine pool by transported cysteine above the level obtainable in sulfate grown cells may indicate that the

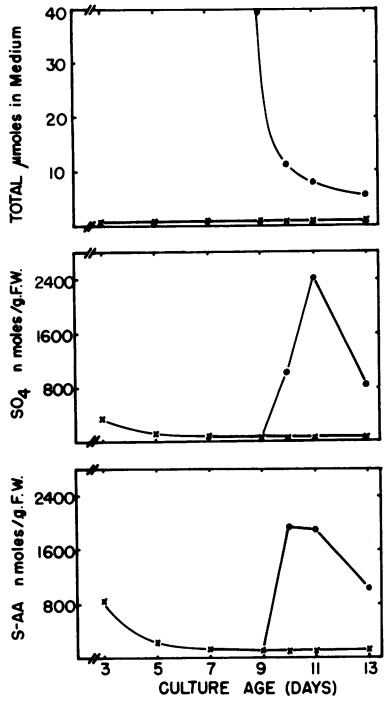

FIG. 8. The effect of L-cysteine on intracellular sulfate and sulfur-containing amino acids. Conditions were as described in Fig. 5. (×——×), cells grown on sulfur-deficient medium; (●——●), cells to which was added 0.5 mM L-cysteine.

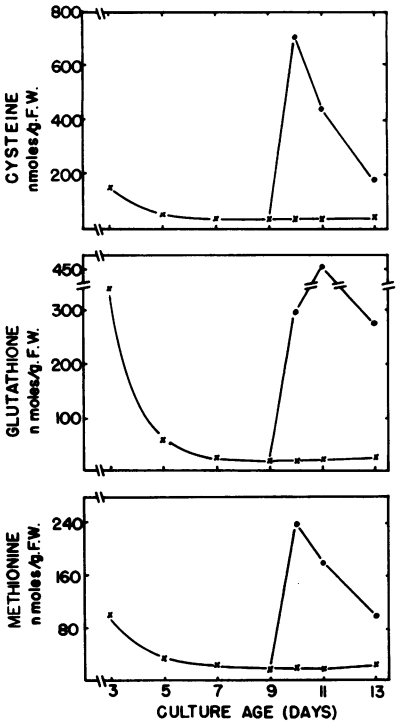

FIG. 9. The effect of L-cysteine on intracellular cysteine, glutathione, and methionine. Conditions were as described in Figure 8.

major sites of regulation of the sulfate assimilation pathway are before cysteine formation. A large fraction of the transported cysteine is degraded to sulfate (Fig. 8), as reported previously (8, 11, 18). The first enzyme of the degradative pathway is an inducible cysteine desulfhydrase (8). Thirty μmol cysteine are transported into the cells in 24 h (Fig. 8); 5 and 10 μmol can be accounted for in sulfate and in the sulfur-containing amino acid fraction, respectively. One-third of the sulfur is not accounted for, assuming that sulfur is incorporated into protein at the same rate as for sulfate grown cells (*i.e.* 5 μmol/day). The method used for the isolation of soluble sulfur-containing compounds does not retain volatile compounds. It is suggested that a major fraction of the material which cannot be accounted for after 24 h is sulfide produced during cysteine degradation. This suggestion is supported by considering the first 48 h. Over a 48-h period, 32 μmol cysteine are transported, 12 μmol are present as sulfate, and 10 μmol are present as sulfur-containing amino acids, leaving 10 μmol for incorporation into insoluble material. Protein synthesis 48 h after addition of cysteine is maintained by the intracellular pools which begin to decline at this time (Fig. 8).

Effect of Nitrogen Nutrition on OAS Sulfhydrylase. OAS sulfhydrylase specific activity declines in cells transferred to nitrogen-deficient medium (Fig. 10). Protein and enzyme synthesis resume upon addition of nitrate/ammonia (25 mM/1 mM, final concentration) to nitrogen-deficient cells (Fig. 11). The enzyme specific activity increases 3-fold and the amount of enzyme/unit fresh weight increases almost 10-fold. It was a similar response of ATP-sulfurylase to nitrogen nutrition that lead Reuveny and Filner (16) to propose that enzymes of the sulfate assimilation pathway are positively controlled by an effector produced by the nitrogen assimilation pathway. The major perturbations of OAS sulfhydrylase in response to nitrogen nutrition (Figs. 10 and 11) may be compared with the absence of a similar response to sulfur nutrition (Figs. 2 and 3).

The addition of nitrate/ammonia to nitrogen-deficient cells has no effect on the rate of sulfate transport which remains low (not shown).

Effect of Nitrogen Nutrition on Sulfur-containing Metabolites. The intracellular sulfate pool is maintained at a high level and the total sulfur-containing amino acid pool declines in cells transferred to nitrogen-deficient medium (Fig. 12). Quantitatively, glutathione is mainly responsible for the latter decline (Fig. 13). The total sulfur-containing amino acid pool is rebuilt when nitrate/ammonia is added to nitrogen-deficient cells (Fig. 12) because of a major

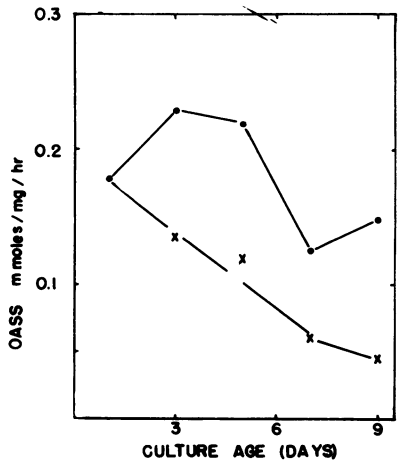

FIG. 10. The effect of nitrogen nutrition on the amount of extractable OASS activity. Cells were inoculated into either modified B-5 or nitrogen-deficient B-5 medium. The OASS from B-5 grown cells (●——●) and nitrogen-deficient cells (×——×) is shown. The changes in fresh weight and protein of the cells are illustrated in Fig. 1.

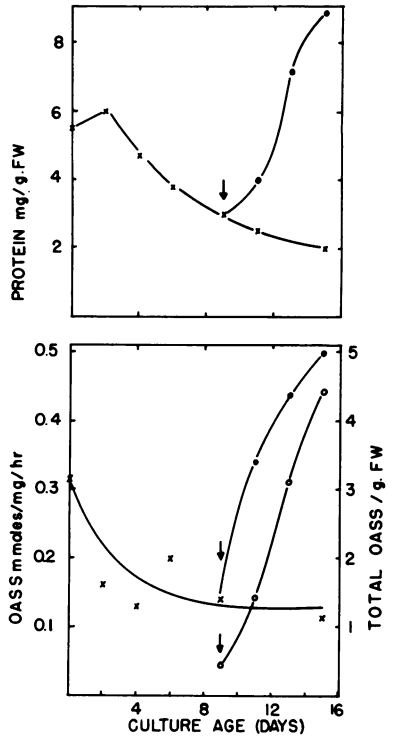

FIG. 11. Effect of NO_3/NH_4 on the amount of extractable OASS activity. Cells were inoculated into nitrogen-deficient medium. At 9 days, NO_3/NH_4 was added (final concentration, 25 mM NO_3/1 mM NH_4) using a syringe with a Millipore filter. The top figure shows changes in protein/g fresh weight upon addition of nitrogen (●——●) to nitrogen-deficient cells (×——×). The lower figure shows the increase in OASS specific activity (●——●) and total activity (○——○) upon addition of nitrogen to nitrogen-deficient cells (×——×).

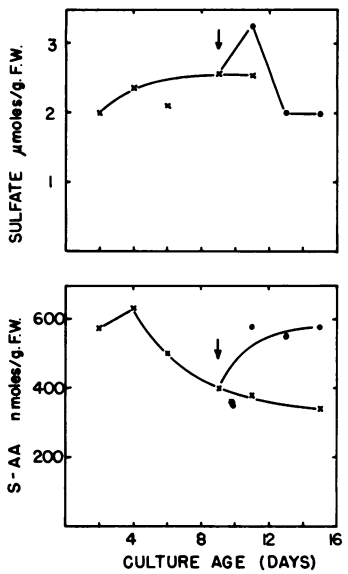

FIG. 12. Effect of NO_3^-/NH_4^+ on intracellular sulfate and sulfur-containing amino acids. Conditions were as described in Fig. 11. (×——×), cells grown on nitrogen-deficient B-5 medium; (●——●), cells to which NO_3/NH_4 was added.

increase in glutathione (Fig. 13). Experiments are in progress to determine whether these perturbations in glutathione are due to the availability of ammonia. Rennenberg and Bergmann (14) recently showed that the release of glutathione into the medium of suspension cultures of tobacco grown photoheterotrophically is

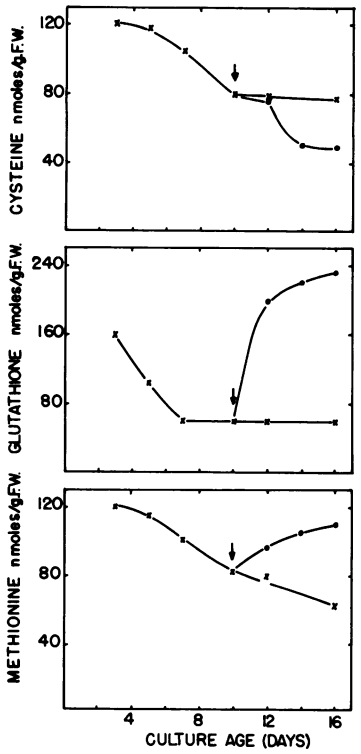

FIG. 13. Effect of NO_3^-/NH_4^+ on intracellular cysteine, glutathione and methionine. Conditions were as described in Figure 12.

dependent upon ammonia. Cultures grown with 60 mM nitrate produced 7 µmol glutathione/l, in contrast to 700 µmol accumulated in the medium of cultures supplied 20 mM ammonia and 40 mM nitrate. The synthesis and excretion of large quantities of glutathione was positively correlated with the glutamine content of the cells, which was 7.0 mM/g dry weight in nitrate-grown cells and 303 mM/g dry weight in ammonia-grown cells. The methionine and cysteine pools decline in nitrogen-deficient cells (Fig. 13). Upon addition of nitrogen, the methionine pool increases and the cysteine pool declines after a 2-day lag. The latter decline is probably due to the demand for cysteine for protein synthesis, which increases during this period (Fig. 11).

Effect of Nitrogen and Sulfur Nutrition on the Cationic Fraction and *O*-Acetylserine. The effect of nitrogen and sulfur nutrition on the total amino acid pool was estimated by growing cells on [^{14}C]sucrose and isolating the material retained by a cation-exchange column. Because compounds other than amino acids are present in this fraction, the values in Figure 14 should not be interpreted as an absolute measure of the amino acid pool, but rather as a reflection of that pool.

The amino acid pool declines as cells become nitrogen-deficient. Upon addition of nitrate/ammonia there is an increase in the amino acid pool (Fig. 14A), a resumption of net protein synthesis, and an increase in OAS sulfhydrylase activity (Fig. 11).

The amino acid pool is maintained at a high level in sulfur-deficient cells and declines when sulfate is added (Fig. 14B). For the first 48 h following sulfate addition, the amino acid pool is higher than in nitrate-sufficient cells (Fig. 14).

The *O*-acetylserine content of the cells was correlated positively with the total amino acid pool (Fig. 14).

CONCLUSION

The previous conclusion that sulfate transport is regulated primarily by the intracellular sulfate pool (18) is supported by the study presented here. The sulfate transport rate increases as cells

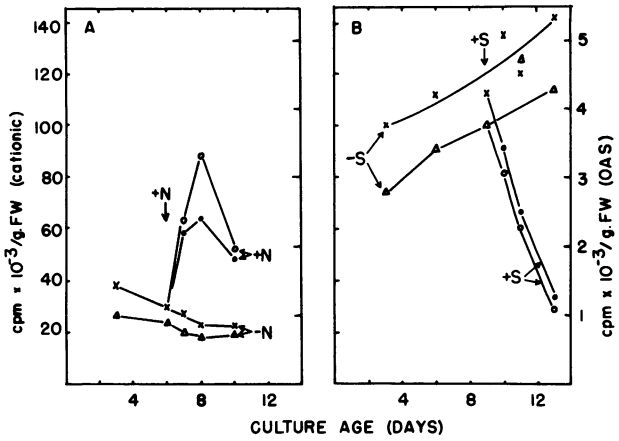

FIG. 14. Effect of nitrogen and sulfur nutrition on the "amino acid" pool and *O*-acetylserine. In A, 25 mM nitrate and 1 mM ammonium was added to cells starved of nitrogen for 6 days. (×——×), amino acid fraction (cationic) of nitrogen-deficient cells; (●——●), cells to which nitrogen was added. (△——△), *O*-acetylserine content of nitrogen-deficient cells; (○——○), cells to which nitrogen was added. In B, 0.5 mM sulfate was added to cells starved of sulfur for 9 days. (×——×), amino acid fraction (cationic) of sulfur-deficient cells; (●——●), cells to which sulfate was added. (△——△), *O*-acetylserine content of sulfur-deficient cells; (○——○), cells to which sulfate was added.

become sulfur-deficient and decreases when either sulfate or L-cysteine is added to sulfur-starved cells. In these experiments, changes in intracellular sulfate were positively correlated with changes in cysteine, glutathione, and methionine. In the former study, however, the sulfate pool was varied without affecting the amino acid pools (18). A similar observation was made by Datko and co-workers (5) in *Lemna*. As reported previously (18), sulfate is more effective than cysteine in reducing sulfate transport. Cysteine-grown cells have higher sulfate pools than sulfate-grown cells. If sulfate is the feedback inhibitor of the sulfate transport system, these two observations are only consistent if more than one pool of sulfate exists. It is proposed that transported sulfate enters a cytoplasmic pool functionally close to the transport sites, whereas a major fraction of the sulfate derived from cysteine enters a pool that is functionally remote from the transport sites, *e.g.* the vacuole. Sulfate transport rates are always low in nitrogen-deficient cells and are not affected by the addition of nitrate/ammonia (data not shown). This is consistent with the interpretation that intracellular sulfate is the major regulator of sulfate transport because the sulfate pool is high in nitrogen-deficient cells and remains high upon addition of nitrate/ammonia. Apparently, terminal metabolites of nitrate assimilation are not positive effectors of the sulfate permease system because they increase upon addition of nitrate/ammonia without affecting sulfate transport. It is possible that the negative feedback regulation by sulfate may override a positive regulatory signal from the nitrate assimilation pathway.

In tobacco cells, OAS sulfhydrylase is apparently not regulated by sulfur nutrition but is produced at constitutive levels. The specific activity was relatively unaffected by growth on sulfur-deficient medium or by addition of either sulfate or L-cysteine to sulfur-starved cells. Similar results were obtained using intact plants (3, 17). The enzyme, however, is positively regulated by a product of nitrate assimilation. The specific activity declines in cells depleted of nitrogen and increases when nitrogen is added to nitrogen-deficient cells. Similar results were obtained by Reuveny and Filner (16) who studied the regulation of ATP-sulfurylase and introduced the concept of positive regulation of sulfate assimilation in plants. Potentially, *O*-acetylserine is the positive effector of some enzymes of sulfate assimilation. This compound is high in cells with high enzyme activity, *i.e.* sulfur-deficient cells and nitrogen-deficient cells to which nitrogen has been added. Under conditions where *O*-acetylserine is high, the total amino acid pool is high. Definitive identification of *O*-acetylserine as the positive effector requires the isolation of mutants with defective serine transacetylase.

The mechanism of regulating OAS sulfhydrylase in bacteria varies. Chambers and Trudinger (4) showed that in several bacteria OAS sulfhydrylase is not correlated with the sulfur source in the growth medium. They reported that regulation of the enzyme by sulfur compounds has been demonstrated only in bacteria that produce excessive amounts of the enzyme. In *Escherichia coli* and *Salmonella typhimurium*, several enzymes of sulfate assimilation, including OAS sulfhydrylase, are controlled by both repression by cysteine and induction by *O*-acetylserine and the product of the cys B gene (9, 21). However, the parallel behavior of sulfite reductase and OAS-sulfhydrylase in *S. typhimurium* characteristic of log-phase growth is lost during stationary phase, when OAS sulfhydrylase increases greatly (2). These and other observations lead Borum and Monty (2) to conclude "that each controlling site can have an independent and possibly different response to the metabolic circumstances of stationary phase". Pasternak and co-workers (13) proposed that the enzymes of sulfate assimilation in bacteria are subject to "differential" rather than "coordinate" repression. Specifically, enzymes early in the pathway are more sensitive to repression by cysteine than enzymes late in the pathway.

This suggestion is partially applicable to tobacco cells. ATP sulfurylase levels are very sensitive to sulfur nutrition (16) whereas OAS sulfhydrylase levels are not. In contrast, both enzymes are equally sensitive to nitrogen nutrition. The consequences of this type of regulation in plants are clear. When cysteine rises above a critical level, cysteine inactivation of adenosine 5-phosphosulfate sulfotransferase (3), cysteine inhibition of serine transacetylase (20), sulfide inhibition of OAS sulfhydrylase (1), and, ultimately, repression of ATP sulfurylase (16) and induction of cysteine desulfhydrase (8) will reduce the cysteine pool. Alternatively, when cysteine synthesis is insufficient *vis a vis* the rate of nitrate assimilation, co-ordinate induction of the enzymes of sulfate assimilation by a terminal metabolite of nitrate assimilation will increase the rate of cysteine synthesis.

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