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Cysteine Does Not Repress Adenosine-5'-Phosphosulfate Reductase Through Its Conversion to Either Sulfate or Glutathione

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Cysteine (Cys) represses the activity of several key regulatory enzymes in the plant sulfate assimilatory pathway. However, it is not clear whether this effect arises from Cys itself or through its conversion to either sulfate or glutathione (GSH). Therefore, we examined this phenomenon by analyzing the activity of adenosine-5'-phosphosulfate (APS) reductase. Both APS reductase (AR) activity and mRNA levels were decreased by treating *Arabidopsis thaliana* roots with 1 mM Cys. The intracellular sulfate concentration was not affected, whereas enzymatic activity and, to some extent, the mRNA level, declined. Cys treatment in sulfur-starved plants also diminished both parameters. However, this response to Cys was more efficient than when plants were treated with an equal amount of sulfate. When Cys was removed from both Cys- and sulfate-fed plants, AR activity was recovered; the same removal of sulfate was not so effective. Moreover, buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, did not influence the repression of AR by Cys. Finally the AR enzyme was inhibited by cysteine *in vitro*. These results indicate that Cys represses AR by inhibiting mRNA expression and by directly repressing enzymatic activity, rather than through its conversion to either sulfate or GSH.

Keywords: APS reductase (AR), *Arabidopsis*, cysteine, glutathione, sulfate

Sulfate assimilation is a fundamental biological process, during which oxidized inorganic sulfate is reduced to sulfide for the biosynthesis of Cys and methionine (Leustek et al., 2000). These two amino acids are essential in maintaining the structure and function of proteins, redox-active cofactors, and coenzymes. Phototrophic organisms, such as plants, algae, cyanobacteria, and a wide range of heterotrophic microorganisms, can use inorganic sulfate as their sole source of sulfur. These organisms serve as nutritional sources for animals that, by themselves, lack the ability to synthesize such amino acids.

The sulfate assimilatory pathway is divided into four steps: uptake, activation, reduction to sulfide, and incorporation of sulfide into Cys. Extracellular inorganic sulfate is taken up into the cell via sulfate transporters (Hawkesford and Belcher, 1991). Sulfate activation is achieved in all organisms by ATP sulfurylase (EC 2.7.7.4) which is found in nearly all organisms and catalyzes the formation of adenosine-5'-phosphosulfate (APS) (Lappartient and Touraine, 1996; Logan et al., 1996). Reduction of sulfate occurs primarily in the plastids of plants, where ferredoxin functions as a reductant. APS is reduced through the formation of an organic thiosulfate with reduced GSH by AR (adenosine-5'-phosphosulfate reductase; EC 1.8.99.2) (Setya et al., 1996). Further reduction to sulfide is catalyzed by a ferroxin-dependent sulfite reductase (E.C 1.8.7.1) (Bruhl et al., 1996). The incorporation of sulfide into Cys is the final step in the pathway, and is catalyzed by Cys synthetase (O-acetylserine thiol lyase; OAS-TL) (EC 4.2.99.8) from O-acetylserine (OAS) and sulfide. OAS is produced by serine acetyltransferase (SAT) (EC 2.3.1.30) from acetyl-CoA and serine. In plants, SAT and OAS-TL exist together as an enzyme complex known as Cys synthetase (Ruffet et al., 1994).

Three AR genes -- *APR1*, *APR2*, and *APR3* (accession nos. U43421, U56921, and U56922) -- were first cloned in *Arabidopsis thaliana* by Setya et al. (1996). Their expression is up-regulated by oxidation, sucrose, and light (Kopriva et al., 1999; Bick et al., 2001). In plants, sulfate transport, ATP sulfurylase, and AR activities are stimulated in response to sulfur starvation, and are repressed when sulfur again becomes available (Smith, 1975; Reuveny and Filner, 1977; Hawkesford et al., 1993; Yildiz et al., 1994; Lappartient and Touraine, 1996; Logan et al., 1996). The end products of sulfate assimilation, Cys and GSH, repress sulfate transport and the activities of ATP sulfurylase and AR (Hart and Filner, 1969; Brunold,

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1978; Wyss and Brunold, 1979; Jenni et al., 1980; Lappartient and Touraine, 1997). Cys is considered a key regulatory compound in bacterial and fungal sulfate assimilatory metabolism (Kopriva and Rennenberg, 2004). ATP sulfurylase in tobacco cells and Indian Mustard is thought to be subject to end-product repression by Cys or GSH, and is activated by growth on more slowly assimilated sulfur sources (Reuveny and Filner, 1977; Lappartient and Touraine, 1996). In *Lemna*, AR (APS reductase) is inhibited *in vitro* by Cys. Treating plants with sulfide results in a decrease in extractable activity of this enzyme (Brunold and Schmidt, 1976).

These observations could lead us to conclude that the intracellular Cys or sulfide pool is a key signal in regulating the sulfate assimilatory pathway. However, other experimental results have implied that the intracellular sulfate pool is more important as a regulatory signal. Cys inhibits sulfate transport into cultured tobacco cells and potato tubers (Hart and Filner, 1969; Maggioni and Renosto, 1977); this Cys effect is correlated with intracellular sulfate. When tobacco cells are incubated with Cys, the intracellular Cys pool is elevated, and excess Cys is oxidized to sulfate. This suggests that sulfate transport is regulated by the intracellular sulfate pool while the Cys pool is regulated by degradative enzymes. Therefore, the effect of Cys repression of sulfate assimilatory enzymes is unclear, because Cys can be metabolized to either sulfate or GSH (Harrington and Smith, 1980). Thus, the central question in this current research was to identify the signal that represses all sulfate assimilation in plants (Smith 1975, 1980; Jensen and Konig, 1982; Datko and Mudd, 1984; Lass and Ullrich-Eberius, 1984; Rennenberg et al., 1988, 1989; Herschbach and Rennenberg, 1991, 1994; Lappartient and Touraine, 1996).

MATERIALS AND METHODS

Plant Material and Growth Conditions

A. thaliana (Columbia) was grown hydroponically for 40 d in polyethylene pots (4 plants each) that contained 500 mL of a nutrient medium. This medium consisted of 1.6 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1 mM KNO_3, 0.4 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM KH_2PO_4, 50 μM $FeCl_2 \cdot 4H_2O$, 50 μM Na_2EDTA, 11 μM H_3BO_4, 2.5 μM $MnCl_2 \cdot 4H_2O$, 0.15 μM $H_2MoO_4 \cdot 2H_2O$, and 0.1 μM $CuCl_2$, 0.2 μM $ZnCl_2$ (adjusted to pH 6.7 with KOH). Plants were raised in a growth chamber at 23°C, under a 12-h photoperiod and with a light intensity of 90 $\mu E\ m^{-2}\ s^{-1}$. The nutrient solution was exchanged every 3 d.

Northern Blot Analysis

Total RNA was isolated from plants using a buffer supplemented with guanidinium thiocyanate (Chomsczynski and Sacchi, 1987). Twenty μg of RNA was electrophoresed on a 1.0% (w/v) agarose gel containing formaldehyde (Lehrach et al., 1977; Cho and Hong, 2004). The gel was rinsed several times with water and then treated for 15 min with a solution of 0.05 N NaOH and 10 mM NaCl. RNA was transferred to a nylon membrane, which was pre-treated with 50 mM Tris-HCl (pH 7.2) for 10 min and then pre-hybridized in a solution of 1.0 M Na^+ (supplied as Na_2HPO_4; pH 7.2), 1 mM EDTA, and 7% (w/v) SDS, at 65°C for 8 h. Hybridization was carried out in a pre-hybridization buffer at 65°C for 16 h. The membrane was then washed at maximum stringency in 100 mM Na^+, 1 mM EDTA, and 1% (w/v) SDS at 65°C. The following cDNA probes were labeled by the random primer method, using [α-^{32}P]dCTP (3000 Ci $mmol^{-1}$): APR1 (Setya et al., 1996), encoding AR (adenosine-5'-phosphosulfate reductase); and EST B64XP (T04000), encoding beta-tubulin. EST clones were provided by the Arabidopsis Biological Resource Center (Ohio State University, USA).

AR Assay

Plant extract was prepared by grinding tissues (frozen in liquid nitrogen) with 50 mM Tris-HCl (pH 8.5). The extract was centrifuged for 20 min at 15,800*g*, and the supernatant was used for protein measurements and enzyme assays. Protein concentrations were measured by the Bradford (1976) assay, using BSA (bovine serum albumin) as a standard. AR was measured at 30°C. The reaction was contained in a 100-μL volume of 100 mM Tris-HCl (pH 8.5), 500 mM Na_2SO_4, 1 mM EDTA, 5 mM DTT, the tissue extract, and 25 μM [^{35}S]APS (~500 Bq·$nmol^{-1}$). This assay measured the formation of [^{35}S]SO^{2-}_3 from APS by its conversion to volatile SO_2 after the addition of acid. At the end of the incubation, Na_2SO_3 was added to 40 mM, then H_2SO_4 was added to 3 M. Uncapped reaction tubes were placed in a scintillation vial containing 1 mL Tri-n-octylamine. The vial was capped tightly and incubated overnight at room temperature to allow volatilized SO_2 to be absorbed by the Tri-n-octylamine. Afterward, the reaction tubes

were removed and 3 mL scintillation fluid was added (Ready-Safe; Beckman, USA). Pure APR3 protein was prepared as described by Bick et al. (2001).

Measurement of Intracellular Sulfate Concentration

Plant material was ground in a mortar with liquid nitrogen, extracted in 0.1 N HCl [1:5 (w/v) ratio], and centrifuged at 4°C for 20 min at 15,800g. The supernatant was diluted 10 times, then injected into an ion chromatograph (Dionex DX-100; Sunnyvale, USA) that was equipped with conductivity detection and an anion-exchange column (IonPac AS9). The eluant was $Na_2CO_3/NaHCO_3$ (2.0 mM : 0.75 mM), at a flow of 2.0 mL min^{-1}. A sulfate standard was made from Na_2SO_4; the sensitivity of this method was 1 μM for sulfate.

RESULTS

Cys Effect on Repression of AR Is Not Correlated with the Intracellular Sulfate Concentration in *A. thaliana*

The mechanism by which the activity of sulfate assimilatory enzymes is repressed by Cys is still not fully understood. This effect may be mediated by Cys directly or by its metabolites sulfate or GSH. Here, we examined whether the effect of Cys on repression of AR activity was mediated by sulfate (Fig. 1A). Although our standard growth medium contained 0.4 mM sulfate, when plants were transferred to media supplemented with 1 mM sulfate (total sulfate concentration), activity was not significantly affected in the first 24 h. However, transfer to a medium with 1 mM Cys caused this activity to diminish rapidly, i.e., within 6 h. The steady-state level of AR mRNA also decreased after Cys treatment (Fig. 1B). However, mRNA did not decline to the extent that enzyme activity did; likewise, it began to recover after 24 h even in the absence of any restoration of enzyme content. These results indicate that Cys probably does not repress AR through metabolism to sulfate in plant tissues. Indeed, the intracellular sulfate concentration was not altered appreciably by Cys-feeding (Fig. 1C).

To further explore this Cys effect, plants were sulfur-starved for 48 h, then transferred to a medium containing either 0.4 mM sulfate or 1 mM Cys. Both AR activity and mRNA levels were measured periodically (Fig. 2A and B). Over the first 6 h, sulfate and Cys

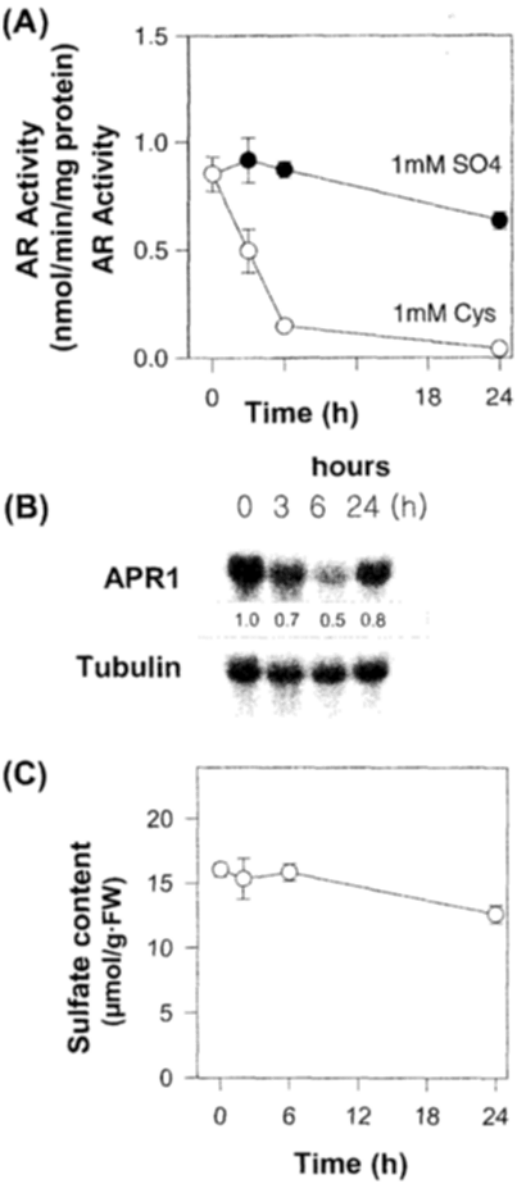

Figure 1. Analysis of AR activity, mRNA level, and sulfate content in sulfate- or Cys-treated roots of *A. thaliana*. **(A)** AR activity was measured at various times after plants grown with 0.4 mM sulfate were transferred to medium with 1 mM sulfate (closed circles) or 1 mM Cys (open circles) as sole sulfur sources. Values are expressed as mean ± standard error of four replicates in nmol min^{-1} mg^{-1} protein. **(B)** AR mRNA level was measured at various times only in 1 mM Cys-treated plants. Eight plants were used for RNA isolation and 20 μg total RNA was electrophoresed, blotted, and sequentially analyzed with probes for AR (*APR1*) and then tubulin. A tubulin probe was used to control for RNA loading. Number below each lane is relative intensity of signal that was corrected with tubulin signal; control was assigned a value of 1.0. **(C)** Analysis of tissue-sulfate levels in roots of *A. thaliana* treated with 1 mM Cys. Plants were sampled at various times after treatment. Values are expressed as mean ± standard error of four replicates.

were equally efficient at repressing enzyme activity; however, the sulfate-induced repression ceased after 6 h while that of Cys continued beyond that point (Fig. 2A). In this second experiment, these treatments also caused the AR mRNA content to decline by 5-fold over the untreated control after 24 h (Fig. 2B). Cys was again more effective than sulfate in decreasing enzymatic activity, with the level being 17-fold lower after 24 h. This strong repression was not correlated with intracellular sulfate concentration, which did not change appreciably after Cys-feeding (Fig. 2C). In addition, AR activity and mRNA levels were not correlated. However, the sulfate concentration was strongly increased by our sulfate treatment.

A third type of experiment was done to explore the differing effects of Cys and sulfate. First, plants were pre-treated for 24 h by feeding with 1 mM Cys in addition to the 0.4 mM sulfate present in the growth medium. The plants were then transferred to a medium lacking either sulfate or Cys. AR activity and mRNA were measured over time (Fig. 3A and B). Removal of Cys caused both AR activity and mRNA levels to recover rapidly whereas the removal of sulfate did not. These treatments had no effect on the tissue sulfate concentration (data not shown). All of our results confirm that the Cys effect on AR repression is not correlated with intracellular sulfate concentrations. The asynchronous behavior of AR activity and mRNA was especially pronounced in this experiment that is, while activity increased by ~20-fold after 24 h, mRNA levels increased by only 0.6-fold.

Cys Can Directly Inhibit AR Activity

Because the repression of AR activity by Cys did not seem to be mediated through its metabolism to sulfate, it is possible that Cys acted directly or through its metabolism to GSH. To explore the hypothesis that GSH is the active regulator, we used BSO (buthionine sulfoximine), an inhibitor of GSH synthesis. Plants were treated for 24 h with BSO, without or with Cys or GSH (Fig. 4). BSO treatment alone increased AR activity by ~2-fold, perhaps because of the inhibition of GSH synthesis and a decrease in the GSH pool (Ruegsegger et al., 1990). Treatment with BSO plus Cys resulted in a decline in AR activity comparable to treatment with Cys alone. GSH, with or without BSO, had the same effect. These results indicate that both Cys and GSH repress AR activity. The Cys effect is due in part to the repression of AR gene expression. However, Cys may also act directly on the AR enzyme. To test this hypothesis, we measured AR activity in cell

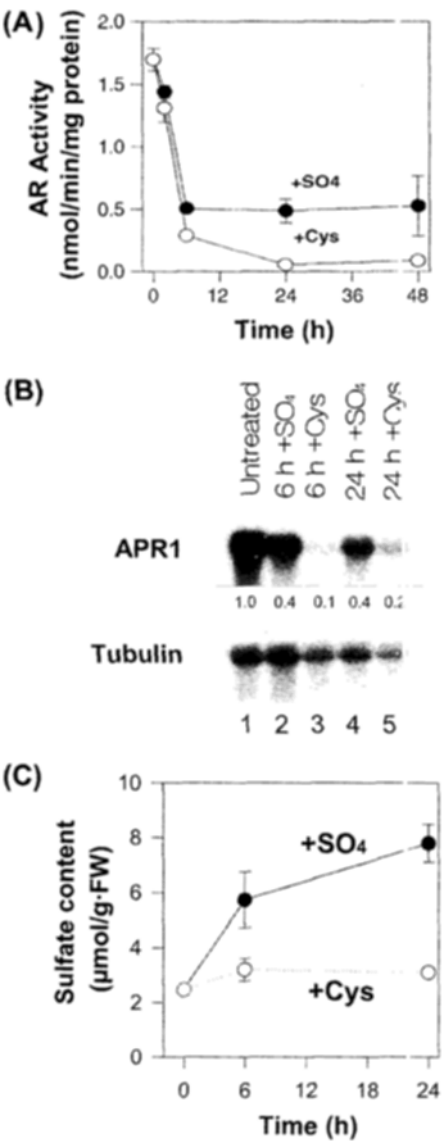

Figure 2. Analysis of AR activity, mRNA level, and sulfate content after feeding of sulfate-starved *A. thaliana* with sulfate or Cys. Forty-d-old plants were starved for sulfate for 2 d. AR activity **(A)** and mRNA level **(B)** were then measured in roots for various times after treatment with 0.4 mM sulfate (closed circles) or 1 mM Cys (open circles). Values are expressed in nmol min^{-1} mg^{-1} protein, and are mean ± standard error of four replicates. Eight plants were used for RNA isolation, and 20 μg total RNA was electrophoresed, blotted, and sequentially analyzed with probes for AR (*APR1*) and then tubulin. The tubulin probe was used to control for RNA loading. Number below each lane is relative intensity of signal, corrected for RNA loading; control was assigned a value of 1.0. **(C)** Intracellular sulfate concentration in roots after feeding of sulfate-starved *A. thaliana* with sulfate or Cys. Sulfate concentration was measured at various times after treatment of 0.4 mM sulfate (closed circles) or 1 mM Cys (open circles) in 2-d sulfur-starved roots of 40-d-old plants. Values are expressed as mean ± standard error of four replicates.

extracts or with purified AR (APR3) that was pre-incubated with Cys at room temperature (Fig. 5). In that experiment, Cys had a marked effect on AR activity, thereby demonstrating that Cys can inhibit AR activity directly.

DISCUSSION

AR activity was significantly decreased when plants were fed 1 mM Cys; 1 mM sulfate had little effect on that activity (Fig. 1A). In sulfur-starved plants, feeding with Cys was more effective than sulfate on AR repression (Fig. 2A). In sulfate/Cys-fed plants, removal of Cys

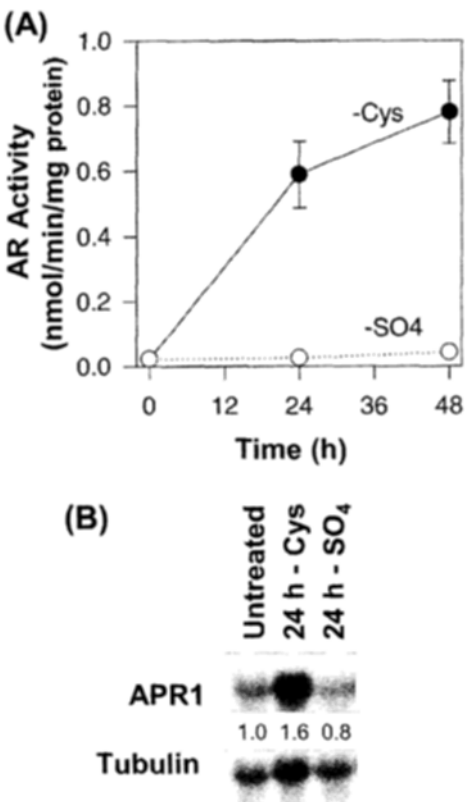

Figure 3. Effect of sulfate and Cys on AR activity and mRNA level in roots of *A. thaliana*. Plants (40-d-old) were pretreated with 1 mM Cys for 24 h. AR activity was measured at various times after removal of either sulfate (open circles) or Cys (closed circles). **(A)** Values are expressed as mean ± standard error of four replicates in nmol min^{-1} mg^{-1} protein. AR mRNA level was measured 24 h after transfer to sulfate- or Cys-free medium. **(B)** Eight plants were used for RNA isolation, and 20 µg total RNA was electrophoresed, blotted, and sequentially analyzed with probes for AR (APR1) and then tubulin. The tubulin probe was used to control for RNA loading. Number below each lane is relative intensity of signal, corrected for RNA loading; control was assigned a value of 1.0.

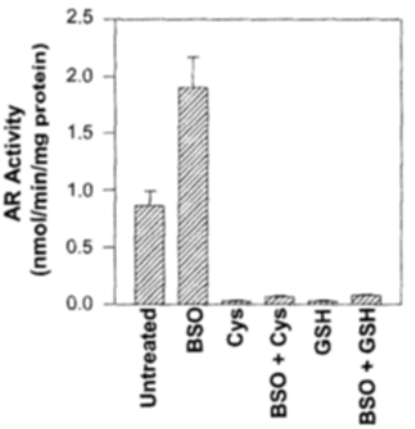

Figure 4. Effect of BSO, Cys, and GSH (alone or in combination) on AR activity in roots of *A. thaliana*. Forty-d-old plants were treated for 24 h with one of the following: 1 mM BSO, 1 mM Cys, 1 mM BSO plus 1 mM Cys, 1 mM GSH, or 1 mM BSO plus 1 mM GSH, as indicated below graph; then assayed for AR activity. Values are expressed as mean ± standard error of four replicates; activity is in nmol min^{-1} mg^{-1} protein.

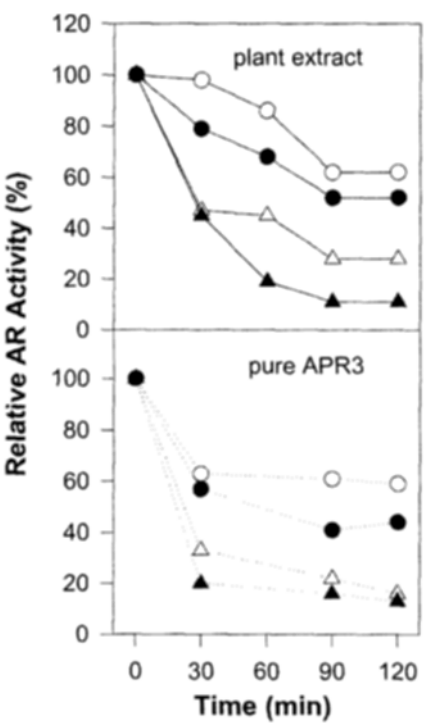

Figure 5. Effect of Cys on AR activity *in vitro*. Plant leaf extract (100 µg) **(A)** or AR (APR3) protein (0.1 µg) **(B)** was pre-incubated at room temperature (23~24°C) over several time periods and with various concentrations of Cys, i.e., 0.1 mM (closed circles), 1 mM (open triangles), 10 mM (closed triangles); open circles indicate untreated control. In reaction mixture, the same concentration of Cys was added as in pre-incubation treatment. Enzyme assay required 15 min. Relative AR activity for control was 100 (%), and values are mean of two replicates.

had a greater influence on AR recovery than did sulfate removal (Fig. 3A). We attributed this to a direct effect by Cys rather than to an indirect impact on intracellular sulfate concentration (Fig. 1C and 2C). Our results contrast with those previously reported, in which sulfate assimilation was thought to be regulated by the intracellular sulfate level (Smith, 1975, 1980; Datko and Mudd, 1984; Lass and Ullrich-Eberius, 1984).

In plant cells, GSH functions in the storage of Cys, and the Cys pool is tightly regulated (de Kok et al., 1981; de Kok and Kuiper, 1986). This may due to the toxic effect of Cys. When we treated plants with a high concentration of Cys (1 mM), the GSH pool was significantly increased in comparison with the Cys pool (Lappartient and Touraine, 1996). That response indicates that the Cys effect on AR repression is due to the metabolism of Cys to GSH. Other supporting evidence was the ~2-fold increase in AR activity by BSO (Fig. 4), which blocked Cys conversion to GSH and finally resulted in a diminished GSH pool. Ruegsegger et al. (1990) have also shown that BSO treatment decreases the GSH pool without eliciting any change in the Cys pool. Furthermore, Lappartient and Touraine (1996) have reported that 1 mM BSO treatment inhibits the effect of 1 mM Cys on the repression of ATP sulfurylase in plants. They also demonstrated that ATP sulfurylase is regulated by the GSH pool, and that the Cys effect is through its conversion to GSH. However, our current research showed that 1 mM BSO did not block that 1 mM Cys effect on AR repression, thereby indicating that Cys also can repress AR by itself, without any metabolism to GSH.

Although Cys caused AR activity to decline, this decrease was not correlated with the steady-state level of mRNA (Fig. 1B, 2B and 3B). The asynchrony between mRNA level and enzyme activity suggests that AR is regulated by allosteric inhibition, or some other enzymatic control. Brunold (1978) showed that AR activity is inhibited by Cys *in vitro*, a conclusion that we confirmed here by testing the effect of Cys on the inhibition of purified AR (Fig. 5).

In conclusion, Cys can repress AR activity without its conversion to either sulfate or GSH. Furthermore, we believe that Cys is an important factor in regulating the sulfate assimilation pathway.

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LITERATURE CITED

Bick JA, Setterdahl AT, Knaff DB, Chen Y, Pitcher LH, Zilinskas BA, Leustek T (2001) Regulation of the plant-type 5'-adenyl sulfate reductase by oxidative stress. Biochem 40: 9040-9048

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72: 248-254

Bruhl A, Haverkamp T, Gisselmann G, Schwenn JD (1996) A cDNA clone from *Arabidopsis thaliana* encoding plastidic ferrodoxin:sulfite reductase. Biochim Biophys Acta 1295: 119-124

Brunold C (1976) Regulation of adenosine-5'-phosphosulfate sulfotransferase activity by H_2S in *Lemna minor* L. Planta 133: 85-88

Brunold C (1978) Regulation of sulfate assimilation in plants. Cysteine inactivation of adenosine 5-phosphosulfate sulfotransferase in *Lemna minor* L. Plant Physiol 61: 342-347

Cho EK, Hong CB (2004) Molecular cloning and expression pattern analyses of heat shock protein 70 genes from *Nicotiana tabacum*. J Plant Biol 47: 149-159

Chomsczynsky P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159

Datko AH, Mudd SH (1984) Sulfate uptake and its regulation in *Lemna paucicostata* Hegelm. Plant Physiol 75: 466-473

de Kok LJ, de Kan PJL, Tanczos OG, Kuiper JC (1981) Sulphate induced accumulation of glutathione and frost-tolerance of spinach leaf tissue. Physiol Plant 53: 435-438

de Kok LJ, Kuiper PJC (1986) Effect of short-term dark incubation with sulfate, chloride and selenate on glutathione content of spinach leaf discs. Physiol Plant 68: 477-482

Harrington HM, Smith IK (1980) Cysteine metabolism in cultured tobacco cells. Plant Physiol 65: 151-155

Hart JW, Filner P (1969) Regulation of sulfate uptake by amino acids in cultured tobacco cells. Plant Physiol 44: 1253-1259

Hawkesford MJ, Belcher AR (1991) Differential protein synthesis in response to sulphate and phosphate deprivation: Identification of possible components of plasma-membrane transport systems in cultured tomato roots. Planta 185: 323-329

Hawkesford MJ, Davidian JC, Grignon C (1993) Sulfate/proton cotransport in plasma-membrane vesicles isolated from roots of *Brassica napus* L.: Increased transport in membranes isolated from sulphur-starved plants. Planta 190: 297-304

Herschbach C, Rennenberg H (1991) Influence of glutathione (GSH) on sulfate influx, xylem loading and exudation in excised tobacco roots. J Exp Bot 42: 1021-1029

Herschbach C, Rennenberg H (1994) Influence of glutathione (GSH) on net uptake of sulphate and sulphate transport in tobacco plants. J Exp Bot 45: 1069-1076

Jenni BE, Brunold C, Zryd JP, Lavanchy P (1980) Properties

and regulation of adenosine 5'-phosphosulfate sulfotransferase from suspension cultures of *Nicotiana sylvestris*. Planta 150: 140-143

Jensen P, Konig T (1982) Development of regulation mechanisms for SO_4^{2-} influx in spring wheat roots. Physiol Plant 55: 459-464

Kopriva S, Muheim R, Trachsel N, Catalano C, Suter M, Brunold C (1999) Light regulation of assimilatory sulphate reduction in *Arabidopsis thaliana*. Plant J 20: 37-44

Kopriva S, Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: Interaction with N and C metabolism. J Exp Bot 55: 1831-1842

Lappartient AG, Touraine B (1996) Demand-driven control of root ATP sulfurylase activity and SO_4^{2-} uptake in intact canola. Plant Physiol 111: 147-157

Lappartient AG, Touraine B (1997) Glutathione-meditated regulation of ATP sulfurylase activity, SO_4^{2-} uptake, and oxidative stress response in intact canola roots. Plant Physiol 114: 177-183

Lass B, Ullrich-Eberius CI (1984) Evidence for proton/sulfate cotransport and its kinetics in *Lemna gibba* G1. Planta 161: 53-60

Lehrach H, Diamond D, Wonzney JM, Boedtker H (1977) RNA molecular weight determinations by gel electrophoresis under denaturing conditions: A critical reexamination. Biochem 16: 4743-4751

Leustek., Martin MN, Bick JA, Davies JP (2000) Pathway and regulation of sulfur metabolism revealed through molecular and genetic studies. Annu Rev Plant Physiol Plant Mol Biol 132: 141-165

Logan HM, Cathala N, Grignon C, Davidian JC (1996) Cloning of a cDNA encoded by a member of the *Arabidopsis thaliana* ATP sulfurylase multigene family. J Biol Chem 271: 12227-12233

Maggioni A, Renosto F (1977) Cysteine and methionine regulation of sulfate uptake in potato tuber discs *Solanum tuberosum*. Physiol Plant 39: 143-147

Rennenberg H, Kemper O, Thoene B (1989) Recovery of sulfate transport into heterotrophic tobacco cells from inhibition by reduced glutathione. Physiol Plant 76: 271-276

Rennenberg H, Polle A, Martin N, Thoene B (1988) Interaction of sulfate and glutathione transport in cultured tobacco cells. Planta 176: 68-74

Reuveny Z, Filner P (1977) Regulation of adenosine triphosphate sulfurylase in cultured tobacco cells. Effects of sulfur and nitrogen sources on the formation and decay of the enzyme. J Biol Chem 252: 1858-1864

Ruegsegger A, Schmutz D, Brunold C (1990) Regulation of glutathione synthesis by cadmium in *Pisum sativum* L. Plant Physiol 93: 1579-1584

Ruffet ML, Droux M, Douce R (1994) Purification and kinetic properties of serine acetyltransferase free of O-acetyl-serine(thiol)lyase from spinach chloroplasts. Plant Physiol 104: 597-604

Setya A, Murillo M, Leustek T (1996) Sulfate reduction in higher plants: Molecular evidence for a novel 5'-adenylsulfate reductase. Proc Natl Acad Sci USA 93: 13383-13388

Smith IK (1975) Sulfate transport in cultured tobacco cells. Plant Physiol 55: 303-307

Smith IK (1980) Regulation of sulfate assimilation in cultured tobacco cells. Effect of nitrogen and sulfur nutrition on sulfate permease and O-acetylserine sulfhydrylase. Plant Physiol 66: 877-883

Wyss HR, Brunold C (1979) Regulation of adenosine 5'-phosphosulfate sulfotransferase activity by H_2S and cysteine in primary leaves of *Phaseolus vulgaris* L. Planta 147: 37-42

Yildiz FH, Davies JP, Grossman AR (1994) Characterization of sulfate transport in *Chlamydomonas reinhardtii* during sulfur-limited and sulfur-sufficient growth. Plant Physiol 104: 981-987