Plant Physiology, July 1999, Vol. 120, pp. 637–643, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Update on Biochemistry

Sulfate Transport and Assimilation in Plants[1]

Thomas Leustek* and Kazuki Saito

Biotechnology Center for Agriculture and the Environment, Rutgers University, New Brunswick, New Jersey 08901–8520 (T.L.); and Faculty of Pharmaceutical Sciences, Chiba University, Yayoi-cho 1–33, Inage-ku, Chiba 263–8522, Japan (K.S.)

Sulfur is one of the six macronutrients required by plants and is found in the amino acids Cys and Met and in a variety of metabolites. When one considers that sulfur in plants is only 3% to 5% as abundant as nitrogen, it is perhaps understandable that sulfur assimilation has been less well studied. As a part of the Cys molecule, the sulfur group, called a thiol, is strongly nucleophilic (electron-donating), making it ideally suited for biological redox processes. When oxidized, two Cys molecules can form a covalent linkage called a disulfide bond, which is readily broken by reduction to form two thiol groups. Disulfide ↔ dithiol interchange is so versatile that nearly all aerobic forms of life, including plants, have evolved to use this reaction as the dominant form of redox control. Redox control regulates enzymes and protects against oxidative damage.

SULFUR IS THE FUNCTIONAL COMPONENT OF GSH AND OTHER REDOX FACTORS

Free Cys is not used for redox control. It is much too readily oxidized to cystine, the disulfide form, which is visible in the laboratory as a white precipitate that is formed within hours after preparing a solution of Cys. A variety of more stable thiol compounds are involved in redox regulation. The most abundant is glutathione, an enzymatically synthesized tripeptide in which Cys is linked via peptide bonds to the γ-carboxyl group of Glu and the α-amino group of Gly. In plants glutathione is thought to be between 3 and 10 mM, and it is present in the major cellular compartments. The reduced form of glutathione is often referred to as GSH, whereas the disulfide form is GSSG. The balance between forms is overwhelmingly maintained in favor of GSH by the enzyme glutathione reductase, using NADPH as an electron source. The result is that the plant cytoplasm, chloroplast stroma, and mitochondrial matrix are highly buffered in the reducing state. Many intracellular enzymes require reducing conditions for activity, just as they require a specific pH or other properties of their chemical environment. The reason is that Cys residues in proteins can also form disulfide bonds, resulting in a disruption of structure and a loss of activity. There are special cases in which specific disulfide bonds are required for formation of tertiary and quaternary structure in a protein, but this is less common, especially for soluble intracellular proteins.

REDOX FACTORS REGULATE PLANT METABOLIC PATHWAYS

Other factors that use the chemistry of disulfide ↔ dithiol interchange to mediate redox reactions include the proteins thioredoxin, glutaredoxin, and protein disulfide isomerase. These proteins are nearly ubiquitous and play fundamental roles in many different types of regulation (Fig. 1). One of the first and best examples of the function of thioredoxin comes from Buchanan (1991). The dark reactions of CO_2 fixation must be strictly coordinated with the light reactions of photosynthesis. The coordination mechanism relies on the reductive activation of specific enzymes by thioredoxin, which is reduced by photosynthetically reduced Fd. New disulfide ↔ dithiol redox regulated processes are being discovered each year, which attests to the prevalent roles that sulfur chemistry plays in biology.

GLUTATHIONE IS IMPORTANT IN STRESS MITIGATION

Because of its nucleophilic properties, glutathione serves as the first line of defense against the products of oxygen metabolism, reactive oxygen species, and other electrophilic compounds such as toxins (herbicides), xenobiotics, and heavy metals (May et al., 1998). When plants encounter reactive oxygen species, glutathione is a direct source of electrons for stress mitigation by the enzyme glutathione peroxidase or an indirect means to maintain a reduced pool of ascorbate, another antioxidant. Glutathione reacts directly with toxins in a reaction mediated by glutathione *S*-transferase. In this way the toxins are inactivated and tagged for transport into the vacuole and for degradation (Kreuz et

[1] This work was supported by the U.S. National Science Foundation (grant nos. IBN 9601146 and MCB 9728661 to T.L.), by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan, and by the Research for the Future Program (grant no. 96I00302) from the Japan Society for the Promotion of Science (to K.S.).

* Corresponding author; e-mail leustek@aesop.rutgers.edu; fax 1–732–932–0312.

Abbreviations: APS, 5′-adenylylsulfate; OAS, *O*-acetylserine; PAPS, 3′-phosphoadenosine-5′-phosphosulfate.

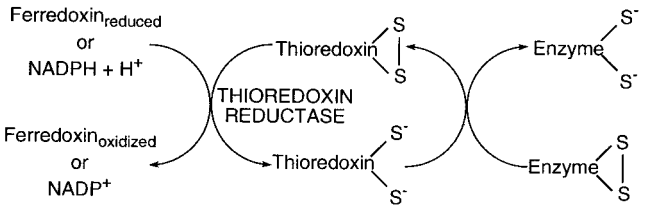

Figure 1. Regulation of metabolism by disulfide ↔ dithiol interchange. The diagram shows how thioredoxin functions as a regulation factor through reduction of a regulatory disulfide on a target enzyme. In the case of C assimilation, the source of electrons for thioredoxin reduction is Fd, reduced via the light reactions of photosynthesis. Thioredoxins also exist in the cytoplasm of plants where NADPH + H^+ serves as an electron source. Recent evidence shows that thioredoxin has the potential to act as an oxidant mediating the formation of a disulfide bond on a target enzyme (Stewart et al., 1998). This activity could be important for activation of antioxidant enzymes during oxidative stress.

al., 1996). In some plants heavy-metal detoxification is mediated by glutathione derivatives called phytochelatins, which have the general structure (γ-glutamylcysteine)$_n$Gly (n = 2–11), and by Cys-rich proteins called metallothioneins. In both molecules thiol groups serve as the metal ion ligand.

PLANT SULFUR ASSIMILATION IMPACTS AGRICULTURE AND THE ENVIRONMENT

The preceding discussion illustrates sulfur's essential, general biological role. However, plants also incorporate sulfur into a wide range of secondary compounds that have an impact, in varied and subtle ways, on our use of plants and on the way that plants influence the environment. For example, the pungent odor and taste of onions, garlic, and cabbage are caused by sulfur-containing secondary compounds. The same compounds can impart an objectionable flavor to canola oil, diminishing its commercial value; but they also have been attributed to disease prevention in humans (Fahey et al., 1997). Some sulfur-containing phytoalexins such as camalexin may be important in combating plant pathogens (Zhao et al., 1998). Although sulfur was long thought not to limit plant productivity, the recent restrictions on emissions of sulfurous air pollutants, the ingredients of acid rain, have resulted in sulfur deficiency in some agricultural areas of the world. Another example is that sulfur assimilation by plants has been implicated as a potential factor in moderating climate. Marine algae are prodigious producers of dimethylsulfoniopropionate, a sulfur-containing analog of betaine (Cooper and Hanson, 1998). Dimethylsulfoniopropionate degradation releases dimethylsulfide, which volatilizes from the ocean and seeds the formation of clouds in the atmosphere. The global scale of this process is such that algal growth may actually influence climate.

OVERVIEW OF THE SO_4^{2-} ASSIMILATION PROCESS IN PLANTS

Sulfur is available to plants primarily in the form of anionic sulfate (SO_4^{2-}) present in soil. It is actively transported into roots and then distributed, mostly unmetabolized, throughout the plant. SO_4^{2-} is a major anionic component of vacuolar sap; therefore, it does not necessarily enter the assimilation stream. Gaseous sulfur dioxide (SO_2) is readily absorbed and assimilated by leaves, but it is significant as a nutrient source only in industrial areas with air pollution. Sulfur is assimilated in one of two oxidation states. SO_4^{2-} can be added to a hydroxyl group of an organic molecule. The reaction is referred to as sulfation and it is catalyzed by sulfotransferases. By contrast, Cys contains reduced sulfur, which is produced from SO_4^{2-} in a multistep pathway in which eight electrons are added to form sulfide (S^{2-}; Reaction 1). The reduction of SO_4^{2-} to S^{2-} consumes 732 kJ mol^{-1}. By comparison, reduction of nitrate to NH_3 requires 347 kJ mol^{-1}. The pathways of SO_4^{2-} assimilation in plants are depicted in Figure 2. The figure shows only those enzymes that are known with certainty by characterization of the defined activity of a purified enzyme and through gene cloning.

$$SO_4^{2-} + ATP + 8\,e^- + 8\,H^+ \rightarrow S^{2-} + 4H_2O + AMP + PPi \quad \text{(Reaction 1)}$$

Cys, the end product of the reductive pathway, is the starting material for production of Met, glutathione, and other metabolites containing reduced sulfur.

In higher plants sulfation is a relatively minor fate for sulfur when compared with the reductive pathway. However, in marine algae, which produce large amounts of sulfated extracellular polysaccharides such as agar, sulfation accounts for a much greater proportion of the total assimilated sulfur.

SO_4^{2-} UPTAKE IS MEDIATED BY A FAMILY OF TRANSPORTERS WITH SPECIALIZED FUNCTIONS

The transport of SO_4^{2-} occurs across several membrane systems as it enters and is distributed throughout the plant and within cells. Transport across the plasma membrane occurs with protons at a ratio of 1 SO_4^{2-}:3 H^+ (symport) and is driven by a proton gradient maintained by a proton ATPase. Transport across the tonoplast membrane is mediated by an unknown mechanism that is driven by the electrical gradient between the vacuole sap and cytoplasm. The phosphate/triose phosphate translocator of the inner chloroplast membrane or a proton/SO_4^{2-} symporter may mediate SO_4^{2-} transport into chloroplasts.

The plasma membrane transporters of plants have been characterized (Smith et al., 1997; Takahashi et al., 1997). The sequences of cDNAs cloned from *Stylosanthes hamata,* Arabidopsis, soybean, barley, maize, resurrection grass, and Indian mustard showed that the plasma membrane transporters of plants are most closely related to fungal and animal proton/SO_4^{2-} cotransporters. Hydropathy analysis revealed that the plant transporters may span the membrane 12 times, a structural feature that is typical of many types of solute symporters.

In most of the species that have been analyzed, SO_4^{2-} transporters are encoded by a gene family. The situation in *S. hamata* is probably typical for most plants. In this species

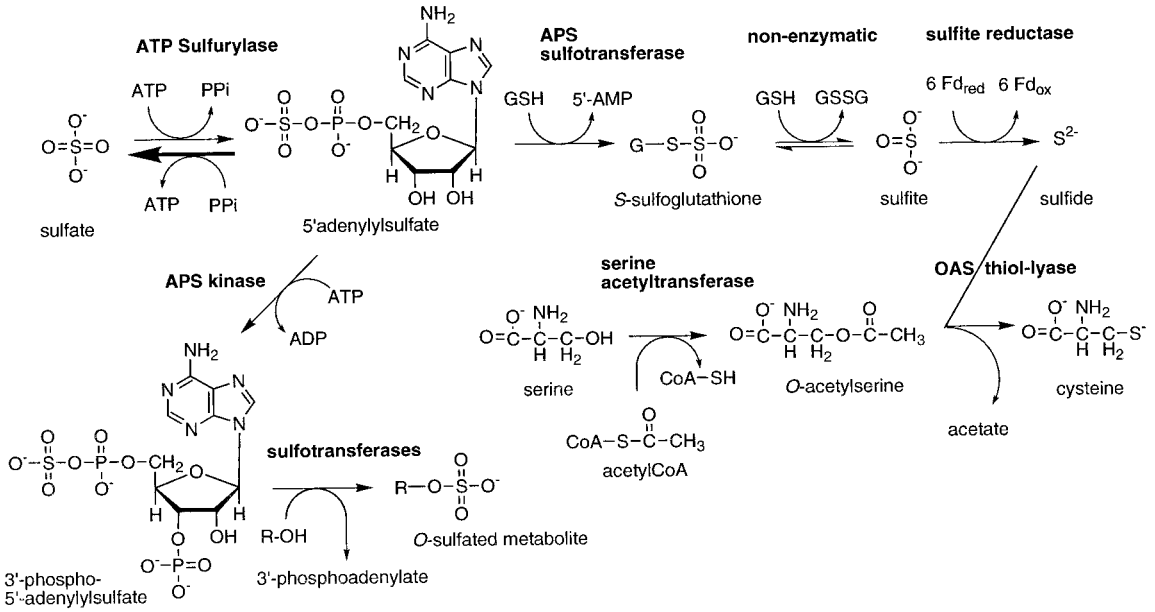

Figure 2. Plant sulfur assimilation pathways showing only those enzymes that have been conclusively demonstrated. The top line shows SO_4^{2-} activation and reduction. The sulfation pathway is shown in the second line on the left and assimilation of reduced sulfur into Cys on the second line on the right. All enzymes are shown in bold above the reaction arrow, whereas intermediates are shown below the chemical structure or in isolation when the chemical structure is not shown. The R- group in sulfated metabolite refers to the metabolite that is sulfated. Fd indicates the reduced and oxidized forms of Fd.

the individual transporters may have specialized functions, since they differ widely in affinity for SO_4^{2-}, and they show distinct spatial and regulated patterns of expression (Smith et al., 1997). High-affinity forms with K_m for SO_4^{2-} of approximately 9 μM are expressed exclusively in roots, whereas the lower-affinity form with K_m for SO_4^{2-} of approximately 100 μM is expressed principally in leaves but also in roots. The steady-state level of mRNA for the high-affinity form increases rapidly after sulfur starvation, whereas the lower-affinity form is unresponsive or responds more slowly to changes in external SO_4^{2-} supply. These results imply that the increase in SO_4^{2-} transport activity observed in roots of sulfur-starved plants is due to an increase in the expression of specific transporters. One of the earliest observations of SO_4^{2-} transport into roots was that the uptake rate varies in relation to the [SO_4^{2-}] of the bathing solution. The results with *S. hamata* suggest that this multiphasic behavior may be due to the activities of separate transporters with different affinities for SO_4^{2-}.

What is the function of multiple SO_4^{2-} transporters? The expression pattern of the high-affinity type suggests that it mediates uptake of SO_4^{2-} into the plant and is a way to adjust to variation in the external sulfur supply. By contrast, low-affinity transporters could function in SO_4^{2-} uptake, both from soil and from the apoplast solution that bathes internal cells. Evidence for specialization of function has been obtained from analysis of an Arabidopsis SO_4^{2-} transporter that is most closely related in sequence to the low-affinity type from *S. hamata* (Takahashi et al., 1997). It is expressed exclusively in the vascular parenchyma of roots and leaves and not in endodermal, cortical, or epidermal cells. The spatial expression pattern of this low-affinity-type transporter indicates that it must be responsible for uptake from the internal apoplastic pool of SO_4^{2-}, not from the soil.

SO_4^{2-} IS AN INERT COMPOUND THAT MUST BE ACTIVATED BEFORE IT CAN BE METABOLIZED

The low reactivity of SO_4^{2-} is a barrier to assimilation that is overcome by formation of a phosphate-SO_4^{2-}-anhydride bond in the compound APS. The reaction is catalyzed by ATP sulfurylase (Reaction 2) and is the sole entry point for metabolism of SO_4^{2-}.

$$SO_4^{2-} + MgATP \rightleftarrows MgPPi + APS \qquad \text{(Reaction 2)}$$

The equilibrium of the adenylation reaction favors the production of SO_4^{2-} and ATP, the reverse reaction. The K_{eq} is 10^{-7} M in vitro, and the forward reaction can be measured only if enzymes that hydrolyze PPi and modify APS are included. Exactly how ATP sulfurylase operates in the forward direction in vivo has yet to be determined, since the conditions do not appear to be in equilibrium. The PPi concentration is approximately 0.3 mM in plant cells. Despite the theoretical difficulty, Arabidopsis ATP sulfurylase is able to produce APS in vivo under apparently nonequilibrium conditions, since it is fully able to substitute for *Escherichia coli* ATP sulfurylase even though the PPi concentration in the *E. coli* cytoplasm is approximately 0.5 mM (Murillo and Leustek, 1995). The Arabidopsis enzyme does not have an intrinsic ability to overcome PPi inhibition, which indicates that extrinsic mechanisms in *E. coli*

facilitate the forward operation of plant ATP sulfurylase, mechanisms that may also function in plant cells.

There are two ATP sulfurylase isoforms in most plants: a major form localized in plastids and a minor form localized in the cytoplasm. Both enzymes have similar kinetic and structural properties. The isoenzymes are encoded by a gene family, and in Arabidopsis there are multiple genes for the plastid enzyme. Arabidopsis contains a cytosolic form of ATP sulfurylase, but the corresponding gene has not yet been identified (Rotte, 1998). The plastid enzyme exists in both leaves and roots and is responsible for initiating the reductive assimilation of SO_4^{2-}, since isolated chloroplasts can form Cys from SO_4^{2-} (Schürmann and Brunold, 1980). The cytoplasmic form probably functions by generating APS for sulfation reactions.

Whether plant ATP sulfurylase plays a role in regulating sulfur assimilation has been studied by a number of investigators (Logan et al., 1996; Lappartient et al., 1999). In general, the activity and steady-state mRNA levels increase when plants are starved for sulfur and decrease when plants are fed reduced forms of sulfur (Cys or glutathione). However, the changes in activity and mRNA, although reproducible, are relatively small; they increase or decrease by approximately 2-fold or less, and the regulation occurs mainly in roots. Two publications report the use of transgenic plants to explore whether ATP sulfurylase regulates SO_4^{2-} assimilation. Hatzfeld et al. (1998) concluded that it is not rate limiting, based on an analysis of a transgenic tobacco cell culture that overexpresses an Arabidopsis ATP sulfurylase but that does not show increased sulfur assimilation. By contrast, transgenic Indian mustard lines that overexpress a different ATP sulfurylase isoenzyme from Arabidopsis accumulate glutathione and show increased resistance to SeO_4^{2-} (Pilon-Smits et al., 1999). SeO_4^{2-} is a toxic analog of SO_4^{2-} that Indian mustard can reduce via the sulfur pathway to a nontoxic, volatile form. The opposite results could be due to differences in experimental systems.

THE SULFATION ROUTE FOR ASSIMILATION: SO_4^{2-} IS DIRECTLY INCORPORATED INTO PLANT METABOLITES

Plant sulfotransferases have been characterized that catalyze the sulfation of flavonol, desulfoglucosinolate, choline, and gallic acid glucoside (Varin et al., 1997). Sulfotransfer is the terminal step in the biosynthesis of these compounds. The function of sulfated flavonol and choline is unknown. Glucosinolates are the compounds responsible for the distinctive taste of mustards. Gallic acid glucoside, also known as turgorin or periodic leaf movement factor, is responsible for triggering nictinastic leaf movement in *Mimosa pudica*. Sulfation may regulate the process by activating the movement factor. The number of sulfated compounds in plants is not known. In contrast, sulfation plays a key role in the production of growth-regulating peptides in animals. However, recently, a sulfated regulator of cell proliferation, phytosulfokine-α, was identified from plants (Matsubayashi et al., 1997).

Several common features of sulfotransferases have emerged from analysis of the enzymes and the encoding cDNAs. The sulfotransferases are strictly dependent upon the phosphorylated APS derivative, PAPS, as a SO_4^{2-} donor, and they all have remarkably high affinity for PAPS. They all contain two highly conserved, sulfotransferase signature sequences that may be involved in PAPS binding. Last, they are all localized in the cytoplasm or, in the case of the gallic acid glucoside sulfotransferase, to the inner surface of the plasma membrane.

Enzymes that synthesize PAPS have been described in plants. But how PAPS is supplied to the sulfotransferases is still to be determined. PAPS is formed through ATP-dependent phosphorylation of the 3′-hydroxyl group of APS, catalyzed by APS kinase (Reaction 3).

$$\text{APS} + \text{ATP} \rightarrow \text{PAPS} + \text{ADP} \qquad \text{(Reaction 3)}$$

Cytoplasmic ATP sulfurylase exists in most plants, but a cytoplasmic form of APS kinase has not yet been specifically identified. However, such an enzyme could exist, or at least cannot be ruled out, because in Arabidopsis there are three different genes that encode APS kinase, and the localization of only one of them has been studied (Lee and Leustek, 1998; Schiffman and Schwenn, 1998). An alternative to in situ, cytoplasmic synthesis of APS and/or PAPS is a system in which the sulfonucleotides are exported from chloroplasts.

SO_4^{2-} IS REDUCED BEFORE INCORPORATION INTO Cys AND THE REDUCTION PATHWAY BEGINS WITH APS

Eight electrons are required to reduce SO_4^{2-} to S^{2-}. The process occurs through the sequential action of two different enzymes. The exact mechanism in plants has been vigorously debated, resulting in a confusing proliferation of hypotheses (Hell, 1997). Rather than reiterating the debate, we discuss here only those enzymes that are known with certainty. Our intention is to simplify a confusing topic.

Several lines of evidence conclusively demonstrate that SO_4^{2-} reduction begins with APS in plants and eukaryotic algae. Schmidt (1972) identified an enzyme he called APS sulfotransferase, which has now been completely purified from a marine red macroalga (Kanno et al., 1996). cDNAs that encode APS sulfotransferase have been cloned from a marine green alga and from several higher plants, most notably Arabidopsis (Bick and Leustek, 1998). That the enzyme encoded by the cloned cDNAs was named APS reductase rather than APS sulfotransferase has inadvertently confounded the subject (Gutierrez-Marcos et al., 1996; Setya et al., 1996). Although there were reasonable arguments for the new name, none of these were conclusive enough to warrant abridgment of the original name. Here we submit to historical precedent and refer to the enzyme as APS sulfotransferase.

The two names derive from two possible catalytic mechanisms, neither of which has yet been confirmed. As proposed by Schmidt (1972), the sulfotransferase transfers SO_4^{2-} from APS to a thiol compound, generating a thiosulfonate. If GSH were used, the product would be *S*-sulfoglutathione (Reaction 4).

$$APS + GSH \rightarrow GS{-}SO_3^- + H^+ + AM \quad \text{(Reaction 4)}$$

By contrast, a reductase would be expected to transfer electrons from two GSH to APS, generating free sulfite (SO_3^{2-}) and GSSG (Reaction 5).

$$APS + 2\,GSH \rightarrow SO_3^{2-} + 2\,H^+ + GSSG + AMP \quad \text{(Reaction 5)}$$

It is clear from the amino acid sequence of APS sulfotransferase that, if it functions as a sulfotransferase, it belongs to a different class than the enzymes described earlier in relation to sulfation. They do not share any common sequence motifs. Rather, based on its amino acid sequence and its function, APS sulfotransferase appears to belong to a family of thiol-dependent reductases and thioltransferases.

GSH PROVIDES ELECTRONS FOR THE FIRST REDUCTION STEP

Mounting evidence indicates that GSH is the most likely in vivo reactant. APS sulfotransferase shows a K_m [GSH] that is consistent with the in vivo concentration of GSH; it appears to be unable to efficiently use other common biological thiol compounds such as thioredoxin (Prior et al., 1999), and a domain of the enzyme is a GSH-dependent reductase that functions in a manner similar to that of glutaredoxin (Bick et al., 1998). Although it is functionally related to glutaredoxin, the amino acid sequence of the domain shows greater homology to thioredoxin. The distinction between thioredoxin and glutaredoxin is more a matter of the preferred electron source than of the sequence. For example, only glutaredoxin is able to use GSH. In this respect, it may be of some significance that glutaredoxin catalyzes reduction of disulfide substrates through a thioltransferase mechanism, i.e. the thiol is transferred with the formation of a glutathione-mixed disulfide intermediate. The analogous reaction for APS sulfotransferase would be the one depicted in Reaction 4. SO_3^{2-} can be produced under the reducing conditions in the chloroplast because *S*-sulfoglutathione is readily reduced nonenzymatically in the presence of excess GSH (Reaction 6) (Schürmann and Brunold, 1980). Based on these considerations we think it is very likely that APS sulfotransferase functions as a GSH:APS sulfotransferase. This hypothesis is depicted in Figure 2.

$$GS^-\,SO_3^{2-} + GSH \leftrightarrow SO_3^{2-} + GSSG + H^+ \quad \text{(Reaction 6)}$$

SO_3^{2+} REDUCTASE COMPLETES THE REDUCTION OF SULFUR WITH ELECTRONS FROM REDUCED Fd

In considering the next reduction step it is significant that *S*-sulfoglutathione and SO_3^{2-} could be available in plastids. Plant SO_3^{2-} reductase catalyzes the reduction of SO_3^{2-} using electrons donated from reduced Fd (Reaction 7). The enzyme has been convincingly demonstrated by purification and cloning of the corresponding gene and cDNA (Bork et al., 1998). SO_3^{2-} reductase shows a high affinity for SO_3^{2-} (K_m = approximately 10 μM), which would serve well for efficient metabolism of SO_3^{2-}.

$$SO_3^{2-} + 6\,Fd_{red} \rightarrow S^{2-} + 6\,Fd_{ox} \quad \text{(Reaction 7)}$$

An Fd-dependent enzyme that reduces thiosulonate to thiosulfide has been measured in cell lysates, but it has not been purified or unambiguously demonstrated. The hypothesis that sulfur is reduced as a thiol-bound form, called the "carrier-bound pathway," has been a tenet of the plant SO_4^{2-}-assimilation field. As indicated by the preceding discussion, the carrier-bound pathway need not be invoked because there is convincing evidence for an efficient SO_3^{2-} reductase. However, the existence of thiosulfonate reductase should not be dismissed as improbable, especially since there has not been a concerted effort to demonstrate it conclusively. Cloning of a thiosulfonate reductase cDNA would be definitive. It is our opinion that an excellent opportunity exists for devising a cloning strategy based on functional complementation of an *E. coli* SO_3^{2-} reductase mutant if the strain is engineered to express plant Fd and NADPH:Fd oxidoreductase.

APS SULFOTRANSFERASE MAY BE A REGULATION POINT IN THE SULFUR-REDUCTION PATHWAY

APS sulfotransferase is encoded by a gene family in Arabidopsis (Bick and Leustek, 1998), all of whose members appear to encode plastid-localized enzymes. APS sulfotransferase is localized only in plastids and not in other cellular compartments (Rotte, 1998). No specialization of function has yet been ascribed to the APS sulfotransferase isoenzymes. SO_3^{2-} reductase is also plastid localized. In Arabidopsis there may be a single gene encoding this enzyme (Bork et al., 1998).

There is a great deal of evidence indicating that APS sulfotransferase is a prime regulation point in SO_4^{2-} assimilation (Brunold and Rennenberg, 1997). The activity of this enzyme changes rapidly in a variety of plant species after sulfur starvation, exposure to reduced sulfur compounds, heavy-metal stress, or other stresses. Heavy metals induce the synthesis of phytochelatins, and high concentrations of metal ions significantly increase the demand for Cys. Recent studies indicate that one potential mechanism for regulating APS sulfotransferase activity may involve changes in the steady-state mRNA level. Sulfur starvation (Gutierrez-Marcos et al., 1996; Takahashi et al., 1997) and heavy-metal treatment (Heiss et al., 1999; Lee and Leustek, 1999) induce the accumulation of APS sulfotransferase mRNA, but the response is limited to roots. By contrast, SO_3^{2-} reductase does not appear to be appreciably regulated at the mRNA level (Bork et al., 1998). The extent to which the regulation of mRNA abundance is responsible for changes in APS sulfotransferase activity has not yet been adequately explored.

INCORPORATION OF REDUCED SULFUR INTO Cys: PROTEIN-PROTEIN INTERACTIONS MAY REGULATE THE PROCESS

The incorporation of S^{2-} into Cys is the last step in reductive SO_4^{2-} assimilation. The reaction is catalyzed by *O*-acetylserine(thiol)lyase from S^{2-} and OAS (Reaction 8).

The synthesis of OAS is catalyzed by Ser acetyltransferase (Reaction 9).

$$OAS + S^{2-} \rightarrow Cys + acetate \quad \text{(Reaction 8)}$$

$$Ser + acetylCoA \rightarrow OAS + CoA \quad \text{(Reaction 9)}$$

Ser acetyltransferase and OAS(thiol)lyase exist in an enzyme complex known as Cys synthase. The stability of the complex is affected by substrates (OAS disrupts it and S^{2-} stabilizes it), and it appears to form through specific protein-protein interactions (Bogdanova and Hell, 1997). Yet, the free form of each enzyme has catalytic activity, and the complex is not required for channeling of OAS (Droux et al., 1998). Moreover, in chloroplasts the ratio of OAS-(thiol)lyase to Ser acetyltransferase is 300:1 (Droux et al., 1998, and refs. therein); therefore, only a fraction of the total OAS(thiol)lyase can be associated in the complex.

One clue to the function of the complex is that association with OAS(thiol)lyase changes the kinetic behavior of Ser acetyltransferase from the Michaelis-Menten type to positive cooperativity with respect to its substrates, Ser and acetyl-CoA (Droux et al., 1998). This suggests that OAS (thiol)lyase functions as a regulatory subunit that regulates Ser acetyltransferase in response to OAS and S^{2-}. Positive cooperativity is a form of allosteric regulation in which the velocity of a bisubstrate enzyme is highly sensitive to small changes in substrate concentration. One can think of the enzyme as having a hair-trigger control mechanism. The idea is appealing because Cys synthesis requires coordination of two converging pathways. If there is insufficient S^{2-} resulting from low activity of SO_4^{2-} reduction, the concentration of OAS will increase, causing dissolution of the Cys synthase complex. By contrast, overactivity of SO_4^{2-} reduction results in overabundance of S^{2-} and a shortage of OAS, a condition that would stabilize the complex. Ser acetyltransferase activity would be regulated, its velocity becoming less or more sensitive to its own substrates. Another possible form of regulation is an increase in the steady-state mRNA level for the plastid form of Ser acetyltransferase after sulfur starvation (Takahashi et al., 1997; Noji et al., 1998). Unlike the earlier steps in the pathway in which mRNA regulation occurs primarily in roots, plastid Ser acetyltransferase mRNA increases primarily in leaves. A third possible regulation mechanism is the feedback inhibition of Ser acetyltransferase by Cys. However, only the cytosolic isoform appears to be regulated in this way (Noji et al., 1998).

Ser acetyltransferase and OAS(thiol)lyase are the only sulfur assimilation enzymes localized in three compartments: the plastids, cytosol, and mitochondria. cDNAs have been cloned from a range of plant species encoding all of the different isoenzymes (Saito et al., 1994). The multifarious localization of Cys synthase enzymes presents a problem in that only plastids contain the full complement of enzymes needed for SO_4^{2-} reduction. It may be that S^{2-} is exported from plastids to supply the substrate required by cytosolic and mitochondrial Cys synthases. Evidence for a specialization of function was obtained by analysis of the cytosolic form of OAS(thiol)lyase from Arabidopsis. It is expressed predominantly in roots, in the vascular parenchyma, and in cortical cells (Gotor et al., 1997). Expression in leaves is concentrated in trichomes. Gotor et al. (1997) noted that the toxic heavy-metal Cd is known to accumulate in trichomes of exposed plants, so it could be that the cytosolic OAS(thiol)lyase has a specialized role, supplying Cys for a detoxification mechanism.

NEGATIVE AND POSITIVE SIGNALS REGULATE SO_4^{2-} ASSIMILATION

From the preceding discussion it is evident that SO_4^{2-} reduction and assimilation into Cys is regulated in plants by a range of mechanisms that include substrate availability, modulation of enzyme activity, and gene expression. Since the exclusive function of SO_4^{2-} reduction is to produce Cys, future studies must concentrate on the question of how the range of regulation mechanisms for individual enzymes is coordinated. Current thinking focuses on both negative and positive signals. It has been known for some time that reduced sulfur compounds such as Cys and glutathione, when applied to plants, repress the activity of the sulfur-assimilation enzymes. Conversely, SO_4^{2-} starvation induces the activity of certain enzymes. With the availability of DNA probes it was discovered that the steady-state levels of mRNA for SO_4^{2-} transporter, ATP sulfurylase, and APS sulfotransferase decline after the application of glutathione or Cys to plant roots (Smith et al., 1997; Lappartient et al., 1999; Lee and Leustek, 1999). In contrast, SO_4^{2-} starvation increases the steady-state level of mRNA for these proteins. Whether these responses are due to transcriptional or posttranscriptional mechanisms is not yet known.

One form of reduced sulfur, glutathione, could act as an endogenous signal because it is known to be transported through the phloem of plants and its level in phloem sap is markedly reduced after short-term sulfur starvation. Further support for this hypothesis comes from the "split-root" experiments of Lappartient et al. (1999) in which a portion of the root system was sulfur starved. In another portion of the root system fed normal levels of sulfate, the steady-state mRNA level and activity for SO_4^{2-} transporter and ATP sulfurylase increased at precisely the time that the level of transported glutathione declined. The implication is that glutathione acts as a negative signal or repressor. Positive regulation could be a derepression phenomenon caused by a decrease in repressor.

Recent experiments indicate, however, that a positive signal may also exist. When OAS was fed to roots of barley, the steady-state mRNA level for the high-affinity transporter increased coordinately with SO_4^{2-}-transport activity (Smith et al., 1997). The response was more rapid than when plants were starved for sulfur, but the magnitude of the increase was smaller. OAS feeding also caused the level of reduced sulfur compounds to increase, possibly explaining why the OAS-induced increase in transporter expression was attenuated compared with SO_4^{2-} starvation. Regulation is probably a balance between negative and positive signals. Other publications have noted that OAS stimulates SO_4^{2-}-reduction enzymes (Smith et al., 1997, and refs. therein). OAS is an appealing candidate as a

positive regulator. It is known to influence the Cys synthase complex. A second effect on the expression of SO_4^{2-}-reduction enzymes could provide a means for coordination of the entire pathway.

Received March 2, 1999; accepted March 23, 1999.

LITERATURE CITED

Bick JA, Åslund F, Chen Y, Leustek T (1998) Glutaredoxin function for the carboxyl terminal domain of the plant-type 5′-adenylylsulfate (APS) reductase. Proc Natl Acad Sci USA **95:** 8404–8409

Bick JA, Leustek T (1998) Plant sulfur metabolism: the reduction of sulfate to sulfite. Curr Opin Plant Biol **1:** 240–244

Bogdanova N, Hell R (1997) Cysteine synthesis in plants: protein-protein interactions of serine acetyltransferase from *Arabidopsis thaliana*. Plant J **11:** 251–262

Bork C, Schwenn JD, Hell R (1998) Isolation and characterization of a gene for assimilatory sulfite reductase from *Arabidopsis thaliana*. Gene **212:** 147–153

Brunold C, Rennenberg H (1997) Regulation of sulfur metabolism in plants: first molecular approaches. Prog Bot **58:** 164–186

Buchanan BB (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspectives on its discovery, present status, and future development. Arch Biochem Biophys **288:** 1–9

Cooper AJL, Hanson AD (1998) Advances in enzymology of the biogeochemical sulfur cycle. Chemtracts-Biochem Mol Biol **11:** 729–747

Droux M, Ruffet ML, Douce R, Job D (1998) Interactions between serine acetyltransferase and *O*-acetylserine(thiol)lyase in higher plants: structural and kinetic properties of the free and bound enzymes. Eur J Biochem **255:** 235–245

Fahey JW, Zhang YS, Talalay P (1997) Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci USA **94:** 10367–10372

Gotor C, Cejudo FJ, Barroso C, Vega JM (1997) Tissue-specific expression of ATCYS-3A, a gene encoding the cytosolic isoform of *O*-acetylserine(thiol)lyase in *Arabidopsis*. Plant J **11:** 347–352

Gutierrez-Marcos JF, Roberts MA, Campbell EI, Wray JL (1996) Three members of a novel small gene family from *Arabidopsis thaliana* able to complement functionally an *Escherichia coli* mutant defective in PAPS reductase activity encode proteins with a thioredoxin-like domain and "APS reductase" activity. Proc Natl Acad Sci USA **93:** 13377–13382

Hatzfeld Y, Cathala N, Grignon C, Davidian JC (1998) Effect of ATP sulfurylase overexpression in bright yellow 2 tobacco cells. Regulation of ATP sulfurylase and SO_4^{2-} transport activities. Plant Physiol **116:** 1307–1313

Heiss S, Schäfer H, Haag-Kerwer A, Rausch T (1999) Cloning sulfur assimilation genes of *Brassica juncea* L.: cadmium differentially affects the expression of a putative low affinity sulfate transporter and isoforms of ATP sulfurylase and APS reductase. Plant Mol Biol **39:** 847–857

Hell R (1997) Molecular physiology of plant sulfur metabolism. Planta **202:** 138–148

Kanno N, Nagahisa E, Sato M, Sato Y (1996) Adenosine 5′-phosphosulfate sulfotransferase from the marine macroalga *Porphyra yezoensis* Ueda (Rhodophyta): stabilization, purification, and properties. Planta **198:** 440–446

Kreuz K, Tommasini R, Martinoia E (1996) Old enzymes for a new job. Herbicide detoxification in plants. Plant Physiol **111:** 349–353

Lappartient AG, Vidmar JJ, Leustek T, Glass ADM, Touraine B (1999) Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compounds. Plant J **18:** 89–95

Lee S, Leustek T (1998) APS kinase from *Arabidopsis thaliana*: genomic organization, expression, and kinetic analysis of the recombinant enzyme. Biochem Biophys Res Commun **247:** 171–175

Lee S, Leustek T (1999) The effect of cadmium on sulfate assimilation enzymes in *Brassica juncea*. Plant Sci **141:** 201–207

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

Matsubayashi Y, Takagi L, Sakagami (1997) Phytosulfokine-α a sulfated pentapeptide, stimulates the proliferation of rice cells by means of specific high- and low-affinity binding sites. Proc Natl Acad Sci USA **94:** 13357–13362

May MJ, Vernoux T, Leaver C, Van Montagu M, Inzé D (1998) Glutathione homeostasis in plants: implications for environmental sensing and plant development. J Exp Bot **49:** 649–667

Murillo M, Leustek T (1995) ATP sulfurylase from *Arabidopsis thaliana* and *Escherichia coli* are functionally equivalent but structurally and kinetically divergent: nucleotide sequence of two ATP sulfurylase cDNAs from *Arabidopsis thaliana* and analysis of a recombinant enzyme. Arch Biochem Biophys **323:** 195–204

Noji M, Inoue K, Kimura N, Gouda A, Saito K (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. J Biol Chem **273:** 32739–32745

Pilon-Smits EAH, Hwang SB, Lytle CM, Zhu YL, Tai JC, Bravo RC, Chen YC, Leustek T, Terry N (1999) Overexpression of ATP sulfurylase in Indian mustard leads to increased selenate uptake, reduction, and tolerance. Plant Physiol **119:** 123–132

Prior A, Uhrig JF, Heins L, Wiesmann A, Lilliq CH, Stolz C, Soll J, Schwenn JD (1999) Structural and kinetic properties of adenylylsulfate reductase from *Catheranthus roseus* cell cultures. Biochim Biophys Acta **1430:** 25–38

Rotte C (1998) Subcellular localization of sulfur assimilation enzymes in *Arabidopsis thaliana* (L.) HEYNH. Diplomarbeit thesis, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany

Saito K, Kurosawa M, Tatsuguchi K, Takagi Y, Murakoshi I (1994) Modulation of cysteine biosynthesis in chloroplasts of transgenic tobacco overexpressing cysteine synthase [*O*-acetylserine(thiol)lyase]. Plant Physiol **106:** 887–895

Schiffmann S, Schwenn JD (1998) Isolation of cDNA clones encoding adenosine-5′-phosphosulfate kinase (EC 2.7.1.25) from *Catharanthus roseus* (accession no. AF044285) and an isoform (akn2) from Arabidopsis (accession no. AF043351) (PGR 98–116). Plant Physiol **117:** 1125

Schmidt A (1972) An APS sulfotransferase from *Chlorella*. Arch Mikrobiol **84:** 77–86

Schürmann P, Brunold C (1980) Formation of cysteine from adenosine 5′-phosphosulfate (APS) in extracts from spinach chloroplasts. Z Pflanzenphysiol **100:** 257–268

Setya A, Murillo M, Leustek T (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5′-adenylylphosphosulfate (APS) reductase. Proc Natl Acad Sci USA **93:** 13383–13388

Smith FW, Hawkesford MJ, Ealing PM, Clarkson DT, Vanden Berg PJ, Belcher AR, Warrilow AG (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter. Plant J **12:** 875–884

Stewart EJ, Åslund F, Beckwith J (1998) Disulfide bond formation in the *Escherichia coli* cytoplasm: an in vivo role reversal for the thioredoxins. EMBO J **17:** 5543–5550

Takahashi H, Yamazaki M, Sasakura N, Watanabe A, Leustek T, de Almeida-Engler J, Engler G, Van Montagu M, Saito K (1997) Regulation of cysteine biosynthesis in higher plants: a sulfate transporter induced in sulfate-starved roots plays a central role in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **94:** 11102–11107

Varin L, Marsolais F, Richard M, Rouleau M (1997) Biochemistry and molecular biology of plant sulfotransferases. FASEB J **11:** 517–525

Zhao JM, Williams CC, Last RL (1998) Induction of *Arabidopsis* tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. Plant Cell **10:** 359–370