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OPEN QUESTIONS ABOUT SULFUR METABOLISM IN PLANTS

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INTRODUCTION

Sulfur in its reduced form plays an important role in plants, being involved in the biosynthesis of primary and secondary metabolites and in the synthesis of coenzymes. Even oxidized sulfur metabolites are necessary to the synthesis of plant sulfolipids in the intact chloroplast membrane. Thus, sulfur plays an important role in plant growth and in the regulation of plant development (see

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books 15, 24a, 30, 31, 49, 59, 73, 74, 85, 97, 109, 111, 112, 132, 136, 143), but little research has so far focused on the primary and secondary steps in sulfur metabolism. Nitrogen metabolism, for example, has received far more attention.

Here we concentrate on unsolved problems in sulfur metabolism, treating areas where no detailed evidence is available so far for oxygenic organisms (higher plants, green algae, or cyanobacteria; we omit phototrophic bacteria) or where current evidence must be questioned owing to the experimental or analytical procedures used. We emphasize areas of sulfate assimilation, including cysteine and glutathione metabolism; we do not discuss in detail methionine synthesis and its further metabolism.

BIOCHEMISTRY AND REGULATION OF SULFUR METABOLISM

Sulfate Uptake and Its Regulation

Sulfate is incorporated into plant constituents after reduction to sulfide (26, 153). The nature of the sulfate carrier is still unknown for plants and green algae; however, evidence from cyanobacteria indicates the involvement of sulfate binding proteins, as in bacteria (70, 71, 80, 128). It is not clear, however, if a sulfate-binding protein is part of the uptake process or if the membrane component itself binds the sulfate molecule. Sulfate uptake increases after prolonged sulfur starvation (11, 14, 26, 153). However, it is unclear if a new sulfate permease is incorporated into the membrane or if only the "normal" permease level increases, leading to a lower apparent K_m for sulfate uptake. Recent evidence from cyanobacteria suggests that a second permease is formed during sulfur starvation (98, 99, 125). The regulation of sulfate uptake itself has two possible regulatory aspects: (*a*) Evidence from bacteria points to cysteine as a regulatory feedback signal controlling sulfate uptake, suggesting that the cysteine molecule itself or a closely related metabolite would be the regulatory signal (45, 58, 77, 85, 91, 121). (*b*) Evidence from green algae and cyanobacteria suggested that the size of the sulfate pool governs sulfate uptake (10–14, 143). This conclusion was based on the observation of increased sulfate uptake in algae even in the presence of cysteine, suggesting that cysteine degradation and oxidation of sulfur to sulfate are limited in these organisms. The situation in higher plants does not contradict this suggestion, since the sulfate pool in plants increases after feeding of reduced sulfur compounds (56, 58). Therefore the size of the sulfate pool could regulate sulfate uptake in higher plants as well.

Sulfate Activation and Metabolism of APS and PAPS

Sulfate is relatively inert; it must be activated for further metabolism (17, 135, 136, 144, 145, 155, 174). Activation is achieved in all cases studied by the

enzymes ATP-sulfurylase forming adenosine-5'-phosphosulfate (APS; Figure 1) and the APS-kinase forming 3'-phosphoadenosine-5'-phosphosulfate (PAPS, (75, 135, 136, 144, 145, 166). These two sulfate-activating enzymes are found in nearly all organisms studied [with the exception of bacterial dissimilatory sulfate reducers, where an APS-kinase has not been detected (129)]. Since the equilibrium for APS formation is far to the left (the phosphate-sulfate anhydride bond in APS is extremely energy rich (about 18 kcal), a secondary pulling reaction for phosphorylation of APS at the 3' position seemed to be necessary for substrate accumulation of PAPS. Obviously this conclusion is not valid for dissimilatory sulfate reducers without PAPS-forming capability (129).

Green algae and higher plants possess multiple ATP-sulfurylases (104). Where are these enzymes located? In spinach the complete pathway of assimilatory sulfate reduction is found within the chloroplast; other organelles have not been ruled out. Since in molds the sulfate reduction pathway is localized within mitochondria (6), one should investigate plant mitochondria

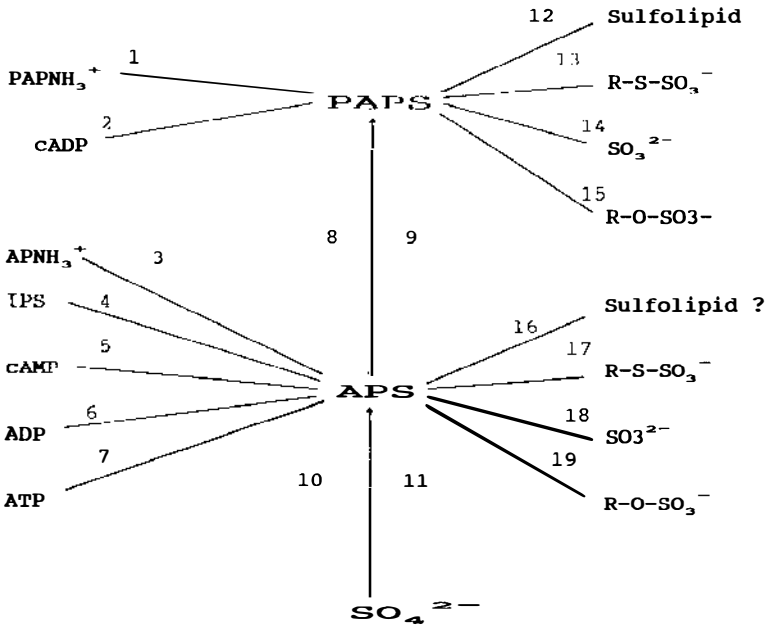

Figure 1 Reactions with sulfonucleotides. 1. PAPS-ammonia-adenyltransferase (hypothetical); 2. PAPC-cADP cyclase (hypothetical); 3. APS-ammonia-adenyltransferase; 4. APS-deaminase; 5. APS-cyclase; 6. ADP-sulfurylase; 7. ATP-sulfurylase; 8. APS-kinase; 9. PAPS-phosphatase; 10. ATP-sulfurylase; 11. APS phosphatase; 12. PAPS-sulfolipid-kinase (uncertain); 13. PAPS-sulfotransferase; 14. PAPS-reductase; 15. PAPS-sulfokinase; 16. APS-sulfolipid-kinase (uncertain); 17. APS-sulfotransferase; 18. APS-reductase; 19. APS-sulfokinase (uncertain)

as well—especially because the enzymes of the sulfate activation sequence have been detected in algal mitochondria (18, 138–140).

Whereas PAPS is the substrate used for sulfate reduction in "normal" bacteria, some phototrophic bacteria, and some cyanobacteria, APS seems to be the preferred substrate for further reduction in higher plants and green algae (47, 103, 146, 148, 153, 155, 172, 182). Two possible mechanisms are found in phototrophic bacteria and cyanobacteria, using either APS or PAPS as substrate for subsequent reduction (149).

Sulfonucleotides are energy-rich in terms of the phosphate-sulfate bond. Thus, the activated sulfate can be transferred to an hydroxyl group forming a sulfate ester (reviewed in 136, 153, 176), which can be hydrolyzed by a sulfatase (arylsulfatase in case of aromatic rings; 30, 31). Transfer to a thiol group forming a thiosulfonate group (thiol-sulfate ester) has been observed (for instance for the APS-sulfotransferase). Transfer to a nitrogen group forming a $N\text{-}SO_3$ linkage should be possible as well, which would change reduction and isotopic exchange reactions known for the thiosulfonate linkage. Thus, no specific information is available on the possible sulfation of amino groups in plants or algae.

We have little information on the function of sulfate esters in the regulation of plant metabolism, although sulfate esters affect certain aspects of metabolic control in animal cells. Tyrosine sulfation has been detected in algae (138). Sulfation of a serine (instead of phosphorylation) seems to tag proteins for export in animals (63, 106, 134).

No such data are available for plants or algae. This situation may follow from two facts: (*a*) that sulfatases are present in green algae and higher plants (30, 31, 40, 105, 117, 119, 165) and (*b*) that such sulfatases increase during sulfate limitation, suggesting a metabolic control related to sulfur starvation. Such a control is evident for the arylsulfatase in green algae, which increases during sulfur starvation (104, 119, 165) and which is found at least to some extent in the periplasmic space, thus increasing possible sulfate sources for growth. Furthermore, sulfate esters seem to regulate the differentiation of the green alga *Volvox* (194), although additional information is needed in this area.

During the last decade, sulfate esters of secondary plant metabolites have been detected; in particular, heterocyclic compounds related to flavins have been found (173, 186, 187). However, no detailed study on the function of the sulfate ester group is currently available. PAPS has been implicated as the primary sulfation donor (46, 72, 186). Speculation suggests better solubility of the sulfated products, but no information concerning specific roles for sulfate esters is available.

Sulfonucleotides may be desulfonated by sulfatases, as has been shown both for animals and plants (30, 31, 136, 153, 176, 182); however, little

information has been obtained either on the specificity of such sulfatases (47, 182) or on specific regulatory signals of such sulfatases. The sulfonucleotide PAPS can be desphosporylated to APS (30, 31, 47, 136, 184), and such phosphatases have been found in algal cells (150, 184); the role of such PAPS-specific phosphorylases is unknown. Recent data show that the sulfonucleotide APS can be deaminated, leading to inosine-5'-phosphosulfate (79).

The high energy bond of APS may be used for the enzymatic replacement of the sulfate group by ammonia (35, 38, 39), leading to an activated amino group in adenosine-5'-phosphoramidate. Such an enzyme was isolated from the green alga *Chlorella* (38). It is not known, whether such an activated amino group is used in any biosynthetic pathway. Chemical side reactions with the phosphate-sulfate group and ammonia are possible (25).

PAPS-specific sulfotransferases seem to be involved in glucosinolate metabolism, a characteristic sulfated compound of the Brassicaceae (46, 72, 164).

Data concerning the APS-sulfotransferase and its regulation have been summarized by Brunold (17, 20). This enzyme is affected by the sulfur demand of the cell, being downregulated by supply of reduced sulfur (e.g. cysteine) and activated by sulfur shortage or a surplus of nitrogen, suggesting a central role of the regulation of assimilatory sulfate reduction at this point. Such an explanation seems sensible, since sulfate ester and sulfolipid formation should be possible without further reduction if reduced sulfur compounds are available. Thus, the regulation of the APS-sulfotransferase occurs precisely at a branching point after sulfate activation, leading either to further reduction or to sulfate ester and sulfolipid formation.

The sulfate group of APS can be transferred to a thiol (1, 20, 104, 146, 147, 185) via an intermediate organic thiosulfate (thiosulfonate); hence the name APS-sulfotransferase (146). A second possibility would be that the APS-sulfotransferase is a thiol-dependent APS-reductase, the oxidized thiol group formed during its reduction being split by free sulfite to a thiosulfonate and a free SH-group, leading to the same results as cell-free artifacts. Both reactions are possible in a cell-free system, and a critical examination of the published data neither excludes nor proves either possibility. Similar questions must be asked regarding PAPS-sulfotransferase and PAPS-reductase (93, 94, 102, 169, 170, 172, 181, 183).

An APS-sulfotransferase acceptor has not been found. Although glutathione will act as an acceptor (171, 185, 195), it may not function as the normal acceptor in vivo and chemical side reactions may also occur, as discussed above. Genetic data are necessary to prove or disprove the concept of a sulfotransferase in relation to a sulfonucleotide reductase. The same requirement applies to the concept of a PAPS-sulfotransferase or PAPS-

reductase (92–94, 102, 182, 183). The main sulfonucleotide metabolic possibilities are summarized in Figure 1.

Sulfolipid and Sulfonic Acid Formation and Degradation

Whereas nitrogen is used only as the reduced product, sulfur is needed also in its oxidized form as a sulfolipid. Here, the sulfur is bound as a sulfonic acid with a direct C-S bond to a quinovose molecule forming a sulfoquinovose (7, 8, 27, 57, 83, 84, 116). Obviously the sulfolipid is necessary to the chloroplast membrane, although no detailed information is available so far about its precise role in photosynthetic membranes.

During the last 20 years little progress has been made concerning the biosynthesis of the C-S bond in the plant sulfolipid (83, 84). Furthermore, data from different organisms seem to contradict the notion of a common biosynthetic pathway. Speculations suggest either a route from PAPS (64) or APS (83) to an unknown acceptor or, alternatively, the oxidation of cysteine (139) to a sulfonic acid with its incorporation into sulfoquinovose (57). At present, no proof exists for an oxidation of cysteine to cysteic acid in higher plants; one can only speculate about the biosynthetic pathway(s) of sulfolipid formation.

The sulfonic acid content of sulfolipids increases rapidly in the light and decreases in the dark (113, 142). Furthermore, green alga can utilize sulfonic acids as their sole sulfur source for growth (10), although the splitting reaction for the C-S bond of sulfonic acids is not known. A permease for sulfonic acids is induced during sulfur starvation in the green alga *Chlorella* (10–13).

Reduction of Bound or Free Sulfite to Bound or Free Sulfide

The concept of a sulfotransferase led to the search for an acceptor for the APS-sulfotransferase; the idea involved a reduction of bound sulfite to bound sulfide. The concept of bound intermediates came from observations of sulfate reduction in intact spinach chloroplasts; these organelles showed no measurable free sulfite and sulfide during photosynthetic sulfate reduction (160) unless an excess of thiols was added. Furthermore, it was possible to use algal proteins in the presence of a purified APS-sulfotransferase to label protein from labeled APS (1, 146, 147, 185). The reduction of such labeled bound sulfite (147, 157) to labeled bound sulfide could be demonstrated in cell-free systems. Again, cell-free systems are susceptible to reactions of free sulfite and sulfide with oxidized thiol groups, forming bound intermediates (Figure 2). A critical examination of my own data (146, 147) does not rule out the possibility that the primary step was the formation of free sulfite which then reacted with disulfide groups to form bound sulfite. This sulfite could then be split easily to free sulfite for further reduction by a sulfite reductase (2, 3, 62, 95, 163, 167, 176, 180, 189, 190), if reduced ferredoxin were

available (which could reduce thiol groups as well). Sulfide could react subsequently with oxidized thiols, forming a persulfide (bound sulfide). It has not been possible so far to clarify this question in cell-free systems, owing to the side-reactions discussed here.

Even the presence of zero-valence sulfur in green algae (78, 87) and spinach chloroplasts is not proof of either a bound or free pathway, since secondary formation of bound (zero-valence) sulfur with free sulfide as an intermediate cannot be ruled out. Thus, the identification of the sulfite or thiosulfonate reductase by genetic methods and its controlled inactivation will be necessary to demonstrate precisely the in vivo function of either the thiosulfonate or sulfite reductase. Recent evidence from higher plants suggests that a sulfite reductase is indeed regulated by the demand for reduced sulfur compounds (163, 189–191); however, again the distinction between sulfite and nitrite reductases must be clarified.

Since bound sulfide can be split by free sulfite to thiosulfate and a free SH-group as a chemical side reaction, one might speculate that the thiosulfate-trithionate pathway reported for microorganisms might be a cell-free artifact as well.

A possible link between nitrite and sulfite reductase and thus nitrogen and sulfur interaction might be possible by the regulation of siroheme. This cofactor is needed for sulfite, and nitrite reduction (118, 176). Sulfite seems to be bound to the iron complexed in siroheme, leading to no free intermediates during the 6-electron reduction step. While genetic data linking siroheme formation and sulfite and nitrite reductase are still missing for plants, evidence from *Escherichia coli* indicates a control of siroheme synthesis by the *cysG* gene product and the *nirB* gene product of the nitrate assimilation pathway (126, 127, 196). Cyanobacteria might be the organisms of choice for studying such possibilities in phototrophic organisms.

Cysteine Metabolism and Its Regulation

Cysteine biosynthesis is catalyzed by the cysteine synthase using O-acetyl-L-serine and sulfide as substrates (43–45, 160, 179). Isoenzymes are found in cyanobacteria (29), green algae (88), and higher plants (36, 37). Measurements of free sulfide in plants and algae by isotopic exchange reactions (interacting with bound sulfides as well) showed normal concentrations in the micromolar range whereas the apparent K_m determined in cell-free systems for sulfide is about 1000 times as high (millimolar range). This apparent discrepancy has not been resolved. Furthermore, the catalytic capacity of the cysteine synthase exceeds sulfur assimilation needs by a factor of several hundred, casting doubt on sulfide as the normal substrate for this enzyme. It might be speculated that a sulfurtransferase—for instance, a rhodanese (188)—could transfer the reduced sulfur from the corresponding reductase to the cysteine synthase, changing possible substrates and

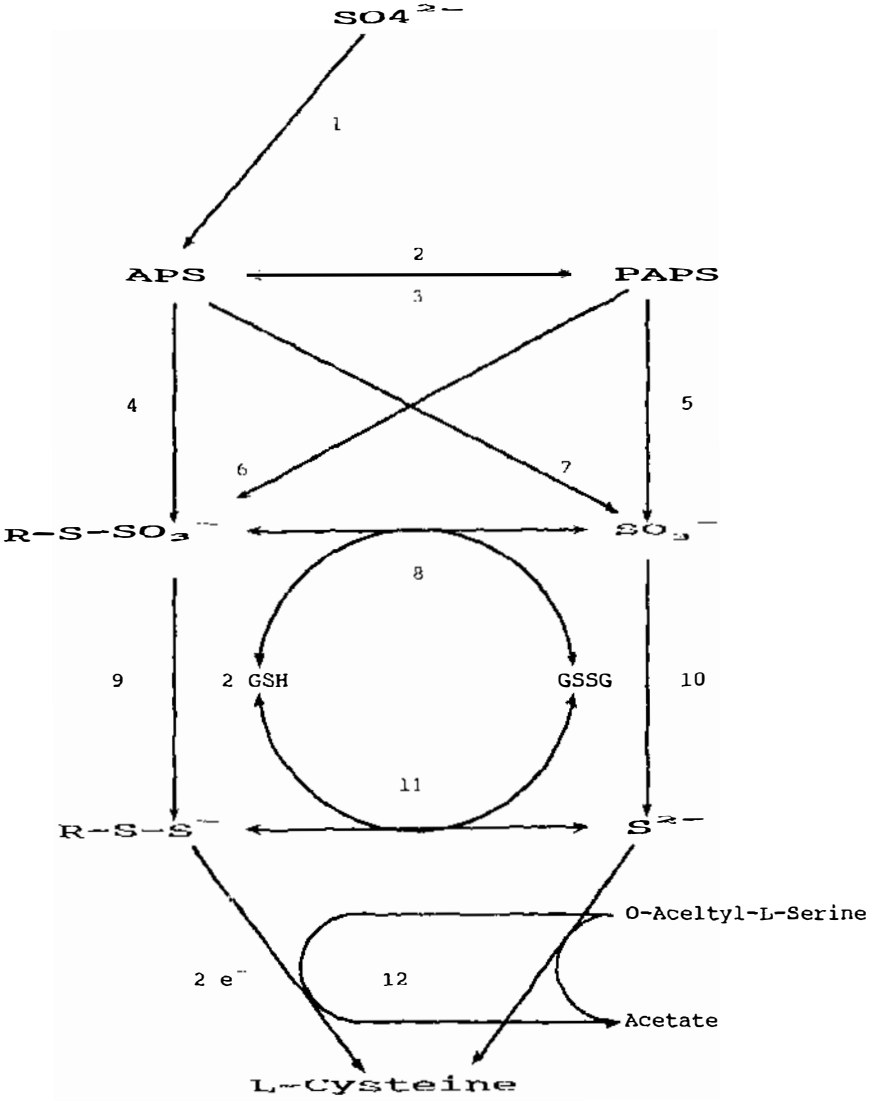

Figure 2 Possible pathways for the reduction of sulfate to L-cysteine. 1. ATP-sulfurylase; 2. APS-kinase; 3. PAPS-sulfatase; 4. APS-sulfotransferase; 5. PAPS-reductase; 6. PAPS-sulfotransferase; 7. APS-reductase; 8. Sulfitolysis (chemical); 9. Thiosulfonate reductase; 10. Sulfite reductase; 11. Sulfidolysis (chemical); 12. Cysteine synthase

catalytic properties. The plant-type cysteine synthase will not accept thiosulfate as sulfur donor, nor was formation of S-sulfo-L-cysteine from O-acetyl-L-serine detected in algae and higher plants. However, coupling of thiosulfate with a thiosulfate reductase and cysteine synthase readily demonstrated the formation of cysteine in a cell-free system (A. Schmidt, unpublished results).

Cysteine synthase is regulated in green algae, although its increase during sulfur starvation can be measured only after prolonged starvation (24 hr); by comparison, sulfate uptake reactions occur within 2–3 hr. This difference may be due to the fact that cysteine can be supplied by degradation of glutathione during sulfur starvation. Clearance of the glutathione pool needs about 15–20 hr after onset of sulfur limitation, showing clearly a different regulatory signal for green algae and cyanobacteria for the oxidized and reduced pathways of sulfur metabolism. Regulation of cysteine synthase can be detected during plant development (162, 191).

Cysteine in higher concentrations (about 0.5 mM) is toxic for green algae and cyanobacteria, causing a bleaching of cells. Plant cells can obviously regulate the cysteine pool precisely to a critical level. If higher levels are supposed, cysteine is either masked or degraded in order to keep its concentration within a tolerable range. Cysteine can be masked, blocking its carboxylic group in γ-linkage by glutamine and its amino group by glycine, forming glutathione (GSH). This masking prevents membrane-catalyzed oxidation of cysteine (86, 159) to cystine by oxygen; this oxidation is not catalyzed with glutathione as a substrate.

Presently, only the pathway leading to methionine (cystathionine, homocysteine, methionine) has been analyzed in detail (see 43–45), whereas other reactions of cysteine have been demonstrated using partially purified protein sources from either plants or algae (Figure 3). However, a clear concept of cysteine metabolism, other than as a precursor of methionine, is missing. Obviously, excess cysteine is split to sulfide in plants and algae, which can be either oxidized to sulfate or emitted as sulfide into the air (85, 130, 131).

However, a clear distinction of possible intermediates leading to free sulfide has not been possible. Formation of free sulfide from L-cysteine could be achieved either by a L-cysteine- or D-cysteine-specific desulfhydrolase with a possible interaction of a racemase (151, 154); by a cystine lyase (178); by intermediate formation of β-mercaptopyruvate (either by transamination or amino acid oxidases) leading to free sulfide by a mercaptopyruvate sulfurtransferase (152); by a side-reaction of a cystathionase (4, 44); or even by a β-cyanoalanine synthase, liberating free sulfide (67–69). Furthermore, a direct oxidation of the SH-group of cysteine to a cysteinesulfinic acid by molecular oxygen (cysteine dioxigenase) has been reported for animals (199), but no such information is available for photosynthetic organisms.

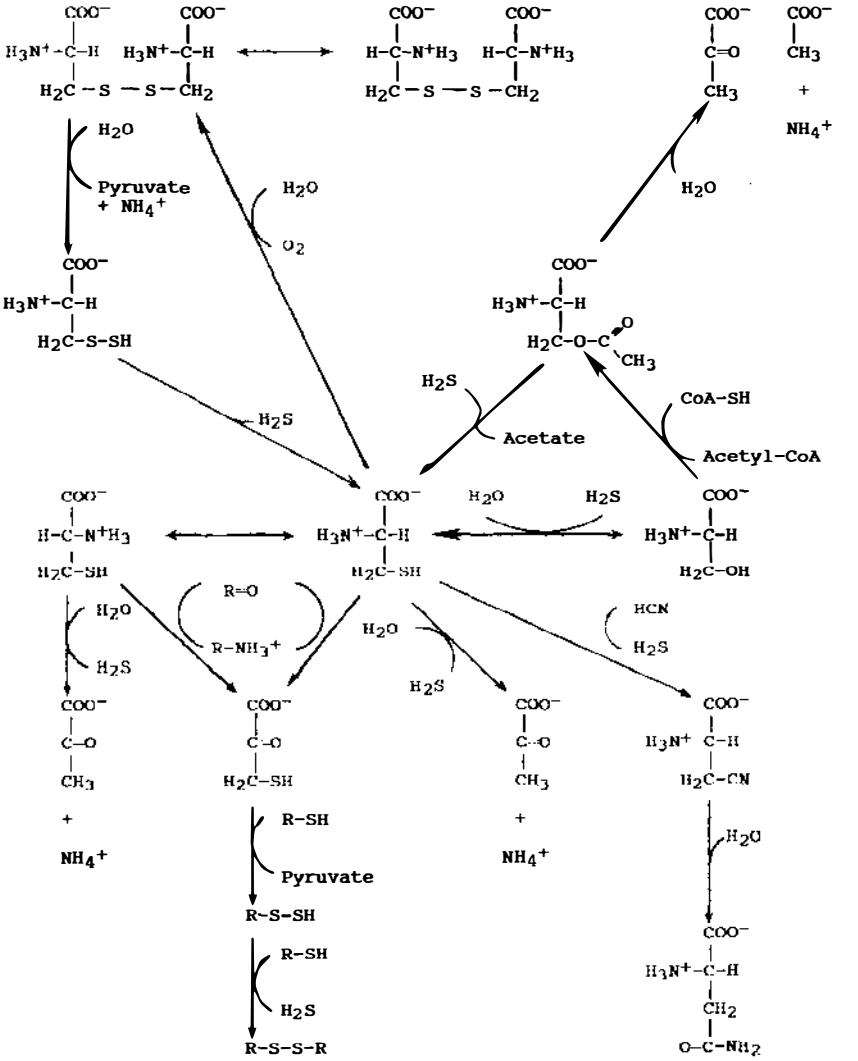

Figure 3 Metabolism of L-cysteine

We must assume that cysteine is a sulfur donor for iron-sulfur clusters and coenzyme biosynthetic pathways; a rhodanese or thiosulfate reductase (depending on the assay conditions; 24, 137, 158, 188) might be involved. Little solid evidence is available for any of the discussed possibilities, with the exception of methionine biosynthesis (43, 45, 107).

Plants do contain the enzyme β-cyanoalanine synthase, converting L-

cysteine to β-cyanoalanine, liberating free sulfide (24a, 67–69, 197, 198). β-Cyanoalanine formed may be metabolized further to asparagine (23). β-Cyanoalanine synthase is not restricted to cyanogenic plants; it is found in normal green plants like spinach, and it can be separated from the cysteine synthase (156). Its demonstration in green algae is not clear as yet, since the algal cysteine synthase will accept cyanide and cysteine forming β-cyanoalanine and sulfide at a rate of about 1% of its biosynthetic capacity (88). The function of this enzyme in plants is obscure, but one might speculate that asparagine is formed by this route, which would provide another element in the balanced control of sulfate and nitrate assimilation.

Glutathione and Phytochelatin Metabolism

The tripeptide glutathione (GSH = γ-glutamyl-cysteinyl-glycine) is the most abundant thiol in plants and algae (81, 96, 133). It serves in control of the thiol-disulfide status of the cell and as an electron donor in the detoxification of H_2O_2 and peroxides that prevents damage by activated oxygen species (see 81, 82, 133). In animals glutathione peroxidase is a selenium-containing enzyme that has not been found in plants; however, the green alga *Chlamydomonas* seems to utilize a selenium-containing glutathione peroxidase (175). Detoxification in plant systems seems to be catalyzed by an ascorbate peroxidase (41). GSH reduces the oxidized ascorbate, although other thiols such as thioredoxin will reduce oxidized ascorbate as well.

Glutathione accumulates after excess feeding of sulfur compounds if the normal regulatory control mechanisms are circumvented (as for instance after feeding sulfide), suggesting that glutathione functions as a storage pool for excess cysteine. Such a function can be demonstrated also by sulfur starvation conditions, where glutathione is slowly degraded and used as a sulfur source (60). Nitrogen starvation does not decrease the GSH pool for the green alga *Chlorella,* suggesting that it is not used as a storage product for reduced nitrogen (60). GSH is synthesized by a γ-glutamyl-cysteine synthase and has been characterized from *Nicotiana tabacum* (9). This compound is condensed with glycine by the glutathione synthase, forming GSH (100). Degradation seems to be catalyzed by a carboxypeptidase with subsequent splitting of the γ-glutamyl-cysteine by a γ-glutamyl-cyclotransferase, forming 5'-oxo-proline, which is split by a 5'-oxo-prolinase, forming glutamate (see 81, 133)

Reduced thiol groups, especially when present in high concentrations as in glutathione, catalyze the activation of oxygen, producing activated oxygen species. These in turn inactivate other proteins, including glutathione reductase itself, leading to specific protein splitting by oxygen radicals (60). Plants contain a small, not yet fully characterized sulfur-containing substance that protects against oxygen radical inactivation (60). Its relation to GSH has not

been established yet, but it is abundant under sulfur starvation and scarce under high GSH load. Its precise role, especially in the chloroplast, needs further characterization.

Related to glutathione metabolism are the phytochelatins (50–55), which have been detected in plants as heavy-metal binding peptides consisting of poly-(γ-glutamyl-cysteinyl)-glycine with a chain length between 2 and 5 (γ-glutamyl-cysteinyl) repetitive sequences. Plants under heavy-metal stress synthesize phytochelatin by a γ-glutamylcysteinyl-dipeptidyl transpeptidase (50) using glutathione as a substrate. Metal-binding phytochelatins may be universal in the plant kingdom (51). Obviously, animal species use a different heavy-metal complexing agent in the metallothioneins. These have not been demonstrated conclusively in plants, and the situation in cyanobacteria is uncertain. One might speculate that phytochelatins are involved in normal heavy-metal metabolism of plant cells as well.

Glutathione plays an important role in the detoxification of drugs and herbicides, as discussed in recent reviews (73, 96). Conjugation is catalyzed by glutathione transferases present in plants; these transferases have not been analyzed carefully in green algae and cyanobacteria.

GENETICS OF SULFUR METABOLISM

The Cysteine Regulon in Prokaryotes

In *Escherichia coli* and *Salmonella typhimurium* the structural genes coding for proteins of the sulfate assimilation pathway are widely scattered over five different regions—for example, from 28 min to 80 min on the *E. coli* genome (5, 141). The cysteine regulon of both organisms consists of at least 16 genes necessary for the synthesis of O-acetyl-L-serine, the uptake and reduction of sulfate to sulfide, and the reaction of sulfide with O-acetyl-L-serine to form L-cysteine [Figure 4; for a detailed review see Kredich 1987 (89)].

Regulation of the *cys* regulon is achieved through feedback inhibition of O-acetyl-L-serine synthesis by cysteine (90, 91), and by a set of positive gene activation factors (121). Full gene expression of the cysteine regulon requires a positive regulatory protein encoded by *cysB* (CysB protein), O-acetyl-L-serine or N-acetyl-L-serine as an inducer, and sulfur limitation. O-acetyl-L-serine is required for transcription initiation and increased binding of the bacterial activator protein to the promoter region. Sulfide inhibits both transcription initiation and binding in a competitive manner and acts as an anti-inducer (115, 122). Only *cysG* and *cysE* are not under positive control of the CysB protein (28, 90, 91, 126).

Sulfate Transport

The sulfate transport systems or sulfate permeases of *E. coli* and *S. typhimurium* are part of the cysteine regulon and subjected to the control of CysB

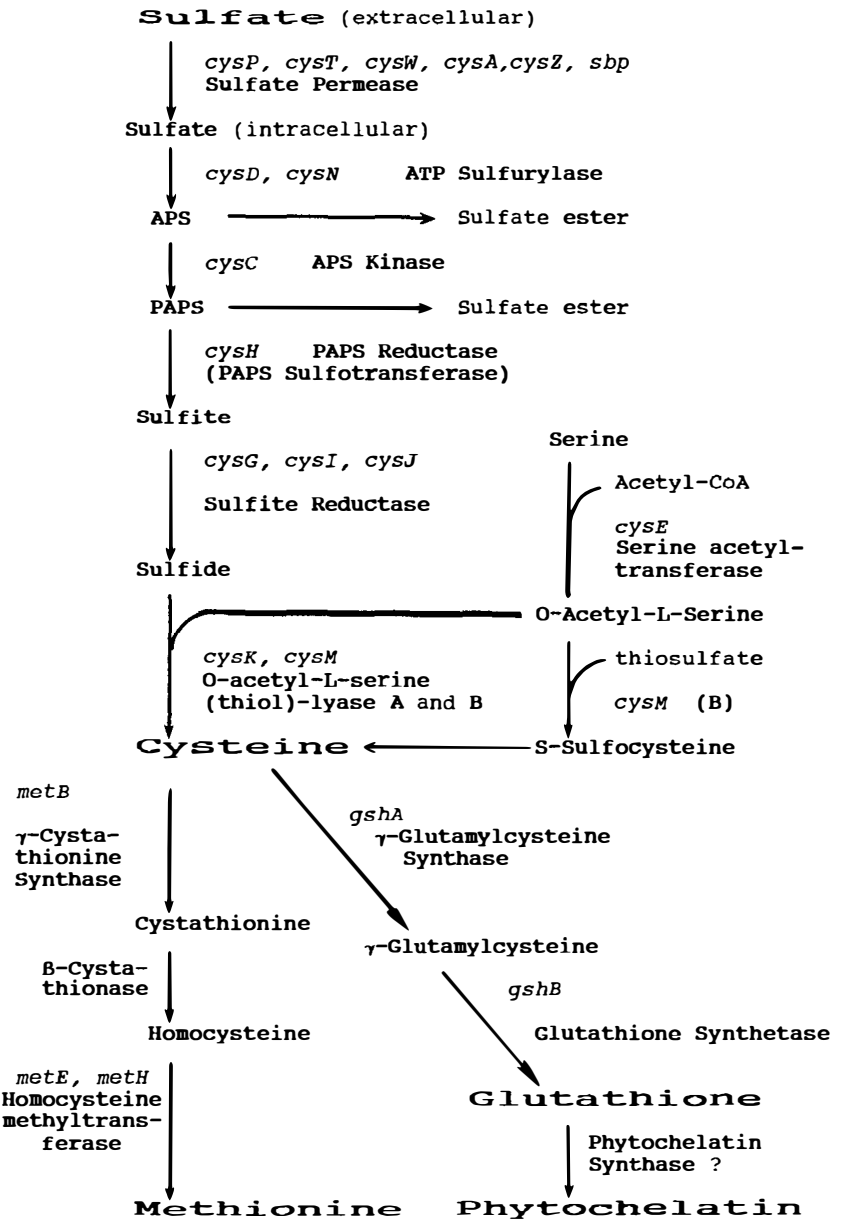

Figure 4 Pathway of cysteine, methionine, and glutathione biosynthesis in reference to the synthesis in *Escherichia coli* and and *Salmonella typhimurium*

protein. Gene expression of this region requires sulfur limitation. Mutations impairing sulfate transport map in the "*cysA*" region (33, 114). In *E. coli*, a second locus, *cysZ* has been reported to be essential for sulfate transport (21, 125). Recently, Sirko et al (177) and Hryniewicz et al (65) cloned and sequenced the "*cysA*" region in *E. coli* K-12, whereby the gene order *cysP cysT cysW cysA cysM* has been established. *cysP*, the first gene of the sulfate transport operon, is expressed from a CysB-dependent promoter and encodes a thiosulfate-binding protein (65). The deduced amino acid sequence indicated the presence of a signal peptide, and the periplasmic location of the mature CysP protein was confirmed experimentally. The corresponding region of the *S. typhimurium* chromosome exhibited a strictly homologous counterpart of the *cysP* operon. The CysP protein showed only 45% sequence similarity with the *S. typhimurium* sulfate-binding protein (SBP; 124), its genetic location is still unknown. The deduced amino acid sequences of CysT, CysW, and CysA are very similar to almost all membrane-spanning osmotic shock-sensitive transport systems, whereby CysT and CysW may represent integral membrane components and CysA a hydrophilic protein with a nucleotide-binding consensus motif (177). The presence of *cysM*, the gene coding for O-acetyl-L-serine (thiol)-lyase B, which is involved in thiosulfate metabolism, argues for the role of CysP as a thiosulfate-binding protein.

A "*cysA*" region of *Synechococcus* PCC 7942 is the only other sulfate permease identified so far (48). Further characterization of the region of DNA adjacent to *cysA* led to the identification of other components of periplasmic transport systems (98, 99). The gene organization in this unicellular cyanobacterium is *rhdA cysV cysU sbpB cysW cysR orf81 cysT sbpA cysA*. Transciption of *cysA* occurs in the direction opposite that of a gene encoding the sulfate-binding protein (*sbpA*). The cyanobacterial CysT, CysW, and CysA proteins are most similar to the functionally analogous proteins in *E. coli*. The products of the *cysT* and *cysW* genes exhibit a high degree of similarity to the MbpY protein, the product of a gene found on the chloroplast genome of the liverwort *Marchantia polymorpha*. A second protein, MbpX is similar to the CysA nucleotide-binding protein. Laudenbach et al (99) postulate that the products of the *mbpX* and *mbpY* genes may represent components of a sulfate permease system in the chloroplast envelope.

There are many differences between the genomic organization of the "*cysA*" region of *E. coli* and that of the cyanobacterium. In *Synechococcus*, a periplasmic protein of 33 kDa that accumulates specifically under sulfur-limiting conditions, was identified as the *rhdA* gene product. The amino acid sequence of this protein shows some similarity (26%) to that of bovine liver rhodanese (thiosulfate-cyanide sulfurtransferase), an enzyme that transfers the thiol group of thiosulfate to a thiophilic acceptor molecule (137). However, a *rhdA* mutant grew well on sulfate, thiosulfate, and tetrathionate. Additional-

ly, the purified protein contained low levels of rhodanese activity but showed no thiosulfate reductase activity. Molecular chaperonins are required for proper folding of rhodanese (108, 110). It is possible that partial denaturation during the purification of the 33-kDa protein lowered its rhodanese activity.

The close location of *rhdA* to genes encoding proteins of the sulfate transport system and its periplasmic localization suggest its possible function as a binding protein for a yet untested sulfur compound. The cyanobacterial genome is large enough to harbor another gene encoding a protein with similar properties. In addition, the "*cysA*" region of *E. coli* lacks a *sbpA* or *cysR* gene. *sbpA* in *E. coli* is found at 89 min on the bacterial genome (5). Located between *cysT* and *cysW* is *cysR* and an unknown small open reading frame (*ORF81*). CysR exhibits some homology to a family of prokaryotic regulatory proteins, containing a potential helix-turn-helix motif, involved in DNA binding (16). *cysR, sbpA, cysT,* and *ORF81* mutants are incapable of growth with thiocyanate as the source of sulfur. A more detailed study of these mutation effects, cspecially with respect to thc transcription of these genes, is definitely needed.

Sulfate Activation

Sulfate is reduced to sulfide by a sequence of enzymatic reactions involving ATP-sulfurylase, APS-kinase, PAPS-sulfotransferase or PAPS-reductase, and NADPH- or ferredoxin-dependent sulfite reductase. Incorporation of sulfide into O-acetyl-L-serine with the action of cysteine synthase completes one form of cysteine biosynthesis. An alternative biosynthesic pathway involves an APS-sulfotransferase and an organic thiosulfate reductase (thiosulfonate reductase; for a review see 17).

Only in *E. coli* and *S. typhimurium* are the structural genes mapped for the above-mentioned enzymes. In both organisms the genes are in the *cysCDHIJ* region, at around 59 min on the chromosome. Sequence and transcription data have become available during the past four years. Similar information has been obtained only for *Rhizobium*.

cysD and *cysN*, encoding a 27-kDa and a 62-kDa protein, respectively, constitute genes of ATP-sulfurylase in *E. coli* K-12 (101). *cysC* encodes a 21-kDa protein and represents APS-kinase. The genes are adjacent and are transcribed counterclockwise on the *E. coli* chromosome in the order *cysDNC*. Expression is, as mentioned above, under the control of the transcriptional activator protein CysB (121). Activity measurements of ATP-sulfurylase of *Lemna* seem to indicate that this type of control might not hold true for this organism (17, 19), whereas CysB regulation might apply for APS-sulfotransferase (see the section above on sulfate activation and metabolism of APS and PAPS).

cysN has been identified in *E.* coli and *Rhizobium meliloti,* where *nodP* and

nodQ correspond to *cysN* and *cysC*. The respective proteins have ATP-sulfurylase activity both in vivo and in vitro (168). The *Rhizobium* NodD protein belongs, like the CysB protein, to the LysR family, a group of proteins known to activate other genes (61). A similar *cysDN* gene arrangement and even multiple copies are likely to be found in other organisms, as the composition of subunits varies in a wide range of organisms (104). ATP-sulfurylase of *Synechococcus* PCC 6301, for example, consists of a dimer comprising a 41-kDa and a 44-kDa subunit (112a).

cysCD are separated from *cysH* by a region of 25 kb (89). *cysH* codes for PAPS-reductase, a 28-kDa protein with only one cysteine residue per molecule (93, 94, 123). Because only one cysteine residue is present, it is difficult to explain how this protein could be a reductase, so we use the term PAPS-sulfotransferase here (see the section above on sulfate activation and metabolism of APS and PAPS).

Subsequent Reduction to Sulfide

The α-subunit of sulfite reductase is coded for by *cysJ* (flavoprotein) and the β-subunit by *cysI* (hemoprotein). A single transription unit in the order: promoter *cysJ cysI cysH* is indicated for *S. typhimurium* and *E. coli* B (123). For *E. coli* K-12 a different organization has been proposed. Here we have two transcription units with the order: promoter *cysI cysJ* and promoter *cysH* (102). This gives a gene order of *cysCNDHIJ* for *E. coli* K-12 compared to *cysJIHDC* in *S. typhimurium* (120, 121, 123). Sequence comparisons indicate a 84–86% DNA sequence identity and an even higher amino acid identity of 91–94%. Necessary for sulfite reductase activity is the *cysG* gene product, an S-adenosylmethionine-dependent uroporphyrinogen III methyltransferase. This enzyme is essential for converting uroporphyrinogen III into siroheme, which is a cofactor of the β-subunit of sulfite reductase (118, 192, 196). In *E. coli cysG* is closely linked to *nirB*, the gene thought to code for nitrite reductase, another siroheme-containing protein (127). Under anaerobic growth *cysG* transcription is regulated by readthrough from the *nirB* promoter (126). Whether *cysG* expression is regulated during aerobic growth is unknown. The *cysG* gene product is also necessary for cobalamin synthesis (vitamin B_{12}), a cofactor for cobalamin-dependent methionine synthetase, coded for by *metH* (76).

No genetic information about enzymes of the alternative sulfate activation pathway, about APS-sulfotransferase, or about organic thiosulfate reductase (thiosulfonate reductase) is available.

Cysteine Synthesis

The genes coding for O-acetyl-L-serine (thio)-lyase A and B (*cysK* and *cysM*, respectively) are closely linked to the permease gene cluster (*cysTWAM*; 21,

66, 177). The *cysK* of *S. typhimurium* and that of *E. coli* exhibit a DNA sequence identity of 85% and an amino acid identity of 96%; this gene's expression is again regulated by the CysB protein (21, 115). The CysM protein can use thiosulfate and O-acetyl-L-serine to form cysteine via S-sulfocysteine (66).

The cysteine synthase of higher plants cannot accept thiosulfate (88), but isoenzymes have been detected in a range of organisms (29, 36, 37). The presence of these isozymes suggests that a *cysM* equivalent is missing. It must be pointed out that the term cysteine synthase refers in higher plants and cyanobacteria only to O-acetyl-L-serine (thiol)-lyase; higher plants exhibit no complex formation with serine acetyltransferase like that in *S. typhimurium* (89, 156).

The regulation of cysteine synthase in higher plants and cyanobacteria is very slow in response to sulfur limitation and seems to be regulated by the availability of a cysteine-derived compound, as indicated above (156). Another regulation mechanism is to be expected as the CysB control.

Of genes required for cysteine biosynthesis, the last so far is the *cysE* gene product (serine-acetyltransferase; 28). Its gene expression is not under control of the CysB protein; it is constitutively expressed in *E. coli* and *S. typhimurium* but subject to feedback inhibition by low levels of cysteine (90, 91). Some amino acid sequence similarities have been reported among the *E. coli* CysE protein, the *E. coli lacA* gene product, and the NodL protein of *Rhizobium leguminosarum* (32), along with a 41% identity to the *nifP* gene product from *Azotobacter chroococcum* (34). In *E. coli,* complementation studies of a cysteine auxotroph defective in *cysE* the NifP protein restored absent serine acetyltransferase activity. On the other hand *nifP* gene disruption in *A. chroococcum* did not affect cysteine auxotrophy (34), which suggests that this organism has two serine acetyltransferase genes (34). The NifP protein appears necessary for optimal N_2 fixation and its presence implicates cysteine as the supplier of metal sulfur clusters, as reported for *E. coli* (193).

Glutathione Synthesis

The *gshA* gene is located at 58 min on the *E. coli* chromosome (5), but beyond the characterization of *E. coli* mutant strains missing glutathione (42) no genetic data are available.

CONCLUDING REMARKS

In order to gain a fuller understanding of the pathway for assimilatory sulfur metabolism, and of all the side-reactions summarized here, attempts have to be made to analyze the genes that transcribe the enzymes involved in these

reactions. A start has been made by comparing data on cyanobacterial sulfate permease genes with information obtained from genetic studies in *Escherichia coli* and *Salmonella typhimurium*. The initial results indicate that we have to expect differences concerning known sequences and regulation events between photosynthetic organisms and *Salmonella*. Identification and analysis of these genes and their transcriptional regulation in the presence of certain sulfur sources will be necessary. In this respect the focus on organisms already subjected to investigations on a biochemical level should be encouraged. Especially organisms that can be used for integrative chromosomal transformation, as for instance the cyanobacterium *Synechocystis* PCC 6803 or to some extent the higher plant *Arabidopsis*, might reward future investigations.

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