Minireview

Photosynthetic Sulphate Reduction

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Dedicated to Professor Achim Trebst on the occasion of his 65th birthday

Comparative Biochemistry, Photosynthetic Sulphate Reduction, Cysteine Biosynthesis, Structural Gene(s), Enzyme Mechanism, ATP Sulphurylase/APS Kinase

Cysteine Biosynthesis: Two Pathways for One Product?

Plants assimilate inorganic sulphate for the biosynthesis of sulphur containing compounds. The enzymes required for reduction are found predominantly in the plastids which provide the necessary energy and the reductants. Sulphate reduction like nitrate or carbon dioxide assimilation is endergonic; for comparison, NO_3 reduction to ammonia requires +347 kJ mol^{-1} and CO_2 reduction +478 kJ mol^{-1} (Thauer *et al.*, 1977). About twice as much energy is necessary to assimilate sulphate under aerobic conditions:

$$SO_4^{2-} + [8\,H] + 2\,H^+ \longrightarrow H_2S + 4\,H_2O.$$
$$\Delta G_o = +733\ \text{kJ mol}^{-1}.$$

Only for sulphidogenic bacteria – under strictly anaerobic conditions, when molecular hydrogen can be used as a source of energy – the reduction becomes exergonic by ΔG_o −152 kJ mol^{-1}.

As sulphur in SO_4^{2-} is hexavalent and divalent in S^{2-}, eight electrons are required for reduction. In plants, these electrons are provided by thioredoxin and ferredoxin whereas non-photosynthetic reduction depends on NADPH. The redox potentials of reductants and oxidants that are involved in sulphate metabolism are listed in Table I. A direct reduction of sulphate to sulphite is not observed under physiological conditions because reductants with a redox potential as low as E_o' −517 mV would be necessary. Thus, inorganic sulphate is "activated" to enable its reduction. Sulphate activation yields adenylylsulphate – an energy-rich mixed anhydride of phosphoric and sulphuric acid that can be reduced to sulphite at $\Delta E_o'$ −60 mV. The reductive step from sulphite to sulphide has a redox potential of $\Delta E_o'$ −117 mV. Dithiols are regenerated from disulphides by electrons with a redox potential of E_o' −260 mV.

Table I. $\Delta E_o'$ of substrates and reductants involved in SO_4 reduction.

Substrates	[mV]	Reductants	[mV]
SO_4^{2-}/SO_3^{2-}	−517	$\text{Ferredoxin}_{red/ox}$	−423
APS/AMP + SO_3^{2-}	−60	$\text{Thioredoxin}_{red/ox}$	−260
PAPS/PAP + SO_3^{2-}	−60	NADPH/NADP$^+$	−320
HSO_3^-/HS^-	−117	2 GSH/GS:SG*	−260
		$MV^{+\cdot}/MV^{2+}$	−444

* Also as substrate.
Standard redox potentials at pH 7.0 of redox pairs involved in sulphate reduction as substrates or reductants; APS, adenylyl sulphate; PAPS, 3-phosphoadenylyl sulphate; MV, methylviologen; GSH, reduced form; GS:SG, oxidized form of glutathione.

For algae and higher plants, two pathways of sulphate assimilation are currently discussed in the literature. Both are compared in the generalized flow scheme below; they will be referred to as APS-bound sulphite pathway and PAPS-free sulphite pathway. The latter will be described in more detail as for the APS-bound pathway the reader is referred to the recent reviews by Anderson (1990) and Schmidt and Jäger (1992). The sum of reactions in the PAPS-free sulphite pathway is:

$$SO_4^{2-} + 2\,ATP + \text{thio}_{red} + 6\,Fd_{red} + [6\,H^+] + \text{O-ASer} \longrightarrow \text{cysteine} + \text{acetate} + ADP + PAP + 2\,P_i + \text{thio}_{ox} + 6\,Fd_{ox} + 3\,H_2O$$

– in the APS-bound sulphite reaction pathway:

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$$SO_4^{2-} + ATP + 6Fd_{red} + [6H^+] + 2e^- + O\text{-ASer} \longrightarrow \text{cysteine} + \text{acetate} + AMP + 2P_i + 6Fd_{ox} + 3H_2O.$$

Six enzymatic steps convert inorganic sulphate into organic sulphur (Fig. 1): uptake, adenylation, phosphorylation, reduction to sulphite, reduction to sulphide and insertion of sulphide into the carbon precursur of cysteine.

a) Uptake

Chloroplasts are considered as the major site of sulphate reduction (Schwenn and Trebst, 1976). On its way into the plastid, sulphate that is taken up by the root system and transported *via* the xylem to the leaf has to pass two membrane systems: the cytoplasmic membrane and the chloroplast envelope. The first step is presumable catalyzed by a SO_4 permease or transporter – sulphate is then imported into the chloroplast stroma in exchange for *ortho*-phosphate. Work with isolated plasma membranes suggested that sulphate is co-transported with protons at a ratio of $3H^+/SO_4^{2-}$ by a permease type of transporter. Clarkson *et al.* (1993) proposed a model based on the proton motive force to explain uptake of sulphate by root cells. Transport into the plastid seems to proceed *via* the phosphate translocator (Flügge *et al.*, 1989). It is not yet clear whether sulphate transport at the tonoplast is

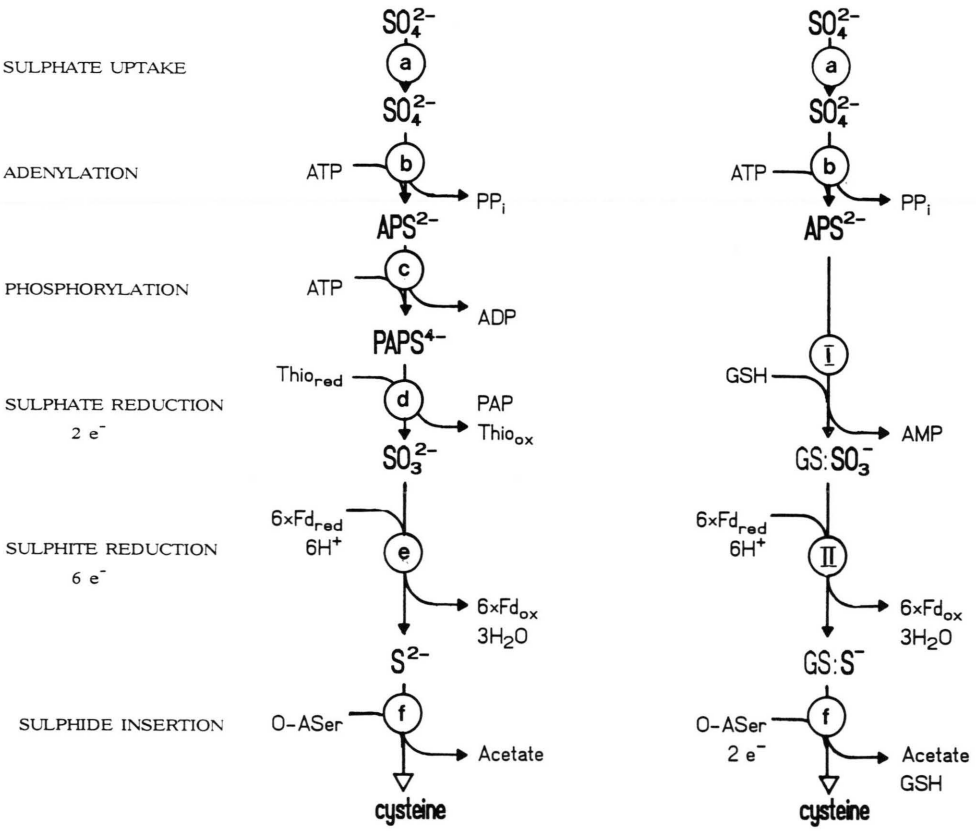


Fig. 1. Photosynthetic sulphate reduction. The path of sulphate currently discussed follows reactions involving free ionic intermediates (*i.e.* sulphite and sulphide in the PAPS sulphite pathway, left scheme) or involving carrier-bound forms (*i.e.* S-sulphoglutathione or glutathione persulphide as in the *Chlorella* APS-bound sulphite pathway on the right). The reaction sequence is catalyzed by a: sulphate transporter, b: ATP sulphurylase, c: APS kinase, d: PAPS reductase, e: ferredoxin:sulphite reductase, f: O-acetyl-L-serine(thiol)lyase, and I. APS:thiol sulphotransferase, II. ferredoxin:thiosulphonate reductase; cosubstrates and reductants are listed on the left of each pathway – products on the right side. Abbreviations: APS, adenylyl sulphate; PAPS, 3′-phosphoadenylyl sulphate; Fd, ferredoxin; O-ASer, O-acetyl-L-serine; Thio, thioredoxin.

purely by diffusion or by specific carrier proteins (*cf.* Cram, 1990).

b) Adenylation

The first enzyme of the pathway forms adenylyl sulphate (APS) which contains the anhydride bond between phosphoric and sulphuric acid.

$$SO_4^{2-} + Mg \times ATP^{2-} \longrightarrow APS^{2-} + Mg \times PP_i^{2-}.$$

ATP sulphurylases have been studied intensively by Segel and coworkers. From spinach leaves, two isozymes were isolated most recently, representing a plastidic and a cytosolic form (Renosto *et al.*, 1993). APS-forming ("assimilatory") ATP sulphurylases have Michaelis constants for SO_4 and ATP in the millimolar and constants for APS or PP_i in the micro- or even submicromolar range. The equilibrium constant K_{eq} is $\approx 10^{-7}$ so that the intracellular concentration of APS is assumed to be extremely low. It may even be lower under physiological conditions because the reaction is not expected to reach the equilibrium. This follows from a powerful product inhibition (K_{iAPS} of 0.04 10^{-6} M) and from a considerable intracellular concentration of inorganic pyrophosphate (Weiner *et al.*, 1987). The concentrations of pyrophosphate that have been found in plants are well above "saturating" with respect to K_m for PP_i of ATP sulphurylase in its reverse reaction.

c) Phosphorylation

In the PAPS sulphite pathway ATP sulphurylase and APS kinase are coupled energetically; inorganic pyrophosphatase usually is included because it has an effect on the equilibrium of ATP sulphurylase. The endergonic reaction of the ATP sulphurylase is balanced by the exergonic reactions catalyzed by APS kinase and PP_iase.

$$APS^{2-} + Mg \times ATP^{2-} \longrightarrow PAPS^{4-} + Mg \times ADP^{-}$$

$$Mg \times PP_i^{2-} + H_2O \longrightarrow 2\,HPO_4^{2-} + Mg^{2+}.$$

APS kinase with its high affinity in the range of 10^{-6} M towards the substrates APS and ATP and its favourable equilibrium constant may be expected to phosphorylate even the lowest concentration of APS to phosphoadenylyl sulphate (PAPS) (Jender and Schwenn, 1984). It was even speculated that by substrate channeling, APS was passed through a complex formed by ATP sulphurylase and APS kinase. This may not be necessary since under physiologically meaningful concentrations of ATP, *ortho*-phosphate and sulphate, $K_{eqoverall}$ is $\approx$ 0.002–0.02 indicating that PAPS could reach millimolar concentrations at equilibrium. In view of the further metabolic fate of PAPS, it is noteworthy that the activity of APS kinase from a green alga is regulated by a dithiol-disulphide redox charge in which thioredoxin plays the key role as activator whereas disulphides like oxidized glutathione deactivate (Schwenn and Schriek, 1984). A redox control of this reaction (Schriek and Schwenn, 1986) appears indispensable as reduction of the activated sulphate ought to proceed only with a sufficient supply of reduced thioredoxin and ferredoxin.

d) Reduction of PAPS

In the third reaction, PAPS (Phosphoadenylyl sulphate) is reduced to sulphite by PAPS reductase using thioredoxin as reductant. The plant enzyme was detected by using bacterial thioredoxin as reductant (Schwenn, 1989). Thioredoxins are small ubiquitous redox proteins that contain two extruding cysteinyl thiols functioning as a dithiol-disulphide redox group (Holmgren, 1989). The enzyme mechanism depends on thioredoxin (Russel *et al.*, 1990). Two electrons from the dithiol group are transferred to the enzyme which, in a second step, reduces its substrate PAPS (Schwenn *et al.*, 1988).

$$PAPS^{4-} + \text{thioredoxin } (SH)_2 \longrightarrow$$
$$HSO_3^{-} + PAP^{4-} + \text{thioredoxin } (S)_2 + H^{+}$$

liberating sulphite and 3′-phospho-adenosine-5′-phosphate (PAP). This sequential mechanism excludes formation of a bound sulphite. Bound sulphite(s) are side-products of a reaction in which the free sulphite is oxidized by a disulphide to give an organic thiosulphate ($RS{:}SO_3$). The enzyme responsible for sulphite formation, contains only one sulphhydryl group per subunit but no chromophore. Site specific mutagenesis of this single cysteine has a deleterious effect on the activity. However, the enzyme is not inactivated completely. As a measurable fraction is retained, the molecular mechanism cannot rely entirely on a dithiol-disulphide redox couple formed by two cysteine residues. From current work, it seems as if PAPS re-

ductase uses a stable organic radical for reduction which in its EPR properties resembles the organic radical of ribonucleotide reductase (Berendt and Schwenn, unpublished).

e) Reduction of Sulphite

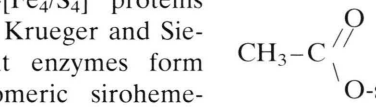
Sulphite generated by PAPS reductase is reduced by either NADPH or ferredoxin-sulphite reductase. Ferredoxin-dependent enzymes found in plastids of higher plants and in cyanobacteria are homopolymeric siroheme-[Fe_4/S_4] proteins (Aketagawa and Tamura, 1980; Krueger and Siegel, 1982). NADPH-dependent enzymes form more complicated heterooligomeric siroheme-[Fe_4/S_4]proteins containing additional subunits which carry flavins (FAD and FMN) as prosthetic groups. Both enzymes transfer six electrons to sulphite that remains in the active center during catalysis:

$$HSO_3^- + 6\,\text{ferredoxin}_{red} + 6\,H^+ \longrightarrow HS^- + 6\,\text{ferredoxin}_{ox} + 3\,H_2O.$$

SO_3^{2-} binds to Fe^{2+} of the heme; it is reduced to sulphide without detectable intermediates presumably by a series of two-electron reductive cleavages of S–O-bonds. The mechanism of reduction using an electron donor like ferredoxin that provides only one electron is still not elucidated. Affinity constants for sulphite and ferredoxin are in the micromolar range (Krueger and Siegel, 1982). The ferredoxin:SO_3 reductase was reported to exist as homodimer with one siroheme and one [Fe_4/S_4] center per catalytically active polypeptide. Data concerning the reaction mechanism are available from the NADPH-dependent enzyme only. It follows a complex sequential mechanism in which sulphite is bound by the siroheme first and NADPH reacts second. For the enterobacterial sulphite reductase, the electrons from NADPH are transferred to the site of sulphite reduction *via* FAD and FMN. As judged from the large ΔE between NADPH and H_2S the equilibrium seems to favour hydrogen sulphide formation. Yet, experimental data suggest also that liberation of sulphide from the enzyme-(siroheme)-sulphide complex may be rate limiting. The model of the active site of sulphite reductase as proposed by Siegel and coworkers is an exchange coupled [Fe_4/S_4]siroheme complex. At present, it is still a matter of debate whether the bridging or the axial ligand is involved in the binding of substrate (McRee *et al.*, 1986; Tan and Cowan, 1991).

f) Insertion of Sulphide

In the final step, sulphide is inserted into O-acetylserine by a thiol-lyase to give cysteine. Except for yeast, which forms homocysteine instead, cysteine is considered the first organic product of sulphate assimilation in plants, algae, and enterobacteria.



Cysteine is formed by a pyridoxal-phosphate-dependent O-acetylserine-(thiol)-lyase. The plant enzyme was reported to have a high affinity for sulphide and O-acetylserine (Ng and Anderson, 1978). It appears that most of the kinetic constants published in previous literature for higher plants represent properties of the plastidic enzyme as this isoenzyme was reported to contribute most of the activity of a leaf extract (Lunn *et al.*, 1990).

The sequence of reactions that led to the proposal of a different pathway specific for photosynthetic organisms (referred to as "APS-bound sulphite pathway") was deduced from work with cell extracts and partially purified protein fractions from the green alga *Chlorella,* from work with *Lemna minor,* higher plant leaf extracts and isolated intact chloroplasts (surveys in Anderson, 1990; Schmidt and Jäger, 1992). The APS-bound sulphite pathway only contains five steps because adenylyl sulphate is used instead of phosphoadenylyl sulphate. The APS-bound sulphite pathway also starts with ATP sulphurylase in the first reaction, but steps following APS formation were proposed to involve carrier-bound intermediates rather than free anionic HSO_3^- or HS^-. These bound forms (designated $XSSO_3H$ or $CarS:SO_3H$ in the literature) were hypothesized to originate from the action of APS sulpho"transferase" activity (reaction I in the flow scheme). It was termed transferase as the reaction mechanism was speculated to involve a transfer of the active sulphate group from APS to a thiol group of a small sulphite carrier. Due to their instability, APS sulphotransferases have not been obtained in a pure state until recently when Li and Schiff (1991)

characterized a homogeneous enzyme preparation from *Euglena*. This sulphotransferase was only active in its tetrameric form – the monomer, with an approximate molecular weight of 24 kDa was inactive. This inactivating monomerization is brought about by the same thiols which are used as substrate. In the earlier literature, glutathione was considered as the physiologically relevant carrier of the sulphate group from APS. However, as oxidized glutathione like any other disulphide is a suitable oxidant for sulphite it forms S-sulphoglutathione ($GS:SO_3H$) as non-enzymatic product. The APS sulphotransferase reaction is followed by a reduction of the bound sulphite to bound sulphide. In *Chlorella* $GS:SO_3H$ is reduced by a ferredoxin-dependent thiosulphonate reductase (reaction II) to glutathione-persulphide (GSSH). This enzyme was proposed to be inactive with free sulphite but used exclusively $GSSO_3H$ as substrate. Thiosulphonate reductases were not characterized further. The assay system developed for ferredoxin:$GSSO_3H$ reductase in fact measured a methylviologen-dependent sulphite reductase (Siegel, 1975). In addition, glutathione reductase reduces $GSSO_3H$ to glutathione and free sulphite – and even if $GSSO_3H$ is reduced to GSSH by a thiosulphonate reductase, how would sulphide be liberated from glutathione-persulphide without auxiliary reductant? Until today, there is no experimental evidence for a reductant required by O-acetylserine-(thiol)-lyase or cysteine synthase.

Even if all the above data can be put together, it is open to dispute whether plants contain two independent side-by-side reactions or use a single set of enzymes for sulphate reduction like the "PAPS-free sulphite" pathway described for bacteria and lower eukaryots, or a sulphate reduction mechanism that is specific for photosynthetic organisms like the "APS-bound sulphite" pathway. Light would be shed upon these conflicting results if each individual enzyme is related with its structural gene(s). However, as the primary structure of the polypeptides as well as the true function of the enzymes was not unambiguously known, the structural genes of sulphate metabolizing enzymes had to be isolated and identified without the need to purify and characterize the gene product.

Of Genes and Enzymes

Plants – in accordance with the endosymbiont hypothesis – have inherited chloroplast genes from prokaryotic organisms like cyanobacteria. Hence, homologous prokaryotic genes were used as probes to search DNA segments of identical or nearly identical sequences. Southern blotting in combination with phenotypic complementation of enterobacterial mutants led to the isolation of ferredoxin-dependent sulphite reductase and thioredoxin-dependent PAPS reductase DNA from *Synechococcus* PCC7942 (Niehaus *et al.*, 1992; Gisselmann *et al.*, 1993). From the same organism, a cluster of sulphur-regulated genes with a sulphate permease and a putative rhodanese was detected by Laudenbach *et al.* (1991 a, b). In the more recent work with higher plant DNA, the structural genes were detected and characterized by complementation of the corresponding yeast mutant (ATP sulphurylase in Klonus *et al.*, in press), degenerate primers to amplify specific DNA (APS kinase (Arz *et al.*, 1994), NADPH and ferredoxin:SO_3 reductase (Gisselmann, unpubl.)), or methods that rely on identified gene products and sequenced peptide fragments (O-acetylserine-(thiol)-lyase (Saito *et al.*, 1992; Römer *et al.*, 1992).

By comparing DNA and polypeptide structure it may be concluded that the genes encoding sulphate assimilating enzymes represent related families. Within these families, the DNA and/or polypeptides deduced from the corresponding reading frames have homologous or highly conserved sequences. Similarities found among gene products from pro- and eukaryotic organisms (Table II) range from nearly 60% identical amino acid residues to less than 30% over the entire gene product. Yet, even at the low homologies, particular segments (presumably functional or structural domains) can be found which remained conserved completely.

Sulphite reductase genes encode for large polypeptides in which homologies are restricted to particular areas of the sequence. The structural gene (acronym *sir*) from *Synechococcus* predicts a gene product of 70 kDa – this finding agrees well with biochemical data where subunits of 63 to 69 kDa were reported for spinach or *Spirulina platensis* (Krueger and Siegel, 1982; Koguchi and Tamura, 1988). The *Synechococcus* polypeptide sequence

Table II. Structural genes and enzymes in assimilatory SO_4 reduction.

Organisms: gene product(s)	Bacteria a	a ≫ b	Yeast b	b ≫ c	Plants c	c ≫a
ATP sulphurylase	*cys* DN	n.r.	*met* 3	29	*sul*	n.r.
APS kinase	*cys* C	47.9	*met* 14	55.4	*akn*	47.5
PAPS reductase	*cys* H	32	*met* 16	30.3	*par*	50.8[a]
NADPH:SO_3 reductase						
Flavoprotein	*cys* J		*met* 10, 18, 20		–	
Hemoprotein	*cys* I		*met* 5[b]		–	
fd:SO_3 reductase	–		–		*sir*	35[c]
OAS-(thiol)-lyase A	*cys* K		–		*cs*	53–56
OAS-(thiol)-lyase B	*cys* M		–		*cs*	42–45
OAHS-(thiol)-lyase	–		*met* 25		–	

Relatedness based on identical amino acid in identical positions of the polypeptide in % obtained from dot matrix comparisons; [a] gene product from *Anacystis nidulans;* [b] *met* 5 is homologous to *cys* I identity of other phenotypes is not yet known (Surdin-Kerjan, pers. commun.); [c] hemoprotein *cys* I is homologous to ferredoxin-dependent sulphite reductase *sir;* genes encoding homologous flavoprotein(s) are not identified; n.r., non-related DNA structure(s), *i.e.* enzyme function is identical but structural gene not related or similar. –, blanks indicate that similarities and relations are neither known nor investigated.

shows significant identities (47.7%) with the higher plant sequence (Gisselmann *et al.,* 1993). This prediction can already be made from the partial DNA sequence of the *sir* gene from *Arabidopsis thaliana* of which at present approximately two thirds are sequenced (Gisselmann, unpubl.). The identity is considerably lower (35%), when the plant type *sir* gene and its product are compared with NADPH-sulphite reductase from *E. coli.* Conserved regions of the polypeptides are restricted to the β-subunit of the bacterial enzyme that contains distinct clusters of identical amino acids. Two of these clusters addressed as "cysteine clusters" are presumably involved in the binding (or orientation) of the catalytically important Fe_4/S_4-siroheme centers (Ostrowski *et al.,* 1989). They are found at corresponding positions in the ferredoxin:SO_3 reductase, in the fd:NO_2 reductase and in the β-subunit of NADPH:SO_3 reductase alike. It is noteworthy, that this limited homology, sufficed to detected the *sir* gene from *Synechococcus* using a fragment of the *cys* I gene from *E. coli* that only included DNA encoding the C-terminal part with its conserved cysteine clusters.

A single conserved cysteine is observed in the PAPS reductases. PAPS reductases (28 kDa per subunit) show rather low sequence identities between bacterial and lower eukaryotic polypeptides (Ostrowski *et al.,* 1989; Krone *et al.,* 1991; Niehaus *et al.,* 1992). The cysteine residue is located within the proximity of the C-terminus. (It is also conserved in yeast. The DNA sequence published by Thomas *et al.* (1990) has to be corrected accordingly (Berendt, unpubl.).) As the gene product is devoid of prosthetic groups that could store the electrons from thioredoxin, a disulphide-dithiol redox pair formed by the enzyme dimer may be involved. PAPS reductase activity is not eliminated when cysteine is replaced by serine, but reduced to ≈10% of the non-mutated protein. This excludes a reaction that depends exclusively on a dithiol-disulphide redox mechanism, yet, in combination with other redox active amino acid residues the thiol(s) may play an important part. A DNA sequence of the PAPS reductase gene from a higher plant is still not established and its relatedness with the cyanobacterial *par* gene encoding PAPS reductase remains to be shown when the cDNA clones from an *Arabidopsis thaliana* library complementing a PAPS reductase mutant *cys* H from *E. coli* or *met* 16 from yeast will be fully characterized (Berendt and Schwenn, unpubl.).

APS kinases are small proteins with a molecular weight ranging from 21 to 30 kDa per subunit; they form homodimeric proteins with remarkable similarities in the polypeptide primary structure of the three organisms compared (47.5 to 55.4% identity (Korch *et al.,* 1991; Satishchandran *et al.,* 1992; Arz *et al.,* 1994)). The plant polypeptide that was deduced from a cDNA clone, was considerably larger (29 kDa) than the gene products from yeast or the enterobacterium (21–22 kDa). It con-

tained an N-terminal extension of 77 amino acid residues; 37 of these amino acids appear to belong to a transit peptide (Gavel and von Heijne, 1990) directing the APS kinase into the chloroplast. The N-terminal extension with homology to plastidic transit peptides is separated from the core protein by a processing site for stromal proteases. Here, the molecular data confirm that a sulphate assimilating enzyme is encoded in the nucleus and that the gene product is imported into the plastid.

The most striking property of plant APS kinase is seen upon expression of the APS kinase gene in *E. coli.* Not only that the APS kinase mutant *cys*C regained the ability to grow on sulphate – indicating a proper function of the plant gene product in the host bacterium – but transformants also acquired APS sulphotransferase activity (Arz *et al.*, 1994). A further investigation of the recombinant APS kinase revealed that APS sulphotransferase activity is a side activity of the APS kinase when no ATP is available (Schiffmann and Schwenn, unpubl.). This finding confirmed kinetic measurements of the partially purified APS kinase from *Chlamydomonas reinhardtii* (Schwenn and Jender, 1981) where an enzyme-bound sulphite was observed as intermediate of the reaction.

The considerable degree of homology that is seen on the basis of nucleotides as well as on the basis of amino acids shows two exceptions: ATP sulphurylase from *E. coli* and O-acetylhomoserine-(thiol)-lyase from *Saccharomyces cerevisiae.* The gene product of *cys*DN which Markham and coworkers found to encode for ATP sulphurylase, is a heteropolymeric polypeptide. DNA and polypeptide from *E. coli* are not related with the gene and product of *met*3 from yeast (Cherest *et al.*, 1987). The latter is distantly related with the plant ATP sulphurylase which is a homooligomer (Renosto *et al.*, 1993). The plant clone encoding for an unusually small protein with ATP sulphurylase activity was isolated by complementation of the yeast mutant *met*3 (Klonus *et al.*, in press). None of the homooligomeric ATP sulphurylases that have been characterized from plants, lower eukaryots or sulphide oxidizers, are related with the sulphurylase that is encoded by *cys*DN (or *nod*PQ in the *Rhizobia*). The gene product *cys*DN that was originally identified by complementation and by function (Leyh *et al.*, 1988) encodes for an enzyme which is activated by GTP. *cys*N, the larger subunit (53 kDa), is homologous to the protein synthesis elongation factors EFTu and G (Leyh *et al.*, 1992) – whereas the smaller subunit *cys*D (35 kDa) is homologous to *nod*P (Schwedock and Long, 1989). Since *cys*DN and *met*3 are not related, these data imply the existence of two different types of ATP sulphurylases: a homooligomeric ATP sulphurylase homologous to *met*3 and heterooligomeric GTP-activated protein from bacteria homologous to *cys*DN and *nod*PQ. With its heterodimeric structure and the control by GTP of enzymatic activity as sulphurylase, the gene product of *cys*DN seems to be unique for the ATP sulphurylase from *E. coli.*

The ATP sulphurylase genes *cys*DN and the APS kinase gene *cys*C form an operon in which *cys*DNC are contiguous with a single *cys*B promoter upstream of *cys*D. Considering the DNA structure, Leyh suggested that both genes are translationally coupled (Leyh *et al.*, 1992).

The homodimeric O-acetylserine-(thiol)-lyases (34 kDa per subunit) are again highly conserved proteins. Bacterial isoforms of O-acetylserine-(thiol)-lyases encoded by *cys*K and *cys*M (Byrne *et al.*, 1988) are identical with the plant proteins by 56% to 42% (Saito *et al.*, 1992; Römer *et al.*, 1992). Although plastids are considered as the major site of sulphate reduction smaller amounts of enzyme can also be detected in chromoplasts, in mitochondria and in the cytosol (Lunn *et al.*, 1990). Its wider distribution in the plant seems to reflect a more general function – a function in cysteine catabolism should be taken into consideration. Yeast is supposed to utilize an O-acetylhomoserine-(thiol)-lyase for sulphide incorporation leading to homocysteine instead of cysteine. *Met*25 is the corresponding gene (Kerjan *et al.*, 1986). The gene product is a polypeptide of 48.7 kDa which shares no homology with the O-acetylserine-(thiol)-lyases.

Our knowledge about the molecular basis of sulphate metabolism in higher organisms is rapidly increasing. Most certainly, the next steps will be directed towards a critical re-examination of the biochemistry using recombinant instead of the "native" enzyme which, in the past, was too difficult to purify in sufficient quantities for a more thorough investigation. Gene expression and activity is the second area that may be reinvestigated. In retrospect, many of the earlier data con-

cerned with regulation can only be considered as circumstantial evidence for the true function of any of the putative genes remained unclear. Many of the questions concerning gene activity in response to sulphur supply, intracellular enzyme function by anti-sense RNA, organ specificity and differential expression, cellular and long-distance transport will soon be answered on a molecular level.

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Due to limited space the list of references is incomplete – apologies to all whose work is not cited directly.