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# Structural biology and regulation of the plant sulfation pathway

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ABSTRACT

In plants, sulfur is an essential nutrient that must be converted into usable metabolic forms for the formation of sulfur-containing amino acids and peptides (primary route) and for the modification of diverse metabolites (secondary route). In plants, the fate of assimilated sulfate depends on the three enzymes – ATP sulfurylase, adenosine-5′-phosphate (APS) reductase, and APS kinase - that form a branchpoint in the pathway. ATP sulfurylase catalyzes the formation of the critical intermediate APS, which can either be used in the primary assimilatory route or be phosphorylated to 3′-phospho-APS (PAPS) for a variety of sulfation reactions. Recent biochemical and structural studies of the branchpoint enzymes in plant sulfur metabolism suggest that redox-regulation may control sulfur partitioning between primary and secondary routes. Disulfide-based redox switches differentially affect APS reductase and APS kinase. Oxidative conditions that promote disulfide formation increase the activity of APS reductase and decreases PAPS production by APS kinase. Here we review recent work on the ATP sulfurylase and APS kinase from plants that provide new insight on the regulation of PAPS formation, the structural evolution of these enzymes in different organisms, and redox-control of this key branchpoint in plant sulfur metabolism.



## 1. Introduction

In all organisms, sulfur is a versatile and essential element used in a variety of metabolic pathways. For plants, sulfur, along with nitrogen, phosphorus, and potassium, is a nutrient and a critical determinant of soil quality [1,2]. Plants uptake inorganic sulfate ($SO_4^{2-}$) from the environment and convert it into physiologically useful forms of sulfur [3,4]. The core sulfur metabolism pathway in plants (Fig. 1) reduces sulfur for the production of cysteine, methionine, iron-sulfur clusters, vitamins, molecules that protect against oxidative stresses (i.e., glutathione and phytochelatin peptides), and compounds involved in biological defenses, such as allylsulfur molecules and glucosinolates [5–10]. Additionally, sulfate can be incorporated into 3′-phospho-adenosine-5′-phosphate (PAPS) to provide a sulfate donor for the modification of brassinosteroid and jasmonate hormones, phytosulfokines, phospholipids, and other sulfonated molecules [11–13]. PAPS is also a precursor of 3′-phosphoadenosine-5′-phosphate, which is a retrograde signal that modulates stress gene expression and plant development [14–17].

Plant thiol metabolism begins with the reductive sulfur assimilatory pathway (Fig. 1). Three enzymes activate sulfate and reduce it to sulfide [18]. ATP sulfurylase (ATP:sulfate adenylyl transferase) catalyzes the committed step of the pathway to form the high-energy mixed anhydride molecule adenosine-5′-phosphosulfate (APS) and inorganic pyrophosphate ($PP_i$) from sulfate and ATP [19–21]. Reduction of APS to sulfite ($SO_3^{2-}$) by APS reductase (5′-adenylylsulfate reductase) requires glutathione as an electron donor [22,23]. In the final step, sulfite reductase converts sulfite to sulfide [24]. Sulfide is then used for formation of cysteine and other peptides in the next stage of plant sulfur metabolism (Fig. 1). Cysteine biosynthesis involves formation of O-acetylserine from acetyl-CoA and serine, which is catalyzed by serine acetyltransferase [25]. Next, the pyridoxal-5′-phosphate-dependent enzyme O-acetylserine sulfhydrylase (O-acetylserine(thiol)lyase) generates cysteine from O-acetylserine and sulfide [26]. In addition to its role as a proteogenic amino acid, cysteine is a precursor for methionine biosynthesis [27,28]. Plants also use cysteine for the production of thiol-containing peptides, such as glutathione, a major redox buffer, and phytochelatins, which are synthesized in response to heavy metal exposure [29–32]. In some plants, cysteine is also used to generate specialized glutathione homologs [33].

Although these pathways comprise primary sulfur metabolism

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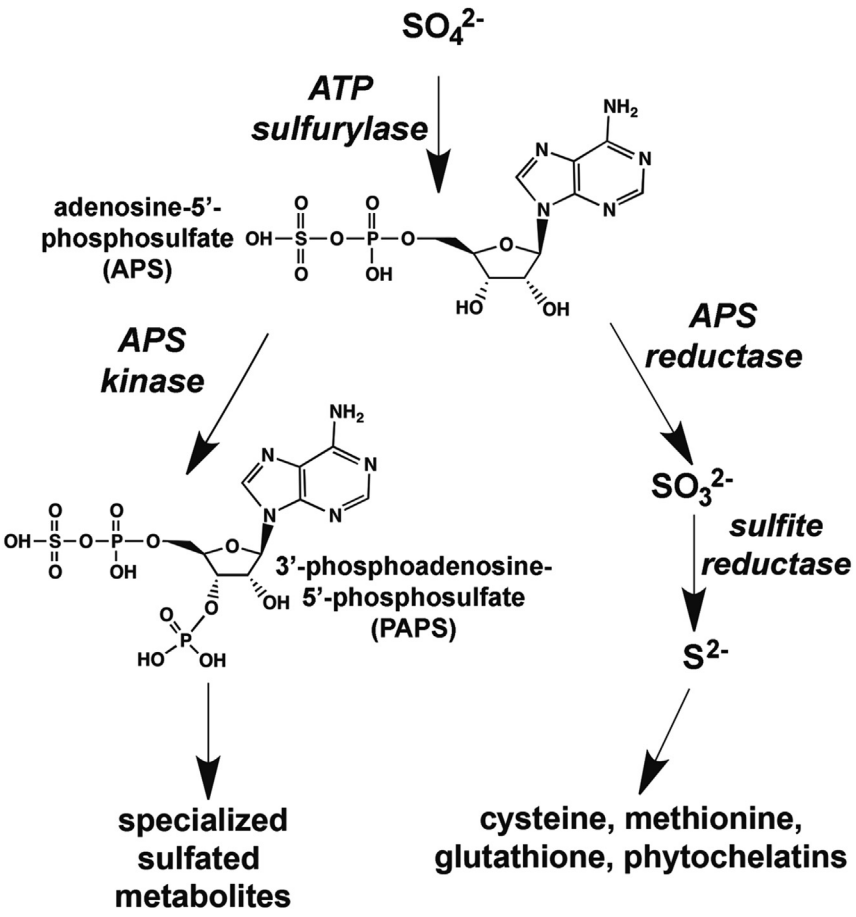


**Fig. 1.** Overview of the plant sulfur assimilation pathway.

in plants, formation of APS by ATP sulfurylase and its subsequent phosphorylation to PAPS by APS kinase constitutes a secondary sulfur metabolic pathway that is critical for the generation of sulfonated metabolites formed by a variety of sulfotransferases [11–13]. A similar diversion of sulfur into PAPS synthesis has been observed in some pathogenic bacteria, such as *Mycobacterium tuberculosis* [34]. It should be noted that other bacteria, fungi, and humans sequentially use both APS kinase and APS reductase in the primary sulfur assimilation pathway to convert APS into PAPS, which is then further metabolized into sulfite by PAPS reductase [34–36].

Recent work demonstrates an essential role for APS kinase and PAPS in plant reproductive viability and development, as well as the partitioning of sulfur between the primary and specialized pathways [16,37–40]. Here we summarize recent structural biology studies on the ATP sulfurylase and APS kinase from plants that provide new insight on the biochemical regulation of PAPS formation and redox-control of this key branchpoint in plant sulfur metabolism.

## 2. Structure and mechanism of plant ATP sulfurylase

ATP sulfurylase is the metabolic entry point of the sulfur assimilation pathway and catalyzes the energetically unfavorable formation of APS (Fig. 1). In plants, multigene families encode multiple ATP sulfurylase isoforms [9,10]. These isoforms are localized to different organelles with the chloroplast providing the primary source of reductive sulfate assimilation [41,42]. Regulation of the plastidic ATP sulfurylases occurs by a sulfur deprivation-inducible microRNA (miR395), which leads to increased translocation of sulfate from the roots to the shoots [43,44]. Moreover, disruption of the ATP sulfurylase 1 in the model plant *Arabidopsis thaliana* (thale cress) results in increased accumulation of sulfate in the leaves, which suggests that this enzyme contributes to metabolic control of sulfur assimilation [44]. Biochemical and structural studies of soybean ATP sulfurylase provide the first molecular insights on this enzyme in plants [20,21].

The 2.48 Å resolution x-ray crystal structure of soybean ATP sulfurylase reveals a dimeric protein with each monomer consisting of two mixed α/β structural domains (Fig. 2A) [21]. Binding of APS in the C-terminal domain of each monomer defines the active site (Fig. 2A–B). APS binds in a cavity with a channel leading from the α-phosphate of APS to the protein surface (Fig. 2B). Multiple interactions anchor APS in the active site with positively-charged residues, including Arg248, His252, His255, and Arg349, providing a possible site for binding of the β- and γ-phosphates of ATP and $PP_i$ during the APS synthesis reaction (Fig. 2B). Structural analysis of the soybean ATP sulfurylase also provided a template for interpreting naturally occurring Arabidopsis haplotype mutations that affect sulfate assimilation [21].

Steady-state kinetic studies of soybean ATP sulfurylase are consistent with a mechanism in which ATP and APS are the first substrates bound in the forward and reverse reactions, respectively [20]. Initial velocity experiments indicate a single-displacement mechanism in the APS synthesis reaction with inhibition by chlorate showing competitive inhibition versus sulfate and noncompetitive inhibition versus APS. For the reverse reaction (i.e., ATP synthesis), initial velocity analysis also suggests a sequential