Regulation of high-affinity sulphate transporters in plants: towards systematic analysis of sulphur signalling and regulation

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Abstract

Plants require the function of plasma membrane-bound sulphate transporters for the initial uptake of inorganic sulphate. Part of this fundamental process is the energy-dependent proton/sulphate co-transport systems that are located in the surface cell layers of roots. During sulphur limitation, plants are able to activate the expression of sulphate transporters that facilitate the uptake of sulphate in roots. SULTR1;1 and SULTR1;2 are suggested to be the essential components of the sulphate uptake system in Arabidopsis roots. The physiological importance of SULTR1;1 and SULTR1;2 is supported by characteristics that can cope with sulphur deficiency: they were (i) functional high-affinity sulphate transporters; (ii) induced by sulphur limitation at the mRNA levels; and (iii) predominantly localized in the root hairs, epidermis, and cortex. The expression of high-affinity sulphate transporters was primarily regulated by sulphur in a promoter-dependent manner. Aside from the sulphur-specific regulation, the induction of SULTR1;1 and SULTR1;2 high-affinity sulphate transporters by sulphur limitation was dependent on the supply of carbon and nitrogen. In this review, the application of SULTR promoter–GFP systems for the analysis of regulatory pathways of sulphate acquisition in plants is described.

Key words: Arabidopsis, gene expression, regulation, sulphate transporter, sulphate uptake, sulphur assimilation.

Introduction

Sulphate (SO₄²⁻) is a macro-nutrient that is required for the synthesis of sulphur-containing amino acids in plants (Leustek and Saito, 1999; Leustek et al., 2000; Saito, 2000). Sulphate is the major form of sulphur that plants can use in the assimilatory pathway, however, most of the sulphur in nature exists as a reduced form. Under these circumstances, sulphate may become limiting for plant growth. In addition, plants may encounter dramatic changes in sulphur status, caused, for example, by the excessive supply of fertilizer and irrigation. To cope with these environmental stresses, plants absorb micromolar sulphate from the soil and concentrate it up to millimolar amounts in the cells. From earlier physiological studies, it was suggested that ion carriers (or transporters) localize at the plasma membranes to complete this function (Leggett and Epstein, 1956; Clarkson et al., 1983). More recently, molecular studies have revealed that most of the higher plants have high-affinity sulphate transporters that potentially facilitate the acquisition of sulphate in roots (Smith et al., 1995a, 1997; Takahashi et al., 1997, 2000; Hawkesford, 2003). In general, transporters must be controlled at the levels of gene expression, protein turnover, or modification of activities for the optimized uptake and assimilation of sulphate. In this review, the focus will be on the nutrient-stress response and regulation of the high-affinity sulphate transporters in Arabidopsis.

When plants are grown under low sulphur (−S) conditions, sulphate uptake and cysteine (Cys) synthesis are activated for survival. It has been reported that sulphate transporters, 5′-adenylylsulphate (APS) reductase and serine acetyltransferase are up-regulated at the mRNA
levels under −S conditions (Smith et al., 1995a, 1997; Gutierrez-Marcos et al., 1996; Takahashi et al., 1997, 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002). Sulphate transporters for the internal translocation of sulphur were also up-regulated under −S (Takahashi et al., 1997; Yoshimoto et al., 2003), suggesting that the re-translocation of sulphate through the vasculature may be important for the efficient utilization of the sulphur source. In addition to these primary responses, prolonged cultures under −S conditions can cause dramatic alterations to sulphur metabolism; the cessation of secondary metabolism and the degradation of the sulphur pool in the organic constituents. Recent transcriptome studies have provided expression profiles of the −S-responsive genes displaying arrays of these typical sulphur responses in plants (Hirai et al., 2003; Nikiforova et al., 2003; Maruyama-Nakashita et al., 2003). Apparently, the −S-responsive genes are co-ordinately regulated by changes in sulphur conditions. As a primary target for the investigation of the −S response, the expression of high-affinity sulphate transporters has been characterized in various plant species in terms of a sulphur-specific response.

High-affinity sulphate transporters in plants

The functional complementation of yeast mutants has been used as a powerful tool to identify ion transporters (Frommer and Ninnemann, 1995; Barbier-Brygoo et al., 2001). Without exception, the molecular cloning of sulphate transporters was performed using this strategy; the yeast selenate-resistant mutant, YSD1, lacking a sulphate transporter was first used for cDNA cloning of sulphate transporters from the tropical legume, Stylosanthes hamata (Smith et al., 1995a, b). The identified clones, SHST1 and SHST2, showed high-affinity kinetics of sulphate uptake activities in the yeast expression system. The mRNA contents of SHST1 and SHST2 were increased in the roots of sulphur-starved plants, suggesting their significance in sulphate acquisition. The SHST1 homologues identified from barley (Smith et al., 1997; Vidmar et al., 1999), Arabidopsis (Takahashi et al., 2000; Vidmar et al., 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002), and tomato (Howarth et al., 2003) showed similar kinetic properties and expression.

The molecular characteristics of high-affinity sulphate transporters have been studied extensively in Arabidopsis. The Arabidopsis SULTR1;1 and SULTR1;2 were localized in the epidermis and cortex of roots, suggesting physiological roles in the initial uptake of sulphate (Takahashi et al., 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002; Maruyama-Nakashita et al., 2004a). SULTR1;1 and SULTR1;2 were able to complement the yeast YSD1 mutant (Smith et al., 1995b), and exhibited high-affinity sulphate uptake activities that were functionally equivalent to the yeast SUL1 sulphate transporter (Takahashi et al., 2000; Vidmar et al., 2000; Shibagaki et al., 2002; Yoshimoto et al., 2002). Both transporters were up-regulated by −S, although SULTR1;2 was abundantly expressed even under high sulphur (+S) conditions, and was less responsive to −S compared with SULTR1;1 (Yoshimoto et al., 2002). From the analysis of sultr1;2 mutants, it is evident that the SULTR1;2 transporter plays a major role in the uptake of sulphate in Arabidopsis roots. The sultr1;2 mutant showed a substantial decrease in sulphate influx activities (Shibagaki et al., 2002). The sultr1;2 knockout showed a compensatory up-regulation of SULTR1;1 mRNA, however, the uptake of sulphate did not fully recover (Maruyama-Nakashita et al., 2003). In addition, the up-regulation of SULTR1;1 mRNA in the mutant was correlated with the decrease in glutathione (GSH) content, suggesting that regulation is dependent to the internal sulphur status.

Sulphur-dependent regulation

Recent experiments suggested that accumulation of SULTR1;1 mRNA under −S is regulated at the level of mRNA transcription (Maruyama-Nakashita et al., 2004a). Furthermore, it has been demonstrated that the up-regulation of SULTR1;1 by −S is dependent on the 5′-promoter region. In SULTR1;1 promoter–GFP plants, the −1944 bp 5′-region was sufficient to drive specific expression of GFP in root hairs, root epidermis, and cortex (Takahashi et al., 2000). However, the −S regulation of SULTR1;1 required a −3031 bp promoter fragment (Maruyama-Nakashita et al., 2004a). It is suggested that putative sulphur-responsive elements are included between −3031 and −1944. This particular region may control the activation of SULTR1;1 gene expression under −S conditions. Further dissection of the SULTR1;1 promoter fragment and comparison with the other −S-inducible genes identified from the microarray studies (Hirai et al., 2003; Nikiforova et al., 2003; Maruyama-Nakashita et al., 2003) may clarify the sulphur-responsive elements necessary for the activation of the −S-responsive genes in plants.

As described previously by Yoshimoto et al. (2002), SULTR1;1 and SULTR1;2 were both regulated by the external supply of sulphate. When compared under the same culture condition, the response of SULTR1;1 was more sensitive to −S, showing drastic differences between 1500 µM and 100 µM in serial dilutions of sulphate conditions (Fig. 1A). SULTR1;2 was also inducible by −S, however, the mRNA content gradually increased following depletion of sulphate from the medium, and exhibited its maximum level when plants were grown with 10 µM sulphate (Fig. 1A). These results suggest that at least two different phases of the sulphur conditions stimulate the induction of SULTR1;1 and SULTR1;2, respectively, in Arabidopsis roots. The analysis of cell type-specific
expression of SULTR1;1 and SULTR1;2 indicated that both were localized in the surface cell layers of roots (Takahashi et al., 2000; Yoshimoto et al., 2002). Accordingly, SULTR1;1 and SULTR1;2 transporters were present in the same cell layers for the uptake, but were induced by −S in slightly different modes. These results suggest that SULTR1;1 and SULTR1;2 are functionally redundant for the uptake of sulphate particularly under −S conditions, but are not simply duplicated in terms of regulation. Expression of these two high-affinity sulphate transporters presumably provides a flexible uptake system suitable for adaptation to a wide range of sulphur conditions in the environment.

In addition to the external sulphate conditions, metabolites of the sulphur assimilatory pathways affected the gene expression of sulphate transporters. Exogenous application of Cys and GSH caused the down-regulation of the HVST1 sulphate transporter in barley that is accompanied by a rapid decrease in sulphate uptake activity (Smith et al., 1997). This negative feedback regulation was applicable to the expression of Arabidopsis sulphate transporters. The expression of SULTR2;1 was significantly repressed by GSH (Lappartient et al., 1999). As shown in Fig. 1B, SULTR1;1 and SULTR1;2 mRNAs were both significantly down-regulated in the presence of Cys and reduced GSH. By contrast with the effects of thiols, O-acetyl-L-serine (OAS), the precursor of Cys synthesis, positively affected the expression of sulphate transporters (Fig. 1C). It is reported that the addition of OAS can override the negative feedback regulation by GSH. In barley, plants fed with OAS exhibited a rapid induction of HVST1 mRNA, nevertheless GSH was abundantly accumulated in the root tissues (Smith et al., 1997).

In general, the mRNA transcripts of sulphur-responsive genes were increased by the OAS treatment (Kim et al., 1999; Koprivova et al., 2000; Hirai et al., 2003). As described above, OAS serves as a precursor of Cys synthesis, and its excessive accumulation causes the up-regulation of sulphur assimilation. In addition, it is reported that sulphur-starved plants abundantly accumulate OAS (Kim et al., 1999; Hirai et al., 2003; Nikiforova et al., 2003). Apparently, OAS acts positively for the induction of sulphate transporters; however, there is still no evidence that OAS is a direct effector molecule interacting with the regulatory factors for the sulphur assimilatory genes in plants. The OAS treatment causes an apparent imbalance of OAS over sulphide in the cell, and may deplete sulphide or sulphate in the reductive assimilatory pathway. Simultaneously, dissociation of the Cys synthase complex is promoted under these circumstances, liberating serine acetyltransferase as an inactive enzyme (Berkowitz et al., 2002). When the OAS pool is depleted by the restoration of sulphide, the Cys synthase complex would be reassembled to operate active OAS synthesis. The reversible regulation of the Cys synthase complex is balanced by the contents of OAS and sulphide, and is postulated to be a pivotal sensing unit of Cys biosynthesis in plants (Hell, 2003).

**Supply of carbon and nitrogen affects the regulation**

The serine biosynthetic pathway in the plastids provides the carbon skeleton for Cys synthesis (Ho and Saito, 2001). The 3-phosphoglycerate derived from glycolysis goes through oxidative conversion and amino-transfer reaction, generating OAS as a substrate of Cys synthase. A corollary
of this metabolic connection between the sulphur and carbon metabolisms is that the expression of APS reductase, the key enzyme of sulphate reduction, was stimulated by feeding sucrose and glucose to plants (Kopriva et al., 1999, 2002; Koprivova et al., 2000; Hesse et al., 2003). Similar to APS reductase, SULTR1;1 and SULTR1;2 mRNA contents were substantially increased by the external supply of sucrose (Fig. 2A). Plants grown with sucrose showed abundant accumulation of SULTR1;1 and SULTR1;2 mRNAs under −S conditions. By contrast, depletion of carbon sources from the media remarkably attenuated the induction of SULTR1;1 and SULTR1;2 by −S. These results suggest that sulphate transporters that associate with the uptake of sulphate in roots are basically regulated by sulphur, but are additionally controlled by the fluctuation of carbon status. It is reported that the expression of nutrient transporters is generally induced by carbon supply in Arabidopsis root (Lejay et al., 2003). The hexokinase-dependent signal is not the most likely pathway involved in this general carbon-dependent regulation of nutrient transporters. Instead, the glycolytic flux is postulated to play the regulatory role. Presumably, the nutrient uptake systems are co-ordinately operated under a common regulatory circuitry to meet the demands of primary metabolism when carbon is sufficiently supplied; however, the exact mechanism remains an open question.

In addition to the effects of carbon status, the expression of SULTR1;1 and SULTR1;2 was significantly influenced by nitrogen supply (Fig. 2B). As in the case of sucrose, limitation of the nitrogen source diminished the response of sulphate transporters, attenuating their expression under −S. Referring to the metabolic connection between the sulphur and nitrogen metabolisms, the pool of OAS may serve for the regulation. As described above, synthesis of OAS originated from 3-phosphoglycerate, and includes an amino transfer reaction catalysed by phosphoserine aminotransferase in the plastids (Ho and Saito, 2001). Thus, the supply of nitrogen is expected to increase the level of the OAS pool. In addition, the rate of reductive sulphur assimilation or Cys synthesis can influence the OAS level. In fact, the accumulation of OAS was shown to be correlated with the relative N/S status of plants (Kim et al., 1999). Furthermore, the −S response of the β-conglycinin gene was dependent on the N/S status and accumulation of OAS (Kim et al., 1999). N-dependent regulation was also evident for APS reductase. The increase of APS reductase mRNA by OAS was significantly stimulated when plants were pre-incubated under −N conditions (Koprivova et al., 2000). These results suggest that regulation of the plant −S response is tightly controlled by the N associated with the synthesis of OAS. By contrast with carbon and nitrogen conditions, fluctuation of the phosphate concentrations in the medium did not exhibit any pronounced effects on the expression of sulphate transporters (Fig. 2C).
Promoter–reporter systems for biological screening of regulatory factors

The expression of the sulphate transporter and the sulphate uptake rate are basically regulated by the sulphur status in conjunction with the metabolic effects of nitrogen and carbon, reflecting the changes of the nutrient status and metabolic fluxes. However, perception of the input nutrient signal and the mechanisms of signal transduction that explain the regulation of transporters are as yet unknown at the molecular level. To fill this gap, a transgenic system was established that displays the expression of sulphate transporter as a GFP fluorescence in Arabidopsis roots (Maruyama-Nakashita et al., 2004a, b).

As a first step in the investigation of the signalling cascades regulating the sulphur response in Arabidopsis, pharmacological studies were performed that examined the effects of inhibitors for protein kinase and phosphatase. It was demonstrated that the expression of SULTR1;1 is significantly influenced by okadaic acid and calyculin A, the inhibitors of protein phosphatase (Maruyama-Nakashita et al., 2004a). The application of these inhibitors to Arabidopsis roots blocked the expression of SULTR1;1 mRNA under −S. The effects of the inhibitors were reproducible in planta, showing the decreased expression of GFP in the roots of SULTR1;1 promoter–GFP transgenic plants. The induction of SULTR1;1 expression under −S condition was promoter-dependent, and was under the control of protein phosphatase sensitive to okadaic acid and calyculin A. In Chlamydomonas, a Snf1-like Ser/Thr kinase, Sac3, is involved in the regulation of arylsulphatase and sulphate uptake activities (Davies et al., 1999). The results indicate that protein phosphorylation and dephosphorylation are involved in the regulation of −S-responsive genes in plants and algae. However, it is not clear whether the de-phosphorylation pathway suggested from the inhibitor studies in Arabidopsis is relevant to the Sac3-mediated regulation.

From the screening of plant hormones, a cytokinin-dependent signalling cascade was identified as a potential target for analysing the regulation of sulphate uptake in Arabidopsis roots (Maruyama-Nakashita et al., 2004b). The expression of SULTR1;1 and SULTR1;2 mRNAs was down-regulated by cytokinin, which is accompanied by a decrease in sulphate uptake activities. Figure 3 shows the typical response of SULTR1;2 to cytokinin treatment, visualized as green fluorescence in SULTR1;2 promoter–GFP transgenic plants (Maruyama-Nakashita et al., 2004b). Signal transduction of cytokinin involves a two-component phosphorelay system including a sensory histidine kinase, histidine phosphotransmitters, and response regulators as essential components (reviewed in Mizuno, 1998; Schmülling, 2001; Hutchinson and Kieber, 2002; Kaki-moto, 2003). CRE1/WOL/AHK4 is the histidine kinase that triggers phosphorelay upon perception of cytokinin (Inoue et al., 2001; Suzuki et al., 2001; Mähönen et al., 2000).

Thus, the cytokinin response 1 (cre1) mutant is insensitive to cytokinin and is unable to transfer the signal to the downstream target genes. The cre1-1 mutant (Inoue et al., 2001) was used to confirm the negative regulation of sulphate uptake by the CRE1/WOL/AHK4-derived cytokinin signal. The results clearly indicated that the cre1-1 mutant is unable to repress the expression of SULTR1;1 and SULTR1;2 mRNA in response to cytokinin (Maruyama-Nakashita et al., 2004b). In addition, the sulphate influx rate of cre1-1 was maintained at nearly the same levels, even after the cytokinin treatment. These results suggested that the CRE1/WOL/AHK4-mediated signal transduction pathway exists in the regulatory circuitry for the expression of SULTR1;1 and SULTR1;2 sulphate transporters in Arabidopsis. The repressive signals are probably transmitted through the response regulators, however, the identification of specific components responsible for the negative regulation of SULTR genes awaits further investigation. Ohkama et al. (2002) reported that the expression of the −S-inducible seed storage protein, β-conglycinin, is positively regulated by cytokinin in Arabidopsis roots. Their findings suggest that an alternative signalling pathway, independent from the one postulated for the repression of sulphate uptake, may activate the expression of β-conglycinin in response to cytokinin.

Future perspectives

In Arabidopsis, genetic screening of selenate- and ethionine-resistant mutants identified the SULTR1;2 sulphate transporter (Shibagaki et al., 2002) and the mto1 region in the cystathionine γ-synthase (Inaba et al., 1994; Chiba et al., 1999), respectively. These attempts were made following the strategies applied to identify the transcriptional regulators of sulphur assimilation in yeast and fungi (Marzufl, 1997; Thomas and Surdin-Kerjan, 1997). However, none of the regulatory proteins that specifically modulate the expression of −S-responsive genes has been
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References


