Altered Sulfate Metabolism of Arabidopsis Caused by Beet Severe Curly Top Virus Infection

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(Received on September 7, 2005; Accepted on November 15, 2005)

Sulfur, an important component of plants, is regulated by a variety of stresses in sulfate assimilation and metabolism. Increase has been observed in the expression of O-acetlyserine(thiol)lyase (OASTL) through two-dimensional electrophoresis with the shoot tips of Arabidopsis infected by beet severe curly top geminivirus (BSCTV). With the three- to six-fold increases in the transcript expression of OASTL, serine acetyltransferase (SAT) and γ-glutamylcysteine synthetase (GSH) were induced over the mock-inoculated organization in each organization through real-time RT-PCR analysis. The expression of these genes might affect the accumulation of anthocyanin in symptomatic tissues and the induction of abnormal callus-like structures formed by additional cell divisions as typical disease symptoms of BSCTV-infected Arabidopsis. This is the first report to describe the collaborative induction of OASTL, SAT, and GSH in virus-infected plants. The changed expressions of OASTL, SAT, and GSH in Arabidopsis infected with BSCTV raises new aspects regarding the biological function of symptomatic tissues related to sulfate metabolism.

Keywords: O-acetlyserine(thiol)lyase, Arabidopsis, BSCTV, γ-glutamylcysteine synthetase, serine acetyltransferase

Sulfur is found in a variety of biomolecules, such as amino acids, oligopeptides, vitamins, cofactors, and secondary metabolites. It also plays a role in maintaining the protein structure or redox cycles (Leustek and Saito, 1999). The quantitative change of sulfate and rate of sulfate assimilation are regulated by a variety of factors, such as nutrition, development, environmental factors, and various stresses (Saito, 2004). It has been reported that a few plant hormones engage in the regulation of genes related to sulfur metabolism. Methyl jasmonate and auxin are related to sulfur-deficiency stress (Hirai et al., 2003; Nikiforova et al., 2003) and cytokinin also is engaged in the down-regulation of sulfate transporter genes in Arabidopsis root (Maruyama-Nakashita et al., 2004). It is also reported that abiotic stresses, such as heavy metals and oxidative stress, affect sulfur assimilation (Saito, 2004). In plants exposed to cadmium and phytochelatins, glutathione is accumulated through the continuous consumption of cysteine. Heavy metal stresses cause an increase in sulfur assimilation or the induction of sulfate transporter genes (Dominguez-Solis et al., 2001; Nocito et al., 2002). The biosynthesis of cysteine and phytochelatin is increased by the up-regulation of serine acetyltransferase (SAT) and O-acetylsereine(thiol)-lyase (OASTL) genes under cadmium stress and the rate of O-acetylsereine (OAS) biosynthesis is increased by the induction of several SAT genes under a highly-concentrated cysteine demand (Howarth et al., 2003). In Arabidopsis trichome cells, OASTL, SAT and glutathione, were specifically expressed and those expressions correlated to a high level of glutathione content in trichome cells to play a possible role as a sink during detoxification processes (Gutierrez-Alcala et al., 2000). However, it has not yet been reported that sulfate metabolism is affected byiotic stresses.

Cysteine biosynthesis, sulfate uptake, and sulfate reduction are regulated in the levels of gene expression and enzyme activation in plants. However, the subcellular, temporal, and spatial localization of enzymes are complicated according to the species of plant (Leustek and Saito, 1999; Saito, 2004). In the absence of sulfate, the expression of genes related to sulfur transporters and adenosine 5'-phosphosulphate reductase (APR) is highly induced in roots and SAT and OASTL are induced in leaf tissue (Leustek and Saito, 1999; Saito, 2004; Takahashi et al., 1997). Sulfur starvation increased the enzymatic activities of sulfate transporters, such as SAT, APR, and OASTL.

Two-dimensional electrophoresis (2-DE) analysis of various symptoms in Arabidopsis caused by BSCTV...
showed that the expression of OASTL was increased (data not shown). Additionally, real-time RT-PCR showed that the transcripts of OASTL genes, key regulators of sulfate metabolism, increased in symptomatic tissues, such as shoot tips and infection origins. It is interesting that the increase of this gene expression is closely related to the anthocyanin accumulation in the tissues with severe disease symptoms, thus showing the interrelationship between symptom development and sulfate metabolism. Many studies have been conducted to determine the expression patterns and the functions of OASTL, SAT, and GSH in the plants grown under a variety of abiotic stresses. This is the first report which shows the induction of genes involved in sulfate assimilation by viral infection. The present results would provide useful information for the study of sulfate metabolism affected by biotic stress.

Materials and Methods

Plant growth and virus strain. *Arabidopsis thaliana* ecotype Col-O was obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, USA). Arabidopsis seeds were planted in flats containing artificial soil. Four- to five-week old plants were inoculated with infectious *Beet severe curly top virus* (BSCTV) DNA by agroinfection of wounds produced in the crown of the rosette by needle puncture as previously described (Park et al., 1999). Three weeks after inoculation, plants were harvested, frozen in liquid nitrogen, and stored at -70°C for further analysis. BSCTV was provided by Dr. Stenger (University of Nebraska, USA) and the same strain was used in our previous studies (Lee et al., 1994).

DNA isolation and Southern blot hybridization. Preparations of total DNA and DNA blot hybridization were prepared as described (Lee et al., 1994) using a 32P-labeled probe prepared by random-primer labeling of pCLC, a pUC8 derivative containing single, tandemly-repeated copies of the BSCTV and *Beet curly top virus* (BCTV) genomes.

Isolation of total RNA and real-time RT-PCR analysis. Total RNA was collected from Arabidopsis organs infected with BSCTV or MOCK-inoculated. RNA was isolated using the RNeasy mini kit (Qiagen, Germany) according to the instructions of the manufacturer. As a quantitative control, we used the *Actin2* gene (GenBank accession no. AY096381) of Arabidopsis (Actin2 forward primer, 5'-TTCCTCAGATGAAAGACGCTGCTG-3' Actin2 reverse primer, 5'-TGCTTGACGTCACCGTTACG-3'). Specific primers for the *AtOAS-7-4* encoding cDNA OASTL (GenBank accession no. X80377), *sat-1* gene encoding cDNA SAT (GenBank accession no. U22964), and *gsh1* gene encoding cDNA γ-glutamylcysteine synthetase (GenBank accession no. Z29420) were as follows: OASTL forward primer, 5'-AAAACCCGACC TCACAAAGA-3'; OASTL reverse primer, 5'-GAGTTCCGGCCATTTTCCAG-3'; SAT forward primer, 5'-GCTCCGGTTCATCTTCTCCAC-3'; SAT reverse primer, 5'-ATGGAGGTTTTTGCTTGG-3'; GSH forward primer, 5'-AATTCCTGTTGTCCTCCGT-3'; and GSH reverse primer, 5'-CCAGTACGCTGTCACGC- TCCA-3'. To generate cDNA from Arabidopsis samples, 2 μg of total RNA was reverse transcribed with oligo (dT) 18 primer (Invitrogen, USA) using M-MLV Reverse Transcriptase (Promega, USA), while ExTag polymerase (Takara, Japan) was used in the subsequent PCR. Real-time PCR was performed using the Light Cycler Fast Start DNA Master SYBR Green I from Roche Diagnostics GmbH according to protocol of the manufacturer (Roche Diagnostics, Japan). The following Light Cycler conditions were used: initial denaturation at 95°C for 3 min, followed by 40 cycles with denaturation at 95°C for 8s, annealing at 60°C for 15s, and elongation at 72°C for 15s. Quantities of specific mRNA in the sample were measured according to the corresponding gene-specific standard curve. Each reaction was performed at least three times.

Results and Discussion

The symptoms of BSCTV-infected Arabidopsis. Severe symptoms were induced from two to three weeks after BSCTV inoculation on the Arabidopsis Col-O ecotype. Inflorescence stalks were severely stunted, the growth of rosette leaves stopped, and floral organs were malformed (Fig. 1) as described previously (Lee et al., 1994). Another symptom was the accumulation of anthocyanin in symptomatic tissues, such as infection origins and rosette leaves at three or four weeks after inoculation with BSCTV (Fig. 1d). Another pathomorphological change was the callus-like structure which also formed in symptomatic tissues, especially in the inflorescence stalks of BSCTV-infected Col-O (Fig. 1f).

Accumulation of viral DNA. To compare the accumulation of BSCTV DNA in BSCTV-infected Arabidopsis, DNA blot hybridization was performed using the total DNA isolated from several organs. Viral DNAs were differentially accumulated in those organs (Fig. 2). As compared to other organs, viral DNAs were much more highly accumulated in shoot tips, infection origins, and inflorescence stems, but accumulated least in rosette leaves. Viral DNA accumulated in the infection origins and in the shoot tips seven times more than in the rosette leaves.
Expression patterns of OSATL, GSH, and SAT genes in Arabidopsis organs. The amounts of OSATL, GSH, and SAT mRNAs were examined through real-time RT-PCR to analyze the expression patterns of OSATL, GSH, and SAT genes involved in sulfate metabolism (Fig. 3). OSATL expression increased in all organs of BSCTV-infected plants. The OSATL expression was three to four times higher in the shoot tips and infection origins than in other organs. Similarly, GSH expression was also three to four times more increased in infection origins than in other organs. Little difference was found between the expression levels in other organs from BSCTV- and mock-infected plants, except the expression of GSH, which only increased in infection origins. On the other hand, the expression patterns of SAT showed that all organs had double the original amount of SAT transcripts, except infection origins. Shoot tips induced the greatest increase of SAT expression compared to other organs of mock-inoculated plants.
OASTL was induced in BSCTV-infected Arabidopsis.

The symptoms of BSCTV-infected Arabidopsis can be used as an important research system which shows the pathophysiological changes in plants by biotic stress. Specifically, the formation of callus-like structures and the accumulation of anthocyanin in BSCTV-infected Arabidopsis suggest that the cell cycle regulation and the anthocyanin metabolism of plants could be changed by BSCTV infection. The formation of abnormal callus-like structures in BSCTV-infected Arabidopsis is also related to viral DNA accumulation (Lee et al., 1994), thereby leading to the change of exterior factors affecting the signal transduction of cell cycle regulation. Callus formation is induced through the balance breakdown of hormones (Che et al., 2002). Additionally, the expression analysis of certain cyclins and CDKs related to cell cycle regulation by virus infection mostly showed increasing and decreasing manners at each stage of cell cycle (data not shown). This suggests that control of the cell cycle could be changed by BSCTV infection. BSCTV infection could change the cell cycle control which causes abnormal plant development and tumorigenic growth in transgenic Nicotiana benthamiana (Latham et al., 1997; Stanley and Latham, 1992). The factors of physiological change have not yet been elucidated, but the abnormal cell division related to BSCTV infection is considered to affect cell cycle regulation and, thus, cause the imbalance of cell cycle control.

Anthocyanin, a kind of flavonoid, acts as a variety of pigments and works as a signal molecule for the protection against UV radiation and plant-microbe interactions (Nesi et al., 2000). BSCTV-infection, a biotic stress, causes the accumulation of anthocyanin in Arabidopsis. Anthocyanin levels are reported to affect those of glutathione-S-transferase (GST) and GSH by confirming the decrease in the total amount of anthocyanin in Bz2 gene mutant of maize (Alfenito et al., 1998), the An2 gene mutant of petunia (Edwards et al., 2000), and the GSH1 mutant of Arabidopsis (Xiang et al., 2001). Specifically, the GSH gene plays an important role in sulfate metabolism, and studies of the abiotic stress have been conducted (Marrs, 1996). The total protein was separated from the shoot tips of BSCTV-infected Arabidopsis three weeks subsequent to BSCTV infection and then analyzed using two-dimensional electrophoresis (2-DE) (data not shown), thus showing an increase in the OASTL protein level referred to as cysteine synthase. As an important enzyme for sulfate metabolism, OASTL is considered to present the interrelatedness of sulfate metabolism by biotic stresses.

OASTL, SAT, and GSH genes were induced by BSCTV.

The expression of OASTL gene at the transcription level was analyzed using real-time RT-PCR with the primers designed for the glutathione (GSH), serine acetyltransferase (SAT), and O-acetylseryl(thiol)lyase (OASTL) genes. The results showed that the transcripts of three genes increased in BSCTV-infected Arabidopsis. All three genes have
independent functions as important factors in sulfate metabolism. The genes investigated in this study were analyzed in other tissues, and for other purposes, by Gutierrez-Alcaela et al. (Gutierrez-Alcaela et al., 2000). OASTL, SAT, and GSH genes were highly expressed in Arabidopsis trichome cells to play a possible role as a sink during detoxification processes.

Here, we investigated the possible biological functions of three genes and their altered expression levels, as well as changes in sulfate metabolism by virus infection. Glutathione is necessary for the glutathionation of anthocyanin by GST, and the formation of glutathione-anthocyanin conjugate is very important for the transportation of anthocyanin to vacuoles (Alfenito et al., 1998; Edwards et al., 2000; Xiang et al., 2001). For example, the mutants of maize’s Bz2 and petunia’s An2 encoding GST show that anthocyanin is not decreased or accumulated (Marrs, 1996; Xiang et al., 2001). Therefore, it is possible that the increase of GSH expression in BSCTV-infected Arabidopsis may be a phenomenon shown in symptoms of anthocyanin accumulation.

Much of the interrelationship between GSH level and cell cycle control has not yet been clarified, but it has been reported that GSH affects the regulation of cell division. For example, the rate of cell division decreased if the ROOTMERISTEMLESS (RML) gene was depleted through the inhibitor related to GSH biosynthesis (Vernoux et al., 2000). It has also been reported that the greater the expression of GSH in Arabidopsis root, the more it is related to proliferating cells, such as epidermal and cortical initials, but that lower expression of GSH shows slowly cycling cells of the quiescent centre that have an extended G (Sanchez-Fernandez et al., 1997). In conclusion, symptom changes, such as callus formation by BSCTV infection, could be closely related to the increased expression of GSH.

The O-acetyls erine(thio)lyase (OASTL) which is called cysteine synthase catalyzes the final step of the cysteine biosynthesis pathway. OASTL is induced under sulfur starvation and different isoforms are localized in plastids, cytosol, and mitochondria (Hesse et al., 1999; Saito, 1999). Along with SAT, OASTL forms the complex referred to as the cysteine synthase complex, thus being included in the regulation of cysteine synthesis. However, OASTL plays a leading role in the regulation of cysteine synthesis, as seen in the experiment in which the overexpression of OASTL could not affect the contents of GSH in several plants (Hofgen et al., 2001). In the absence of sulfate, the genes related to sulfur transporters and APR are predominantly induced in roots and SAT and OASTL genes are induced in leaf tissues (Leustek and Saito, 1999; Saito, 1999; Takahashi et al., 1997). In regards to the enzyme activity, the effects of sulfur starvation increase the activity of sulfate transporters such as SAT, APR, and OASTL. Similarly, the expression of SAT gene increased in most organs of BSCTV-infected Arabidopsis. This is closely related to the expression of OASTL gene related to sulfate metabolism.

Prior to the formation of cysteine catalyzed by O-acetyls erine(thio)lyase (OASTL), serine acetyltransferase (SAT) acts on the activation of serine through O-acetylation. Different isoforms of SAT are localized in plastids, cytosol, and mitochondria (Saito, 2004). In contrast to OASTL, its binding partner, SAT is known to be a target fit for manipulation of metabolite flux through a pathway related to the change of sulfur contained in plants, and is reported to directly play an important role in cysteine synthesis (Hofgen et al., 2001). The change of SAT gene expression shows a similar pattern to the change of viral accumulation in each organ. The expression amount of SAT increased five or more times in the shoot tip of BSCTV-infected Arabidopsis than in mock-inoculated plants, and two or more times than in the infected stalk. However, in contrast to viral accumulation, the expression amount of SAT increased in cauline (?) leaves compared to the mock-inoculated plants (data not shown). Therefore, in organs other than the cauline leaf, the expression of SAT may be related to the accumulation of viral DNA. However, in all organs except the infection origin, OASTL shows an increase in the amount of expression regardless of viral accumulation. As compared to the result of 2-DE, the increases in expression amounts of both proteins and transcripts by real-time RT-PCR in the shoot tip of BSCTV-infected Arabidopsis are all considered to be due to viral infection. The expression of OASTL is thought to be affected by the increase of SAT expression through viral DNA accumulation rather than by the direct correlation with viral DNA. The expression extent of GSH is also considered to be similar to that of OASTL. Similarly, all organs except the infection origins show some increase in the expression level compared to mock-inoculated plants. Therefore, the change of sulfate metabolism by viral DNA accumulation seems to affect the expression of SAT, OASTL, and GSH, since sulfur metabolism is changed due to (1) the sulfur starvation within cells by sudden viral DNA accumulation after viral infection and (2) the possible detoxification processes of some toxic components accumulated by viral infection.

References

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