RESEARCH PAPER

Isolation of a novel ABC-transporter gene from soybean induced by salicylic acid

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Abstract

This paper reports on the identification and characterization of a new ATP-binding cassette (ABC) transporter which was identified as a salicylic acid-induced gene from soybean (Glycine max cv. Williams 82) in a subtractive suppression hybridization approach. A fragment of an ABC-transporter gene was used to isolate a full-length cDNA clone for this gene with a length of 4750 bp. The encoded protein has a length of 1447 amino acids and is composed of two similar repeat units typical of full-size ABC transporters. The sequence displays a close relationship to plant pleiotropic drug resistance (PDR)-type transporters and, on a homology basis, clusters together with the Arabidopsis thaliana PDR12 gene, suggesting GmPDR12 as a name for the gene isolated from soybean. GmPDR12 is rapidly responsive to salicylic acid and methyl jasmonate. The mRNA starts to accumulate 30 min after the addition of the signalling compounds. Salicylic acid is required for the execution of the hypersensitive reaction in soybean cell suspension cultures inoculated with Pseudomonas syringae pv. glycinea. It has been demonstrated previously that salicylic acid can be substituted by a variety of functional analogues of salicylic acid. All of these compounds lead to a strong and rapid transcriptional activation of GmPDR12, suggesting a shared signalling pathway.

Key words: ABC transporter gene, Glycine max, salicylic acid, soybean, Subtractive suppression hybridization.

Introduction

Plant responses to pathogens are a multilayer network of defence reactions, which try to limit and eventually stop the invading microbial pathogen. The reactions include the rapid generation of reactive oxygen species, cross-linking of cell wall polymers, the production of antimicrobial pathogenesis-related proteins, and low molecular weight phytoalexins (Heath, 2000; Dangl and Jones, 2001). The network of responses requires common signalling pathways and one key compound is salicylic acid (SA). When invaded by pathogens, resistant plants induce defence reactions both locally and in distant organs. Local defence is commonly associated with the hypersensitive reaction (HR), a form of programmed cell death in plants (Heath, 2000). Very often, SA is present and required at higher local concentrations (50–100 μM) to perform the cell death programme (Klessig and Malamy, 1994; Tenhaken and Rubel, 1997). Transgenic plants expressing the SA-metabolizing enzyme salicylate hydroxylase (nahG gene) or mutants of the biosynthetic pathway are therefore incapable of executing the cell death response (Delaney et al., 1994; Shirasu and Schulze-Lefert, 2000). Plant cells need SA downstream of the recognition process of pathogens (Shah, 2003).

The model system for analysing SA-dependent plant defence responses is the soybean–Pseudomonas syringae pv. glycinea interaction. The soybean cultivar Williams 82 recognizes the bacteria when they functionally express the avrA gene and triggers a cell death programme. When using a plant cell suspension culture of the same soybean cultivar, the HR often requires the external addition of physiological concentrations of SA to execute the cell death...
programme. Thereby, SA can be functionally replaced by a number of different chemicals (Tenhaken et al., 2001).

Of interest in this study is the regulation of gene expression by SA and its analogues which are useful tools for elucidating SA-signalling pathways. The soybean cell culture system allows the signalling pathways to be easily manipulated and tested, and it responds to pathogens in a gene-for-gene interaction (Levine et al., 1994). A screen for such genes identified a gene encoding for an ATP-binding cassette (ABC)-transporter from soybean, which is rapidly induced by SA. The ABC transporter belongs to a large family of similar proteins in plants. In Arabidopsis the genome project had identified >130 different genes for this gene family (Jasinski et al., 2003; Garcia et al., 2004). On the basis of structural features, two separation lines can be drawn. Half-size transporters have a single nucleotide binding domain and one cluster of transmembrane helices. By contrast to that the full-size transporters show an internal duplication consisting of two half-size sites (Higgins, 1992). The other structural feature is the location of the transmembrane helices either at the beginning (forward organization) or at the end of each subunit (reserve organization), the latter being characteristic of the pleiotropic drug resistance (PDR) subfamily of ABC transporters.

ABC transporters are well known for their ability to confer drug resistance of xenobiotics to yeast, animal, and plant cells. The largest and best characterized subfamilies, PDR, multidrug resistance (MDR), and multidrug resistance-associated proteins (MRP), all refer to xenobiotic substrates for these transporters. A comprehensive overview of the Arabidopsis PDR transporters was recently published (Martinoia et al., 2002; van den Brule and Smart, 2002). However, there is growing evidence that endogenous metabolites are natural substrates for many ABC transporters that need to be identified in the future. For example, the cer5-mutant of Arabidopsis was recently shown to encode for an ABC transporter responsible for lipid transport of cuticular waxes (Pighin et al., 2004). Some transporters like the Arabidopsis AtMRP2/3 can simultaneously transport chlorophyll catabolites as natural substrates and xenobiotic-glutathione conjugates (Lu et al., 1998; Rea et al., 1998). A comprehensive review of the transport of secondary metabolites by ABC transporters was recently published by Yazaki (2006).

ABC transporters have been identified in several membrane systems in plant cells, the most prominent being the tonoplast membrane of the vacuole and the plasma membrane. The PDR-type ABC transporters are often found in the plasma membrane. One function seems to be the export of terpenoids as anti-insecticidal compounds (Jasinski et al., 2001; van den Brule et al., 2002; Stukkens et al., 2005). Genes for ABC transporters in plants can be induced by a variety of external signals, indicating a far broader role in many aspects of the plant life cycle than is currently known. For example, the tobacco ABC transporter NtPDR1 is induced by elicitors like yeast extract or flagellin, indicating a role in plant defence (Sasabe et al., 2002). A loss of Arabidopsis AtPDR8 leads to increased cell death after pathogen infection (Kobae et al., 2006). Using microarrays, Glombitza et al. (2004) showed the induction of ABC transporters by a variety of stress factors and pathogens like Pseudomonas syringae. Similarly, the tobacco NpPDR1 gene is induced by pathogens and jasmonate (Grec et al., 2003). The OsPDR9 ABC transporter from rice roots is induced by hyperosmotic stress or heavy metal treatment such as abiotic stress factors, suggesting an advantage for coping with these stresses (Moons, 2003). Iron deficiency in tobacco is another abiotic stress factor, which leads to the induction of the NtPDR3 ABC transporter (Ducos et al., 2005).

This paper reports on the identification and characterization of a novel ABC transporter gene from soybean, which is a member of the PDR-subfamily. The gene is rapidly induced by SA and jasmonic acid and exhibits a transient expression pattern after treatment with the signalling compounds. In addition, functional analogues of SA, which complement pathogen-triggered cell death programmes that otherwise require SA, show a very similar gene induction pattern.

Materials and methods

Biological material

Soybean cell suspension cultures were grown in the dark at 26 °C in MS-medium and subcultured every week by a 1:6 dilution into fresh medium as described (Levine et al., 1994). For experiments, cells were used 3–4 d after subculturing.

Chemicals

The functional analogues of SA were provided by Alexis (LY-171,883; WY-14,643) or Sigma. Bay X1005 was a kind gift of Dr Müller-Peddinghaus, Bayer AG Wuppertal, Germany. Bion® was bought at a local store for agriculture supplies.

Subtractive suppression hybridization

Soybean cells were treated with 200 µM SA (or H2O in the control) for 4 h and total RNA was subsequently isolated by the guanidinium thiocyanate/acetic phenol method as described by Chomczynski and Sacchi (1987). mRNA was isolated from the total RNA by hybridization with a biotinylated oligo-d(T) primer and purified with streptavidin-coated magnetic beads (Merck Biosciences, Darmstadt, Germany) according to the manufacturer’s protocol. Subtractive suppression hybridization from Rsal-digested ds-cDNA was performed according to the protocol from Clontech (Heidelberg, Germany). After the suppression, hybridization-amplified products were cloned into pBluescript and individual clones were sequenced (MWG, Ebersberg or Seqlab Göttingen, Germany).

Isolation of a full-length cDNA clone of GmPDR12

A 250 bp fragment with homology to the 3’-end of ABC transporters was used as a hybridization probe to screen the λ-phage cDNA library from soybean (Tenhaken and Thulke, 1996). Two phage clones were purified and converted into pBluescript plasmids by in vivo excision (Stratagene, La Jolla, CA, USA). As both
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Soybean cell suspension cultures were treated with SA for 4 h. Using subtractive suppression hybridization, several differentially expressed genes, which are strongly induced by SA-treatment, were identified. They include several isoforms of the glutathione-S-transferase gene family (identical to GenBank AF243365), members of the glutathione peroxidase gene family (similar to GenBank AY237655), a glucosyl transferase (identical to GenBank BE346477), a putative extensin (GenBank AA903043), and an ABC-transporter gene (similar to GenBank Z70524). As the available sequence information from SSH-clones is rather limited, a cDNA library for a full-length cDNA of the ABC-transporter gene was screened. The cDNA is 4770 bp long and has 5'-UTR of 118 bp with several in-frame stop codons. The 3'-UTR sequence is 290 bp long followed by a poly(A)-tail. The open reading frame (ORF) encodes for a protein of 1447 amino acids with a predicted molecular weight of 163 kDa, which is the molecular weight range, typically found for full-size transporters (Higgins, 1992). A scheme of the domain structure of the transporter is shown in Fig. 1B. Generally the protein consists of two halves which show a similar organization (Fig. 1B). Each half has a highly conserved Walker A and B motif, involved in ATP-binding, and an additional ABC-signature motif close to the Walker B domain. The ATP-binding site is followed by several transmembrane helices. Typically for this sub-family of ABC proteins each half of the transporter has six membrane-spanning helices (Fig. 1A). The model for the location of the trans-membrane helices predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) is shown in Fig. 1B, C. The location also becomes evident by the hydrophobicity plot in Fig. 1A, which shows appropriate long hydrophobic stretches for each membrane span.

The cDNA of the soybean ABC-transporter gene is supported by seven EST (expressed sequence tag) clones. The positions of the ESTs are shown as short bars below the gene in Fig. 1C. The EST sequences match largely to the 3'-end of the cDNA.

Amino acid sequence comparison using BLASTP (http://www.ncbi.nlm.nih.gov/BLAST) revealed the high degree of similarity between the predicted soybean ABC transporter and other ABC transporters from plants. The sequence corresponds to the PDR subgroup of ABC transporters. This family has 15 members in Arabidopsis thaliana according to the Arabidopsis Information Resource (http://www.arabidopsis.org/info/genefamily/pdr.html; van den Brule and Smart, 2002). However, the top-ranking BLASTP homologues were NtPDR1 from Nicotiana tabacum (accession number BAB92011, 69% identity) and NpABC1 from Nicotiana plumaginifolia (accession number CAC40990, 68% identity). This is shown in the phylogenetic tree in Fig. 2, which was generated with the predicted protein sequences from 15 Arabidopsis PDR, the two tobacco, and the soybean GmPDR12. The two tobacco sequences and the soybean ABC-transporter protein cluster together with the protein encoded by the At1g15520 gene, which is AtPDR12 according to the Arabidopsis Information Resource (Martinoia et al. 2002; van den Brule and Smart, 2002). Therefore the new ABC-transporter gene was named GmPDR12.
It is well known that ABC transporters belong to a large family of different but still quite similar proteins (van den Brule and Smart, 2002; Jasinski et al., 2003). To address the question whether a close homologue of the transporter is present in the soybean genome, which might cross-hybridize with *GmPDR12*, a Southern blot analysis was performed. Genomic DNA was restricted with several restriction endonucleases, gel separated, and hybridized with an 800 bp *BamHI* fragment from the 5'-end of *GmPDR12* under conditions of moderate stringency (62 °C) (Fig. 3). A single band is visible in each lane of restricted DNA, indicating that hybridization with moderate stringency will only detect the *GmPDR12* gene.

The gene induction of *GmPDR12* was investigated in RNA gel blots. A time kinetic for the gene induction of *GmPDR12* after treatment with SA is shown in Fig. 4. Whereas no change in the expression level was observed in controls, the SA-treatment leads to a rapid and strong induction of the *GmPDR12* gene. The mRNA already accumulates 30 min after application of SA with a peak at around 1.5 h post induction. The decline of the mRNA level to background levels occurs within 24 h when a relatively high dose (200 μM) of SA is applied. Independent experiments were performed with lower concentrations of SA (50 μM) which also lead to a rapid induction of the *GmPDR12* gene but show a much more transient induction pattern. Under these conditions, background levels for the *GmPDR12* mRNA were reached after 6–8 h (data not shown). This prompted the analysis of the level of SA in the soybean cells. As indicated in Fig. 4, the amount of unconjugated SA remains high in the cytoplasm for several hours and starts to decline 6 h after treatment with SA. This decline is caused by the accumulation of the SA-glucoside in the vacuole (data not shown; compare also Tenhaken and Ruebel, 1997). Time-course experiments with a lower starting concentration of SA indicate that a decline of *GmPDR12* mRNA is closely correlated with the availability of free SA in the cytoplasm (data not shown).

The dose-dependent induction of *GmPDR12* by SA and methyl jasmonate was further investigated by quantitative PCR in RNA samples of cells treated for 2 h with the different signalling compounds (Fig. 5). Low concentrations of SA (25 μM) lead to a clear induction of the *GmPDR12* gene, which is further enhanced in samples of cells treated with higher concentrations (100–200 μM). Interestingly, methyl jasmonate is also a powerful inducer of the ABC-transporter gene especially at lower concentrations (Fig. 5).

Inoculation of soybean cell suspension cultures with avirulent *Pseudomonas syringae pv. glycinea* bacteria triggers an HR. This cell death programme is dependent on the presence of the *avrA* gene in the bacteria and on SA. Although the exact function of SA for the cell death programme is not known it has been worked out previously that SA can be substituted by a range of other compounds (Tenhaken et al., 2001). Therefore the response of the...
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GmPDR12 gene towards functional analogues of SA was investigated. A gel blot analysis with RNA isolated from soybean cells treated with the different analogues is shown in Fig. 6. All of the compounds tested (details in Fig. 6A) rapidly induce GmPDR12 whereas no detectable signal was observed in H2O or solvent (DMSO)-treated cells. The observed kinetics of gene induction is very similar for SA and its functional analogues (data not shown). In the screen for SA-induced genes, a gene encoding a glutathione peroxidase (Gpx1; similar to GenBank AY237655) was also identified. For a comparison of the response to the functional analogues of SA a parallel gel blot analysis (with the same RNA) was performed and probed with a 200 bp fragment of Gpx1 (Fig. 6B). The gene is almost not expressed in untreated controls but is strongly up-regulated upon treatment with SA or functional analogues of SA (FAS compounds). With respect to the induction level and the specificity of the treatment, a very similar response was observed for both the Gpx1 and the GmPDR12 genes.

Transcriptional regulation of SA-up-regulated genes by as-1, W-box, and DOF elements is well known (Chen and Singh, 1999; Yang et al., 1999). To answer the question whether GmPDR12 expression could also be subject to such regulation, a promoter fragment of the GmPDR12 gene was isolated by a primer walking strategy. An 820 bp fragment upstream of the ATG was sequenced and analysed for potential SA-responsive elements. As the exact position of the transcriptional start side is unknown, all numbering of the promoter refers to position +1 as being the ATG of the ORF for the ABC transporter. Two elements commonly found in SA-responsive genes are W-boxes and DOF (DNA binding with one zinc finger)-binding sites, which are both present in the GmPDR12 promoter. The position of the elements is shown in Fig. 7. One palindromic W-box (TGAG-X15-GTCA) is located at position –731 and a second box (TGAC-X12-GTCA) was identified at position –453. In addition, the non-palindromic W-box motif (TTTGAC) is present at position –236 and a further core-motif (TGAC) is located at position –197.
The second cis element that was identified at position –754 was the AAAG motif. This motif meets the perfect consensus sequence for binding of DOF proteins. Since the element is small and thus likely to occur manifold in the soybean genome by chance, it was questioned whether there were further DOF elements in the GmPDR12 promoter. Indeed 15 additional elements on the (+) and (−) strands could be identified at position –820 to –160 within the GmPDR12 promoter which could function as binding sites for a zinc finger transcription factor (Kang et al., 2003).

**Discussion**

Using a subtractive hybridization approach, several SA-induced genes were identified from soybean (Glycine max). Most interesting was a fragment which showed homology to an ABC-transporter gene, which was chosen for further studies. By screening a cDNA library, a full-length cDNA clone of this novel transporter was identified which was designated GmPDR12. The deduced cDNA sequence of GmPDR12 encodes for a continuous ORF of 1447 amino acids. The sequence is highly homologous to other ABC transporters of plants. Within the GmPDR12 protein, an ABC signature could be identified at positions 320 and 999 which is most characteristic for ABC proteins (Higgins, 2001). Moreover, complete Walker A and Walker B motifs were identified at positions 181 and 340, respectively. These motifs are hallmark motifs of ABC transporters and are required to energize the transport of compounds (Higgins, 2001).

The organization of the ABC transporter is reminiscent of the PDR-type subfamily, and a protein comparison shows a
high degree of similarity to the previously characterized transporter NpABC1 from *Nicotiana plumbaginifolia* (accession number CAC40990) and NiPDR1 from *Nicotiana tabacum* (accession number BAB92011). Based on the derived protein sequences of *GmPDR12*, a cluster analysis has been performed to classify the transporter further (Fig. 2). Fifteen members of the *Arabidopsis thaliana* PDR-transporter protein family, as well as the two transporters from *Nicotiana* species and the GmPDR12 protein sequence, were aligned to calculate a non-rooted tree [CLUSTALX (Thompson et al., 1997); MEGA2.1 (Kumar et al., 2001)]. The ABC transporter is most similar to the *Arabidopsis* protein encoded by At1g15520, which was assigned as AtPDR12 (Martinioa et al., 2002; van den Brule and Smart, 2002). This suggests that the orthologous gene has been isolated from soybean and is therefore named *GmPDR12*.

Interestingly, the sequences of ABC transporters are very similar at the protein level but they are distinct enough to give specific hybridization signals that could be demonstrated by Southern blot analysis (Fig. 3).

The induction of the *GmPDR12* expression by SA is very rapid and the mRNA starts to accumulate 15–30 min after SA treatment. This puts the *GmPDR12* in the group of early immediate SA-response genes by contrast to many pathogenesis-related protein genes like PR-1 from tobacco, which starts to accumulate 6–12 h after treatment with SA (Horvath et al., 1998). Whereas SA leads to a prolonged gene induction of PR genes, the *GmPDR12* is only very transiently induced. The *Arabidopsis* AtPDR12 gene was shown to be induced by high concentrations of SA, leading to a broad maximum of gene induction between 3 h and 24 h after spraying the plants with 5 mM SA. This concentration is about a 100-fold higher than the SA concentration used in the present experiments with soybean cells. The ABC transporter from tobacco (NpPDR1) does not respond to SA treatment up to 1 mM, which is sufficient to induce PR genes. However, treatment with jasmonate at higher concentrations induces the gene (Grec et al., 2003). Thus the soybean *GmPDR12* gene is transcriptionally induced by low concentrations of both SA and jasmonate, which are representative of the key signalling molecules in the two pathogen-defence pathways in plants.

It was found that a group of chemicals, which functionally substitute for SA (FAS compounds) in programmed cell death of the HR (Tenhaken et al., 2001), are potent inducers of the *GmPDR12* gene. Interestingly, the FAS compounds do not induce SA-responsive PR genes in tobacco, indicating several SA-signalling pathways. The promoter of the *GmPDR12* gene contains two WRKY motifs, which are commonly found in the promoters of SA and/or pathogen-induced genes (Eulgem et al., 2000). In addition, many DOF elements are present in the *GmPDR12* promoter, exceeding the statistical expectation about 3-fold. Although the role of DOF-transcription factors in SA signalling is not fully worked out so far, accumulating evidence supports a role of DOF proteins in SA signalling. For instance, the AtGst6 gene induced by SA was initially thought to be mediated by a TGA transcription factor binding to an *as1* element (Chen et al., 1996). However, more detailed studies identified a functional promoter sequence motif with two DOF-binding sites, which also mediate SA responsiveness (Chen and Singh, 1999). Furthermore, the DOF3 gene from *Arabidopsis* is induced by SA and by this may amplify the response (Kang and Singh, 2000).

A common signalling pathway by SA and FAS compounds is also suggested by the very similar gene induction of the *GmPDR12* gene and the *Gpx1* gene, encoding a glutathione peroxidase (compare Fig. 6B). Eight different FAS compounds, which can all substitute SA during the programmed cell death in pathogen-inoculated soybean cells (Tenhaken et al., 2001), lead to the same gene induction of *GmPDR12*. These compounds include activating ligands for animal transcription factors of the PPAR-type (Kliewer et al., 1994), suggesting that a similar mechanism might by operating in plants, although this type of transcription factor is not present in the *Arabidopsis* gene on a homology basis. Whereas SA treatment triggers a transient induction of *GmPDR12* (compare Fig. 4), the FAS compounds lead to a longer lasting accumulation of *GmPDR12* mRNA (compare 4 h time point in Fig. 6B with 2 h in Fig. 6A; also data not shown). SA is rapidly metabolized to SA-glucoside in soybean cells, which will cause a decline of the SA signal (Tenhaken and Ruebel, 1997). By contrast to SA, the FAS compounds are likely to be more stable.

**Possible functions of *GmPDR12***

The *GmPDR12* transporter shares several characteristics with the transporter NpABC1 from tobacco and AtPDR12 from *Arabidopsis*. Based on fractionation studies and
antibody localization, these transporters are likely to be found in the plasma membrane (Jasinski et al., 2001). Antibodies, which have been generated against the first hydrophilic domain of the GmPDR12 transporter, specifically recognize the recombinant protein but are not sensitive enough to detect the protein in membrane fractions prepared from stimulated soybean cells (data not shown). It was also not possible to detect a GFP-fusion protein after transient expression in protoplasts, probably because of the low amount of the protein and the high background fluorescence of the plant cells. Jasinski et al. (2001) suggested that the export of terpenoids like sclareol is mediated by NpABC1; similar conclusions were drawn from insertion mutants in the Arabidopsis AtPDR12 gene, which are more sensitive to toxic sclareol than the wild-type plants (Campbell et al., 2003). The major terpenoid compounds in soybean are soyasapogenols, which are known to modify the taste of soybean food (Okubo et al., 1992). However, no antimicrobial activities of these terpenoids have been reported, except the inhibition of HIV virus replication in vitro (Nakashima et al., 1989).

ABC transporters can frequently use diverse substrates, suggesting additional metabolites to be exported by the PDR12 transporters. An enhanced tolerance of lead was recently associated with AtPDR12 (Lee et al., 2001). Often the genes of ABC transporters are induced by the compounds that they transport. In Saccharomyces cerevisiae, a knock-out in the PDR12 gene renders the cell sensitive to organic acids like benzoic acid, which is structurally very similar to SA (2-hydroxybenzoic acid) (Piper et al., 1998). Therefore it might be possible that plant transporters of the PDR12 type are involved in the export of SA from cells. This hypothesis has been tested by trying to express the GmPDR12 gene in the yeast PDR12 deletion mutant (Piper et al., 1998). Only in some experiments was a partial complementation of the benzoic acid hypersensitivity phenotype of the yeast mutant (data not shown) obtained. However, SA export from plant cells has been found in ozone-treated plants or after inoculation with pathogens. Here labelling experiments have shown that a part of the locally synthesized SA is exported and distributed systemically throughout the plant (Shulaev et al., 1995), which is a part of the signalling pathway to systemic-acquired resistance in plants.

The induction of ABC transporters during plant–pathogen interaction points to an important role in limiting a microbical attack. It is tempting to speculate that the transport of secondary (phenolic) metabolites into the cell wall to reinforce the mechanical strength locally might be one function of the pathogen-responsive ABC transporters in the plasma membrane. The identification of transported substrates, which are critical for defence of the plants, will be a major next step in understanding more details of the interaction between plants and pathogens.

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References


Lee M, Lee K, Lee J, Noh EW, Lee Y.

Lu YP, Li ZS, Drozdowicz YM, Hortensteiner S, Martinoia E, Klein M, Geisler M, Bovet L, Forestier C.

Kobae Y, Sekino T, Yoshioka H, Nakagawa T, Martinoia, E, Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Kumar S, Tamura K, Jakobsen IB, Nei M.


