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Research article

Molecular cloning and expression analysis of PDR1-like gene in ginseng subjected to salt and cold stresses or hormonal treatment

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ABSTRACT

The plant pleiotropic drug resistance (PDR) family of ATP-binding cassette (ABC) transporters is potentially involved in diverse biological processes. Currently, little is known about their actual physiological functions. A *Panax ginseng* PDR transporter gene (*PgPDR1*) was cloned and the cDNA has an open reading frame of 4344 bp. The deduced amino acid sequence contained the characteristic domains of PDR transporters: Walker A, Walker B, and ABC signature. Genomic DNA hybridization analysis indicated that one copy of *PgPDR1* gene was present in *P. ginseng*. Subcellular localization showed that PgPDR1-GFP fusion protein was specifically localized in the cell membrane. Promoter region analysis revealed the presence of cis-acting elements, some of which are putatively involved in response to hormone, light and stress. To understand the functional roles of *PgPDR1*, we investigated the expression patterns of *PgPDR1* in different tissues and under various conditions. Quantitative real-time PCR (qRT-PCR) and Western blotting analysis showed that *PgPDR1* was expressed at a high level in the roots and leaves compared to seeds and stems. The expression of *PgPDR1* was up-regulated by salicylic acid (SA) or chilling, down-regulated by ABA, and regulated differently at transcript and protein levels by MeJA. These results suggest that *PgPDR1* might be involved in responding to environmental stresses and hormones.

1. Introduction

ATP-binding cassette (ABC) transporters constitute one of the largest protein families and play a major role in many cellular processes, including lipid translocation and transport of a variety of compounds across the biological membranes of organisms from all kingdoms of life [1,2]. The proteins typically contain a common basic structure formed by the repetition of two structural domains: a hydrophobic transmembrane domain (TMD) consisting of multiple membrane spanning segments (usually six), and a hydrophilic nucleotide binding domain (NBD) containing the Walker A, Walker B, and ABC signature. On the basis of the organization of these structural elements, the full-length ABC transporters can be subdivided into four major subfamilies: pleiotropic drug resistance (PDR), multidrug resistance (MDR), MDR-associated protein (MRP), and ABCA [3,4]. Among these subfamilies, PDR family is unusual in that its members are found only in fungi and plants, and the nucleotide binding domain of the protein precedes the transmembrane domain (NBD-TMD) which is reverse form of the configuration in MDR, MRP and ABCA proteins (TMD-NBD) [5].

The plant PDR transporters have been known to participate in various biological processes, including defense against pathogens and salinity stresses, secondary metabolites and plant hormones transporting [6–8]. The PDR transporter *SpTUR2* from *Spirodela polyrrhiza* is closely related to environmental stresses and induced by cold, NaCl, and abscisic acid (ABA) [9]. The *Arabidopsis AtPDR12* was reported to be tightly regulated by elicitor treatments, such as methyl jasmonate (MeJA), suggesting its direct involvement in the pathogen resistance processes by transporting antimicrobial metabolites. The AtPDR12 was implicated in transporting phytohormone ABA [10]. NpPDR1 of *Nicotiana plumbaginifolia*, shown to export the antifungal diterpenoid sclareol from epidermal cells, is

Abbreviations: ABA, abscisic acid; ABC, ATP-binding cassette; CaMV, cauliflower mosaic virus; DIG, digoxigenin; GFP, green fluorescent protein; JA, jasmonic acid; MDR, multidrug resistance; MeJA, methyl jasmonate; MS, Murashige and Skoog; MRP, MDR-associated protein; NBD, nucleotide binding domain; ORF, open reading frame; PDR, pleiotropic drug resistance; qRT-RCR, quantitative real-time PCR; RACE, rapid-amplification of cDNA end; SA, salicylic acid; SAR, systemic acquired resistance; TMD, transmembrane domain.

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essential for pathogen defense [11]. Some of PDRs were shown to be able to transport cadmium (Cd) [12] and auxin precursor indole-3-butyric acid (IBA) [13,14]. In addition, *NtPDR1* and *NtPDR3*, from the *Nicotiana tabacum* up-regulated by MeJA and various elicitors indicate that these genes might also be involved in plant defense [15,16]. Therefore, the expression of PDR genes is highly regulated by various factors. Transcription of PDR genes is typically induced by conditions in which their substrate is present and their activity is required. For instance, transcriptional profiling of PDR genes in rice reveals that the expression of plant PDRs is significantly induced by hormones such as jasmonates, SA and ABA [17]. *OsPDR9* encoding a PDR-type ABC transporter is regulated by heavy metals, hypoxic stress and redox perturbations in rice roots [18]. These observations suggest the involvement of PDRs in responses to stresses and hormones, but there is little experimental evidence regarding the physiological role of PDR transporters.

Ginseng (*Panax ginseng* C.A. Meyer) is one of the most valuable traditional medicines in Asian countries for thousands of years. The major pharmacologically active ingredients of ginseng are ginsenosides that have important pharmacological effects in human health [19,20]. However, cultivation of ginseng has encountered a lot of difficulties because of its vulnerability to environmental stresses. Many of stresses-related genes are influenced by environmental factors such as plant hormones, temperature and salinity during the development of ginseng [21,22]. In order to understand the important roles of PDR-type ABC transporter in response to various stresses and hormones, we isolated the cDNA of *PgPDR1* from 4-year-old *P. ginseng* roots. This is the first report about the isolation and characterization of a novel PDR1 gene from *P. ginseng*. We also conducted the expression profiling of *PgPDR1* in response to abiotic stresses and hormones.

2. Results and discussion

2.1. Cloning and sequencing of PgPDR1

Using a pair of degenerate primers corresponding to conserved plant PDR genes, we isolated a partial PDR cDNA from ginseng roots by RT-PCR. 3′ RACE (rapid-amplification of cDNA end) and 5′ RACE were carried out using the corresponding gene specific primers based on the cloned partial cDNA sequences. The full cDNA was obtained by RT-PCR, and the gene was named *PgPDR1* (*P. ginseng* PDR) with a GenBank accession number KC013236. The *PgPDR1* cDNA is 4652 base pairs (bp) long and has 5′-UTR (119 bp) and 3′-UTR (189 bp) sequences. The open reading frame (ORF) encodes for a protein with 1447 amino acid residues with a predicted molecular weight of 162.5 kDa. PgPDR1 protein consists of two halves with similar organizations. Each half has the highly conserved Walker A and B motif, and the ABC-signature motif near the Walker B motif. Each half of the transporter has several membrane-spanning helices and the conserved domains that are the characteristic domain of plant PDR transporter [4]. The alignment in the conserved regions of the ABC transporter is shown in Fig. 1 comparison of the Walker A and B motif, and the ABC-signature motif. The results show that the PgPDR1 contains perfectly conserved domains with other plant PDRs.

The location of the putative trans-membrane helices predicted by TMHMM 2.0 is shown in Fig. 2a. Hydropathy analysis shows that PgPDR1 is an integral membrane protein containing 12 putative membrane-spanning domains. These domains are located at both the N- and C-terminal ends of the PgPDR1 respectively. The predicated three dimensional structure of PgPDR1 protein using protein homology/analogy recognition engine (Phyre) is shown in Fig. 2b. We established a structural model for PgPDR1 (Fig. 2c) based on the comparison to other plant PDR transporters and the hydrophobicity plot presented in Figs. 1 and 2a, b.

2.2. Homology analysis

In our study, we have cloned three ginseng PDR genes (*PgPDR1*, *PgPDR2* and *PgPDR3*, unpublished) that share high sequence homology. In order to further understand the structural characteristics of PDR genes in *P. ginseng*, the amino acids were aligned with the PDR proteins from other plants by using ClustalX and MEGA 4.1. In plants, PDR genes have been arbitrarily divided into five clusters [5]. Phylogenetic analysis revealed that *PgPDR1* belongs to cluster I (Fig. 3). The closest PgPDR1 homologs are NtPDR1 (BAD07483), GmPDR12 (CAK03587), NpPDR1 (CAC40990), AtPDR12 (AAF71978) and GmPDR1 (CAK03587) with 83.97%, 71.94%, 71.69%, 69.65% and 68.16% identity, respectively.

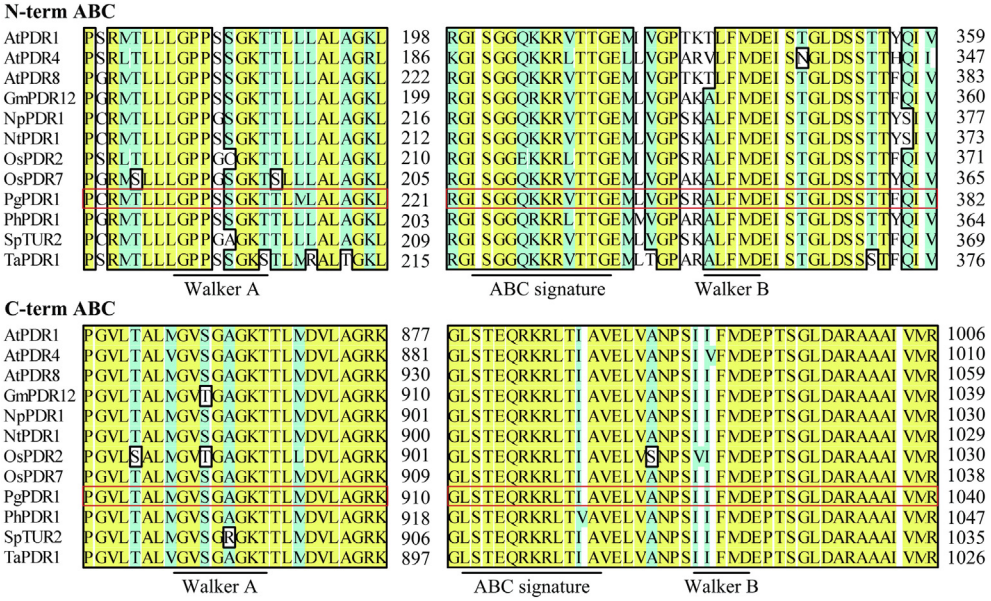

Fig. 1. Amino acid alignment of the ABC domains of the plant PDR transporters.