A potato cDNA encoding a homologue of mammalian multidrug resistant P-glycoprotein

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Abstract

A homologue of the multidrug resistance (MDR) gene was obtained while screening a potato stolon tip cDNA expression library with 35S-labeled calmodulin. The mammalian MDR gene codes for a membrane-bound P-glycoprotein (170–180 kDa) which imparts multidrug resistance to cancerous cells. The potato cDNA (PMDR1) codes for a polypeptide of 1313 amino acid residues (ca. 144 kDa) and its structural features are very similar to the MDR P-glycoprotein. The N-terminal half of the PMDR1-encoded protein shares striking homology with its C-terminal half, and each half contains a conserved ATP-binding site and six putative transmembrane domains. Southern blot analysis indicated that potato has one or two MDR-like genes. PMDR1 mRNA is constitutively expressed in all organs studied with higher expression in the stem and stolon tip. The PMDR1 expression was highest during tuber initiation and decreased during tuber development.
Figure 1. Nucleotide and deduced amino acid sequences of the PMD1R1 gene. Putative transmembrane segments S1—S12 are underlined. Two potential ATP-binding sites (each consisting of two parts) are indicated by hatched regions. The dotted lines indicate the potential N-linked glycosylation sites corresponding to the sequences NXS/T. The HmdIII site is indicated by a double line above the sequence.
Figure 2. A. Hydropathy plot of the predicted amino acid sequence of the N-terminal half (top) and C-terminal half (bottom) of PMDR1. Kyte-Doolittle hydrophobicity values are marked on the left for a window of 11 amino acid residues. Putative transmembrane segments of S1–S12 are indicated. B. Dot matrix comparison showing internal duplication in the PMDR1 polypeptide. C. Dot matrix comparison of human MDR1 P-glycoprotein (HUMDR1) to the potato MDR-like gene product (PMDR1). Dot matrix analysis was performed for a window of 19 amino acid residues. D. Dot matrix comparison of Arabidopsis P-glycoprotein (ATPGP1) to PMDR1. E. Comparison of the potential ATP-binding sites of PMDR1 to those of Arabidopsis ATPGP1 and human MDR1 genes. Identical and functionally similar amino acid residues are boxed. PO, AR, and HU indicate potato, Arabidopsis, and human, respectively. NNB and CNB represent the conserved sequences in the ATP-binding sites of the N-terminal half and C-terminal half, respectively.
upstream ATG at position 23 is followed by a termination codon after only three nucleotides, we assign the ATG at nucleotide position 33 as the translation initiation codon, which is located 2 bases downstream from a purine residue in agreement with the eukaryotic initiation site [9]. The 186 bp of the 3'-untranslated region sequence does not contain an intact polyadenylation signal and an obvious poly(dA) tail and they may be present in the downstream region. The cDNA codes for a polypeptide of 1313 amino acid residues. The complete nucleotide sequence and the deduced amino acid sequence of PMDR1 are presented in Fig. 1.

The hydropathy profiles and the dot matrix comparison revealed that the N-terminal half of PMDR1 is similar to its C-terminal half (Fig. 2A, 2B), and their amino acid sequences share 59.8% identity and 92.4% similarity. However, a distinct variation exists in their N-terminal ends; the PMDR1 polypeptide has an extra 26 amino acid stretch in the N-terminal end as compared to Arabidopsis ATPGPl-encoded protein. The deduced amino acid sequence of PMDR1 revealed several significant structural features. The hydropathy plot presented in Fig. 2A shows a series of highly hydrophobic domains in the N-terminal and C-terminal halves and their hydropathy profiles are very similar (Fig. 2A). Each half of the PMDR1 polypeptide contains six putative transmembrane segments (Fig. 1, 2A) which are conserved in the corresponding regions of other MDR homologues. Another structural feature of PMDR1 polypeptide is the presence of two putative ATP-binding sites (Fig. 1). Two pairs of conserved amino acid sequences are present in the polypeptide at positions 422–437/545–558 in the N-terminal half and positions 1077–1092/1200–1213 in the C-terminal half. These amino acid sequences of the putative ATP-binding sites are highly conserved in proteins coded by MDR-like genes (Fig. 2E).

To determine the approximate copy number of PMDR1, Southern blot analysis of potato genomic DNA was carried out using the random-primed 32P-labeled probe of the cDNA fragment (nt 953–3302, Fig. 1) [13]. Depending on the restriction enzymes, one or three hybridizing bands were observed, indicating that potato has one or two MDR-like gene(s) (Fig. 3A). To study the expression of the PMDR1 gene in different organs, total RNA was isolated from different organs of potato plants and developing tubers at two different stages of development [19]. The RNase protection assays were performed by using the standard protocol [13]. An Spe/II/HindIII fragment of the PMDR1 coding region (nt 1711–2133, Fig. 1) was subcloned into pBluescript II KS(+) plasmid and used as a template for making the 32P-labeled antisense RNA probe, and the reaction mixture was analyzed on a 4% polyacrylamide gel containing 7 M urea. The 32P-labeled antisense RNA probe for the potato calmodulin PCMc6, which exhibits constitutive mRNA expression [16], was used for comparison. The results indicate that PMDR1 is constitutively expressed in all organs studied, with the higher expression in the stem and stolon tip (Fig. 3B). The expression was highest in the stolon tip during tuber initiation and decreased during tuber development (Fig. 3C).

A number of MDR or MDR-like genes have been isolated from different organisms [6, 14]. Although the functions of some of these genes have not been clearly identified, they are most likely to be membrane-bound transporter proteins with a wide substrate specificity.

Figure 3. A. Southern analysis of PMDR1. 10 μg of potato DNA was digested with restriction enzymes and transferred onto a nylon membrane. The membrane was hybridized at 42 °C with 32P-labeled probe in a solution containing 50% formamide, 6× SSPE, 5× Denhardt's solution, 0.1% w/v SDS, and 100 μg/ml herring sperm DNA. The membrane was washed at 60 °C in 0.5× SSC and 0.1% w/v SDS. The size of the standard markers is shown in kb. B. BamHI; EcoRI; H, HindIII. B. RNase protection assay showing the expression of PMDR1 in different tissues of potato plants. 20 μg of total RNA was used in each reaction. L, leaf; S, stem; R, root; St, stolon tip. The expression of potato calmodulin isoform PCMc6 is shown for comparison. It has been observed that PCMc6 expression is lower in leaf as compared to other organs [16]. C. RNase protection assay showing the expression of PMDR1 during the early stages of tuber development. St, stolon tip; Dt, developing tuber; T, tuber. The expression of potato calmodulin isoform (PCMc6) is shown for comparison.
Most of the studies on the functions and substrates of MDR P-glycoprotein were conducted with the tumor cells, which are invariably associated with increased production of P-glycoprotein. However, several studies have consistently shown that the MDR P-glycoprotein is expressed in many normal organs, suggesting that they may play a protective role in the transport or secretion and keep toxic metabolites and xenobiotics out of these normal tissues [2, 5, 6]. Since many cytotoxic compounds transported by P-glycoproteins of mammals and other organisms are hydrophobic natural compounds derived from plants [2, 6], it is likely that similar transport systems may also exist in plants. Because the deduced amino acid sequence and the structural features of potato MDR-like gene (PMDR1) share a striking similarity to the mammalian P-glycoprotein, it is possible that its function is also conserved.

Calcium channel blockers and calmodulin antagonists have been known to reverse the MDR phenomenon [5, 8, 17, 18]. However, the mechanism by which these agents reverse MDR effect is not fully understood [5]. Since the PMDR1 cDNA clone was isolated by screening a potato expression library using [35S]-labeled calmodulin, we believe that there is a direct interaction of Ca2+/calmodulin with the PMDR1 gene product. Recently, Schlemmer et al. [15] reported that the murine MDR3 P-glycoprotein function is down-modulated by Ca2+/calmodulin. Their results suggest that the murine MDR3 P-glycoprotein is a calmodulin-binding protein. These results and our present study suggest that Ca2+/calmodulin may play a regulatory role in the function of the MDR P-glycoprotein. Ca2+ and calmodulin regulate many cellular processes and growth and development in plants [10, 12]. Balamani et al. [1] were able to block tuber induction by using Ca2+ chelators and calmodulin antagonists, suggesting a role for Ca2+/calmodulin in tuberization. The high expression of PMDR1 mRNA in the stem and stolon tip (Fig. 3B and 3C) raises the possibility that calmodulin and its modulated proteins play a role in the tuberization process.

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References
