

Plant Gene Register

Isolation and Characterization of a cDNA Clone Encoding a Small Wound-Inducible Protein from *Phaseolus vulgaris*¹Jinsong Sheng² and Mona C. Mehdy*

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The metabolic activities of plants are dramatically altered upon mechanical injury or pathogen attack. A large number of proteins accumulate at wound or infection sites, including a heterogeneous class of proteins termed pathogenesis-related proteins (Carr and Klessig, 1990). We report here the isolation of a cDNA clone designated PvPR4 encoding a novel protein that resembles other pathogenesis-related proteins by virtue of its small size and basic isoelectric point (Table I).

PvPR4 was identified by screening a cDNA library made from fungal elicitor-treated bean (*Phaseolus vulgaris* L.) cell suspensions (Mehdy and Lamb, 1987) at low stringency with a rabbit Cyt P450 cDNA clone, P-450-4 (Okino et al., 1985). The cDNA contains a 329-bp insert and the size of the corresponding mRNA is about 300 to 350 bases, indicating that PvPR4 is a full-length clone. The PvPR4 cDNA contains a 24-bp 5' untranslated region, followed by an open reading frame encoding a 47-amino acid protein with a calculated molecular mass of 5.4 kD. The 3' untranslated region contains 164 bp including a 68-bp poly(A) tail. The encoded protein is highly hydrophilic and rich in Lys (14.9%) and Arg (6.8%), resulting in a basic protein with an isoelectric point of 10.2. Sequence comparisons revealed no homology with Cyts P450 or any other sequences in the GenBank, EMBL, or SwissProt data bases. It is notable that PvPR4 is one of the smallest wound-induced proteins reported. Most pathogenesis-related proteins range between 10 and 40 kD in size (Carr and Klessig, 1990). In addition, several barley antifungal thionins are about 5 kD and the tomato wound-inducible systemin is about 2 kD, but these polypeptides are cleaved from much larger precursors (Bohlmann and Apel, 1991; McGurl et al., 1992). Based on its size, we suggest that the PvPR4 protein is more likely to interact with other proteins or to be a subunit of an enzyme rather than have enzymic properties itself.

The pattern of PvPR4 mRNA expression was examined during development and in response to wounding. In 2-d-old bean seedlings, the PvPR4 mRNA was abundant in radicles and epicotyls and 10-fold lower in the cotyledons. In mature plants, the mRNA level was higher in roots than in stems and leaves. In all mature plant organs, the levels of

Table I. Characteristics of PvPR4 cDNA from *Phaseolus vulgaris*

Organism:

Phaseolus vulgaris L. cv Canadian Wonder.

Techniques:

A cDNA library constructed from poly(A)⁺ RNA of bean cell cultures treated with fungal elicitor for 3.5 h has been previously described (Mehdy and Lamb, 1987) and was used for screening. The PvPR4 insert was subcloned into a plasmid and sequenced.

Features of the Encoded Protein:

47 amino acids, 5.4 kD, no signal peptide. Hydrophilic and basic (isoelectric point = 10.2).

mRNA Expression:

Abundant in radicles and epicotyls of 2-d-old seedlings; low levels of expression in mature plants; mature plant roots have somewhat higher mRNA levels than stems and leaves. The PvPR4 mRNA accumulates 10-fold in wounded hypocotyls from 8-d-old etiolated seedlings.

PvPR4 mRNA were considerably lower than in 2-d-old seedling organs. In hypocotyls of 8-d-old etiolated seedlings, the PvPR4 mRNA increased 10-fold upon wounding over a period of 24 h. Genomic blot analysis using five restriction enzymes suggested that there is a single gene of PvPR4 in the genome. Based on its mRNA expression, the encoded PvPR4 protein may be important in early seedling development and may function in protection or healing at wound sites.

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The GenBank accession number for the sequence reported in this article is L00625.

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