Nucleotide Sequence of a Pumpkin Phloem Lectin cDNA

Dwight E. Bostwick and Gary A. Thompson*
University of Arizona, Department of Plant Sciences, Tucson, Arizona 85721

One of the characteristic events that occurs during phloem differentiation in dicotyledonous plants is the appearance of phloem protein within the sieve elements and companion cells of the phloem tissue (Esau and Cronshaw, 1967). Two very abundant phloem proteins, PP1 and PP2, have been isolated from phloem exudates of pumpkin (Cucurbita maxima) (Read and Northcote, 1983). PP1 is a 96-kD structural protein that forms polymeric filaments, and PP2 is a 48-kD lectin that is covalently linked to the PP1 polymers (Sabnis and Hart, 1979; Read and Northcote, 1983). PP2 is composed of two subunits, α (Mr, 26,500) and β (Mr, 25,000), joined by disulfide linkages between Cys residues (Read and Northcote, 1983). It is unclear whether the two subunits are encoded by different genes or are modifications of a single gene product.

cDNAs encoding phloem proteins were isolated by screening a pumpkin seedling cDNA library with a complex anti-serum raised against pumpkin phloem exudate proteins (Table I). We subsequently demonstrated that two of these cDNAs, cPC13 (868 bp) and cPC20 (792 bp), encoded a functional PP2 subunit (Bostwick et al., 1992). Nucleotide sequence analysis of the cDNAs showed that the entire sequence of cPC20 was identical to nucleotides 46–837 of cPC13. Northern blot analysis demonstrated that the mRNA encoding a PP2 subunit migrates as a single species of approximately 1000 nucleotides (Sham and Northcote, 1987; Bostwick et al., 1992). Although cPC13 is not a full-length copy of the PP2 mRNA, we identified an ORF of 654 nucleotides that extends from nucleotide 31 to 684. The translation initiation sequence (5'-TGCAATGGA-3') for the deduced polypeptide matched six of nine nucleotides from the consensus sequence for plant genes (5'-AACAATGGC-3') (Lutcke et al., 1987). The ORF of cPC13 encoded a deduced polypeptide of 218 amino acids with a calculated molecular mass of 24,478 D. This coincides with the apparent molecular mass of 25,000 D for the β subunit of PP2 (Read and Northcote, 1983). Hydropathy analysis of the deduced polypeptide did not indicate a hydrophobic signal peptide or other hydrophobic domains, supporting previous observations (Allen, 1979) that PP2 is a cytosolic protein that is not glycosylated.

Table I. Characteristics of the cDNA clone cPC13

| Organism: | Pumpkin (Cucurbita maxima [Duch.] cv Big Max). |
| Function: | Unknown. PP2 is a lectin that binds oligomers of N-acetylglucosamine and appears to be a component of phloem filaments (Read and Northcote, 1983). |
| Expression: | PP2 has been localized within the sieve element and companion cells of the phloem tissue (Smith et al., 1987). PP2 mRNA has been localized within the companion cells of pumpkin phloem tissues (Bostwick et al., 1992). |
| Methods of Identification: | cDNAs encoding phloem proteins were identified by screening a cDNA library with antibodies raised against the total phloem exudate of pumpkin seedlings. Affinity-purified antibodies against PP2 were used to identify several cDNAs encoding PP2 subunits. A fusion protein encoded by the ORF exhibited the carbohydrate-binding characteristics of PP2 (Read and Northcote, 1983). |
| Techniques: | A cDNA expression library was constructed from poly(A)+ RNA isolated from 3-d-old pumpkin seedlings. cDNAs encoding PP2 subunits were identified and the two largest cDNAs, cPC13 and cPC20, were sequenced by the dideoxynucleotide method. Sequence analysis revealed a complete ORF within cPC13. |
| cDNA Features: | cPC13 is 868 bp in length. The length of the 5' noncoding region is 30 bp. The 152 bp 3' noncoding region contains a single polyadenylation signal sequence (AATAAG) located 132 bp from the translation termination codon (TGA). A previous report of 980 bp for cPC13 (Bostwick et al., 1992) included a 112-bp repeated region at the 5' end of the cDNA that was determined to be an artifact of cDNA synthesis. |
| Structural Features of the Deduced Protein: | The 654-bp ORF encoded a deduced polypeptide of 218 amino acids with a calculated molecular mass of 24,478 D. The deduced protein has several similarities to previous biochemical analyses of PP2 (Beyenbach et al., 1974; Allen, 1979). The deduced protein and PP2 are rich in Gly, Leu, and Lys; both contain six Cys residues; and both lack Thr. |
| Antibodies: | Antibodies were raised in chickens against the total phloem exudate of pumpkin seedlings. |

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The EMBL accession number for the sequence reported in this article is Z17331.
LITERATURE CITED

Allen AK (1979) A lectin from the exudate of the fruit of the vegetable marrow (Cucurbita pepo) that has a specificity for a \( \beta \)-1,4-linked N-acetylglucosamine oligosaccharide. Biochem J 183: 133–137


