

Purification, Characterization, and cDNA Cloning of Profilin from *Phaseolus vulgaris*¹

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Profilin from common bean (*Phaseolus vulgaris* L.) was purified to homogeneity by poly-L-Pro affinity chromatography and gel filtration. The hypocotyl and symbiotic root nodule protein was detected as a single isoform with a 14.4-kD molecular mass and an isoelectric point of 5.3. Partial amino acid and DNA sequencing of a full-length cDNA clone confirmed its identity as profilin. An antibody generated against the purified protein binds to a protein with the same molecular mass in leaves and nodules. Immunolocalization of the protein showed a diffuse distribution in the cytoplasm of hypocotyls and nodules but enhanced staining at the vascular bundles. The strong identity of the sequence among the profilins of birch, maize, and bean suggests that it may play an important role in the signal transduction mechanism of plant cells and plant-bacterial symbioses.

Profilin is a low mol wt actin- and poly-L-Pro-binding protein that is ubiquitous in eukaryotic cells (Haarer and Brown, 1990; Valenta et al., 1991; Machesky and Pollard, 1993). At high concentrations, profilin can inhibit actin nucleation and polymerization in vitro (Reichstein and Korn, 1979; Tseng et al., 1984; Lindberg et al., 1988). At low concentrations, it catalytically enhances the rate of actin-ATP exchange (Mockrin and Korn, 1980; Goldschmidt-Clermont et al., 1991), thus enhancing actin polymerization. Some profilins can also bind PIP and PIP₂ with high affinity (Lassing and Lindberg, 1988; Machesky and Pollard, 1993) and inhibit phospholipase C- γ activity (Goldschmidt-Clermont et al., 1990). Therefore, this multisubstrate binding protein might play a central role in actin and cell regulation. The in vivo activity of profilin profoundly affects the actin cytoskeleton. Mutants in profilin show altered actin morphology (Haarer and Brown, 1990; Magdolen et al., 1993), and the extent to which actin is found in the form of F-actin is changed after profilin microinjection (Cao et al., 1992). Furthermore, very recently it was re-

ported that microinjected profilin affects cytoplasmic streaming in plant cells by rapidly depolymerizing actin microfilaments (Staiger et al., 1994). However, the ultimate regulatory events still remain to be elucidated.

In plants, profilin has been identified as an important and common allergen from different pollens (Valenta et al., 1991; Vallier et al., 1992). The plant profilin can bind to poly-L-Pro (Valenta et al., 1991; Staiger et al., 1993), a characteristic found in all other profilins reported to date (Machesky and Pollard, 1993). Its ability to bind actin was demonstrated by filter binding assays (Valenta et al., 1993).

In an animal system, the phenomenon of bacterial entry and permanence in an intracellular environment has been documented (Falkow, 1991). Perhaps one of the most interesting examples of an intracellular growing bacterium is *Listeria monocytogenes*. Within a few hours after infection, host cell actin filaments form a dense cloud around the intracytoplasmic bacteria and then rearrange to form a polarized comet tail that is associated with moving bacteria (Cossart and Kocks, 1994). It has been demonstrated recently that in a cell-free extract system capable of reconstituting *L. monocytogenes* motility, profilin is necessary to support comet-tail formation or bacterial motility (Theriot et al., 1994). In addition to the modulation of cytoskeletal functions, exploitation of host signal transduction pathways by invasive bacteria is well documented (Rosenshine and Finlay, 1993).

We have considered the possibility that similar events could take place in the plant symbiosis. It has been postulated that the cytoskeleton plays a central role in the development and establishment of the symbiotic root nodule (Bakhuizen, 1988; Brewin, 1991; van Brussel et al., 1991; Pérez et al., 1994). The infection threads have been compared with the phragmosome formation in wounded tissue (Goodbody and Lloyd, 1990). The cytoskeleton seems to play a pivotal role in the development of this cellular structure (Clayton and Lloyd, 1985; Kakimoto and Shibaoka, 1987; Zhang et al., 1993). Our ultimate aim is to understand how the cytoskeleton participates in the symbiotic phenomenon between *Rhizobium* and the legumes; therefore, it is important to characterize, in detail, the cytoskeletal components and their role in this symbiotic interaction.

Abbreviations: PIP, L- α -phosphatidyl-D-myo-inositol-4-phosphate; PIP₂, L- α -phosphatidyl-D-myo-inositol-4,5-bisphosphate.

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MATERIALS AND METHODS

Materials

Poly-L-Pro and cyanogen bromide-activated Sepharose 4B were obtained from Sigma. Triton X-100 and urea were obtained from Bio-Rad, as were all electrophoresis reagents. The goat anti-rabbit antibody conjugated with alkaline phosphatase was obtained from Boehringer-Mannheim. The rest of the chemicals were reagent grade.

Biochemical Material

All biological material was obtained from *Phaseolus vulgaris* L. biovar Negro Jamapa.

Seeds were washed and sterilized with a 10% (v/v) solution of commercial bleach and rinsed, and the excess water was decanted. Five hundred milliliters of seeds were placed in a 4-L flask, and cheesecloth was tightened with a rubber band. Twice each day seeds were rinsed and decanted. On the 5th d the hypocotyls were harvested and frozen at -70°C until use. Symbiotic nodules were obtained from greenhouse-grown plants 21 d after inoculation with *Rhizobium tropici*, strain CIAT 899.

Extracts

Homogenization was performed with the frozen tissue in a coffee mill. The powder was extracted at 4°C for 1 h with 2 volumes of extraction buffer (100 mM Gly, 100 mM KCl, 10 mM Tris-HCl, pH 8, 1 mM DTT, 1% Triton X-100) per gram of hypocotyls, centrifuged at 30,000g for 1 h, and filtered through Miracloth (Calbiochem).

Poly-L-Pro Affinity Chromatography

The coupling was performed according to Lindberg et al. (1988). Five hundred milligrams of poly-L-Pro (M_r 10,000–30,000) were dissolved in 50 mL of coupling buffer (0.5 mM NaCl, 0.1 mM NaHCO_3), and 10 g of cyanogen bromide-activated Sepharose was rinsed and coupled for 12 h at 4°C in coupling buffer, washed with 100 mM Tris-HCl, pH 8, for 2 h, and equilibrated with extraction buffer without Triton.

The plant supernatant was incubated for 1 h with 0.1 volume of the poly-L-Pro-Sepharose, and the matrix was rinsed with several volumes of extraction buffer, washed with 3 M urea, packed into a column, and eluted with 8 M urea. This material was concentrated with a Centriprep device (Amicon, Beverly, MA) and further chromatographed on a Sephadex G-100 column to remove minor contaminants. The 14-kD protein containing fractions were pooled and concentrated using a Centricon 10 device (Amicon).

Antibody Production and Immunodetection

Profilin was run on a 15% polyacrylamide gel, and the Coomassie blue-stained band was cut in pieces and emulsified with 1 mL of 0.01 M sodium phosphate, 0.14 M NaCl, pH 7.5 (PBS), and complete Freund's adjuvant. The rabbit was bled to get the preimmune serum and injected with

100 μg of the emulsion. Boost injections were prepared in exactly the same manner but with incomplete adjuvant and administered every 2 weeks, at the same time the animal was bled to test for antibodies.

Proteins for immunodetection were run on 15% PAGE and electrotransferred to Immobilon polyvinylidene difluoride membranes (Millipore) following the recommendations from Hoefer Scientific Instruments (San Francisco, CA), blocked with 1% BSA, incubated with a 1:10,000 dilution of the first antibody in TBST (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 8), and developed with a 1:3,000 dilution of a second antibody against rabbit IgGs in TBST conjugated to alkaline phosphatase. Immunoquantification of western blots was performed by serial dilution of protein (equal amounts of protein: 10, 5, and 2.5 μg in each case), which was separated on 15% PAGE. Internal standards of purified profilin were included in each polyacrylamide gel (there was a linear correlation factor higher than 0.96 within a 2- to 50-ng range). Bands were quantified using a computer program (Whole Band Analyzer Program; Bio Image Applications, Millipore). For immunostaining, the antibody was purified according to Lillie and Brown (1987), and the tissue was fixed with 4% formaldehyde in PBS for 1 h, extensively washed and dehydrated through an ethanol series, and embedded in Paraplast. Eight-micrometer sections were cut and deparaffinated, and the immunoaffinity-purified antibody in TBST plus 5% BSA was added for 1 h. The sections were washed and incubated with a 1:500 dilution of the second antibody coupled to alkaline phosphatase in TBST plus 5% BSA for 1 h. 4-Nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate were used as substrates. The sections were dried and mounted in Poly/Mount (Polysciences, Inc., Warrington, PA). Images were taken with 10X and 40X (Zeiss) dry objective lenses and photographed with T-Max ASA 100 film (Kodak).

Amino Acid Analysis and Sequencing

The purified protein amino acid composition was determined by HPLC and a DEAE column. Cys, Trp, and Pro were not determined (data not shown).

The protein sequence was performed on a V8-digest peptide because the amino end was blocked. The protein was partially digested and a peptide with a 6-kD mass was purified by SDS-PAGE, eluted overnight with the smallest possible volume of 100 mM NH_4CO_3 , 0.1% SDS, concentrated under a vacuum, and loaded onto a polyvinylidene difluoride membrane. The sequencing was done in an automated liquid phase sequencer (Millipore).

Other Methods

Proteins were separated by PAGE in the presence of SDS (Laemmli, 1970) and stained with Coomassie blue. Two-dimensional gels were done according to O'Farrell (1975), with the modifications for minigels recommended by Hoefer Scientific Instruments. The protein was quantified according to Bradford (1976).

cDNA Library Screening

Twenty-day-old root nodules were used to construct a cDNA library in λ Zap (Stratagene). For the screening, four plates (150 mm, 20,000 plaque-forming units/plate) were probed with the purified profilin antibody using standard procedures (Sambrook et al., 1989). After the third screening, 16 positive isolated plaques were picked and rescued into plasmid form (Bluescript SK-) by in vivo excision with the ExAssist helper phage, as described by the manufacturer (Stratagene). Plasmids were isolated (Birnboim, 1983), the clones were digested with *Eco*RI and *Xho*I to size the inserts, and the longest one was selected for further characterization. All the enzymes were purchased from Boehringer-Mannheim.

DNA Sequence Analysis

A restriction map of the clones was obtained, and after *Bst*XI/*Eco*RI digestion, nested deletions were made by the exonuclease III procedure (Henikoff, 1984). The deletions and the original clone were sequenced by the dideoxy chain-termination protocol (Sanger et al., 1977), using a single- or a double-stranded template with the Sequenase 2.0 kit (United States Biochemical). The sequence was obtained from both strands of the clones. The DNA sequence was analyzed and compared using the GeneWorks program, version 2.3.1, and associated data banks (Intelligence, Mountain View, CA).

RESULTS

Affinity Purification of a Poly-L-Pro Binding Protein from *Phaseolus vulgaris*

A major protein component was obtained after poly-L-Pro affinity chromatography of *P. vulgaris* hypocotyl, nodule, or leaf extracts. The polypeptide accounted for more than 90% of the eluted protein and had a molecular mass of 14,400 D, as determined by SDS-PAGE (Fig. 1a, lanes 5–7). Prominent bands in this molecular mass range in the leaf and nodule crude extracts correspond to the small subunit of Rubisco and leghemoglobin, respectively. The protein can be purified further by gel-filtration chromatography on a Sephadex G-100 column. After the gel-filtration step, the poly-L-Pro binding protein is homogeneous by the criterion of two-dimensional PAGE. This protein was purified about 900-fold after this step (see "Materials and Methods").

Polyclonal antibodies were raised against the hypocotyl protein and used for western blot analysis of crude extracts from hypocotyls, nodules, and leaves (Fig. 1b). To further characterize the nodule isoform, two-dimensional PAGE of a crude nodule extract was run. The antibodies detected a single protein with a pI identical to that of the hypocotyl polypeptide (Fig. 2b). The protein was a single isoform, with an estimated pI of 5.3 (Fig. 2a). With these criteria, we concluded that the poly-L-Pro binding protein is most likely profilin and will be called so henceforth.

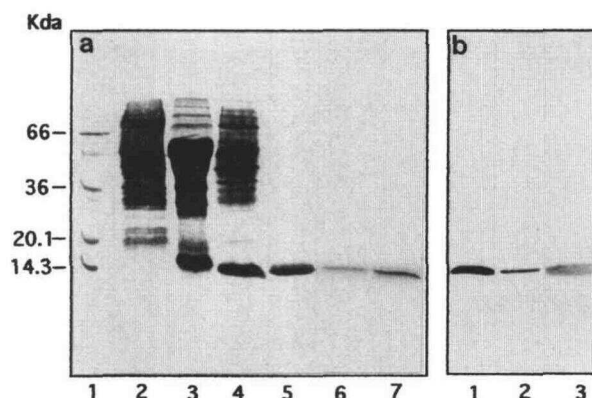


Figure 1. a, Coomassie blue-stained proteins separated by SDS-PAGE. Lane 1, Mol wt markers. From top to bottom: BSA, M_r 66,000; glyceraldehyde-3-phosphate dehydrogenase, M_r 36,000; soybean trypsin inhibitor, M_r 20,100; lysozyme, M_r 14,300. Lane 2, Hypocotyl extract, 40 μ g; lane 3, leaf extract, 40 μ g; lane 4, nodule extract, 40 μ g; lane 5, urea elution from the poly-L-Pro column of a hypocotyl extract. Lane 6, Same as lane 5 but from leaf; lane 7, same as lane 5 but from nodule. b, Western blot of 19 μ g of crude extract from hypocotyl (lane 1), leaf (lane 2), and nodule (lane 3) probed with the hypocotyl antiserum (1:10,000 dilution) against the hypocotyl profilin.

Profilin Cellular Concentration and Purification Yield

Profilin cellular concentration was determined through immunquantification of western blots. The standard curves showed a linear correlation coefficient of 0.98 or better in the 5- to 100-ng range of purified protein. We estimated the hypocotyl profilin concentration to be between 1 and 3 mg of profilin in 1 g of total protein, an amount equivalent to 0.1 to 0.3% of the total Triton X-100 soluble protein in the cell. In symbiotic root nodules the concentration was lower, as estimated by this technique. We calculated 0.06 to 0.08% of profilin of total Triton X-100 soluble protein in this tissue.

Since our purification yield was between 200 and 500 μ g of pure protein for 1 g of extracted protein, we estimated a 10 to 30% recovery. More than 80% of the profilin originally in the extract was bound irreversibly to the affinity matrix or was degraded. We could elute only 10 to 30% of the initial protein from the matrix, and even with very stringent elution conditions (boiling with 2% SDS and 5% β -mercaptoethanol), no more than 40% could be eluted.

Immunolocalization

To further characterize *P. vulgaris* profilin, immunolocalization was performed in paraffin-embedded hypocotyls and symbiotic root nodules. The immunostain showed that it was present in many of the cellular types we analyzed. In the hypocotyls, the distribution was cytoplasmic without any preferential distribution (Fig. 3a). The immunolocalization was done in different parts of the hypocotyl with the same results (data not shown). Parallel negative controls were made with the preimmune serum (Fig. 3b). Only the primary xylem, because of its thick secondary walls, was visible under the bright field.

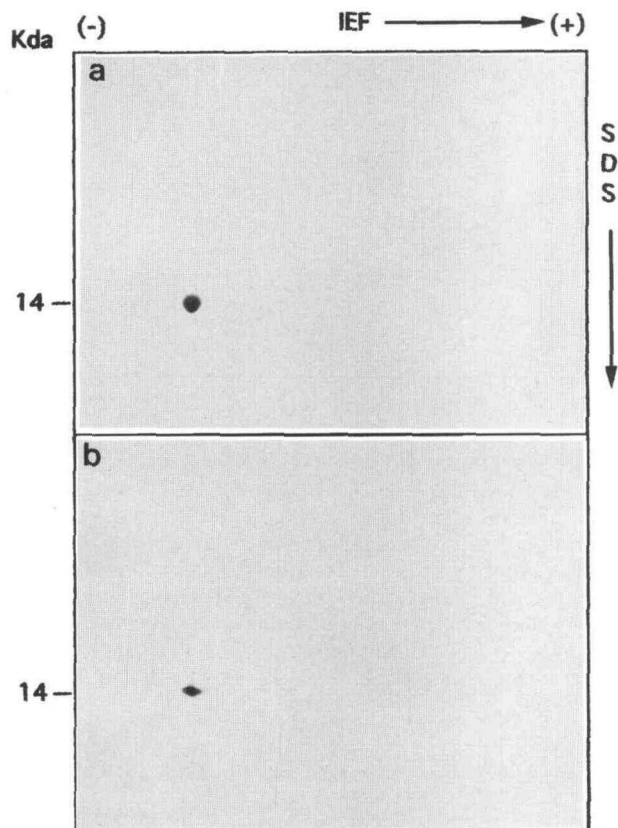


Figure 2. a, Two-dimensional PAGE of the gel-filtrated purified profilin. The protein was stained with Coomassie blue. b, Two-dimensional PAGE of 100 μ g from a crude extract of root nodules stained with polyclonal antiserum against the hypocotyl profilin. The molecular mass is indicated in kD.

In the root nodules, the antigen was present in all cells examined but was most abundant in the vascular tissue as well as in the endodermis of that organ (Fig. 4, a and b). The central zone showed discrete staining of both infected and noninfected cellular types, with staining at the periphery of amyloplasts in noninfected cells and in the nucleus of infected cells. At the vascular bundles, there was a lot of signal in all phloem cells (Fig. 4b), and the endodermis of the nodule showed more signal when compared with the cortex and the epidermis.

As a positive control, a polyclonal antibody against purified leghemoglobin was used in the same way (Fig. 4c). The antibody clearly stained the infected zone, showing a similar pattern of nuclear and cytoplasmic staining in infected cells. In this control, there was no staining at the vascular bundles, endodermis, cortex, or epidermis of the organ. The preimmune serum control gave no staining at all (Fig. 4d).

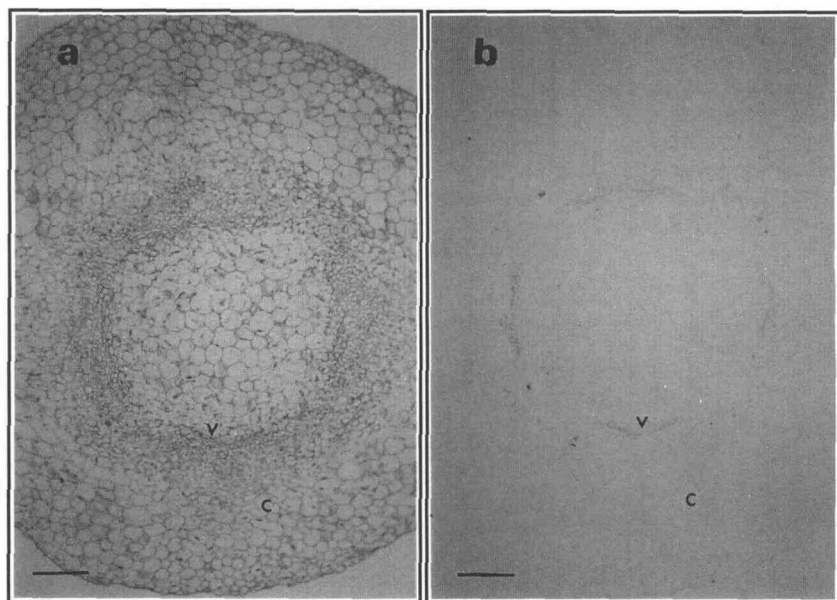
Protein Sequencing

The initial attempt to sequence the amino terminus of the polypeptide was not possible, probably because it is blocked, as reported in other systems (Ampe et al., 1985). After a limited V8 digest in an ammonium buffer, several peptides were obtained. A 6-kD peptide was run by SDS-PAGE, eluted, and sequenced. A 30-amino acid sequence was obtained that completely matched the corresponding region deduced from the cDNA clone (Fig. 5).

Sequence Analysis of the Profilin cDNA Clone

As indicated in "Materials and Methods," after the third immunoscreening we isolated at least one full-length clone, which was shown to have more than 700 bp, as indicated by restriction analysis. This clone was completely sequenced. As shown in Figure 5, the cDNA contained an open reading frame of 131 amino acids, beginning with an

Figure 3. Transverse section of hypocotyls stained with the purified polyclonal antiserum against the hypocotyl profilin (a) and preimmune serum (b). v, Vascular tissue; c, cortex. Bar = 10 μ m.



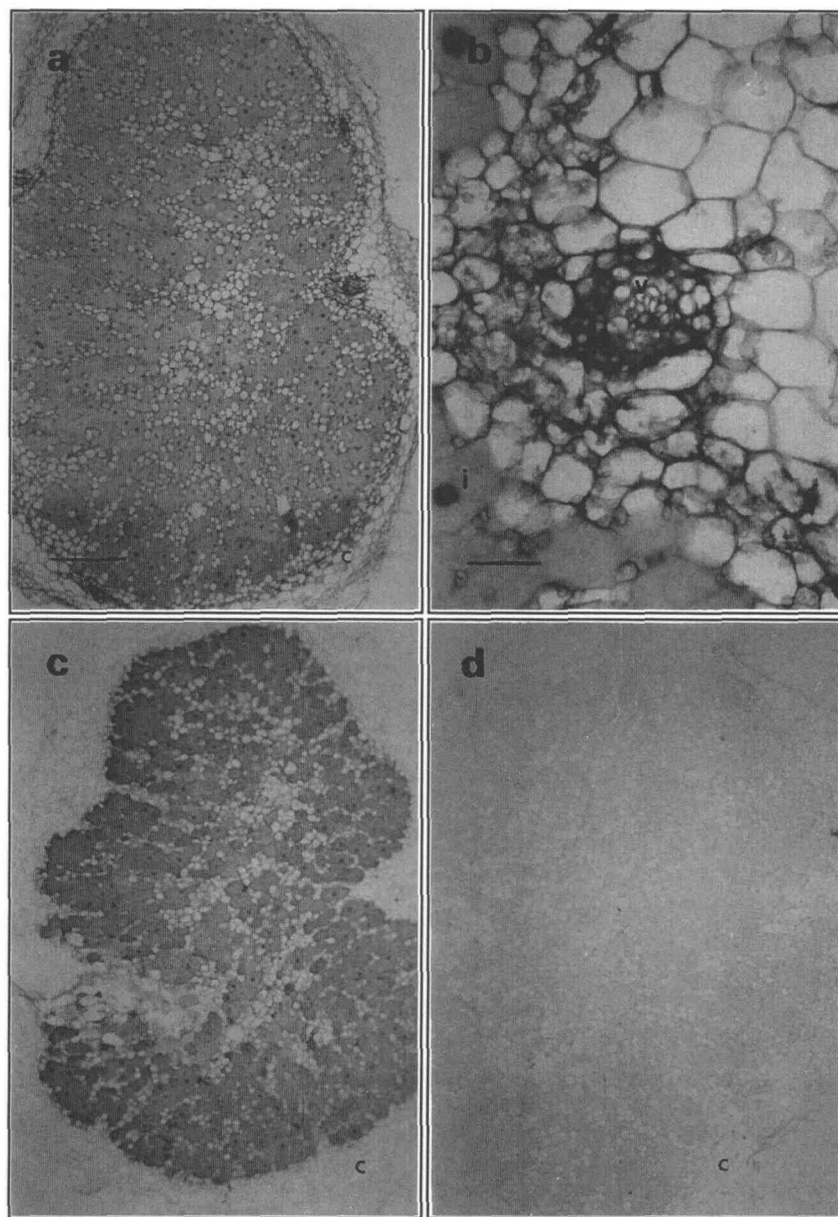


Figure 4. Radial section of a 20-d-old root nodule stained with the purified polyclonal anti-serum against the hypocotyl profilin (a and b), anti-leghemoglobin (c), or preimmune serum (d). b is the same as a, but at a higher magnification for a clear view of a nodule vascular bundle. c, Cortex; v, vascular bundle; i, infected cell. Bar = 10 μ m (a) and 40 μ m (b).

ATG at +51 and terminating with TAA at +446. The translated amino acid sequence completely contained the corresponding sequenced peptide from position 47 to 76 (100% match), confirming the identity of the isolated protein and the cDNA clone (Fig. 5). Upstream of the initiation codon a 50-bp leader sequence was located. A long 294-bp trailer sequence followed the stop codon. Notwithstanding the fact that the cDNA ends with a string of A's, the putative polyadenylation signal showed some deviation with respect to the plant consensus sequence (Heidecker and Messing, 1986). In addition, as in other plant genes, a second cryptic polyadenylation signal was located at 537 to 543 bp. Computer-aided analysis failed to show any significant hairpin structure in this region. The translation of the polypeptide predicted a molecular mass of 14,181 D. The comparative hydrophobic analysis of the reported ho-

mologous plant profilins showed an almost identical profile (data not shown).

Homology to Other Profilin Sequences

As shown in Figure 6, the predicted bean nodule amino acid sequence was clearly homologous to other selected plant profilin sequences. Similarities among the different proteins were apparent throughout the entire sequence, although several regions of high sequence conservation were obvious in the alignment. Pairwise alignments of these sequences indicated 72 to 78% identical amino acids and an additional 11 to 15% of similar amino acids, indicating homology and function conservation. The derived amino acid sequence was also compared with some non-plant profilins showing the following identities: *Dictyoste-*

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ATTTTCAGAGAGAAGCAAAGCAGTGTGTTGGTGAGAAATCAGAAGC 50
ATGTCGTGGCAAAACGTACGTCGACGACACCTCTCTGTGAGATCGAAGG 100
M S W Q T Y V D D H L L C E I E G
TAACCACTCTCACTACGCGCCATCTCGGCCAAGACGCGCAGCCTTGGG 150
N H L T H A A I L G Q D G S V W A
CTAAGAGCGCCAGCTTCCCTCAGTTCAACCGGAAGAAATAACTGGGATC 200
K S A S F P Q F K P E E I T G T
ATGAATGATTTTAAATGAGCCTGGAACTTGCCTCTAGGATGTACAT 250
M N D F N E P G T L A P T G L Y I
TGGTGGCACTAATATATAGGTATCCAAAGGTGAACCCGGCTCTGTCTATC 300
G G T K Y M V I Q G E P G S V I R
GAGGCAAGAGGGTCTGGTGGTGTACTGTGAAGAGACGAATCTGGCC 350
G K R G P G G V T V K K T N L A
TTGGTATAGGCAATATATAGGATGACCTCCAGGTCAATGCAACAT 400
L V I G I Y D E P M T P G Q C N H
GATAGTTGAAGGCTTGGTGTATCTCTCACTGAACAGGCTCTTATGCC 450
I V E R L G D Y L I E Q Q L
CTTGTATGATGGTTATAGTGCATATTTCATTGGCTCTGTACAGTTT 500
TTGCATCGACCCCTGACGTGAATGCTTGTATTCAGGTTTATAAATCTTG 550
TGGGTGTCAAAAGTCAGGGGATCTGCGTGTAGTGAAGAAAGTGTTTGAT 600
GCTTGAGAAATGATGACTATATATCTCTATCTGTACTTTTATGTTGGG 650
TACTGTATACATTACAGTGTATATTTTATGCTATGATATATGTTGGGA 700
ATATTAAACAAACTTCCAGATCTCTCTCTATCTCGCAAAAAAAAAA 750
AAAAAAAAA 759

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Figure 5. Nucleotide sequence of bean nodule profilin cDNA and derived amino acid sequence of 131 residues. The start and stop codons are underlined. Putative polyadenylation signals are boxed. The shaded amino acid region corresponds to the sequenced peptide.

lium discoideum, 45%; *Physarum polycephalum*, 41%; *Drosophila melanogaster*, 38%; *Acanthamoeba castellanii*, 37%; *Saccharomyces cerevisiae*, 30%; and human, 32%.

DISCUSSION

Purification

Profilin was purified (800- to 900-fold) to homogeneity with poly-L-Pro affinity chromatography and Sephadex G-100 gel filtration. After the affinity step, a major contaminant of 66 kD could be detected when the gel was over-

loaded. This contaminant could be separated from the profilin with the Sephadex G-100 step. Interestingly, this contaminant fractionates as a multimeric protein. The purified profilin migrates as a single spot in a two-dimensional gel.

Profilin accounts for 0.1 to 0.3% of the total protein, and the yield was 10 to 30% of the initial profilin. We think that a significant amount of profilin was degraded during the chromatography, even in the presence of diverse protease inhibitors, despite having done the purification as quickly as possible. Indeed, this was the major problem encountered when we tried to purify actin from root and root nodules of *P. vulgaris* (Pérez et al., 1994). Alternatively, some of the protein might have been adsorbed irreversibly to the poly-L-Pro matrix.

Actin was also purified by DNase I affinity chromatography of pea roots (Andersland et al., 1992). In this tissue, around 1% of the total cellular protein was actin, as determined by DNase I inhibition assay. Following the above rationale, one would expect that 0.1 to 0.2% of the total protein would be profilin, a range that was in close agreement with our results.

Characterization

The purified protein had an M_r of 14,400 and a pI of 5.3, values that were in accord with those of the *Betula* profilin and other plant profilins (Staiger et al., 1993). It was interesting that *P. vulgaris* showed just one isoform in hypocotyls and root nodules, in contrast to maize, where at least three isoforms were detected in pollen (Staiger et al., 1993). Therefore, there was no nodule-regulated expression or repression of profilin isoforms. This contrasts with the actin results in which a differential expression of isoforms was observed (Pérez et al., 1994).

Figure 6. Multiple alignment of the bean nodule profilin predicted amino acid sequence with plant homologous proteins. Dashes represent gaps in the alignment. The consensus sequence at the lower part of the alignment indicates 100% conserved residues. Closed circles indicate nine highly conserved amino acids in all eukaryotic profilins (Staiger et al., 1993). Asterisks indicate conserved solvent-exposed hydrophobic residues (Schutt et al., 1993). Shaded bars, Putative α -helix region. Hatched bars, Probable β -strand region. The putative actin-binding region (Staiger et al., 1993) is boxed. PROF_PHLPR, *Pleum pratense* (common timothy) (EMBL accession number X77583). PROFBETVE, *Betula verrucosa* (Valenta et al., 1991). PRO1-, PRO2-, and PRO3 MAIZE, *Zea mays* (Staiger et al., 1993). PROF_BEAN, *P. vulgaris*, this paper.

PRO2_MAIZE	MSDRKMSWQ	AYVDEHLMCE	IEGH--HLAA	AAIVGHGDA	WAQSTAFPEF	48
PRO1_MAIZE	-----MSWQ	TYVDEHLMCE	IEGH--HLTS	AAIVGHGDA	WAQSTAFPEF	42
PRO3_MAIZE	-----MSWQ	TYVDEHLMCE	IEGH--HLSS	AAIVGHGDA	WAQSTAFPEF	42
PROF_PHLPR	-----MSWQ	TYVDEHLMCE	IEGH--HLAS	AAIVGHGDA	WAQSTAFPEF	42
PROF_BETVE	-----MSWQ	TYVDEHLMCD	IDGQASNSLA	SAIVGHGDSV	WAQSSFPQF	44
PROF_BEAN	-----MSWQ	TYVDDHLLCE	IEGN--HLTH	AAIVGHGDSV	WAKSASFQF	42
Consensus	MSWQ	.YVD.HL.C.	I.G.....	.AI.G.DG..	WA.S..FP.F	50
		*	*	*	*	
		████████		████████	████████	
PRO2_MAIZE	KTEDMANIMK	DFDEPGHLAP	TGLFLGPTKY	MVIQGEPEGAV	IRGKKGSGGI	98
PRO1_MAIZE	KPEEMAAMIK	DFDEPGHLAP	TGLILGCTKY	MVIQGEPEGAV	IRGKKGSGGI	92
PRO3_MAIZE	KPEEMTNIIK	DFDEPGFLAP	TGLFLGPTKY	MVIQGEPEGAV	IRGKKGSGGI	92
PROF_PHLPR	KPEEITGIMK	DFDEPGHLAP	TGMFVAGAKY	MVIQGEPEGAV	IRGKKGSGGI	92
PROF_BETVE	KPQEITGIMK	DFDEPGHLAP	TGLHLGGIKY	MVIQGEAGAV	IRGKKGSGGI	94
PROF_BEAN	KPEEITGIMN	DFNEPGTLAP	TGLYIGCTKY	MVIQGEPEGAV	IRGKKGSGGI	92
Consensus	K.....I..	DF.EPG.LAP	.G.....KY	MVIQGE.G.V	IRGKKG.GG.	100
	████████████████	████████	████████	████████████████	████████████████	
PRO2_MAIZE	TVKKTQALV	VGIYDEPMTF	GQCNMVVERL	GDYLLQGM		137
PRO1_MAIZE	TVKKTQSLI	IGIYDEPMTF	GQCNLVVERL	GDYLLQGM		131
PRO3_MAIZE	TVKKTQALV	IGIYDEPMTF	GQCNMVVERL	GDYLVQGL		131
PROF_PHLPR	TIKKTQALV	VGIYDEPMTF	GQCNMVVERL	GDYLVQGM		131
PROF_BETVE	TIKKTQALV	PGIYDEPMTF	GQCNMVVERL	GDYLLDQGL		133
PROF_BEAN	TVKKTNLALV	IGIYDEPMTF	GQCNMIVERL	GDYLLQGL		131
Consensus	T.KKT...L.	.GIY.EP.TP	GQCN..VERL	GDYL..QG.		139
		*	*	*	*	
	████████	████████	████████████████	████████████████		

Immunolocalization

All tissues examined had the polypeptide (data not shown), and hypocotyls as well as nodules reacted strongly to the antibody in paraffin sections (Figs. 3 and 4). It was important to note that the paraffin sections reveal only the overall distribution of the antigen. Any subcellular localization was uncertain because the plant cytoplasm was extremely thin and had a large vacuole that made it difficult to preserve the paraffin sections.

The staining of all the cellular types in the hypocotyls (Fig. 3a) suggests that profilin is important for the organization of the plant cytoplasm, probably by enhancing actin polymerization. Controls were performed in some sections, so we could discriminate any nonspecific reaction. No positive signal was obtained when staining was done with the preimmune serum (Fig. 3b), and appeared only with the second antibody, or anti-leghemoglobin (data not shown).

In the root nodules, there was strong staining at the vascular bundles. These cells are very active in transport because they provide the carbon needed for respiration to the bacteroids and transport the ureides produced by the nodule to the rest of the plant. We think that the actin cytoskeleton, by regulating the streaming, is a modulator of this process. If this is the case, the interaction of profilin and actin could be critical for adequate transport.

The strong nuclear signal in the infected cells is difficult to interpret, but the same signal was obtained with the anti-leghemoglobin antibody. This phenomenon has already been described, presumably due to the small size of this protein, relative to the diameter of the nuclear pores (Robertson et al., 1984). Also, redistribution of such a small, soluble protein during chemical fixation is possible (Melan and Sluder, 1992).

Sequence Structural Analysis

At the nucleotide level, an open reading frame of 131 amino acids was detected. Comparison of the translated protein with other reported profilin sequences indicated that the isolated clone corresponds to bean profilin. The alignment with other plant profilins (Fig. 6) indicated that, overall, 54% of the residues were identical. At the protein level, the amino acid composition showed that all 20 of the standard protein amino acids were used in normal proportions, although there was an elevated proportion of Gly (13.7%). Also, two Cys's were found to occur near the amino and carboxyl terminus, at positions 13 and 115, respectively. These residues could be located very near each other in the three-dimensional structure (see below). Of particular interest were two sets of residues: (a) nine amino acids that are highly conserved in all eukaryotic profilins (Staiger et al., 1993) and (b) six conserved solvent-exposed hydrophobic residues (Schutt et al., 1993). These two groups partially overlap and can be located in all the plant sequences, including bean profilin. In addition, the probable secondary structure regions were determined by structural equivalence alignment (Fig. 6), in which we used the reported profilin secondary- and three-dimensional structures of *Acanthamoeba* (Archer et al., 1993; Vinson et

al., 1993), bovine (Schutt et al., 1993), and human (Metzler et al., 1993). In general, our proposed secondary-structure regions for plant profilin correspond well with the characteristics of the sequences, suggesting a similar three-dimensional structure. A putative, actin-binding sequence (VER-LGDYL) (Staiger et al., 1993) near the carboxyl terminus was located in the bean sequence. However, the structural equivalence alignment with the published contact region of bovine actin and profilin (Schutt et al., 1993) suggested that, in plants, this actin-binding region could start at about residue 109, which is located after the last β sheet and spans through the middle of the last α -helix, including the above-mentioned sequence. At position 89 of the bean sequence, an unusual Pro was located where none is found in the other sequences. However, this residue seems to be located precisely between two β -structures (positions 6 and 7). Similarly, the unusual residues Asn⁹⁸ and Leu⁹⁹ were located between the proposed location of the sixth and seventh β -sheets. It has been proposed that the highly conserved region that interacts with PIP₂ is rich in basic residues (Machesky et al., 1990; Vinson et al., 1993). An equivalent of this region was located between amino acids 83 and 97 in the bean sequence.

Future Prospects

We have been unable to demonstrate that the plant profilin is an actin-binding protein. These experiments have been difficult to perform due to intrinsic problems similar to those encountered when purifying the *P. vulgaris* actin. Thus, no profilactin complex has been obtained after poly-L-Pro chromatography, as is the case of pollen (Staiger et al., 1993; Valenta et al., 1993). Assays were performed to evaluate plant profilin association with the muscular actin, but we could not detect any unambiguous association (Vidali, 1993).

We could not demonstrate the interaction between the bean profilin and PIP₂, probably due to partial denaturation induced by the solvent used to elute the protein from the affinity column. However, as recently reported, recombinant plant profilin is able to interact with PIP₂ (Drøbak et al., 1994).

We are working also on purifying preparative amounts of plant actin, so that we can evaluate profilin affinity for this polypeptide, as well as on the immunogold localization of the actin and profilin at the subcellular level to assess the distribution of both proteins. This will enable us to extend our observations from light microscopic immunolocalization.

We expect that since the nodule and the hypocotyl proteins appear to be the same, both are probably encoded by the same gene. With the profilin clone that we have available now, it will be possible to determine the number of genes in *P. vulgaris*. Finally, the strong identity of the sequence between the profilins of birch, maize, and bean suggests that it may play an important role in the signal transduction mechanisms of the plant cell, including symbiosis.

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