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Molecular Characterization of a Polygalacturonase Inhibitor from *Pyrus communis* L. cv Bartlett[1]

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A polygalacturonase inhibitor glycoprotein with an apparent molecular mass of 43 kD was purified from pear (*Pyrus communis* L. cv Bartlett) fruit. Chemical deglycosylation of this protein decreased the molecular mass to 34 kD. Gas chromatographic analysis suggests that N-linked glycosylation accounts for the majority of sugar moieties. Partial amino acid sequence analysis of the purified polygalacturonase inhibitor protein provided information used to amplify a corresponding cDNA by polymerase chain reactions. Multiple cloned products of these reactions were sequenced and the same open reading frame was identified in all of the products. It encodes a 36.5-kD polypeptide containing the amino acid sequences determined by protein sequencing and predicts a putative signal sequence of 24 amino acids and seven potential N-glycosylation sites. The expression of polygalacturonase inhibitor is regulated in a tissue-specific manner. Activity and mRNA level were much higher in fruit than in flowers or leaves.

PGIPs have been defined by their inhibition in vitro of fungal polygalacturonase [poly(1,4-α-D-galacturonide) glycanohydrolase; EC3.2.1.15] activity and have been found in infected (Fielding, 1981; Abu-Goukh and Labavitch, 1983) and uninfected dicotyledonous plant tissues (Albersheim and Anderson, 1971; Abu-Goukh et al., 1983b; Hoffmann and Turner, 1984; Degra et al., 1988). Biochemical characterization of PGIPs has shown that they are relatively heat stable (Albersheim and Anderson, 1971; Abu-Goukh, 1982) glycoproteins (Lafitte et al., 1984; Egli, 1987). Several plant PGIPs display isoform charge heterogeneity (Fielding, 1981), and, in the case of pear (*Pyrus communis*) fruit, isoforms with pIs of 4.5, 6.6, and 7.7 have been identified (Abu-Goukh et al., 1983a). Pear PGIPs have a molecular mass of 44 kD when separated by SDS-PAGE (Abu-Goukh et al., 1983a). Purified pear PGIPs inhibit different fungal PGs, including that of *Botrytis cinerea*, but do not affect endogenous pear fruit PG activity, suggesting an interaction with exogenous PGs rather than those involved in ripening-related processes (Abu-Goukh and Labavitch, 1983). Kinetic studies of the most abundant PGIP isoform from pear fruit (PG inhibitor I, pI 6.6) indicate that this PGIP competitively inhibits *B. cinerea* PG activity (Abu-Goukh et al., 1983a).

PGIPs have been considered to contribute to the general defense response of the host against pathogens (Abu-Goukh and Labavitch, 1983). PGIPs from a single plant source are capable of differentially inhibiting PGs from several different fungal sources (Albersheim and Anderson, 1971; Abu-Goukh and Labavitch, 1983; Brown and Adikaram, 1983; Hoffman and Turner, 1984), suggesting that PGIPs can discriminate between PGs. PGIPs from different plant species are likely to differ in their inhibition kinetics and target-PG specificity (Albersheim and Anderson, 1971; Abu-Goukh and Labavitch, 1983; Brown and Adikaram, 1983). Therefore, expression of heterologous PGIPs in plants could potentially be exploited to improve the resistance of crops.

Recently, a genomic clone encoding a bean PGIP has been isolated and used to study its expression in different organs of the plant (Toubart et al., 1992). The results are in agreement with the presence of PGIP activity throughout plant development and in diverse organs of bean (Salvi et al., 1990). In pears, PGIP activity was observed in fruit throughout development (4–14 weeks after anthesis) and ripening, although the extractability of PGIP activity changes (Abu-Goukh et al., 1983b).

Although PGIPs from different plants have been characterized biochemically, little information is available about the structure and regulation of expression of PGIPs, which is crucial for the understanding of the in vivo function of this class of proteins. Therefore, we have initiated studies to characterize the physical properties of pear PGIP and to isolate its cDNA. Here we report the carbohydrate composition and partial protein sequence of purified pear PGIP, and the amino acid sequence deduced from its cDNA. The cDNA clone was used to estimate the number of PGIP genes in the genome and to determine the organ-specific regulation of PGIP expression.

MATERIALS AND METHODS

Plant Material

Mature green pear (*Pyrus communis* L. cv Bartlett) fruits from orchard trees at the University of California, Davis, were harvested during the 1990 growing season and served

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Abbreviations: FPLC, fast-performance liquid chromatography; PCR, polymerase chain reaction; PG, polygalacturonase; PGIP, polygalacturonase inhibitor protein; pI, isoelectric point; poly(U), polyuridylate; RACE-PCR, amplification of cDNA ends by PCR; RNA-PCR, PCR after reverse transcription of RNA; SSC, sodium chloride-citrate buffer; SSPE, sodium chloride-phosphate-EDTA buffer; TFMS, trifluoromethane sulfonic acid.

as the source for the purification of PGIP. If not used immediately, fruits were stored either in air or under controlled atmosphere (2% O_2, 5% CO_2) at 0°C. There was no apparent effect of storage on recovered PGIP activity. Flowers and leaves from young pear trees were collected at Armstrong Field Station, University of California, Davis.

PGIP Purification

PGIP was purified according to Abu-Goukh et al. (1983a) with modifications. Five kilograms of fruit flesh were homogenized in an equal volume of extraction buffer (1 M sodium acetate, pH 6, 1 M NaCl, 1% [w/v] PVP-40, 0.2% [w/v] sodium bisulfite). The homogenate was stirred on ice for 1 h and then centrifuged at 15,000*g* for 20 min. The supernatant was stored at 4°C, and the pellet was resuspended in 1 volume of extraction buffer and stirred again for 1 h at 4°C. After centrifugation (15,000*g*, 20 min), the two supernatants were combined. The protein precipitating between 50 and 100% saturated ammonium sulfate was collected, resuspended in 0.1 M sodium acetate, pH 6, and extensively dialyzed at 4°C against 10 mM sodium acetate, pH 6.

The dialyzed ammonium sulfate fraction was mixed with an equal volume of 0.2 M sodium acetate, pH 6, 2 M NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM $MnCl_2$ (2× Con A buffer) and applied to a column of Con A-Sepharose 4B. Chromatography was performed at 4°C. Protein bound by the column was eluted using 250 mM α-methyl mannoside in Con A buffer. The eluent was dialyzed against 50 mM sodium acetate, pH 4.5 (buffer A), and then concentrated by ultrafiltration using a pressure cell fitted with a PM-10 membrane (Amicon, Danvers, MA).

PGIP was further purified by cation-exchange FPLC (Pharmacia, Uppsala, Sweden) using a Mono S column run at a flow rate of 0.5 mL/min. The column was equilibrated with buffer A and eluted with a linear NaCl gradient (to a final concentration of 0.5 M NaCl in buffer A). The fractions containing PGIP activity were pooled and the buffer of the active material changed to 50 mM sodium phosphate, pH 7 (buffer B) using a Centricon-10 microconcentrator. An equal volume of 3.4 M ammonium sulfate in buffer B was added to the sample, which was then separated by hydrophobic interaction FPLC (Phenyl-Superose HR 5/5) at a flow rate of 0.5 mL/min. A linear, declining gradient was created by mixing buffer B with and without 1.7 M ammonium sulfate. Active fractions were pooled and stored at −80°C after freezing in liquid nitrogen.

PGIP Activity Assay

Inhibition of endo-PG activity from the culture filtrate (Egli, 1987) of *B. cinerea* DEL 11 (obtained from Richard M. Bostock, Department of Plant Pathology, University of California, Davis) was determined by an agarose diffusion assay (Taylor and Secor, 1988) in the presence of 6.5 nmol/min of PG and 0.01% sodium polypectate (Sigma). Alternatively, inhibition was determined by a reducing sugar assay (Gross, 1982) using 0.65 μmol/min of PG and 0.05% sodium polypectate in a buffer containing 37.5 mM sodium acetate, pH 4.5, and 10 mM EDTA. One unit of PGIP activity was defined as the amount of inhibitor needed to reach 50% of complete inhibition of *B. cinerea* PG activity.

Carbohydrate Analysis

Dried, purified PGIP (600 μg) was dissolved in 2 N TFA. The solution was saturated with helium before hydrolysis at 121°C for 1 h. The monosaccharides generated during the TFA treatment were converted into alditol acetates (Blakeney et al., 1983) and examined by GLC and GC-MS (Greve and Labavitch, 1991). The analysis was performed on duplicate samples.

Protein Gel Electrophoresis

Native pear fruit PGIP and PGIP that had been chemically deglycosylated using TFMS according to Karp et al. (1982) were separated by SDS-PAGE as described by Laemmli (1970). Polyacrylamide gels (10%) were stained with Coomassie blue.

Protein Assay

Protein was determined by the method of Bradford (1976) using a Bio-Rad protein assay kit and BSA as a standard.

Protein Sequencing

The purified PGIP and its CNBr fragments were sequenced. The CNBr peptide fragments were separated by SDS-PAGE according to Promega Probe-Design Technical Manual (Titus, 1990). After electroblotting to ProBlott membrane (Applied Biosystems, Foster City, CA) and Coomassie staining of the peptide fragments, individual bands were cut out and subjected to automated Edman degradation by the Protein Structure Laboratory at the University of California, Davis.

PGIP Gene Isolation

N-terminal (DLXNPDDKKVLLQIKKAFGDPYVLA) and internal amino acid sequences (DFTSIDLSRNKLEGDAXVIFGLXKTTQIVDL) were determined. Primer 1 (5′-GGAATTCAAYCCNGAYGAYAARAARGT-3′, 128-fold degeneracy), primer 2 (5′-GCTCTAGATCDATNGANGTRAARTCCAT-3′, 192-fold degeneracy), and primer 3 (5′-GGAATTCCARATHAARAARGCNTTYGG-3′, 192-fold degeneracy) were synthesized (Applied Biosystems). (N indicates A, T, C, or G; Y indicates T or C; R indicates A or G; H indicates A, T, or C; D indicates A, T, or G.) cDNA was obtained by reverse transcription of 100 ng of poly(A)$^+$ RNA from immature pear fruits using an oligo(dT_{17}) primer (Maniatis et al., 1982). One-fifth of the first strand cDNA reaction or 1 μg of genomic DNA was used as a template together with primers 1 and 2 in subsequent PCR. The reactions were carried out in a Perkin-Elmer Cetus (Norwalk, CT) DNA Thermal Cycler using *Taq* DNA polymerase (Perkin-Elmer Cetus) for 40 cycles (one cycle = 1 min at 94°C, 2 min at 48°C, and 3.5 min at 65°C) according to the manufacturer's instructions. The identity of the PCR product was verified by amplifying 150 pg of DNA of the first PCR in a second PCR

utilizing primers 2 and 3. The reaction conditions were the same as described above, although 25 cycles were used. The resulting 569-bp PCR product was gel purified and ligated into pCR1000 (Invitrogen, San Diego, CA).

The 5′ end of the PGIP transcript was obtained by RACE-PCR (Frohman et al., 1988). Oligo(dT_{17}) was used to reverse transcribe 5 μg of poly(A)$^+$ RNA. The DNA templates were G-tailed using terminal deoxynucleotidyl transferase. An adaptor primer (5′-TTGTCGACGGATCCTTC$_{17}$-3′) was used together with a gene-specific primer 4 (5′-TAGACGAA-GAGCGCCGA-3′) in subsequent PCR. The reactions were carried out for 40 cycles (one cycle = 45 s at 94°C, 25 s at 45°C, 3 min at 72°C). One-fiftieth of the reaction was used in a second round of PCR with the same primers for 40 cycles under identical conditions. The products were cloned into pCR1000.

RACE-PCR was also employed to obtain the 3′ end of the PGIP transcript. An adaptor primer (5′-GACTCGAGTCG-ACATCGAT$_{17}$-3′) was used for first-strand cDNA synthesis. This primer, together with gene-specific primer 5 (5′-AGG-ACTCAAGTCTCTCAG-3′), was used in 40 cycles of PCR (one cycle = 45 s at 94°C, 25 s at 50°C, 3 min at 72°C). The products were cloned into pCR1000.

The complete PGIP coding sequence was amplified by 30 cycles of PCR (one cycle = 1 min at 94°C, 2 min at 58°C, 2 min at 72°C) with gene-specific primers 6 (5 ′-ACATCTCT-CAGGCTCTCAACC-3′) and 7 (5′-AAATTGCTGGCCAA-ATCTGCAG-3′). A single 1059-bp PCR product was obtained and cloned using pCR1000. All cloned PCR products were sequenced in the pCR1000 vector using the dideoxy sequencing method (Sanger et al., 1977) with the Sequenase kit (United States Biochemical) employing plasmid- and cDNA-specific primers.

DNA Gel Blot Analysis

Genomic DNA was isolated according to Bendich et al. (1980) from pear leaves. Genomic DNA was digested with the indicated restriction endonucleases, separated on 0.8% (w/v) agarose gels in Tris-borate-EDTA buffer (Maniatis et al., 1982), depurinated, denatured, and transferred to Hybond-N nylon membranes (Amersham) by capillary blotting. Filters were prehybridized in hybridization solution (50% formamide, 5× Denhardt's solution, 5× SSPE [Maniatis et al., 1982], 0.5% SDS, 200 μg/mL of single-stranded herring sperm DNA, 0.1% sodium pyrophosphate) before adding a ^{32}P-radiolabeled RNA probe (1.8 × 10^6 cpm/mL, a 365-bp transcript corresponding to position 332 to 697; see Fig. 2) for hybridization at 52°C for 48 h. Radiolabeling followed the procedures described for the Maxiscript in vitro transcription kit (Ambion, Austin, TX) and used the T_7 promoter of pCR1000. After hybridization, filters were subjected to low (Tm −25°C) stringency washes followed by autoradiography at −80°C using a preflashed Kodak XAR-5 film and an intensifying screen (Cronex, DuPont, Newtown, CT).

RNA Gel Blot Analysis

Total RNA was isolated from pear fruit by the method of Dong et al. (1991) or by a modification of the procedure of Reid et al. (1988). Total RNA was separated by electrophoresis in a 1.2% agarose gel containing 2.2 M formaldehyde in Mops running buffer (Maniatis et al., 1982) and blotted to Hybond-N nylon membranes. The blots were baked in a vacuum oven at 80°C, prehybridized in 5× SSPE, 50% formamide, 0.5% SDS, prehybridized in hybridization solution (as described above) with the addition of 10 ng/mL of poly(U) for 5 h, and then hybridized in fresh hybridization solution, without poly(U), with the ^{32}P-radiolabeled RNA probe (a full-length, 1059-bp transcript, 2 × 10^6 cpm/mL) for 20 h at 30°C below the Tm. After hybridization, the filters were sequentially washed at 20°C and 8°C below the Tm and autoradiographed as described for the DNA gel blot analysis. The total amount of RNA on the blot was estimated by hybridization to an 18S rRNA probe from radish (Delseny et al., 1983). Individual bands were quantified by densitometer scanning using a BioImage analyzer.

RESULTS

Carbohydrate Characteristics of PGIP from Pear Fruit

Purification of PGIP resulted in an approximately 250-fold increase in PGIP specific activity as measured by the agarose diffusion assay. PGIP activity was bound by Con A, indicating the presence of glucosyl and/or mannosyl residues. Based on the yield of purified PGIP, it appears to be an abundant protein in fruit, representing about 0.4% of the protein in the initial salt extract. The purified glycoprotein has a molecular mass of 43 kD when analyzed by SDS-PAGE (Fig. 1). TFMS treatment of purified pear PGIP decreased its molecular mass to 34 kD, indicating that carbohydrates contribute approximately 20% of the total mass of the mature protein.

The carbohydrate composition of purified pear PGIP was determined by GC-MS analysis. Most of the sugar residues (Man, GlcNAc, Glc, Fuc, Xyl) associated with pear PGIP are typical of those found in N-linked glycans of plant glycoproteins (Table I) and the presence of Xyl and Fuc indicates that, in the case of pear PGIP, some of them are complex. The

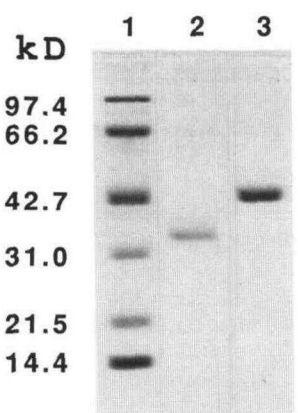

Figure 1. SDS-PAGE analysis of purified pear fruit PGIP before (lane 3, 0.5 μg) and after chemical deglycosylation (lane 2, 0.2 μg). Molecular mass standards are shown in lane 1. The gel was stained with Coomassie blue.

Table I. *Gas chromatographic analysis of the carbohydrate composition of PGIP from pear fruit*

Averages of duplicate samples are presented.

Sugar	Amount
	μg/mg protein
Man	182
Fuc	27
GlcNAc	24
Xyl	23
Glc	9
Ara	8

presence of high quantities of Man is in agreement with the binding of PGIP to Con A.

PGIP cDNA and Genomic DNA Analysis

Amino acid sequences of the N terminus and the CNBr-cleavage products of purified pear PGIP were determined and used to design three degenerate oligonucleotide primers. These primers were used in PCR of reverse-transcribed RNA or genomic DNA to amplify a 569-bp PCR product (Fig. 2). Based on the sequence of this product, gene-specific primers were used in RACE-PCR to amplify the 5′ and the 3′ ends of the PGIP transcript. Using the sequence information from each of the RACE-PCR products, the entire PGIP coding region was amplified by RNA-PCR to determine whether both RACE products belonged to the same mRNA. The nucleotide sequence of the PGIP coding region is the consensus of sequencing of both strands of three 1059-bp clones, two clones from the 3′ RACE reactions, and two 569-bp clones of RNA-PCR products, and of single-stranded sequencing of four clones from the 5′ RACE reaction and two 569-bp clones of genomic DNA-PCR products. Sequencing of multiple clones of many different PCR reactions makes it unlikely that errors from *Taq* DNA polymerase activity are included in the reported sequence.

Computer analysis indicated significant sequence similarities to only one other gene or protein in the GenBank and SwissProt data banks. A comparison with the recently published protein sequence for *Phaseolus vulgaris* PGIP is illustrated in Figure 3. Pear PGIP is approximately 50% identical and 65% similar to bean PGIP at the amino acid level. The largest open reading frame beginning with an ATG (Met) in the pear PGIP cDNA sequence predicts a polypeptide with 330 amino acids and a molecular mass of 36.5 kD. The nucleotide sequence accurately codes for the peptide sequences, which were determined from the purified, mature pear PGIP. Alignment of the sequences obtained by amino acid and nucleic acid sequence analyses demonstrates that the amino terminus of the mature pear PGIP is preceded by a typical 24-amino acid signal sequence, indicating probable

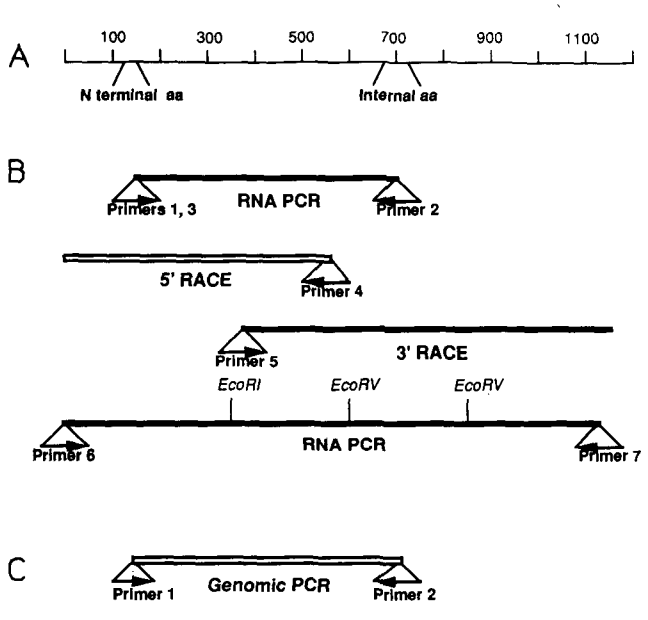

Figure 2. Cloning strategy for pear PGIP. A, Nucleic acid number based on the sequence of all the RNA-PCR and RACE-PCR products. The location of the original peptides from the purified pear PGIP used for amino acid sequencing is indicated on the scaled line. B, Location of the oligonucleotide primers used for RNA-PCR and RACE-PCR and the PCR products. The sequence of each of the primers is given in "Materials and Methods." C, The location and orientation of the 569-bp product obtained by PCR of pear genomic DNA. Closed bars indicate cloned PCR products for which sequence was determined from both strands.

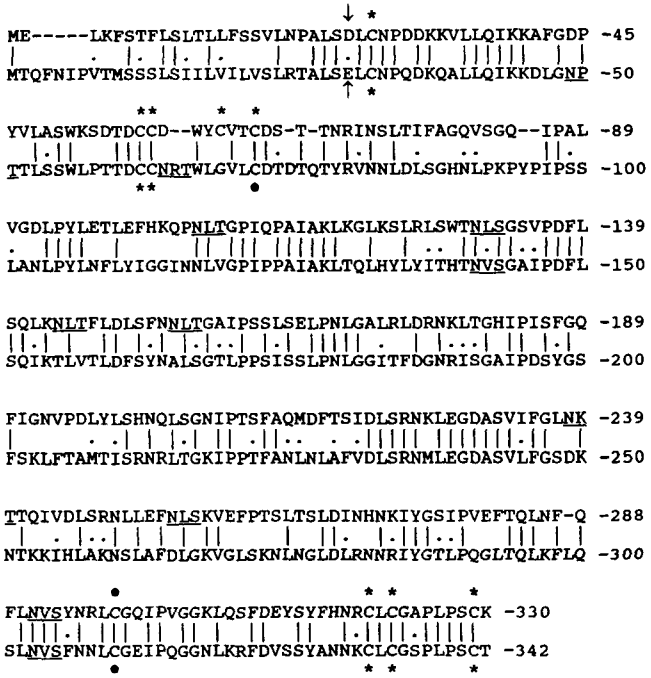

Figure 3. Amino acid sequence comparison of PGIPs. The deduced sequence from pear is shown on the upper line and that from *P. vulgaris* (Toubart et al., 1992) on the lower line. The site of potential signal peptide cleavage is marked with arrows. The identical amino acids in the pear and bean sequences are indicated by vertical lines and conserved amino acids by a point. Cys residues in both sequences are indicated by asterisks. Potential glycosylation sites (N-X-T/S) are underlined.

targeting of PGIP through the endomembrane system. The potential processing site for the signal peptidase is conserved between pear and bean PGIPs. The sequence for the processed pear PGIP has a predicted molecular mass of 33.9 kD, which is in close agreement with the observed molecular mass of the purified, deglycosylated PGIP (34 kD, Fig. 1). The pear PGIP coding sequence contains seven potential N-glycosylation sites (Asn-X-Ser/Thr), only two of which are shared with the bean PGIP. It is interesting that all nine Cys residues of the pear PGIP are clustered at the N- and C-terminal ends of the mature protein. Eight of these are conserved with the bean PGIP. These Cys residues may affect the protein's tertiary structure. The pI of the predicted processed protein was calculated to be 6.2. All these data support the conclusion that this is an authentic PGIP cDNA sequence.

DNA gel blot analysis of pear genomic DNA demonstrated two hybridizing bands when DNA was digested with *Nsi*I and *Nco*I and probed at low (Tm −25°C) stringencies. The *Nsi*I fragments were 1.25 and 3.7 kb and the *Nco*I fragments were 2 and 21 kb in size (data not shown). Because *Nsi*I and *Nco*I did not cut within the cloned PGIP cDNA sequence and the radiolabeled probe did not span an intron, the presence of, most likely, two genes in the pear genome is suggested.

Organ-Specific Expression of PGIP

Tissues from different organs of pear were assessed for PGIP expression by RNA gel blot analysis and by measuring the inhibition of *B. cinerea* PG activity. PGIP mRNA was approximately 100-fold more abundant in fruit than in flowers and was not detectable in pear leaves (Fig. 4). PGIP specific activity in fruit was approximately 200-fold higher than in flowers and approximately 1400-fold higher than in leaves. On RNA gel blots, the PGIP cDNA hybridizes to an mRNA at 1.35 to 1.4 kb in total and poly(A)$^+$ but not in poly(A)$^-$ RNA (data not shown). No other hybridizing species were detected in polyadenylated RNA even at low (Tm −30°C) stringencies.

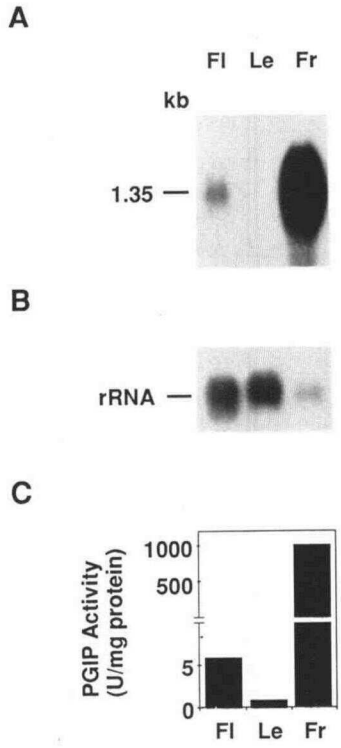

Figure 4. PGIP activity and mRNA expression in pear tissues. Total RNA from flower (Fl, 8 μg), leaf (Lf, 8 μg), and mature fruit (Fr, 3 μg) was analyzed for hybridization to the ^{32}P-labeled PGIP probe described in "Materials and Methods" (A). The size of the PGIP mRNA is indicated. B, Confirmation of the amount loaded was determined by densitometer scanning of hybridization to rRNA and used to normalize the scanning of PGIP-specific hybridization. C, PGIP-specific activity in salt extracts from flowers, leaves, and mature fruit. Inhibition of *B. cinerea* PG was determined by duplicate reducing sugar assays.

DISCUSSION

The reported PGIP cDNA comprises an open reading frame of 330 amino acids, including a typical signal sequence for targeting to the endomembrane system (von Heijne, 1985), consistent with its proposed cell wall localization (Abu-Goukh et al., 1983b). The PGIP cDNA coding sequence predicts several properties that were observed in the purified PGIP: molecular mass of the processed polypeptide of approximately 34 kD, two colinear amino acid sequences that are present in the purified protein, sites for extensive glycosylation, and a pI of 6.2, which is in close agreement with the pI reported for the most abundant PGIP isoform in pear (Abu-Goukh et al., 1983a). Based on these observations, we conclude that we have isolated an authentic cDNA clone for pear PGIP.

Comparison of the pear PGIP amino acid sequence with that recently published for bean PGIP (Toubart et al., 1992) shows only moderate amino acid sequence identity of 50%, with three regions of higher similarity (Fig. 3). Just two of seven potential glycosylation sites of pear PGIP are shared with bean PGIP. Yet, both sequences have highly conserved sites of potential signal peptide processing, and all eight Cys residues of the bean PGIP are conserved in pear PGIP, suggesting that they may be involved in stabilizing the tertiary structure of plant PGIPs. The observed differences in PGIP primary structures may be responsible for differences in kinetics and specificity toward fungal PGs (Albersheim and Anderson, 1971). This may be the reason why pear PGIP had little effect on *Aspergillus niger* pectinase (Sigma) activity unlike the *Phaseolus* PGIP (Cervone et al., 1987). In addition, bean PGIP exhibits noncompetitive inhibition of *Colletotrichum lindemuthianum* endo-PG (Lafitte et al., 1984), whereas pear PGIP competitively inhibits *B. cinerea* PG (Abu-Goukh et al., 1983a).

The organ-specific accumulation of pear PGIP is different from that reported for PGIP mRNA accumulation in bean (Toubart et al., 1992). Pear PGIP mRNA was detected at low levels in flowers and abundantly in fruits but was not detected in leaves, which could partially explain the differences in PGIP activity that were found in these different organs. Bean PGIP mRNA was observed in flowers, leaves, hypocotyls, and more abundantly in cell-suspension cultures. The

pear PGIP promoter may differ from the bean promoter to allow for high levels of fruit-specific expression of the gene.

DNA gel blot analysis suggests the presence of probably two PGIP genes in pear. Although only a small number of PGIP genes and a single mRNA species were detected in pear using hybridization probes, it is possible that other more distantly related PGIPs exist or that other PGIPs are far less abundant in this species. Multiple PGIPs have been identified in plants (Fielding, 1981; Abu-Goukh et al., 1983a). Some are expressed at low levels throughout the plant, such as the *Phaseolus* PGIP. Others, such as the pear PGIP, show high expression in Pomoideae fruit but low or undetectable expression in other tissues. The differences in structure and expression of PGIPs from pear and bean suggest that, although these proteins inhibit fungal PGs in vitro, they may differ in their role in pectin metabolism during pathogen challenge in vivo. Expression of PGIPs in transgenic plants may resolve these important differences and lead to a better understanding of the function of these proteins in plants.

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