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THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 270, No. 42, Issue of October 20, pp. 24839–24843, 1995
Printed in U.S.A.

Molecular Cloning and Pattern of Expression of an α-L-Fucosidase Gene from Pea Seedlings*

(Received for publication, June 6, 1995)

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α-L-Fucosidase is a cell wall protein purified from pea (*Pisum sativum*) epicotyls. The α-L-fucosidase hydrolyzes terminal fucosyl residues from oligosaccharides of plant cell wall xyloglucan. α-L-Fucosidase may be an important factor in plant growth regulation, as it inactivates fucose-containing xyloglucan oligosaccharides that inhibit growth of pea stem segments. The amino acid sequences of the NH_2-terminal region and one internal peptide were used to design redundant oligonucleotides that were utilized as primers in a polymerase chain reaction (PCR) with cDNA, generated from pea mRNA, as the template. A specific PCR amplification product containing 357 base pairs was isolated, cloned, and sequenced. The deduced amino acid sequence included the two peptides used to design the primers for PCR plus two other peptides obtained by proteinase digestion of α-L-fucosidase. No sequence homology to other α-L-fucosidases was apparent, although the NH_2-terminal region is strongly homologous to Kunitz-type trypsin inhibitors. cDNA and genomic copies were isolated and sequenced. In pea, the gene is present in two or three copies. Its mRNA is present in roots, leaves, and elongating shoots. The spatial pattern of expression of the α-L-fucosidase was determined by *in situ* hybridization.

Xyloglucan (XG)[1] is a hemicellulosic polysaccharide present in the primary cell walls of all of the higher plants that have been examined. XG forms strong noncovalent bonds to cellulose microfibrils and is believed to strengthen the cell walls by cross-linking the microfibrils (1). XG appears to have a regulatory as well as a structural role, as there is evidence that XG oligosaccharides regulate the rate of plant cell growth (2–4). Indeed, fucosylated subunits of XG inhibit auxin-stimulated growth in pea stem segments (4–7). These oligosaccharins (oligosaccharides with biological regulatory properties) have relatively strict structural requirements for activity, including a critical terminal fucosyl residue. Hydrolysis of the α-L-fucosidic bonds abolishes the growth-inhibiting activity of the XG oligosaccharides. Thus, we hypothesized that plants have an α-L-fucosidase that participates in the regulation of plant growth by controlling the concentration of fucosylated XG oligosaccharides. This hypothesis led to the demonstration that pea stems have a developmentally regulated α-L-fucosidase with the ability to cleave the fucosyl residue of XG oligosaccharides. Indeed, the α-L-fucosidase, which was shown to reside in the primary cell walls of pea stems, has been purified to homogeneity (5).

cDNAs encoding α-L-fucosidases have previously been isolated from human (8, 9) and rat (10) livers. The human and rat liver α-L-fucosidases have subunit molecular masses of approximately 50,100 and 55,000 Da, respectively. Both of these α-L-fucosidases hydrolyze artificial substrates such as *p*-nitrophenyl-α-L-fucoside and 4-methylumbelliferyl fucoside. Both of these fucosidases have broad aglycon specificities such that they hydrolyze α-1,2-, α-1,3-, α-1,4-, and α-1,6-L-fucosidic linkages. In contrast, the α-L-fucosidase from pea epicotyls, which hydrolyzes the terminal α-1,2-fucosidic linkages of xyloglucan oligosaccharides, has a molecular mass of 20,000 Da and does not cleave *p*-nitrophenyl-α-L-fucoside. The α-L-fucosidase from pea epicotyls is also unable to hydrolyze fucosyl linkages of intact plant cell wall polysaccharides including XG. We now report the isolation of cDNA and genomic transcripts encoding the α-L-fucosidase from pea. We show that the gene is expressed in elongating tissues (leaf, root, and stem) but is absent in fully elongated stems. We also describe the tissue- and position-dependent accumulation for transcripts encoding the α-L-fucosidase in pea.

EXPERIMENTAL PROCEDURES

Isolation and Sequencing of Peptides from α-L-Fucosidase—The pea stem α-L-fucosidase was purified as described (5). Homogeneous α-L-fucosidase was used to obtain its amino acid composition and the sequences of four peptides. The amino acid composition analysis was performed at the University of Georgia sequencing facility on an Applied Biosystems 420A amino acid analyzer. α-L-Fucosidase (≈75 pmol) was hydrolyzed prior to composition analysis in 6 N HCl at 155 °C for 75 min, as recommended by the manufacturer.

Three internal peptide fragments of the α-L-fucosidase and the NH_2-terminal peptide were prepared by incubating the α-L-fucosidase (16 μg) at 37 °C for 3 h in 0.1 ml of 600 mM ammonium bicarbonate (pH 8.0), 5 mM $CaCl_2$, and 1 μg of *Pseudomonas gingivalis* H66 (50 kDa) cysteine proteinase (11). The resulting digest was loaded on a C-8 reversed phase Aquapore RP 300 column (Brownlee) equilibrated with 0.1% trifluoroacetic acid. The peptides, which were separated using a binary gradient (solvent A = 0.1% trifluoroacetic acid in H_2O, solvent B = 0.085% trifluoroacetic acid in 80% acetonitrile) at a flow rate of 0.2 ml/min, were detected by their absorbance at 220 nm. Each peptide peak was manually collected, dried, and sequenced. Amino acid sequence analysis of the α-L-fucosidase peptides was performed at the University of Georgia protein sequencing facility by automated Edman degradation on an Applied Biosystems 470A protein sequencer.

* This work was supported in part by United States Department of Energy (DOE) Grant DE-FG05-93ER20114 (to P. A.) and by the DOE-funded (DE-FG05–93ER20097) Center for Plant and Microbial Complex Carbohydrates. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by an European Economic Community postdoctoral fellowship within the BRIDGE program.

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[1] The abbreviations used are: XG, xyloglucan; bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction.

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TABLE I
Amino acid composition of pea stem α-L-fucosidase
Calculated molecular weight: 20,018; residues: 185. Values shown represent the result of a single analysis.

Residue	Number	Mole percent[a]
Asx	18.2	9.93
Glx	21.2	11.12
Ser	7.4	3.97
Gly	26.2	14.03
His	2.0	1.06
Arg	8.9	4.81
Thr	8.9	4.89
Ala	6.9	3.68
Pro	15.4	8.38
Tyr	4.9	2.65
Val	16.5	8.82
Cys	1.4	0.81
Ile	12.4	6.72
Leu	13.0	7.09
Phe	10.0	5.42
Lys	12.2	6.61

[a] Based on an estimated molecular mass of 20,000 Da.

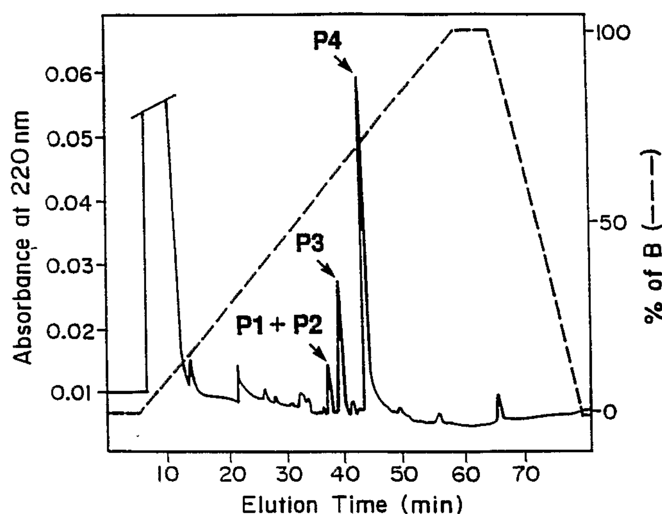

FIG. 1. **Peptide fragments of pea stem α-L-fucosidase generated by cleavage with *P. gingivalis* H66 cysteine proteinase.** The mixture of α-L-fucosidase was separated into peptides P1 through P4 by high performance liquid chromatography on an Aquapore RP 300 macroporous C-8 reversed phase column. Solvent B: 0.085% trifluoroacetic acid in 80% acetonitrile. Experimental details are described under "Experimental Procedures."

Isolation of the α-L-Fucosidase Gene—α-L-Fucosidase-specific degenerate oligonucleotide primers for use in PCR reactions were synthesized by New England Biolabs.

Total RNA was isolated from pea tissue as described (12). Poly(A)$^+$ RNA was prepared using oligo(dT) column chromatography as described (13). Single-stranded cDNA was synthesized using oligo(dT) primers and a kit from Clontech. Amplification of cDNA sequences, flanked by the α-L-fucosidase-specific degenerate oligonucleotide primers, was carried out in two sequential PCR experiments using a DNA thermal cycler (Perkin-Elmer). The reaction mixture, in a final volume of 50 μl, contained 2 ng of cDNA, 64 pmol of each primer, 50 mM potassium chloride, 10 mM Tris-HCl (pH 8.3), 1.5 mM magnesium chloride, 0.01% (w/v) gelatin, 200 mM of each dNTP, and 2.5 units of *Taq* polymerase (Perkin-Elmer). The initial PCR amplification consisted of 26 cycles; each cycle was as follows: 94 °C for 1.5 min, 45 °C for 1.5 min, and 72 °C for 3 min. The products of the reaction were separated on a 2% agarose gel, and the major product (≈350 bp) was eluted and used for the second PCR which used the same conditions. The major PCR product was eluted, purified, blunt-ended with T4 DNA polymerase (New England Biolabs), and blunt-end-ligated into the *Sma*I site of the plasmid vector pBluescript II SK$^+$ (Stratagene). The cloning procedure, including transformation of the cloning vector and selection of transformants, is described in the Stratagene pBluescript II instruction manual.

The amplified α-L-fucosidase cDNA (in λGT11) fragment was sequenced as described (14) by the Sanger dideoxy method using *Taq* polymerase in the thermal cycler.

A pea cDNA library from 7-day-old etiolated pea stems was constructed according to the manufacturer's specifications (in λGT11 vector, Stratagene). A pea genomic library (in λEMBL3) was purchased from Clontech. Both libraries were screened with the 357-bp α-L-fucosidase clone by standard methods (13, 15). A cDNA clone was isolated and sequenced. A 6.5-kb genomic clone hybridizing with the probe was partially sequenced using the dideoxy method after subcloning in M13mp18 and M13mp19 (16). Both strands of one of the subclones containing the complete α-L-fucosidase coding region (1.0 kb) were sequenced in their entirety.

Northern Blot Analysis—Tissue was collected from 7-day-old etiolated peas. Root and leaf tissues were frozen in liquid nitrogen and kept at −70 °C until use. Stem tissue (1-cm sections collected from the apical hook region (referred to as "elongating stem") and 1-cm sections collected from the base of the stem (referred to as "elongated stem")) was collected and frozen at −70 °C until use.

RNA was extracted from 7-day-old etiolated pea tissue as described (13). Total RNA (10 μg) was size-fractionated by electrophoresis on a formaldehyde-agarose gel and blotted on a Hybond-N filter (Amersham Corp.). Hybridization conditions with ^{32}P-labeled cDNA clones (the 357-bp partial cDNA clone of the α-L-fucosidase and maize ribosomal cDNA as a control) as well as washing conditions were the same as described for Southern analysis.

Genomic Blot Analysis—Genomic DNA was prepared from stems of 7-day-old etiolated pea seedlings as described by Burr and Burr (17). The DNA was digested with restriction enzymes, separated by size in an 0.8% agarose gel, and blotted onto a nylon membrane as recommended by the manufacturer (Hybond N, Amersham). Hybridization was carried out at 65 °C in 200 mM sodium phosphate (pH 7.2), 1 mM EDTA, 7% SDS, 1% bovine serum albumin, and 0.5 mg/ml of sonicated salmon sperm DNA. The DNA was labeled with ^{32}P to a specific activity of 10^9 cpm/μg by random priming (Boehringer Mannheim). Final washes were carried out at 65 °C in 20 mM sodium phosphate (pH 7.2), 1% SDS, and 1 mM EDTA. The membrane was then exposed to Kodak XAR5 film with intensifying screens (Du Pont Lighting Plus) at −70 °C.

In Situ Hybridization—To obtain an α-L-fucosidase-specific probe, the 357-bp fragment was cloned into a pBluescript SK$^+$ vector (Stratagene) and used as a linear template for synthesis of riboprobes. Sense and antisense digoxigenin-labeled α-L-fucosidase RNAs were synthesized according to the instructions of Boehringer Mannheim.

Pea tissue (stem, leaf, root) was collected from 7-day-old etiolated peas and fixed in 3:1 ethanol/acetic acid for 1 h at room temperature. Once the fixative was removed, the samples could be stored in 70% ethanol at 40 °C indefinitely. The fixed tissue was dehydrated through an ethanol series and embedded in paraffin. Sections (8 μm) were cut and mounted on poly-L-lysine-coated slides. Treatment of the sections prior to hybridization was performed as described previously (18). The sections were deparaffinized by rinsing in xylene and hydrated by passing through an alcohol series. The hydrated sections were then incubated with 0.5 ml of proteinase K (1 μg ml^{-1}) in 0.5 M Tris/HCl (pH 7.6) for 30 min at 37 °C. The proteinase K was removed by rinsing in phosphate-buffered saline and blocking with 2 mg/ml glycine in phosphate-buffered saline. Subsequently, the sections were refixed for 20 min at room temperature in freshly prepared 4% formaldehyde followed by rinsing two times in phosphate-buffered saline. The sections were then treated with 0.25% acetic anhydride in 100 mM Tris ethyl acetate buffer (pH 8.0, freshly made) followed by rinsing three times in H_2O. Finally, the sections were dehydrated through an alcohol series to 100% ethanol and dried.

The fixed, deproteinated sections were hybridized by incubating at 40 °C overnight in hybridization buffer while enclosed under a coverslip. Hybridization buffer consists of 200–400 ng ml^{-1} digoxigenin-labeled probe, 50% formamide, 300 mM NaCl, 10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 10% dextran sulfate, and 10 mM dithiothreitol. After the hybridization, the coverslip was removed in 2 × SSC at room temperature, and the sections were washed three times for 10 min at 55 °C with 0.2 M SSC. Subsequently, an RNase A treatment (20 μg ml^{-1} in 500 mM NaCl/Tris-ethanolamine (pH 8.0)) was performed at 37 °C for 30 min. The RNase-treated sections were stained overnight at room temperature with alkaline phosphatase-conjugated anti-digoxigenin antibodies according to the protocol of Boehringer Mannheim, using nitro blue tetrazolium and X-phosphate as a substrate. Color development was monitored microscopically.

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  1  gatatcaagcttaaaaggcttttgttaactcaataacttaacacgtgtgtgaaacatttg
 61  tcacatgcttgttctaaattaaagggtgcttcactaaaagggtgcttcacttcattacta
121  gtattatatatagaggtatagttaaccaacaaaatcacaaacataaaaacatccccttaa

181  ttacctaacaATGAAACCTCTTTCACCACTCACCCTTTCCTTTTTCCTCTTTGTTTTCAT
               M  K  P  L  S  P  L  T  L  S  F  F  L  F  V  F  I    17
                                ↓
241  CACCAATCTTTCACTAGCTTTCTCAAATGAAGATGTTGAGCAAGTATTGGACATTAACGG
       T  N  L  S  L  A  F  S  N  E  D  V  E  Q  V  L  D  I  N  G    37

301  CAACCCCATTTTCCCCGGTGGCCAATACTACATCTTGCCAGCAATCCGTGGTCCTCCCGG
       N  P  I  F  P  G  G  Q  Y  Y  I  L  P  A  I  R  G  P  P  G    57
     ------P1----------------------------------          ----------
361  CGGCGGAGTAAGACTCGGAAGAACCGGTGATTTAACCTGTCCAGTCACCGTCCTACAAGA
       G  G  V  R  L  G  R  T  G  D  L  T  C  P  V  T  V  L  Q  D    77
     ---------------------------------P3---------------------------
421  CCGCCAAGAAGTTAAATACGGTCTACCAGTTAAGTTCGTTATCCCAGAAATATCTCCTGG
       R  Q  E  V  K  Y  G  L  P  V  K  F  V  I  P  E  I  S  P  G    97

481  TATAATTTTCACTGGAACACCAATTGAAATAGAGTATGCAAAGAAACCTAATTGTGCAAA
       I  I  F  T  G  T  P  I  E  I  E  Y  A  K  K  P  N  C  A  K   117

541  ATCATCAAAATGGTTAGTATTTGTTGACAATCTTATTGAAAAGGCATATGTTGGTATTGG
       S  S  K  W  L  V  F  V  D  N  L  I  E  K  A  Y  V  G  I  G   137
               ----------P2------------------
601  TGGTCCTGAAAATTACCCTGGTGTGCAAACATTGAGTGGATTATTTAAGATTGAGAAGCA
       G  P  E  N  Y  P  G  V  Q  T  L  S  G  L  F  K  I  E  K  H   157
          ---------P4--------------
661  TGAATCTGGATTTGGCTATAAGCTTGGATTTTGTATTAAGGGTTCTCCTACTTGTTTGGG
       E  S  G  F  G  Y  K  L  G  F  C  I  K  G  S  P  T  C  L  G   177

721  TGTTGGAAGATTTGATAATGATGAAAATGGAAGAAGGTTGAATTTGACTGAGCATGAGTC
       V  G  R  F  D  N  D  E  N  G  R  R  L  N  L  T  E  H  E  S   197

781  TTTTCAAGTTGTGTTTATTCAAGCTGAGGCTAATGATGCCGAGTTTATTAAGTCTGTTGT
       F  Q  V  V  F  I  Q  A  E  A  N  D  A  E  F  I  K  S  V  V   217

841  TTGAattttagtttaataattaagctagaattgtgtgagttttagttcttgcaaagtttt
      -*-
901  agcatataataaagtggaaataatttgtgttcaatgtttgctctcttttaatattgttgt
961  ctctctctctatatatatcattcattatactctctctctagaag
```

FIG. 2. **Nucleotide and deduced amino acid sequence of the α-L-fucosidase gene.** A putative TATA box (nucleotides 125–132) and three polyadenylation signals are highlighted. The stop codon is indicated by an *asterisk*. The deduced amino acid sequence for the α-L-fucosidase is depicted as a single letter code. The *numbers* at the *right* margin are amino acid residues. The site of processing of the mature protein is indicated by a *vertical arrow*. The *underlined* regions, as indicated, are the sequences of peptides P1–P4 obtained by amino acid sequencing of protease-digested α-L-fucosidase. The reported genomic sequence has been deposited in the GenBank® data base under the accession number X82595.

```
                                          10          20          30        40
A. Aden. p. trypsin inhibitor    NFLRNGGSYY IVPAFRGK.. GGGLELARTG SETCPRTVVQ
B. Pea α-Fucosidase              NPIFPGGQYY ILPAGIRGPP GGGVRLGRTG DLTCPVTVLQ
C. Psopho. t. trypsin inhib.     ELVRNGGTYY ILPDRWAL.. GGGIEAAATG TETCPLTVVR
                                                                     ↑

                                          50          60          70        80
                                 APAEQSRGLP ARLSTPPRIR YIGPEFYLTI EFEECKPPSC
                                 DRREVKNGLP VKFVIQNISW YNFHWH..TI EIEYTKKPNC
                                 SPNEVSVGEP LRISSQLRSG FIPDYSVVRI GFAN..PPKC
                                                                          ↑
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FIG. 3. **Comparison of the deduced NH_2-terminal amino acid sequence of pea stem α-L-fucosidase with the NH_2-terminal sequences of two trypsin inhibitors of the Kunitz family (Carolina and Psophocarpus).** *A*, inhibitor DE-5 from the Brazilian Carolina tree (*Adenanthera pavonina*) (25). *B*, deduced amino acid sequence from pea stem α-L-fucosidase. *C*, inhibitor from winged bean *Psophocarpus tetragonolobus* (26). *Arrows* indicate the positions of conserved cysteine residues. The *boxed* amino acids represent regions of identity between the α-L-fucosidase sequence and each of the Kunitz inhibitor sequences (*A* and *C*).

A B

FIG. 4. **Determination of the number of α-L-fucosidase genes in the pea genome.** Autoradiogram of the 357-bp α-L-fucosidase probe hybridized to digests of genomic DNA from pea. *Lane A*, *Eco*RI digest; *lane B*, *Hin*dIII digest.

RESULTS AND DISCUSSION

Amino Acid Composition and Amino Acid Sequencing—The lysine and arginine content of the α-L-fucosidase (Table I) led to the expectation of the presence of several tryptic peptides. However, 24-h digestion of the α-L-fucosidase with L-(1-tosylamino-2-phenyl) ethyl chloromethyl ketone-treated trypsin failed to produce any peptides. Therefore, a portion of the purified α-L-fucosidase (16 μg) was subjected to *P. gingivalis* H66 cysteine proteinase, which generated peptide fragments that were separated by reversed phase high performance liquid chromatography (Fig. 1). Fractions containing peptides P1 + P2, P3, and P4 (see Fig. 1) were collected separately and sequenced. P1 and P2 did not separate, but sequence analysis of the peak containing P1 and P2 yielded a major and a minor sequence that were readily distinguishable. A second undigested portion of the α-L-fucosidase (3 μg) was used to determine its NH_2-terminal sequence. The amino acid sequences of peptides P1, P2, P3, P4, and of the NH_2 terminus are illustrated in Fig. 2. The amino acid sequences of peptide P1 and the NH_2 terminus are identical.

PCR, Cloning, and Nucleotide Sequencing—An oligonucleotide was synthesized in its sense orientation on the basis of the NH_2-terminal sequence. The oligonucleotide was a 17-mer with 64-fold redundancy corresponding to the first six amino acids of P1 (Fig. 2). A second oligonucleotide was synthesized corresponding to the sequence of the fourth to the ninth amino acids of peptide P4. The oligonucleotide, which was constructed in its antisense orientation, was a 17-mer with 128-fold redundancy.

The redundant oligonucleotides were used as primers for the PCR using a single-stranded cDNA template synthesized from total pea RNA by reverse transcriptase. A prominent DNA band of ~0.35 kb was visualized upon ethidium bromide staining of the reaction products that had been fractionated in an agarose gel (data not shown). The band was absent in control reactions containing one or none of the oligonucleotide primers. The 0.35-kb band was eluted from the agarose gel and further amplified by PCR using the same oligonucleotide primers. The PCR-generated fragment was purified and cloned. One recombinant clone, which was shown after size fractionation in an agarose gel to contain the PCR-amplified fragment, was sequenced; its deduced amino acid sequence consisted of a reading frame coding for 119 amino acids (starting at amino acid 27, Fig. 2), representing 65% of the estimated mature α-L-fucosidase sequence (5). The amino acid sequence of the α-L-fucosidase fragment included the sequences corresponding to the NH_2 terminus and peptide P4, which were used to generate the primers. The partial cloned fragment also contained sequences encoding peptides P2 and P3 (Fig. 2) not used in the design of the PCR primers. This indicated that the PCR-amplified product was a partial cDNA encoding the α-L-fucosidase.

cDNA and Genomic Cloning—Several genomic clones that hybridized to the partial sequence of the α-L-fucosidase cDNA were isolated from a commercial λEMBL3 library (see "Experimental Procedures"), and one was selected for sequence analysis. The DNA sequence of 1.0 kb, including the α-L-fucosidase gene, is shown in Fig. 2. A TATA box is found, as expected, at

−66 bp relative to the initial ATG in the 5′-flanking region of the α-L-fucosidase coding region. There are no introns within the coding region.

Comparison of the α-L-fucosidase genomic sequence with the determined cDNA sequence revealed minor base mismatches but exhibited an overall identity of 97%. Sequence examination of the coding region established that the cDNA previously characterized is an mRNA of this gene. The cDNA does not contain a poly(A) tail, even though the genomic sequence contains polyadenylation signals starting at positions 855, 908, and 919.

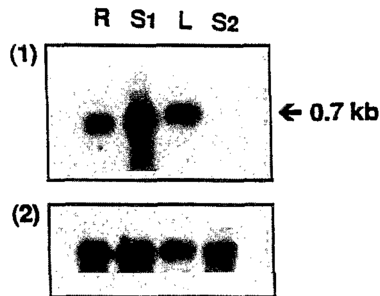

FIG. 5. **Northern blot analysis showing α-L-fucosidase mRNA distribution in various pea tissues.** RNA isolated from roots, stems, and leaves of 7-day-old etiolated pea seedlings was blotted and hybridized against a radioactive 357-bp α-fucosidase probe (1) and against a radioactive ribosomal probe (2). *R*, root; *S1*, elongating stem from apical hook region; *L*, young etiolated leaves; *S2*, elongated stem from basal stem region.

The protein predicted by the genomic sequence is identical to that predicted by the cDNA, with the exception of an additional methionine residue at the amino terminus which was missing in the cDNA. The predicted protein begins at the NH_2 terminus with a hydrophobic stretch having features typical of a signal peptide. The putative signal peptide is absent in the mature protein, presumably due to processing that accompanies passage through the endoplasmic reticulum (19, 20). The presence of a secretion signal in the nascent α-L-fucosidase is expected, as the α-L-fucosidase is located in the extracellular matrix in pea epicotyls (5). As confirmed by NH_2-terminal amino acid sequencing of the α-L-fucosidase itself, the amino-terminal end of the mature protein begins with the glutamate residue at position 27. Therefore, the signal peptide processing site is at the Asn-Glu junction between positions 26 and 27 of the predicted protein. This would make the molecular mass of the processed protein 19,943 Da, in agreement with the estimation of 20 kDa for purified, denatured α-L-fucosidase (5).

No strong homology was found when the sequence of the α-L-fucosidase cDNA clone and the encoded protein were compared with the sequences of human and rat liver α-L-fucosidases present in the GenBank® nucleic acid data base (release 71.0) and the NBRF protein data base (release 31.0). However, the NH_2 terminus of the pea stem α-L-fucosidase, including amino acids 1–80 (see Fig. 3), has 43 and 33% sequence iden-

FIG. 6. **Localization of α-L-fucosidase mRNAs in 7-day-old etiolated peas.** Plant material was fixed, embedded, and cut into 8-μm sections. Hybridization was performed with digoxigenin-labeled single-stranded antisense RNA (*A–C*) or sense RNA (*D*), as outlined under "Experimental Procedures." Sections were photographed by bright field microscopy. *A*, transverse section through pea leaf and stem. *Bar* = 700 μm. *B*, longitudinal section of pea stem in the apical hook region. *Bar* = 300 μm. *C*, transverse section through a pea stem under the node closest to the apical hook. *Bar* = 300 μm. *D*, transverse section through a pea stem in the apical hook region. *Bar* = 300 μm. *L*, leaf; *S*, stem.

tity to the NH_2 terminus of two Kunitz-type trypsin inhibitors (Fig. 3, first and third sequences, respectively). The Kunitz trypsin inhibitors have a molecular mass of about 21 kDa and include four cysteines forming two disulfide bridges (21). Two cysteine residues are present at identical positions in both the α-L-fucosidase and Kunitz inhibitor sequences (Fig. 3, amino acids 34 and 80). However, we could not detect trypsin inhibitor activity in the purified pea stem α-L-fucosidase.[2] On the other hand, it may not be coincidental that the pea stem α-L-fucosidase is not cleaved into peptides by trypsin, whereas the α-L-fucosidase is cleaved by a protease that works differently from trypsin.

We performed a genomic Southern blot analysis to estimate the number of α-L-fucosidase sequences in the pea genome. Aliquots of genomic DNA of pea were digested with one of several restriction enzymes, blotted to nylon membrane, and probed with the 357-bp partial cDNA (see "Experimental Procedures"). The results show (Fig. 4) that the α-L-fucosidase gene is present in two or three copies in the pea genome. Therefore, pea α-L-fucosidases are comprised of a small gene family with at least two genes.

A single α-L-fucosidase mRNA species (0.7 kb) was detected by Northern blot analysis of total RNA extracted from pea roots, leaves, and elongating stems (Fig. 5). α-L-Fucosidase transcripts were undetected in fully elongated stem tissue. The observed pattern of expression is consistent with the hypothesis that the α-L-fucosidase has a role in growth regulation (5).

The NH_2-terminal region of α-L-fucosidase (amino acids 1–50) has 28% sequence identity to sweet potato sporamins A and B (data not shown). Sweet potato sporamins are a group of proteins with molecular weight of 20,000 (22) that account for 60–80% of the soluble protein in mature tubers. The amino acid sequences of the sporamins are also homologous to the Kunitz-type trypsin inhibitors of leguminosae seeds (23). Three regions (see Fig. 3, amino acids 6–7, 9–10, and 21–23) and the positions of the two cysteine residues (Fig. 3, amino acids 34 and 80) are conserved in the pea stem α-L-fucosidase, Kunitz trypsin inhibitors, and sweet potato sporamins. α-L-Fucosidase is located in the cell wall, whereas the Kunitz inhibitors reside in the lysosome. The sequence homology between these proteins, with distinct localization patterns and with apparently different functions, suggests these genes have evolved by duplication and mutation of an ancestral genetic domain.

Localization of α-Fucosidase Transcripts—We showed above that mRNA encoding the α-L-fucosidase is present in several pea tissues, all of which have some cells that are elongating. α-L-Fucosidase mRNA was not detected in elongated stems. To localize more precisely the accumulation of α-L-fucosidase mRNA, we used the 357-bp cDNA fragment as a probe for *in situ* hybridization with elongating tissues (young leaf, hook region of the stem, stem region below hook). The α-L-fucosidase mRNA is localized to the epidermal layers of the elongating stem (Figs. 6, *A* and *B*) and the vascular system of the stem and leaf (Figs. 6, *A–C*). The α-L-fucosidase transcripts accumulated in the epidermal layer of the stem section closest to the shoot apex (Fig. 6*A*). However, in stem sections below the apical hook, the α-L-fucosidase transcript is absent from the epidermal layer but still present in the vascular system (Fig. 6*C*). The transcript is also present in the meristematic zone of root (data not shown), where differentiation is in progress. Hybridization with a sense control probe of stem and leaf sections similar to that in Fig. 6*A* exhibited only background signal and no organized pattern (Fig. 6*D*). The pattern of transcript accumulation in the epidermal layer and its absence in the elongated stem tissue reinforce the hypothesis that α-L-fucosidase plays a role in the control of cell elongation by regulating the levels of bioactive fucosylated xyloglucan oligosaccharides generated within the growing cell wall (24). The localized accumulation of α-L-fucosidase mRNA in the highly dividing meristematic tissue (both root and shoot) may also be interpreted as its product's having a regulatory function. Thus, considerable evidence supports the hypothesis that α-L-fucosidase functions in plant growth regulation, although the role of the enzyme *in vivo* remains to be established.

[2] C. Augur, V. Stiefel, A. Darvill, P. Albersheim, and P. Puigdomenech, unpublished results.

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