Plant Physiol. (1993) 103: 289–290

Plant Gene Register

Ripening-Related Polygalacturonase cDNA from Avocado[1]

Sally Y. Kutsunai, An-Chi Lin, Frank W. Percival[2], George G. Laties, and Rolf E. Christoffersen*

Department of Biology University of California, Los Angeles, California (S.Y.K., G.G.L.); and Department of Biological Sciences, University of California, Santa Barbara, California (A.-C.L., F.W.P., R.E.C.)

During the ripening of avocado (*Persea americana*) fruit, extensive cell wall degradation leads to a dramatic softening of the mesocarp tissue. Two enzymes have been suggested to play a critical role in this ripening-associated cell wall degradation in many fruits, including avocado, viz. cellulase (endo-β-1,4-glucanase) and endo-PG (Awad and Young, 1979; Fischer and Bennett, 1991). Although the avocado cellulase cDNA (Tucker et al., 1987) and gene (Cass et al., 1990) have been cloned and sequenced, the avocado PG has not previously been characterized at the molecular level. The ripe avocado fruit is known to contain numerous electrophoretic variants of the PG protein (Kanellis et al., 1991), but whether these represent different gene products or posttranslational modifications of one or more primary polypeptides is unknown. In tomato fruit, the various PG isoforms that accumulate during ripening (PG1, PG2A, and PG2B) are derived from a single gene (Fischer and Bennett, 1991).

Using heterologous hybridization with a tomato cDNA probe, we have identified a putative avocado PG cDNA (pAVOpg) from a library generated with RNA from ripe avocado fruit (Table I). The cDNA contains an insert of 1725 bp that detects a 1900-nucleotide mRNA on hybridization to ripe fruit RNA and no detectable signal in mRNA from unripe fruit. This pattern of expression during ripening in avocado parallels that previously reported for PG enzyme activity (Awad and Young, 1979).

Sequence analysis of the pAVOpg cDNA revealed an open reading frame of 453 amino acids, which is similar in size to that reported for avocado PG protein (Kanellis et al., 1991). This putative avocado PG sequence shows close similarity to PG sequences derived from both tomato and corn, 52 and 36% identity, respectively. In addition, it contains an octapeptide motif that is conserved in PG sequences derived from plant, fungal, and bacterial species (Bairoch, 1992). This octapeptide is contained within a span of 14 residues (TCGPGHGISIGSLG) that is identical among the known plant PG sequences and thus should allow characterization of other plant PG genes using degenerate oligonucleotide primers in polymerase chain reaction experiments.

Table I. *Characteristics of pAVOpg from avocado*

Organism:
Avocado (*Persea americana* cv Hass).
Techniques:
Plasmid sequencing; restriction fragment subclones, and synthetic oligonucleotide primers used to completely sequence both strands.
Method of Identification:
The pAVOpg clone was isolated by heterologous hybridization to a cDNA library constructed from mRNA isolated from ripe avocado fruit. The probe used was from tomato (Della Penna et al., 1986). Sequence comparison with tomato PG shows a high level of similarity.
Expression Characteristics:
Expression is correlated with the climacteric ripening of fruit. The mRNA is undetectable in unripe avocado fruit and represents an abundant mRNA species in the ripe fruit.
Features of cDNA Structure:
A total of 1725 base insert in the *Pst*I site of pUC18 using the GC tailing method. Translation start site at base 34. Stop site at position 1393.
Structural Feature of Deduced Protein:
Open reading frame predicts 453 residues; mol wt of unprocessed polypeptide, 49,061; predicted isoelectric point = 5.50; NH_2-terminus signal peptide; three potential glycosylation sites at positions 173, 294, and 358; Gly-rich octapeptide found in PG sequences from plant, fungal, and bacterial sources at positions 300 to 307.
Antibodies:
Rabbit polyclonal to purified protein from avocado fruit.
Subcellular Localization:
Unknown.

NOTE ADDED IN PROOF

A similar sequence has recently been reported by Dopico et al. (Plant Mol Biol **21:** 437–449 [1993]).

ACKNOWLEDGMENT

The authors thank Alan B. Bennett for providing the tomato PG cDNA.

Received January 15, 1993; accepted January 19, 1993.
Copyright Clearance Center: 0032-0889/93/103/0289/02.
The GenBank accession number for the sequence reported in this article is L06094.

[1] Supported in part by a grant from the National Science Foundation to G.G.L. and R.E.C. and a grant from the University of California, Los Angeles Academic Senate Committee on Research to G.G.L.

[2] Permanent address: Biology Department, Westmont College, Montecito, CA.

* Corresponding author; fax 1–805–893–4724.

Abbreviation: PG, polygalacturonidase.

LITERATURE CITED

Awad M, Young RE (1979) Postharvest variation in cellulase, polygalacturonase, and pectinmethylesterase in avocado (*Persea americana* Mill, cv Fuerte) fruits in relation to respiration and ethylene production. Plant Physiol **64:** 306–308

Bairoch A (1992) PROSITE: a dictionary of sites and patterns in proteins. Nucleic Acids Res **20:** S2013–S2018

Cass LG, Kirven KA, Christoffersen RE (1990) Isolation and characterization of a cellulase gene family member expressed during avocado fruit ripening. Mol Gen Genet **223:** 76–86

Della Penna D, Alexander D, Bennett AB (1986) Molecular cloning of tomato fruit polygalacturonase: analysis of polygalacturonase mRNA levels during ripening. Proc Natl Acad Sci USA **83:** 6420–6424

Fischer RL, Bennett AB (1991) Role of cell wall hydrolases in fruit ripening. Annu Rev Plant Physiol Plant Mol Biol **42:** 675–703

Kanellis AK, Solomos T, Roubelakis-Angelakis KA (1991) Suppression of cellulase and polygalacturonase and induction of alcohol dehydrogenase isoenzymes in avocado fruit mesocarp subjected to low oxygen stress. Plant Physiol **96:** 269–274

Tucker ML, Durbin ML, Clegg MT, Lewis LN (1987) Avocado cellulase: nucleotide sequence of a putative full-length cDNA clone and evidence for a small gene family. Plant Mol Biol **9:** 197–203