

Short communication

Molecular characterisation of cDNA clones representing pectin esterase isozymes from tomato

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Abstract

Two pectin esterase cDNA clones representing different isozymes with ca. 95% homology were isolated from an early ripening tomato fruit cDNA library. Both clones were longer than previously published sequences, and the encoded proteins possessed extended (229–233 amino acid) putative N-terminal extensions. In addition, the mRNA species corresponding to the two clones showed differential levels of expression in fruit.

Pectin esterase (PE; EC3.1.1.11) is a cell wall-metabolising enzyme responsible for the demethylation (deesterification) of galacturonic acid residues in high-molecular-weight pectin. It is characterised by the existence of multiple isoforms, and up to eight different isoenzymes have been detected in tomato fruit, depending on the variety, and method of isolation and detection employed [2, 8, 9]. Two isoforms are routinely isolated from tomato fruit of the variety Ailsa Craig [15]. We have previously isolated a pectin esterase cDNA clone for one of these isoforms

from tomato [10] using degenerate oligonucleotide probes based on a published tomato pectin esterase amino acid sequence [5, 6]. Although the deduced amino acid sequence of this cDNA clone was not identical to the published amino acid sequence, it was very similar, and differences were attributed to the existence of several genes, or to isoenzyme differences between different tomato varieties [10]. In this report we describe the characterisation of two further PE cDNA clones, one of which corresponds to a longer version of the original cDNA, the second to a novel sequence

The nucleotide sequence data reported will appear in the EMBI, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X74638 and X74639.

Fig. 1. Comparison of the nucleotide sequences of cDNA clones B8 and B16. Nucleic acid sequence of B8 cDNA and B16 cDNA. Identical bases are indicated by dashes (-). Absence of bases is indicated by colons (:).

in length respectively (Fig. 1) with 93.7% homology in the coding regions. Open reading frames in the two clones encoded proteins of 550 and 546 amino acids (Fig. 2). The sequence of clone pB16 was different from the cDNA sequence published by Ray *et al.* [10] at four positions. However, within the section of the 3' untranslated region used to generate probes to distinguish pB8 from pB16, the sequences of pB16 and Ray *et al.* [10] were identical. The new clone (pB16) may, therefore, represent a longer version of the original clone. The deduced amino acid sequence of pB8 was more similar to the published amino acid sequence of purified tomato PE [6] than was the original cDNA clone (Fig. 2). The sequences of neither clone are identical to a partial PE cDNA obtained by Harriman *et al.* [4].

Based on the N-terminus of the mature protein

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MANPQQPLLKTHKQNPISFKILSFVITLFLVALFL:::VAPYQVEIKHSNLCKTAQDS 1
--T-----T-----S-----T--V-----VVFL-----F-----2
          *
QLCLSYVSDLSINEIVTTESDGHSLMKPLVNYVHQMNNAIPVVRKMNQINDIRQHGAL 1
          SD--L--K--YS-----I-----E-----2
TDCLELLDQSVDFASDSIAAIDKRSRSEHANAQSWLSGVLTNHVTCLDELDSPTKAMING 1
          L--LVC-----2
          #
TNLEELISRAKVALAMLASLTQDEDVFMTVLGKMPGWSVSSMDRKLMESSGKDIANAVV 1
--D-----V--PNDE-LRPG-----R-----G-----2
          3
          4
AQDGTGDYQTLAEAVAAAPDKSKTRYVIYVKGRTYKENVEVASNKMNLIMVGDGMATYTI 1
-K--K-R-----S-R-----I-----I-----2
-K--N-----I-----I-----3
          4
TGSLNVVDGSTTFRSATLAAGQGQFILDICIQNTAGPAKQQAVALRVGADMSVINRCRI 1
          H-----K-----H-----K-----D-----2
          E-----TL-----3
          4
DAYQDTLYAHSQRQFYRDSYVTGTVDFFIGNAAVVFQKQCLVARKPGKYQQNMVTAQGRT 1
          I-----I-----I-----I-----2
          D-----G-----3
          4
DPNQATGTSIQFCNIIASSDLEFVLKEFPTYLGRPWKEYSRTVMESYLGLINPAGWAE 1
--D--P--K--V-----K-----D--S-----2
--A-----N-----3
          P--K--V-----4
WDGDFALKTLTYGFMNNGPGAGTSKRVRKWPFGYHVITDPAKAMPFTVAKLIQGGSWLRST 1
-H-----E--S-----2
          K-----4
GVAYVDGLYD 1
D-----2
          4

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Fig. 2. Comparison of tomato PE amino acid sequences from different sources, showing residue differences. Amino acid sequence from: 1, B8 cDNA; 2, B16 cDNA; 3, PET1 cDNA (Harriman *et al.* [4a]); 4, Marković and Jörnval [6]. The N-terminus of the mature protein is underlined and marked with a #. The putative point of cleavage of a signal peptidase is marked with a *. Residue positions which remain inconclusive in the Marković and Jörnval amino acid sequence are indicated in bold.

identified by Marković and Jörnval [5, 6], the proteins encoded by pB16 and pB8 had N-terminal extensions of 233 and 229 amino acids respectively which both contained hydrophobic domains of ca. 25 residues within the first 40–45 amino acids. In both cases this region had the characteristics of a eukaryotic signal sequence suggesting it was probably the signal for co-translational translocation into the endoplasmic

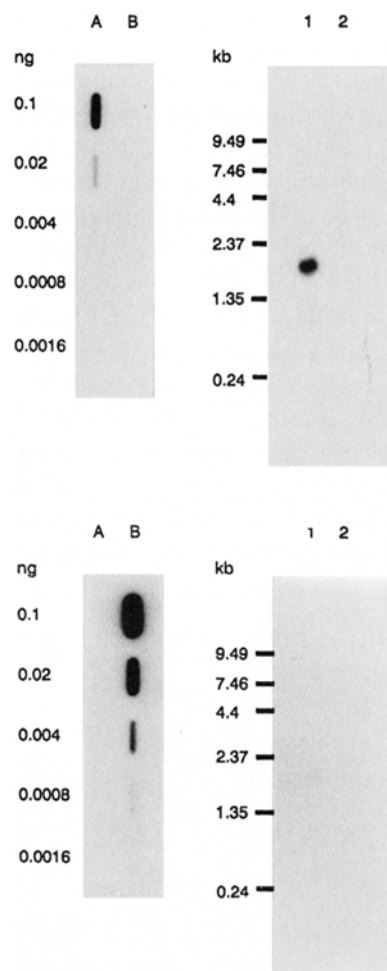


Fig. 3. Relative expression of genes encoding mRNAs corresponding to cDNA clones B8 and B16. 20 µg mature green fruit RNA per sample (Lane 1, Ailsa Craig; Lane 2, PE antisense) was denatured, fractionated through a 1% (w/v) agarose gel and blotted. The filters were hybridised with oligonucleotide probes specific to the 3' ends of B8 (a, top) or B16 (b, bottom). DNA standards (A, B8 cDNA; B, PE1 cDNA [10] were included to determine probe specificity and quantify relative expression levels.

reticulum. Based on the predictions of von Heijne [16] the most likely point of cleavage by a signal peptidase for B8 and B16 is after the proline residues at positions 39 and 43 respectively (Fig. 2). In both cases there was also a potential N-glycosylation site within the N-terminal pro-sequence region (175 and 179 amino acids for B8 and B16 respectively). After cleavage of the N-terminal extensions, clones B8 and B16 would both encode 317 amino acid mature proteins with molecular weights of 34 554 and 34 721 and pIs of 7.8 and 9.4 respectively.

The unusually long N-terminal extension represents ca. two-thirds of the length of the mature protein. It may be involved in stabilising the protein or inhibiting its enzymic function during transport through the endomembrane system. Alternatively, it may be involved in targeting the protein to the surface of the cell, and the cell wall. It is not possible to rule out the suggestion that the N-terminal extension forms a second chain in the active pectin esterase enzyme. It is not clear why pectin esterase is synthesised with an N-terminal extension that is more than 3 times the length of the 71 amino acid N-terminal extension of polygalacturonase [3], another tomato fruit enzyme that is targeted to the cell wall. There

were no identifiable regions of homology between the polygalacturonase and pectin esterase N-terminal extensions.

To analyse the relative expression of mRNAs corresponding to the two cDNA clones, a sequence specific to each clone was identified in the 3'-untranslated regions. Template oligonucleotides were synthesised to these regions (pB16, 1705–1749 bp; pB8, 1909–1957 bp) and used to probe RNA blots (Fig. 3). Both oligonucleotide probes were specific to their respective cDNA clones, and hybridised to a single mRNA species of ca. 2 kb (Fig. 3a and 3b). However, the expression of the mRNA corresponding to the novel cDNA (B8; Fig. 3a) was about 40-fold greater than that of the original cDNA (B16; Fig. 3b). Transgenic tomatoes in which major fruit PE isoforms were inhibited with an antisense gene [4] had no detectable mRNA that hybridised to either cDNA (Fig. 3). When the hybridisation was repeated for total RNA extracted from fruit at different developmental stages, the expression of the mRNA corresponding to B16 was minimal at all times (Fig. 4b), while the expression of the mRNA corresponding to B8 (Fig. 4a) increased during fruit ripening and development to a maximum as the fruit changed from green to orange

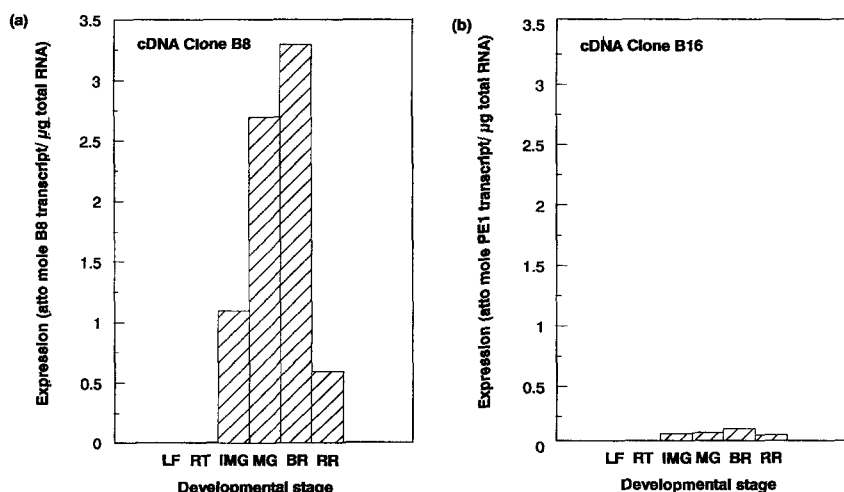
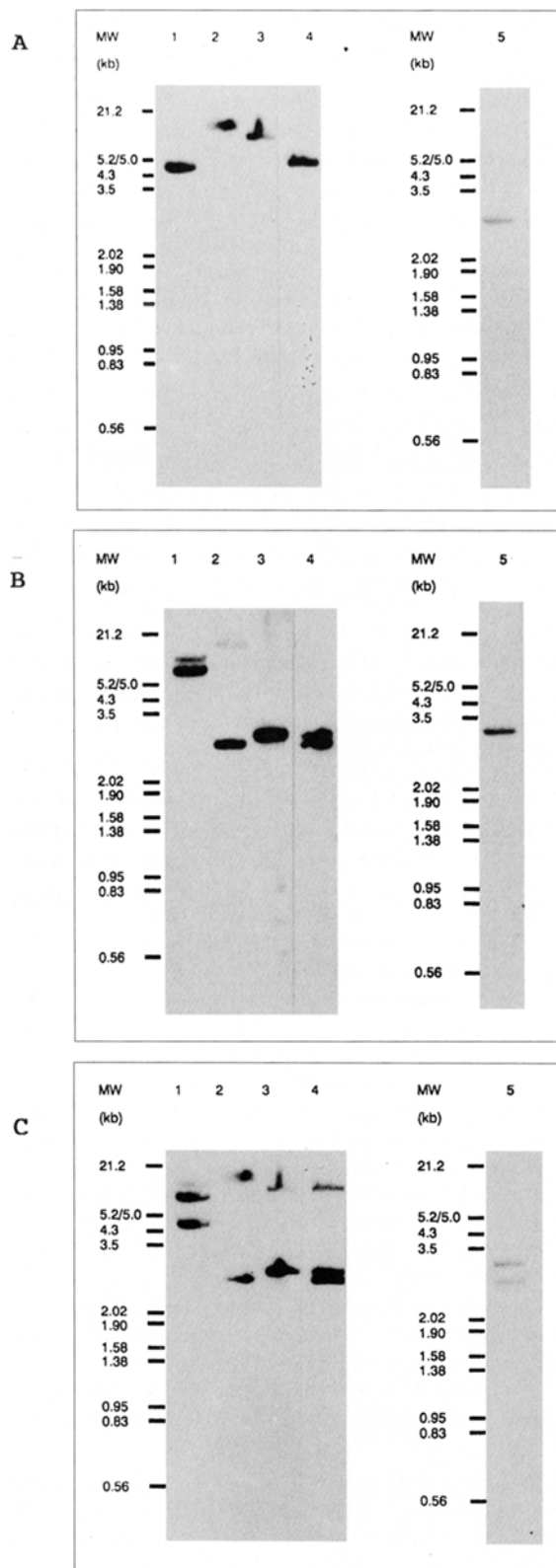


Fig. 4. Temporal expression of genes encoding mRNAs corresponding to cDNA clones B8 and B16. 20 μg of total RNA per sample was glyoxylated, fractionated through a 1.2% (w/v) agarose gel, blotted and hybridised with probes specific to the 3' end of B8 (a) and B16 (b). LF, leaf; RT, root; IMG, immature green fruit; MG, mature green fruit; BR, breaker fruit; RR, 7 days post-breaker fruit. Expression was quantified by densitometry of the autoradiograph film.



(breaker stage of fruit development). No detectable expression of mRNA homologous to either clone was found in leaves or roots (Fig. 4).

When the same oligonucleotide probes were hybridised to genomic Southern the B8 specific probe hybridised to single bands in digests with 5 different restriction enzymes (Fig. 5a); however, the probe specific to B16 hybridised to two bands in three out of five digests with the same restriction enzymes, suggesting the presence of more than one gene homologous to this clone (Fig. 5b). When the filters were stripped and reprobed with a mixed probe prepared from the 3'-untranslated regions of the two clones, a minimum of two bands were observed in each digest (Fig. 5c). The bands observed correspond to those observed with the individual clone-specific probes. These data, together with the previously published data [10] suggest the presence of three similar pectin esterase genes in tomato, only one of which has a 3'-end similar to cDNA clone B8.

A tomato genomic library constructed in λ EMBL3 [1] was screened with the original cDNA [10]. Characterisation of one hybridising clone of 13.5 kb revealed one full-length and two partial pectin esterase genes in tandem. Parts of this clone were subcloned and sequenced. The novel, highly expressed cDNA (B8) was found to correspond to the first gene in the series, and the original cDNA [10] and cDNA B16 were found to correspond to the second gene in the series. The third gene was different from the other two, although it shared ca. 86% and 88% homology to clones pB8 and pB16 respectively over the coding region for which sequence data was available (573 nucleotide). In addition to the PE sequences described here, there is now good evidence for the existence of different PE isoforms

Fig. 5. Genomic Southern blots to estimate copy numbers of genes homologous to the 3' ends of cDNA clones B8 and B16. 10 μ g Tomato (cv. Ailsa Craig) leaf DNA was digested with one of 5 restriction enzymes, fractionated through a 0.8% (w/v) agarose gel, blotted and probed with a 3' probe: B8 (a); B16 (b); mixed probe (c) prepared from 3'-untranslated regions of B8 and PE1 [10]. 1. *Hind* III; 2. *Xba* I; 3. *Pvu* II; 4. *Pst* I; 5. *Eco* R I.

which are expressed in roots and leaves, and to a minor extent in fruit [4, 14].

The existence of several pectin esterase genes, which has also been reported by Harriman *et al.* [4a], is a possible explanation for the discrepancies noted between the published amino acid sequence [5, 6] and sequences derived from cDNA clones [4, 10]. It is apparent that more than one of these genes is expressed in ripening tomato fruit, since different cDNA species have been isolated which correspond to different genes present on genomic clones (cDNA clones B8 and B16). Since these genes encode very similar protein sequences, it is likely that their protein products will be co-purified resulting in the sequence of a mixture of gene products.

It is interesting that the mRNA species corresponding to the different cDNA clones described here show differential expression in fruit. The explanation for this is unclear. It is possible that the promoter strengths of the genes or stabilities of the mRNAs are different. Alternatively, it is possible that more than one gene may be required to synthesise sufficient gene product; or the different genes may encode proteins with slightly different functions in the cell wall. However, further work will be required to elucidate this.

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