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Pectate lyases, cell wall degradation and fruit softening

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Abstract

This is a brief review of what is known about the role of pectate lyases in plants. The mode of action and three-dimensional structure of microbial pectate lyases is discussed first and then the limited information on the plant proteins is presented. Pectate lyase-like genes have been isolated from a wide range of plant tissues including germinating seeds, pollen, cell cultures, and ripening fruits. The abundance of ESTs for these genes in tomato and the presence of pectate lyase-like transcripts in many other fruits may indicate that these enzymes have a more important role in ripening than previously suspected.

Keywords: Cell wall, fruit softening, pectate lyase, pectin.

Introduction

Pectate lyases (PL, EC 4.2.2.2), otherwise known as pectate transeliminases, catalyse the eliminative cleavage of de-esterified pectin, which is a major component of the primary cell walls of many higher plants (Carpita and Gibeaut, 1993). The backbone of pectic polysaccharides is built of blocks of α-1,4 linked polygalactosyluronic acid residues interspersed with regions of alternating galactosyluronic acid and rhamnosyl residues (Willats *et al*., 2001). Cleavage by PL requires the presence of calcium ions and generates oligosaccharides with unsaturated galacturonosyl residues at their non-reducing ends.

Until recently, it was thought that PLs were secreted mainly by plant pathogens, their action resulting in the maceration of plant tissues. However, the abundance of PL-like sequences in plant genomes (currently 27 genes in the *Arabidopsis* genome are thought to encode PL-like proteins, www.tigr.org, *Arabidopsis* database, July 2002) strongly suggests an important role for these enzymes in various plant developmental processes.

Microbial PLs: mode of action and three-dimensional structures

PL activity was first discovered in 1962 in cultures of *Erwinia carotovora* and *Bacillus sp*. (Starr and Morán, 1962) and their secretion by plant pathogenic bacteria is today well-documented (Collmer and Keen, 1986; Kotoujansky, 1987; Nakajima *et al*., 1999). PL action results not only in plant cell-wall degradation, but also in the activation of defence systems, presumably through the release of oligogalacturonides from the plant cell wall, which then function as defence elicitors (De Lorenzo *et al*., 1991).

The best-studied microbial PLs to date are those from *Erwinia chrysanthemi*, which causes devastating diseases involving maceration of parenchymatous tissues of various dicot plants (Pérombelon and Kelman, 1980; Keen *et al*., 1984; Kelemu and Collmer, 1993). These enzymes act by depolymerizing cell-wall polygalacturonides in the presence of calcium ions, thus destroying the integrity of the plant tissues (Collmer and Keen, 1986; Barras *et al*., 1994). This bacterium has been shown to express up to five independently regulated PL genes (*pelA*, *B*, *C*, *D*, and *E*) coding for five isozymes of PL (first reported by Lietzke *et al*., 1994, 1996). *Erwinia* isoforms, obtained by expression in *E. coli*, have been shown to act synergistically to extend the range of pectin substrates that the bacterium can degrade (Bartling *et al*., 1995).

The three-dimensional structures of various extracellular PLs (family 1 lyases as defined by http://afmb.cnrsmrs.fr/~pedro/CAZY/lya.html) have been deter-

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mined, including PelC (Yoder *et al*., 1993*a*, *b*; Yoder and Jurnak, 1995), and PelE (Lietzke *et al*., 1994) from *Erwinia chrysanthemi* and BsPel from *Bacillus subtilis* (Pickersgill *et al*., 1994). All these enzymes share an unusual structure termed 'the parallel β helix' in which β-strands are folded into a large, right-handed superhelix (Fig. 1). Two PLs, which cleave methylated pectin, also belong to this family (Mayans *et al*., 1997; Vitali *et al*., 1998). There is also a brief preliminary description of the *E. chrysanthemi* PL, which also has parallel β-helix architecture and PL activity, but no sequence similarity with the family 1 enzymes (Jenkins and Pickersgill, 2001). The PL structures differ in the size and conformation of the loops that protrude from the parallel β-helix core, nevertheless they all share the same basic structure. It can be deduced from sequence similarity, the position of the calcium-binding site in BsPel and from site-directed mutagenesis (Kita *et al*., 1996), that the protruding loops on one side of the parallel β helix form the pectolytic active site. The structural differences in the loops are probably related to subtle differences in the enzymatic and maceration properties of the proteins (Scavetta *et al*., 1999). Recent work on *E. chrysanthemi* PelC, the first protein in which the parallel β-helix structure was recognized, shows that it appears to consist of two domains that strongly interact and unfold at pH 7, the co-operativity decreasing at higher and at lower pH (Kamen *et al*., 2000). However, the crystal structure of PelC only reveals a single domain.

It is likely that the PLs secreted by plant pathogens share a common enzymatic mechanism but, unfortunately, the catalytic roles of the amino acid residues in the active site have not yet been identified. The crystal structure of a PelC mutant complexed to a plant cell-wall fragment has recently been published (Scavetta *et al*., 1999). The substrate binds in a cleft, interacting primarily with positively charged groups; either lysine or arginine amino acids on PelC or the four Ca^{2+} ions found in the complex (Scavetta *et al*., 1999). The suggestion made by the authors is that an arginine, which is invariant in the PL superfamily, is the amino acid that initiates proton abstraction during β elimination cleavage of polygalacturonic acid.

Plant PLs

PL-like sequences from higher plants were first reported from pollen (Wing *et al*., 1989). Two genes with sequence similarity to *Erwinia* PLs were expressed at maximal levels in mature tomato flowers, anthers and pollen. Since then, many other similar sequences have been shown to be expressed in pollen, anthers and pistils (Kulikauskas and McCormick, 1997) and a Japanese cedar pollen allergen has been positively identified as having PL activity (Taniguchi *et al*., 1995). Functions suggested for PL in pollen include the initial loosening of the pollen cell wall to enable pollen tube emergence and growth and breakdown of the cell wall of transmitting tissue in the style to facilitate penetration of pollen (Taniguchi *et al*., 1995; Wu *et al*., 1996). Genes encoding a variety of cell-wall-degrading enzymes, including polygalacturonase (Brown and Crouch, 1990; Niogret *et al*., 1991; Allen and Lonsdale, 1993), pectinesterase (Albani *et al*., 1991) and

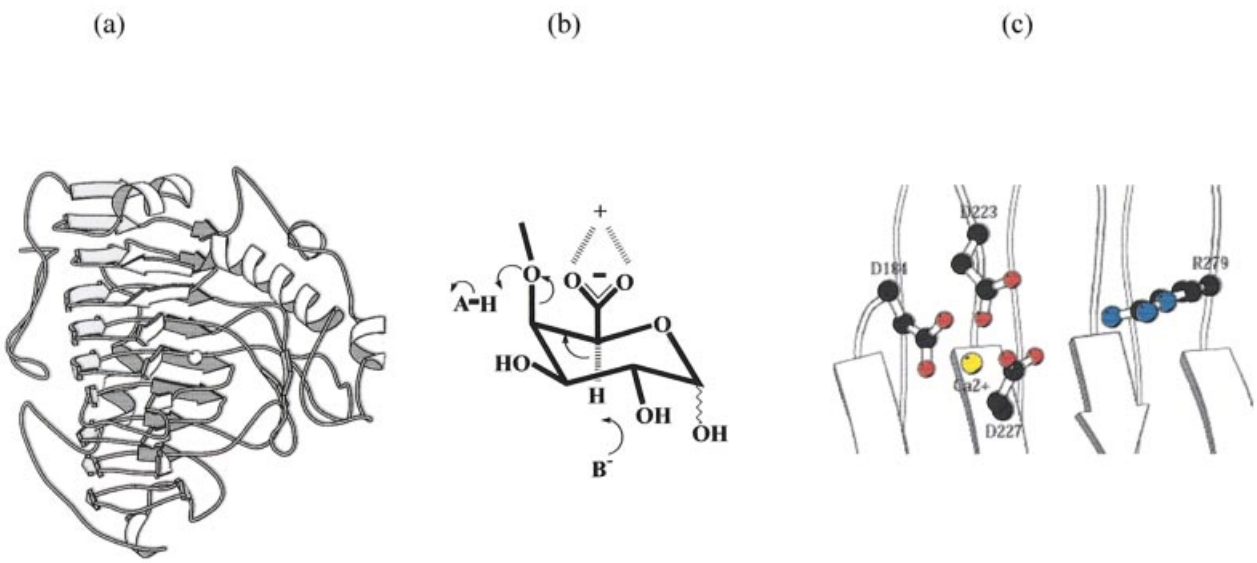

Fig. 1. (a) The overall fold of *Bacillus subtilis* pectate lyase with calcium shown as a sphere. (b) The essentials for catalysis are a base to abstract the C5 proton, an acid to protonate the glycosidic oxygen and a positive environment to decrease the pK_a of the α-proton at C5. (c) The active site of pectate lyase, looking down onto parallel β-sheet one (PB1) with strands 3 through 6 shown. Note the conserved carboxylates on or close to strands 3 and 4 and invariant arginine before strand 6. (Figure kindly provided by Professor R Pickersgill.)

β-galactosidase (Rogers *et al*., 2001) have also been reported in pollen.

In a search for genes involved in cell wall modifications during trans-differentiation of *Zinnia elegans* cells, two PL-like genes were isolated recently (Domingo *et al*., 1998; Milioni *et al*., 2001). The detailed study of *ZePel1* (Domingo *et al*., 1998) showed that it was active at a very early stage in tracheary element induction and its expression was modulated by auxin. Furthermore, the recombinant protein made in *E. coli* exhibited calcium-dependent PL activity. The authors speculated that these enzymes may assist in the removal and modification of the existing pectin matrix to allow the deposition of newly synthesized wall polymers for a specialized function.

In the capsules of the opium poppy, latex-containing laticifers are abundant and the laticifer system develops through the gradual disappearance of adjacent cell walls between differentiating laticifer elements throughout the plant. Sequences with homology to PL and other cell-wall-degrading enzymes have recently been isolated from an opium poppy latex cDNA library and PL activity has also been observed in the latex (Pilatzke-Wunderlich and Nessler, 2001).

A search of the tomato EST database (http://www.tigr.org/tdb/lgi/index.html) shows that PL-like sequences were isolated from a host of cDNA libraries including those made from germinating seeds, developing flowers, ovaries, pollen, trichomes, and ripening fruits suggesting that PL gene expression is widespread, if not ubiquitous in plant tissues.

PL and fruit softening

Perishable horticultural commodities such as fleshy fruits have a relatively short post-harvest shelf life during which the fruit tissues undergo profound changes in texture, colour and flavour, as well as becoming more susceptible to pathogenic attack (Seymour *et al*., 1993). Fruit softening is associated with cell wall disassembly (Seymour and Gross, 1996) and modifications to the pectin fraction are some of the most apparent changes that take place in the cell wall during ripening.

The majority of work on the disassembly of fruit cell walls has focused on ripening in tomatoes. The ripe pericarp of these fruit is rich in polygalacturonase activity and it was long assumed that this was the principal enzyme responsible for fruit softening. However, transgenic experiments in which the accumulation of polygalacturonase mRNA was suppressed still softened normally (Smith *et al*., 1989*a*). Also, in other fruits such as strawberry and banana, polygalacturonase activity is very low or absent despite evidence for pectin solubilization and degradation (Huber, 1984; Smith *et al*., 1989*b*). Early experiments to measure the presence of PL activity in tomato fruit proved unsuccessful (Besford and Hobson, 1972). However, the tomato EST programme (http://www.tigr.org/tdb/lgi/index.html) suggests a high level of PL-like gene expression in ripe tomato fruits.

PL sequences have also been reported from banana (Domínguez-Puigjaner *et al*., 1997; Medina-Suárez *et al*., 1997; Pilatzke-Wunderlich and Nessler, 2001; Marín-Rodríguez, 2001) and strawberry fruits (Medina-Escobar *et al*., 1997) and from ripening grape berries (Nunan *et al*., 2001). In bananas, the expression of two distinct PL-like genes (*Pel I* and *Pel II*) has been detected during ripening. Both show different levels of expression in ripening pulp and peel, with *Pel* I predominating. An active PL protein was produced by expression of banana *Pel* I in yeast. More importantly, for the first time from fruit tissue, PL activity has been obtained directly from banana pulp with a substantial increase in activity during ripening (Marín-Rodríguez, 2001). Additionally, a PL sequence from strawberry has also been expressed in yeast giving an active protein, although the authors were unable to observe any endogenous enzyme activity in the fruits themselves (Medina-Escobar *et al*., 1997). More recently PL gene expression has been manipulated in transgenic strawberry fruits and suppression of the PL mRNA during ripening resulted in significantly firmer fruits (Jiménez-Bermúdez *et al*., 2002). The highest reduction in softening occurring during the transition from the white to the red stage.

Conclusion

The likely importance of PLs in plant development has been appreciated only recently as a result of genome sequencing and EST programmes and from biochemical studies where PL activity has been measured in various plant tissue extracts. While pectin degrading enzymes such as polygalacturonase have been the focus of significant research, PLs have been less well studied. However, the extent of PL-like gene expression in ripening fruits suggests that these enzymes could play a more important role in fruit softening than previously thought.

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References

Albani D, Altosaar I, Arnison PG, Fabijanski SF. 1991. A gene showing sequence similarity to pectin esterase is specifically expressed in developing pollen of *Brassica napus* sequences in its 5′ flanking region are conserved in other pollen-specific promoters. *Plant Molecular Biology* **16,** 501–513.

Allen RL, Lonsdale DM. 1993. Molecular characterization of one of the maize polygalacturonase gene family members, which are expressed during late pollen development. *The Plant Journal* **3,** 261–271.

Barras F, van Gijsegem F, Chatterjee AK. 1994. Extracellular enzymes and soft-rot *Erwinia*. *Annual Reviews on Phytopathology* **32,** 201–234.

Bartling S, Wegener C, Olsen O. 1995. Synergism between *Erwinia* pectate lyase isozymes that depolymerise both pectate and pectin. *Microbiology* **141,** 873–881.

Besford RT, Hobson GE. 1972. Pectic enzymes associated with softening of tomato fruit. *Phytochemistry* **11,** 2201–2205.

Brown SM, Crouch ML. 1990. Characterization of a gene family abundantly expressed in *Oenothera* organogensis pollen that slows sequence similarity to polygalacturonase. *The Plant Cell* **2,** 263–274.

Carpita NC, Gibeaut DM. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal* **3,** 1–30.

Collmer A, Keen NT. 1986. The role of pectic enzymes in plant pathogenesis. *Annual Reviews on Phytopathology* **24,** 383–409.

Collmer A, Ried JL, Mount MS. 1988. Assay methods for pectic enzymes. *Methods in Enzymology* **161,** 329–335.

De Lorenzo G, Cervone F, Hahn MG, Darvill A, Albersheim P. 1991. Bacterial endopectate lyase: evidence that plant cell wall pH prevents tissue maceration and increases the half-life of elicitor-active oligogalacturonides. *Physiology and Molecular Plant Pathology* **39,** 335–344.

Domingo C, Roberts K, Stacey NJ, Connerton I, Ruíz-Terán F, McCann MC. 1998. A pectate lyase from *Zinnia elegans* is auxin inducible. *The Plant Journal* **13,** 17–28.

Domínguez-Puigjaner E, Llop I, Vendrell M, Prat S. 1997. A cDNA clone highly expressed in ripe banana fruit shows homology to pectate lyases. *Plant Physiology* **114,** 1071–1076.

Huber DJ. 1984. Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. *Journal of Food Science* **47,** 1310–1315.

Jenkins J, Pickersgill R. 2001. The arhitecture of parallel β-helices and related folds. *Progress in Biophysics and Molecular Biology* **77,** 111–175.

Jiménez-Bermúdez S, Redondo-Nevado J, Muòoz-Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA. 2002. Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. *Plant Physiology* **128,** 751–759.

Kamen DE, Griko Y, Woody RW. 2000. The stability, structural organization and denaturation of pectate lyase C, a parallel beta-helix protein. *Biochemistry* **39,** 15932–15943.

Keen NT, Dahlbeck D, Staskwicz B, Belser W. 1984. Molecular cloning of pectate lyase genes from *Erwinia chrysanthemi* and their expression in *Escherichia coli*. *Journal of Bacteriology* **159,** 825–831.

Kelemu S, Collmer A. 1993. *Erwinia chrysanthemi* EC16 produces a second set of plant-inducible pectate lyase isozymes. *Applied and Environmental Microbiology* **59,** 1756–1761.

Kita N, Boyd CM, Garrett MR, Jurnak F, Keen NT. 1996. Differential effect of site-directed mutations in Pel C of pectate lyase activity, plant tissue maceration, and elicitor activity. *Journal of Biological Chemistry* **271,** 26529–26535.

Kotoujansky A. 1987. Molecular genetics of pathogenesis by soft rot *Erwinias*. *Annual Reviews on Phytopathology* **25,** 405–430.

Kulikauskas R, McCormick S. 1997. Identification of the tobacco and *Arabidopsis* homologues of the pollen-expressed LAT59 gene of tomato. *Plant Molecular Biology* **34,** 809–814.

Lietzke SE, Scavetta RD, Yoder MD, Jurnak F. 1996. The refined three-dimensional structure of pectate lyase E from *Erwinia chrysanthemi* at 2.2 Å resolution. *Plant Physiology* **111,** 73–92.

Lietzke SE, Yoder MD, Keen NT, Jurnak F. 1994. The three-dimensional structure of pectate lyase E, a plant virulence factor from *Erwinia chrysanthemi*. *Plant Physiology* **106,** 849–862.

Marín-Rodríguez MC. 2001. Investigation of the role of pectate lyase in banana fruit softening. PhD thesis, University of Greenwich.

Mayans O, Scott M, Connerton I, Gravesen T, Benen J, Visser J, Pickersgill R, Jenkins J. 1997. Two crystal structures of pectin lyase A from *Aspergillus niger* reveal a pH driven conformational change and striking divergence in the substrate-binding clefts of pectin and pectate lyases. *Structure* **5,** 677–689.

Medina-Escobar N, Cárdenas J, Moyano E, Caballero JL, Muñoz-Blanco J. 1997. Cloning molecular characterisation and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology* **34,** 867–877.

Medina-Suárez R, Manning K, Fletcher J, Aked, J, Bird CR, Seymour GB. 1997. Gene expression in the pulp of ripening bananas. *Plant Physiology* **115,** 453–461.

Milioni D, Sado P-E, Stacey NJ, Domingo C, Roberts K, McCann MC. 2001. Differential expression of cell-wall-related genes during the formation of tracheary elements in the *Zinnia* mesophyll cell system. *Plant Molecular Biology* **47,** 221–238.

Nakajima N, Ishihara K, Tanabe M, Matsubara K, Matsura Y. 1999. Degradation of pectic substances by two pectate lyases from a human intestinal bacterium, *Clostridium butyricum-beijerinckii* group. *Journal of Bioscience and Bioengineering* **88,** 331–333.

Niogret MF, Dubald M, Mandaron P, Mache R. 1991. Characterization of pollen polygalacturonase encoded by several cDNA clones in maize. *Plant Molecular Biology* **17,** 1155–1164.

Nunan KJ, Davies C, Robinson SP, Fincher GB. 2001. Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta* **214,** 257–264.

Pérombelon MCM, Kelman A. 1980. Ecology of the soft rot *Erwinias*. *Annual Reviews on Phytopathology* **18,** 361–387.

Pickersgill R, Jenkins J, Harris G, Nasser W, Robert-Boudoy J. 1994. The structure of *Bacillus subtilis* pectate lyase in complex with calcium. *Nature Structural Biology* **1,** 717–723.

Pilatzke-Wunderlich I, Nessler CL. 2001. Expression and activity of cell-wall-degrading enzymes in the latex of opium poppy, *Papaver somniferum* L. *Plant Molecular Biology* **45,** 567–576.

Rogers HJ, Maund SL, Johnson LH. 2001. A beta-galactosidase-like gene is expressed during tobacco pollen development. *Journal of Experimental Botany* **52,** 67–75.

Scavetta RD, Herron SR, Hotchkiss AT, Kita N, Keen NT, Benen JA, Kester HCM, Visser J, Jurnak F. 1999. Structure of a plant cell wall fragment complexed to pectate lyase C. *The Plant Cell* **11,** 1081–1092.

Seymour GB, Gross KC. 1996. Cell wall disassembly and fruit softening. *Postharvest News and Information* **7,** 45N–52N.

Seymour GB, Taylor JE, Tucker GA. (eds) 1993. *Biochemistry of fruit ripening*. London, New York: Chapman and Hall.

Smith CJS, Watson CF, Morris PC, Bird CR, Seymour GB, Gray JE, Harding SE, Tucker GA, Schuch W, Grierson D. 1989a. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. *Plant Molecular Biology* **14,** 369–379.

Smith NJ, Seymour GB, Tucker GA, Jeger M. 1989b. Cell wall changes in bananas and plantains. *Acta Horticulturae* **269,** 283–289.

Starr ME, Morán P. 1962. Eliminative split of pectic substances by phytopathogenic soft-rot bacteria. *Science* **135,** 920–921.

Taniguchi Y, Ono A, Sawatani M, Nanba M, Kohno K, Usui M, Kurimoto M, Matuhasi T. 1995. *Cry j* I, a major allergen of Japanese cedar pollen, has a pectate lyase enzyme activity. *Allergy* **50,** 90–93.

Vitali J, Schick B, Kester HCM, Visser J, Jurnak J. 1998. The three-dimensional structure of *Aspergillus niger* pectin lyase B at 1.7 Å resolution. *Plant Physiology* **116,** 69–80.

Willats WGT, McCartney L, Mackey W, Knox JP. 2001. Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology* **47,** 9–27.

Wing RA, Yamaguchi J, Larabell SK, Ursin VM, McCormick S. 1989. Molecular and genetic characterization of two pollen-expressed genes that have sequence similarity to pectate lyases of the plant pathogen *Erwinia*. *Plant Molecular Biology* **14,** 17–28.

Weissbach A, Hurwitz J. 1959. The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia coli*. *British Journal of Biological Chemistry* **234,** 705–709.

Wu Y, Qiu X, Du S, Erickson L. 1996. *PO149*, a new member of pollen pectate lyase-like gene family from alfalfa. *Plant Molecular Biology* **32,** 1037–1042.

Yoder MD, Jurnak F. 1995. The refined three-dimensional structure of pectate lyase C from *Erwinia chrysanthemi* at 2.2 Å resolution. Implications for an enzymatic mechanism. *Plant Physiology* **107,** 349–364.

Yoder MD, Keen NT, Jurnak F. 1993*a*. New domain motif: the structure of pectate lyase C, a secreted plant virulence factor. *Science* **260,** 1503–1507.

Yoder MD, Lietzke SE, Jurnak F. 1993*b*. Unusual structural features of the parallel β helix of the pectate lyases. *Structure* **1,** 241–251.