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REVIEW

Biological effects of pectic fragments in plant cells

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INTRODUCTION

The cell wall is a complex structure, whose properties depend on the species, the environment, the tissue type and the stage of development of the plant. The physical characteristics and biological functions of the cell wall depend on its constituent

Abbreviations: ELISA, enzyme linked immunosorbent assay; IAA, indole acetic acid; PGA, polygalacturonic acid; DP, degree of polymerization; IP_3, inositol-(1,4,5)-trisphosphate; PAL, phenylalanine ammonia lyase; Pri, proteinase inhibitor; RGI, rhamnogalacturonan I; 4CL, 4-coumarate-CoA ligase; PR, proteins, pathogenesis-related proteins; HRGP, hydroxiproline-rich glycoprotein; cAMP, cyclic adenosine monophosphate; CREB, cAMP responsive binding protein.

structural polymers and the way they interact with each other to form the wall matrix. Higher plant cell walls are composed mainly of three types of polysaccharides: cellulose, hemicelluloses and pectins.

Pectins play an important role in different physiological processes in plants. Although present also in monocots (Jarvis *et al.* 1988) and lower plants, pectins have mostly been studied in dicots. As structural polysaccharides, pectins contribute to the strength and flexibility of cell walls from non-lignified organs and ripening fruits. They are the first targets of enzymes released by fungal or bacterial invading pathogens, and pectic oligomers released by exo- or endogenous enzymes appear to be signal molecules involved in triggering plant defence mechanisms and morphogenesis.

STRUCTURE OF PECTINS

Chemical structure

Pectins consist mainly of homopolygalacturonic acid (PGA) sequences that alternate with rhamnogalacturonan I (RGI). The so-called 'smooth' chains of PGA alternate with 'hairy' regions of RGI. PGA molecules that can reach 100 nm long, have a backbone resulting essentially from 1,4-polymerization of up to 200 α-D galacturonic acid residues interspersed with rhamnose (Carpita & Gibeaut 1993). Results from small-angle neutron and X-ray scattering indicated a persistence length (parameter of chain flexibility) of about 20 monomers, which indicates a relatively rigid chain as compared for example to amylose (Axelos & Thibault 1991).

The carboxylic acids of the secreted molecule are largely methylesterified in the Golgi system (>70–80%), and acetylation may occur on the secondary alcohols. Pectic polymers of actively growing cells are largely methylesterified, in contrast to very acidic pectins of mature cells (Yamaoka & Chiba 1983; Asamizu *et al.* 1984; Goldberg *et al.* 1986). The methyl-ester groups seem to be distributed randomly along PGA (de Vries *et al.* 1983; Westerlund *et al.* 1991; Renard & Thibault 1993). The degree of acetylation is generally lower than 10%, but in a few cases such as sugar-beet, higher values ($\simeq$30%) have been measured. These acetyl and methyl groups are of paramount importance to explain the physico-chemical properties, especially the gelling behaviour, of pectic polymers.

The basic RGI structure consists of alternating α-1,4-linked methylgalacturonosyl and α-1,2-linked rhamnosyl residues. About 50% of the rhamnoses bear side-chains similar to arabinans, galactans and arabinogalactans attached to the C4 of rhamnose. Thirty different types of side chains (McNeil *et al.* 1984) 8–20 residues long (van Buren 1991) have been studied.

Rhamnogalacturonan II molecules are more complex and contribute little to the pectin content of the cell walls.

Supramolecular structure of PGA

Nonesterified homopolygalacturonic blocks of sufficient length are able to associate intermolecularly in the presence of calcium ions. The 1,4-linked monomeric units lead to the formation of polar cavities that can be occupied by calcium ions (Kohn 1975, 1987). Morris *et al.* (1982) and Powell *et al.* (1982) have proposed a two-step model of cooperative association between homopolygalacturonic acid chains through calcium bonds. In that model, an initial dimerization of pectin chains of 2_1-helical symmetry

occurs by cooperative binding of calcium to the inner faces of the chains. The binding site is composed of two carboxyl groups and four coordinating oxygens (O-6, O-5, O-4 and O-2′) on either of the chains. The association would be cooperative because binding of the first cation between any pair of chains causes alignment which facilitates binding of the next, and so on along the sequence. This form of association has been named 'egg-box' (Grant *et al.* 1973).

Subsequent calcium-induced aggregation of these preformed dimers in tetramers, hexamers, etc. could occur, but the binding energy of these subsequent associations would be lower than for dimer formation. The association between dimers would thus be weaker and more tenuous than those between participating chains within dimers.

There are at least two other models: (i) the model of Walkinshaw & Arnott (1981) in which the $Ca^{2+}/RCOO^-$ ratio = 1/3; and (ii) the model of Mackie *et al.* (1983) in which the calcium binding site includes the two carboxyl groups and 3 coordinating oxygens (O-6, O-3′ and O-2′). Whatever the model, the cooperative binding of calcium by pectin decreases the calcium activity in solution (Köhn & Lùknar 1977; Thibault & Rinaudo 1985).

Liners *et al.* (1989, 1992) have proposed, using a monoclonal antibody against a calcium-induced supramolecular conformation of PGA, that an uninterrupted sequence of at least nine galacturonic residues is necessary on each of two chains to dimerize by binding five calcium ions. Randomly de-esterified pectins are labelled by this 2F4 antibody up to a degree of methylesterification (DM) of 30%. Pectins deesterified sequentially using orange peel methylesterase adopt the dimeric conformation as recognized by the antibody up to a higher DM of 40%, which confirms previous results obtained by potentiometry and conductimetry (Thibault & Rinaudo 1985, 1986).

Homopolygalacturonic fragments detached from PGA induce different physiological processes involved in defence, growth, differentiation, and morphogenesis in plants (Aldington & Fry 1993).

PLANT RESPONSES

Elicitation

Many pathogens secrete pectolytic enzymes (polygalacturonase, pectate lyase and pectin methyl esterase) during the first phase of host–pathogen interactions, leading to tissue maceration (Basham & Bateman 1975; Collmer & Keen 1986; Ryan 1988). Although these enzymes are bound to release oligomers, which in turn can act on the plant cell, we shall focus our attention on the interactions between purified oligomers and the cells or tissues, since the enzymes themselves and/or unidentified enzyme products can interfere with the cell responses.

The pectic fragments regulate different defence responses of the host-plant cells during pathogen invasion. Nothnagel *et al.* (1983) found that a fraction rich in dodeca-α-(1,4)-D-galacturonide was the most active in eliciting phytoalexin synthesis in a soybean cotyledon bioassay. Jin & West (1984) showed that pectic fragments enhanced casbene synthase in castor bean seedlings. Casbene synthase was activated by oligopectates with a degree of polymerization (DP) >8, the dodecamer being the most active and shorter oligomers were inactive. Broekaert & Peumans (1988) reported that pectic polysaccharides increased chitinase levels of tobacco leaves. Oligogalacturonide preparations elicited the accumulation of lignin in suspension cultures of castor bean,

with 7 being the most effective average DP. Oligomer fractions with size averages of less than 6 or more than 20 were essentially inactive as elicitors of lignin biosynthesis (Bruce & West 1989). Pectic fragments with DP 9–13 also initiated hyperlignification and water peroxide production in cucumber hypocotyls, while shorter and longer oligomers were inactive (Robertsen & Svalheim 1990; Svalheim & Robertsen 1993). Forrest & Lyon (1990) reported that unsaturated oligopectates with DP 10 and 11 released by a *Bacillus polymyxa* pectate lyase had the highest elicitor activity on soybean cotyledons. Messiaen *et al.* (1993) showed that unsaturated oligogalacturonides with DP≥9 in a calcium-induced conformation triggered PAL activation in carrot protoplasts. Only oligogalacturonides with DP≥9 in the same conformation triggered the accumulation of RNA transcripts from defence-related genes in carrot cell suspensions (Messiaen & Van Cutsem 1993). Within 1–5 min of DP 12 addition, cultured soybean cells were observed to release a burst of H_2O_2, termed the oxidative burst, that has been proposed both to retard pathogen invasion and to stimulate subsequent defence pathways (Legendre *et al.* 1992, 1993).

Oxidized oligogalacturonides known as galactaric acid oligomers were shown to activate cell wall peroxidases (Pressey 1991). Although oligogalacturonide oxidation *in vivo* by uronic acid oxidase remains to be evaluated, auxin oxidation by oligogalacturonide-activated peroxidases could explain the antagonism between the effect of oligogalacturonides and auxins on plant morphogenesis. Indeed, classical secretory guaiacol peroxidases show low substrate specificity, and since auxin crosses cell walls during its polar transportation, one could imagine some degree of control of auxin metabolism by the wall. Considering the interference of auxins with the elicitation of defence responses (Jouanneau *et al.* 1991), the wall peroxidases could emerge in the future as a key factor in the interactions between plant cells and pectin fragments.

Inhibitors of animal proteinases (Prl) are synthesized by many plants and accumulated in vacuoles in response to mechanical damage by, for example, leaf-chewing insects (Walker-Simmons & Ryan 1977). This wound signal is transported systematically to the rest of the plant through action potentials (Wildon *et al.* 1992). Even small oligogalacturonides applied exogenously have a potent induction effect that can be reversed by agents such as aspirin (Doherty & Bowles 1990), but their mobility in the apoplasm is still being evaluated.

Morphogenesis

Oligogalacturonides have a regulatory effect on hormone action and synthesis. Branca *et al.* (1988) showed that oligogalacturonides with a DP greater than 8 could inhibit the auxin-induced elongation of pea stem segments. The oligopectates did not affect stem elongation in absence of IAA. The IAA dose–effect curve was shifted upon addition of these oligogalacturonides indicating that they acted as competitive antagonists of IAA.

Pectic fragments have the property of enhancing flowering. Marfà *et al.* (1991) observed that endopolygalacturonase-released sycamore cell wall components were able to regulate the induction of flower formation on tobacco thin-cell layer explants. They purified the active cell wall components as α-1,4-oligogalacturonides with a DP ranging from 10 to 14. The DP 10 and 11 needed slightly higher concentrations to achieve full activity in inducing flowers.

Bellincampi *et al.* (1993) studied the formation of roots on tobacco explants in presence of oligogalacturonide fragments. DPs between 10 and 14 were most active inhibiting the formation of roots in tobacco thin cell layers, DP 9 showing a moderate

activity at higher concentrations. The authors hypothesized that oligogalacturonides had an effect opposite to that of auxins.

ΔGalA-(1→2)-Rha disaccharides appeared to promote *Amaranthus* hypocotyl elongation (Hasegawa *et al.* 1992) and it has been suggested that these oligomers could originate from enzymic hydrolysis of pectic RGI (Fry *et al.* 1993).

Ripening

Pectic fragments are involved in two related aspects of fruit ripening: ethylene synthesis and wall softening. Cell wall fragments isolated by digestion of pear cells by a pectinase-containing enzyme mixture were shown to induce a rapid and transient production of ethylene by the cells (Tong *et al.* 1986). Brecht & Huber (1988) found a ripening-promotive activity in the larger pectin fragments released enzymically from isolated cell walls from ripe tomato pericarp. Only fragments with DP>8 did stimulate ripening when infiltrated into preclimacteric tomato fruits. Pectic oligomers were also described as the ethylene-inducing component in the pear cell suspension model (Campbell & Labavitch 1991a). These last authors also suggested (1991b) that the rate of ripening of endocarp tomato tissues may be regulated, in part, by the release of pectic oligomers from the cell walls of adjacent exocarp tissues.

CHARACTERISTICS OF THE PECTIC SIGNAL

Dose–response relationship

The concentrations of oligogalacturonides that are effective in plant bioassays seem to fall into two categories.

Higher concentrations ($\sim 10^{-4}$ M, i.e. $\sim 0{\cdot}2$ mg/ml^{-1}) have been used to elicit defence reactions in different systems (Table 1). This range of concentration is about the same as the one tested with tyrosine hydrazone derivatives of DP 12 receptor-mediated endocytosis. About $3{\cdot}10^{-5}$ M ($\sim 0{\cdot}06$ mg/ml^{-1}) of pectic fragments were necessary to saturate the cell surface binding sites of soybean cells in suspension cultures (Low *et al.* 1993).

Morphogenic effects such as flowering or fruit ripening required oligogalacturonide concentrations in the range of 10^{-8}–10^{-6} M, which poses the question as to how the cells discriminate between different signals carried by the same molecule.

In non-pathological conditions, oligogalacturonides could be detached from the wall, for example by endogenous polygalacturonases, at concentrations too low to elicit defence responses, but high enough to trigger morphogenic responses. Conversely, invading pathogens de-esterify and degrade pectins *in situ*. For example, the sites of infection of potato tissues by *Erwinia carotovora* are heavily labelled by 2F4 antibodies directed against dimerized fragments of DP≥9 (unpublished results), suggesting high local concentrations of pectic fragments, close to the plasmalemma. The discrimination between the morphogenic and the pathogenic signals mediated by the same pectic fragments could thus be dose-dependent.

Structure–function relationship

Only proteinase inhibitor (Prl) synthesis is known to be activated by di- and trigalacturonic acids: the production of Prl does not depend on the length of the released

Table 1. Size and conformation of pectic fragments active in triggering defence or morphogenic responses in different systems

Plant material	Physiological effect	Size of active oligomers	Pectic fragment concentration	References
Defence reactions				
Soybean cotyledons	Glyceollin accumulation	DP 12	0·28 mg/ml^{-1}	Nothnagel *et al.* (1983)
		DP >10	1·0 mg/ml^{-1}	Mau & West (1993)
		DP 10–11	0·06 mg/ml^{-1}	Forrest & Lyon (1990)
	Casbene synthase activation	DP >8	1·0 mg/ml^{-1}	Jin & West (1984)
Tomato plants	PI accumulation and membrane depolarization	DP 10–20	1·0 mg/ml^{-1}	Doherty & Bowles (1990)
Castor bean	Casbene synthase activation	average DP 13	1·0 mg/ml^{-1}	Lois & West (1990)
Carrot cell cultures	PAL activation	DP ≥9 dimers	1·0 mg/ml^{-1}	Messiaen *et al* (1993)
	Defence gene activation	DP ≥9 dimers	0·2 mg/ml^{-1}	Messiaen & Van Cutsem (1993)
Tobacco leaves	Chitinase accumulation	average DP 14	0·04 mg/ml^{-1}	Broekaert & Peumans (1988)
Castor bean cell cultures	Lignification	average DP 7	0·3 mg/ml^{-1}	Bruce & West (1989)
Soybean cell cultures	H_2O_2 production	DP 12	0·01 mg/ml^{-1}	Legendre *et al.* (1993)
		DP 9–16	0·1 mg/ml^{-1}	Svalheim & Robertsen (1993)
Lithospermum cell cultures	Shikonin production	DP 12–20	0·1 mg/ml^{-1}	Tani *et al.* (1993)
Soybean cell cultures	Endocytosis	DP 12	0·06 mg/ml^{-1}	Low *et al.* (1993)
Morphogenesis				
Tobacco thin-cell layers	Inhibition of root formation	DP 10–14	0·001–0·01 mg/ml^{-1}	Bellincampi *et al.* (1993)
Tobacco thin-cell layers	Induction of flower formation	DP 10–14	0·001–0·01 mg/ml^{-1}	Marfà *et al.* (1991)
Pear cell cultures	Ethylene synthesis	DP 7–12	0·001–0·1 mg/ml^{-1}	Campbell & Labavitch (1991a)
Tomato fruit	Induction of fruit ripening	DP >8	<0·05 mg/ml^{-1}	Brecht & Huber (1988)
Pea stem segments	Inhibition of auxin-induced elongation	DP >8	0·15 mg/ml^{-1}	Branca *et al.* (1988)

elicitors (Thain *et al.* 1990; Ryan 1992). In all other cases, DP 9 seems to be the minimum length for homopolygalacturonic oligomers to act.

This length requirement is necessary but not sufficient for biological activity: the elicitating activity of oligogalacturonides is diminished when the -COOH groups are neutralized by either methylesterification (Jin & West 1984), reduction (Davis *et al.* 1986) or when the reducing terminus of the oligosaccharide is reduced with $NaBH_4$ to the alditol. No elicitor or morphogenetic activity is observed with non-pectic polyanions (West *et al.* 1984; Marfà *et al.* 1991; Bellincampi *et al.* 1993). From these observations, Aldington *et al.* (1991) concluded that there was no evidence that oligogalacturonides acted by binding to a specific receptor, but rather that they acted through a relatively simple interaction between the acidic molecules and the target cells.

However, Marfà *et al.* (1991) suggested that the sharp size dependency of the biologically active oligogalacturonides could be due to the fact that a minimum size is required for the oligogalacturonides to adopt the conformation recognized by their receptor, while the maximum size for bioactivity may reflect the ability of oligogalacturonides to reach and/or fit into their receptor.

Farmer *et al.* (1989, 1991) stressed that PGA forms intermolecular 'egg-box' complexes that bind Ca^{2+} very tightly as bridging ions; the correlation of the solution chemistry of pectic oligomers with their *in vitro* and *in vivo* biological activities supports the hypothesis that the formation of such complexes may be necessary for their biological activities.

Messiaen *et al.* (1993) showed by fluorescence imaging that only oligogalacturonides with $DP \geq 9$ in a calcium-induced ('egg-box') conformation were able to trigger an increase in cytosolic calcium in indo-1 loaded carrot protoplasts. The same molecules in a single chain conformation did not modify the cytosolic free calcium. This observation strongly suggested that oligogalacturonides were only active if they were dimerized by calcium ions.

When PAL activity was elicited by DPs 9–16 in the presence of increasing $CaCl_2$/NaCl ratios (Messiaen & Van Cutsem 1993), a clear optimum was observed at the same 1/150 equivalent ratio necessary for optimum recognition of the oligopectates by the antibody described by Liners *et al.* (1992). A linear regression with $r^2=0{\cdot}946$ is obtained when plotting the PAL activity measured on carrot cells elicited at different Ca/Na ratios as a function of the ELISA test readings at the same ratios. This shows that the elicitating structure and the epitope of the 2F4 antibody both belong to the same supramolecular ('egg-box') conformation of polygalacturonic acid induced by a Ca/Na equivalent ratio of about 1/150. Not only PAL was activated, but mRNAs coding for different proteins related to pathogenesis and ethylene synthesis were transcribed: PAL, 4CL, PR proteins, HRGP, peroxidase (Fig. 1). Transcription of casbene synthase mRNA had already been shown on castor bean seedlings with DP 13 oligomers (Lois & West 1990).

Above DP 16, the solubility of larger homopolygalacturonic acid chains as well as their diffusion coefficient decrease, and they will reach plasmalemma less easily. These longer fragments should also be able to form multimetric associations, perhaps with pectic chains of the matrix, provided enough calcium is available. As the ion exchange capacity of dicot walls is fairly high (Jarvis *et al.* 1988) and calcium is usually the prevalent cation in apoplastic fluid, this last condition should be fulfilled *in vivo*. However, the existence of these multimeric complexes has not been proved *in situ*, but their formation could contribute to the reduction of the elicitor or morphogenic activity of longer DPs supplied *in vitro*.

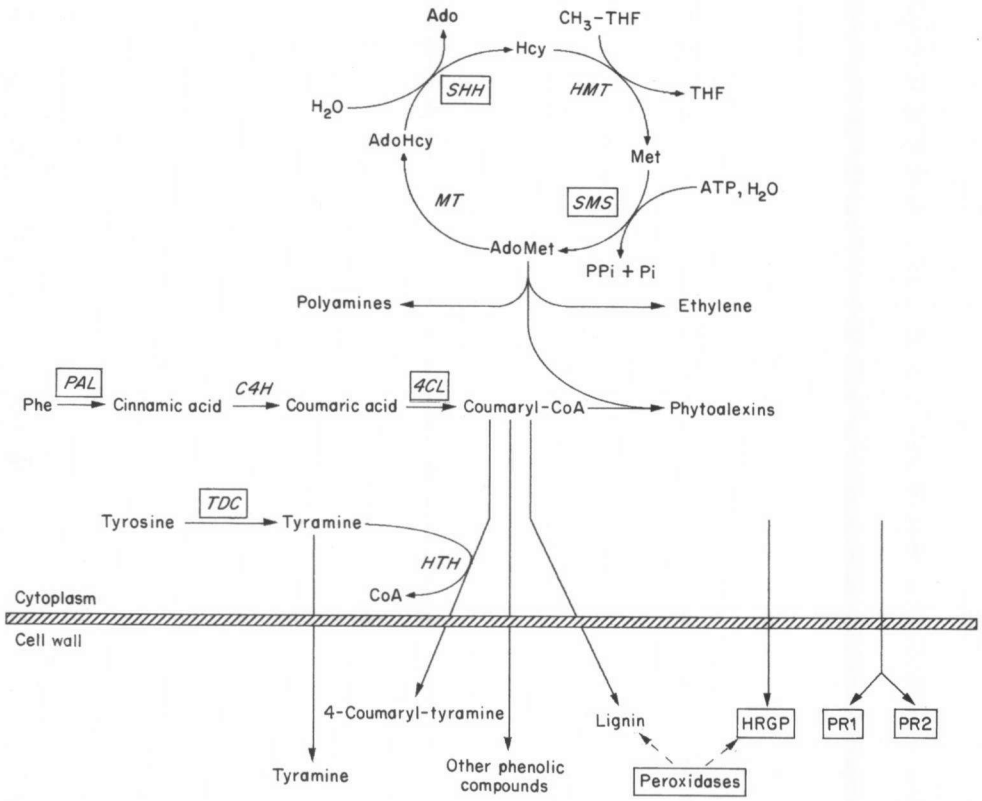

Fig. 1. Main biochemical pathways involved in defence responses. Several phytoalexins such as the parsley furanocoumarins are synthesized through the general phenylpropanoid pathway. The boxed genes have been used for *in vitro* nucelar run-off transcription experiments. Phe, L-phenylalanine; Ado, adenosine; AdoMet, S-adenosyl-L-methionine; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; HTH, hydroxycinnamoyl-CoA, tyramine–hydroxycinnamoyl transferase; Met, methionine; Hcy, homocysteine; AdoHcy, S-adenosyl-L-homocysteine; HMT, Hcy methyl transferase; PAL, phenylalanine ammonia lyase; SMS, AdoMet synthetase; MT, methyl transferase; SHH, AdoMet hydrolase; TDC, tyrosin decarboxylase; THF, tetrahydrofolate; HRGP, hydroxyproline-rich glycoprotein; PR-1 and PR-2, pathogenesis-related proteins 1 and 2.

RESPONSES AT THE MEMBRANE SURFACE

Membrane potential and ion fluxes

Changes in membrane properties seem to be important in signal transduction. Independently of any receptor, physical changes at the plasma membrane level could act as primary signals: local pressure changes at the surface of the cells or ionic imbalances might be of paramount importance in pathogen recognition (Boller 1989). The presence of cationic ion exchangers such as oligopectates in the close vicinity of the plasmalemma could disturb the membrane integrity by chelating membrane-bound calcium.

The depolarization of the plasma membrane by short (DP 1–7) and large (DP 10–20) pectic elicitors was shown by Thain *et al.* (1990). Changes in proton transport concomitant with other ion fluxes appeared to link the oligogalacturonide signal to changes in gene expression (Doherty & Bowles 1990).

On treatment of tobacco cells with oligogalacturonides, Mathieu *et al.* (1991) observed the uptake of calcium from the culture medium, the leakage of potassium from

the cells and an alkalinization of the incubation medium simultaneously to membrane depolarization and cytoplasm acidification. Only DPs >9 and <16 were active. Messiaen *et al.* (1993) using fluorescence imaging of carrot protoplasts, measured a cytosolic calcium mobilization induced by dimerized oligogalacturonides and inhibited by verapamil, a calcium channel blocker. This direct observation is important first because it has been shown that phytoalexin synthesis was calcium-dependent (Stab & Ebel 1987; Kurosaki *et al.* 1987a), and second because calcium is a well-known second messenger, not only in animal cells but also in plant cells: several membrane responses have been described in the literature involving calcium channels and calcium as the second messenger.

Results obtained on carrot protoplasts (Messiaen & Van Cutsem 1994) show that short as well as long fragments and PGA all depolarize the plasma membrane. The DPs ≥ 9 dimerized by Ca^{2+} ions, but not PGA nor short DPs, acidify the cytosol by 0·2 pH units within 25 min and the effect is completely nullified by calmidazolium, a calmodulin inhibitor. Only dimerized DPs ≥ 9 increase the cytosolic calcium concentration. The Ca^{2+} rise induced by the long oligomers is much quicker and stronger than when protoplasts are depolarized with KCl, ruling out any simple effect of membrane depolarization on voltage-gated calcium channels. The membrane depolarization itself is not affected by verapamil but completely prevented by calmidazolium. The membrane depolarization and the cytosolic pH drop seem to be linked since they are both inhibited by fusicoccine, a proton pump activator that prevents defence responses to pectic oligomers.

Endocytosis

In animal cells, when a ligand binds to a surface receptor, parallel to the usual signal transduction pathway, the receptor–ligand complexes are commonly internalized via receptor-mediated endocytosis. This mechanism allows cells to remove the ligand from its receptors after signal transduction and to regulate the number of receptors at the cell surface.

As oligogalacturonides and other signalling molecules are too large and too polar to freely permeate the lipid membranes, Horn *et al.* (1989) used fluorescein and ^{125}I-labelled elicitors to monitor the cellular distribution and movements of these molecules in soybean suspension-cultured cells. The labelled elicitors (polygalacturonic acid and glycoproteins) first bound to the cell surface and were then internalized in a temperature- and energy-dependent endocytotic way. The internalized probe accumulated in the vacuole within a few hours. The saturable character of the binding of the pectic molecules pointed to a receptor-mediated process (Low *et al.* 1993). However, no oligogalacturonide binding protein has hitherto been identified in any plant species, although other authors have more or less explicitly postulated the existence of membrane receptors for pectic fragments (Marfà *et al.* 1991; Bellincampi *et al.* 1993; Messiaen & Van Cutsem 1993).

We have observed by immunocytochemistry (unpublished) that pectin molecules released from the cell wall were internalized by potato stem and carrot root cells in multivesicular invaginations of the plasma membrane, also known as plasmalemmasomes (Herman & Lamb 1992). The vesicles fused with the vacuole, releasing large amounts of pectin that could be labelled by the 2F4 antibody after de-esterification. This could be a way for the cell to modify its wall composition, to remove elicitors from the surface of the plasmalemma and to increase its vacuolar calcium store.

Moreover, since it needs pectic fragments to be in contact with cells during at least 4 hours to trigger full defence responses (J. Messiaen, unpublished), a possibility exists that the internalization of pectic fragments plays a role in signal transduction similar to the one mediated by caveolae in animal cells (Travis 1993).

MECHANISM OF SIGNAL TRANSDUCTION

Involvement of the phosphatidyl inositol pathway

The phosphoinositide cascade is present in plants (Einspahr & Thompson 1989). G-proteins (Hasunama & Funadera 1987; Blum *et al.* 1988; Lee *et al.* 1993; Neuhaus *et al.* 1993), phospholipase C (Dr Øbak 1993), inositol phospholipids (Van Breemen *et al.* 1990) and PKC-like protein kinases (Dr Øbak 1993) have been described. The photoactivation of caged IP_3 increased the cytosolic calcium activity and initiated stomatal closure (Gilroy *et al.* 1991) and etiolated protoplast swelling (Shacklock *et al.* 1992).

Concerning pectic fragments, some of the steps of a putative phospho-inositide cascade have been investigated. In the following paragraphs, we shall see that IP_3 and Ca^{2+}, calmodulin and phosphorylation play a role in the transmission of the pectic signal.

Cytosolic Ca^{2+} increase

The aforementioned cytosolic Ca^{2+} increase of elicitated carrot cells observed by fluorescence imaging was of small mean amplitude (less than 100 nM) but lasted for at least 30 min. Such small but sustained elevations in Ca^{2+} are characteristic of many responses in mammalian cells (Bush 1993).

The photoactivation of caged IP_3 electrophorated in carrot protoplasts was successful in increasing transiently the cytosolic calcium activity and in inducing PAL activation to a similar level as DPs ≥ 9 (J. Messiaen & P. Van Cutsem, unpublished). The addition of calcium ionophore A23187 to carrot cell cultures induced the production of phytoalexin in carrot, in a dose-dependent manner (Kurosaki *et al.* 1987a). Calcium seems thus to be a common second messenger of different elicitors on which depend different transduction chains leading to activation of multiple defence reactions (Renelt *et al.* 1993).

Curiously enough, membrane depolarization and cytosolic acidification caused by large DPs were inhibited by calmodulin inhibitors, but not by the calcium channel blocker verapamil (Messiaen & Van Cutsem 1994). This is interesting because the induction of Prl activity by pectic fragments, although sensitive to agents which affect proton transport, was not affected by verapamil (Doherty & Bowles 1990). Either calcium entered the cytosol by verapamil-insensitive channels, or Prl induction, like membrane depolarization, responded to a parallel calcium-independent signal transduction chain. Incidentally, the involvement of a lipid-based signal transduction system in the wound induction of Prl has been ruled out since the use of a specific inhibitor of lipoxigenase did not suppress the response to oligouronides (Ryan 1992). However, lipid peroxidation is still a step of the signal cascade that leads to plant defence induction (Degousée *et al.* 1994).

Phosphorylations

Certain protein kinases are Ca^{2+}-dependent, and pectic fragments like other elicitors could thus act indirectly on protein phosphorylation (Felix *et al.* 1991). Farmer *et al.* (1989, 1991) report an oligogalacturonide-induced modification of the *in vitro* phosphorylation pattern of tomato and potato proteins. A plasma membrane protein called pp34 was phosphorylated at one or more Thr residues, suggesting the activation of a Ser/Thr protein kinase. Only fractions with DP about 14 and higher were active.

Jacinto *et al.* (1993) suggested that pp34 might be a component of the signal transduction pathway and stressed the fact that protein phosphorylation in plants occurs in parallel with the activation of defined defence genes in response to elicitor treatment. There is little doubt that phosphorylations play a crucial role in pectic signal amplification and integration and the use of molecular methods to clone the pp34 sequence will hopefully open the way to a detailed study of membrane-bound components involved in elicitor signal transduction.

Role of cAMP

In plants, the role of cAMP is unclear. Nougarède *et al.* (1985) have localized adenylate cyclase activity in pea cotyledonary node using cytoenzymologic methods during the G_1-S or G_2M transitions. Kurosaki *et al.* (1987a,b) have shown that cAMP or analogues induced the synthesis of phytoalexins in carrot cells and Bolwell *et al.* (1991) activated PAL and phytoalexin synthesis in beans by using forskolin, an activator of adenylate cyclase in animal cells.

Wheat and tobacco proteins have been found to contain amino acid sequences similar to the ones found in cAMP responsive binding proteins (CREB) and c-Jun/c-Fos of animal cells (Tabata *et al.* 1989; Katagiri *et al.* 1989). More recently, Ehrlich *et al.* (1992) working with beans, have isolated a protein that bound the 5'-TGACGTCA sequence specifically recognized by mammalian CREBs.

However, to our knowledge, assaying the cAMP content in plant cells stimulated by pectic fragments has never been performed, and the implication of this cyclic nucleotide in oligopectate signalling has still to be proven.

CONCLUSIONS

In conclusion, pectin appears to be probably the most important polysaccharide of the plant cell wall. As a structural polysaccharide, it contributes to the cell wall strength and plasticity. New roles for pectins have been discovered, confirmed or reassessed: signalling and elicitation in wound response and host–pathogen interactions, morphogenetic effects and intercellular adhesion. Pectin has now to be considered not only as a structural but also probably as the main informative plant cell wall polymer.

The mode of action of pectic fragments is still largely unknown. From the current literature, a rough picture of its early signal transduction mechanisms progressively emerges, and points to an involvement of the phosphoinositide signal-transducing system. Pectic fragments of a minimum length under a proper conformation, whether 'egg-boxes' or a more or less similar conformation induced by calcium ions, bind to plasmalemma receptors. The membrane depolarizes, and IP_3 is simultaneously hydrolysed from plasmalemma phosolipids. The cytosolic calcium activity increases due to the opening of calcium channels, and calcium binds to calmodulin. Protein phosphorylation

occurs, the plasmalemma permeability to ions changes, leading to cytosolic acidification, and defence-specific genes are finally transcribed and translated.

This text-book scheme represents only an attempt to delineate a general transduction cascade for pectic signals. Branchings into parallel processing pathways such as cAMP, membrane lipid derived products or auxin metabolism and cross-talk with other elicitating signals will certainly be investigated in the near future.

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