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STRUCTURES AND FUNCTIONS OF OLIGOSACCHARINS

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Dr. Peter Albersheim

Complex Carbohydrate Research Center
The University of Georgia
Athens, Georgia 30602-4712

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STRUCTURES AND FUNCTIONS OF OLIGOSACCHARINS
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1. **Augur, C., N. Benhamou, A. Darvill, and P. Albersheim. 1993. Purification, characterization, and cell wall localization of an α-fucosidase that inactivates a xyloglucan oligosaccharin. *The Plant J.* 3: 415-426.**

An α-fucosidase that releases fucosyl residues from oligosaccharide fragments of xyloglucan, a plant cell wall hemicellulosic polysaccharide, was purified to homogeneity from pea (*Psium sativum*) epicotyls using a combination of cation exchange chromatography and isoelectric focusing. The α-fucosidase has a molecular mass of 20 kDa according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The α-fucosidase has an isoelectric point of 5.5. The substrate specificity of the α-fucosidase was determined by high performance anion exchange chromatographic analysis of oligosaccharide substrates and products. The enzyme hydrolyzes the terminal α-1,2-fucosidic linkage of oligosaccharides and does not cleave *p*-nitrophenyl-α-L-fucoside. The enzyme does not release measurable amounts of fucosyl residues from large polysaccharides. The subcellular localization of α-fucosidase in pea stems and leaves has been studied by immunogold cytochemistry. The α-fucosidase accumulates in primary cell walls and is not detectable in the middle lamella or in the cytoplasm of 8-day-old stem tissue and 14-day-old leaf tissue. α-Fucosidase activity was readily detected in extracts of 8-day-old stem tissue. No significant α-fucosidase activity or immunogold labeling of the a-fucosidase was detected in 2- and 4-day-old stem tissue indicating that production of α-fucosidase is developmentally regulated.

2. **Bellincampi, D., G. Salvi, G. De Lorenzo, F. Cervone, V. Marfà, S. Eberhard, A. Darvill, and P. Albersheim. 1993. Oligogalacturonides inhibit the formation of roots on tobacco explants. *The Plant J.* 4: 207-213.**

α-1,4-Oligogalacturonides with degrees of polymerization (DPs) ranging from 6 to 18 or 2 to 8 were added to tobacco leaf explants and root formation was evaluated after 15 days of incubation. Auxin-induced formation of roots was inhibited by oligogalacturonides with DPs 6-18 but not by the oligogalacturonides with DPs 2-8. The inhibition of root formation by the larger oligogalacturonides was prevented by increasing the amount of auxin present in the medium. Oligogalacturonides (DPs 6-18) also inhibited root formation when added to tobacco thin cell-layer (TCL) explants in a medium that is known to induce the formation of roots. The addition of size-homogeneous oligogalacturonides, to either tobacco leaf explants or TCLs, established that oligogalacturonides with DPs between 10 and 14 were most active in inhibiting the formation of roots. These data suggest that oligogalactuornides of the same size as those known to elicit plant defense responses, and to affect floral development and membrane functions, also inhibit the induction of root morphogenesis in tobacco.

3. **Brady, K.P., A.G. Darvill, and P. Albersheim. Activation of a tobacco glycine-rich protein gene by a fungal glucan preparation. *The Plant J.* 4: 517-524.**

A *Phytophthora megasperma* f.sp. *glycinea* cell wall glucan preparation was previously shown to protect tobacco plants against viral infection. Eleven plant defense-related genes were assayed for elevated mRNA accumulation levels in response to glucan treatment of tobacco plants. The expression of only one of these genes, a glycine-rich protein (GRP) gene, was induced by glucan application. Elevated GRP gene mRNA levels could be detected within 15 min of glucan treatment and reached maximum levels at 4 h post-treatment followed by a slow decline to 8 h. The maximum induction of the GRP gene was approximately ninefold above H_2O-treated control plants. Northern blot analysis showed that a single mRNA species of 1.4 kb was responding to the glucan treatment. GRP genes occur in tobacco as members of a multigene family, but only one specific GRP gene was induced by the glucan treatment. A genomic copy of this responding GRP gene was cloned and sequenced. This tobacco GRP gene is homologous to the petunia *ptGRP1* gene and the French bean *GRP1.8* gene, but is not closely related to the French bean *GRP1.0* gene. GRP gene expression has previously been associated with disease resistance in plants, but it remains to be determined whether β-glucan activation of the tobacco GRP gene results in the observed resistance to virus.

4. **Caprari, C., C. Bergmann, Q. Migheli, G. Salvi, P. Albersheim, A. Darvill, F. Cervone, and G. De Lorenzo. 1993. *Fusarium moniliforme* secretes four *endo*polygalacturonases derived from a single gene product. *Physiol. Mol. Plant Path.* 43: 453-462.**

Extracellular *endo*polygalacturonase, purified from the pathogenic fungus *Fusarium moniliforme*, consists of four molecular forms (38, 41·5, 45, and 48·5 kDa, respectively). Three forms (38, 41·5, and 45 kDa) were purified to homogeneity by FPLC on a Mono S column followed by electroelution after SDS-PAGE. The N-terminal amino acid sequences of each of the three forms, and of a mixture containing all four forms were shown to be identical to that predicted from the nucleotide sequence of the *endo*polygalacturonase gene previously cloned from *F. moniliforme*. Enzymatic deglycosylation experiments revealed the presence of N-linked, high mannose oligosaccharide side-chains on all four forms of *endo*polygalacturonase. Hydrogen fluoride catalysed chemical deglycosylation of the polygalacturonase mixture yielded a single polypeptide with an apparent molecular mass of 36·2 kDa. Southern blot analysis, carried out at high stringency with an *endo*polygalacturonase-specific probe on genomic DNA digested with three different restriction enzymes, showed a single hybridizing restriction fragment in all three digests. A single 2·0 Mb chromosome hybridized with the *endo*polygalacturonase-specific probe, as shown by Southern blot analysis of *F. moniliforme* chromosomes separated by CHEF electrophoresis. Northern blot analysis revealed only one mRNA species (1350 nt) encoding *endo*polygalacturonase. These data indicate that a single gene encodes the *endo*polygalacturonases of *F. moniliforme*.

5. **Bergmann, C.W., Y. Ito, D. Singer, P. Albersheim, and A.G. Darvill. 1994. Polygalacturonase-inhibiting protein accumulates in *Phaseolus vulgaris* L. in response to wounding, elicitors and fungal infection. *The Plant J.* 5: 625-634.**

Polygalacturonase-inhibiting protein (PGIP) is a cell wall-associated protein that specifically binds to and inhibits the activity of fungal *endo*polygalacturonases. The *Phaseolus vulgaris* gene encoding PGIP has been cloned and characterized. Using a fragment of the cloned *pgip* gene as a probe in Northern blot experiments, it is demonstrated that the *pgip* mRNA accumulates in suspension-cultured bean cells following addition of elicitor-active oligogalacturonides or fungal glucan to the medium. Rabbit polyclonal antibodies specific for PGIP were generated against a synthetic peptide designed from the N-terminal region of PGIP; the antigenicity of the peptide was enhanced by coupling to KLH. Using the antibodies and the cloned *pgip* gene fragment as probes in Western and Northern blot experiments, respecitvely, it is shown that the levels of PGIP and its mRNA are increased in *P. vulgaris* hypocotyls in response to wounding or treatment with salicylic acid. Using gold-labeled goat-anti-rabbit secondary antibodies in EM studies, it has also been demonstrated that, in bean hypocotyls infected with *Colletotrichum lindemuthianum*, the level of PGIP preferentially increases in those cells immediately surrounding the infection site. The data support the hypothesis that synthesis of PGIP constitutes an active defense mechanism of plants that is elicited by signal molecules known to induce plant defense genes.

6. **Ham, K.-S., P. Albersheim, and A.G. Darvill. 1995. Generation of β-glucan elicitors by plant enzymes and inhibition of the enzymes by a fungal protein. *Can J. Bot.* In press.**

The biosynthesis and accumulation of phytoalexins is a well-studied plant defense response. Plant synthesize and accumulate phytoalexins in response to microbial infection or elicitor treatment. The β-glucan heptaglucoside is a well-studied phytoalexin elicitor isolated from partial acid hydrolysates of *Phytophthora sojae* f.sp. *glycines* (Psg) mycelial walls. Using the soybean-Psg system, we have demonstrated that *endo*-β-1,3-glucanases (EC 3.2.1.39) are the principal soybean enzymes involved in generating phytoalexin oligoglucoside elicitors from mycelial walls. We have also recently observed that Psg secretes a protein that inhibits the soybean *endo*-β-1,3-glucanse activity that could release elicitors from fungal mycelial walls. The inhibitor protein, which has been purified to homogeneity, does not inhibit *endo*-β-1,3-glucanases of the fungus or a tobacco pathogenesis-related *endo*-β-1,3-glucanases. The existence of the inhibitor protein in Psg suggests that pathogens have evolved specific proteins to inhibit the fungal wall-degrading enzymes of their host plants, just as plants have evolved proteins (e.g., pectic enzyme inhibitors) to inhibit plant cell wall-degrading enzymes secreted by their pathogens. It seems possible that pathogens secrete inhibitors of other pathogenesis-related proteins (e.g. chitinases) and that the interplay of hydrolases and their inhibitors could determine the outcome of plant-pathogen interactions.

7. **Stotz, H.U., C.W. Bergmann, P. Albersheim, A.G. Darvill, and J.M. Labavitch. 1995. Polygalacturonase inhibitor proteins from bean (*Phaseolus vulgaris* L.), Pear (*Pyrus communis* L.) and tomato (*Lycopersicon esculentum*: Mill.): Immunological relatedness and specificity of polygalacturonase inhibition. Submitted.**

Purified polygalacturonase inhibitor proteins (PGIPs) from pods of bean (*Phaseolus vulgaris* L.) and from fruits of tomato (*Lycopersicon esculentum* Mill.) and pear (*Pyrus communis* L.) were tested for inhibition of polygalacturonases (PGs) from *Aspergillus niger*, *Fusarium moniliforme* and *Botrytis cinerea*. Bean PGIP equally inhibited all three fungal PGs. Tomato PGIP was less active against PGs from *A. niger* and *B. cinerea* than bean PGIP. Pear PGIP only inhibited PG from *B. cinerea*. Tomato PGIP, which noncompetitively inhibited *A. niger* PG, is kinetically more similar to bean (Lafitte *et al.*, 1984, *Physiol. Plant Pathol.* 25: 39-53) than pear PGIP (AbuGoukh *et al.*, 1983, *Physiol. Plant Pathol.* 23: 111-122). Polyclonal antibodies raised against PGIPs from bean and pear were used to determine the immunological relatedness of the three PGIPs. Antibodies raised against deglycosylated pear PGIP cross-reacted weakly with bean and tomato PGIPs, while antibodies raised against the bean glycoprotein cross-reacted weakly with pear and tomato PGIPs. Antibodies raised against an amino-terminal peptide from bean did not recognize pear PGIP. These data suggest that differences in the specific interaction of plant PGIPs with fungal PGs may be due to differences in PGIP structure.

8. **Ham, K.-S., A.G. Darvill, and P. Albersheim. 1995. Fungi protect themselves against plant pathogenesis-related glycanases. Submitted.**

Plants defend themselves against attempted infection by microbes by synthesizing pathogenesis-related proteins that include families of *endo*-β-1,3-glucanases and chitinases. These enzymes kill fungi *in vitro* by acting concertedly to depolymerize mycelial wall polysaccharides. Overexpression of these enzymes in transgenic plants provides in some cases increased protection against fungal pathogens. The inconsistent effect of the transgenic enzymes may be explained by the discovery that a fungal pathogen secretes a family of proteins each of which has the ability to inhibit individual members of the *endo*-β-1,3-glucanase family of its host. Thus, the glycanase products of plant defense genes and the glycanase-inhibitor products of fungal virulence genes may be determinants of the battles between fungi and plants.

9. **Wu, S.-C., S. Kauffmann, A.G. Darvill, and P. Albersheim. 1995. Purification, cloning, and characterization of two xylanases from *Magnaporthe grisea*, the rice blast fungus. Submitted.**

Magnaporthe grisea, the fungal pathogen that causes rice blast disease, secretes two *endo*-β-1,4-D-xylanases (E.C. 3.2.1.8) when grown on rice cell walls as the only carbon source. One of the xylanases, XYN33, is a 33 kD protein on SDS-polyacrylamide gel and accounts for approximately 70% of the *endo*xylanase activity in the culture filtrate. The second xylanase, XYN22, is a 22 kD protein and accounts for approximately 30% of the xylanase activity. The two proteins were purified, cloned, and sequenced. XYN33 and XYN22 are both basic proteins with calculated isoelectric points of 9.95 and 9.71, respectively. The amino acid sequences of XYN33 and XYN22 are not

homologous, but they are similar, respectively, to family F and family G xylanases from other microorganisms. The genes encoding XYN33 and XYN22, designated *xyn33* and *xyn22*, are single-copy in the haploid genome of *M. grisea* and are expressed when *M. grisea* is grown on rice cell walls or on oatspelt xylan, but not when grown on sucrose.

10. **Augur, C., V. Stiefel, A. Darvill, P. Albersheim, and P. Puigdomenech. 1995. Molecular cloning and expression pattern of an a-fucosidase gene from pea seedlings. In preparation.**

α-Fucosidase is a cell wall protein purified from pea (*Pisum sativum*) epicotyles. The α-fucosidase hydrolyzes terminal fucosyl residues from oligosaccharide fragments of xyloglucan, a plant cell wall polysaccharide. α-Fucosidase may be an important factor in plant growth regulation as it inactivates oligosaccharides that inhibit growth of pea stem segments. The *N*-terminal region and several proteinase-released internal peptides of the α-fucosidase were sequenced by automated Edman degradation. The amino acid sequences of the *N*-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 that were used to design the primers for the PCR as well as two other peptides obtained by proteinase digestion of α-fucosidase. No sequence homology to other α-fucosidases was apparent, although the *N*-terminal region of the α-fucosidase is strongly homologous to Kunitz-type trypsin inhibitors. cDNA and genomic copies of the pea α-fucosidase were isolated and sequenced. The genomic sequence contains an open reading frame of 217 amino acids, which comprises the mature protein in addition to a hydrophobic signal peptide sequence. There are no introns present. In pea, the gene is present in one or two copies. Its mRNA is present in roots, leaves and elongating shoots. No mRNA accumulation was observed in elongated stem. The spatial pattern of expression of the α-fucosidase was determined by *in situ* hybridization. In early developmental stages of roots, shoots and leaves, the expression of the gene was particularly high in epidermal cells and in the vascular tissue.