Oligosaccharins: Oligosaccharide Regulatory Molecules

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Carbohydrates are the building blocks of many of the structural polymers that give form to living cells and organisms, and they play important roles in the interactions of cells with one another as well as with their environment. The extensive stereochemistry, multiple hydroxyls and oxygen atoms, and accessible hydrophobic regions characteristic of glycosyl residues make oligosaccharides ideal ligands for precise interactions with recognition sites on proteins. These sites can interact with as many as six glycosyl residues at once. Between 10^4 and 10^5 different tetrasaccharides can be formed from interconnecting four glycosyl residues, with each tetrasaccharide adopting one or a few characteristic three-dimensional shapes and each requiring only a few specific enzymes for its biosynthesis. This means that proteins distinguish among a large range of information-carrying oligosaccharides, information being transmitted to cells through oligosaccharide-specific receptors.

Organisms utilize in a variety of ways the ability of proteins to recognize oligosaccharides. Prime examples are immunodominant oligosaccharide antigens on animal cell surfaces, which include the classical A, B, and O antigenic determinants of red blood cells. Biologists use carbohydrate antigens to characterize the various developmental stages of embryos and to identify cancer cells. Other oligosaccharides are bound by cell-surface-localized lectins, thereby attaching animal cells to their substrates, which are often extracellular matrix polysaccharides or glycoproteins. Cell-surface oligosaccharides are also the specific receptors for viruses, bacteria, peptide hormones, and toxins. Another oligosaccharide activates proteins that inhibit blood clotting. This is just a partial list of the known biological functions of oligosaccharides.

Plants and animals have evolved signaling mechanisms to regulate the expression of genes that are essential for their growth, development, and defense against pests. Some of these signals or regulatory molecules are oligosaccharides. Oligosaccharides with regulatory activities are called *oligosaccharins*, the subject of this Account.

Plant cells are encased in an extracellular matrix rich in complex carbohydrates. The extracellular matrix of the cells of the growing or succulent tissues of plants is referred to as the primary cell wall. The structures of primary cell wall polymers are evolutionarily conserved. Primary cell walls are about 90% complex carbohydrates, approximately one-fourth of which is crystalline cellulose microfiber that functions somewhat like steel rods in reinforced concrete: the cellulose microfibers are responsible for much of the strength of the walls. The remaining primary cell wall polysaccharides are structurally more complex than cellulose. There are 13 known sugars in primary cell walls interconnected in six structurally complex polysaccharides and several heavily glycosylated structural proteins. The glycosyl residues of the primary cell wall polysaccharides are frequently substituted with nonglycosyl groups.

Two of the three oligosaccharins we describe below are believed to originate as components of polysaccharides of the primary cell walls of plants, while the third originates from a wall polysaccharide of a fungal pathogen of plants. Progress in elucidating the structures of these oligosaccharins and in determining the molecular basis for their bioactivity is the primary subject of this Account.

Oligoglucoside Elicitors

Structure and Biological Activity. The biosynthesis and accumulation of antimicrobial phytoalexins is one of the best-studied plant defense mechanisms.[1,2] Plants synthesize and accumulate low molecular weight antibiotics called phytoalexins in response to microbial infection and after treatment with elicitors. The phytoalexins elicitor-active oligosaccharides were first detected in the culture filtrates and then in the mycelial walls of *Phytophthora megasperma* f. sp. *glycinea*, a fungal pathogen of soybean.[3-6] The phytoalexins of soybean are pterocarpans that can be conveniently analyzed by their absorbance of UV light at 285 nm. A hepta-β-glucoside (compound 1, Figure 1) purified from

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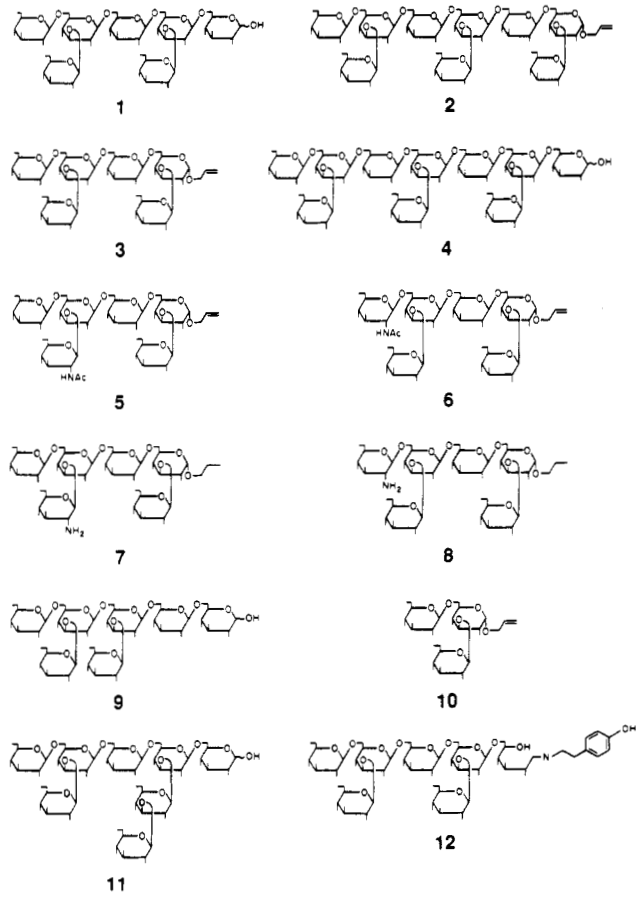

Figure 1. Structure of the hepta-β-glucoside elicitor (compound 1) and structurally related oligoglucosides. Oxygen atoms (e.g., hydroxyl groups) not involved in glycosidic linkages or located at the reducing ends have been omitted for clarity.

the mixture of oligoglucosides generated by partial acid hydrolysis of *P. megasperma* mycelial walls was the smallest oligoglucoside that could induce soybean seedlings to accumulate phytoalexins.[6] From the number of peaks observed in the various chromatographic steps, it was estimated that 300 structurally distinct, elicitor-inactive heptaglucosides were present in the original oligoglucoside mixture. Homogeneous preparations of the aldehyde-reduced forms (i.e., the hexa-β-glucosyl glucitols) of the elicitor-active hepta-β-glucoside and of seven other elicitor-inactive hepta-β-glucosides were obtained in amounts sufficient to determine their structures.[6,7] The structure of the elicitor-active hepta-β-glucoside[8,9] was confirmed by chemical synthesis.[10]

The abilities of the active hexa-β-glucosyl glucitol purified from fungal wall hydrolyzates and of the corresponding chemically synthesized, unreduced hepta-β-glucoside 1 to induce phytoalexin accumulation in soybean cotyledons were identical.[10] Both are active at concentrations of ≈10 nM, making them the most active elicitors of phytoalexin accumulation yet observed. The seven other hexa-β-glucosyl glucitols that were purified had no elicitor activity over the limited concentration range that could be tested (≤400 μM).[6] These results provided the first evidence that specific structural features are required for an oligo-β-glucoside to be an effective elicitor of the phytoalexin accumulation.

Ten oligo-β-glucosides (**2**–**11**, see Figure 1), structurally related to elicitor-active hepta-β-glucoside **1**, were chemically synthesized,[8,9,11-13] and structural features essential for effective elicitation of phytoalexin accumulation in soybean cotyledon tissue were identified.[14] Hexa-β-glucoside **3** (Figure 1) is the minimum elicitor-active structure.[14] The four most active oligoglucosides [compounds **1**–**4**; concentration required for half-maximum induction of phytoalexin accumulation (EC_{50}) ≈ 10 nM] have, as part of their structures, the branching pattern identified previously for elicitor-active hepta-β-glucoside **1**.[6] Oligoglucosides **5**–**10**, even though they are structurally related to oligoglucosides **1**–**4**, are not effective elicitors. In hexa-β-glucoside **3**, replacement of the side-chain glucosyl residue adjacent to the nonreducing terminus with a β-glucosaminyl (compound **7**) or *N*-acetyl-β-glucosaminyl residue (compound **5**) reduced the elicitor activity ≈10- and 1000-fold, respectively. The corresponding modifications of the nonreducing terminal backbone glucosyl residue (compounds **8** and **6**, respectively) resulted in even greater decreases in elicitor activity (≈100- and 10 000-fold, respectively). The importance of the presence of the side-chain glucosyl residues for elicitor activity was confirmed by the demonstration that a linear, 6-linked hepta-β-glucoside is inactive as an elicitor. In addition, the different arrangement of side chains in hepta-β-glucoside **9** reduced elicitor activity ≈800-fold, confirming previous findings.[6] These structure–activity studies confirmed that specific structural features are required for an oligoglucoside to effectively elicit phytoalexin accumulation in soybean.

Phytoalexin elicitor assays of reducing-end derivatives of hepta-β-glucoside **1** demonstrated that attachment of an alkyl or aromatic group to the oligosaccharide (e.g., compound **12**) had no significant effect on the EC_{50}.[14] A tyramine-coupled derivative of hepta-β-glucoside **9** was slightly more active (≈2.5-fold) than underivatized hepta-β-glucoside **9**. Coupling of aromatic groups to maltoheptaose, a structurally unrelated hepta-α-glucoside, yielded derivatives with no detectable elicitor activity. More importantly, a tyramine derivative of biologically inactive hepta-β-glucosides did not activate those oligosaccharides. Thus, it was possible to prepare a radioiodinated form of compound **12** that was used to demonstrate the presence of specific, high-affinity binding sites for the elicitor in soybean membranes (see following section).

Receptor Studies. The first step in the signal transduction pathway induced by the hepta-β-glucoside elicitor of phytoalexins is likely to be recognition of the oligoglucosides by a specific receptor. Several studies utilizing heterogeneous mixtures of fungal glucan fragments suggested that binding sites for such fragments exist in plant membranes.[15-18] More recently,

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Table I
Biological Activities of α-1,4-Oligogalacturonides

activity	dp[a]	molar concn[b]	plant	ref
Plant Defense Responses				
induction of phytoalexins	8–13 (12)[c]	10^{-5}	soybean	28, 29, 31
	9–15 (13)	10^{-4}	castor bean	30
	ND[d]		parsley	33
induction of proteinase	2–20	$\leq 10^{-4}$	tomato	38
inhibitors	≈20	$\approx 10^{-5}$	tomato	55
induction of lignin	8–11 (11)	10^{-7}	cucumber	35
	ND		castor bean	36
induction of β-1,3-glucanase	ND		parsley	33
induction of chitinase	ND		tobacco	34
induction of isoperoxidases	ND		castor bean	36
inhibition of hypersensitive response	ND		tobacco	80
elicitation of necrosis	ND		cowpea	81
Development and Growth				
inhibition of auxin-induced elongation	>8		pea stem	48
regulation of TCL morphogenesis: flower formation	10–14 (12–14)	10^{-7}	tobacco	47
induction of ethylene	>8		tomato	50
	≥2		pear	51
enhancement of cell expansion and separation	≈100		soybean	49
Rapid Responses at the Plasma Membrane and Cell Surface				
efflux of K^+ and influx of Ca^{2+}	12–15	10^{-6}	tobacco	54
rapid depolarization of plasma membrane	1–7 and 10–20		tobacco	53
induction of H_2O_2 and oxidative burst	ND		soybean	39
enhancement of in vitro phosphorylation of 34-kDa protein	14–20	$\approx 10^{-7}$	tomato	55
			potato	55

[a] Dp (degree of polymerization) range of oligogalacturonides that show the designated biological activity. [b] Order of magnitude estimation of the concentration of oligogalacturonides that give the half-maximum biological response. The concentration is included only where purified oligogalacturonides were assayed. [c] Numbers in parentheses represent dp of most active oligogalactouronide. [d] ND: dp of active oligogalacturonides not determined.

membrane-localized binding sites were demonstrated for the hepta-β-glucoside elicitor that had been coupled either to aminophenethylamine[19] or tyramine[20] and subsequently radioiodinated. The binding sites were found in membranes isolated from all the major organs of young soybean plants. Binding of the radiolabeled hepta-β-glucoside elicitor to the root membranes is saturable over a ligand concentration range of 0.1–5 nM, a range comparable to, albeit somewhat lower than, the range of hepta-β-glucoside concentrations (6–200 nM) required to saturate the bioassay for phytoalexin accumulation.[6,10,14] The root membranes possess only a single class of high-affinity binding sites for the hepta-β-glucoside elicitor (apparent $K_d \approx 1$ nM). The hepta-β-glucoside binding site(s) are inactivated by heat or pronase treatment,[20] suggesting that the molecule(s) responsible for the binding are proteinaceous. Binding of the hepta-β-glucoside elicitor to the membrane preparation is reversible, indicating that the elicitor does not become covalently attached to the binding protein(s).

The membrane-localized, elicitor-binding protein exhibits a high degree of specificity with respect to the oligoglucosides that it binds. More importantly, the ability of an oligoglucoside to bind to soybean root membranes correlates with its ability to induce phytoalexin accumulation.[20] Those oligoglucosides with high elicitor activity are efficient competitors of the radiolabeled ligand, while biologically less active oligoglucosides are less efficient. Thus, four oligoglucosides ranging in size from hexamer to decamer (compounds **1**–**4**), indistinguishable in their abilities to induce phytoalexin accumulation,[14] are equally effective competitive inhibitors of binding of radiolabeled hepta-β-glucoside **12** to soybean root membranes. The abilities of structurally modified oligoglucosides **5**–**11** to compete with the radiolabeled ligand are reduced to an extent comparable to the reduction in the biological activities of these oligoglucosides.

The hepta-β-glucoside elicitor-binding sites were solubilized from soybean root microsomal membranes with the aid of detergents.[21,22] The solubilized binding protein retained its high affinity for the hepta-β-glucoside elicitor (apparent $K_d = 1.4$ nM) and, more importantly, retained the specificity for elicitor-active oligoglucosides characteristic of the membrane-localized protein.[22]

The results of the structure–activity[14] and binding[20] studies demonstrated that the structural elements of the hepta-β-glucoside elicitor required for its high biological activity are also essential for efficient binding of the elicitor to its putative receptor. The combined results of the biological assays[14] and the binding studies[20] provide evidence that the binding protein is the physiological receptor of the hepta-β-glucoside elicitor.

Bioactive Oligogalacturonides

Structure of Bioactive Oligogalacturonides. Those plant cell wall polysaccharides rich in galacturonic acid are called pectic polysaccharides. They

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include rhamnogalacturonan I, rhamnogalacturonan II, and homogalacturonan.[23] Homogalacturonan, a linear polymer composed solely of 1,4-linked α-D-galacturonic acid residues, has long been considered a structural component of higher plant cell walls, in part because of its ability to form gels in the presence of calcium ions.[24,25] Homogalacturonan fragments elicit defense responses, affect plant cell elongation, regulate development, and cause rapid changes in plasma membrane functions (Table I).[26,27] The dodeca-α-1,4-D-galacturonide shows the greatest elicitor activity.[28-30]

Bioactive oligogalacturonides are generated by treating plant cell walls or homogalacturonan [polygalacturonic acid (PGA)] with acid, pectate lyase, or *endo*-polygalacturonase (EPG). The identification of modified oligogalacturonides in bioactive oligogalacturonide preparations[31] prompted the development of procedures to further purify oligogalacturonides. High-performance anion-exchange chromatography with pulsed amperometric detection demonstrated that oligogalacturonides purified according to their degree of polymerization (dp) (by conventional anion-exchange chromatography of EPG-digested PGA) contain at least seven components, one of which is the expected homooligo-α-1,4-galacturonide. Six modified oligogalacturonides accounted for approximately 15% of a trideca-α-1,4-galacturonide-enriched fraction. Four of the six oligogalacturonides contained modified reducing termini. They were Gal*p*A-α-1,4-[Gal*p*A]$_7$-galactaric acid, Gal*p*A-α-1,4-[Gal*p*A]$_7$-tetraric acid, Gal*p*A-α-1,4-[Gal*p*A]$_8$-galactaric acid, and Gal*p*A-α-1,4-[Gal*p*A]$_9$-galactaric acid (unpublished results of the authors). The two remaining modified oligogalacturonides were shown, by fast atom bombardment mass spectrometry (FABMS), to be composed of 12 and 13 hexuronic acid residues, respectively. How their structures differ from the homotrideca-α-1,4-galacturonide has not been elucidated, but since they did not cochromatograph with the authentic homotridecagalacturonide, either they contain a differently linked galacturonic acid residue or one of the glycosyluronic acid residues is replaced with a different glycuronic acid residue. Similarly modified oligogalacturonides are present in all of the conventional anion-exchange fractions containing bioactive homogalacturonides (dp 10–15). The modified oligogalacturonides appear to have little or no biological activity.

Elicitation of Plant Defense Responses by Oligogalacturonides. Microbial phytopathogens generally secrete EPG and/or *endo*-polygalacturonic acid lyase that releases oligogalacturonide fragments from plant cell walls.[32] The oligogalacturonide fragments elicit several plant defense responses (Table I), including accumulation of phytoalexins,[28-30,33] synthesis of β-1,3-glucanases and chitinases that degrade fungal cell walls and inhibit fungal growth,[33,34] and deposition in plant cell walls of lignin that serves as a physical barrier to fungal penetration.[35,36] Oligogalacturonides are also involved when plants, in response to insect attack, produce proteins that inhibit insect-secreted proteases.[37,38] Furthermore, oligogalacturonides induce suspension-cultured cells to secrete extracellular isoperoxidases[36] and H_2O_2.[39] (The oxidative burst induced in plant cells by oligogalacturonides is comparable to the defense-related production of activated oxygen species that cause lipid peroxidation, membrane damage, and localized tissue inflammation in animal cells.[39,40])

Oligogalacturonides of specific sizes are usually required to induce plant defense responses (see Table I). However, the dp of oligogalacturonides required to elicit a response varies with the assay. Oligogalacturonides with dp between 10 and 15 elicit defense responses to pathogenic fungi.[28-30,33] In contrast, oligogalacturonides with dp between 2 and 20 induce tomatoes to produce inhibitors of insect proteinases.[38] The reason for the size requirement, when it exists, has not been ascertained.

Regulation of Plant Growth and Development by Oligogalacturonides. Most undifferentiated plant cells are totipotent, that is, each cell has the potential to develop into a mature plant. The ability of plant cells to differentiate and develop into specialized organs is controlled by a number of factors, including hormones, light, and temperature.[41,42] A tobacco explant bioassay[43,44] was used to study the effects of phytohormones and cell wall derived fragments on the development of organs.[45,46] The tobacco explants used consist of floral stem tissue that, when incubated for 24 days on media containing the plant hormones auxin and cytokinin, form flowers, vegetative shoots, or roots;[44] the type of organ that forms depends on the concentrations of auxin and cytokinin. Oligogalacturonides, with dp 10–14, induce the formation of flowers and inhibit the formation of roots in tobacco explants when the explants are grown in media containing auxin and cytokinin at concentrations that, without the oligogalacturonides, either cause roots or no organs to

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form.[44,46,47] Oligogalacturonides with dp 12–14 are active at about 10^{-7} M.[47] Oligogalacturonides of the same size and at the same concentration also inhibit the formation of roots in tobacco leaf disc explants (F. Cervone, University of Rome, personal communication).

Evidence is increasing that plant cell wall derived fragments regulate other developmental processes such as cell elongation and fruit ripening. A mixture of oligogalacturonides at $\approx 10^{-4}$ M inhibited auxin-induced elongation of pea stem segments.[48] A pure xyloglucan-derived nonasaccharide (see below) also inhibited auxin-induced growth but at $\approx 10^{-8}$ M. Larger oligogalacturonide-enriched material (dp $\approx$ 100) enhanced cell expansion and cell separation of suspension-cultured soybean cells that were treated with colchicine to inhibit cell division.[49] Oligogalacturonides induce ethylene production in both ripening tomato fruit[50] and suspension-cultured pear cells.[51] Ethylene, a phytohormone, induces many responses in plants, including the stimulation of fruit ripening.[52]

Oligogalacturonides Induce Rapid Responses at the Plasma Membrane and Cell Surface. The surfaces of plant cells exhibit a variety of responses within 5 min of exposure to oligogalacturonides (Table I). Suspension-cultured soybean cells produce H_2O_2.[39] The membrane potential of tomato leaf mesophyll cells[53] depolarizes, although a relatively high concentration ($\approx 10^{-3}$ M) of a mixture of oligogalacturonides (dp 1–7 or 10–20) is required to elicit this response. In contrast, lower concentrations ($\approx 10^{-6}$ M) of size-specific oligogalacturonides (dp 12–15) induce a large, transient K^+ efflux, Ca^{2+} influx, cytoplasmic acidification, and depolarization of the plasma membrane in suspension-cultured tobacco cells.[54] The cells return to normal about 2 h after exposure to the oligogalacturonides. Size-specific oligogalacturonides (dp 14 and 15) enhance the in vitro phosphorylation of a 34-kDa protein associated with plasma membranes prepared from potato and tomato leaves.[55] The rapid effects of oligogalacturonides at the cell surface have yet to be directly correlated with any of the demonstrated biological activities of oligogalacturonides. Characterizing oligogalacturonide receptors should facilitate elucidation of the mechanisms by which oligogalacturonides can have so many biological effects.

Xyloglucan Oligosaccharides

Structure of Xyloglucans. The cell walls of higher plants contain a family of highly branched polysaccharides called hemicelluloses. Hemicelluloses are functionally defined as those polysaccharides that form strong noncovalent associations with cellulose microfibers.[56] The predominant hemicelluloses in the primary cell walls of higher plants are arabinoxylan (including glucuronoarabinoxylan) and xyloglucan. Xyloglucan is thought to be a load-bearing structure in the primary cell wall because of its role in noncovalently cross-linking cellulose microfibers. The dynamic nature of this cross-linking is proposed as the major factor that controls the rate of cell wall expansion, thereby regulating plant cell growth.[56,57]

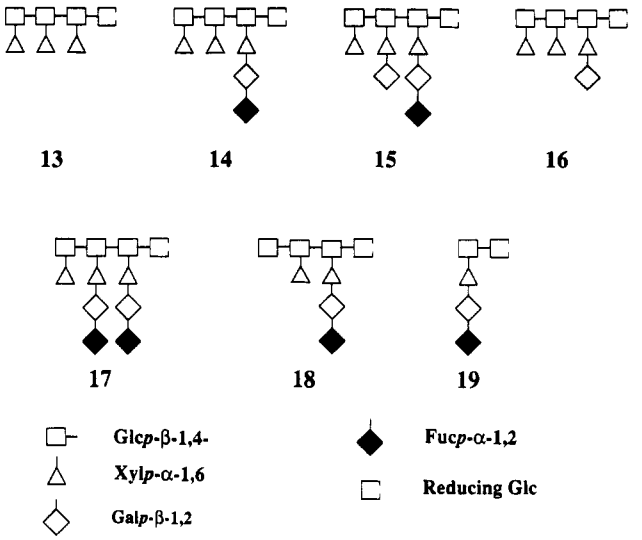

Figure 2. Structure of xyloglucan oligosaccharides. Compounds 13–16 are released by the *endo*-glucanase from the polymeric xyloglucan. Compounds 18 and 19 can be prepared by enzyme treatment of 14.

Xyloglucan has a backbone composed of 1,4-linked β-D-glucopyranosyl residues, approximately three-quarters of which are substituted at C-6 with α-D-xylopyranosyl residues. One-third to one-half of the α-D-xylopyranosyl residues in xyloglucans isolated from the cell walls of dicotyledonous plants are substituted at C-2 with a β-D-galactopyranosyl or an α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl moiety. The galactopyranosyl residue can be substituted with either one or two *O*-acetyl groups.[58] β-Linked xylopyranosyl or α-linked arabinofuranosyl residues are attached, in sycamore cell xyloglucan, to C-2 of a few of the backbone 4-linked β-D-glucopyranosyl residues that are already substituted at C-6 with an α-linked xylopyranosyl residue.[59,60]

Oligosaccharide fragments of xyloglucan are generated by treatment of the polysaccharide with a purified *endo*-β-1,4-glucanase isolated from the fungus *Trichoderma reesei*.[56,61] This enzyme cleaves the unbranched 4-linked β-glucopyranosyl residues of the backbone. Typically, every fourth residue in the main chain of a xyloglucan is unbranched. Therefore, the major oligosaccharide products obtained by enzymatic digestion

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contain a cellotetraose backbone.

The three nonreducing β-D-glucosyl residues of the cellotetraose backbone of the *endo*-glucanase-generated oligosaccharides are each substituted at C-6 with a glycosyl side chain. Heptasaccharide **13**, nonasaccharide **14**, and decasaccharide **15** (Figure 2) are the most commonly observed fragments generated by *endo*-glucanase.[62,63] Compounds **16** and **17** (Figure 2) are present in low levels in the *endo*-β-1,4-glucanase digests of xyloglucan isolated from suspension-cultured sycamore cells and from rapeseed hulls.[64]

Sodium borohydride reduction of xyloglucan oligosaccharides to their corresponding oligoglycosyl alditols facilitates their purification and structural characterization by ^{1}H-NMR spectroscopy.[60,61] Correlations were established between specific substructures of various xyloglucan oligoglycosyl alditols and a subset of their ^{1}H-NMR resonances. These correlations allow most of the structures of xyloglucan oligoglycosyl alditols to be deduced by inspection of their one-dimensional ^{1}H-NMR spectra.[60,61]

Ambiguities in the structures remaining after ^{1}H-NMR analysis can be resolved by FABMS.[61] The negative-ion FAB mass spectra of underivatized xyloglucan oligoglycosyl alditols typically include an abundant pseudomolecular $[M - H]^-$ ion and fragment ions derived from the alditol end of the molecule. An abundant pseudomolecular $[M + NH_4]^+$ ion is present in the positive-ion FAB mass spectra of acetylated xyloglucan oligoglycosyl alditols, but the fragment ions in these mass spectra are derived from the nonreducing end of the molecule. Thus, the sequence of xyloglucan oligoglycosyl alditols can be unambiguously deduced from the complementary information present in these two types of FAB mass spectra.[59-61]

Bioactive Xyloglucan Oligosaccharides. Xyloglucan oligosaccharide **14**, at $\approx 10^{-8}$ M, inhibits 2,4-dichlorophenoxyacetic acid (2,4-D) stimulated growth of pea stem segments.[65-67] 2,4-D is an analogue of the plant hormone known as auxin. The ability of **14** to inhibit growth of pea stems is maximum at $\approx 10^{-8}$ M; inhibition is diminished at higher and lower concentrations. Compound **13**, an oligosaccharide that lacks the fucosyl-galactosyl side chain of **14** (Figure 2), does not inhibit 2,4-D-stimulated growth.

The structural features required for xyloglucan oligosaccharides to inhibit 2,4-D-stimulated growth were investigated. A closely related oligosaccharide, **16**, which lacks only the terminal fucosyl residue of **14**, does not inhibit 2,4-D-stimulated growth, whereas another related oligosaccharide, **18**, which retains the fucosyl residue but lacks a terminal xylosyl residue (unpublished results of this laboratory), is as active in inhibiting growth as **14** (Figure 2). Thus, the terminal fucosyl residue of **14** is essential for its growth-inhibiting activity, whereas the terminal xylosyl residue farthest from the reducing terminus of the oligosaccharide is not required.

Compound **17** is more effective than **14** in inhibiting 2,4-D-stimulated growth (unpublished results of this laboratory). Therefore, it was surprising that **15**, which contains a terminal fucosyl residue but, unlike **14**, possesses an additional galactosyl residue (Figure 2), is apparently unable to inhibit 2,4-D-stimulated growth.[68] A pentasaccharide (**19**, Figure 2) and a trisaccharide (fucosyl lactose) were reported to inhibit 2,4-D-stimulated growth of pea stems, although they are less effective inhibitors than **14**.[66] Compound **19**, unlike **14**, also retains biological activity at concentrations higher than 10^{-8} M, that is, inhibition of pea stem elongation by **19** does not show a clear concentration optimum.

Oligosaccharides **13**, **14**, and **19** were chemically synthesized.[69] Synthetic **14** and **19** inhibit 2,4-D-stimulated growth of pea stems to the same extent and at corresponding concentrations of the same oligosaccharides isolated from xyloglucan. Chemically synthesized **13**, like **13** isolated from xyloglucan, did not significantly inhibit 2,4-D-stimulated growth. Thus, the structures and biological activities of **14** and **19** were confirmed, as was the structure of **13**.

The general biological significance of the abilities of xyloglucan-derived oligosaccharides **14** and **17–19** to inhibit 2,4-D-induced growth remains to be ascertained. However, available data are consistent with a feedback control loop hypothesis[65] in which elevated amounts of auxin promote the formation of xyloglucan-derived oligosaccharides that inhibit the growth-promoting effect of auxin.

Concluding Remarks

Three oligosaccharins are described that regulate plant growth, organogenesis, and defense against pathogens. Other oligosaccharins are known to be active in plant tissues. For example, chitosan oligosaccharides isolated from fungi or insects elicit plant defense responses.[70-72] More recently, a sulfated lipooligosaccharide, isolated from the nitrogen-fixing bacterium *Rhizobium meliloti*, was shown to elicit, by itself, nodule organogenesis in the roots of its symbiont host alfalfa.[73,74] This oligosaccharin determines which legume host the bacterium can nodulate.

The results of the oligosaccharin research described in this Account provide evidence that plants utilize the structural complexities of oligosaccharides to regulate important physiological processes. The cell wall polysaccharides of plants and microbes are a rich source of oligosaccharins, and the walls also contain glycanases and glycosidases capable of generating oligosaccharins. Animal cells also use oligosaccharides generated from extracellular matrix polysaccharides as regulatory molecules.

Although considerable evidence supports the hypothesis that oligosaccharins are important regulatory

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molecules in plants, much work still remains to evaluate the in vivo role and importance of these molecules. Most of the data regarding the biological activities of oligosaccharins was obtained in bioassays. Studies with intact plants are needed, perhaps using plants transformed with genes encoding enzymes or other proteins that alter the in situ activity of an oligosaccharin.

The existence of oligosaccharins in plant tissues was recently established by the demonstration that 14 (Figure 2) is present in the medium of suspension-cultured spinach cells[75] and bioactive oligogalacturonides are present in the medium of suspension-cultured sycamore cells (unpublished results). The xyloglucan nonasaccharide present in the medium of suspension-cultured cells is apparently formed by enzymatic cleavage of the xyloglucan polymer.[76] Furthermore, secreted enzymes induced in plant cells by pathogens release oligo-β-glucosides from the mycelial walls of fungi that elicit phytoalexins to accumulate in the cells of the plant.[77–79] Studies on the enzymes that release and process oligosaccharins, on receptors of oligosaccharins, and on the effects oligosaccharins have on membranes and membrane-associated proteins should elucidate the initial events that regulate oligosaccharin activities and lead to a better understanding of the signal pathways initiated by these regulatory molecules.

The progress in this new area of biology is partly due to the development of sophisticated analytical techniques and the cooperation of physiologists, molecular biologists, and organic chemists. The results of this interdisciplinary research are prompting plant scientists to reevaluate their concepts of development, defense mechanisms, and the function of cell walls. These studies may also lead to biotechnology-based, environmentally friendly approaches to improve plant resistance to microbial and insect pests and to control the growth and development of plants.

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Development of Organopalladium(IV) Chemistry: Fundamental Aspects and Systems for Studies of Mechanism in Organometallic Chemistry and Catalysis

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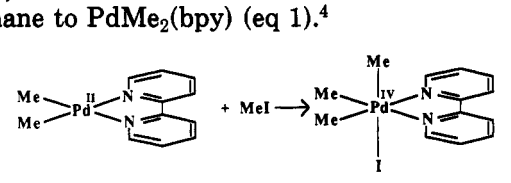
Palladium is one of the most widely studied elements in organometallic chemistry, partly owing to the important role of palladium complexes in organic synthesis and catalysis.[1] Platinum has an extensive organometallic chemistry in oxidation state +IV, commencing with the report of $[PtIMe_3]_4$ in 1907,[2] but synthetic organopalladium chemistry has, until recently, been confined to the formal oxidation states 0, +I, and +II. In 1975 and 1977 several (pentafluorophenyl)palladium(IV) complexes containing bidentate nitrogen donor ligands were reported;[3] e.g., $PdCl_3(C_6F_5)$(bpy) and $PdCl_2(C_6F_5)_2$(bpy) (bpy = 2,2′-bipyridyl) were obtained on the oxidation of Pd^{II} complexes with chlorine.[3a,b] In 1986 the first alkylpalladium(IV) complex, $PdIMe_3$(bpy), was obtained on the oxidative addition of iodomethane to $PdMe_2$(bpy) (eq 1).[4]



(1)

Since the initial report of $PdIMe_3$(bpy), a rich and diverse organopalladium(IV) chemistry has evolved,[5–8,9a,b,10] including reaction systems that are ideal

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