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Update on Signaling

Oligosaccharides as Signals and Substrates in the Plant Cell Wall[1]

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OLIGOSACCHARIDES AND OLIGOSACCHARINS

An oligosaccharide is any short chain of sugar residues interconnected by glycosidic linkages. A few select oligosaccharides can, at very low concentrations, exert "signaling" effects on plant tissues. Such oligosaccharides are termed "oligosaccharins" (Darvill et al., 1992; Aldington and Fry, 1993), and their discovery has provoked much research.

Most known oligosaccharins have been produced artificially by the acid- or enzyme-catalyzed fragmentation of cell wall polysaccharides. Glycoproteins have also been implicated as sources of oligosaccharins. The concept has thus arisen that polysaccharides and glycoproteins, many of which have structural and/or enzymic roles (Bacic et al., 1988), also act as the locked-up form of novel signaling molecules.

Some oligosaccharins are "elicitors," i.e. they induce responses that may help the plant to resist disease. The same and other oligosaccharins, however, also have effects on growth and development that are not obviously related to disease resistance. These non-elicitor effects will be the emphasis of the present article.

Not surprisingly, some oligosaccharins have turned out to be substrates for specific plant enzymes. Indeed, studies of oligosaccharins have led to the discovery of new cell wall enzymes. This aspect will also be explored because it may in some cases shed light on the mode of action of oligosaccharins.

XYLOGLUCAN-DERIVED OLIGOSACCHARINS

Xyloglucan is a structural polysaccharide of the primary cell wall (Levy et al., 1991). Its backbone is a long (0.15–1.5 μm) chain of β-(1→4)-linked D-Glc units, to which side chains composed of α-D-Xyl, β-D-Gal, and α-L-Fuc, and smaller amounts of α-L-Ara and β-D-Xyl, are attached. Xyloglucan chains can hydrogen-bond to cellulose, so, in the cell wall, they may tether adjacent microfibrils and thus restrain cell expansion. Specific oligosaccharides can be produced from xyloglucan by partial digestion with cellulase [β-(1→4)-D-glucanase]. Some of the oligosaccharides thereby produced are shown in Figure 1; some of these have turned out to be oligosaccharins.

Growth-Inhibiting Effects of Xyloglucan Oligosaccharins

One such oligosaccharin, XXFG (formerly XG9), will, at about 10^{-9} M, antagonize the growth promotion induced in pea stem segments by the auxin 2,4-D (York et al., 1984). Related oligosaccharides lacking a Fuc residue, e.g. XXLG and XXXG, are ineffectual (McDougall and Fry, 1989). Neither the reducing nor the nonreducing end of the backbone of XXFG is crucial, as shown by the fact that XXFGol and GXFG are both about as effective as XXFG (Augur et al., 1992). FG, which possesses the all-important Fuc residue but has a smaller backbone, is also effective, but methyl α-L-fucopyranoside has no effect (McDougall and Fry, 1989). Thus, the minimal requirement may be for the Fuc-Gal moiety. It will be interesting to see whether the L-Fuc of XXFG can be functionally replaced by a metabolically related unit such as L-Gal. It also remains to be seen whether fucosylated compounds other than xyloglucan (e.g. RG-I, *N*-linked glycoproteins, or phytoglycolipids) can mimic the growth-inhibiting activity of XXFG.

The inhibitory effect of 1 nM XXFG is lost at 100 nM (York et al., 1984; McDougall and Fry, 1989), but this does not seem to occur with FG (McDougall and Fry, 1989). Apparently the $Xyl_3 \cdot Glc_4$ backbone of XXFG is needed for the occurrence of a supraoptimum. It seems plausible that the loss of inhibitory effect is connected with the fact that oligosaccharins possessing a $Xyl_3 \cdot Glc_4$ backbone acquire a growth-promoting effect at concentrations above about 100 nM (see below).

Fucosylated xyloglucan oligosaccharins also inhibit endogenous growth and the growth induced by H^+, GA_3, and fusicoccin (e.g. Pavlova et al., 1992). The growth-inhibiting effect of XXFG is not countermanded by increasing the concentration of 2,4-D, so XXFG does not act as a classic anti-auxin.

It is not known how nanomolar concentrations of XXFG are perceived by plant cells. The requirement for a Fuc-Gal group and effectiveness at 10^{-9} M suggest that it interacts with a specific receptor. ^{3}H-Labeled XGOs do not readily enter the protoplast (Smith and Fry, 1991), so the most likely location for a receptor is in the plasma membrane.

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Abbreviations: ΔGalA, 4,5-unsaturated derivative of D-galacturonic acid; DP, degree of polymerization; RG, rhamnogalacturonan; XET, xyloglucan endotransglycosylase; XXFG (etc.), for abbreviated names of xyloglucan oligosaccharides, see Fig. 1.

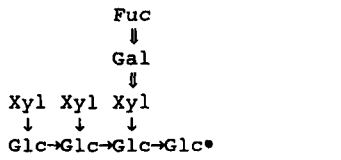
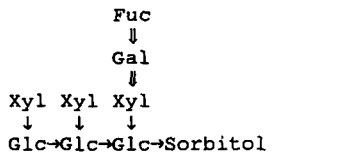
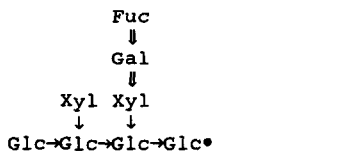
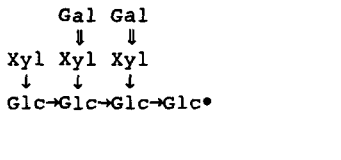
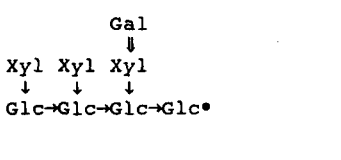
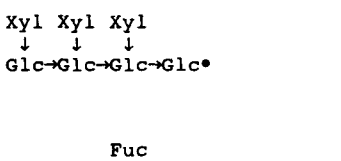
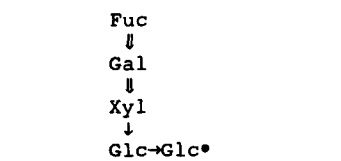
STRUCTURE	ABBREVIATED NAME	
	NEW	OLD
	XXFG	XG9
	XXFGol	XG9-ol
	GXFG	-
	XLLG	XG9n
	XXLG	XG8
	XXXG	XG7
	FG	XG5

Figure 1. Simplified structures and abbreviated names of xyloglucan oligosaccharides mentioned in the text. In the structures, arrows indicate glycosidic bonds: →, (1→4)-linkage; ↓, (1→6)-linkage; ⇓, (1→2)-linkage; •, reducing terminus. For further details of this revised nomenclature, see Fry et al. (1993).

Effects of Xyloglucan Oligosaccharins on Morphogenesis

Few effects on morphogenesis have been rigorously documented. Pavlova et al. (1992) showed that in cultured wheat embryos, FG evokes two responses. In the absence of 2,4-D, 10 nM FG greatly increases the number of adventitious roots, whereas in the presence of 2,4-D, 10 nM FG increases callus proliferation. Thus, further studies of the effects of XGOs on morphogenesis are urgently required.

Growth-Promoting Effects of Xyloglucan Oligosaccharins: The Role of XET

Another set of xyloglucan oligosaccharins can promote the elongation of pea stem segments in the absence of 2,4-D (McDougall and Fry, 1990). This effect differs in several important respects from the growth-inhibiting effect of 10^{-9} M XXFG. For example, the optimal concentration for growth promotion is approximately 10^{-6} M, the Fuc residue is not required, and some or all of the $Xyl_3 \cdot Glc_4$ backbone is required.

The order of effectiveness of three oligosaccharins (XLLG > XXXG > XXFG) is the same for growth promotion (McDougall and Fry, 1990) as for action as the acceptor substrate of a newly discovered enzyme, XET (Smith and Fry, 1991; Farkaš et al., 1992; Fry et al., 1992; Nishitani and Tominaga, 1992). This suggests that these oligosaccharins have their effect on growth by acting as substrates of XET. To see how this might work, it is necessary to consider the action and physiological significance of XET (see Fig. 2).

XET cuts a mid-chain Glc–Glc bond within one xyloglucan molecule and may form a transient xyloglucan–XET complex (Fig. 2A). The XET then transfers its portion of xyloglucan on to the nonreducing end of another xyloglucan molecule (the acceptor substrate) (Fig. 2B), thus forming a new Glc–Glc bond identical with the one that had been cut. The net result is polysaccharide-to-polysaccharide transglycosylation (McDougall and Fry, 1990) (Fig. 2C). If xyloglucan chains act as intermicrofibrillar tethers as proposed, this transient breaking of xyloglucan backbones may cause a temporary loosening of the cell wall, thereby facilitating cell expansion.

A small xyloglucan oligosaccharide (e.g. XXXG) added to the system could interfere in the process by acting as a competing acceptor substrate so that polysaccharide-to-oligosaccharide transglycosylation occurs (Smith and Fry, 1991; Fry et al., 1992) (Fig. 2D). Whereas the strength of the cell wall is usually restored after transglycosylation by the reformation of an intact polysaccharide chain, in the presence of XXXG the polysaccharide is effectively cut. It seems likely that this "cutting" would weaken the cell wall and could lead to an enhancement of cell expansion. Therefore, this might provide the answer to the question of how $Glc_4 \cdot Xyl_3$-based XGOs, such as XXXG, can act as growth-promoting oligosaccharins (McDougall and Fry, 1990).

Interaction with XET seems unlikely to account for the mechanism by which 10^{-9} M XXFG inhibits growth because (a) the K_m of XET for XGOs is about 2×10^{-5} to 5×10^{-5} M, and (b) XET does not require a Fuc residue in its substrates.

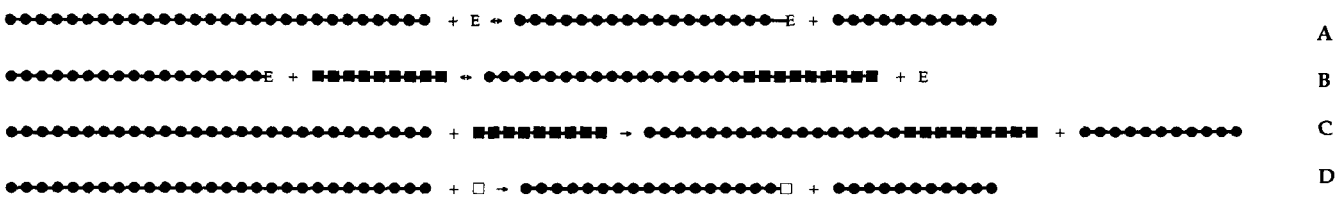


Figure 2. The proposed action of XET. A, Cleavage of a xyloglucan chain (donor substrate; ●–●–●–●. . .) by the enzyme (E) to form a polysaccharide-enzyme complex; B, transfer of the enzyme-linked polysaccharide fragment onto a second xyloglucan chain (acceptor substrate; ■–■–■–■. . .); C, the sum of reactions A and B, showing that no net change in chain lengths need be involved; D, the equivalent of reaction C in which a xyloglucan oligosaccharide (□) acts as the acceptor substrate. *Note*: In these diagrams, each building block (●, ■, or □) represents one XGO unit, such as XXXG or XXFG.

Biosynthesis and Biodegradation of Xyloglucan Oligosaccharins

XXFG and related oligosaccharins accumulate extracellularly to approximately 10^{-7} M in spinach and other cell cultures. This indicates that oligosaccharins are not just test-tube artifacts. By analysis of kinetic experiments involving the in vivo feeding of [^{3}H]Fuc, it has been shown that [^{3}H]XXFG arises by the partial hydrolysis of preformed polysaccharide (McDougall and Fry, 1991), and not by direct synthesis of the free oligosaccharin. The evidence indicates that xyloglucan oligosaccharides could well be novel, naturally occurring signaling molecules. It remains to be seen to what extent XGOs (and other oligosaccharins) are generated within intact plants.

Biological signals are often degraded within the organism so that the information that they convey does not persist when it is no longer relevant. An α-L-fucosidase activity exists in some plant tissues that would be capable of inactivating the growth-inhibiting activity of XXFG. However, when [*fucosyl*-^{3}H]XXFG was fed to cultured spinach cells, it underwent little hydrolysis; instead it was "sequestered" by covalent binding to polymeric xyloglucan. Therefore, the action of α-L-fucosidase and related enzymes in vivo requires further investigation.

PECTIC OLIGOSACCHARINS

Pectins are primary cell wall polysaccharides rich in α-(1→4)-linked D-galacturonic acid residues. Digestion with pure pectinase (endopolygalacturonase) yields three major fragments: oligogalacturonides, RG-I, and RG-II. Most work on biological effects has dealt with simple oligogalacturonides.

In addition to their well-known effects as elicitors of defense responses including phytoalexin synthesis, oligogalacturonides also influence plant growth (LoSchiavo et al., 1991; Filippini et al., 1992), especially auxin-induced growth in pea stem segments. The inhibitory effect of oligogalacturonides contrasted in four major ways with that of 10^{-9} M XXFG: (a) the concentration of oligogalacturonides required for maximal effect was about 10^{-4} M; (b) oligogalacturonides did not exhibit a supraoptimal concentration; (c) the effect of the oligogalacturonides could be overcome ("competitively") by use of a higher auxin concentration; and (d) the oligogalacturonides did not interfere with gibberellin-stimulated growth. The most effective oligogalacturonides appeared to be those of DP ≥ 9.

In other bioassays, the optimal DP for oligogalacturonides often is approximately 12. These bioassays concern several aspects of plant development, including the control of organogenesis in thin cell layers of tobacco (Mohnen et al., 1990), the inhibition of auxin-stimulated induction of new auxin-binding sites (LoSchiavo et al., 1991), the inhibition of auxin-stimulated rooting in tobacco leaf explants, and the inhibition of auxin-dependent embryogenesis in carrot cultures (Filippini et al., 1992). Many of these effects appear to be examples of oligogalacturonides antagonizing the multiple actions of auxin.

In agreement with the idea that oligogalacturonides are anti-auxins, they were found to interact with auxin-binding sites in plant membrane preparations (Filippini et al., 1992). Thus, it may be suggested that oligogalacturonides act at the membrane level. This idea is supported by the ability of oligogalacturonides to evoke rapid changes in ion flux (seen in membrane polarization, K^+ efflux, Ca^{2+} influx, and alkalinization of the medium [Mathieu et al., 1991]).

It has recently been reported that cress roots secrete an allelopathic substance that modifies the growth of neighboring *Amaranthus* seedlings (Hasegawa et al., 1992). The active principle appeared to be an unusual disaccharide, ΔGalA-(1→2)-Rha, containing a 4,5-unsaturated derivative of galacturonic acid (ΔGalA). ΔGalA-(1→2)-Rha at 3 μM promoted *Amaranthus* hypocotyl elongation; there was a dramatic 5-fold promotion at 1 mM. The biosynthetic origin of ΔGalA-(1→2)-Rha was not investigated; however, its structure tempts one to suggest that it is formed by the cleavage of a pectic polysaccharide such as RG-I, which contains the repeating unit . . .4)-Gal*p*A-(1→2)-Rha*p*-(1→. . . . Two enzymes would be required to produce ΔGalA-(1→2)-Rha—a lyase and an endorhamnosidase. It will be of great interest to determine whether these enzymes exist in cress roots and whether ΔGalA-(1→2)-Rha is the first of a novel class of oligosaccharins.

GLUCOSAMINE-CONTAINING OLIGOMERS

Defense-related effects of oligosaccharins derived from chitin and chitosan have been well documented. However, additional effects of more complex GlcN-containing frag-

ments have recently come to light and seem set to become a focus of intensive research.

Basse et al. (1992) showed that yeast invertase can be digested to yield a glycopeptide that elicits ethylene and Phe ammonia-lyase synthesis in cultured tomato cells. The simplest active compound was $Man_{10}\cdot GlcNAc_2\cdot Asn\cdot Arg$, which had a one-half maximal effect at 5 to 10 nM; $Man_8\cdot GlcNAc_2\cdot Asn\cdot Arg$ was 100-fold less effective. $Man_{10}\cdot GlcNAc_1$ (released from $Man_{10}\cdot GlcNAc_2\cdot Asn\cdot Arg$ by endo-β-*N*-acetylglucosaminidase H) was a suppressor of the elicitor effect, whereas $Man_8\cdot GlcNAc_1$ was not. Therefore, it was proposed that the elicitor and suppressor compete for a common recognition site.

Other effects of somewhat similar oligomers are the modulation of flax seedling growth by an oligosaccharin with the composition $Man_3\cdot Xyl\cdot GlcNAc_2\cdot Fuc$ (Priem et al., 1990) and the promotion of tomato fruit ripening by an isomer of $Man_5\cdot GlcNAc$ (Priem and Gross, 1992). If such oligomers are, as proposed, biologically relevant oligosaccharins, it is of interest to establish their presence in plants in vivo. There is a growing number of reports that oligomeric *N*-glycans with the correct structure do indeed occur both in the spent media of cultured cells (Priem et al., 1990) and in mature, green tomato fruit pericarp (Priem et al., 1993). The biosynthetic origin of these oligomers has not been established, but one strong possibility is that they arise by the partial hydrolysis of apoplastic *N*-linked glycoproteins such as peroxidase.

Chemically very different GlcN-containing oligosaccharins are the "nodulation factors" produced by *Rhizobium*. These are β-(1→4)-linked oligosaccharides of GlcN bearing various substituents including a fatty acid residue (Lerouge et al., 1990). Nodulation factors as dilute as 10^{-12} M induce the early stages of root nodule formation. Surprisingly, at least one nodulation factor (known as NodRlv-V[Ac,C18:4], produced by *Rhizobium leguminosarum*), also has a completely different effect. At 10^{-9} to 10^{-7} M, it restores the ability of nonembryogenic mutant carrot cells to undergo somatic embryogenesis (de Jong et al., 1992). Embryogenesis is also restored by the addition of a specific 32-kD isoenzyme of chitinase (de Jong et al., 1992), raising the novel possibility that this chitinase may solubilize embryogenesis-inducing fragments from an unknown GlcN-containing component of the carrot cells themselves.

CONCLUSIONS

The oligosaccharin concept is here to stay. This is ensured by the large number of biological effects exerted by a wide range of oligosaccharides. Despite this, there is an urgent need to add flesh to our skeletal understanding of oligosaccharins, including the discovery of new biological effects, the more thorough physiological description of known effects, the definition of structure-activity relationships, and documentation of the biosynthesis, transport, binding, action, and turnover of oligosaccharins in the plant.

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