

Oligosaccharide Signaling of Plant Cells

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Abstract A variety of oligosaccharide signals have been identified that function in the regulation of plant development, defense, and other interactions of plants with the environment. Some of these oligosaccharides are produced by various pathogens or symbionts, whereas others are synthesized by the plant itself. This mini-review summarizes our present state of information on these oligosaccharide signals and provides an overview of approaches being used to identify receptors for these signals and gain an understanding of the mechanism(s) by which these signals activate downstream events. Possible biotechnological applications of future work in this field are also considered. *J. Cell. Biochem. Suppl.* 30/31:123–128, 1998. © 1998 Wiley-Liss, Inc.

The past 25 years have witnessed the growing recognition of the importance of oligosaccharide signals in the regulation of plant development, defense and other interactions of plants with the environment. Early evidence of such signals was obtained during studies of plant–pathogen interactions when oligosaccharides were identified as some of the “elicitors” in cell-free fungal extracts that could stimulate plants to produce phytoalexins as a defense response to the pathogen [Ayers et al., 1976]. Subsequent studies established that other plant defense responses were also activated by various oligosaccharides and that some of these oligosaccharides are produced by enzymatic degradation of the plant cell wall, itself [for reviews, see Ryan and Farmer, 1991; John et al., 1997]. In addition to stimulating plant defense responses, partial hydrolysates of plant cell walls were soon found to have effects on plant growth and organogenesis [Mohnen and Hahn, 1993]. A number of these biologically active oligosaccharides have now been identified and are called “oligosaccharins” [Darvill et al., 1992].

Concurrent with the above studies, work in another arena identified a series of nodulation genes in rhizobia involved in the production of signals, called Nod factors, that are necessary for the initiation of the nitrogen-fixing symbiosis that occurs between these bacteria and the roots of legumes [for review, see Denarie et al.,

1996]. These Nod factors are responsible for the host-strain specificity of this symbiosis and stimulate the host plant to produce a new organ, the nodule, and initiate steps that result in the entry of the rhizobia into this nodule. In 1990, these Nod factors were found to be lipochitooligosaccharides [LeRouge et al., 1990], thus identifying yet a new category of oligosaccharide signals.

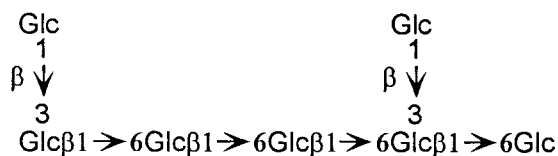
Although a number of oligosaccharins have been characterized to date, little is known of the mechanism(s) by which these signals activate downstream events in the plant. This mini-review provides a summary of our current state of information in this field as well as a consideration of some of the present challenges and the approaches by which we seek to overcome them as we attempt to unravel the mechanism(s) of oligosaccharide signal transduction. The implications of these studies on future biotechnological developments are also discussed.

OLIGOSACCHARIDE SIGNALS

The types of oligosaccharides that have been found to participate in various signaling processes in plants are summarized in Figure 1. A number of these oligosaccharins have been found to be active at concentrations in the nano- to picomolar range [Cote and Hahn, 1994; Denarie et al., 1996]. The identification and isolation of these active molecules from the heterogeneous mixtures of oligosaccharides present in fungal, bacterial, and plant extracts and the determination of the structures of these compounds have been enormous tasks that remain important ongoing efforts. For example, the

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OLIGOGLUCOSIDES**Defense**Hepta- β -glucoside**XYLOGLUCANS****Development**

Xyloglucan nonasaccharide

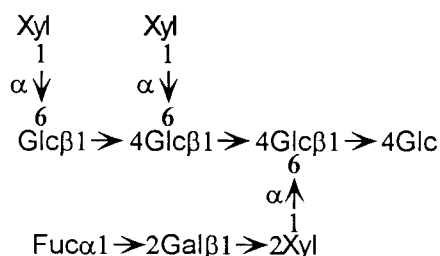
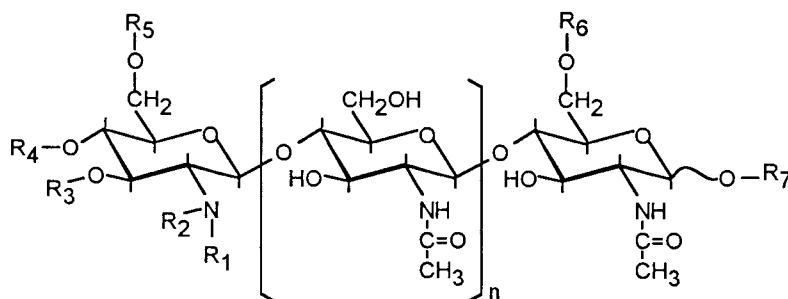
**OLIGOGALACTURONIDES****Defense
Development****CHITIN OLIGOSACCHARIDES****Defense****CHITOSAN OLIGOSACCHARIDES****Defense****NOD FACTORS****Development**

Fig. 1. Types of oligosaccharides known to function in the signaling of plant cells. The types of plant responses triggered by each of these types of signals are shown at the right. The generic structure of a Nod factor is shown; the number of GlcNAc residues in the backbone have been found to vary from 3 to 5.

Sites of potential modification are represented by $R_1 - R_7$. Modifications that have been identified to date include methylation (R_1), acylation, usually with a C_{16} or C_{18} fatty acid (R_2), acetylation or carbamylation (R_3, R_4, R_5), sulfation or glycosylation (R_6), and the addition of a glycerol (R_7).

hepta- β -glucoside shown in Figure 1 was purified from a mixture estimated to contain about 300 inactive other structural isomers of this compound [Darvill et al., 1992].

The structural prerequisites for the ability of oligoglucosides to elicit a defense response were established by comparisons of the biological activity of the hepta- β -glucoside with the activi-

ties of other isolated or chemically synthesized isomers and various derivatives of these molecules. Both the size and position of the branches were found to be essential for activity. Increasing the length of the oligosaccharide at the reducing end had no appreciable effect on activity, and most activity was retained upon removal of a single glucose from the reducing

end; however, the hexa- β -glucoside was found to be the minimal structure that gave appreciable activity [for reviews, see Darvill et al., 1992; Cote and Hahn, 1994]. Similar types of experiments established the importance of the positions and structures of the branches for activity of the xyloglucan nonosaccharide shown in Figure 1 and other members of this oligosaccharin category [Mohnen and Hahn, 1994].

The oligogalacturonides, chitin oligosaccharides, and chitosan oligosaccharides each represent linear homopolymers of various lengths. Studies on these oligosaccharins have focused primarily on the degree of polymerization (DP) necessary for biological activity. Such studies on the oligogalacturonides have led to the suggestion that more than 10 galactosyluronic acid residues are required for a biologically active conformation, whereas biological activity has been found with DPs of >4 or >7 in the case of oligochitins and oligochitosans, respectively [Darvill et al., 1992]. A recent study has shown that modifications of the reducing ends of several oligogalacturonides resulted in a reduction of biological activity, thus implying that this end of the polymer plays an important role in signaling [Spiro et al., 1998].

All the above oligosaccharides can be produced by enzymatic degradation of components of the cell walls of fungi, bacteria, or plants. Indeed, a number of pathogens have been found to secrete various glycosidases upon contact with the plant. It is of interest that the plants in turn appear to have developed mechanisms to regulate some of these enzymes to favor the production of the biologically active oligosaccharides that function in the plant defense response [Cote and Hahn, 1994]. Glycosidases involved in degradation of cell wall components are also produced by the plants, often in response to pathogen infection or changes in plant hormone concentrations [Mohnen and Hahn, 1993; Cote and Hahn, 1994]. The latter type of control may be of particular importance in regulating the production of oligosaccharide signals that function in plant development.

In contrast to the above oligosaccharins, the Nod factors represent lipooligosaccharide signals that are synthesized *de novo*. This synthesis is under the control of a set of rhizobial nodulation genes that are induced by flavonoids secreted by the legume roots. Some of these genes, present in all rhizobia, encode enzymes involved in the synthesis of the core of

the Nod factor; other genes, which vary among different rhizobial species or biovars, are involved in the synthesis or addition of various decorations to the core structure as shown in Figure 1 [Denarie et al., 1996]. The net result is the production by various rhizobial strains of highly specific Nod factors that can be recognized only by particular host legumes.

The host/strain specificity of rhizobial-legume symbiosis and the vast amount of information available on the molecular genetics of the production of the Nod factor signals involved present enormous advantages to the study of the molecular prerequisites necessary for the function of these oligosaccharide signals. The diversity in Nod factors produced by various rhizobial strains and mutants of these strains provides a wide variety of chemical analogues for studies of structure-function relationships. Recent attempts to produce "recombinant" Nod factors in *Escherichia coli* may result in the ability to achieve an even wider range of analogues, as well as sufficient quantities of these materials [Samain et al., 1997].

OLIGOSACCHARIN RECEPTORS

The specific structural requirements for oligosaccharin activity and the low concentrations of these signals that can elicit a response led to early speculation of the existence of specific plant receptors for these signals. This hypothesis has been confirmed with the finding of high-affinity sites for various oligosaccharins in membrane fractions isolated from the plants. High-affinity binding sites for β -glucan fragments, including the hepta- β -glucoside, have been identified in microsomal/plasma membrane fractions of soybeans [Schmidt and Ebel, 1987; Cosio et al., 1990; Cheong and Hahn, 1991; Yoshikawa and Sugimoto, 1993], and other members of this family of plants [Cosio et al., 1996]. A high-affinity binding site for a glycopeptide elicitor has been found in tomato plasma membranes [Basse et al., 1993]; chitin oligosaccharides have been found to bind to these membranes [Baureithel et al., 1994], as well as to the plasma membranes of rice cell suspension cultures [Shibuya et al., 1996]. The specificities of these membrane binding sites for various oligosaccharides have been found to closely match the specificities required for the plant responses to the oligosaccharins.

Two high-affinity binding sites for Nod factor were identified in *Medicago varia* membrane

preparations [Niebel et al., 1997]. The specificity of one of these sites resembled that obtained for the tomato membrane site above as well as for a site previously identified in *Medicago truncatula* [Bono et al., 1995]; the second site has a higher affinity for Nod factor, but its specificity has not yet been established. The existence of these two types of Nod factor binding sites serves to demonstrate the complexity of this signaling process and supports previous work that suggested the participation of multiple receptors in Nod factor signaling [Ardourel et al., 1994]. Differences in specificity of the β -glucan sites discussed above also support this possibility [Yoskikawa and Sugimoto, 1993].

Recent attempts have been made to identify proteins in the above membrane preparations that are responsible for the binding activity. Photoaffinity labeling experiments have identified a 70 kDa protein with high affinity for the hepta- β -glucoside [Frey et al., 1993], and a 75-kDa binding protein for chitooligosaccharides [Ito et al., 1997]. Although the hepta- β -glucoside binding protein has been solubilized and purified by affinity chromatography [Mithofer et al., 1996], relatively little information is available about either of the above proteins.

By definition, the oligosaccharin binding proteins identified above fit into that category of proteins known as lectins, which are defined on the basis of their ability to specifically recognize particular carbohydrate residues. Lectins were first discovered in plants more than 100 years ago, and a large number of these proteins have been isolated and characterized from a wide variety of plants [Lis and Sharon, 1998]. Most of the conventional plant lectins described to date are soluble proteins and although there have been many attempts to implicate them in some of the roles regulated by the above oligosaccharins, we do not as yet know the biological role of any of these proteins. However, a novel lectin isolated from the roots of the legume, *Dolichos biflorus*, has recently been found to be localized on the surface of the root hairs and to bind Nod factors. This lectin also has phosphatase activity and its specificity for nucleoside di- and triphosphates places it in the category of enzymes known as apyrases. The enzymatic activity is enhanced in the presence of Nod factors and other ligands that bind to its carbohydrate binding site [Etzler, 1998]. The presence of both activities on a single protein and the apparent interaction between the active

sites provide a possible means of signal transduction. The existence of other such plant lectin/enzymes is suggested by the recent finding of a cDNA from *Arabidopsis thaliana* that encodes a legume seed lectin-like domain as well as a receptor-like serine/threonine kinase [Herve et al., 1996].

It must be recognized that although the carbohydrate binding properties and localizations of the above proteins have made them promising candidates for oligosaccharin receptors, the verification of such a role will come only after it is established that upon binding the signal they can initiate the activation of downstream events, either through a direct signal transduction mechanism or indirectly by perhaps transporting the signal to a separate transducer. Such verification could be obtained by a variety of approaches, including direct biochemical studies of the effect of the signal on the receptor, reconstitution signaling experiments in artificial membranes, complementation studies with mutants, or the use of antisense technology to block the expression of the protein.

SIGNAL TRANSDUCTION

Although we have considerable information on the structures of a number of oligosaccharins and of the plant responses to these signals, at present we know little about the mechanism(s) by which these signals are transduced. Initial attempts to gain insights into this area have come through studies directed at identifying initial responses of the plant cell to the oligosaccharide signal. For example, oligogalacturonides have been found to enhance the *in vitro* phosphorylation of several proteins isolated from the plasma membranes of tomato and potato leaves; these oligosaccharins have also been found to cause membrane depolarization and ion fluxes in tobacco cell cultures and carrot protoplasts as well as an oxidative burst resulting in H_2O_2 accumulation in soybean cell suspension cultures [for review, see Mohnen and Hahn, 1993]. Many of these same responses are found in the defense responses of plants to other elicitors [Cote and Hahn, 1994]. Nod factors have also been found to induce membrane depolarization and ion fluxes, including calcium oscillations in root hairs as well as rearrangements of actin microfilaments [Ehrhardt and Long, 1994; Felle et al., 1995; Cardenas et al., 1998].

A variety of signal transduction mechanisms and pathways have been found in plants [for reviews, see Bowler and Chua, 1994; Trewavas and Malho, 1997]. By identifying possible secondary messengers and other elements that may be involved in the oligosaccharin signaling processes, the above types of studies are aiding in the consideration of which, if any, of these mechanisms may be involved in oligosaccharide signaling. Indeed, several studies have already been initiated using various signal transduction inhibitors [Cote and Hahn, 1994].

The above information on plant responses, coupled with information on the specificities of the signals and emerging information on receptors, will be of value in eventually elucidating the molecular basis of transduction of these oligosaccharide signals. It is also anticipated that genetic studies will play a wider role in these investigations as mutants in the receptor genes are identified.

One factor to consider as work proceeds on these signal transduction mechanisms is that many of the oligosaccharin signals are oligomeric in nature and may thus behave as multivalent ligands. Even the Nod factors may be presented to the plant in a multivalent form, such as micelles or adsorbed to the rhizobial surface. Such multivalent ligands could be anticipated to promote the clustering of receptors on the plant cell surface, a process that could constitute part of a signal transduction mechanism.

FUTURE PROSPECTS

The oligosaccharides identified to date that participate in signaling events may represent only the tip of an iceberg of many such signals. For example, a variety of studies have shown that Nod factors appear to have effects on non-leguminous plants and have led to the suggestion that the plant may produce similar signals to regulate growth and organogenesis [Spaink et al., 1993]. Such types of signals have also recently been implicated in animal embryogenesis [Semino et al., 1996; Bakkers et al., 1997], suggesting that this type of signaling mechanism may have been highly conserved during evolution and may play a basic role in development.

Although our understanding of oligosaccharide signal transduction is still in its infancy, it is expected that this area will undergo rapid

progress as receptors for oligosaccharins are identified. In addition to enhancing our basic understanding of this signaling process, the identification of specific oligosaccharin receptors has profound biotechnological implications. For example, the transfer of genes encoding receptors that recognize pathogen oligosaccharins to other plants could help confer pathogen resistance to these plants. Similarly, the transfer of genes encoding the receptors for Nod factors to nonleguminous food crops may enable the extension of the nitrogen-fixing rhizobial symbiosis to these plants and thus have an enormous impact on the world food supply and the environment. It is clear that the oligosaccharide signaling field that has emerged since the inception of this journal is now poised for rapid growth. It will be of great interest to follow the advances in this area over the next 25 years.

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