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STRUCTURE AND FUNCTION OF COMPLEX CARBOHYDRATES ACTIVE IN REGULATING PLANT-MICROBE INTERACTIONS

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Abstract - A key regulatory role of complex carbohydrates in the interactions between plants and microbes has been established. The complex carbohydrates act as regulatory molecules or hormones in that the carbohydrates induce de novo protein synthesis in receptive cells. [1] The first complex carbohydrate recognized to possess such regulatory properties is a polysaccharide (PS) present in the walls of fungi (2). Hormonal concentrations of this PS elicit plant cells to accumulate phytoalexins (antibiotics). [2] More recently we have recognized that a PS in the walls of growing plant cells also elicits phytoalexin accumulation; microbes and viruses may cause the release of active fragments of this endogenous elicitor. [3] Another PS in plant cell walls is the Proteinase Inhibitor Inducing Factor (PIIF) (53). This hormone appears to protect plants by inducing synthesis in plants of proteins which specifically inhibit digestive enzymes of insects and bacteria. [4] Glycoproteins secreted by incompatible races (races that do not infect the plant) of a fungal pathogen of soybeans protect seedlings from attack by compatible races. Glycoproteins from compatible races do not protect the seedlings (61). [5] The acidic PS secreted by the nitrogen-fixing rhizobia appear to function in the infection of legumes by the rhizobia. W.D. Bauer and his co-workers have evidence that these PS are required for the development of root hairs capable of being infected by symbiont rhizobia. Current knowledge of the structures of these biologically active complex carbohydrates will be presented.

INTRODUCTION

Our laboratory has recently come to realize that complex carbohydrates of higher plants, fungi and bacteria can act as regulatory molecules, that is, as molecules which in minute quantities alter the metabolism of receptive cells by causing the synthesis of specific proteins. It is not surprising that these structurally complex and exquisitely specific molecules can possess regulatory properties as many diverse classes of molecules including glycoproteins, proteins, peptides, steroids and a variety of smaller molecules such as epinephrine, indoleacetic acid, gibberellic acid, cytokinins, and even ethylene, are known to possess regulatory properties.

The carbohydrate portions of glycoconjugates are critically involved in recognition phenomena in biology (see, for example, 1, 46, 48). One of the earliest known functions of such carbohydrates in recognition processes is defining the blood group substances of mammals. The carbohydrate portions of glycoconjugates act on the cell surface of bacteria as serological determinants and as receptors for phage and bacteriocins. More recently, the carbohydrate portions of glycoconjugates have been recognized as cell surface specific antigens of at least some fungi, and the receptors of hormones and toxins in eucaryotic cells. The carbohydrate portions of glycoconjugates participate critically in determining the movement of glycoproteins within and between cells by acting as signals for directed trans-

port of these molecules. Cell surface glycoconjugates are also important in differentiation of invertebrates, and they are the receptors for mitogenic lectins. Thus, the complex carbohydrates of glycoconjugates participate in a wide range of critical recognition phenomena. Evidence has even been obtained that the carbohydrate portion of a low molecular weight glycoconjugate has the ability to induce cell differentiation in a slime mold (57), a function related to those which will be described in this paper.

In spite of knowing this wide range of recognition functions of the carbohydrate portion of glycoconjugates, there has been no suggestion until recently that carbohydrates themselves can act as regulatory molecules that alter protein synthesis in receptive cells, but recent evidence, accumulated with several experimental systems, establishes that carbohydrates can do just that. We describe below several plant and plant-microbe systems in which carbohydrates act as regulatory molecules.

PLANTS, WHEN EXPOSED TO CERTAIN β-GLUCAN FRAGMENTS OF FUNGAL ORIGIN, DEFEND THEMSELVES BY SYNTHESIZING AND ACCUMULATING PHYTOALEXINS

Many plants respond to invasion by a pathogenic or a nonpathogenic microorganism, whether a fungus, a bacterium, or a virus, by accumulating phytoalexins. Phytoalexins are defined as low molecular weight antimicrobial compounds that are both synthesized by and accumulate in plants after exposure to microorganisms. Many plants attempt to defend themselves against microbes and, perhaps, against other pests (2, 6, 22, 30, 37, 41, 43, 44, 58) by producing phytoalexins. Molecules which trigger phytoalexin production in plants have been called elicitors (42).

The best characterized and most effective known elicitor of biological origin is composed of fragments of β-glucans present in the mycelial walls of many fungi (12, 54). β-Glucan Elicitor can be obtained by partial acid hydrolysis of purified mycelial walls of the fungal pathogen of soybeans, Phytophthora megasperma f.sp. glycinea which causes root stem rot. The β-Glucan Elicitor is very active in stimulating phytoalexin accumulation in soybean tissues. The smallest β-Glucan fragments which have elicitor activity contain approximately nine β-glucosyl residues interconnected by 3-, 6-, and 3,6-glucosidic linkages.

The β-Glucan Elicitors isolated from different races of Phytophthora megasperma f.sp. glycinea (2) and from the yeast Saccharomyces cerevisiae (31) do not differ significantly in their elicitation of phytoalexin accumulation in several soybean cultivars (2, 31), in French beans (18), and in potatoes (18). Thus, as is common for regulatory molecules, elicitors are not species specific with regard to their source nor with regard to the cells whose metabolism they regulate. Also like other regulatory molecules, the β-Glucan Elicitor is effective in very small concentrations; approximately ten nanograms of the β-Glucan Elicitor stimulates accumulation in a single soybean cotyledon of more than sufficient amounts of phytoalexins to stop the growth of a variety of microorganisms in vitro.

Evidence has been obtained that the β-Glucan Elicitor, like many other regulatory molecules, stimulates de novo enzyme synthesis in receptive plant cells. The β-Glucan Elicitor causes soybean cells to accumulate at least five chemically and metabolically related pterocarpan phytoalexins. Ebel, Hahlbrock, and Grisebach and their coworkers (26, 64, 65) have studied the biosynthesis of these soybean phytoalexins. Apparently, synthesis of these phenylpropanoid compounds is a result of de novo synthesis of the necessary enzymes. Dixon and his coworkers have shown that enzymes responsible for the biosynthesis of the phytoalexin phaseollin in French beans (Phaseolus vulgaris) are also synthesized de novo as a result of elicitation by the β-Glucan Elicitor (18, 23, 24). Thus, the β-Glucan Elicitor causes receptive cells to synthesize new proteins.

POLYSACCHARIDE FRAGMENTS FROM THE WALLS OF PLANT CELLS ELICIT PHYTOALEXIN ACCUMULATION IN PLANT CELLS

Realization that the polysaccharides of the walls of growing plant cells are extremely complex structures (16, 19, 20, 49) has made us wonder about the function of these molecules. Until recently, these complex molecules had been thought to have only a structural function, but it is difficult to

believe that such complex molecules have evolved for only structural requirements. This skepticism proved well founded for we have recently demonstrated that at least two plant cell wall polysaccharides, or fragments thereof, serve as regulatory molecules.

We have shown that one of these cell-wall derived regulatory molecules, which elicits phytoalexins in soybean cotyledons, is a component of isolated cell walls of soybean stems and of the walls of suspension-cultured cells of tobacco, sycamore, and wheat. This elicitor can be released from the isolated walls by partial acid hydrolysis, and purified by ion exchange and gel filtration chromatography. The elicitor-active fragments thus obtained are size heterogeneous. Their gel chromatography elution volume suggests that many of the elicitor-active fragments consist of 10-15 glycosyl residues. These elicitor-active fragments are called the Endogenous Elicitor.

The Endogenous Elicitor originates from a galacturonic acid-rich cell wall polysaccharide; treatment of the Endogenous Elicitor with an endopolygalacturonase destroys its elicitor activity. The Endogenous Elicitor of soybean cell walls does not appear to originate from either rhamnogalacturonan I or rhamnogalacturonan II, the two pectic polysaccharides which have been partially characterized in this laboratory (19, 49). This is not surprising as more than half of the pectic polysaccharides of the walls of growing cells have yet to be characterized.

We were not the first to discover that plants have an Endogenous Elicitor. Bailey, Hargreaves and Selby (8, 32, 33) found a heat stable, dialyzable component which is released from damaged pea or bean tissues and which elicits phytoalexin accumulation in these tissues. It is not known whether the "Constitutive" Elicitor discovered by Bailey, Hargreaves and Selby is the same as the Endogenous Elicitor present in cell walls, but it seems likely that both elicitors are the same.

The realization that the Endogenous Elicitor is a fragment of a cell wall pectic polysaccharide is made more intriguing by observations that two enzymes which degrade pectic polysaccharides are elicitors. Stekoll and West (56) have studied an elicitor of casbene, a castor bean phytoalexin. The elicitor, present in culture filtrates of the pathogenic fungus Rhizopus stolonifer, is a pectic-degrading enzyme, an endopolygalacturonase. More recently, we (G. Lyon and P. Albersheim, unpublished results) have obtained evidence that an elicitor secreted by the bacterial pathogen, Erwinia carotovora which causes soft rot, is a polygalacturonic acid lyase, another pectic-degrading enzyme. Partially purified preparations of this enzyme are effective elicitors of phytoalexin accumulation in soybean cotyledons.

The ability of pectic-degrading enzymes secreted by Rhizopus stolonifer and Erwinia carotovora to stimulate phytoalexin accumulation suggests that these enzymes could release the Endogenous Elicitor present in the cell walls of plants. However, we have not successfully released the Endogenous Elicitor of soybean cell walls by treatment of the walls with the E. carotovora polygalacturonic acid lyase. An alternative mechanism by which the pectic-degrading enzymes may elicit phytoalexin accumulation is indirectly by damaging plant cells (13, 14, 29, 62). The damaged cells might release or activate a plant enzyme which liberates the Endogenous Elicitor. We have experimental support for this alternative explanation (G. Lyon and P. Albersheim, unpublished results). We have solubilized and partially purified an enzyme from soybean stems that elicits phytoalexin accumulation in soybean cotyledons. This enzyme has only been isolated from stems whose cells had been damaged by a freeze-thaw procedure. Experiments have not yet been carried out to determine whether this enzyme works by releasing the Endogeneous Elicitor. The activation of an elicitor-releasing enzyme in damaged cells could explain the manner by which phytoalexin accumulation is stimulated by a variety of abiotic elicitors such as U.V. light, freeze-thawing, heavy metals and antibiotics, and perhaps even by the β-Glucan Elicitor.

The Endogenous Elicitor is likely to be distributed throughout the plant. The enzyme, putatively responsible for releasing the Endogenous Elicitor, must be regulated in some manner. The enzyme might be compartmentalized, such as in lysosomes, or bound to the cell membrane, or stored in an inactive form, perhaps as a zymogen. If this putative enzyme is released or activated by cell damage and if this enzyme is also distributed throughout the plant, all parts of the plant would, as observed, be capable of localized phytoalexin accumulation in response to any stimulus which causes

cell damage.

THE PROTEINASE INHIBITOR INDUCING FACTOR (PIIF) IS A FRAGMENT OF A CELL WALL POLYSACCHARIDE

A third complex carbohydrate found to be a regulatory molecule is the plant hormone known as "PIIF" - the Proteinase Inhibitor Inducing Factor - which, like the Endogenous Elicitor, is a fragment of a polysaccharide present in the walls of growing plant cells. Ryan and his coworkers (53) discovered 10 years ago that the leaves of potato and tomato plants that had been attacked by the Colorado potato beetle rapidly accumulate two proteinase inhibitors. The proteinase inhibitors accumulate even in unattacked leaves distant from the site of attack. The proteinase inhibitors are proteins which have been purified to homogeneity and well-characterized (53).

Ryan and his coworkers found that insects are not necessary for stimulation of inhibitors. Virtually any type of extensive crushing or tearing of the vegetative tissues of tomato, potato and other dicotyledonous plants releases PIIF into the vascular system of the plant where it is transported to other tissues of the plant and initiates accumulation of proteinase inhibitors (53).

Ryan and his colleagues found that PIIF was heat stable, but they were unable to purify PIIF to homogeneity. Nevertheless, the properties of their partially purified preparations suggested that PIIF might be a carbohydrate. Our laboratory formed a collaboration with Ryan's group and analyzed their PIIF-active fractions for carbohydrate constituents.

The first of Ryan's preparations of PIIF-active material that our laboratory examined were impure and contained a variety of different glycosyl residues connected by a still larger variety of glycosyl linkages. However, this mixture contained those characteristically-linked glycosyl residues present in rhamnogalacturonan I (49), a pectic polysaccharide accounting for approximately 7% of the walls of suspension-cultured sycamore cells. Assay of several different highly purified plant cell wall components for PIIF activity showed that rhamnogalacturonan I was the only component tested in tomatoes which possessed PIIF activity. Studies of more purified preparations of PIIF-active material extracted from tomato plants, and of other rhamnogalacturonan I preparations from sycamore have demonstrated that PIIF is, in fact, a fragment of rhamnogalacturonan I. Thus, just as with the Endogenous Elicitor, it is evident that damage of plant cells releases fragments of a cell wall polysaccharide, in this case rhamnogalacturonan I or fragments thereof, which induces the synthesis in plant cells of proteins involved in defense of the plant.

PIIF-active rhamnogalacturonan I can be released from isolated cell walls by the action of a highly purified fungal endopolygalacturonase. The PIIF-active rhamnogalacturonan I has been purified by ion exchange and gel filtration chromatography. Purified rhamnogalacturonan I has a molecular weight of approximately 200,000 and is composed of L-rhamnosyl, D-galacturonosyl, L-arabinosyl, and D-galactosyl residues in the ratio of approximately 2:5:3:3. The backbone of rhamnogalacturonan is composed predominantly, if not entirely, of D-galacturonosyl and L-rhamnosyl residues. There are about 500 glycosyl residues in the backbone, but it is not known whether the backbone is a single contiguous glycan or whether each molecule contains a number of interconnected backbone chains. About half of the rhamnosyl residues of rhamnogalacturonan I are 2-linked, have a galacturonosyl residue attached to C-2, and are glycosidically attached to C-4 of a galacturonosyl residue. The other half of the rhamnosyl residues are 2,4-linked, have a galacturonosyl residue glycosidically attached at C-2, and are glycosidically attached to C-4 of a galacturonosyl residue. Sidechains averaging six glycosyl residues in length are attached to C-4 of the 2,4-linked rhamnosyl residues. There are many different sidechains containing variously linked L-arabinosyl and/or D-galactosyl residues. The size or even the composition of the smallest rhamnogalacturonan I fragment which possesses PIIF activity is not known.

GLYCOPROTEINS SECRETED BY INCOMPATIBLE RACES (RACES THAT CAN NOT INFECT THE PLANT) OF A FUNGAL PATHOGEN OF SOYBEANS ACT AS REGULATORY MOLECULES AND PROTECT THE PLANT FROM ATTACK BY COMPATIBLE RACES

Almost all the microorganisms and other pests with which a plant comes in contact cannot successfully pathogenize the plant. The few microorganisms which are plant pathogens are often highly specialized and are pathogenic on only one or a few species of plants. Most "host-specific" pathogen species have a number of races, each of which is distinct from the others in its ability to attack various varieties (cultivars) of its host plant species. In other words, race 1 of a pathogen of a particular crop may attack variety A but not variety B, while race 2 of the pathogen may attack variety B but not variety A. Both races might be able to attack variety C and neither variety D, and so on. In this type of host-pathogen system, for each gene that governs resistance in the host plant there is a corresponding gene in the fungal pathogen that governs avirulence. This type of relationship is referred to in the plant pathology literature as a gene-for-gene host-pathogen system (21, 27, 28).

Gene-for-gene resistance in plants is determined by dominant Mendelian genes (21, 27, 28, 36). Each such resistance gene that a plant possesses can make the plant totally resistant to one or more races of at least one of its pathogens. However, a resistance gene is effective in protecting a plant against only those pathogen races which produce molecules capable of a specific interaction with the product of the resistance gene. Since these molecules of the pathogen cause the pathogen to be avirulent, the genes responsible for the synthesis of these molecules are called avirulence genes rather than virulence genes.

The interdependence of resistance and avirulence genes leads to the conclusion that the products of specific resistance genes of the host must recognize (interact with) the products of specific avirulence genes of the pathogen. This recognition reaction is the key to whether a race of a gene-for-gene pathogen will be compatible with (virulent on) a variety of its host (1, 21). A positive interaction of a product of a resistance gene with the product of an avirulence gene initiates a resistance or incompatible response in the plant.

We have hypothesized that the avirulence genes of a gene-for-gene pathogen are manifest as cell surface or extracellular structures (1). The only fungi whose surface structures have been extensively studied are the yeasts. Ballou *et al.* (9, 10) have demonstrated that in yeast the immunodominant species-specific cell surface structures are portions of mannan-containing glycoproteins. The species-specific differences in the glycoproteins reside in small differences in the structures of the carbohydrate portion of these glycoproteins.

At least some of the enzymes secreted by yeast are themelves mannan-containing glycoproteins, and the structures of the mannan portions include the same antigenically active carbohydrate structures as the species-specific cell surface mannan-containing glycoproteins (17, 55). The carbohydrate portions of the cell surface and extracellular glycoproteins are synthesized by the same glycosyl transferases (9). Thus, each species of yeast has a unique set of glycosyl transferases that is responsible for the synthesis of these species-specific antigens.

We have suggested that the products of the avirulence genes of fungal pathogens are glycosyl transferases, enzymes which function in the synthesis of complex carbohydrates which are present both on the fungal cell surface and on at least some secreted glycoproteins. We think of the products of a plant's resistance genes as receptors for the glycoproteins synthesized by the avirulence-gene encoded glycosyl transferases of the pathogen. Thus, we propose that complex carbohydrates, present on the cell surface and/or extracellular glycoproteins of the pathogen, are recognized by receptors in resistant varieties of the pathogen's host and that this interaction activates the host's defenses. If the hypothesis is correct and if the plant pathogenic fungi are similar in this respect to yeast, at least some of the glycoproteins secreted by a pathogen will contain race-specific complex carbohydrates.

We have been studying the race- and cultivar-specific interaction of soybeans and *Phytophthora megasperma* f.sp. *glycinea*, the causal agent of

root and stem rot. This host-pathogen system appears to be a gene-for-gene system, since there exist at least 16 fungal races and many differently susceptible cultivars of the host plant (45). Invertase, which is one of many proteins secreted by this pathogen, was chosen for study as a typical extracellular protein of this pathogen. As with yeast, the invertases secreted by races 1, 2, and 3 of Phytophthora megasperma f.sp. glycinea are mannan-containing glycoproteins (66). The glycosyl linkage compositions of the carbohydrate portions of the invertases produced by three different Phytophthora races are clearly different (66). The demonstration of race-specific carbohydrate structures in differentially virulent Phytophthora races provides support for the hypothesis that such complex carbohydrates are involved in determining specificity in gene-for-gene host-pathogen systems, for the only known way to discriminate between the races and the only known selection pressure to cause differences in the races is by their differing abilities to infect the various soybean cultivars.

We reasoned that if the race-specific carbohydrates of the extracellular glycoprotein population determine host-pathogen specificity, the biological activity of these molecules should be demonstrable. In other words, the extracellular glycoproteins from incompatible (avirulent) races of Phytophthora megasperma f.sp.glycinea, but not those from compatible (virulent) races, should be capable of activating a defense reaction in seedlings of a soybean cultivar which would thereby protect the seedlings from attack by compatible races of this fungal pathogen.

Our approach to demonstrating the biological activity of the extracellular glycoproteins was first to partially purify the glycoprotein fraction from the extracellular culture medium of three races of Phytophthora megasperma f.sp. glycinea. The macromolecules obtained were composed on the average of 81.5% protein and 18.5% carbohydrate. Analysis of the carbohydrate fractions showed quantitative but not qualitative differences in their composition (61). This result is similar to that obtained for the carbohydrate fractions of the extracellular invertases of these three races (66).

The important question was whether the extracellular glycoproteins of incompatible Phytophthora races can protect soybean cultivars from compatible races of this fungal pathogen. This would be expected if these glycoproteins are the race-specific determinants, that is, the biochemical expression of the avirulence genes. Experiments to answer this important question were carried out with three races of Phytophthora megasperma f.sp. glycinea and four soybean cultivars that are differentially susceptible or resistant to the races of Phytophthora. In the combinations tested, the extracellular glycoproteins from incompatible, but not from compatible, races of Phytophthora megasperma f.sp. glycinea protect seedlings from infection by compatible races of the pathogen. For example, the extracellular glycoproteins from races 1 or 2 protect the cultivar Harosoy 63, with which races 1 and 2 are incompatible, from infection by race 3, while the extracellular glycoproteins from compatible race 3 do not protect the Harosoy 63 seedlings from race 3 fungus (61). On the other hand, the extracellular glycoproteins from races 1 or 3 protect the cultivar Sanga, with which races 1 and 3 are incompatible, from the compatible race 2 fungus, although the extracellular glycoproteins from race 2, which do protect Harosoy 63 from race 3, do not protect Sanga from race 2 (61).

These positive results encouraged us. We are even more encouraged by results of our first protection experiments with the alkali-released carbohydrate portion of the race-specific glycoproteins. These experiments tentatively indicate that the carbohydrate portions, by themselves, are more effective race-specific protection factors than the intact glycoproteins.

A long term goal is to look for receptors in the soybean seedlings for the specificity factors. If our hypothesis is correct, the receptors should be present in those plants which interact with the race-specific glycoproteins, that is, in resistant cultivars, but should not be present in plants which do not interact with the race-specific glycoproteins, that is, in susceptible cultivars. The receptors are likely to be the products of the resistance genes of the soybean cultivars.

ACIDIC POLYSACCHARIDES SECRETED BY THE SYMBIOTIC NITROGEN-FIXING RHIZOBIA APPEAR TO REGULATE THE ENTRY OF THESE BACTERIA INTO THE ROOTS OF LEGUMES

A great many publications have demonstrated the essential function of the cell surface and extracellular polysaccharides of Gram-negative bacteria in the interaction of these bacteria with other cells, including the cells of both plants and animals (1, 38, 51). The nitrogen-fixing Rhizobium are Gram-negative bacteria, therefore their surface and extracellular polysaccharides are likely to be active as regulatory molecules by determining with which higher plants the Rhizobium can form symbiotic nitrogen-fixing relationships. This hypothesis has been supported by the results described in this section.

Numerous extracellular polysaccharides of Gram-negative bacteria have been structurally characterized, and these polysaccharides are, in general, serotype or species specific. This also appears to be generally true for Rhizobium species for, with the possible exception of R. leguminosarum and R. trifolii, the acidic polysaccharides secreted by the various Rhizobium species appear to be nodulation group specific, that is, Rhizobium which nodulate different legumes secrete different extracellular polysaccharides. For example, R. japonicum (25) which nodulates and fixes nitrogen in soybeans secretes a markedly different acidic polysaccharide than does R. meliloti (39) which nodulates alfalfa. Rhizobium leguminosarum, the pea symbiont, R. trifolii, the clover symbiont, and R. phaseoli, the true bean symbiont, are the most closely related Rhizobium species (40, 60). We have found that R. phaseoli secretes an acidic polysaccharide with a different structure than that secreted by R. leguminosarum and R. trifolii (P. Åman, L.-E. Franzén, M. McNeil, A. Darvill and P. Albersheim, unpublished results). However, we have also shown that the acidic polysaccharides secreted by R. leguminosarum and R. trifolii have basically the same structures (52). The structure of the acidic extracellular polysaccharides produced by R. leguminosarum and R. trifolii, both fast-growing rhizobia, has some similarities to the acidic polysaccharides secreted by R. meliloti and R. phaseoli, which are also fast-growing species. However, there is no relationship between the structures of the acidic polysaccharides secreted by the fast-growing Rhizobium species and the acidic polysaccharide secreted by slow-growing R. japonicum.

We have established that the glycosyl residue sequence and the anomeric configurations of the glycosyl linkages of the acidic polysaccharides secreted by two R. trifolii and two R. leguminosarum strains are identical. We have not, however, investigated the possibility of differently substituted acetyl, succinyl, or other alkali-labile residues in these polysaccharides. Therefore, it is not yet established that the acidic extracellular polysaccharides from R. leguminosarum and R. trifolii have identical structures. Analyses for alkali-labile substituents are important, for Jansson et al. (39) have determined that the acidic polysaccharide secreted by R. trifolii U226 possesses at least one O-acetyl residue per repeating unit; the O-acetyl residue(s) is attached to C-2 and/or C-3 of a 4-linked glucosyl residue(s). It remains to be ascertained whether the R. leguminosarum polysaccharide has the same acetyl substitution.

Rhizobium leguminosarum is a symbiont of pea (Pisum sativum) and R. trifolii a symbiont of clover (Trifolium pratense). In some instances these two species cross nodulate their legume hosts. Some strains of R. leguminosarum nodulate Trifolium species and some strains of R. trifolii nodulate Pisum species (34, 35, 50, 59), although the nodules formed in each case are unable to fix nitrogen. Both R. leguminosarum and R. trifolii cause curling and branching of root hairs in a host of R. trifolii, Trifolium glomeratum, phenomena generally induced only by Rhizobium capable of forming a symbiosis with that legume (59, 63). Rhizobium leguminosarum and R. trifolii have also been reported to have a high degree of homology between their DNA molecules (40). Thus, it is not very surprising that the polysaccharides secreted by R. leguminosarum and R. trifolii are very similar if not identical. The facts that R. leguminosarum and R. trifolii can in some instances cross nodulate legumes and that these two Rhizobium species secrete identical or nearly identical acidic polysaccharides supports the hypothesis that the secreted acidic polysaccharides participate as regulatory molecules in the recognition processes which permit rhizobia to enter their host legumes.

Strong support for a regulatory function of acidic polysaccharides secreted

by Rhizobium species is provided by findings of W.D. Bauer and his coworkers at the Charles Kettering Institute. They have obtained evidence that the acidic polysaccharides are required for the development of legume root hairs capable of being infected by symbiont rhizobia; root hairs developed in the absence of these polysaccharides can not be infected (15, and personal communication). Therefore, these polysaccharides constitute a good example of complex carbohydrates with regulatory properties, in this case an ability to cause a specific differentiation of the epidermal cells of legume roots.

RHAMNOGALACTURONAN II - AN EXTRAORDINARILY COMPLEX POLYSACCHARIDE PRESENT IN THE WALLS OF GROWING PLANT CELLS

We have recently isolated, from the walls of suspension-cultured sycamore cells, a previously unknown pectic polysaccharide called rhamnogalacturonan II (19). Rhamnogalacturonan II, which accounts for about 4% of the cell wall, is completely solubilized from the walls of suspension-cultured sycamore cells by the action of an endo-α-1,4-polygalacturonase and separated from the other solubilized pectic polysaccharides by anion exchange and gel permeation chromatography.

A brief description of rhamnogalacturonan II is included here because the extreme structural complexity of this molecule suggests that it too will be found to function as a regulatory molecule. This polysaccharide, which has been purified to apparent homogeneity, possesses a well-defined structure and molecular size. As isolated, rhamnogalacturonan II contains a total of about 50 glycosyl residues. It contains nine different glycosyl constituents including the rarely observed sugars 2-O-methyl fucose, 2-O-methyl xylose, which have nevertheless previously been recognized to be trace components of pectic polymers (3, 4, 5, 11), and apiose, a branched pentose, which has also been recognized as a component of the pectic polysaccharides of Lemna species, but the Lemna-type apiose-containing pectic polysaccharide has not been found to be widespread in nature and is not structurally related to rhamnogalacturonan II. Apiose and the 2-O-methyl derivatives of fucose and xylose have never previously been recognized to be associated in a single polysaccharide, although all three sugars have been isolated from leaves of deciduous trees (7).

Rhamnogalacturonan II is characterized by many different terminal glycosyl residues including terminal galacturonosyl, terminal galactosyl, terminal arabinosyl, terminal 2-O-methyl xylosyl, terminal 2-O-methyl fucosyl, and terminal rhamnosyl residues. The large content of terminal glycosyl residues and of a variety of branched glycosyl residues indicates a highly branched structure. Rhamnogalacturonan II also contains a number of unusually linked glycosyl residues including 2-linked glucuronosyl, 3'-linked apiosyl, 3-linked rhamnosyl, 2,4-linked galactosyl, and 3,4-linked fucosyl residues. The glycosyl composition of rhamnogalacturonan II remains constant throughout the lag, log and stationary phases of growth of suspension-cultured sycamore cells. We also have evidence that a molecule very similar or identical to rhamnogalacturonan II is present in the primary cell walls of the four other dicots examined; namely, pea, French bean and tomato seedlings, and suspension-cultured tobacco cells.

It is interesting to consider how a polysaccharide as complex as rhamnogalacturonan II is synthesized; synthesis by any of the known pathways would require on the order of 100 enzymes. This is an enormous investment by the cell to achieve structural complexity in a polymer that represents only 4% of the wall. Why is there such an investment? We can't help but think that the reason has been to evolve a molecule with regulatory functions. We are very curious to learn the function of this molecule.

CONCLUDING REMARKS

It has been an exciting experience for us to realize that the plant cell wall polysaccharides, whose structures we have been struggling to decipher, are functioning not only as structural polymers but also in a regulatory capacity. At least two of the complex polysaccharides which are present in the walls of growing plant cells contain fragments which possess the remarkable properties of hormones, that is, molecules formed by one cell which in minute amounts, stimulate receptive cells to synthesize specific proteins. Two different pectic polysaccharides contain within themselves the glycosyl sequences which constitute either the plant hormone known as PIIF or the Endogeneous Elicitor. PIIF and the Endogenous Elicitor are

apparently released from the cell walls surrounding injured cells and then stimulate receptive cells to synthesize proteins involved in defense of the plant.

Two of the other complex carbohydrates described in this paper, the β-Glucan Elicitor and the acidic polysaccharides secreted by rhizobia, also appear to possess the attributes of hormones, except that these regulatory carbohydrates are produced by one organism and affect receptive cells in another organism.

PIIF, the Endogeneous Elicitor, and the β-Glucan Elicitor have, in addition to being carbohydrates with regulatory properties, two other characteristics in common. They are "stored" as insoluble cell wall polysaccharides; and they are released or "activated" by cleavage of the wall polysaccharides, presumably by specific enzymes.

The fact that the walls of growing plant cells contain these oligosaccharide "hormones" means that the walls function as a "pseudogland" containing regulatory molecules which can be released as needed. We can envision the cell wall containing a variety of messages capable of controlling physiological processes of a developing plant.

The fact that these oligosaccharide hormones originate as portions of larger polymers is strikingly similar to the origin of a number of animal peptide hormones. For example, several polypeptide hormones synthesized in the pituitary gland originate in a common precursor polypeptide (47). A 16,000 dalton fragment of the precursor polypeptide is removed and the remaining polypeptide cleaved to produce the hormones corticotropin and β-lipotropin. The corticotropin can be further cleaved to yield α-melanotropin, and the β-lipotropin can be cleaved to yield α-lipotropin and β-endorphin. This process is analogous to cleavage of a wall polysaccharide to yield biologically active fragments.

We hope that the knowledge that plant cell wall polysaccharides possess a number of interesting biological functions will stimulate other laboratories to focus on unraveling their complex structures. Certainly, the increasing realization that complex carbohydrates play key roles in biological recognition processes will stimulate efforts to develop rapid and efficient methods for the structural characterization and synthesis of these molecules.

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REFERENCES

1. P. Albersheim and A.J. Anderson-Prouty, Ann. Rev. Plant Pathol. 26, 31-52 (1975).
2. P. Albersheim and B.S. Valent, J. Cell Biol. 78, 627-643 (1978).
3. G.O. Aspinall and A. Canas-Rodriguez. J. Chem. Soc. 4020 (1958).
4. G.O. Aspinall and R.S. Fanshawe, J. Chem. Soc. (C), 4215 (1961).
5. G.O. Aspinall, K. Hunt and I.M. Morrison, J. Chem Soc. (C), 1945-1949 (1966).
6. A.R. Ayers, J. Ebel, B. Valent, P. Albersheim, Plant Physl. 57, 760-765 (1976).
7. J.S.D. Bacon and M.V. Cheshire, Biochem. J. 124, 555-562 (1971).
8. J.A. Bailey, J. Gen. Micro. 61, 409-415 (1970).
9. C.E. Ballou, Advances in Enzymology, 40, 239-270, Wiley, New York (1974).
10. C.E. Ballou and W.C. Raschke, Science 184, 127-134 (1974).
11. A.J. Barrett and D.H. Northcote, Biochem. J. 94, 617-627 (1965).
12. S. Bartnicki-Garcia, Ann. R. Micro. 22, 87-108 (1968).
13. D.F. Bateman, Biochemical Aspects of Plant-Parasite Relationship, pp.

103, Academic Press, London, (1976).
14. D.F. Bateman and H.G. Basham, Encyclopedia of Plant Physiology, Springer-Verlag, Berlin, 4, 316-355 (1976).
15. W.D. Bauer, T.V. Bhuvaneswari, A.J. Mort and G. Turgeon, Plant Physl. supplement 63(5), 135 (1979).
16. W.D. Bauer, K. Talmadge, K. Keegstra and P. Albersheim, Plant Physl. 51, 174-187 (1973).
17. P. Biely, Z. Krátký and S. Bauer, Eur. J. Bioch. 70, 75-81 (1976).
18. K. Cline, M. Wade and P. Albersheim, Plant Physl. 62, 918-921 (1978).
19. A. Darvill, M. McNeil and P. Albersheim, Plant Physl. 62, 418-422 (1978).
20. J. Darvill, M. McNeil, A.G. Darvill and P. Albersheim, submitted for publication (1980).
21. P.R. Day, Genetics of Host-Parasite Interaction, Freeman, San Francisco (1974).
22. B. Deverall, Defense Mechanisms of Plants, Cambridge Univ. Press, London (1977).
23. R.A. Dixon and D.S. Bendall, Physl.Pl. P. 13, 295-306 (1978).
24. R.A. Dixon and C.J. Lamb. 1979. Bioc. Biop. A. 586, 453-463 (1979).
25. W.F. Dudman, Carbohy. Res. 66, 9-23 (1978).
26. J. Ebel, B. Schaller-Hekeler, K.-H. Knobloch, E. Wellman, H. Grisebach and K. Hahlbrock, Bioc. Biop. A. 362, 417-424 (1974).
27. H.H. Flor, Advan. Genet. 8, 29-54 (1956).
28. H.H. Flor, Ann. R. Phyto. 9, 275-296 (1971).
29. J.M. Gardner and C.I. Kado, J. Bact. 127, 451-460 (1976).
30. H. Grisebach and J. Ebel, Angew. Chem. 17, 635-647 (1978).
31. M. Hahn and P. Albersheim, Plant Physl. 62, 107-111 (1978).
32. J.A. Hargreaves and J.A. Bailey, Physl. Pl. P. 13, 89-100 (1978).
33. J.A. Hargreaves and C. Selby, Phytochem. 17, 1099-1102 (1978).
34. C.M. Hepper, Ann. Bot. 42, 109-115 (1978).
35. C.M. Hepper and L. Lee, Plant and Soil 51, 441-445 (1979).
36. A.L. Hooker and K.M.S. Saxena, Ann. R. Genet. 5, 407-423 (1971).
37. J.L. Ingham, Botan. Rev. 38, 343-424 (1972).
38. K. Jann and O. Westphal, The Antigens, Academic Press, London (1975).
39. P.-E. Jansson, L. Kenne, B. Lindberg, H. Ljunggren, J. Lönngren, U. Rudén and S. Svensson, J. Am. Chem. S. 99, 3812-3815 (1977).
40. A.W.B. Johnston and J.E. Beringer, Nature 267, 611-613 (1977).
41. N. Keen and B. Bruegger, ACS Symp. S. 62, Washington, D.C. (1977).
42. N. Keen, J. Partridge and A. Zaki, Phytopathol. 62, 768 (1972).
43. J. Kuć, Ann. R. Phyto. 10, 207-232 (1972).
44. J. Kuć and W. Currier, Adv. Chem. Se. 149, 356-368 (1976).
45. F.A. Laviolette and K.L. Athow, Phytopathol. 67, 267-268 (1977).
46. R.A. Lerner and D. Bergsma, The Molecular Basis of Cell-Cell Interaction, XIV (2), Liss, New York (1978).
47. A.S. Liotta, C. Loudes, J.F. McKelvy and D.T. Krieger, PNAS USA 77, 1880-1884 (1980).
48. V.T. Marchesi, V. Ginsburg, P.W. Robbins and C.F. Fox, Cell Surface Carbohydrates and Biological Recognition, Liss, New York (1978).
49. M. McNeil, A.G. Darvill and P. Albersheim, Plant Physl., in press (1980).
50. J. Mleczhowska, P.S. Nutman and G. Bond. J. Bact. 48, 673-675 (1944).
51. I. Ørskov, F. Ørskov, B. Jann and K. Jann, Bact. Rev. 41, 667-710 (1977).
52. B. Robertsen, P. Aman, A.G. Darvill, M. McNeil and P. Albersheim, submitted for publication (1980).
53. C.A. Ryan, Trends Bioc. July, 148-150 (1978).
54. J.H. Sietsma and J.G.H. Wessels, Bioc. Biop. A. 496, 225-239 (1977).
55. W.L. Smith and C.E. Ballou, Biochem. 13, 355-361 (1974).
56. M. Stekoll and C.A. West, Plant Physl. 61, 38-45 (1978).
57. C. Town and E. Stanford, PNAS USA 76, 308-312 (1979).
58. H. VanEtten and S. Pueppke, Biochemical Aspects of Plant-Parasitic Relationships, pp. 239-289, Academic Press, London (1976).
59. J.M. Vincent, The Biology of Nitrogen Fixation, pp. 265-341, Amer. Elsevier, New York (1974).
60. J.M. Vincent, A Treatise on Dinitrogen Fixation, pp. 277-366, Wiley, New York, (1977).
61. M. Wade and P. Albersheim, PNAS USA 76, 4433-4437 (1979).
62. R.K.S. Wood, Proc. Symp. Hungar. Acad. Sci., pp. 107-115 (1977).
63. P.Y. Yao and J.M. Vincent, Aust. J. Biol. Sci. 22, 413-423 (1969).
64. U. Zähringer, J. Ebel and H. Grisebach, Arch. Bioch. 188, 450-455 (1978).
65. U. Zähringer, J. Ebel, L.J. Mulheirn, R.L. Lyne and H. Grisebach, FEBS Letters 101, 90-92 (1979).
66. E. Ziegler and P. Albersheim, Plant Physl. 59, 1104-1110 (1977).