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Update on Plant Defense Proteins

Lectins as Plant Defense Proteins[1]

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Many plant species contain carbohydrate-binding proteins, which are commonly referred to as either lectins or agglutinins. Generally speaking, lectins are proteins that bind reversibly to specific mono- or oligosaccharides. Since the initial discovery of a hemagglutinating factor in castor bean extracts by Stillmark in 1888, several hundred of these proteins have been isolated and characterized in some detail with respect to their carbohydrate-binding specificity, molecular structure, and biochemical properties. Lectins from different plant species often differ with respect to their molecular structure and specificity. It is important, therefore, to realize that all plant lectins are artificially classified together solely on the basis of their ability to recognize and bind carbohydrates. Moreover, the question arises whether proteins with a completely different structure and sugar-binding specificity fulfill the same physiological role. No conclusive answer can be given to this question as yet, for the simple reason that the role of most plant lectins is not known with certainty. There is, however, growing evidence that most lectins play a role in the plant's defense against different kinds of plant-eating organisms. The idea that lectins may be involved in plant defense is not new. In an earlier review, Chrispeels and Raikhel (1991) critically assessed the defensive role of the phytohemagglutinin family and a number of chitin-binding proteins. During the last few years important progress has been made in the study of plant lectins in general and in the understanding of their effects on other organisms in particular. In this *Update* we summarize the recent developments that support the defensive role of plant lectins and, in addition, discuss earlier work in this field against the background of our present knowledge of this group of plant proteins.

TERMINOLOGY AND DEFINITION

Before discussing their role in plant defense we have to delineate the group of proteins that fall within the limits of the concept lectin. The term lectin initially referred to the ability of some carbohydrate-binding proteins to selectively agglutinate erythrocytes of a particular human blood group (from the Latin verb *legere*, which means "to select"). Since the term lectin was not strictly adhered to and was widely applied to all proteins showing more general agglutination behavior, the original meaning of the word "lectin" was lost. From this point of view the name "agglutinin," which is used as a synonym for lectin, may be more correct, because it refers to the ability of the carbohydrate-binding proteins to agglutinate erythrocytes or other cells.

Earlier definitions of lectins as carbohydrate-binding proteins of nonimmune origin that agglutinate cells or as carbohydrate-binding proteins other than antibodies or enzymes require an update, since the molecular cloning of lectins and lectin-related proteins has led to new insights. First, some plant enzymes are fusion proteins composed of a carbohydrate-binding and a catalytic domain. Class I chitinases, for instance, are built up of a chitin-binding hevein domain and a catalytic domain, which are separated by a hinge region (Collinge et al., 1993). Similarly, the so-called type 2 RIPs, such as ricin and abrin, are fusion products of a toxic A chain (which has the *N*-glycosidase activity characteristic of all RIPs) and a carbohydrate-binding B chain (Barbieri et al., 1993). Second, several carbohydrate-binding proteins possess only one binding site and, therefore, are not capable of precipitating glycoconjugates or agglutinating cells. For instance, the nonagglutinating Man-binding proteins from orchids are very similar to the dimeric Man-specific lectins from the same species except that they occur as monomers (Van Damme et al., 1994). Third, several legume species contain proteins that are clearly related to the lectins but are devoid of carbohydrate-binding activity. Well-known examples of this group of proteins are the *Phaseolus vulgaris* arcelins and the α-amylase inhibitor (Mirkov et al., 1994).

In our opinion the presence of at least one noncatalytic domain that binds reversibly to a specific carbohydrate is the only prerequisite for a protein to be named a lectin. Consequently, plant lectins can be defined as all plant proteins that possess at least one noncatalytic domain that binds reversibly to a specific mono- or oligosaccharide. This new definition includes a broad range of proteins that behave quite differently from the point of view of their agglutination and/or glycoconjugate precipitation properties. Based on their overall structure three major types of

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Abbreviations: PHA, *Phaseolus vulgaris* agglutinin; RIP, ribosome-inactivating protein; WGA, wheat germ agglutinin.

lectins are distinguished, namely "merolectins," "hololectins," and "chimerolectins" (Fig. 1). Merolectins are proteins that are built exclusively of a single carbohydrate-binding domain. They are small, single polypeptide proteins, which because of their monovalent nature are incapable of precipitating glycoconjugates or agglutinating cells. Examples of this group are hevein (Van Parijs et al., 1991) and the monomeric Man-binding proteins from orchids. Hololectins also are built exclusively of carbohydrate-binding domains but contain two or more such domains that are either identical or very homologous. This group comprises all lectins that have multiple binding sites and, hence, are capable of agglutinating cells or precipitating glycoconjugates. Obviously, the majority of all known plant lectins are hololectins, because they behave as hemagglutinins. Chimerolectins are fusion proteins possessing a carbohydrate-binding domain tandemly arrayed with an unrelated domain, which has a well-defined catalytic activity (or another biological activity) that acts independently of the carbohydrate-binding domain. Depending on the number of sugar-binding sites, chimerolectins behave as merolectins or hololectins. For instance, type 2 RIPs with two carbohydrate-binding sites on their B chain (e.g. ricin) agglutinate cells, whereas class I plant chitinases with a single chitin-binding domain do not.

SOME GENERAL CONSIDERATIONS ABOUT THE PHYSIOLOGICAL ROLE OF PLANT LECTINS

Ever since the discovery of lectins, scientists have been intrigued by their possible roles. A breakthrough occurred when it was understood that most plant lectins may not only play a role in the plant itself, for instance, as a store of nitrogen or as a specific recognition factor, but also interact with glycoconjugates of other organisms. They interfere with the normal functioning of that organism. It is unlikely that all plant lectins will be found to play a role in plant defense. Lectins that occur at low concentrations may be involved in specific recognition processes either within or outside the plant. Legume root lectins, for instance, may be involved in the recognition and/or binding of *Rhizobium* and *Bradyrhizobium* sp. for the purpose of establishing symbioses (Diaz et al., 1989; Bohlool and Schmidt, 1974).

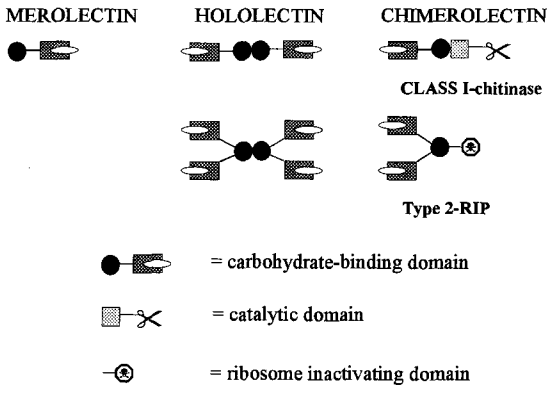

Figure 1. Schematic representation of the three types of plant lectins: merolectins, hololectins, and chimerolectins.

INDIRECT EVIDENCE FOR A DEFENSIVE ROLE OF PLANT LECTINS

Since lectins distinguish themselves from all other plant proteins by their specific carbohydrate-binding activity, one can reasonably assume that their physiological role involves their sugar-binding properties. In principle, any lectin-mediated reaction or process relies on the specific binding of the lectin to a glycoconjugate receptor (irrespective of whether this receptor is located within or outside the plant). Therefore, the search for the physiological role of plant lectins was always intimately linked to the search for their natural receptors. In the case of lectins, receptors can be defined as glycoconjugates that possess a carbohydrate moiety with a structure complementary to that of the binding site of the lectin. This implies that glycoconjugates of different nature (e.g. glycoproteins, glycolipids, and polysaccharides) but with identical (or structurally similar) carbohydrates can act as receptors for the same lectin.

Although the first discovery of a plant lectin was based on the binding of the castor bean lectin to glycoconjugates on the membranes of human red blood cells, it was assumed that lectin receptors must be present in the plant itself. However, since the search for endogenous receptors (also called lectin binders) did not yield significant data, this idea was gradually abandoned. With the exception of some enzymes, e.g. some types of chitinases, glucanases, and glycosidases, lectins are the only plant proteins that are capable of recognizing and binding glycoconjugates present on the surface of microorganisms (i.e. bacteria and fungi) or exposed along the intestinal tract of insect or mammalian herbivores. Given the diversity of microbial and animal glycans, the broad spectrum of carbohydrate-binding specificities of lectins can be interpreted as an indication of the plant's successful development of recognition/reaction molecules against different types of sugar-containing receptors.

Molecular, biochemical, cellular, physiological, and evolutionary arguments indicate that lectins have a role in plant defense. A major argument for this role is the observation that plant lectins bind glycoconjugates of other organisms. Although many plant lectins are able to bind simple sugars such as Glc, Man, or Gal, they have a much higher affinity for oligosaccharides, which are not common or totally absent in plants. For instance, chitin-binding plant lectins recognize a carbohydrate that is a typical constituent of the cell wall of fungi and the exoskeleton of invertebrates. Similarly, the sialic acid-binding lectins from elderberry (*Sambucus* sp.) (Shibuya et al., 1987) and *Maackia amurensis* (Knibbs et al., 1991) bind to a sugar that is absent in plants but is a major carbohydrate component of animal glycoproteins. The same holds true for all lectins that bind exclusively to the complex (modified) oligosaccharide side chains of typical animal glycoproteins.

A circumstantial argument in favor of a defense role of plant lectins is their marked stability under unfavorable conditions. Most lectins are stable over a wide pH range, are able to withstand heat, and are resistant to animal and insect proteases. In these respects, they strongly resemble other defense-related proteins such as some pathogenesis-

related proteins, protease inhibitors, chitinases and glycanases, RIPs, α-amylase inhibitors, antifungal proteins, and thionins. However, lectins are degraded in vivo, and some plant herbivores possess gut proteases capable of digesting the plant lectins present in their diet.

The preferential association of lectins with those parts of the plant that are most susceptible to attack by foreign organisms is also an argument for a protective role. Although the whole plant is exposed to a continuous threat of pests and diseases, some tissues or organs need extra protection, since they play a key role in the survival of the individual or the species. Resting storage organs and seeds are particularly vulnerable, since they are most attractive to potential parasites and predators and may lack an active defense system (because of their inactive metabolic state). For instance, seeds infested by a seed-borne insect or bulbs devoured by a vole are usually not viable anymore. In contrast, a growing plant that is half-eaten by insects may survive and even produce viable offspring. Taking into account the evolutionary adaptation of plants, one can reasonably argue that they have developed (passive) defense systems to protect their storage organs and seeds. From this point of view the preferential accumulation of lectins in typical storage organs is certainly indicative. Moreover, since most of these lectins are present in large quantities and, in addition, behave as storage proteins, some plants accumulate a part of their nitrogen reserve as carbohydrate-binding proteins.

DIRECT EVIDENCE THAT LECTINS PLAY A ROLE IN PLANT DEFENSE

Although the ideas discussed above favor a defense-related role for plant lectins, they would be greatly strengthened by direct evidence obtained using purified protein in artificial diets or by using transgenic plants. The effects of a special class of lectins, namely the type 2 RIPs, are briefly reviewed first, followed by a discussion of other lectins that show some selectivity for certain classes of organisms.

TYPE 2 RIPS: A SPECIAL CLASS OF CHIMEROLECTINS WITH A GENERAL TOXICITY TOWARD ALL EUKARYOTES

Type 2 RIPs are known to be potent cytotoxic agents. The sugar-binding B chain binds to a (glycoconjugate) receptor on the cell surface, thereby promoting the uptake of the A chain. After its entry into the cell the A chain catalytically inactivates eukaryotic ribosomes by cleaving the *N*-glycosidic bond of a single adenosine residue of the large rRNA. In principle, type 2 RIPs are extremely toxic to all eukaryotes if they reach the cytoplasm.

The deadly effect of type 2 RIPs on higher animals (including humans) has existed since ancient times. Insects seem to react differentially upon feeding type 2 RIPs. Ricin was highly toxic to the coleoptera *Callosobruchus maculatus* and *Anthonomus grandis* but had no effect on the lepidoptera *Spodoptera litoralis* and *Heliothis virescens* (Gatehouse et al., 1990). The fact that some insects survive a ricin-containing diet indicates that they either can inactivate the toxin or do not bind the toxin. Another type 2 RIP, namely the lectin from winter aconite (*Eranthis hyemalis*) (Kumar et al., 1993), was very toxic to larvae of *Diabrotica undecimpunctata* (a major insect pest of maize). It seems likely, therefore, that type 2 RIPs offer the plant good protection against animals and probably also against some insects.

In principle, type 2 RIPs are also toxic to fungi. However, since they cannot penetrate the cytoplasm, it is difficult to imagine that they have a direct deleterious effect on invading fungi. Bacterial ribosomes are insensitive to type 2 RIPs, which implies that these toxins cannot exert any direct effect on bacteria. Surprisingly, type 2 RIPs exhibit an inhibitory activity against plant viruses, although the mechanism of action is unknown. Possibly the RIPs included in the virus suspension used for the infectivity tests kill the wounded plant cells by inactivating the ribosomes and thereby provoke a form of hypersensitive response.

THE ANTIVIRAL ACTIVITY OF PLANT LECTINS

With the exception of the above-described type 2 RIPs no other plant lectins have been reported to inhibit viral infection, replication, or systemic spread. Although this lack of evidence does not preclude a possible antiviral effect of plant lectins, it seems logical in view of the absence of glycans on plant viruses. It is worth mentioning in this context that several plant lectins are potent inhibitors in vitro of animal and human viruses, which have glycoproteins in their virions (Balzarini et al., 1992). Some plant lectins may have an indirect antiviral role. For instance, the presence of insecticidal lectins may prevent and/or reduce the spread of insect-transmitted viral diseases.

THE ANTIBACTERIAL ACTIVITY OF PLANT LECTINS

The cell wall of bacteria not only precludes any interaction between the glycoconjugates on their membrane and carbohydrate-binding proteins but also prevents these proteins from penetrating the cytoplasm. Therefore, plant lectins cannot alter the structure and/or permeability of the membrane or disturb the normal intracellular processes of invading microbes. Therfore, if lectins play a role in the plant's defense against bacteria, it must be through an indirect mechanism that is based on interactions with cell wall carbohydrates or extracellular glycans. It has been suggested, for instance, that the potato lectin (which is considered as a cell wall protein) immobilized avirulent strains of *Pseudomonas solanacearum* in the cell wall (Sequeira and Graham, 1977). Virulent strains were not recognized by the lectin, escaped attachment to the cell wall, and therefore were able to multiply and spread over the plant. Unfortunately, the presumed antibacterial activity of the potato lectin was inferred from in vitro experiments with lectin preparations of unknown purity. Consequently, the results have to be interpreted with care.

Another indirect defense mechanism is the blocking of the movements of normally motile bacteria at the air-water interface by the thorn apple (*Datura stramonium*) seed lectin

(Broekaert and Peumans, 1986). Since essentially pure (affinity-purified) lectin preparations were used in these experiments and the effects were fully reversed by fetuin (which is firmly bound by the lectin), the loss of motility could be ascribed with near certainty to the lectin. The lectin-mediated block of bacterial motility in vitro was correlated with the rapid and highly specific release (during imbibition) of the lectin from the seed coat and the seed epidermis. By counteracting the chemotactic movement of soil bacteria toward the germinating seed, the lectin may prevent invasion of the seedling roots by potentially harmful bacteria. Since recent studies of the binding of plant lectins to bacterial cell wall peptidoglycans indicated that several legume seed lectins strongly interact with muramic acid, *N*-acetylmuramic acid, and muramyl dipeptide, the involvement of lectins in the plant's defense against microbes may have been underestimated (Ayouba et al., 1994).

THE ANTIFUNGAL ACTIVITY OF PLANT LECTINS

Since plant lectins cannot bind to glycoconjugates on the fungal membranes or penetrate the cytoplasm of the cells because of the presence of a thick and rigid cell wall, a direct interference with the growth and development of these organisms (i.e. through an alteration of the structure and/or permeability of the membrane or a disturbance of the normal intracellular processes) seems unlikely. However, indirect effects based on the binding of lectins to carbohydrates exposed on the surface of the fungal cell wall are possible.

By virtue of their specificity, chitin-binding lectins seemed likely to have a role in the plant's defense against fungi (and insects). In vitro studies, demonstrating that WGA inhibited spore germination and hyphal growth of *Trichoderma viride*, strongly supported the hypothesis of the antifungal role of the chitin-binding plant lectins, until it was shown that the inhibition of fungal growth was due to contaminating chitinases in the lectin preparation (Schlumbaum et al., 1986). Although this finding at first compromised the proposed antifungal activity of plant lectins, more definitive proof followed when it was demonstrated that chitinase-free lectin from stinging nettle (*Urtica dioica*) inhibited the growth of *Botrytis cinerea*, *Trichoderma hamatum*, and *Phycomyces blakesleeanus* (Broekaert et al., 1989). The exact mechanism of the nettle lectin has not been elucidated yet, but it is certainly not based on a chitinase activity and it does not affect the normal metabolism of the fungal cells. Only the synthesis of the cell wall appears to be affected as a result of disturbed chitin synthesis and/or deposition (Van Parijs et al., 1992). In spite of the in vitro antifungal activity of the nettle lectin, it is still unknown whether it has any protective activity in vivo, since the lectin is not capable of killing germinating spores or mycelium. Considering the modifying effects of the nettle lectin on the fungal cell wall and the morphology of the hyphal structure, we believe that the nettle lectin is involved in the control of the colonization of the rhizomes by endomycorrhiza. Such a role is in partial agreement with the location of the lectin in rhizomes and seeds.

Several other chitin-binding plant proteins, which according to our definition have to be regarded as lectins, have antifungal properties. The first group is the chitin-binding merolectins, which are small proteins composed of a single chitin-binding domain. Hevein, a 43-amino acid polypeptide from the latex of the rubber tree (*Hevea brasiliensis*), has an antifungal activity comparable to that of the nettle lectin (Van Parijs et al., 1991). Other proteins of the same group, e.g. the 30-amino acid chitin-binding polypeptide from seeds of *Amaranthus caudatus*, have more potent antifungal properties but still are unable to kill the fungi (Broekaert et al., 1992). The only plant lectins that can be considered fungicidal proteins are the chimerolectins belonging to the class I chitinases. In vitro tests with the purified enzymes as well as experiments with transgenic plants have demonstrated that class I chitinases confer resistance against plant pathogenic fungi. However, since the antifungal properties of these proteins reside in their catalytic rather than carbohydrate-binding domain, a detailed description of their protective role falls beyond the scope of this *Update* (but see Collinge et al., 1993).

THE ANTI-INSECT ACTIVITY OF PLANT LECTINS

The epithelial cells along the digestive tract of phytophagous insects are directly exposed to the contents of the diet and, therefore, are possible target sites for plant defense proteins. Since glycoproteins are major constituents of these membranes, the luminal side of the gut is literally covered with potential binding sites for dietary lectins. One can easily imagine that when the binding of a lectin to a glycoprotein receptor provokes a local or systemic deleterious effect the insect may be repelled, retarded in its growth, or even killed.

The PHA was the first lectin to which anti-insect properties were ascribed on the basis of its deleterious effect on the larvae of bruchid beetle *Callosobruchus maculatus* (cowpea weevil). Ironically, this first indication for a protective role of lectins against insects was based on a false-positive result, since the effects were due to a contaminating α-amylase inhibitor (Huesing et al., 1991b). More recent experiments indicated that the lectins from wheat germ, potato tuber, and seeds from peanut, thorn apple (*Datura stramonium*), and osage orange (*Maclura pomifera*) had an inhibitory effect on the development of larvae of the cowpea weevil. However, only WGA was active at a physiological concentration (Murdock et al., 1990). The chitin-binding lectins from rice (*Oryza sativa*) and stinging nettle also inhibited larval growth of the cowpea weevil (Huesing et al., 1991a), but it appears that this typical seed predator is insensitive to most plant lectins and is only moderately affected by the presumed toxic lectins (such as WGA and the rice and nettle lectins).

The European corn borer (*Ostrinia nubilalis*) and the Southern corn rootworm (*Diabrotica undecimpunctata*) both feed on maize plants (Czapla and Lang, 1990). WGA and the *Bauhinia purpurea* seed lectin were lethal to neonate *Ostrinia nubilalis* larvae at fairly low concentrations. Similarly, the pokeweed (*Phytolacca americana*) lectin killed larvae of the Southern corn rootworm, whereas several other

lectins, including WGA, inhibited larval growth. Therefore, both maize insects appear to be much more sensitive to dietary lectins than the seed predator cowpea weevil. Promising results have also been obtained with a few Man-binding lectins from monocotyledonous plants. Feeding trials with purified lectins from snowdrop (*Galanthus nivalis*) and garlic (*Allium sativum*) indicated that they are moderately active against chewing insects, such as the cowpea weevil and the tobacco horn worm (*Spodoptera litoralis*). More importantly, however, the snowdrop lectin showed a high toxicity toward sucking insects not only in tests with artificial diets but also in experiments with transgenic plants (Hilder et al., 1995).

Although it is very likely that their toxicity is based on a specific binding to glycoconjugates somewhere in the gut of the insect, the exact mechanism of action of plant lectins is not known. Three types of interactions are possible, namely (a) binding of lectins to the chitin in the peritrophic membrane (only for chitin-binding lectins), (b) binding of lectins to glycoconjugates exposed on the epithelial cells along the digestive tract, and (c) binding of lectins to glycosylated digestive enzymes.

THE TOXICITY OF PLANT LECTINS FOR HIGHER ANIMALS

Like in insects, the epithelial cells along the digestive tract of higher animals are fully exposed to the contents of the diet. It can be expected, therefore, that plants developed defense proteins against predators that act through an interaction at this site. Since the cells at the luminal site of the digestive tract are covered with membrane glycoproteins and highly glycosylated mucins, there are countless targets for interactions with dietary plant lectins. Taking into consideration the diversity of lectins and their specificity for animal glycans, one can predict that these proteins protect plants against animal predators. Fortunately, the toxicity of lectins toward higher animals is well documented because of studies assessing the possible health risk of lectins present in plants used in food and feed production.

A great deal of our present knowledge about the toxic properties of plant lectins and the effects they provoke in animals and humans have been obtained from feeding experiments with purified PHA and accidental poisoning of humans by raw or insufficiently cooked beans. Ingested PHA, which is highly resistant to gut proteases, binds to the brush border cells of the intestine where it is rapidly endocytosed. When the lectin enters the cells, it induces an enhanced metabolic activity that eventually leads to hyperplasia and hypertrophy of the small intestine (Pusztai et al., 1990). Moreover, ingestion of PHA or raw beans causes acute nausea followed by vomiting and diarrhea. The discomfort is so severe that experimental animals are very reluctant to consume a diet containing PHA, and in some instances they starve. The severe effects of PHA illustrate the potential of lectins in protection against predators.

Although most of the research on toxic lectins has been done with PHA, there is plenty of evidence that other lectins provoke similar effects. For instance, the lectins of black locust (*Robinia pseudoacacia*) and elderberry (*Sambucus nigra*) bark cause the same severe toxicity symptoms as PHA. Since both bark lectins are abundant, elderberry and black locust are never attacked by rodents, deer, or other wildlife, whereas the bark of surrounding lectin-free species, e.g. poplar, willow, and wild apple, is a favorite food for the same animals (Fig. 2).

Several other lectins from seeds and vegetative storage organs bind to the intestinal mucosa of rats and thus dis-

Figure 2. Illustration of the protective effect of toxic bark lectins against rodents.

turb the normal function of the intestine. In addition, some cause systemic effects such as an enlargement of the pancreas (Pusztai et al., 1993). These lectins may also play a role in the defense against predators. The presence of moderately toxic proteins in seeds and vegetative storage organs may be an evolutionary adaptation. Although the presence of toxic lectins may not completely protect a seed or plant part from consumption, the reaction of avoidance by the animal may be beneficial for the survival of the species.

CONCLUSIONS

Most plant lectins are probably involved in the plant's defense. Whereas direct interference with viruses and microorganisms are rather exceptional, the deleterious effects of plant lectins on predatory invertebrates and higher animals are obvious. Considering the abundance of lectins in storage organs and their storage protein-like behavior, we believe that plants accumulate part of their nitrogen reserve in the form of carbohydrate-binding proteins, which can be used as passive-defense proteins.

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